الجمهورية الجزائرية الديمقراطية الشعبية
وزارة التعليم العالي والبحث العلمي

Université Ferhat Abbas Sétif 1
Faculté des Sciences de la
Nature et de la Vie

جامعة فرحات عباس، سطيف 1
كلية علوم الطبيعة والحياة

Department of Biology And Animal Physiology

N°...../SNV/2020

THESIS

Presented by

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For the fulfilment of the requirements for the degree of

DOCTORATE 3rd cycle

BIOLOGY

Special filed**: ANIMAL PHYSIOLOGY**

Topic

Anti-inflammatory and antioxidant effect of *Oxalis cernua* areal part and root methanolic extracts

Presented publically in: 14/ 01 /2021

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Dedication

We dedicate this modest work to the scientific community

hoping that it would be useful to them.

Acknowledgements

First and foremost, I thank *Almighty Allah* for having given me the strength, and patience to achieve this study.

This work was funded by the Ministry of Higher Education and Scientific Research, and was carried out at tow laboratories of the University of Setif: The Laboratory of Phytotherapy Applied to Chronic Diseases under the responsibility of Pr. S. KHENOUF, and the Laboratory of Applied Biochemistry under the responsibility of Pr. L. ARAAR. I deeply thank both of them for welcoming me to within laboratories and for ensuring that this thesis was completed in best conditions.

I would like warmly to thank Pr. A. BAGHIANI and S. KHENOUF respectively for having supervised and co-supervised this subject. For the patience, advice and rereading of the manuscript, which were essential to the success of this thesis. I have particularly appreciated the confidence they were able to give me and the freedom of choice toward of my work. These are the conditions that reinforced my will to do scientific research, find here the expression of my deep gratitude and my deepest respect.

I would like also to show my sincere appreciation to Pr. Dahamna Saliha, Pr. Necib Youcef, and Dr. Bouaziz Amel for their time, interest and helpful comments.

My sincere gratitude goes to those who have faith in me and my intellect even when I didn't have faith in myself; who provided all the facilities to achieve this study, I appreciate all their contributions of time and ideas. They make my Ph.D. experience productive and stimulating.

I owe a great debt of gratitude to all my teachers, as time goes on, I realize more and more clearly the huge impact that they have had on my academic career. I express all my gratitude to all those who have fuelled my scientific curiosity, for their availability, and also for their great human values and precious advices.

My thanks are also extended to all my colleagues in laboratories (too many to list here but you know who you are!). The joy and enthusiasm they have for research were contagious and motivational for me, even during tough times in the Doctorate pursuit. Special thanks to all those who work with me throughout this period, for their help and encouragement.

I am deeply thankful to my twin CHAMCE; to my brothers; and also to my mom and dad who raised me with a love of morals and science, and supported me in all my pursuits. I owe a great debt of gratitude for their patience, encouragement, sacrifices, and unconditional love. Their role in my life was, and remains, immense. They have been with me all these years and have made them the best years of my life.

List of publication

- **Meriama belghoul,** Abderrahmane Baghiani, Seddik Khennouf, Lakhmici Arrar. 2020. Sub-acute oral toxicity and *in vivo* antioxidant properties of *Oxalis cernua*. South African Journal of Botany. Vol (133): 91-97.

List of communications

- **Meriama belghoul,** Abderrahmane Baghiani, Seddik Khennouf, Lakhmici Arrar. Phenolic content and antioxidant activity of *Oxalis sernua* extract. *9*^{es} Intarnational Scientific Bioressource Development. 3- 6 may 2018. Monastir, Tunisia.

- **Meriama belghoul,** Abderrahmane Baghiani, Seddik Khennouf, Lakhmici Arrar. *Oxalis sernua a*ntioxidant activity and phenolic content of chloroform, ethyl acetate and aqueous extract. Agriculture and biology Meetings (RAB). 6-7 may 2018. Constantine, Algeria.

- **Meriama belghoul,** Abderrahmane Baghiani, Seddik Khennouf, Lakhmici Arrar. *Oxalis cernua* antioxidant activity and phenolic content of hydromethanolic extract. 1er International Congress on Cancer Prevention and Screening. 23 June, 2018. Ferhat Abbas University. Setif-Algeria.

- **Meriama belghoul,** Abderrahmane Baghiani, Seddik Khennouf, Lakhmici Arrar. Antioxidant activity and phenolic content of *Oxalis sernua* methanolic root extract. 2nd International Conference on Bioanalysis: food and health. 15 th Decembre 2018. Institute of Applied Sciences and Technology. Mahdia-Tunisia.

الملخص

تهدف هذه الدراسة إلى تقييم السمية، النشاطية المضادة الأكسدة والخواص المضادة للإلتهابات للمستخلص الميثانولي لكل من الجزء الهوائي وجذور النبتة *Oxalis cernua*. تم استخلاص عديدات الفينول متبوعا بفصل اولي لإعطاء المستخلص الخام للجزء الهوائي AME و مستخلص الكلوروفورم CHE مستخلص الايثيل اسيتات EAE و المستخلص المائي AqE و بالإضافة الى المستخلص الخام للجزء الترابي RME. لسيقان وأوراق هذه النبتة على قيمة علاجية عالية، ولها أيضًا وظائف مضادة للإلتهابات وإزالة السمية، في حين يتم استخدام الجذور بشكل خاص بسبب خصائصها المدرة للبول. أظهر القياس الكمي لعديدات الفينول والفلافونويدات أن المستخلص الميثانولي للجزء الهوائي (AME) يحتوي على أعلى كمية منهما. تم تقييم نشاطها المضاد للأسدة مخبريا باستخدام عدة اختبارات مختلفة :مثل DPPH، ABTS، جذور الهيدروكسيل، قدرة الإرجاع، واستخلاب أيونات الحديد، وكذلك فحوصات تبييض البيثاكاروتين؛ والإختبار المضاد للإنحلال الدموي. علاوة على ذلك، تم تقييم النشاطية المضادة للإلتهابات في المختبر باستخدام اختبار تثبيط تحلل البروتينBSA وألبومين البيض. بينت نتائج هذه الدراسة أن المستخلصات التالية: EAE، AME، CHE، CHE، AqE ؛ CHE، و AME أظهرت أعلى نشاطية مضادة للأكسدة على التوالي. بالإضافة إلى ذلك، كان كل من مستخلصي AME وRME أكثر تثبيطا لتحلل البروتين. في السمية الحادة، لم تظهر المستخلصات الميثانولية لـ *Oxalis cernua* عند استعمال الجرعتين (2000 و5000 ملغم/كغم من وزن الجسم) مرة واحدة أي علامات سمية بيوكيميائية، ونسيجية طوال فترة 14 يومًا. عند استعمال الجرعات (100، 200، 400 ملغم/كغم من وزن الجسم) أثناء دراسة السمية الحادة خلال 21 يومًا على التوالي، لوحظ أن النشاطية المضادة للأكسدة التي تم تقييمها باستخدام اختبارDPPH وقدرة الإرجاع لم تعمل على تحسين حالة الأكسدة والإرجاع للبلازما. كما لوحظت بعض التغييرات للمؤشرات البيوكيميائية والنسيجية، إلا أنها كانت ضمن النطاق الفيزيولوجي. بينت نتائج سمية الكبد والتسمم الكلوي الناجم عن CCl_4، النشاطية المضادة للأكسدة لـــ *Oxalis cernua* عملت على إرجاع المعايير البيوكيميائية بشكل كبير إلى مستوياتها الطبيعية، كما أيد الفحص النسيجي نتائج الدراسة البيوكيميائية. بينت النشاطية المضادة للإلتهابات في الجسم الحي، أن مستخلص RME *Oxalis cernua* بجرعة (200 ملغم/كغم من وزن الجسم)، له تأثير قوي كمضاد للإلتهابات يقلل من وذمة الأذن التي يسببها Xylen. في نموذج carageennan، أظهر مستخلص AME *Oxalis ceernua* بجرعة (200 ملغم/كغم من وزن الجسم) تأثير قوي كمضاد للإلتهابات (80٪). إلى جانب ذلك، أظهر كلى المستخلصين تأثيرًا مؤيدًا للإلتهابات في نموذج ألبومين البيض. النتائج المتحصل عليها تعزز استخدام النبتة في الطب التقليدي.

الكلمات المفتاحية: *Oxalis cernua*، حمايضة، عديدات اليفينول، الفلافونويدات، السمية، الإجهاد التأكسدي، النشاطية المضادة للالتهابات.

Abstract

The aim of this study is to evaluate the toxicity, antioxidant and anti-inflammatory properties of *Oxalis cernua* areal part and root methanolic extracts. In folk therapy, *Oxalis cornea*'s stems and leaves have high medicinal values, and also have the functions of anti-inflammation and removing toxicity, whereas, the roots are used particularly due to their diuretic properties. Polyphenols extraction was followed by fractionation to give the crude extract of the aerial part AME, chloroform extract CHE, ethyl acetate extract EAE and aqueous extract AqE, in addition to the crude extract of the roots RME. Quantification of polyphenols and flavonoids revealed that AME contained the highest amounts. The evaluation of *in vitro* antioxidant activity was performed using several different methods: DPPH, ABTS, hydroxyl radicals; reducing power, ferrous ion chelating, and also β- carotene bleaching assays; and anti-haemolytic test. Furthermore, *in vitro* anti-inflammatory activity was assessed using BSA and egg albumin anti-denaturation assays. The findings of this study exhibited that EAE, AME, CHE, CHE, AqE; and CHE, AME noted the highest antioxidant activities respectively. Additionally, in *in vitro* ant-inflammatory both of AME and RME were the most potent anti-protein denaturation extracts. In acute toxicity, *Oxalis cernua* methanolic extracts were administered in a single highest dose of (2000 and 5000 mg/kg bw) did not cause any behavioural, biochemical, and histological signs of toxicity throughout a period of 14 days. In subacute toxicity study doses (100, 200, 400 mg/kg bw) were administered *per os* during 21 days successively. Antioxidant activity assessed with DPPH and reducing power tests did not improve plasma redox status. Biochemical and histological parameters showed some changes which remain inside physiological range. In hepatotoxicity and nephrotoxicity induced by CCl_4, *Oxalis cernua* antioxidant and biochemical parameters were significantly restored to near-normal levels. The histological examination endorsed the results of the biochemical study. In *in vivo* anti-inflammatory activity, *oxalis cernua* RME at dose (200 mg/kg bw), administered *per os*, revealed a potent anti-inflammatory effect reducing ear oedema induced wby xylene. In carrageenan model, *Oxalis cernua* AME at dose (200 mg/kg bw) displayed potential anti-inflammatory effect (80%). Besides, both extracts exhibited a pro-inflammatory effect in egg albumin model. The results obtained endorse the use of the plant in traditional medicine

Key words: *Oxalis cernua*, Hamaydha, polyphenols, flavonoids, toxicity, oxidative stress, anti-inflammatory activity.

Résumé

L'objectif de cette étude est d'évaluer la toxicité, les propriétés antioxydantes et anti-inflammatoires des extraits methanoliques de la partie aérienne et des racines de la plante *Oxalis cernua*. En thérapie traditionnelle, les tiges et les feuilles d'*Oxalis cernua* ont des valeurs médicinales cruciales et ont également des fonctions anti-inflammatoires en éliminant la toxicité, tandis que les racines sont utilisées en particulier en raison de leurs propriétés diurétiques. L'extraction des polyphénols a été suivi par fractionnement pour donner l'extrait brut de la partie aérienne AME, l'extrait chloroformique CHE, l'extrait d'acétate d'éthyle EAE et l'extrait aqueux AqE, en plus de l'extrait brut des racines RME. La quantification des polyphénols et des flavonoïdes a révélé que l'AME en contenait la plus grande quantité. L'évaluation de l'activité antioxydante, *in vitro*, a été réalisée en utilisant plusieurs méthodes différentes : DPPH, ABTS, radicaux hydroxyles ; pouvoir réducteur, chélation des ions ferreux, et également les tests de blanchissement de β-carotène ; et de l'activité anti-hémolytique. De plus, l'activité anti-inflammatoire *in vitro* a été évaluée en utilisant des tests de l'anti-dénaturation protéique de la BSA et de l'albumine d'œuf. Les résultats de cette étude ont montré que EAE, AME, CHE, CHE, AqE; et CHE, AME ont eu respectivement l'activité antioxydante la plus élevée. De plus, l'activité anti-inflammatoires, *in vitro*, des extraits AME et de RME était puissante. Concernant la toxicité aiguë, les extraits méthanoliques d'*Oxalis cernua*, lorsqu'ils sont administrés en une seule dose maximale de (2000 et 5000 mg/kg pc), n'ont présenté aucun signe de toxicité comportementale, biochimique et histologique durant une période de 14 jours. Au cours de la toxicité subaiguë les doses (100, 200, 400 mg/kg pc) ont été administrées *per os* pendant 21 jours successifs. L'activité antioxydante évaluée par le test DPPH et le pouvoir réducteur n'a pas amélioré le statut redox plasmatique. Les paramètres biochimiques et histologiques ont noté quelques changements qui restent dans l'intervalle physiologique. Dans l'hépatotoxicité et la néphrotoxicité induites par CCl_4, les paramètres antioxydants et biochimiques *d'Oxalis cernua* ont été significativement restaurés à des niveaux presque normaux. L'examen anatomique et histologique a confirmé les résultats de l'étude biochimique. Dans l'activité anti-inflammatoire *in vivo*, *Oxalis cernua* RME à la dose (200 mg/kg pc) administré *per os*, a révélé un puissant effet anti-inflammatoire réduisant l'œdème de l'oreille induit par le xylène. Dans le modèle de carageennan, *Oxalis cernua* AME à la dose (200 mg/kg pc) a présenté un effet anti-inflammatoire potentiel (80%). En outre, les deux extraits ont montré un effet pro-inflammatoire dans le modèle d'albumine d'œuf. Les resultats obtenus support l'utilisation de cette plante en médecine traditionnelle.

Mots clés : *Oxalis cernua*, Hamaydha, polyphénols, flavonoïdes, toxicité, stress oxydatif, activité anti-inflammatoire.

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List of abbreviations

AAPH: 2,2-azobis amidinopropane hydrochloride

ABTS: Acid 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonique)

ALP: alkaline phosphatase

ALT: Alanine aminotransferase

AME: Areal part methanolic extract

AqE: Aqueous extract

Asp: Aspirin

AST: Aspartate aminotransferase

BHT: 2,6-di-tert-butyl-4-methylfenol

BSA: Bovine serum albumin

CAT: Catalase

CHE: Chloroform extract

COX: Cyclooxygenase

DDT: Dichlorodiphenyltrichloroethane

Dic: Diclofenac Sodium

DNTB: 5,5 0- dithiobis-2-nitrobenzoic acid

DPPH: 1,1-diphenyl-2-picryl-hydrazil

DUOX: Dual oxidase

dw: Dry weight

EAE: Ethylacetate extract

EC_{50}: Effective concentration of 50%

EDTA: Ethylenediaminetetraacetic acid

Fe: Iron

Fe^{2+}: Ferrous iron

Fe^{3+}: Ferric iron

GAE: Gallic acid equivalent

GCs: Glucocorticoids

GPx: Peroxidase

GR: Glutathione reductase

GSH: Glutathione

GSSG: Glutathione disulfide

GSTs: Glutathione S-Transferases

HT_{50}: Half-time of 50% of haemolysis

I%: Inhibition percentage.

IC_{50}: Inhibitory concentration of 50% of activity

IL: Interleukin

LD_{50}: Lethal the dose 50

LOX: Lipoxygenase

MDA: Malondialdehyde

NADH/ NAD^+ /NAD: Nicotinamide adenine dinucleotide

NADPH: Nicotinamide adenine dinucleotide phosphate

$NO^{\cdot}$: Nitric oxide

NOX: NADPH oxidase

NSAIDs: Nonsteroidal anti-inflammatory drugs

$O^{\bullet}_2$: Superoxide anion

OCDE: Organisation for Economic Co-operation and Development

$OH^{\cdot}$: Hydroxyl

QE: Quercetin equivalents

RME: Root methanolic extract

ROS: Reactive oxygen species

SOD: Superoxide dismutase

TBA: 2-thiobarbituric acid

TCA: Trichloroacetic acid

TNF-α: Tumor necrosis factor alpha

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Introduction

Introduction

Excessive generation and accumulation of reactive oxygen species characterize the reduction of the basic homeostatic state of the cell or tissue and causes oxidative stress. The latter provides a major contribution to a wide range of illnesses including inflammatory diseases. Hence, the balance between oxidants and antioxidants is vital for cell function, regulation, adaptation and growth.

However, the mammalian system is naturally equipped to effectively deal with oxidative stress using endogenous and exogenous antioxidant defence mechanisms. Those act simultaneously against ROS such as catalase, superoxide dismutase, lipoic acid, melatonin, uric acid, glutathione, bilirubin, vitamins (vitamin A, C and E) as well as carotenoids, trace elements, flavonoids, and polyphenols, which help to maintain the cellular redox environment.

Since ancient times, phytotherapy has been used to cure a variety of diseases. Recently, it is getting worldwide interest through scientific evidences. Medicinal plants contain diverse compounds with significant biological and antioxidant activities. Their particular structures enable them to neutralize both of the oxidants and the drugs' side-effects through different mechanises.

Oxalis cernua was introduced into the Mediterranean basin from the Cape Region (South Africa) most probably multiple times, then it has spread widely across many parts of the world afterwards. Its sour taste derives from the high content in oxalic acid. In folk prescription, the roots are used owing to their diuretic properties, stems and leaves have neuroprotective and antihypertensive effects. It has the properties of anti-inflammation, clearing heart, removing toxicity and analgesia.

The evidences describing the biological effects of *Oxalis cernua* are increasing, besides, to the best of our knowledge, there are no reports concerning its toxicological profile, antioxidant, and anti-inflammatory effects. Therefore, in an attempt to explore the scientific basis of this properties, it appears fundamental to formulate the following objectives:

- Quantification of phenolic compounds of *Oxalis cernua* areal part and root methanolic extracts.
- Evaluation of *in vitro* antioxidant activity of *Oxalis cernua* areal part and root methanolic extracts.
- Evaluation of *in vitro* anti-inflammatory activity of *Oxalis cernua* methanolic areal part and root extracts.
- Assessment of acute toxicity of *Oxalis cernua* areal part and root methanolic extracts.
- Assessment of subacute toxicity of *Oxalis cernua* areal part and root methanolic extracts.
- Assessment of *in vivo* antioxidant activity of *Oxalis cernua* areal part and root methanolic extracts.
- Assessment of anti-hepatotoxicity and anti-nephrotoxicity of *Oxalis cernua* areal part and root methanolic extracts induced by CCl_4.
- Assessment of *in vivo* anti-inflammatory activity of *Oxalis cernua* methanolic areal part and root extracts.

Review of literature

1. Redox state and oxidative stress

Electron flow is the basis of life on earth, whether it is driven by light in photosynthesis or through chemical redox couples in respiration (Foyer, 2018). All forms of life maintain a steady state concentration of reactive oxygen species (ROS) determined by the balance between their rates of production and removal by various antioxidants. Each cell is characterized by a particular concentration of reducing species, stored in many cellular constituents, which determine the redox state of a cell. By definition, redox state is the total reduction potential or the reducing capacity of all the redox couples such as glutathione disulfide (GSSG/2GSH) found in biological fluids, organelles, cells or tissues. This state not only describes the state of a redox pair, but also the redox environment of a cell. Under normal conditions, the redox state of a biological system is maintained towards more negative redox potential values. However, this balance can be disturbed when the level of ROS exceed and/or the levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cellular damage or apoptosis of normal cells if the stress is massive and prolonged (Singh et *al*., 2018).

1.1. Definition of oxidative stress

Oxidative stress is an imbalance between the production of free radicals and the defence mechanisms of associated antioxidants (Samet and Wages, 2018). It is also defined as a disturbance of the equilibrium between the excessive production and/or the reduction of the clearance of highly reactive molecules including in particular ROS, and the mechanisms of elimination or defence of antioxidants and/or the ability of cells and tissues to detoxify or trap them (Singh et *al*., 2018; Cannavò et *al*., 2019).

Despite the absence of an unpaired electron, some molecules can easily react with other molecules, leading to the formation of additional oxidizing substances, which are not radicals, but reveal oxidative properties, generated from oxygen and nitrogen such as, ozone (O_3),

nitrogen acid (HNO_2), dinitrogen trioxide (N_2O_3), hypochlorous acid (HOCl), and hypobromous acid (HOBr) (Singh et *al*., 2018).

1.2. Types of oxidative stress

The 21st century toxicology paradigm "The dose makes the poison" is appropriately applicable to oxidative stress. It is the dose in spatiotemporal context which is crucial. From the definition above, oxidative stress could be classified into two categories, according to the intensity to: acute (or eustress) and chronic (or distress) stress (Cannavò et *al*., 2019): the first is related to the temporary increase in ROS that returns to normal levels. It is considered as a physiological oxidative stress (eustress). In this case, low exposure of cells and organisms is utilized for redox signalling by addressing specific targets. Besides, this phenomenon is transient and does not have significant changes in humans' health. The second is more harmful and related to the difficulty of cells to neutralize increased amounts of ROS that cause chain reactions creating new radicals. This toxic oxidative burden which damages biomolecules (distress), whereas high exposure results in disruption of redox signalling and/or damage to unspecific targets. It is behind the onset of diseases such as diabetes (Chacko et *al*., 2016; Wojtunik-Kulesza et *al*., 2016; Ighodaro, 2018; Cannavò et *al*., 2019).

1.3. Free radicals

The small bio-regulator molecule defining aerobic life forms performing hundreds of tasks at tissues level is effectively delivered to cells despite its limited solubility (Asmat et *al*., 2016; Cortese-Krott et *al*., 2017; Bordt, 2018). Under physiological conditions and due to its reactive nature, each cell, *in vivo*, diverts approximately four percent of the oxygen consumed, every 24h, to form nearly 1 billion molecules of ROS. This process is largely modulated by mitochondria, which uses oxygen as the terminal electron acceptor to produce energy through the electron transport chain via the biochemical process of oxidative phosphorylation (Dzal et *al*., 2015; Jagannathan et *al*., 2016; Sanchez-Morillo et *al*., 2017). Thereby, oxygen remains a fundamental biological necessity for energy production.

1.3.1. Definition of free radicals

Free radicals are atomic or molecular species capable of independent existence with one or more unpaired electrons in their structure. These reactive chemical entities are generated as oxygen by-products, performing several cellular functions at low concentrations and exerting oxidative stress when they are produced at excess amount (Arman et *al*., 2019). Free radicals are generally unstable, produced under physiological and pathological conditions (Table 1), among which: superoxide anion radical, the hydroxyl radical, singlet oxygen, hydrogen peroxide, nitric oxide, and peroxynitrite too (Aslani and Ghobadi, 2016).

Table 1: Examples of reactive oxygen species forms (Wojtunik-Kulesza et *al*., 2016).

Free radicals	Non-radicalss
Hydroxyl radical ($^{\bullet}OH$)	Hydrogen peroxide (H_2O_2)
Superoxide anion ($^{\bullet}O_2^-$)	Singlet oxygen ($^1O_2$)
Lipid peroxyl ($^{\bullet}LOO^-$)	Ozone (O_3)
Nitric oxide ($^{\bullet}NO^-$)	Lipid peroxide ($LOO^{\bullet}$)
Nitrous acid (HNO_2^-)	Peroxynitrite ($ONOO^-$)

1.4. Sources of reactive oxygen species

In aerobic environment, 90% of consumed oxygen is reduced to water, while the remaining 10% is reduced to a superoxide anion radical or a hydrogen peroxide molecule. The reactive molecules are of endogenous and exogenous origin (Wojtunik-Kulesza et *al*., 2016).

1.4.1. Exogenous sources

Exogenous sources of free radicals are numerous namely: ionizing or solar radiation (UV, visible, infrared-A), ozone, increased caloric intake due to lifestyle changes, alcoholism,

smoking, metals (such as: iron, copper, chromium, lead, mercury, nickel and vanadium), and xenobiotics (such as: food additives, drugs, poisons, pesticides and certain pollutants). Some factors are unavoidable, while others generate huge amounts of harmful molecules, can be avoided (Sies, 2018; Umm-e-Ammara Warraich and Kayani, 2020).

1.4.2. Endogenous sources

1.4.2.1. NADPH oxidases

Nicotinamide adenine dinucleotide phosphate oxidases (NADPH) are membrane-bound enzyme complexes that transfer electrons from NADPH to oxygen, generating superoxide. Currently, NADPH has seven isoforms located in the plasma membrane, phagosomes and also widely expressed in various tissues. A ligand-initiated enzyme with NADPH/NADH oxidase (NOX) action has been appearing to be available in an assortment of non-phagocytic cells including endothelial cells, chondrocytes, fibroblasts and smooth muscle cells. NOX1, NOX2, NOX3 require additional cytosolic activators; whereas NOX4 is constitutively active and independent of an activator protein, NOX5, dual oxidase DUOX1/2 need intracellular calcium to activate ROS generation (Han, 2016; Phull et *al*., 2018).

1.4.2.2. Xanthine oxidoreductase

Xanthine oxidoreductase (XOR), member of molybdenum hydroxylase, is responsible for purine catabolism, catalysing the oxidation of hypoxanthine to xanthine and of xanthine to uric acid, which, *in vivo*, possesses both antioxidant and pro-oxidant activities. In mammals, there is two interconvertible forms: xanthine dehydrogenase, which prefers NAD^+ as an electron acceptor, and xanthine oxidase, which transfers the electrons directly to molecular oxygen, with the production of the ROS superoxide anion and hydrogen peroxide. The production rate depends on O_2 tension, pH and purine concentration. Circulating XOR may bind to endothelial cells, where it is responsible for remote organ injury and has been implicated in the development

of endothelial dysfunction. The dysregulation of these enzymes is associated with impaired vascular function and cardiovascular diseases. Hence, the precipitation of uric acid crystals in the soft joints and tissues causes gout, a chronic inflammatory condition (Battelli et *al*., 2014; Dong, Chao et *al*., 2020).

1.4.2.3. Mitochondria

Generally, the rate of mitochondrial ROS generation is rather low and results in minimal damage, because mitochondria have a highly efficient antioxidant defence system. Though, in numerous conditions, high rates of ROS production occur, and may escape the scavenging systems and alter mitochondrial functions (Di Meo et *al*., 2016).

It is considered to be the major site of ROS production, where the oxygen used in this process ranges from 0.15% to 4%, due to the presence of the electron transport chain during oxidative phosphorylation by maintaining basal adenosine triphosphate levels. Free radicals are also produced in the endoplasmic reticulum, plasma and nuclear membranes, lysosomes and peroxisomes; during activation of inflammatory cells; reactions related to phagocytosis, prostaglandin synthesis and metabolism; during the activity of certain enzymes including oxidases, cyclooxygenases, lipoxygenases, dehydrogenases and peroxidases as well as cytochrome P450 (Görlach et *al*., 2015; Czarny et *al*., 2018).

1.4.2.4. Myeloperoxidase (MPO)

Myeloperoxidase is a pro-inflammatory enzyme stored in the azurophilic granules of neutrophilic granulocytes. MPO catalyses the formation of hypochlorous acid from hydrogen peroxide, and generates several intermediates of ROS, which play physiological role in innate host defences including: singlet oxygen, chlorine, tyrosyl radicals, cross-links proteins, chloramines, hydroxyl radicals, and ozone. Conversely, these same toxic agents can be released to the outside of the cell, where they can attack normal tissue and thus contribute to the

pathogenesis of several diseases such as atherosclerosis, myocardial infarction, atrial fibrillation, multiple sclerosis, Alzheimer's disease, lung cancer, and transplant rejection (Vita and Brennan, 2004; Klebanoff, 2005; Pulli et *al*., 2013).

1.5. The biological roles of ROS

ROS are produced and scavenged at different rates in different cellular compartment continuously in the biological system, and play an essential role in physiological processes including: cell differentiation, protein phosphorylation, activation of transcription factors, apoptosis, steroidogenesis and cellular defence against microorganisms. Besides, energy production, cell signalling, cytoskeletal regulation, cell migration and contraction, cellular communication, significant changes in gene expression, and stimulation or inhibition of signal transduction (Aslani and Ghobadi, 2016; Zandalinas and Mittler, 2018).

1.6. The harmful effects of ROS on biomolecules

1.6.1. Effects on carbohydrates

Excessive ROS production results in several deleterious events, including irreversible modification of carbohydrates. Subsequently, the damage can result in the impairment of the cellular receptor functions such as those associated with hormonal and neurotransmitter responses, IL (interleukin) activities and prostaglandin formation (Mohamed et *al*., 2016; El-haskoury et *al*., 2018).

1.6.2. Effects on lipids

The interaction between lipids and ROS produce the peroxidation of fatty acids, which alters lipid bilayer of cell membrane and generates free radicals. This process is potentially dangerous to cells, leading to changes in membrane fluidity, permeability, transport properties and incorporated proteins. In mitochondria, phospholipids may alter the integrity of

permeability of transition pores, disaggregate the respiratory chain's complexes, and enhance electron leakage within the intermembrane too (Perillo et *al*., 2020).

1.6.3. Effects on DNA

Excessive production of ROS can lead to overexpression of oncogenic or mutagenic genes such as those involved in cell cycle control, metabolic rate and cellular response. The most common deoxyribonucleic acid (DNA) alterations are 8-oxoguanine and/or guanosine. Mitochondrial DNA is more sensitive than nuclear DNA as it is not covered by histones, so the probability of oxidative changes in the coding region is high (Mikhed et *al*., 2015; Aslani and Ghobadi, 2016; Czarny et *al*., 2018).

1.6.4. Effects on proteins

Several posttranslational protein modifications including: oxidation of sulfur-containing side chains of cysteine and methionine residues; oxidation of tryptophans, histidines, dityrosine and others are the results of ROS harmful effects. Modified proteins can lead to aggregation, oligomerization, destabilization, fragmentation, and/or enhanced proteins' degradation, while some irreversible protein modifications may accumulate and lead to cell dysfunction (Dahl et *al*., 2015; Tramutola et *al*., 2017).

1.6.5. Homeostasis and diseases related to oxidative stress

Reduced levels of ROS play a major role in organism's homeostasis carrying out the vital physiological processes. Nonetheless, the excess production of ROS alters the dynamic equilibrium, which can deteriorate biomolecules and cause several acute and chronic pathological conditions such as cardiovascular diseases, acute and chronic kidney disease, neurodegenerative diseases, macular degeneration, biliary diseases, cancer, obesity, diabetes, hypertension, atherosclerosis, inflammation and also aging. Nevertheless, the ability of

antioxidants to protect cells from ROS and to minimize their deleterious effects became a scientific basis that increased its worldwide acceptance with regards to its beneficial effects (Umm-e-Ammara Warraich and Kayani, 2020).

2. Antioxidants

Antioxidants are compounds that cover different classes of molecules, and also that can safely interact with free radicals and terminate the chain reaction or delay oxidative damage before vital molecules are damaged. Their effectiveness depends on their chemical characteristics as well as their physical location. They can use numerous mechanisms such as free radicals' trapping, chelation by metals, breaking the auto-oxidation chain reaction, the reduction of localized concentrations of $O^{\bullet 2}$, and enzyme induction also (Oroian and Escriche, 2015; Pisoschi and Pop, 2015).

2.1. Enzymatic antioxidants

2.1.1. Glutathione peroxidase (GPx)

Glutathione Peroxidase is an intracellular enzyme that use, most times, selenium as a cofactor. In humans, there are at least eight GPx enzymes (GPx1– GPx8), which are respectively mapped to chromosomes 3, 14, 5, 19, 6, 6, 1 and 5. In mitochondria and cytosol, glutathione peroxidase catalyses hydrogen peroxides to water, and lipid peroxides to their corresponding alcohols to inhibit lipid peroxidation process protecting cells from oxidative stress. Conversely, lower GPx activity is susceptible to impair antioxidant protective defences leading the development of many common and complex diseases, including cancer and cardiovascular disease (Ighodaro, 2018).

2.1.2. Glutathione reductase (GR)

Glutathione reductase is an essential enzyme that belongs to the family of NADPH-dependent oxidoreductase, which recycles oxidised glutathione back to the reduced form. It tends to accumulate in cellular regions of high electron flux, where reactive species are generated. Besides, it plays an important role in ROS detoxification, and GSH regeneration.

The detoxification of peroxides and the maintenance of the proper GSH-GSSG redox cycling are crucial events to avoid extreme oxidative events toward lipids peroxidation, protein and nucleic acids as well. Additionally, it has been reported that GR homeostasis alteration and continuous oxidative stress has been frequently associated with the development and progression of numerous illnesses, including, autism, cancer, diabetes mellitus, neurodegenerative disorders, cystic fibrosis and aging (Farina and Aschner, 2019).

2.1.3. Glutathione S-transferases (GSTs)

Glutathione-S-transferases are a functionally varied family of enzymes that utilize GSH in conjugation reactions. In this multigene enzyme family, there are currently seven human cytosolic GSTs (known as alpha, mu, pi, sigma, theta, zeta and omega). Moreover, the genes controlling their synthesis have polymorphic variants (Kolesnikova et *al*., 2017; Rae and Williams, 2017).

GSTs detoxify a large number of electrophilic exogenous toxic agents like carcinogens, drugs (chemotherapeutic agents of cancer such as adriamycin, busulfan, carmustine, chlorambucil, melphalan, mitozantrone, thiotepa, cyclophosphamide and ethacrynic acid), and environmental pollutants and their metabolites (acrolein, atrazine, DDT ' dichlorodiphenyltrichloroethane', inorganic arsenic, lindane, malathion, methyl parathion, muconaldehyde and tridiphane) by their conjugation with glutathione. Thereby, the imbalance in the detoxification system can be the cause of various pathological processes including infertility (Dasari et *al*., 2017; Kolesnikova et *al*., 2017; Rae and Williams, 2017).

2.1.4. Superoxide dismutase (SOD)

Superoxide dismutase is the first detoxification enzyme and the most powerful antioxidant in the cell. It protects cell from excessive ROS, the harmful agents that promote aging or cell death through catalysing the dismutation of two molecules of superoxide anion ($O^{\bullet}_2$) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Several SOD isoforms differ in the nature of the active metal centre: (iron (Fe), zinc (Zn) copper (Cu) and manganese (Mn)). They are found in prokaryotes and chloroplasts of some plants, in eukaryotes, and in prokaryotes and mitochondria of eukaryotes respectively. These isozymes are encoded by different genes mapping to chromosome 21, 6, and 4. In animals and humans, a number of pathologies has been attributed to SOD deficiency such as myocardial injury, cerebral vascular hypertrophy, and neurodegeneration (Ighodaro and Akinloye, 2018).

2.1.5. Catalase (CAT)

Catalase has a prime role as a member of the enzymatic antioxidant defence system in regulating the cellular level of hydrogen peroxide protecting the cells from oxidative stress damage. In mammalian, CAT is expressed in the majority of cells and has high concentrations in the liver and erythrocytes. According to the structural and functional similarities, CATs are divided into three groups: typical or mono-functional catalases, catalase peroxidases, and non-heme catalases. Their main function is to utilize the heme group to reduce/oxidize hydrogen peroxide (H_2O_2) to water and molecular oxygen with rates up to 40 million molecules per second in a two-step process (Tehrani and Moosavi-Movahedi, 2018; Gebicka and Krych-Madej, 2019).

$$\text{Catalase} + H_2O_2 \longrightarrow \text{Compound I} + H_2O \qquad (1)$$

$$\text{Compound I} + H_2O_2 \longrightarrow \text{Catalase} + H_2O + O_2 \qquad (2)$$

Numerous ailments are associated with catalase deficiency or malfunctioning namely: cardiovascular diseases, diabetes mellitus, hypertension, anaemia, some dermatological disorders, vitiligo, Alzheimer's disease, bipolar disorder, and schizophrenia. Moreover, it has been reported that an anomaly of catalase activity is inherited in acatalasemia (Nandi et *al*., 2019).

2.1.6. Peroxiredoxin (Prx)

Peroxidases, an evolutionarily conserved family, are antioxidant proteins with a key role in detoxification and protection from oxidative stress as well as in signalling. These abundant thiol proteins are highly efficient at reducing hydrogen peroxide, peroxynitrite and other hydroperoxides up to one million times higher than for most other thiol proteins. Mammals have 6 isoforms divided into three subgroups distributed between different organelles (cytosol, mitochondria, nucleus, endoplasmic reticulum, peroxisomes). Perturbation in the expression level of several Prxs under the alteration of the redox state balance has been reported in human aging, cancer, Down's syndrome, neurodegenerative disease, Alzheimer disease, inflammation and Parkinson disease as well (Bell and Hardingham, 2011; Hampton et *al*., 2018).

2.1.7. Lipoxygenase (LOX)

Lipoxygenase is a member of the non-heme iron-containing enzyme family, and it stimulates the insertion of molecular oxygen into polyunsaturated fatty acids, including arachidonic, linoleic acids, and eicosapentaenoic acid. In humans, several types based on the position of the carbon atom where the molecular oxygen is incorporated, therefore, lipoxygenases are classified as 5-, 8-, 12-, and 15-lipoxygenase (Dong, Changqing et *al*., 2020). Oxygenated lipids can initiate subsequent biological reactions and activate cell signalling mechanisms via specific cell surface or metabolic receptors. A large body of evidences has

proven the activity and the role of LOXs in inflammation, sensitization and many sicknesses such as atherosclerosis, neuronal disorder, and tumor (Mashima and Okuyama, 2015).

2.2. Non-enzymatic antioxidants

2.2.1. Glutathione (GSH)

Glutathione is a tripeptide that is synthesized in cytosol (0.5–10 mM) from glutamic acid, cysteine, and glycine. It exists in thiol-reduced form (GSH) (98%, except in endoplasmic reticulum) and disulfide-oxidized forms (GSSG) (2%, potentially toxic), both of them are used as primary indicator of cellular redox status. GSH is the cell's major antioxidant owing to its numerous cellular processes, comprising scavenging ROS, modulation of cell proliferation, detoxification of xenobiotic and endogenous compounds, transport and storage of cysteine and maintenance of redox status, regulate redox-dependent cell signalling, and also involved in several metabolic processes (synthesis of proteins and DNA, enzyme activity, metabolism, gene expression, signal transduction, and the intensification of cytoplasmic and transmembrane transport). Its disequilibrium is involved in numerous pathological pathways and thus plays an important role in cancer and regulation, cell cycle, survival, growth, and death (Corso and Acco, 2018; Ferguson and Bridge, 2019).

2.2.2. Vitamin C

Vitamin C (ascorbic acid or ascorbate) is the most important water soluble antioxidant obtained by diet, its main biologically active form is L-ascorbic acid. However, ascorbate has a varied chemistry, enabling the following functions: regenerate the reduced state of vitamin E in membranes and lipoproteins; protect thiol groups of proteins against oxidation and remove H_2O_2 in organisms; detoxify radicals via trapping superoxide anions, hydroxyl radicals,

hydrogen peroxide, nitrogen species and singlet oxygen; and the reduction of Fe^{3+} to facilitate Fe uptake (Smirnoff, 2018).

2.2.3. Carotenoids

Carotenoids are common pigments (with yellow, orange, and red colours) with antioxidant activities. Their structures may be cyclized and have different numbers of hydrogen atoms, or possess oxygen-containing functional groups, the latter named as xanthophylls. Common carotenes in the human diet include beta-carotene, alfa-carotene and lycopene, whereas xanthophylls include: lutein, zeaxanthine, cryptoxanthin, canthaxanthin, astaxanthin and fucoxanthin (Chandrasekara and Shahidi, 2018).

2.2.4. Polyphenols

Polyphenols represent one of the largest categories of plant components, with more than 8000 phenolic structures that have been reported. They are ubiquitous in all parts of the plant, the main groups are flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans. Combinations of different types of polyphenols or polyphenols in combination with other phytochemicals / nutrients may also confer superior efficacy (Oyenihi and Smith, 2019).

Polyphenols inactivating free radicals according to two mechanisms: the first is the mechanism of hydrogen atoms transfer which assumes that the antioxidant reacts with the free radical by transfer of hydrogen atoms, and the products of this reaction are a harmless species and an oxidized radical. The second is the mechanism of single electron transfer, which assumes that the oxidant gives an electron to the antioxidant molecule whose reaction products are a cation radical and an energetically stable species (Zeb, 2020).

2.2.4.1. Types of polyphenols

- **Flavonoids**

Flavonoids are characterized by the presence of a flavone structure of 15 carbons in their skeleton, containing aromatic and heterocyclic rings (amino acids phenylalanine and tyrosine). They can be divided into several subclasses, the most representative of which are: flavones, flavonols, flavanols, flavanones, anthocyanidins and isoflavones. Their hydroxyl groups are responsible for trapping free radicals on which the antioxidant activity depends (Maan et *al*., 2020).

- **Phenolic acids**

These compounds consist of a benzene ring linked to a carboxylic group (benzoic acid) or to a propenoic (cinnamic), which showed a high potential as antioxidants. They can be divided into two categories according to their structure: hydroxybenzoic acids and hydroxycinnamic acids. Phenolic acids, readily absorbed through intestinal tract walls, are beneficial to human health due to their potential antioxidants and avert the damage of cells resulted from free-radical oxidation reactions. They also promote the anti-inflammation capacity of human beings (Kumar and Goel, 2019).

- **Tannins**

They are produced by plants as secondary metabolites with a wide diversity in structure and have the ability to bind and precipitate proteins. Depending on their structure, tannins can be divided into two classes of macromolecules called condensed tannins, hydrolysable tannins and complex tannins (Chandrasekara and Shahidi, 2018).

2.2.4.2. Biological activity of polyphenols

Currently, the interest in dietary phenolic compounds has increased due to their anti-free radical capacities, antioxidant and anti-inflammatory properties, modulation of signal transduction, antimicrobial activities, anti-proliferation and inhibition of lipoprotein oxidation low density. They can also exert an indirect antioxidant effect, protecting endogenous antioxidant enzymes in humans' body, which have a role in the prevention of degenerative diseases (Aslani and Ghobadi, 2016).

3. Toxicity of plants

Traditional medicine has relied on phytotherapy for millennia, in addition mankind has learnt by experience and observation to use plants correctly, building up an extensive traditional culture of their use. Over the last hundred years or so, scientific and technical progress and specifically the pharmacognosy studies of many of these plants has allowed the discovery of a large number of new molecules and their mechanisms of action. Modern scientific research has confirmed the importance of these molecules and the ability of extracts to work in synergy with all components, focusing on improving the efficacy and safety of their use in patient care (Colalto, 2018). The use of herbal medicine and the interest in related products by patients has increased around the world due to its presumptive efficiency, availability, and common acceptance (Alonso-Castro et *al*., 2017).

The important and irreplaceable sources of phytotherapy in both developed and developing countries are commonly employed for the treatment of various diseases. Medicinal plants are considered as the first line remedies and the primary form of healthcare for up to 80% of the world's population. On the other hand, the long history of treatment using medicinal plants gives us a false feeling of safety and an assumption of minimal side effects. The plants materials used include seeds, berries, roots, rhizomes, leaves, bark and flowers. Usually they

have a lower rate of side effects than synthetic drugs, as teas, powdered medicines, or alcoholic extracts (tinctures, drops) for decades or even centuries. Despite the fact that side effects and interactions cannot be excluded (Awodele et *al*., 2013; Kopp, 2017; Ekar and Kreft, 2019).

Toxicity was defined as the potential of substances, extract of plants or toxin compounds, to exert a harmful effect on humans or animals (cell, organ or the entire body) (Kharchoufa et *al*., 2018). In humans, medicinal plants' toxicity is influenced by toxic metabolites' concentration, solubility, and the frequency of intoxication. Also, chronic diseases, patient's age, and nutritional status. Undesirable side effects of medicinal plants can be trigger as a consequence of the presence of contaminants (drugs, hormones, heavy metals) and pathogenic microorganisms, intrinsic effects, pharmacokinetic and pharmacodynamic interaction with prescribed drugs (Bagnis et *al*., 2004; Jha, 2010; Alonso-Castro et *al*., 2017).

Some plant's secondary metabolites such as alkaloids, glycosides, saponins, oxalates, and tannins are considered toxic through contact, inhalation or ingestion. They are capable of causing injury, disease and even death. Plants' toxicity depends on several factors, namely the different chemicals that characterize theme, the part of the plant ingested, their concentration, the chemical substances present, the age, the physical conditions of development, and the ripening state of its fruits are aspects to consider (Serrano, 2018).

Plants' toxicity can affect the entire spectrum of organ systems: such as digestive (main route of administration), liver (site of degradation), skin, renal (the main route of degradation and excretion), nervous (highly perfused by blood), respiratory and cardiovascular systems. Whereas, some plants have a large spectrum of toxicity affecting more than one organ system (Strickland et *al*., 2018).

The plants' harmful effects range from minor symptoms such as mild headache and abdominal discomfort to much more serious outcomes that can cause major organ damage and

even death namely: in the gastro-intestine (diarrhoea, vomiting, nausea), the brain (depression, anorexia, convulsions, paralysis, dizziness, shivers, coma, stimulation, tremors, convulsions, paresis, and abnormal behaviour), the kidney (necrosis, acute renal failure, urination) and the liver (dysfunction, cell destruction, necrosis, lobar hepatitis) (Hamm et *al*., 2017; Serrano, 2018).

3. 1. Acute and sub-acute toxicity

For animals, oral route, intraperitoneal and intravenous are the most common mods of administration. Acute oral toxicity determination is a preliminary screening phase in the evaluation and estimation of toxic characteristics of plants' constituents. Besides, the duration is the feature that determine the experiment as acute, subacute, sub-chronic and chronic. However, it was reported in the literature that toxicity levels could be as following: mild (non-life- threatening), moderate (life-threatening, with adverse reactions), and severe (death occurrence). Acute toxicity of medicinal plants could recognise their ability to trigger harmful effects upon exposure to a single dose at a distant point from the site of administration. In rodents, the data collected will allow to calculate doses for longer-term experiments, to recognise and classify the target organs for toxicity, and to evaluate the effects of unintended ingestion too. Since all substances are potentially toxic at sufficiently high doses, acute toxicity is involved to determine the level of toxicity through the determination of Lethal Dose fifty (LD_{50}), which is the expected dose of the medicinal plant that kill 50% of the animals tested (Table 2) (Bhardwaj and Gupta, 2012; Hamm et *al*., 2017; Strickland et *al*., 2018).

Table 2: Toxicity classes with respect to LD_{50} determination in rats (single *Per Os* administration) (Bhardwaj and Gupta, 2012).

Class	Description	LD_{50} (mg/kg)
1	Extreme toxicity	<1
2	High toxicity	1-50
3	Moderate toxicity	50-500
4	Low or slight toxicity	500-5000
5	Practically non-toxic	50000-15000
6	Relatively harmless	>15000

In sub-acute toxicity, well-defined doses of medicinal plant extract are daily administered *per os* for short to long-term periods of time ranging from 2 weeks to 1 year. During this study, detailed behavioural and clinical observations, and pathological examinations are conducted and collected to characterize the toxic effects of the extract (Bhardwaj and Gupta, 2012).

3.2. Targeted systems

3.2.1. Liver

3.2.1.1. Structure and function

The liver is a vital organ only found in vertebrates. In humans, it measures roughly 1500 g. Suspended by ligaments, it occupies the space inferior to the right and a portion of the left diaphragm and is enveloped by a fibrous membrane (Glisson's capsule). Except for a bare area where it directly abuts the diaphragm, the liver is covered by visceral peritoneum (Juza and Pauli, 2014).

It has 4 lobes with comparable fundamental portal and hepatic venous systems and also individual variation (Figure 1). Histologically, no less than 15 different cell types were found. Hepatocytes are the most frequent and comprise 60% of the total cells. Sinusoidal endothelial cells, Kupffer cells (specialized liver macrophages with immunological and phagocytic functions), hepatic stellate cells (perisinusoidal or Ito cells), and biliary epithelium. To complete thousands of vital functions, the hepatocyte contains smooth and rough endoplasmic reticulum (15% of the cell volume), and approximately 30 lysosomes, 500 peroxisomes, 1000 mitochondria per cell, numerous free ribosomes, Golgi complex, cytoskeleton elements, and varying levels of cytoplasmic lipid and glycogen. Mice and humans have a gall bladder (not the rats), whereas the bile excretion average is about 15 ml/kg/day in humans (Malarkey et *al*., 2005).

Liver is involved in biochemical processes of nutrients provision, energy supplying, growth and reproduction. Furthermore, its foremost physiological processes include metabolism, proteins secretion, excretion, storage and detoxification (Khan, Haroon et *al*., 2019). Liver homeostasis is a fundamental point in the organism, its metabolic and hepatocellular dysfunctions may be induced by drug administration, viral infection and exposure to high levels of environmental toxins (Hashemzadeh et *al*., 2018; Hozzein et *al*., 2019).

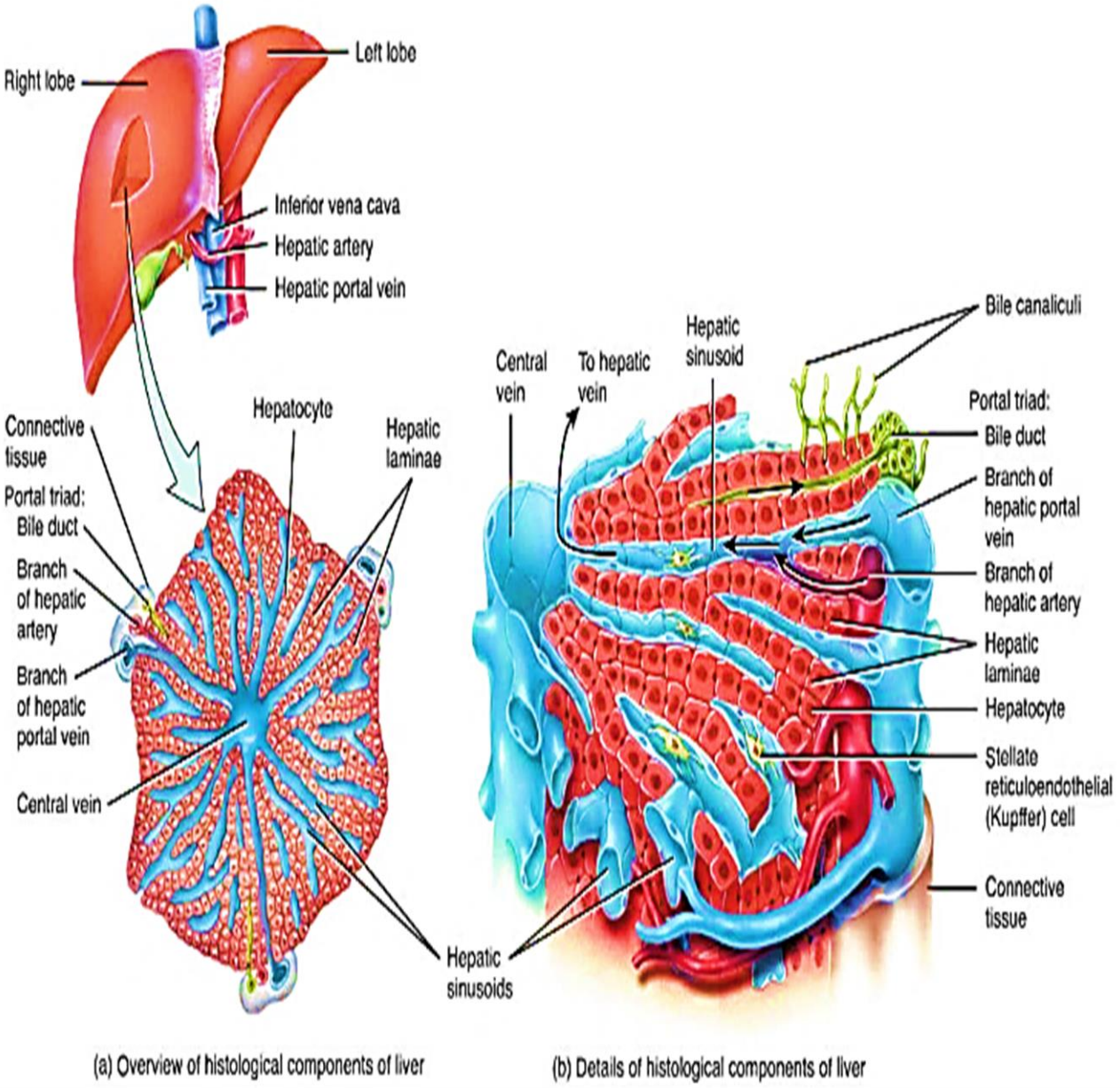


Figure 1: Anatomy and microstructure of the liver (Tortora and Derrickson, 2009).

3.2.1.2. Hepatotoxicity

3.2.1.2.1. Hepatotoxicant agents and diseases

The liver is exposed to high concentration of toxic chemicals and their metabolites which may damage the cells, tissues, structures, as well liver function, namely certain antibiotics, high doses of paracetamol and anti-tubercular drugs, chemotherapeutics, peroxidised oil, aflatoxin, carbon-tetrachloride (CCl_4), chlorinated hydrocarbons, thioacetamide, and excessive consumption of alcohol. Consequently, hepatic damage range from the elevation of liver enzymes, inappropriate digestion of nutrients, steatosis, necrosis, jaundice, fibrosis, cirrhosis, hepatitis, liver carcinoma, obesity related fatty liver disease, inherited and genetic defects related liver diseases, and autoimmune disorder, to hepatocellular carcinoma and even apoptosis (Al-Asmari et *al*., 2014; Madrigal-Santillán et *al*., 2014; Shakya, 2020).

According to several studies, most of the hepatotoxic chemicals damage liver cells primarily by producing reactive species, then, oxidative stress plays a crucial role in the initiation and progression of hepatic damage. For instance, the excess generation of free radicals modulate the metabolic pathways involved in hepatotoxicity, and also lead to lipid peroxidation of the membrane fatty acids causing destruction of the hepatocyte, and cells' intracellular organelles. Furthermore, directly or through oxidative stress mechanism, the toxins could trigger inflammatory response in the liver through the increment of pro-inflammatory cytokines (Kumar et *al*., 2011; Al-Asmari et *al*., 2014; Shakya, 2020).

3.2.1.2.2. Hepatoprotective agents

The use of natural therapies to treat hepatic ailments has a long history. Some of the phytochemicals extracted from medicinal plants exhibited antioxidant and anti-inflammatory potential, they have shown their ability to suppress oxidative stress and inflammation. In

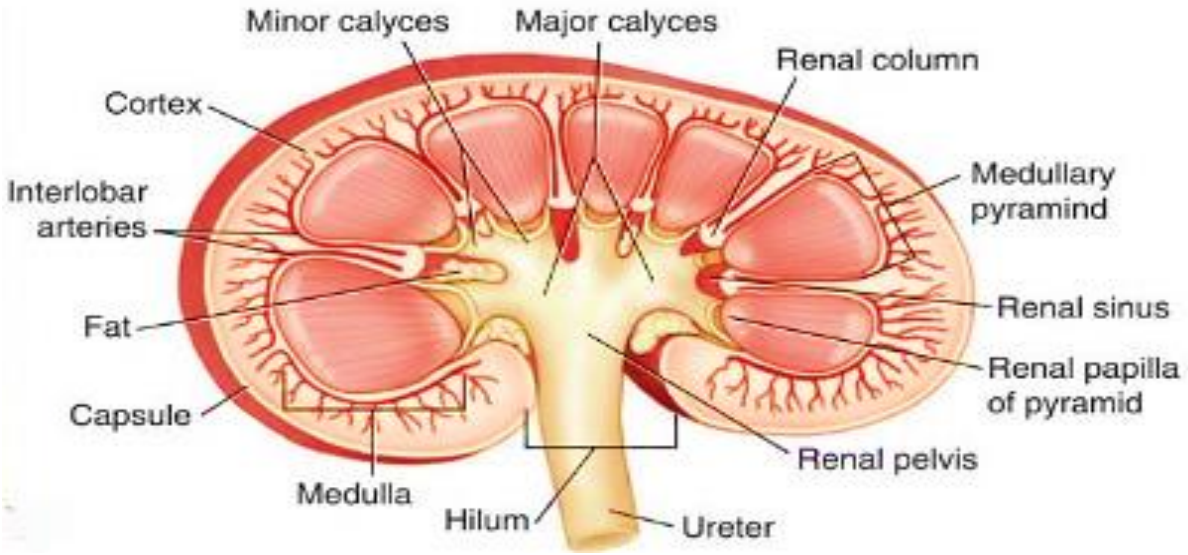
general, hepatoprotective herbs, as well as plants, contain a variety of chemical compounds such as phenols, coumarins, terpenoids, carotenoids, lignans, essential oils, monoterpenes, glycosides, alkaloids, lipids, carotenoids, flavonoids, organic acids, and xanthines (Al-Asmari et *al*., 2014; Madrigal-Santillán et *al*., 2014).

3.2.2. Kidneys

3.2.2.1. Structure and function

In most mammals, kidneys are paired, bean-shaped, the right kidney is lower than the left kidney, situated in the posterior part of the abdomen on each side of the vertebral column and lie in the retro-peritoneum. Their weight is relative to body's weight (0.51% to 1.08%), receive 25% of the cardiac output, and are surrounded by a fibrous capsule (perinephric fat). Kidney's parenchyma has four anatomical sections: the superficial cortex (contains renal corpuscles), the deep medulla (contains conical medullary pyramids), renal papilla, and renal pelvis (Figure 2). Nephrons, the kidney's functional and structural units, are present in the cortex and medulla, they consist of glomeruli, renal tubules (proximal tubules, loop of Henle, distal tubules, connecting segment, collecting ducts), interstitium, and juxtaglomerular apparatus (Radi, 2019).

Kidneys have a pivotal role in hormones' regulation, enzymes and acid-base balance, extracellular fluid volume, blood pressure and pH, erythropoiesis, electrolytes, urine formation, excretion of the waste products of metabolism, and metabolic activities (Esmail and Ali, 2019; Radi, 2019).



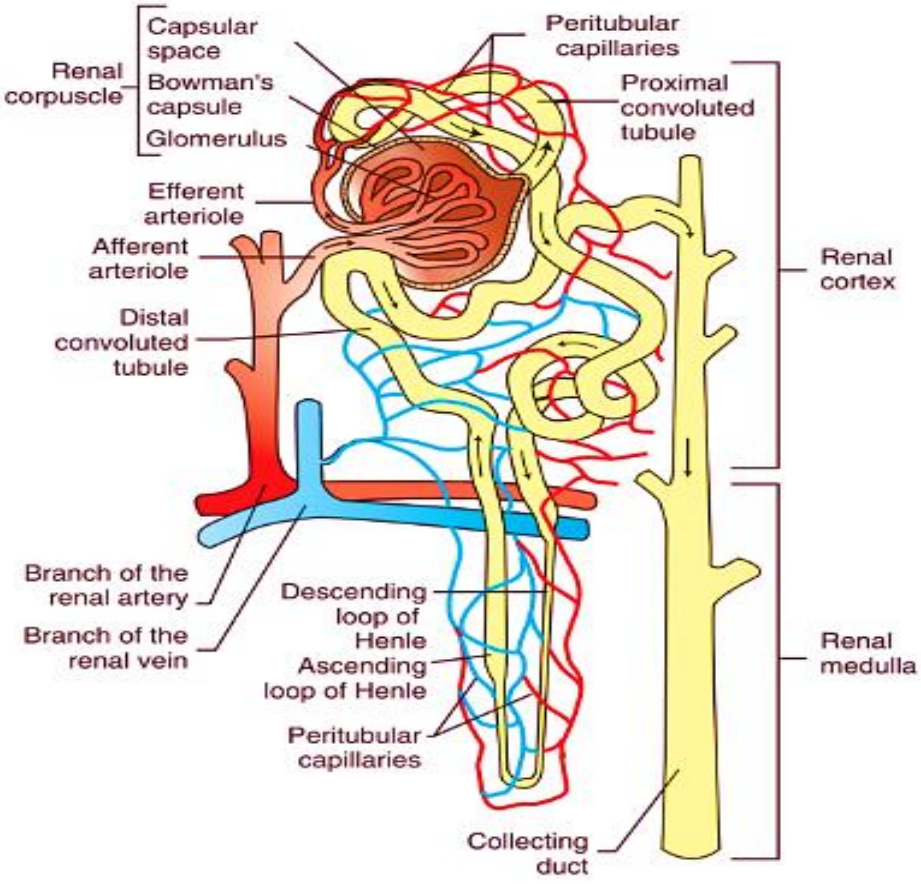


Figure 2: Anatomy of the kidney and nephron **(Colville and Bassert, 2015).**

3.2.2.2. Nephrotoxicity

Kidney performs a variety of crucial functions for the organism homeostasis such as excreting metabolic substances and toxic agents. Accordingly, it is highly susceptible to impairment caused by ROS (Ozbek, 2012).

Physiologically, several factors make the kidney more vulnerable to toxins namely the kidney's high blood flow rate, the large surface area of renal tubular epithelium, and metabolic processes (site for toxin interaction and uptake), the concentrating mechanism, and the specific transport mechanisms that mediate cellular uptakes (Vaya et *al*., 2017).

3.2.2.2.1. Nephrotoxicant agents and diseases

It was reported that drugs such as antineoplastic agents (alkylating agent, Antimetabolites), anti-tumour, antibiotics, antimicrobial agents, aminoglycosides, miscellaneous); heavy metals (lead, bismuth, mercury, arsenic and cadmium); chemical and diagnostic agents like ethylene glycol; carbon tetra chloride; and sodium oxalate are well known to be nephrotoxic agents. Affecting the kidney, they could induce acute renal failure, chronic interstitial nephritis and nephritic syndrome (Gaikwad et *al*., 2012; Vaya et *al*., 2017).

On the other side, medicinal plants may be a risk for the kidneys as numerous renal conditions were noted after their use including: acute tubular necrosis, acute interstitial nephritis, Fanconi syndrome, hypokalaemia, hypertension, papillary necrosis, chronic interstitial nephritis, nephrolithiasis, urinary retention, and cancer of the urinary tract (Bagnis et *al*., 2004).

3.2.2.2.2. Nephroprotective agents

Medicinal plants' antioxidants have been shown to ameliorate oxidative stress, which induce kidney damage, by the reduction of lipid peroxidation and the enhancement of scavenging ability of antioxidant defence system. Nephroprotective agents possess protective activity against nephrotoxicity due to the presence of various complexes chemical substances such as tocotrienol, ligustrazine, troxerutin (Gaikwad et *al*., 2012; Rafieian-kopaei, 2013). Although the antioxidants activities of medicinal plants and their beneficial effects in preventing and treating numerous syndromes, there is also a growing evidence pointing to their pro-oxidant hazardous effects, too (Tamadon et *al*., 2013).

4. Inflammation

Infectious microorganisms such as fungi, bacteria, and viruses invade the body, reside in particular tissues and/or circulate in the blood, and trigger inflammation usually. Moreover, tissue injury, cancer, ischemia, cell death, degeneration, irritants, and harmful stimuli are also activators of the inflammatory response. The latter is characterized by redness, warmth, swelling and pain (Ribeiro et *al*., 2018).

One of the most vital processes needed in animal cells defence is the inflammation. This term was taken from the Latin word "inflammare" and it means "to burn". The inflammation aims to localize and remove injurious stimuli; then, to remove damaged tissue components; and to initiate the healing process (Adegbola et *al*., 2017; Abdulkhaleq et *al*., 2018).

4.1. Phases of inflammation

Inflammation is either acute or chronic. Acute inflammation may be an initial response of the body to harmful stimuli. It can be divided into three main phases: vascular phase, which

is characterized by an increase in blood flow, and a raise in blood vessels permeability (including: prostaglandins, thromboxanes and leukotrienes). Then, followed by a cellular phase characterized by the infiltration of cells in the irritated area (granulocytes, monocytes, macrophages, lymphocytes and plasma cells) to eliminate all of microorganisms, pathogens, and damaged tissues. The last phase is the resolution of the inflammation and healing. It is well known that this phase is influenced by several anti-inflammatory mediators besides the recruitment of monocytes for the removal of cell or tissue debris and restoration of tissues integrity. However, persistent inflammatory stimuli or dysregulation of mechanisms of the resolution phase for weeks, months or even years can lead to chronic inflammation. The latter is associated with numerous pathological conditions like obesity-associated diabetes secondary to insulin resistance (Cato and Wade, 1996; Lawrence and Gilroy, 2007; Maione et *al*., 2016; Ribeiro et *al*., 2018).

At cellular level, inflammatory mechanisms could be activated by free radicals. For instance, oxidative stress could activate nuclear factor kappa B to promote cytokine production through genes' transcription, then, cytokines enhance the inflammatory response. In distant organs, xanthine oxidase and circulating neutrophils generate free radicals into systemic circulation and activate the endothelial cells. Next, in the second phase of the inflammatory response, adhesion molecules, cytokines generation, and additional inflammatory mediators will be triggered. Finlay, neutrophils' free radicals, as noxious agents, react with molecular components, enhance the inflammatory process and affect cell viability. For that reason, free radicals are mostly remarkable therapeutic target to prevent cell damage from oxidant, and also to modulate the inflammatory response (Closa and Folch-Puy, 2004).

4.2. Anti-inflammatory agents

4.2.1. Nonsteroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) treat a variety of illnesses namely mild-to-moderate pain caused by inflammation and tissue injury, pyrexia, ileus and renal colic, headaches and migraines, metastatic bone pain, rheumatoid arthritis, postoperative pain, osteoarthritis, inflammatory arthropathies, acute gout, and dysmenorrhea. Accordingly, NSAIDs are one of the most prescribed classes of medication (McLean and Khan, 2018).

Most NSAIDs, based on structure or mechanism of action, are divided into two main classes enolic acid and derivatives: compromise the pyrazolones (phenylbutazone), and the oxicams (meloxicam and piroxicam); and carboxylic acid and derivatives: include salicylates (aspirin), propionic acids (ibuprofen, naproxen, carprofen, ketoprofen, and vedaprofen), acetic acids, anthranilic acids (tolfenamic and meclofenamic acids), phenylacetic acids (acetaminophen), fenamic acid, and aminonicotinic acids (flunixin) (Castagnetti and Mariella, 2015).

Cyclooxygenase's (COX) inhibition is the main mechanism of action of NSAIDs. COX-1 and COX-2 are isoenzymes forms that catalyse the metabolism of arachidonic acid generating inflammatory mediators. Under physiological conditions, COX-1 is expressed and responsible for the formation of prostaglandins. However, under pathophysiological conditions COX-2 is induced. Consequently, the arachidonic acid is converted into prostaglandins, thromboxane, and prostacyclin. Hence, NSAIDs block the production of prostaglandins and exert anti-inflammatory, analgesic, antipyretic, anti-endotoxic, and antithrombotic effects. Furthermore, decreasing oxidants, scavenging oxygen-derived free radicals, and chelating iron are other NSAIDs' mechanisms of actions (Castagnetti and Mariella, 2015; Braun et *al*., 2020).

Through their anti-nociceptive, antithrombotic, anti-pyretic, anti-inflammatory and anti-cancer properties, NSAIDs which possess nonspecific cytotoxic effects lead to multiple organ pathologies such as intracerebral haemorrhage, hepatotoxicity, respiratory tract inflammation and infection. For instance, in hepatotoxicity NSAIDs can actually lead to acute hepatitis (characterized by jaundice, nausea and fever together with elevated serum levels of transaminases), and also to chronic active hepatitis (characterized by serological and histopathological abnormalities). The underlying cytotoxic effects of NSAIDs on human body are presented in table 3 (Bindu et *al*., 2020).

Table 3: NSAIDs' common adverse effects profile (Wongrakpanich et *al*., 2018).

System	Adverse effects
Gastrointestinal toxicity	✓ Dyspepsia ✓ Gastroduodenal ulcers ✓ Bleeding and perforation
Cardiovascular adverse effects	✓ Oedema ✓ Hypertension ✓ Congestive heart failure ✓ Myocardial infarction ✓ Stroke and other Thrombotic events

Nephrotoxicity	✓ Electrolyte imbalance ✓ Sodium retention ✓ Oedema ✓ Reduce glomerular filtration rate ✓ Nephrotic syndrome ✓ Acute interstitial nephritis ✓ Renal papillary necrosis ✓ Chronic kidney disease

4.2.2. Steroidal anti-inflammatory drugs

Glucocorticoids (GCs) (cortisol) or hydrocortisone when given as a pharmaceutical are steroid hormones produced in the zona fasciculata of the adrenal gland. Classically, being lipophilic, they pass directly across the cell membrane into the cytoplasm to regulate the transcription of target genes directly or indirectly through their binding to cytoplasmic receptors. As an adrenal hormone secreted diurnally, it is also produced under the stimulus of adrenocorticotrophic hormone and also during stress (Schäcke et *al*., 2006; Hardy et *al*. 2012). Depending upon the circumstances, they can promote life and successful adaptation to stress or can contribute to disease and premature death (Wolkowitz et *al*., 2009), and have pleiotropic effects in the body including anti-inflammatory effect; carbohydrate (a decrease in the uptake and utilisation of glucose, an increase in hepatic gluconeogenesis, favouring high glycaemia level), protein and lipid metabolism (increased muscle proteolysis and lipolysis); maintenance of fluid and electrolytic balance; influence the immune system, the cardiovascular system, the kidney, the endocrine system, the skeletal muscle, the regulation of growth and development, and the nervous systems (Castagnetti and Mariella, 2015). They are highly effective in the

treatments of inflammatory illnesses, autoimmune allergic disorders namely: rheumatoid arthritis, hypertension, obesity, type II diabetes and depression too (Buckingham, 2006; Hardy et *al*. 2012).

The inflammation process is inhibited by glucocorticoids through the redirection of lymphocyte traffic; the inhibition of cytokine gene expression; and the inhibition of the expression of adhesion molecules. Generally, glucocorticoids increase the synthesis of lipocortin-1, which inhibits phospholipase A2 action. Then, inhibit the production of lipid mediators (leukotrienes and prostaglandins) through the suppression of eicosanoid generation or through the inhibition of leukocyte migration. Furthermore, they have direct effect on the repression of the activity of the inducible form of nitric oxide synthase, and also on lipid mediators by inhibiting the expression of the enzymes involved in their biosynthesis (Cato and Wade, 1996).

In the acute phase of inflammation, GCs inhibit vasodilatation and increase vascular permeability, and decrease leukocyte emigration into inflamed sites. In the chronic phase of inflammation, GCs altered leukocyte distribution, death and survival, and altered cellular differentiation programmes, which result in effective long-term suppression of inflammation by suppressing key inflammatory cytokines such as tumour necrosis factor a, interleukin-1, -6 and -7 (Güler-Yüksel et *al*., 2018).

Prolonged administration of exogenous corticoids has a plethora of unwanted pathologies including a significant redistribution of fat, protein wasting and associated muscle weakness; hyperglycaemia and insulin resistant diabetes mellitus (steroid diabetes) and at times diabetes, hypertension, raised cholesterol, altered serum lipids, sodium and water retention, excretion of potassium, loss of mineral from bone leading to osteoporosis and fractures; immunodeficiency, poor wound healing and loss of connective tissue leading to easy bruising; impaired growth and

development; osteoporosis; personality changes, gastric ulcers and skin atrophy associated with telangiectasia; menstrual irregularities, infertility and other endocrine-related changes; depression and, sometimes, impaired cognitive function (Buckingham, 2006). Nevertheless, there are a number of possible approaches towards reducing these side effects for instance: co-administration of anabolic steroids; co-administration of retinoic acid derivatives (Cato and Wade, 1996).

4.3. Anti-inflammatory drugs of plant origin

It was reported that inflammation is at the basis of various diseases of advanced age such as cancer, heart attacks, inflammatory skin disorders, arthritis, cardiovascular inflammation, ulcerative colitis, Crohn's disease and Alzheimer's diseases too. Moreover, long-term uses of NSAID used to reduce the swelling and inflammatory pain, yet, cause adverse side effects and damage human biological systems such as gastrointestinal tract, and liver (Murugesan and Deviponnuswamy, 2014; Maione et *al*., 2016).

Therefore, medicinal plants' biodiversity is a valuable source of a wide range of secondary metabolites. They possess a variety of compounds with promising biological activities, which are less likely to cause side effects. They have been used as pharmaceuticals and have had anti-inflammatory effects since ancient times (Al-Snafi, 2015). In the literature, there are several active compounds isolated from medicinal plants extracts and exert their anti-inflammatory activity through their anti-oxidative potential as hydrogen donating compounds, metal ion chelators, singlet oxygen quenchers and free radical scavengers, which are attributed to the phenolic hydroxyl groups attached to ring structures. Moreover, several research reported the anti-inflammatory effects of these secondary metabolites such as: polyphenolic, flavonoids (myricitrin, myricetin, quercetin and luteolin), steroid, alkaloid (bukittinggine, berberine, spectaline, and milonine), proantho-cyanidin, terpenoid (lupeol), diterpenes (copalic acid,

Sesquiterpene, 6a-acetoxygedunin), and monoterpenes (b-myrcene and limonene)) (Ribeiro et *al*., 2018).

The anti-inflammatory potential of medicinal plants is exerted through several mechanisms. Usually, it is associated to their antioxidant effects, signalling cascades involving cytokines and regulatory transcription factors, and also to their interactions with several key enzymes. However, the major target for anti-inflammatory activity is the inhibition of eicosanoid generating enzymes including phospholipase A2, cyclooxygenases and lipoxygenases, which lead to the reduction of prostanoids and leukotrienes. Furthermore, additional mechanisms comprise the inhibition of phosphodiesterase, protein kinases, histamine release, and activation of transcriptases (Al-Snafi, 2018; Ribeiro et *al*., 2018).

As a result of their safety and effectiveness in phytotherapy, medicinal plants' constituents with anti-inflammatory effects are preferred. For instance: kaempferol, apigenin, luteolin, and galangin could inhibit cyclooxygenases and prostaglandins production; whereas, quercetin and myricetin inhibited lipoxygenase and leukotrienes production. Besides, some plants crude extracts could inhibit both arms of arachidonic acid, cyclooxygenases and lipoxygenase (Al-Snafi, 2018).

5. The plant *Oxalis cernua*

The genus Oxalis comprises of more than 900 species. *Oxalis cernua* Thunb (synonyme: *Oxalis pes-caprae* L.) is a member of the Oxalidaceae family, and also an important species of this genus. Its commun name in english is Bermuda buttercup, because of its pleasant sour taste, it is also called as "sourgrass" or soursob. However, its local name is "hamayda" (Te Beest et *al*., 2011; Ibrahim et *al*., 2013; Signorini et *al*., 2013; Edrah et *al*., 2018).

5.1. Origin and geographic repartition

As an ornamental plant, *Oxalis cernua* was introduced in Europe and the Mediterranean basin in the second half of the eighteenth century (1796) and later in Africa, North America, Australia and other continents (Sala et *al*., 2007; Signorini et *al*., 2013). Currently, it is widely naturalized in many countries, especially in Mediterranean (including Algeria), tropics and subtropical areas of the world (Te Beest et *al*., 2011; Ibrahim et *al*., 2013). According to the scientific literature, the plant usually grows in undisturbed habitats as a synanthropic weed. It is found in cultivated ground, grassland, landscaped areas orchards, pastures, gardens, roadsides and different kinds of disturbed habitats. Moreover, it has become a very troublesome invasive weed in many countries (Bogdanović et *al*., 2003; Signorini et *al*., 2013; Licata et *al*., 2016).

5.2. Botanical description of the plant

Oxalis belongs to root herb. It has the characteristics of low plant height, same growth speed, long flowering stage, and can inhibit the growth of weed. In addition, its cytosol is highly acidic (pH < 3) (Loureiro et *al*., 2006).

Oxalis cernua is a perennial geophyte, caespitose, that can grow up to more than 40 cm (10–50 cm) high, sparsely pubescent plant with bulbous roots, which emit an annual, ascending, subterranean stem, which bears bulbils and a basal rosette of leaves at ground level (Figure 3). In the native range, it flowers from May to August, while in the invaded area of the Mediterranean basin, flowering occurs from December to April. The leaves are divided into three heart-shaped leaflets, 8–20 x 12–30 mm, and the surface of each leaflet is slightly hairy, often with a number of black specks. Petioles are up to 20 cm long (Bogdanović et *al*., 2003; Costa et *al*., 2017). Six to twelve infudibuliform yellow tristylous flowers form umbellate cymes. The flowers present a yellow simpetalous corolla composed by five petals, 20–25 mm long, and 5 sepals bicallous at the top, and are up to 7 mm long. The petals bear two rounds of

five stamens and one round of five stigmas arranged in three levels according to the floral morphotype of each individual. Despite the energy spent on flowering, it rarely forms capsules or produces seeds (Bogdanović et *al*., 2003; Castro et *al*., 2007).

Oxalis cernua has a great capacity of asexual reproduction via the production of numerous bulbs associated with the contractile capacities of its roots. Sexual reproduction is only possible after legitimate crosses between floral morphs and compatible ploidal levels. However, in its native range, the species reproduces both sexually (via seeds) and asexually (via bulbils). This plant is also existing in Jijel (Algeria) from where it was collected (Te Beest et *al*., 2011; Costa et *al*., 2017).

Systematic of *Oxalis Cernua* Thunb taxonomy is as following (Peirce, 1997):

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledoae

Order: Geraniales

Family: Oxalidaceae

Genus: Oxalis

Species: *Oxalis cernua* Thunb / *Oxalis pes-caprae* L.

5.3. Traditional use of *Oxalis cernua*

Oxalis cernua has been used for various purposes such as in food and also in treating many illnesses. Several studies have reported that *oxalis pescaprea* is eaten in many countries of the Mediterranean. For instance, it is eaten in North Africa especially in salads (Lentini and Venza, 2007; Van Wyk, 2011). This culinary herb is harvested only in the wet season and either eaten fresh or more typically baked in hot ash or cooked in milk (Van Wyk, 2011), or as additives with grilled or cooked meats. Besides, flowers are used in traditional dyeing (BOUSSAHA et *al*., 2014).

In folk medicine, the roots are used particularly due to their diuretic properties. Stems and leaves have high medicinal values, and have the functions of anti-inflammation, antihypertensive effects, analgesia, clearing heat, removing toxicity. Moreover, Oxalis is widely used to cure diarrhoea due to dampheat, dysentery, stranguria, leucorrhoea, hematemesis, haematuria, irregular menstruation, traumatic injury, sore throat, eczema, acariasis, haemorrhoids, measles, snake and insect bites. In clinical practice, oxalis has significant effects on curing inflammation. For example, the entire plant was converted into a paste to treat swelling (Zhongyao and Yingzi, 2014; Edrah et *al*., 2018; Gaspar et *al*., 2018).

5.4. Chemical composition of *Oxalis cernua*

It can contain up to 16% of the dry weight as oxalic acid (E)-4-(1-(4-(1-hydroxymethyl) phenoxy) ethyl) phenyl 3,4,5-trimethoxycinnamate, which gives them a sharp flavour, present in both photosynthetic and non-photosynthetic tissues (DellaGreca et *al*., 2010; Benouali et *al*., 2014; Edrah et *al*., 2018). A toxic compound that might cause nervous system paralysis in large herbivores when consumed in great quantities (Gaspar et *al*., 2018). Sheep, after eating a great amount of this plant, died or aborted. The sick animals showed: colic, tympanitis, paralysis for the limbs, renal failure and coma too. The herb is sometimes harmful for goats, but innocuous

for bovines and horses. Consumption of this plant provokes intestinal inflammations, blood in the urines and often death by collapse due to the high amount of calcium oxalate (Evans et *al*., 1973; Passalacqua et *al*., 2006).

At the present time, the exact effective components of oxalis are still unclear, so additional studies are still required. Among them, the structure of some chemical components were identified, which were: daucosterol, βsitosterol, vitexin, isovitexin, acacetin, acaciin, β tocopherol, tartaric acid, vanillic acid luteolin, oxalicacid, malic acid and citric acid. Luteolin derivatives, apigenin derivatives, homoorientin, vitexin derivatives, as for the main flavones, flavonols, flavone, 7,3′-dimethoxyl-2″-O-glycosylorientin. 7,3′-dimethoxyl-6-desoxyhexoseorientin. Besides, ester and phenyl cinnamate derivatives, aromatic compounds and phenols were found in the leaves and twigs such as flavonoids namely the glycosyl flavones, isoorientin, isovitexin and swertisin (Gaspar et *al*., 2018).

5.5. Pharmacological properties of the plant

According to the literature, *Oxalis cernua* has been reported to be considered (particularly the leaves) as an important and inexpensive source of bioactive compounds which had several health benefits such as antioxidant activity, vascular activity (antispasmodic), bacteriostatic activity, anti-inflammatory and neuroprotective activity (Zhongyao and Yingzi, 2014; Gaspar et *al*., 2018). Furthermore, their polyphenols are associated with beneficial cardiovascular effects (Gaspar et *al*., 2018). Several studies found out that its natural compounds have the functions of antibiosis, and antitumor (Zhongyao and Yingzi, 2014).

Material and methods

1. Material

1.1. Collection of plant

Oxalis cernua was collected from Tassouste (36° 45′ 12″ Nord, 5° 50′ 59″ Est), Jijel (Algeria) in April-May 2017. This plant identified by Professor Oudjhih Bachir, Institut of nutrition and agronomy, Batna (Algeria). The voucher specimen was deposited at the institute herbarium under the number (225/ISVSA/DA/UHLB1/17). The plant was washed with water and air dried in shadow for six weeks, the dried plant material was ground to a fine powder.

Figure 3: *Oxalis cernua* whole plant with flower, leaves and roots.

1.2. Experimental animals

Female Wistar Albino rats and *Swiss albino* mice (nulliparous and non-pregnant) with a mass weight of 182±20 g, and 24±6 g respectively, were purchased from Pasteur institute (Algiers) and housed in plastic cages under standard conditions (natural photoperiod and temperature) and acclimatized for 15 days prior to the experiments. Water and food were made available *ad libitum*. The experiments were conducted in accordance with the internationally acceptable guidelines for evaluating the safety and efficacy of medicinal plants (Organisation for Economic Co-operation and Development) (OCDE, 2008).

2. Methods

2.1. Preparation of the crude extract

Powdered plant material was soaked in pure methanol for 5 days (at a ratio of 1 :10 (w/v)) at room temperature with renewal of the solvent and with intermittent shaking (Markham, 1982). The methanol extract was then filtered and concentrated at 45 °C under reduced pressure using a rotary evaporator (Büchi 461). *Oxalis cernua* areal part methanolic extract (AME) and root methanolic extract (RME) were further concentrated to dryness. The yield of extraction was calculated from the initial weight of the used plant powder.

A part of AME was subjected to fractionation using liquid- liquid extraction. AME was successively fractionated with different solvents of increasing polarity: hexane for defatting, chloroform for aglycone flavonoids extraction and ethyl acetate for glycosidic flavonoids extraction, as shown in figure 4. The combined organic layer of each fraction was evaporated under reduced pressure on a rotavapor below 45°C to dryness and resulted chloroform (CHE), ethyle acetate (EAE) and aqueous (AqE) fractions. Extracts were stored at -20 °C until use.

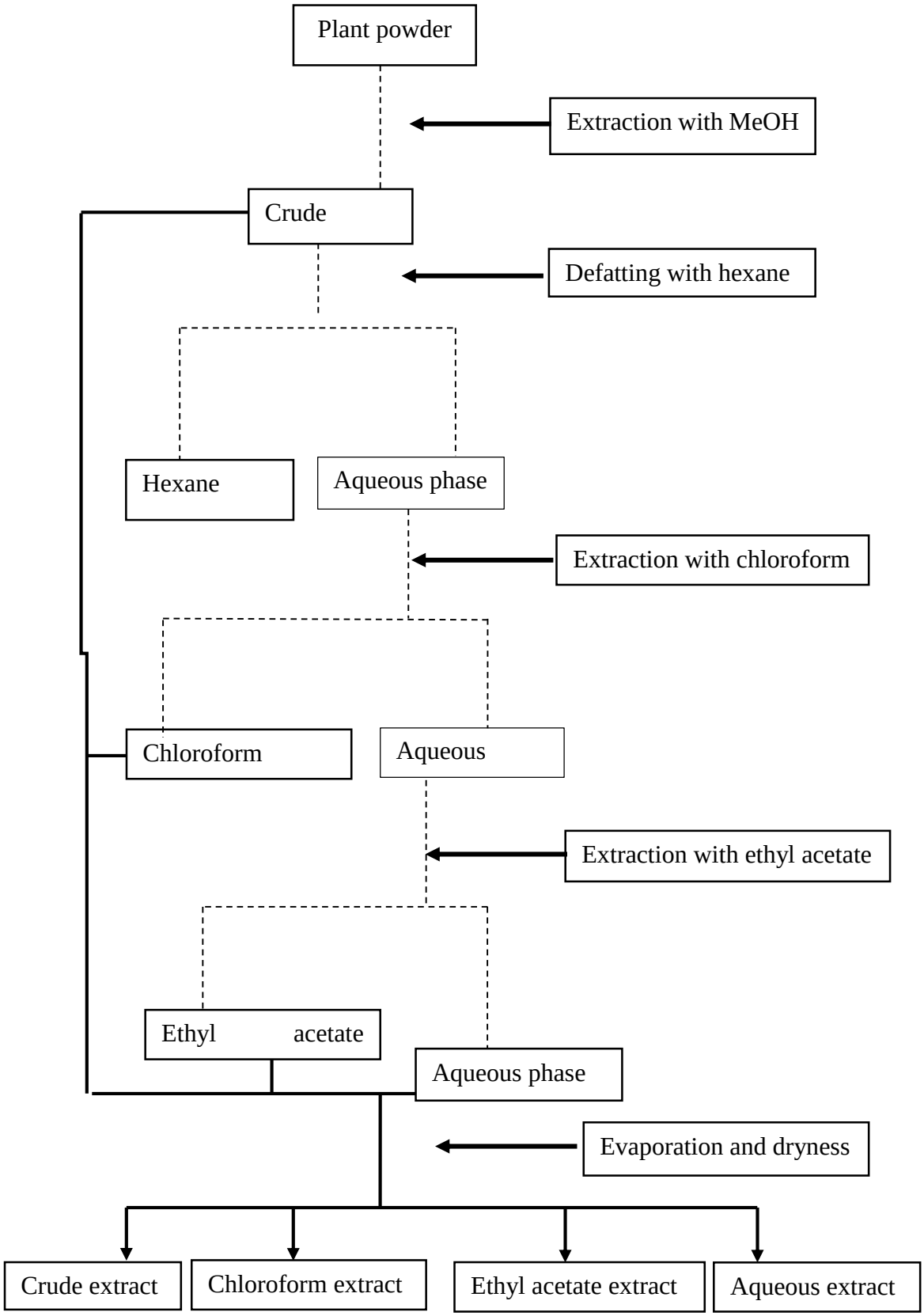


Figure 4: Schematic diagram represents the process of extraction and fractionation of *Oxalis cernua*. According to Markham (1982) with slight modifications.

2.2. Determination of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteau colorimetric method (Bentahar et *al*., 2016), gallic acid was used as standard. Briefly, 0.2 ml of the extract was mixed with 1 ml of Folin-Ciocalteu reagent (10- fold diluted). After 4 min, 0.8 ml of saturated sodium carbonate Na_2CO_3 (75g/l) solution was added and absorbance was measured at 765 nm after 2 h. The same procedure was repeated for gallic acid solutions (10-160 µg/ml). Total polyphenols content was expressed as µg of gallic acid equivalent (GAE)/ mg extract (Figure 5).

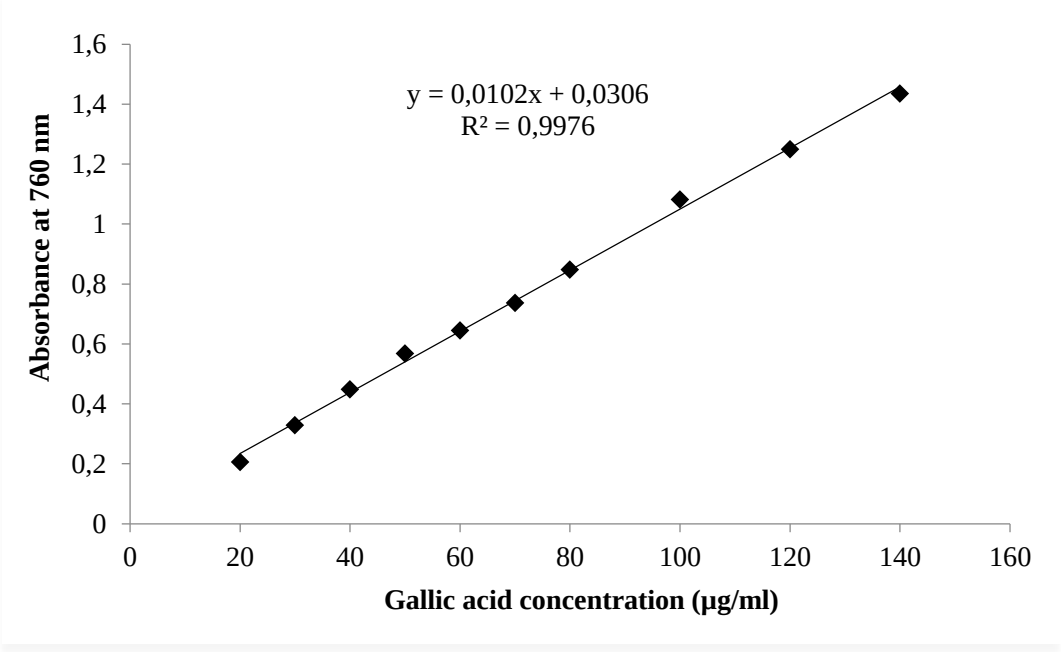


Figure 5: Standard curve of gallic acid for the determination of total polyphenols in various plant extracts. Each value represents mean ± SD (n = 3).

2.3. Determination of total flavonoids content

Flavonoids were quantified using aluminium chloride reagent ($AlCl_3$) (Bouaziz et *al*., 2014), and expressed as quercetin equivalents (QE). Briefly, 1 ml of the extract, dissolved in corresponding solvent was added to 1 ml of $AlCl_3$ (2% in methanol). The absorbance was measured at 430 nm after incubation at room temperature for 10 min. Results were expressed as milligrams of quercetin equivalent per gram dried weight (mg QE/g dw) (Figure 6).

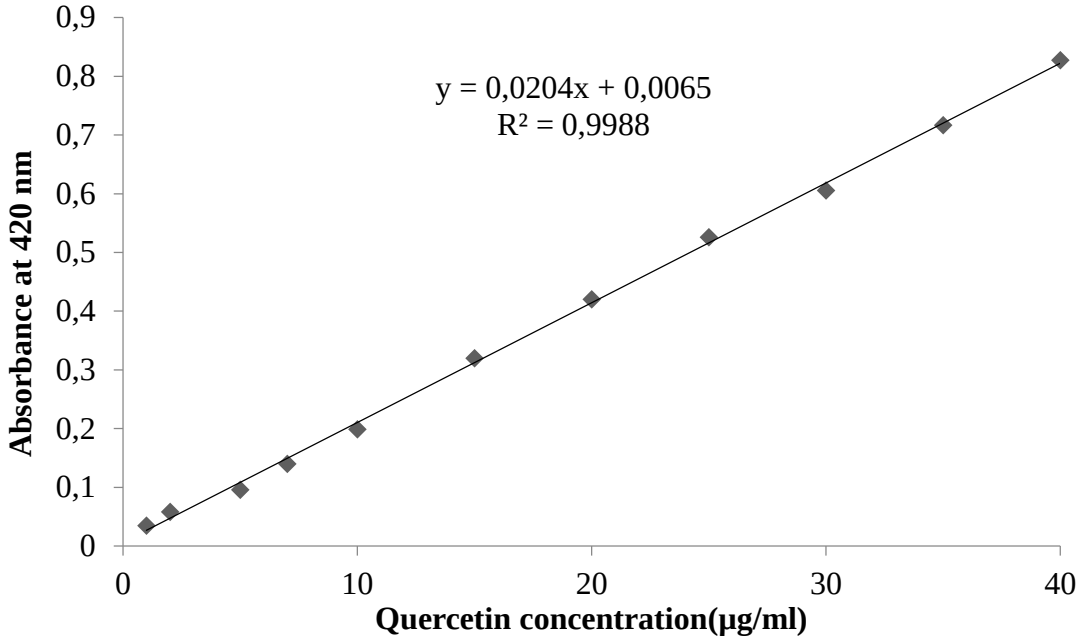


Figure 6: Standard curve of quercetin for the determination of total flavonoids in various plant extracts. Each value represents mean ± SD (n=3).

2.4. Antioxidant activity of plant extracts (*in vitro* assays)

2.4.1. DPPH radical scavenging assay

Free radical scavenging activity against 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) radical was measured (Belkhiri et *al*., 2017). Briefly, 50 µl of the extracts and BHT were mixed with 1250 µl of a 0.004% methanol solution of DPPH. The absorbance of the samples was read at 517 nm after an incubation period of 30 min in dark at room temperature. Lower absorbance indicated higher free radical-scavenging activity. The DPPH scavenging activity in percentage was determined according to the following formula:

Scavenging effect % = [(AC – AS) / AC] × 100.

Ac: was the control absorbance;

AS: was the absorbance in presence of sample.

The extract concentration required for a 50% (IC_{50}) reduction of DPPH radical was determined graphically.

2.4.2. ABTS radical scavenging activity assay

ABTS radical scavenging activity was assessed via spectrophotometric analysis according to the method of Re et *al*., (1999) with slight modifications. This method is based on the ability of antioxidants to quench the ABTS radical cation. The ABTS radical stock solution was generated by the reaction of 7 mM of ABTS solution in 2.45 mM potassium persulfate ($K_2S_2O_8$) (final concentration). The mixture was kept in the dark at room temperature for 24 h before use. Then, it was diluted with methanol to give an absorbance of 0.70 ± 0.02 at 734 nm. Briefly, 50 µl of sample was mixed with 1ml of ABTS solution and kept at dark for 30 min at room temperature. The absorbance of reaction mixture was measured at 734 nm. Trolox was used as a standard and all the experiments were conducted in triplicate. The ABTS radical scavenging ability was calculated according to the same equation as that in the DPPH assay. Effective concentration (EC_{50}) defined as the concentration of a drug (commonly quantified as the EC_{50}) that gives 50% maximal response (or the logarithm of the EC_{50}). Generally, EC_{50} values are used to reflect the antioxidant capacity of various materials (Sridhar and Charles, 2019).

2.4.3. Reducing power assay

The ability of extracts to reduce ferric iron (Fe^{3+}) was evaluated according to Aouachria et *al*. (2017). Briefly, 400 µl of extract was mixed with 400 µl of phosphate buffer (0.2 M, pH=6.6) and 400 µl of a 1% potassium ferricyanide [$K_3Fe(CN)_6$], then the mixture was incubated at 50°C for 20 min. After that, 400 µl (10%) of trichloroacetic acid (TCA) was added to the mixture and centrifuged for 10 min (3000 rpm). Finally, 400 µl of the supernatant solution was mixed with 400 µl of distilled water and 80 µl $FeCl_3$ (0.1%), and the absorbance was

recorded at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The results were expressed as µg ascorbic acid equivalent/mg extract. BHT was used as positive control and the reducing power was calculated using the same equation as that in the DPPH assay. The EC_{50} value defined as the effective concentration of test material at which the absorbance was 0.5.

2.4.4. β-carotene/linoleic acid bleaching assay

In this assay, the antioxidant capacity of the extract was determined by measuring the inhibition of the conjugated diene hydro-peroxides arising from linoleic acid oxidation (Madoui et *al*., 2019). A stock solution of β-carotene/linoleic acid mixture was prepared as follow: 0.5 mg β-carotene was dissolved in 1ml of chloroform, and then 25 µl linoleic acid and 200 mg Tween 40 were added in round-bottomed flask. Chloroform was evaporated using a rotavapor. Then, 100 ml distilled water saturated with oxygen (30 min, 100 ml/min) was added with vigorous shaking to form emulsion. A volume of 350 µl of the extract, prepared in methanol at a concentration of 2 mg/ml, was added to 2500 µl aliquot of the reaction mixture, and the emulsion system was incubated up to 24h in dark at room temperature. Control samples (2 mg/ml) contained only the emulsion without sample, while blank consisted only of corresponding extract or control. After this incubation period, absorbance of the mixtures was measured at 490 nm at 0h and after 1h, 2h, 4h, 6h, 12h and 24h of incubation. The bleaching rate of β-carotene was calculated as antioxidant activity and calculated using the equation:

Antioxidant activity % = (Ae × 100)/Ac.

Ac was the control absorbance (BHT);

Ae was the absorbance in the presence of extract.

2.4.5. Ferrous iron chelating power of extracts

Ferrous iron-chelating activity was measured through the inhibition of the formation of Fe^{2+}–ferrozine complex after treatment of test extract with ferrous iron (Fe^{2+}) (Bouaziz et *al*., 2015). The reaction mixture contained 250 µl extract, 50 µl $FeCl_2$ (0.6 mM in water) and 450 µl methanol. The control tube contained all the reaction reagents except the extract. The mixture was shaken and allowed to stand for 5 min at room temperature. Then,100 µl of ferrozine (5mM in methanol) were added, the mixture shaken again, followed by further standing period at room temperature for 10 min to complex the residual Fe^{2+} ion. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm against a blank containing all the reaction reagents except ferrozine. Lower absorbance indicates a higher chelating power. Ethylenediaminetetraacetic acid (EDTA) was used as reference chelator. The chelating effect was calculated as a percentage, using the same equation as that in the DPPH assay. IC_{50} value defined as the concentration of test material that is required to chelate 50% of iron ions.

2.4.6. Hydroxyl radical scavenging activity of extracts

The hydroxyl radical scavenging activity was examined according to the method of Smirnoff and Cumbes (1989). The Principe of this method is based on the production of $OH^{\bullet}$ in the reaction medium through the reaction of Fenton. Then, the OH product reacted with sodium salicylate to produce the hydroxyl salicylate complex. Briefly, a mixture of 1.0 ml of $FeSO_4$ (1.5 mM), 0.7 mL of hydrogen peroxide (6 mM), 0.3 mL of sodium Salicylate (20 mM) was prepared. After that, 1 ml of the various concentrations of extracts or ascorbic acid as standard were added and incubated at 37 °C for 60 min. The absorbance was measured at 562 nm. The inhibition percentage of $OH^{\bullet}$ was calculated using the following equation:

$$\text{Inhibition\%} = [1- (A1-A2) / A0] \times 100.$$

A0: Control absorbance (without extract/standard);

A1: Extract or standard absorbance;

A2: Absorbance without sodium salicylate.

2.4.7. Anti-haemolytic activity of plant extracts

The resistance to free radical damage was tested and measured as the capacity of erythrocytes to withstand free radical induced haemolysis (Trabsa et *al*., 2014). Mice erythrocytes were obtained by centrifugation at 3000 rpm for 10 min, and washed three times with phosphate buffer (10 mM, pH=7.4). In order to induce free-radical chain oxidation in erythrocytes, a suspension of (2% haematocrit) were treated by (50 mM) of AAPH. Then, 80 µl of erythrocytes was added to 20 µl of extract (0.1 mg/ml), the mixture was treated by 136 µl of AAPH (300 mM). The haemolysis was quantified using a 96-well microplate reader device. The kinetics of erythrocytes resistance to haemolysis was determined at 37°C by continuous monitoring by measuring the rate of decrease at 630 nm. Results were expressed as the time corresponding to 50% of maximal haemolysis (half-haemolysis time, HT_{50} / min).

2.5. Anti-inflammatory activity of plant extracts (*in vitro* assays)

2.5.1. BSA anti-denaturation assay

A solution of 0.2% of BSA was prepared in Tris buffer saline and pH was adjusted to 6.8. Two different concentrations (0.312 and 0. 625 mg/ml) were prepared by using methanol as a solvent. After that, 50ml of each extract was transferred to tubes. Then, 5ml of 0.2% BSA solution was added. The control consists of 5 ml of 0.2% BSA solution with 50 µl of methanol. The standard consists of 100 µg/ml of diclofenac sodium in methanol with 5ml of 0.2% BSA solution. The test tubes were incubated at 72° C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined at 660 nm. The percentage inhibition of

precipitation (denaturation of the protein) was determined on a % basis relative to the control using the following formula (Verma et *al*., 2011):

I% = 100 × (Absorbance of control – Absorbance sample)/Absorbance of control.

2.5.2. Egg-albumin anti-denaturation assay

The anti-inflammatory activity of *Oxalis cernua* (areal part, fractions, and roots) was studied by using inhibition of protein denaturation method (Sangeetha and Vidhya, 2016). Briefly, the reaction mixture (5ml) comprised of 0.2 ml of egg albumin (from fresh hen's egg), 2.8ml freshly prepared phosphate buffered saline (PBS, pH: 6.4) and 2ml of varying concentration of plant extracts (1.25 and 2.5 mg/ml). Similar volume of distilled water was used as negative control. Diclofenac at the final concentration of (100 µg/ml) was used as the positive control. Subsequently, the mixtures were incubated at 37±2 °C in an incubator for 15 minutes and then heated at 70 ºC for 5 minutes. Afterward, the samples were allowed to cool down to room temperature, and the absorbance was measured at 660 nm. The Percentage inhibition of protein denaturation was calculated by using the following formula:

I% = 100 × (Absorbance of control – Absorbance sample) / Absorbance of control.

2.6. Acute toxicity study

Female Albino Wistar rats selected through random sampling technique were used for acute toxicity study, five groups of five animals each with similar average body weight were used. *Oxalis cernua* (AME and RME) were orally administered to treatment groups with doses of (2000 and 5000 mg/kg bw), respectively. Whereas, the control group received distilled water (OCDE, 2008).

2.6.1. Observation

The animals were observed periodically during the first 24 hours after administering the extracts and then once a day for 14 days. Body weights were recorded at the beginning, then once after every seven days during the period of study. At the end of the observation period, all animals were sacrificed.

2.6.2. Plasma preparation and biochemical parameters estimation

After 14 days, animals were anesthetized and sacrificed by cervical dislocation and blood was obtained in heparin tubes collection; then was centrifuged at 3000 rpm for 10 min. Plasma biochemical parameters: urea, creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analysed using Diatron pictus 920 Random Access Clinical Analyser (Bechman), and commercial kits (Spinreact, Spain), at the laboratory of CAC-Sétif, Cancer Center.

2.6.3. Histological investigation of liver and kidney

Internal organs were removed rapidly and washed with an isotonic sodium chloride solution. The organ pieces were fixed in 10% formalin. Sections at 5µm were stained with haematoxylin and eosin and examined under light microscope. The analyses of the different organs were performed using an Optika B-500T i-5 microscope in order to verify alterations in the tissues, at the pathological anatomy laboratory of the CHU of Sétif, Algeria.

2.7. Estimation of subacute toxicity and *in vivo* antioxidant activity of plant extracts

2.7.1. Experimental design

Female *Swiss albino* mice (nulliparous and non-pregnant) were used. The animals were randomly distributed into seven groups of five animals each with similar average body weight. Control Group: served as a neutral control and received distilled water.

Group Vitamin C: received 100 mg/kg bw of vitamin C.

Group 1: received 100 mg/kg bw of *Oxalis cernua* AME.

Group 2: received 200 mg/kg bw of *Oxalis cernua* AME.

Group 3: received 400 mg/kg bw of *Oxalis cernua* AME.

Group 4: received 100 mg/kg bw of *Oxalis cernua* RME.

Group 5: received 200 mg/kg bw of *Oxalis cernua* RME.

Group 6: received 400 mg/kg bw of *Oxalis cernua* RME.

Distilled water, vitamin C (100 mg/kg bw) and extracts (100, 200 and 400 mg/kg bw) were given orally once a day for 21 days. Weight of mice was noted before treatment (day 0), on day 7, day 14 and day 21 of treatment (Baghiani et *al*., 2013).

2.7.2. Preparation of plasma and homogenate of tissues

After the 21st day, animals were sacrificed, blood was collected in heparin tube from anesthetized mice, then centrifuged at 3000 rpm for 10 min to obtain the plasma. Livers and kidneys were removed rapidly and washed with an isotonic sodium chloride solution. Some of the organ pieces were fixed in 10% formalin. Sections at 5µm were stained with haematoxylin and eosin and examined under light microscope. The analyses of the different organs were performed using an Optika B-500T i-5 microscope in order to verify alterations in the tissues, at the pathological anatomy laboratory of the CHU of Sétif, Algeria. The other organ pieces of livers and kidneys, were homogenized in ice-cold KCl (1.15%). The supernatant was separated by centrifugation at 4000 rpm for 10 min at 4C° and was used for the determination of MDA as a lipid peroxidation marker, Catalase and reduced glutathione (Sapakal et *al*., 2008).

2.7.3. DPPH Radical-scavenging activity of plasma

In this assay, the capacity of plasma to trap the DPPH radical was evaluated according to the same method described in *in vitro*. An aliquot of 25 µl of plasma was added to 625 µl of

DPPH solution (0.004%), and the reaction mixture was shaken vigorously. After incubation at room temperature for 30 min, the absorbance of this solution was determined at 517 nm.

2.7.4. Plasma reducing power determination

The reducing power of plasma was measured according to the same method described *in vitro*. An aliquot of 400 µl of plasma was mixed with 400 µl phosphate buffer (0.2 M, pH=6.6) and 400 µl of 1% potassium ferricyanide [$K_3Fe(CN)_6$], then the mixture was incubated at 50°C for 20 min. 400 µl of TCA (10%) was added to the mixture, and centrifuged for 10 min (3000 rpm). Finally, 400 µl of the supernatant solution was mixed with 400 µl water and 80 µl of $FeCl_3$ (0.1%), and the absorbance was recorded at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.7.5. Lipid peroxidation assessment

Lipid peroxidation process was determined in supernatant of homogenate of liver and kidneys using TBA (Guemmaz et *al*., 2018). 125 µl of TCA (20%) and 250 µl of TBA (0.67%) were added to 125 µl of tissue homogenate. The mixture was incubated at 100°C for 20 min, then the sample was cooled and MDA-TBA complex was extracted with 1ml of butanol. By centrifugation for 15 min at 3000 rpm, organic phase was separated and measured at 530 nm. The MDA concentration was calculated by the absorbance of MDA-TBA complex (1.56×10^5 $M^{-1}cm^{-1}$). Lipid peroxidation was expressed as nanomols MDA/g tissue.

2.7.6. Catalase activity

The catalase activity was determined in tissue homogenate of liver and kidneys (Aebi, 1984). In brief, to start the reaction, 50 µl of tissue homogenate was added to a quartz cuvette containing 2950 µl of H_2O_2 (19 mM) dissolved in sodium phosphate buffer (50 mM, pH=7.4). The organs CAT activity was measured using spectrophotometer at 240 nm by calculating the

degradation rate of H_2O_2. The H_2O_2 molar extinction coefficient (43.6 M^{-1} $cm^{-}1$) was used to determine the catalase activity which was expressed as units per milligram of protein (one unit of activity equal to one µmol of H_2O_2).

2.7.7. Glutathione contents

The estimation of reduced glutathione contents (GSH) in supernatant was performed (Zerargui et *al*., 2016). A volume of 5 ml of sodium phosphate buffer (0.1M, pH=8) was added to 25 µl of supernatant homogenate, then 3 ml of the mixture was added to 20 µl DTNB (0.01 M). The reaction of DTNB with GSH and yield was the base to determine GSH on a yellow coloured chromophore with a maximum absorbance at 412 nm. The amount of GSH present in the tissue was calculated as nanomols of GSH/g tissue.

2.8. Hepato-protective and nephron-protective effect of plant crude extracts

2.8.1. Experimental design

Female Wistar rats (nulliparous and non-pregnant) were used. The animals were randomly distributed into nine groups of 5 animals each with similar average body weight.

Group1: served as a neutral control, received distilled water, and olive oil in the 7 day.

Group 2: received CCl_4 with olive oil in the 7^{th} day.

Group 3: received 100 mg/kg bw of vitamin C, and CCl4 with olive oil in the 7^{th} day.

Group 4: received 100 mg/kg bw of *Oxalis cernua* AME, and CCl_4 with olive oil in the 7^{th} day.

Group 5: received 200 mg/kg bw of *Oxalis cernua* AME, and CCl_4 with olive oil in the 7^{th} day.

Group 6: received 400 mg/kg bw of *Oxalis cernua* AME, and CCl_4 with olive oil in the 7^{th} day.

Group 7: received 100 mg/kg bw of *Oxalis cernua* RME, and CCl_4 with olive oil in the 7^{th} day.

Group 8 received 200 mg/kg bw of *Oxalis cernua* RME, and CCl_4 with olive oil in the 7^{th} day.

Group 9: received 400 mg/kg bw of *Oxalis cernua* RME, and CCl_4 with olive oil in the 7^{th} day.

Distilled water, vitamin C and extracts (100, 200 et 400 mg/kg bw) were given orally once a day for 6 days, CCl_4 (2ml/kg) (Kamisan et *al*., 2013).

2.8.2. Biochemical parameters estimation

After 24h, all animals were anesthetized and sacrificed by cervical dislocation, and the blood was obtained in heparin tubes collection. After that, blood was centrifuged at 3000 rpm for 10 min. Plasma biochemical parameters: urea, creatinine, alkaline phosphatase (AP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analysed using Diatron pictus 920 Random Access Clinical Analyser (Bechman), and commercial kits (Spinreact, Spain), at the laboratory of CAC-Sétif, Cancer Center. DPPH and reducing power as antioxidant marker were determined.

2.8.3. DPPH Radical-scavenging activity of plasma

In this assay, the capacity of plasma to trap the DPPH radical was evaluated according to Cuendet, et *al*., (1997). An aliquot of 25µl of plasma was added to 625µl of DPPH solution (0.004%), and the reaction mixture was shaken vigorously. After incubation at room temperature for 30 min, the absorbance of this solution was determined at 517 nm.

2.8.4. Plasma reducing power determination

The reducing power of plasma was measured according to Aouachria et *al*., (2017). An aliquot of 400 µl of plasma was mixed with 400 µl phosphate buffer (0.2 M, pH=6.6) and 400 µl of 1% potassium ferricyanide [$K_3Fe(CN)_6$], then the mixture was incubated at 50°C for 20 min. 400 µl of TCA (10%) was added to the mixture, and centrifuged for 10 min (3000 rpm). Finally, 400 µl of the supernatant solution was mixed with 400 µl water and 80 µl of $FeCl_3$ (0.1%), and the absorbance was recorded at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.8.5. Homogenate tissue preparation

Livers were removed rapidly and were homogenized in ice-cold KCl (1.15%). The supernatant was separated by centrifugation at 4000 rpm for 10 min at 4C° and was used for the determination of MDA as a lipid peroxidation marker, Catalase and reduced glutathione (Sapakal et *al*., 2008).

2.8.6. Histological examination of liver and kidney

Livers and kidneys were removed rapidly and washed with an isotonic sodium chloride solution. The organ pieces were fixed in 10% formalin. Sections at 5µm were stained with haematoxylin and eosin and examined under light microscope. The analyses of the different organs were performed using an Optika B-500T i-5 microscope in order to verify alterations in the tissues, at the pathological anatomy laboratory of the CHU of Setif, Algeria.

2.9. Evaluation of anti-inflammatory activities (*in vivo*)

To investigate the anti-inflammatory effect of *Oxalis cernua* (AME, and RME), three experimental models of inflammation have been used in *in vivo* examinations. The effect of *per os* treatment with *Oxalis cernua* extracts was experienced by the model of paw oedema induced in rats by both carrageenan and albumin, and ear oedema induced by xylene.

2.9.1. Xylene-induced ear oedema

The anti-inflammatory activity of *Oxalis cernua* methanolic extracts was investigated on xylene induced inflammation ear (Rotelli et *al*., 2003; Mulla et *al*., 2010). Female *Swiss albino* mice were used. They were randomly distributed into eight groups of five animals each with similar average body weight. The animals were treated *per os*, control group received distilled water, aspirin group received (100 mg/Kg bw) of aspirin, and treated groups with *Oxalis cernua* received (100, 200 and 400 mg/kg bw) of both extracts AME and ARE. Acute inflammation of

the outer surface of the right ear of experimental animals is induced by the application of 30 µl of xylene, an hour after oral administration, while the left ear was considered as a control. A digital calliper was used to measure the thickness of the ear after 2 h. Anti-inflammatory activity is expressed as a percentage inhibition of oedema following the formula:

$$I\% = 100 \times (Vc - Vt) / Vc.$$

Vc: represents the increase in ear oedema of control group;

Vt: represents the increase in ear oedema of treated group (standard/extract).

2.9.2. Carrageenan-induced rat paw oedema

The anti-inflammatory activity of *Oxalis cernua* methanolic extracts was investigated on carrageenan induced inflammation in rat paw (Sarveswaran et *al*., 2017). Female rats were used in this study. They were randomly divided into eight groups of five animals each with similar average body weight. *Per os*, control group received distilled water, diclofenac group received 20 mg/kg bw of diclofenac dissolved in 0.9% NaCl, and treated groups with *Oxalis cernua* received (100, 200 and 400 mg/kg bw) of both extracts AME and ARE. Then, the initial thickness of the left paw was measured.

Subsequently,1 h after treatment, 0.1 ml suspension of carrageenan was freshly prepared in normal saline, then it was injected into the sub-planter tissue of the right hind paw of the experimental animals to induce oedema. The paw oedema up to ankle joint was measured using a digital calliper at 0, 60, 90, 120, 150 and 180 min after the carrageenan injection. The difference between the initial and subsequent values gave the actual oedema which was compared with control. The inhibition percentage (I%) of inflammation was calculated using these paw volumes, with respect to their controls according to the following formula:

$$I\% = 100 \times (Vc - Vt) / Vc.$$

Vc: represents mean oedema in control group;

Vt: represents mean oedema in group treated with standard/extract.

At the end of the experiment, the removed paws were fixed in 10% formalin. The paws were decalcified using 10% of formic acid for the histological study. Sections at 5µm were stained with haematoxylin and eosin, then, examined under light microscope. The analyses of the paws were performed using an Optika B-500T i-5 microscope in order to verify alterations in the tissues, at the pathological anatomy laboratory of the CHU of Sétif, Algeria.

2.9.3. Egg albumin-induced rat paw oedema

The anti-inflammatory activity of *Oxalis cernua* methanolic extracts was investigated on egg albumin induced inflammation in rat paw (Essien et *al*., 2017). Female rats were used in this study too. They were randomly divided into eight groups of five animals each with similar average body weight. The animals were treated *per os*, control group received distilled water, standard group received (20 mg/Kg bw) of aspirin (Asp), and treated groups with *Oxalis cernua* received (100, 200 and 400 mg/kg bw) of both methanolic extracts (AME and ARE). After 30 min, the initial volume (size) of their right hind paw was measured and recorded before inducing an inflammatory oedema by injecting subcutaneously 0.2 mL of fresh egg-albumin into the sub-plantar of the right hind paw. The right-paw volumes were measured and recorded at 30 min intervals and for 2 h using Calliper.

The difference between the initial and subsequent values gave the actual oedema which was compared with control. The inhibition percentage (I%) of inflammation was calculated using these paw volumes, with respect to their controls according to the following formula:

$$I\% = 100 \times (Vc - Vt) / Vc.$$

Vc: represents the increase in paw oedema of control group;

Vt: represents the increase in paw oedema of treated group (standard/extract.)

At the end of the experiment, the removed paws were fixed in 10% formalin. They were decalcified using 10% of formic acid for the histological study. Sections at 5µm were stained with haematoxylin and eosin and examined under light microscope. The analyses of the paws were performed using an Optika B-500T i-5 microscope in order to verify alterations in the tissues, at the pathological anatomy laboratory of the CHU of Setif, Algeria.

3. Statistical analysis

Statistical analysis was performed using Graph Pad Prism (version 5.01 for Windows). In *in vitro*, experimental results are expressed as mean ± standard deviation (SD) of triplicate. The data were analysed via *t*-test to determine statistical significance. *p*-values less than 0.05 ($p<0.05$) were considered as indicative of significance. The IC_{50} and EC_{50} values were calculated from linear regression analysis.

In *in vivo*, experimental results were expressed as mean ± standard error of mean (SEM). Statistical analysis of data was accomplished using analysis of variance (ANOVA). *p*-values less than 0.05 ($p<0.05$) were considered as indicative of significance.

Results and discussion

1. Extraction process and fractions preparation

Extraction is the primary step of any medicinal plant study to ensure that the active ingredients within the plants are not lost or destroyed during preparation which affects the final result. The matrix properties of the plant part, solvent, temperature, pressure and time are the most important factors in the course of this process (Pandey and Tripathi, 2014; Panja, 2018; Yahya et *al*., 2018). This is why all the extraction techniques aim to enhance overall yield and selectivity of bioactive components, to increase sensitivity of bioassay by increasing the concentration of targeted compounds; to convert the later one into a more suitable form for detection and separation yield (Azmir et *al*., 2013; Pandey and Tripathi, 2014).

Maceration is a common and inexpensive method to acquire bioactive compounds, it consists of several steps. Grinding of plant materials into small particle is used to increase the surface area for proper mixing with solvent. The later diffuse into the solid plant material and solubilise compounds with similar polarity. Occasional shaking in maceration facilitate extraction by two ways; increase diffusion, and remove concentrated solution from the sample surface for bringing new solvent to the menstruum for more extraction yield (Azmir et *al*., 2013; Pandey and Tripathi, 2014).

Among the key considerations prior to an extraction process is the selection of appropriate solvents for extracting bioactive compound from the plants. The basis of the solvent selection depends on the specific nature of the bioactive compound(s) to be isolated, so, the solvents used must have the same polarity as the solute of interest. In the present work, the isolation of phenolic as well as secondary plant compounds necessitates the selection of polar solvents with lower boiling points such as methanol. These solvents for most, are suitable for extracting the polar and semi-polar constituents (Panja, 2018; Yahya et *al*., 2018).

Bioactive compounds can be restricted by the successive use of multiple solvents of increasing polarity: hexane, chlorophorm and ethyl acetate during fractionation of crude extract.

Methanol extraction products contain anthocyanin, terpenoids, saponins, tannins, flavones, polyphenols. Chlorophorm extraction products consist of terpenoids and flavonoids. Hexane extraction products involve non-polar or mildly hydrophobic compounds with extremely high lipophilicity. Ethyl acetate extraction products comprises moderately hydrophobic compounds, including low and moderately polar, neutral, basic and acidic solutes, of which, alkaloids, wax, steroids, polar-chain carbonated polymers and fatty acids. Water extraction product covers metals, ions, high hydrophilic compounds, and water-soluble proteins/enzymes, glycoproteins, peptides, amino acids, nucleotides, sugars, and polysaccharides (Pandey and Tripathi, 2014; Yahya et *al*., 2018).

2. Determination of phenolic content

Based on the ethno-pharmacological and modern evidence, polyphenols represent one of the largest categories of secondary metabolites that are consumed in great amounts on a daily basis and studied extensively for their health-promoting properties. The increased interest on phenolic compounds has arisen, mainly because of their antioxidant capacity which can be attributed by radical scavenging mechanism (Carmo et *al*., 2018; Oyenihi and Smith, 2018). Therefore, the optimal extraction conditions for *Oxalis cernua* are important because the properties that are favourable to health are determined by the biological evaluation of this extract.

The extracts obtained from *Oxalis cernua* are characterized by their yields, which have been determined in relation to the dry weight of the sample (100g). The results obtained in the present study (Table 4) revealed that the highest yield percentage was that of *Oxalis ernua* AME (14.05 %), while RME, AqE, EAE and CHE registered 4.55%, 3.48%, 1.62% and 0.40% respectively.

The total phenolic content in the extracts was determined by the Folin–Ciocalteu method, whereas the flavonoids content quantified using $AlCl_3$ method. Total phenolic content was expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/ g of extract) and total flavonoids content as mg quercetin equivalents per gram of dry extract (mg QE/ g of extract).

As seen in Table 4, the obtained results revealed that the level of these phenolic compounds in *Oxalis cernua* extracts and fractions was considerable. CHE noted the highest amount of polyphenols with a value of (127.93±0.05mg GAE/g dw). Whereas, *Oxalis cernua* AME showed the highest amount of flavonoids (43.69±0.61mg QE/g dw).

The technic and the solvent are key factors to maximize recovery selectively and avoiding undesired substances when plant-derived compounds are studied. However, the yield and the phenolic content are relative and may vary with the variation in the extraction method. Therefore, several aspects are considered, among which the period of collection, contaminants, solvent polarity, extraction time and temperature, genetic background and/or pedo-climatic conditions which may influence the dry matter, this is why it is difficult to compare this results with those of bibliography (Ekar and Kreft, 2018; Rocchetti et *al*., 2019; Shahid-Ud-Daula et *al*., 2019).

Table 4: Yields, total polyphenols and flavonoids contents in various extracts of *Oxalis cernua*.

EXTRACTS	Yields (%)	Polyphenols[(a)]	Flavonoids[(b)]
AME	14.05±0.60	79.00±0.10	43.69±0.61
CHE	0.40±0.00	127.93±0.05	9.20±0.13
EAE	1.62±0.06	72.00±0.78	33.91±0.65
AqE	3.84±0.73	30.86±0.95	16.67±0.13
RME	4.55±0.00	26.20±0.45	9.95±0.10

AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract., RME; root methanolic extract. (a) mg gallic acid Equivalents/g of dry extract; (b) mg Quercetin Equivalents/g of dry extract. Results are expressed as means ± SD, (n = 3).

3. *In vitro* antioxidant activity

The large body of knowledge on antioxidants fuelled research activity on their role of preventing and interrupting the propagation of free radical through scavenging species that initiate peroxidation, chelating metal ions or breaking the auto-oxidative chain reaction (Fierascu et *al*., 2018). The antioxidant potential of phenolic compounds of *Oxalis cernua* depends on the redox properties of their phenolic hydroxyl groups, which act as oxygen quenchers reducing / or hydrogen donating agents. Regardless of the different reaction systems and complexity of different methods utilized to quantify the antioxidant activity of the extract, none of them can fully describe its potential (Pires et *al*., 2017).

Since the antioxidant capacity of a medicinal plant cannot be fully described by a single method, although there are several, it is recommended that each evaluation of the antioxidant activity must be performed using several different methods. Thus, DPPH and ABTS scavenging, reducing power, β- carotene bleaching, ferrous ion chelating, hydroxyl radical,

anti-haemolytic, and anti-denaturation protein assays are used to evaluate the antioxidant activity of *Oxalis cernua* extracts in the present study.

3.1. DPPH radical scavenging activity of extracts

A wide variety of colorimetric techniques have been improved for the estimation of antioxidants potential. Moreover, studies have assumed a widely used common assay to investigate the proton radical scavenging activity of bioactive compounds like phenols is (2,2 diphenyl-1-picrylhydrazyl) DPPH (Sridhar and Charles, 2019).

DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals (Figure 7). The delocalization of electron also gives rise to the deep violet colour. When a solution of DPPH is mixed with that of a substrate that can donate a hydrogen atom, this gives rise to the reduced form with the loss of this violet colour (Alam et *al*., 2013).

It accepts electrons or hydrogen radicals from donor compounds and shows a strong absorption band between 515-520 nm. The inhibition percentage of DPPH assay is expressed using inhibition concentration (IC_{50}) values, which is reported as the amount of antioxidants required to decrease the initial concentration of scavenging radicals by 50%. In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored (Sridhar and Charles, 2019).

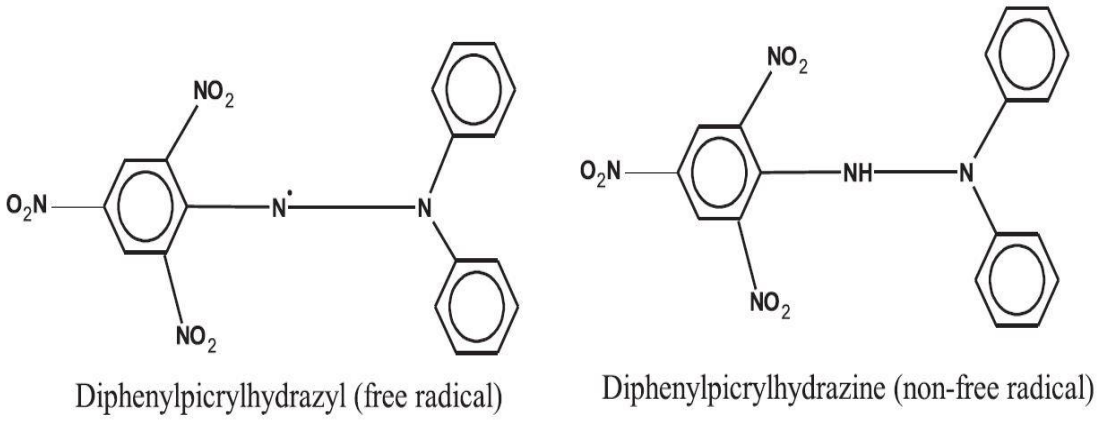


Figure 7: The basic structures for diphenylpicrylhydrazyl (free radical) and diphenylpicrylhydrazine (non-free radical) (Sridhar and Charles, 2019).

The results showed that all extracts exhibited a scavenging activity against DPPH radical. However, comparing IC_{50} values between different plant extracts and BHT, as a standard, demonstrated that the later noted the highest scavenging activity (IC_{50} = 8.58 ± 0.00 µg/ml) as showed in figure 8, because, the lower is IC_{50}, the higher is the antioxidant activity.

The EAE was found to revel significantly ($p < 0.01$) a scavenger activity (IC_{50} = 67.84 ± 0.00 µg/ml). The DPPH scavenging activity decreased, after that, in the following order: AME (IC_{50} = 83.43 ± 0.00 µg/ml) > CHE (IC_{50} = 85.84 ± 0.00 µg/ml) > AqE (IC_{50} = 186.35 ± 0.02 µg/ml) > RME (IC_{50} = 250.37 ± 0.02 µg/ml) respectively. Otherwise, BHT and all the previous extracts scavenge DPPH radical in concentration dependent manner.

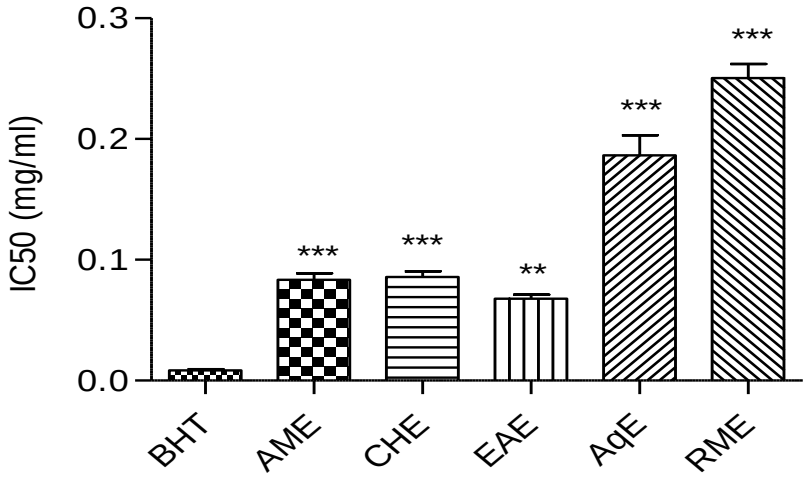


Figure 8: A comparison between IC_{50} values of different plant extracts in DPPH free radical scavenging activity. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract, RME; root methanolic extract, and BHT; bautylated hydroxytoluene. Values were expressed as IC_{50} mean ± SD; n = 3. Comparisons are made with respect to BHT. (**: $p < 0.01$, ***: $p < 0.001$).

Pearson's correlation coefficient was evaluated between DPPH scavenging activity and total phenolic and flavonoids content, the values were (R^2 =0.005) and (R^2 =0.360) respectively. According to the given results, no significant correlation ($p<0.05$) was observed between them. Even though, the extract was able to quench the DPPH radicals so, it can be assumed that polyphenols and flavonoids act as hydrogen donors with good antioxidant principles.

Phenolic compounds are ubiquitous in plant organs, consisting of an aromatic ring with different degrees of hydroxylation, and known for their ability to inhibit or delay the oxidation of other molecules. They are protective against oxidative stress via different mechanisms and modes of action that are often independent of their antioxidant effect and may render their effects cooperatively via several mechanisms (Chandrasekara and Shahidi, 2018).

Flavonoids' hydroxyl groups are able to donate electrons and hydrogen where position and number of hydroxylation correlate reasonably to anti-oxidation of flavonoids. Numerous

studies have attributed flavonoids' anti-oxidant activity to free radical scavenging mechanism. Nevertheless, the results are often ambiguous and incomparable based on different oxidant species or analytical methods applied. On the other side, it may be also assumed that most flavonoids were in their glycoside forms and consequently less effective compared to their aglycone forms. Accordingly, flavonoids had no influence in antioxidant activity by DPPH scavenging method (Wang et *al*., 2018).

In the present study, the obtained results are in agreement with Gaspar and their collaborators (2018). It was reported that the screening for free radical scavenging bioactivity by DPPH displayed an IC_{50} = 17.93 µg/ml. The data confirms that the extract may probably be an interesting source of antioxidant compounds based on the results from other natural extracts (Gaspar et *al*., 2018). However, another study reported that *Oxalis corniculata* methanolic extract and fractions had exhibited a potent DPPH scavenging ability too. Their IC_{50} values were: aqueous fraction (78.5 ± 1.65µg/ml), chloroform fraction (52.4 ± 1.03µg/ml), ethyl acetat fraction (34.4 ± 1.17µg/ml), methanol extract (68.5 ± 0.96µg/ml) (Jain et *al*., 2018).

3.2. ABTS radical scavenging activity of extracts

The ABTS (2, 2'-bis-azino (3ethylbenz-thiazoline-6-sulfonic acid)) is a free radical soluble in both organic solvents and water. It is widely used to evaluate the antioxidant activity of natural products. In this assay, ABTS is oxidized to its radical cation ($ABTS^{\bullet+}$**)** (blue colour) light-absorbing at 734nm directly using potassium per-sulphate as the oxidizing agent. Then, antioxidants react only with $ABTS^{\bullet+}$ by only one reaction, during that it converts to its colourless form neutral (Figure 9) (Schaich et *al*., 2015; Kontogiorgis et *al*., 2016).

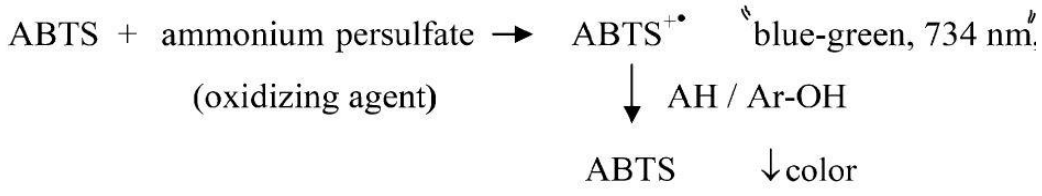


Figure 9: The oxidation of ABTS to its radical cation ($ABTS^{\bullet+}$) (Schaich et *al*., 2015).

The radical cation of ABTS is used for classification of relative inhibition capacity of flavonoids and phenolic compounds through their ability as electron or proton donors. When the radical interacts with antioxidants, its concentration is reduced, showing a decrease of the absorption thus the higher is the antioxidant activity of the tested substances, the lower is the absorption since antioxidants interact with the coloured cation in proportion to their concentration in each sample (Kontogiorgis et *al*., 2016).

In the current study, the given results (Figure 10) revealed that all *Oxalis cernua* extracts and fractions have good ABTS radical scavenging activities. In addition, they all showed a dose-dependent $ABTS^{\cdot+}$ radical scavenging activity. However, statistically there was a significant difference ($p<0.001$) when comparing TROLOX as a standard to plant extracts and fractions.

The result of ABTS inhibition activity revealed that TROLOX shown an IC_{50} value of (0.0026±0.000007mg/ml). Moreover, the scavenging activity of *Oxalis cernua* extracts and fractions decreased in the following order: AME >EAE >AqE >RME >CHE, whereas IC_{50} values were: (0.0179±0.00023 mg/ml), (0.0241±0.0012 mg/ml), (0.0246±0.0005 mg/ml), (0.0299±0.0028 mg/ml), (0.0318±0.0016 mg/ml) respectively. In addition, in the previous sections, a similar effect of the antioxidant capacity (with the DPPH assay) was observed.

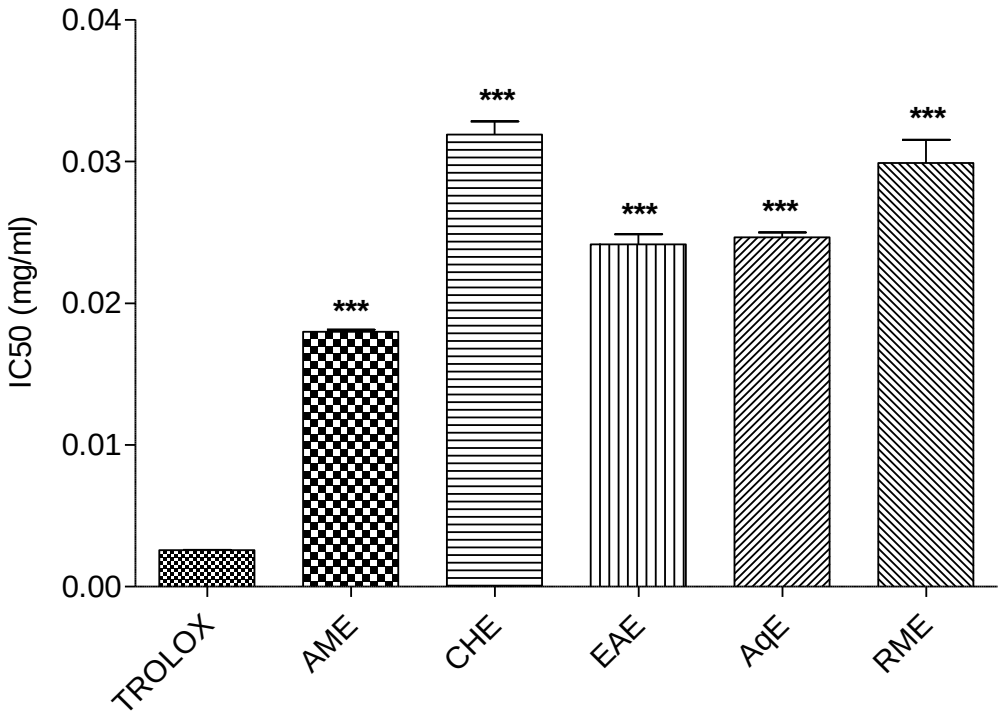


Figure 10: A comparison between IC_{50} values of different plant extracts in ABTS free radical scavenging activity. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract, RME; root methanolic extract and Trolox as standard. Data were presented as IC_{50} mean ± SD; n = 3; (***: $p < 0.001$).

The comparison between total flavonoids content and the ABTS test results demonstrated a significant correlation R^2= 0.85. This could indicate that the scavenging potential of *Oxalis cernua* was possibly due to the hydrogen and electron donating ability of the flavonoids compounds present in these extracts which made them antioxidants acting as free radical inhibitor or scavengers.

Comparing the ABTS free radical scavenging activity of our study with those of Khyadea and their collaborators, the IC_{50} (0.021 mg/ml) of *Oxalis corniculata* Linn aerial part methanolic extract from India was comparable to that noted in the present study. However, the difference could be due to the geographical origin (Khyadea et *al*., 2016).

Furthermore, this finding was consistent with the results of different studies on the antioxidant activity and bioactive compounds. However, it is likely that the scavenging capacity of the extracts is due to the characteristics of the chemical structure of polyphenolic compounds as hydrogen or electron donors acting as free radical's scavengers (Beta and Hwang, 2018).

3.3. Reducing power capacity of extracts

The reducing power capacity of the extracts may provide a significant indication about the potential antioxidant capacity of the plant to donate an electron (Raj et *al*., 2019). It was established using the reduction of Fe^{3+} to Fe^{2+}. In this assay, the yellow colour of the $FeCl_3/K_3Fe(CN)_6$ present in the solution changes to various shades of green and blue, depending on the reducing power of test solution. The presence of the antioxidants in the samples leads to Fe^{3+}/ferricyanide complex reduced to the Fe^{2+} form formation of Perl's Prussian blue at 700 nm. Therefore, the increased absorbance is an indication of increased reducing powers, and increased reducing powers of various plant extracts indicated strong antioxidant potential (Sharma and Vig, 2014; Khorasani Esmaeili et *al*., 2015).

The reducing power of the extracts and standard were augmented in a concentration dependent manner. However, the reducing ability of the extracts were significantly different from that of the BHT ($p<0.001$) (Figure 11). They showed some degree of electron donation, and were in the following order BHT (EC_{50}= 0.045 ± 0.00 mg/ml) > CHE (EC_{50}= 0.254 ± 0.00 mg/ml) > EAE (EC_{50}= 0.327 ± 0.05 mg/ml) > AME (EC_{50} = 0.332 ± 0.09 mg/ml) > AqE (EC_{50}= 0.437 ± 0.03 mg/ml) > RME (EC_{50}= 0.483 ± 0.02 mg/ml).

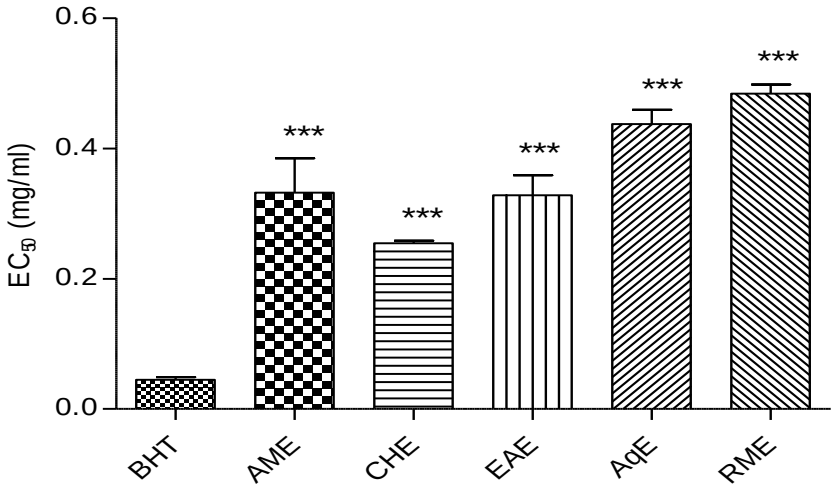


Figure 11: A comparison between EC_{50} values of different plant extracts in reducing power assay. Comparisons are made with respect to BHT. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract., RME; root methanolic extract, BHT; bautylated hydroxytoluene. Values were expressed as EC_{50} mean ± SD; n = 3; (***: p <0.001).

According to the results, CHE had the highest reducing power among the extracts tested, and also the highest content of polyphenolic compounds, and that cause the greater reducing power. The decreasing order of the values of EC_{50} of the extracts was in agreement with their content on polyphenols.

The reducing power assay is based on the reduction of Fe^{3+} to Fe^{2+}. It is also attributed to the presence of reductones that exert antioxidant action by breaking the free radical chain reaction donating a hydrogen atom and eventually form a stable product (Sridevi et *al*., 2018).

The reducing power activity of the extracts was shown in figure 11. The reducing power of reference compound (BHT) was found to be better than extracts at the same concentrations. It exhibited that BHT was the powerful electron donors, able to break the radical chain reaction more than the extracts, which showed a considerable antioxidant activity, indicating that it can act as electron donors and also could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions.

The comparison between total phenolic and flavonoids content and the reducing power test results revealed a non-significant correlation with (R^2= 0.01) and (R^2= 0.06) respectively. This could suggest that the reducing capacities of *Oxalis cernua* were possibly not due to the electron donating ability of the phenolic and flavonoids constituents present in these extracts. It was speculated that the reducing power could be attributed to the synergy of different bioactive constituents, or to nonphenolic compounds (Arina and Azrina, 2016).

The study of Khan and their colleagues demonstrated that *Oxalis corniculata* methanolic extract and its different fractions exhibited varied degrees of reducing power. Among all the extracts, Aqueous fraction (0.515 ± 0.006 µg/ml) exhibited the strongest reducing power. The values of absorbance of crude extract was (0.641 ± 0.004 µg/ml), ethyl acetate fraction (0.595 ± 0.014 µg/ml), and Chloroform fraction (0.565 ± 0.013 µg/ml). Comparing the reducing power results obtained in this study with those of Khan and their colleagues, it appears that *Oxalis cernua* methanolic extract and its different fractions have a remarkable potency to donate electron to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction (Khan et *al*., 2012).

3.4. Antioxidant activity of extract using β –Carotene / linoleic acid bleaching assay

The antioxidant activity of *Oxalis cernua* was also assessed using the β-carotene linoleate bleaching test. In this assay, the oxidation of linoleic acid produces peroxyl free radicals due to the abstraction of hydrogen atom from diallylic methylene groups of linoleic acid. Then the highly unsaturated β-carotene will be oxidised by free radical. The antioxidants present in the extract will reduce the oxidation of β-carotene by hydroperoxides. The later will be decomposed by the antioxidants from the extracts. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts Effect (Abdelwahab et *al*., 2011).

This *in vitro* assay had been based on the loss of yellowish-orange colour of β-carotene reacting with radicals which resulted from the linoleic acid oxidation that occurred in the emulsions (Hassani et *al*., 2019; Prasad et *al*., 2013). The presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.

Accordingly, the zone of discoloration increased rapidly in samples without antioxidant whereas, in the presence of an antioxidant, they retained their colour, and thus the colour retention is greater. The result of this test corresponds to their free radical scavenging activity, which may be due to the presence of higher phenolic content (Kalaivani et *al*., 2011).

Figure 12 demonstrated the changes in the percentage of inhibition of linoleic acid oxidation under the influence of the different extracts of *Oxalis cernua* (2mg/ml) compared to that of synthetic antioxidant BHT as a positive control during 24 under the same condition. In this assay, the extracts were capable to prevent beta carotene oxidation, and to exhibit antioxidant potential by virtue of their radical scavenging activity.

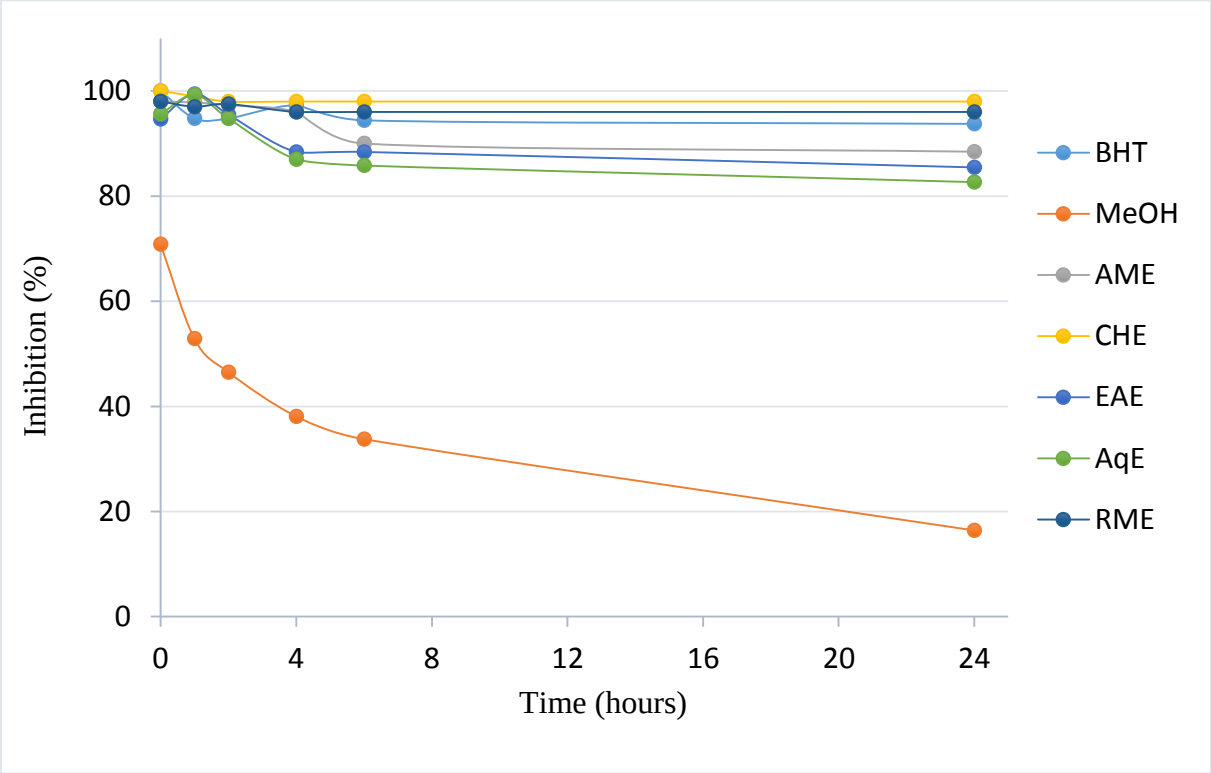


Figure 12: Percentage changes of the inhibition ratios of linoleic acid oxidation of different plant extracts (2mg/ml) using β-carotene bleaching method, compared to BHT as a positive control during 24 h. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract., RME; root methanolic extract, and BHT; bautylated hydroxytoluene. Values were expressed as inhibition percentage mean ± SD; n = 3.

As shown in figure 13 all the extracts inhibit the oxidation of beta-carotene in a very important level no significant differences were recorded ($p<0.05$) compared to BHT (98±0.49%). CHE had the best β-carotene bleaching inhibition activity whit (98±0.01%), which was as potent as the standard. Then it was followed by RME (96.21±6.56%), AME (88.44±0.46 %), EAE (85.45±2.06%) and AqE (82.66±0.14%) respectively.

The antioxidant activities of the phenolic compounds may depend on structural factors, such as the number of phenolic hydroxyl or methoxyl groups, flavone hydroxyl, keto groups, free carboxylic groups, and other structural features. Recent reports indicated that several bioactive compounds present in plants have strong antioxidant activity. Chromophores such as β-carotene have alternate double and single carbon-carbon bonds which are known as

conjugated system. Carotenoids undergo bleaching (loss of colour) when exposed to radicals or to oxidizing species which involves interruption of the conjugated double bond system either by cleavage or by addition to one of the double bonds (Kumar et *al*., 2014). The antioxidant activity of the extracts in the present investigation is probably due to the transfer of hydrogen atom from phenolic compounds to free radical, therefore inhibiting bleaching of beta carotene (Prasad et *al*., 2013).

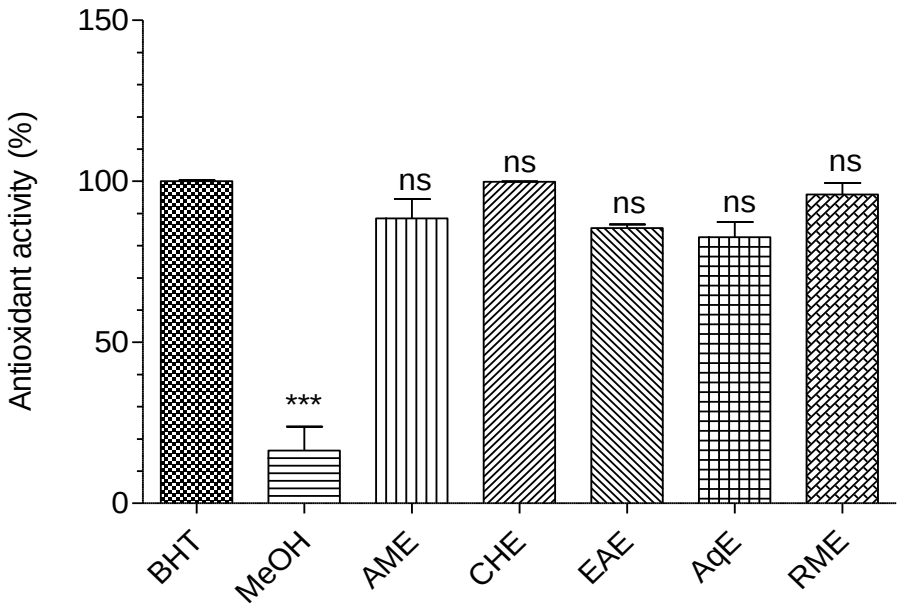


Figure 13: Antioxidant activities of different plant extracts measured by β-carotene bleaching method. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract., RME; root methanolic extract, and BHT; bautylated hydroxytoluene was used as reference antioxidant. Values were expressed as mean ± SD; n = 3; (ns: no significant difference $p > 0.05$, *** $p < 0.001$).

All the extracts showed a potent ability to inhibit free radical mediated lipid peroxidation. Though, the presence of antioxidants minimizes the oxidation of beta-carotene which may be strongly correlated with their content of polyphenols (R^2 =0.95) (Armentano et *al*., 2015). Considering the flavonoids content of the extracts and fractions, it did not show appreciable anti-bleaching activities (R^2=0.06). It could be possible that the types of flavonoids present in

extract are not appropriate for these functional activities as they are structure dependent. The chemical nature depends on the structure class, degree of hydroxylation, conjugations and substitutions, and degree of polymerization (Abdelwahab et *al*., 2011; Mishra et *al*., 2013).

3.5. Metal chelating activity of extracts

The ferrous iron Fe^{2+} bivalent transition metal ion has an important role. It can transfer electrons spontaneously to initiate radical reaction. It is thought to be effective pro-oxidants, through forming oxygen-derived free radicals which could induce lipid peroxidation mainly through the Fenton reaction or by accelerating the transformation of lipid hydroperoxides to the respective radicals. Therefore, the chelating effect of free iron by chelating agents or antioxidants from natural source can prevent the formation of these free radicals (Ahmad Wani and Tirumale, 2018; Fu et *al*., 2019).

Assessment of iron chelating capacity of antioxidant is significant as excess iron causes superoxide anion and hydrogen peroxide to convert into the extremely reactive hydroxyl radicals that cause severe injury to proteins, DNA and also accelerate lipid peroxidation (Jain et *al*., 2019).

In the metal chelating activity, Ferrozine quantitatively chelates with Fe^{2+} forming a red coloured complex. In presence of other chelating agents, the reaction gets limited which leads to the decrease in the colour intensity of the ferrozine-Fe^{2+} complexes. Chelating activity of the sample and standard (EDTA) to compete with ferrozine for the ferrous ions can be estimated by the measurement of the colour reduction. The Ferrozine-Fe^{2+} complex formation was disrupted by the presence of chelating agents in the sample. Thus, the decreased absorbance at 562 nm indicates high levels of iron binding potential (Jain et *al*., 2019; Sohal et *al*., 2019).

The capacity of samples to chelate ferrous ion is presented in figure 14, all the extracts demonstrated Fe^{2+} chelating activity in a dose-dependent manner. However, EDTA is perfectly

effective on sequestering the active iron like Fe^{3+} to form a metal complex along with diminish of the oxidation capacity (Guo et *al*., 2018). There was a significant difference between the standard EDTA which is a known metal ion chelator (IC_{50} = 0.01 ± 00 mg/ml) and all of AME (IC_{50} = 0.16 ± 0.00 mg/ml), CHE (IC_{50}= 0.19 ± 0.02 mg/ml), AE (IC_{50} = 0.18 ± 0.02 mg/ml) and RME (IC_{50} = 0.07 ± 0.01 mg/ml) with ($p < 0.01$). Whereas, AqE (IC_{50} = 0.13± 0.03 mg/ml) showed a better activity with a difference statistically significant ($p<0.05$) compared to the standard.

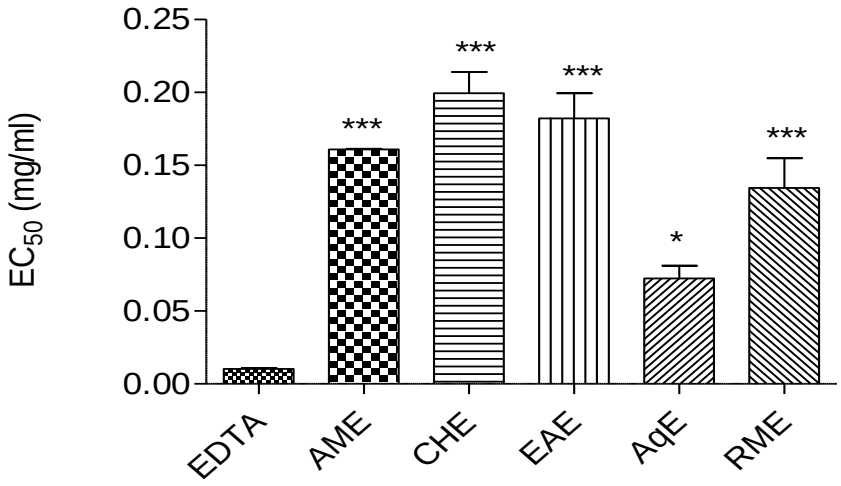


Figure 14: A comparison between different plant extracts in metal chelating activity. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract., RME; root methanolic extract, and EDTA; ethylenediaminetetraacetic acid was used as standard. Data were presented as EC_{50} mean ± SD; n = 3; (*: $p< 0.05$, ***: $p < 0.001$).

In this assay both the samples, and standard EDTA compounds interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity and captures the ferrous ion before ferrozine. The result indicates that the sample has good iron binding capacity, suggesting its action as an antioxidant relating to its iron binding capacity (Jain et *al*., 2019; Pešić et *al*., 2019; Sohal et *al*., 2019).

Several studies reported that metal ion chelating activity was related to the ability of some phenolic compounds to disrupt the formation of Ferrosine-Fe^{2+} complex. These findings may be attributed to the presence of functional groups present in polysaccharides and EDTA (Wei et *al*., 2019).

The analysis of the capacity of extracts to chelate ferrous ion demonstrated a weak correlation of polyphenols (R^2= 0.7) and flavonoids (R^2=0.6). These findings may indicate that metal chelation activity did not depend on the quantity of phenolic present in the extracts, but the specific type of phenolic compounds and the number of hydroxyl groups and their arrangement in the molecules of interest were most important (Rahman et *al*., 2018).

The results of the present study are in accordance with previous published reports which indicate that the antioxidant activity of ethyl acetate and chloroform fractions are almost the same (201.7 ± 3.5 µg/ml) and (100.9 ± 2.6 µg/ml) respectively. while, methanolic extract (52.8 ± 2.4 µg/ml) and Aqueous extract (57.8 ± 2.2 µg/ml) were potent effective in Fe^{2+} chelating compared to the current findings (Khan et *al*., 2012).

3.6. Hydroxyl radical scavenging activity of extracts

Hydroxyl radicals are highly reactive and are short-lived. They can be generated by biochemical reaction. Superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently produce extremely reactive hydroxyl radicals in the presence of divalent metal ions (Figure 15), such as iron and copper by a reaction known as the Fenton reaction (Lalhminghlui and Jagetia, 2018):

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

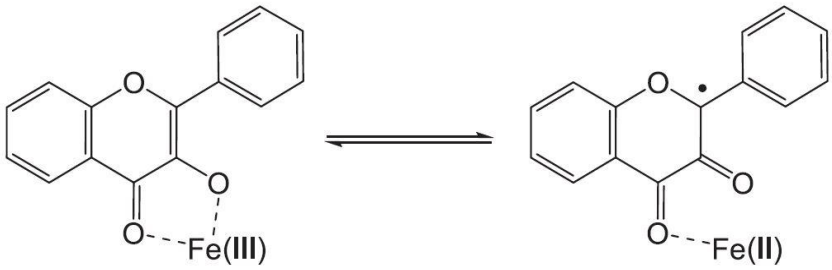


Figure 15: Proposed route for generation of Fe^{2+}- flavonoid complex from Fe^{3+}-flavonoid complex (Procházková et *al*., 2011).

Antioxidant compounds are capable of sequestering and disrupting the radical reactions triggered by the OH radical. The hydroxyl radicals can be eliminated by antioxidant substances through three types of mechanisms (Anugraha et *al*., 2019; Theis et *al*., 2019):

- ✓ Electron transfer: $OH^+ + R \longrightarrow OH^- + R^+$;
- ✓ Hydrogen abstraction: $RH + OH \longrightarrow R + H_2O$;
- ✓ In addition to an aromatic ring or double bond breaking, which generate an addition product: $OH^- + R = R \longrightarrow HO\text{-}R\text{-}R$.

Hydroxyl radical is one of the strongest oxidative free radicals formed in biological systems. It is concerned as a highly destructive species in free radical pathology, capable of damaging almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and can cause strand breakage, leading to cytotoxicity, mutagenesis, carcinogenesis and other diseases (Saravanakumar et al., 2019; Yu et *al*., 2019).

Salicylic acid detection method is currently the most widely used. The advantage of this method is that: it can simply simulate antioxidants scavenging hydroxyl radicals in tubes (Hou et *al*., 2019). In this assay, the test and standard compound colour changed to various shades of pink (Jothy et *al*., 2012).

In this experiment, the ability of the extract and standard to reduce hydroxyl radical was in a dose dependant manner. The results demonstrated that the extracts of *Oxalis cernua* had appreciable hydroxyl radical scavenging activity when compared with standard antioxidant ascorbic acid (AA). However, both of CHE and RME were potent to quench radical reactions more than the AA. Furthermore; AME, AqE and EAE displayed a strong effect as similar as the standard. There were no statistically significant differences between the standard AA (IC_{50} = 0.16 ± 0.07 mg/ml) and all of AME (IC_{50} = 0.13 ± 0.00 mg/ml), AqE (IC_{50} = 0.17 ± 0.01 mg/ml) and EAE (IC_{50} = 0.18 ± 0.01 mg/ml). Whereas, a significant difference ($p<0.001$) was noted concerning CHE (IC_{50} = 0.06 ± 0.00 mg/ml) and RME (IC_{50} = 0.09 ± 0.02 mg/ml) with respect to the standard (Figure 16).

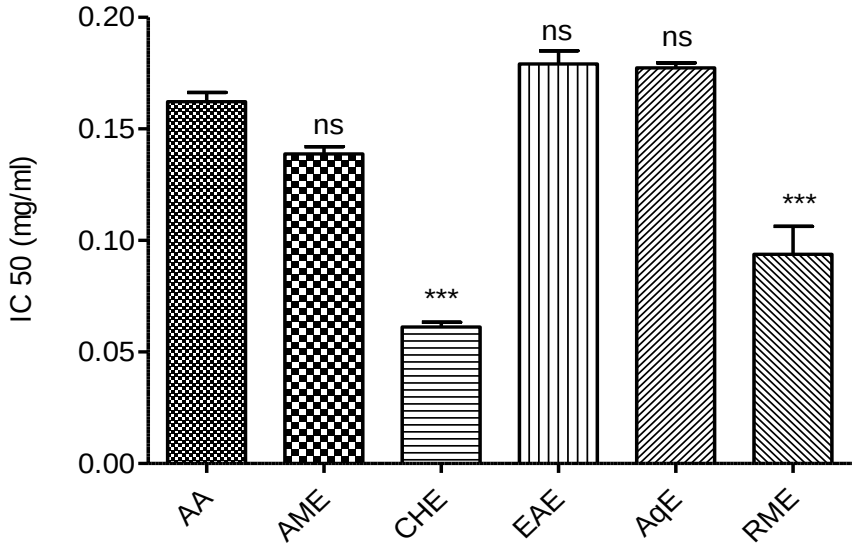


Figure 16: A comparison between different plant extracts in hydroxyl radical scavenging activity. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract; RME; root methanolic extract, AA; ascorbic acid was used as standard. Data were presented as IC_{50} mean ± SD; n = 3; (ns: no significant difference $p> 0.05$; *** $p<0.001$).

Hydroxyl radical scavenging activity is an important index to evaluate the antioxidant properties of the extracts to disrupt the radical reactions. It is well established that the hydroxyl radical scavenging capacity of samples is directly related to its antioxidant activity. The ability of the extracts to quench hydroxyl radicals seems to be directly related to the prevention of

propagation of lipid peroxidation (Bajpai et *al*., 2017). Moreover, it seemed to be a good scavenger of active oxygen species, thus reducing the rate of chain reaction (Jothy et *al*., 2012).

The correlation analysis between the hydroxyl radical scavenging assay noted with respect to the content of polyphenols ($R^2 = 0.21$) and flavonoids ($R^2 = 0.31$). It has been reported that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging activity of phenolic than are specific functional groups. Consequently, several reports revealed that polyphenols and flavonoids have a major effect on the ability of medicinal plants to scavenge radicals initiated by hydroxyl radical assay (Lim et *al*., 2019; Theis et *al*., 2019).

Oxalis corniculata methanolic extract and fractions displayed strong scavenging effects against hydroxyl radical. The IC_{50} values were (26.7 ± 1.5 µg/ml), (11.6 ± 0.9 µg/ml), (10.5 ± 0.7 µg/ml) and (26.0±1.4 µg/ml) for *Oxalis corniculata* methanolic, ethyl acetate fraction, chloroform fraction and aqueous fraction, respectively. Comparing the obtained result from the current study with those of *Oxalis corniculata*, it seems evident that the later phyto-components were more potent to scavenge hydroxyl radicals (Jain et *al*., 2018).

Many secondary metabolites and flavonoids have been found to scavenge hydroxyl free radicals in a concentration-dependent manner. Also, the antioxidants can either scavenge or block the precursor formation for their effective antioxidant activity (Lalhminghlui and Jagetia, 2018; Wani and Tirumale, 2018; Lim et *al*., 2019).

3.7. Anti-haemolytic assay

Erythrocytes deliver oxygen to body tissues. Consequently, they are under continuous stress by oxygenation and deoxygenation cycles, strong shearing forces in narrow blood vessels and endogenously generated ROS during their lifecycle. Additionally, erythrocytes are extremely vulnerable to oxidative damage because of the high cellular concentration of oxygen

and haemoglobin, and high content of membrane polyunsaturated fatty acid. Oxidative damage of the lipid membrane of erythrocytes is implicated in haemolysis that is associated with some hemoglobinopathies, radiation, exposure to transition metals, and dearth in the antioxidant defence system (Yang et *al*., 2017).

In the present haemolytic assay, AAPH was used as a free radical-induced erythrocyte lysis. The antiradical efficiency was defined as the percentage of the compound-mediated HT_{50} increase as compared to control. The anti-haemolysis activity of extracts was tested and measured as the capacity of erythrocytes to inhibit AAPH radical to induced haemolysis.

Results illustrated in figure 17 were expressed for the time corresponding to 50% of maximal haemolysis (half-haemolysis time, HT_{50} in min). In this assay, the extracts AME (HT_{50} = 109.86 ± 3.90 min), and CHE (HT_{50} = 118.31 ± 3.24 min) displayed a significance ($p<0.05$ and $p< 0.001$ respectively) statistically different. They increase significantly the half time of erythrocytes haemolysis exhibiting a potent antihemolytic activity and can effectively protect erythrocytes against AAPH-induced haemolytic injury compared to the control (aspirin) (HT_{50} = 77.31 ± 4.03 min), however, that of the ascorbic acid (AA) was the highest (HT_{50} = 138.47 ± 6.59 min) as the correspondent standard. Whereas, both of EAE (HT_{50} = 80.13 ± 2.79 min) and RME (HT_{50} = 93.68± 5.93 min) revealed no significant difference ($p>0.05$) with respect to the control. Furthermore, AqE (HT_{50} = 2.49 ± 0.83 min) showed a significance ($p<0.001$) statistically different. It may decrease significantly the half time of erythrocytes haemolysis exhibiting a weak anti-haemolytic activity.

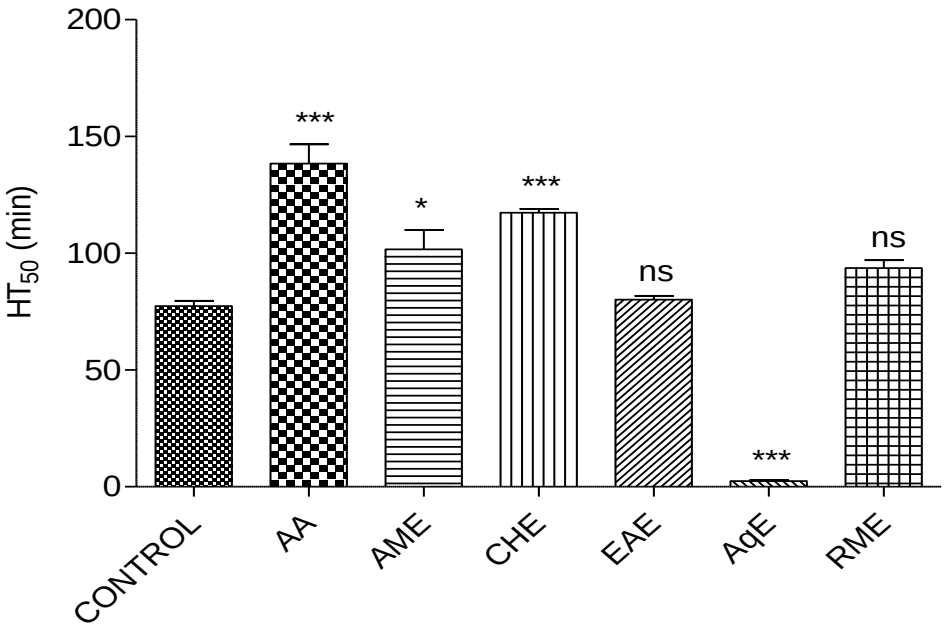


Figure 17: Antihemolytic activity of *Oxalis cernua* extracts expressed by the half-hemolysis time (HT_{50}). AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract., RME; root methanolic extract, and AA; ascorbic acid was used as standard. Values were expressed as HT_{50} means ± SD of triplicate; (ns: $p > 0.05$, *: $p < 0.05$, ***: $p < 0.001$).

Oxidative damage of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), a water-soluble free radical generator, induced haemolysis, enhances membrane lipid peroxidation, and architectural damage of membrane proteins, leading to erythrocyte disorders and other diseases, including Alzheimer's disease, sickle cell anaemia, renal failure, and beta-thalassemia, cellular aging, and haemolysis. The exposure of erythrocytes to AAPH caused maximum utilization of endogenous antioxidants, weakening the antioxidant defence system and leading to an enormous increase of haemolysis (Silva et *al*., 2014; Yang et *al*., 2017).

The current result exhibited that the antioxidant properties of the extracts are probably related to its bioactive compounds which, have potential capacity to quench free radicals and thereby attenuate oxidative haemolysis. It has been reported in the literature that polyphenols and flavonoids, via hydrogen donation, are able to capture peroxyl radicals generated by AAPH,

resulting in the protection of erythrocytes' membrane (Campos et *al*., 2015; dos Santos et *al*., 2017).

The correlation analysis indicated that there is no significant correlation between total phenolic content and HT_{50} of anti-haemolytic activity (R^2= 0.41). On the other hand, Person's correlation did not show association between flavonoids content and HT_{50} of anti-haemolytic activity (R^2= 0.02). The erythrocytes' membrane stability as mentioned by various authors can be justified by the involvement of extracts compounds (Bammou et *al*., 2016; Pereira-Freire et *al*., 2018).

4. Evaluation of *in-vitro* anti-inflammatory activity

Protein denaturation is a process in which proteins lose their secondary and tertiary structure without breaking or hydrolysis of peptide bonds by application of external stress or compound, such as strong acid or base, concentrated inorganic salt, organic solvent or heat. Under ideal condition, denaturation may be reversible, and its original native structure will retain by refolding when the denaturating agent is removed. However, most protein, once de-natured, remains permanently disordered. Denatured proteins are often insoluble and therefore precipitate which increases the activity of macrophase in the protein de-naturation site within the tissue leading to some diseases (Sarvaka et *al*., 2016; Nagendra et *al*., 2019).

The most of the proteins lose their biological properties and their functions when they heat denatured. Numerous studies reported that denaturation has a complex mechanism which comprises alteration in electrostatic hydrogen, hydrophobic and disulphide bonding (Alamgeer et *al*., 2019). Protein denaturation is a well-documented cause of inflammation, it has been correlated with the formation of inflammatory disorders like rheumatoid arthritis, neurodegenerative diseases, diabetes and cancer. Therefore, the ability of phyto-constituents to

prevent the protein denaturation may also help to prevent the inflammatory disorders (Sarveswaran et *al*., 2017; Ketnawa and Ogawa, 2019).

The ability of *Oxalis cernua* methanolic extracts to inhibit protein denaturation was evaluated through two different protein denaturation bioassay. Bovine serum albumin (BSA) and egg albumin denaturation techniques. They are widely used validated, reliable and sensitive tests to evaluate *in vitro* anti-inflammatory potential. The absorbance changes were identified by UV-Visible spectrophotometric methods. Diclofenac sodium was used as a reference drug. The denaturation of protein was tested with concentrations (0.625 and 0.312 mg/ml) and (2.5 and 1.25 mg/ml) for BSA and egg albumin assay respectively. It is believed that agent such as nonsteroidal anti-inflammatory drugs are the major pharmacological agents that can help in anti-inflammatory and pain-relief management due to their capacity in inhibiting protein denaturation (Kariawasam et *al*., 2017; Djova et *al*., 2018).

4.1. BSA anti-denaturation activity of plant extracts

Albumin alone constituted 60% of total protein content in animal serum, and is commonly used in cell culture, particularly when protein supplementation is necessary. Thus, BSA anti-denaturation assay was used to evaluate the anti-inflammatory potential of the methanolic extracts (Agarwal and Shanmugam, 2019). Moreover, protein denaturation is one of the fundamental features of inflammatory tissue, occurring under stressful conditions, expressing antigens which are associated with type-III hypersensitivity reaction, which is allied to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus (Djova et *al*., 2018; Dongmo et *al*., 2019).

The results of different extracts and fractions were illustrated in figure18 and also were compared to the standard drug diclofenac, where BSA anti-denaturation inhibition percentage displayed (39.22±2.44%). At concentration (0.312 mg/ml), *Oxalis cernua* AME and RME

revealed a weak inhibition percentage of protein denaturation (9.42±1.86% and15.61±1.54%) significantly different ($p<0.001$). Meanwhile, CHE fraction (43.38±2.02%) had the same potential of inhibition as diclofenac. Moreover, both of EAE and AQE exhibited the highest activity more than the other tested fractions and standard, with an increase significantly difference ($p<0.001$) in the inhibition percentage, the values were (72.83±0.76%, and 72.68±2.69%) respectively.

However, it was found that at concentration (0.625 mg/ml) the effect of the extracts exhibited almost the same results, nonetheless, the values were different. *Oxalis cernua* AME and RME shown minimum inhibition percentage of protein denaturation in BSA assay (30.13±1.48% and 8.77±0.89%), which was significantly different ($p<0.001$). Meanwhile, the potential of inhibition of CHE fraction (38.58±0.27%) was comparable to diclofenac. Moreover, both of EAE and AQE fractions exhibited an increment significantly difference ($p<0.001$) at the inhibition percentage as illustrated in figure 19, while the values were (68.22±2.47%, and 68.90±0.61%) respectively. All extracts hindered the denaturation of BSA with doses 0.312 and 0.625 mg/ml in a nondependent manner. Based on the values of inhibition percentage (I %), both fractions of EAE and AqE inhibited heat induced protein denaturation and may be one of the reason of possessing anti-inflammatory properties.

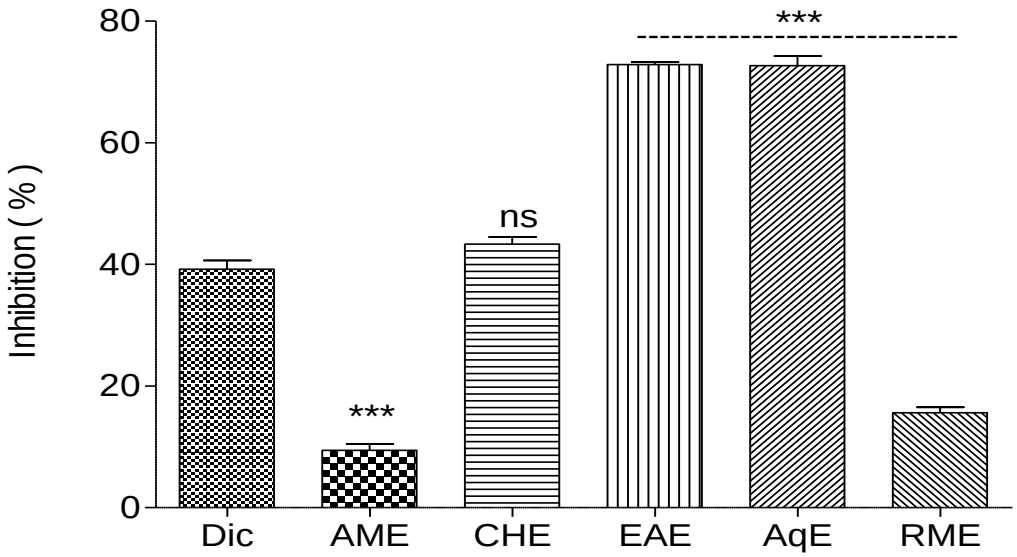


Figure 18: A comparison between different plant extracts in BSA anti-denaturation assay. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract; RME; root methanolic extract, Diclofenac (Dic) was used as standard. Data were presented as inhibition percentage mean ± SD; n = 3; (ns: no significant difference $p > 0.05$; *** $p<0.001$).

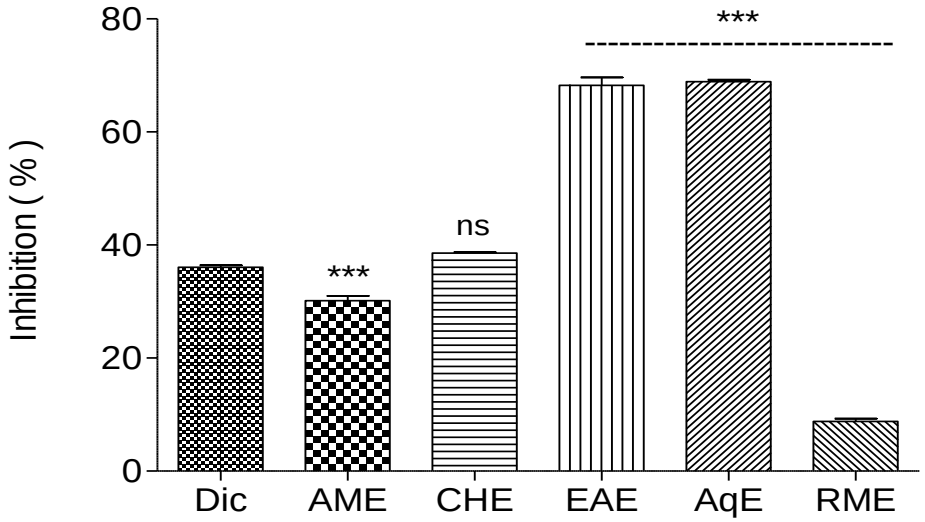


Figure 19: A comparison between different plant extracts in BSA anti-denaturation assay. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract; RME; root methanolic extract, Diclofenac (Dic) was used as standard. Data were presented as inhibition percentage mean ± SD; n = 3; (ns: no significant difference $p > 0.05$; *** $p<0.001$).

A weak correlation has been noted between egg albumin protein denaturation inhibition and polyphenols content with both concentrations (0.312, and 0.625 mg/ml) were respectively (R^2 = 0.001, and R^2=0.001). Besides, the correlation analysis with flavonoids content were (R^2 = 0.001, and R^2=0.04). The findings from the present assay indicate that the extracts possess anti-inflammatory properties. However, *Oxalis cernua* methanolic extracts which have inhibitory effect on protein denaturation had a weak correlation to the phenolic content.

It is well known that the generation of free radical species caused oxidative stress, a hallmark of various chronic inflammatory ailments. Polyphenols and flavonoids are commonly acclaimed to reduce the oxidative stress associated to inflammatory conditions. This result supports the traditional use of the selected medicinal plants because of their great significance in therapeutic treatments. Concerning the weak correlation, several studies reported that the anti-denaturation effect might be due to the presence of secondary metabolites contained in this plant. They have interesting biological activities as the active principles such as triterpenoids, alkaloids, steroids, sinapic acid, coumaric acid, vanillic acid, kaempferol, ferulic acid and benzoic acid, which, had exhibited significant anti-inflammatory and antioxidant activities (Kiranmayi, 2018; Mahdjar et *al*., 2020; Saleem et *al*., 2020).

4.2. Egg-albumin anti-denaturation activity of plant extracts

Thermal treatments applied to egg white causes undesirable modifications to their physicochemical and functional properties. Coagulation and/or precipitation of egg white protein has been observed at around 74°C for 20 min. From the aforementioned results, it can be delineated that the inhibition of protein denaturation was not in a concentration-dependent manner. It has been stated that the denaturation increases viscosities of protein solutions. These anti-denaturation effects were further supported by the changes in viscosities. In the current

assay, the low viscosity of reference drug and the different extracts supported this fact (Osman and Idrees, 2017).

In this term, *Oxalis cernua* extracts have demonstrated their capability as an anti-inflammatory agent. All extracts have given more than 70% of average percent inhibition. At test concentration 1.25 mg/ml in egg albumin assay, EAE exhibited superior activity and the value (88.03±0.35%) was statistically significant ($p<0.001$). Then, it was followed by AqE (79.73±1.09%), where no significant difference was recorded ($p> 0.05$). Nevertheless, *Oxalis cernua* AME (70.24±2.60%), RME (76.46±1.74 %), and CHE fraction (71.06±0.27%) exhibited a decrease in protein denaturation percentage with respect to diclofenac (79.72 ±2.40%). On the basis of these results, it is conceivable that EAE exhibits a strong potential in reducing protein denaturation as depicted in Figure 20.

The ability of the extracts to inhibit heat induced protein denaturation at the test concentration 2.5 mg/ml in egg albumin assay were statistically significant ($p<0.001$) compared to diclofenac a standard drug at a concentration of 100 µg/ml showed the highest percentage (79.72 ±2.40%) of protection. As represented in in figure 21, different *Oxalis cernua* extracts values were ordered increasingly RMA (70.58±1.16%), AME (71.36±1.66%), CHE (80.07±0.16%), AqE (82.84±1.75%), and EAE (85.41±0.37%). They were effective in limiting heat induced albumin denaturation *in vitro*. Probably, owing to their noticeable anti-inflammatory compound.

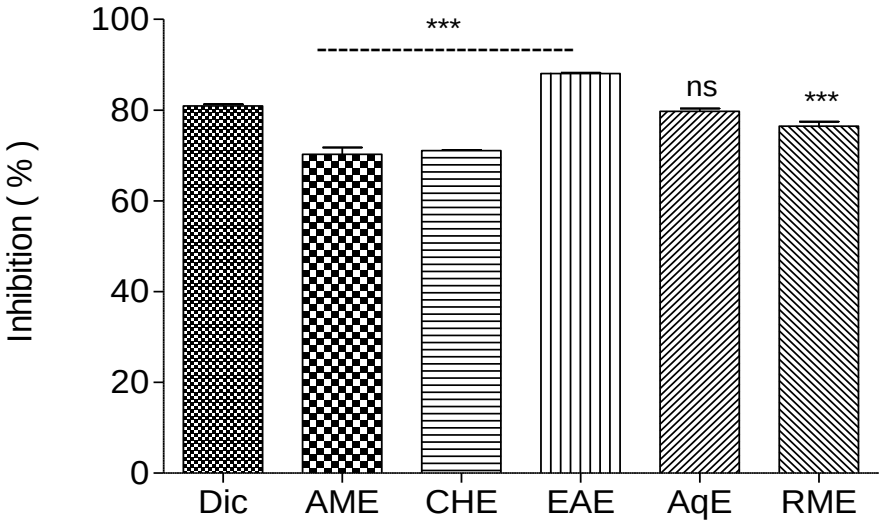


Figure 20: A comparison between different plant extracts in Egg-albumin anti-denaturation assay. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract; RME; root methanolic extract, Diclofenac (Dic) was used as standard. Data were presented as inhibition percentage mean ± SD; n = 3; (ns: no significant difference $p > 0.05$; *** $p < 0.001$).

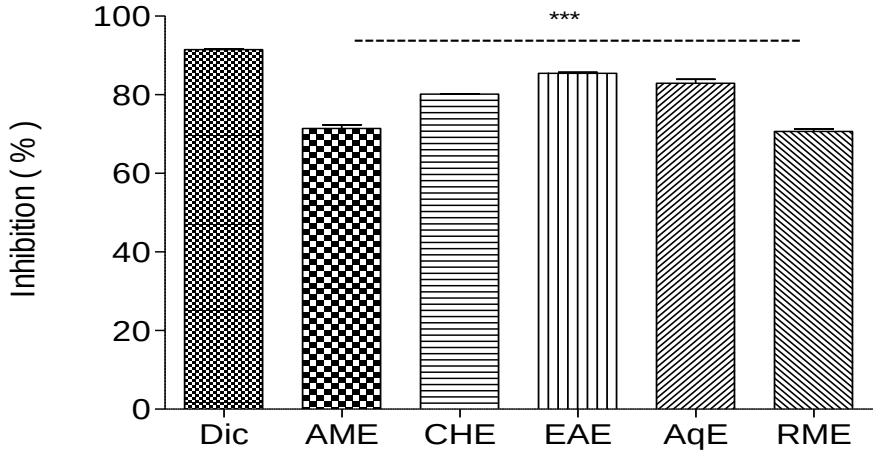


Figure 21: A comparison between different plant extracts in Egg-albumin anti-denaturation assay. AME; areal part methanolic extract, CHE; chloroform extract, EAE; ethyl acetate extract, AqE; aqueous extract; RME; root methanolic extract, Diclofenac (Dic) was used as standard. Data were presented as inhibition percentage mean ± SD; n = 3; *** $p < 0.001$.

The weak correlation has been noted between egg albumin protein denaturation inhibition and polyphenols content with both concentrations (1.25, and 2.5mg/ml) were respectively (R^2 = 0.15, and R^2 =0.03). Besides, the correlation analysis with flavonoids content were (R^2 = 0.01, and R^2 = 0.002). The findings from the present assay indicate that the extracts possess anti-inflammatory properties. However, *Oxalis cernua* methanolic extracts which have inhibitory effect on protein denaturation had a weak correlation to the phenolic content.

It has been reported that conventional non-steroidal anti-inflammatory drugs can inhibit the heat induced protein denaturation. Additionally, to their inhibitory effect on prostaglandin synthesis by blocking the cyclooxygenase pathway, they also have the ability to prevent protein denaturation which contributes to their anti-inflammatory effects (Modak et *al*., 2017; Henneh et *al*., 2018).

The anti-inflammatory activities may be due to the strong existence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, phenols, sterols, and carbohydrate. Among these bioactive compounds several have potential biological properties. They serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation. Consequently, the protein's anti-denaturation properties of *Oxalis cernua* methanolic extracts may be due to the occurrence of these secondary metabolites. Moreover, the anti-denaturation effect may be synergistic rather than single one (De et *al*., 2017; Henneh et *al*., 2018; Nagendra et *al*., 2019).

Currently, polyphenols antioxidant ability is comprehensively assessed using *in vitro* methods which are rapid, cheap, analysing a large number of samples in parallel and have no ethical restrictions. Nonetheless, comprehensive *in vivo* antioxidant assays are relatively lacking. It thus remains to be tested whether these effects have sufficient clinical significance in a physiologically relevant environment, where bioavailability limitations come into play (Oyenihi and Smith, 2018; Patrignani et *al*., 2019).

5. Acute toxicity examination

It is important to note that toxicology, as a scientific discipline identifies and provide the necessary data and knowledge that facilitates translation of *in vitro* and *in vivo* toxicity test findings into more confident and robust evidence-based estimates of real-world human risks (Bus, 2017). The OECD panel of experts defines the study of acute toxicity as ''an adverse effect that occurs within a short period of (oral) administration of a single dose of a substance or multiple doses within 24 h'', and the subacute toxicity as '' advance effects resulting from repeated daily (oral) dosage of a chemical to an experimental annual part (not exceeding 10 percent) of the line span'' (Amudha, and Vanitha, 2019).

Acute toxicity tests provide preliminary information on the toxic nature of a material for which no other toxicological information is available. Sub-acute toxicity studies give informative about the cumulative toxic effects of the drug, physiological organ and metabolites of a compound at minimum dose for prolonged exposure. The adverse effect of different substances can be analysed. From such studies can provide data which will be helpful in fixing the level of doses (Amudha, and Vanitha, 2019).

5.1. Behavioural observation and mortality pattern

The *per os* administration of single doses of *Oxalis cernua* extracts (2000 and 5000 mg/kg bw) in treated female rats during 14 days, according to OECD guideline 425, revealed that *Oxalis cernua* AME and RME did not affect any behavioural changes in rats during the study period comparing the treated animals with the control group. Furthermore, no mortality case was recorded throughout two weeks and, thus, the lethal dose of half of animals (LD_{50}) is higher than (5000 mg/kg bw).

The present study highlighted the acute toxicity of *Oxalis cernua* AME and RME administered in a single dose by gavage in female rats. The toxicity of plant is very important and measured by the lethal dose that killed 50% of the organism population (LD_{50}). The results obtained indicated that oral administration at the dose of (2000 and 5000 mg/kg bw) induce no mortality or toxicity sign, an LD_{50} value was upper than (5000 mg/kg bw), according to OECD (2008), therefor, the plant extracts are considered as relatively safe.

5.2. Body weight evolution

During the period of 14 days, body weights of animals treated with *Oxalis cernua* AME (2000 and 5000 mg/kg bw) decreased gradually throughout the first week, then increased through the second week (Figure 22). The differences were statistically significant ($p < 0.05$, and $p < 0.001$) with respect to the control group. Besides, the comparison between initial and final body weight did not indicate any significant differences ($p>0.05$) as shown in table 5. Whereas, through the same period and with respect to the control group, as illustrated in figure 23, rats received *Oxalis cernua* RME (2000 mg/kg bw) *per os* exhibited a decrease followed by an increment over the two successive weeks respectively. However, the results were not statistically significant ($p>0.05$). In addition, treated animals with *Oxalis cernua* RME (5000 mg/kg bw) displayed an increase over all the course, nevertheless, a significant difference was recorded between initial and final body weights ($p < 0.01$) (Table 6).

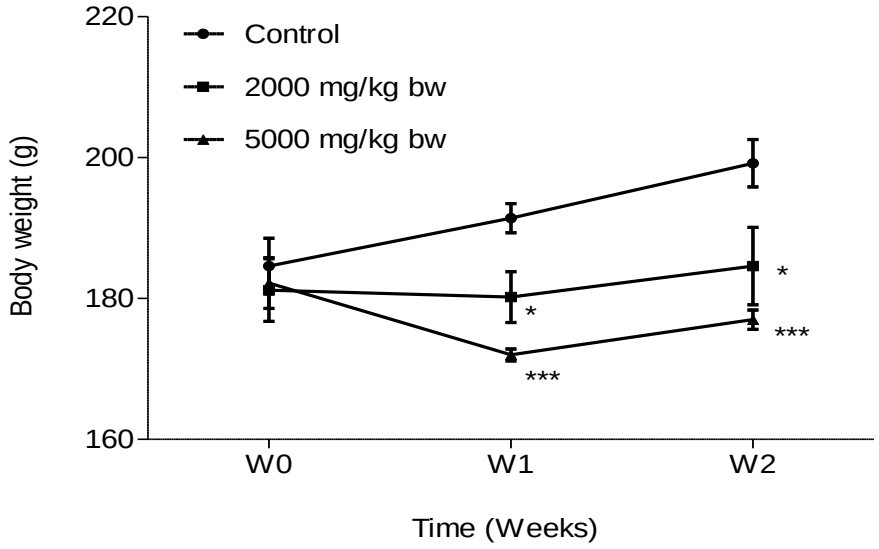


Figure 22: Body weight of female rats administered in a single dose orally. The treated animals received *Oxalis cernua* AME (2000 and 5000 mg/kg bw) and control (vehicle; distilled water). All data are reported as mean ± SEM; n=5. *p < 0.05, and ***p < 0.001. Significant differences were compared with control group.

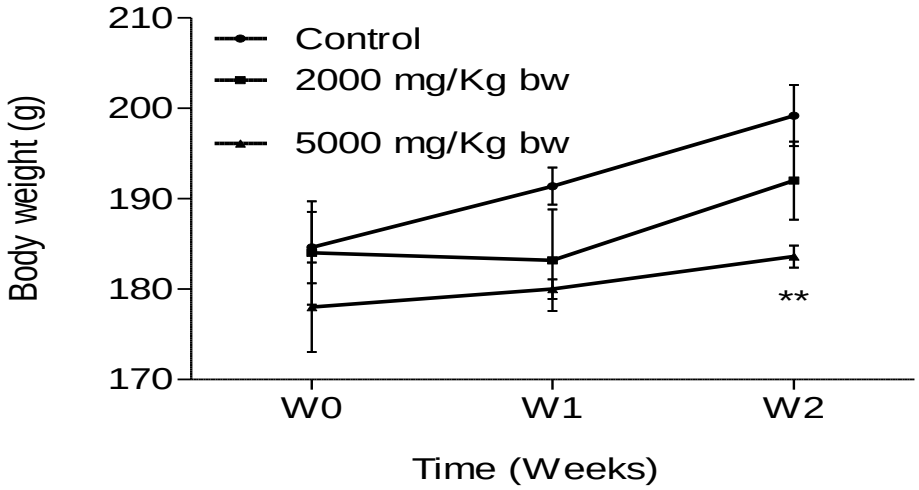


Figure 23: Body weight of female rats administered in a single dose orally. The treated animals received *Oxalis cernua* RME (2000 and 5000 mg/kg bw) and control (vehicle; distilled water). All data are reported as mean ± SEM; n=5. **p < 0.01. Significant differences were compared with control group.

Changes in body weights are indicators of global health state of animals. Their decrease or increase are associated with toxic effects of plant compounds. Relatively to the present study, the administration of *Oxalis cernua* AME did not lead to any meaningful abnormal changes in the initial and final body weights of treated female rats when compared to the control. Meanwhile, there was a significant decrease in body weights gain compared to the control in the course of the first weak.

This body weight loss could be due to disturbances in metabolism of macronutrients as carbohydrate, protein or fat, and even to hydro-mineral deregulation or intra, and extra-cellular dehydration. It is also proven that toxic effects of substances could lead to decrease appetite and hence, lower caloric intake by the animal. The body weight loss could mean that, the extract could also act on the inhibition of the proliferation of adipocytes and consequently reduce fat content (Dongmo et *al*., 2019).

5.3. Relative organ weights

Relative organ weights were summarized in tables 5 and 6, some significant differences were recorded when comparing the organs of treated animals with *Oxalis cernua* methanolic extracts with the control group. Liver, spleen, and lung of female rats treated with *Oxalis cernua* AME (2000 mg/kg bw) revealed differences statically significant ($p<0.05$, and $p < 0.01$). Similarly, kidney and lung of those received the dose (5000 mg/kg bw) shown a significant difference ($p < 0.01$, and $p < 0.001$). On the other hand, only lung of female animals treated with *Oxalis cernua* RME exhibited significant differences ($p <0.01$, and $p < 0.001$).

Changes in organ weights are also an important and sensitive parameter for detecting harmful effects of medicinal plants. It's often associated with treatment related effects. It is possible that medicinal plant could be toxic when administrated *in vivo*. The lack of harmful effects on the studied organs did not attest total absence of toxicity because it's known that the manifestations of toxicity occur at the molecular level before expanding to the whole tissues

and organs. Relatively to this, *Oxalis cernua* methanolic extracts constituents could present harmful effect on some biochemical markers of vital organs functions (Dongmo et *al*., 2019).

Table 5: Effects of single administration of *Oxalis cernua* AME on body and relative organs weights.

	Control (H_2O)	**2000 mg/kg bw**	**5000 mg/kg bw**
Initial body weight	184,6±3,94	181,20±4,46^{ns}	182,20±3,61^{ns}
Final body weight	199,20±3,36	199,20±5,48^{ns}	177±1,37^{ns}
Liver	33.48±0,60	40.03±3.72*	39.71±0,79^{ns}
Kidney	5.22±1.12	6.29±0,25^{ns}	7.45±1.19**
Stomach	6.57±0,24	6.72±0,050^{ns}	6.58±0,29^{ns}
Spleen	2.97±0,10	3.64±0,25**	3.39±0,14^{ns}
Heart	2.92±0,14	3.51±0,36^{ns}	3.42±0,33^{ns}
Lung	5.79±0,19	8.45±1.40**	9.58±0,48***

All data are reported as mean ± SEM; n = 5. (ns: $p > 0.05$, *: $p<0.05$, **: $p<0.01$, ***: $p < 0.001$).

Table 6: Effects of single administration of *Oxalis cernua* RME on body and relative organs weights.

	Control (H_2O)	**2000 mg/kg bw**	**5000 mg/kg bw**
Initial body weight	184,6±3,94	184±5.71	178±4,93
Final body weight	199,20±3,36	192±4,30 ^{ns}	183.6±1,20 *
Liver	33.48±0,60	38.62±3.91 ^{ns}	35.67±5.62 ^{ns}
Kidney	5.22±1.12	5.68±0,31 ^{ns}	5.93±0,84 ^{ns}
Stomach	6.57±0,24	6.35±0,51 ^{ns}	6.68±0,88 ^{ns}
Spleen	2.97±0,10	3.40±0,29 ^{ns}	3.26±0,18 ^{ns}
Heart	2.92±0,14	3.00±0,28 ^{ns}	3.16±0,33 ^{ns}
Lung	5.79±0,19	7.45±0,73**	9.84±1.07 ***

All data are reported as mean ± SEM; n = 5. (ns : non-significant, p> 0.05, *: p<0.05, **: p < 0.01, ***: p < 0.001).

5.4. Biochemical parameters

As demonstrated in table 7 and 8, evaluation of biochemical parameters revealed that there were significant differences between treated rats and the control group. However, a significant increase (p < 0.05) in ALP and AST with treated groups, and a significant decrease in urea (p<0.05) with 2000 mg/kg group were recorded but they were not dose dependently.

Another important aspect analysed in the toxicological evaluation was the biochemical parameters, which are efficient indicators of organ dysfunction damage. As can be observed in table (7 and 8). The role of liver and kidney functions are important for the survival of animals. Their functionality can be measured by serum biochemical analysis, which are crucial in the toxicological evaluation of xenobiotics.

Liver plays significant role in metabolic activity as the main organ of detoxification and biotransformation of xenobiotics. The important hepatic damage markers are aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), located in the cytosol and describe its cellular integrity. Their release indicates hepatocytes damage and an increase level in the serum. High levels of liver enzymes are signs of hepatocellular toxicity, especially in ALT levels, which is a strong indicator of hepatic injury, whereas a decrease may indicate enzyme inhibition (Dongmo et *al*., 2019; Ugwah-Oguejiofor et *al*., 2019).

Table 7: Biochemical parameters in plasma of rats treated with *Oxalis cernua* AME.

Parameters	Control group	2000 mg/kg bw	5000 mg/kg bw
ALP (U/L)	169.7±3.96	217.3±3.56***	208.40±3.66***
AST (U/l)	190.50±3.39	214.6±3.33**	234±3.36***
ALT (U/l)	35.78±2.09	36.28±1.43 ns	35.38±1.46 ns
Urea (g/L)	0.60±0.061	0.25±0.00***	0.59±0.06 ns
Creatinine (mg/L)	5.29±0.35	5.05±0.36 ns	5.38±0.18 ns

Values were expressed as mean ± SEM; n = 5. (ns: non-significant, $p > 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Table 8: Biochemical parameters in plasma of rats treated with *Oxalis cernua* RME.

Parameters	Control group	2000 mg/kg bw	5000 mg/kg bw
ALP (U/L)	169.7±3.96	211.62±3.44***	188.94±3.80 ns
AST (U/l)	190.50±3.39	213.82±2.74**	260.2±2.42***
ALT (U/l)	35.78±2.09	39.17±2.41 ns	40.65±1.50 ns
Urea (g/L)	0.60±0.061	0.25±0.00 ***	0.30±0.06 ns
Creatinine (mg/L)	5.29±0.35	5.58±0.14 ns	5.86±0.35 ns

Values were expressed as mean ± SEM; n = 5, ns: non-significant $p > 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

In this study, there was a significant increase in the alkaline phosphatase (ALP) and AST of both groups (2000 and 5000 mg/kg bw) (Table 7 and 8). Therefore, it may be inferred that extract could slightly cause cytolysis of hepatic tissue since it causes elevation of liver enzymes' level.

As concern renal functions, the kidneys are highly vulnerable organs to toxic compounds due to the important volume of blood flows throughout it, urea and creatinine levels are index to evaluate renal damage. A high serum level of urea indicates that the kidneys may not be working properly, or that the animal is dehydrated. Whereas, low urea levels are seen in acute liver failure or over-hydration. In this study, the urea level at dose (2000 mg/kg bw) was significantly decreased, which may due to the kidneys dysfunction.

Creatinine clearance is an indicator of damage of nephrons function, glomerular filtration rate and renal failure. As presented in table (7 and 8), the *Oxalis cernua* methanolic extracts did not promote any significant alterations in the creatinine levels when compared with the control group, suggesting that the extract may not be toxic to the kidney (Dongmo et *al*., 2019; Ugwah-Oguejiofor et *al*., 2019).

5.5. Histological examination

The histological sections were carried out to determine the biological response factors (Figure 24 and 25). The macroscopic analysis of liver, kidneys, stomach, spleen, heart and lungs did not show any significant alteration when compared with the control group. The assessment of histology of treated rats showed a preservation of the cellular architecture of the organs compared with control group, no damaging changes and morphological alterations were observed. However, some particularities have been considered. A slight spleen congestion, and a gastritis was recorded in tissues of dose (2000 mg/kg bw).

The histological examination of the main organs was performed at the end of acute toxicity study period, it revealed that *Oxalis cernua* extracts did not have any major effects in the tissue morphology, although slight congestion could be due to the vasoconstriction action of the extract on the walls of blood vessels (Fonseca et *al*., 2019), and gastritis to the plant high content in oxalic acid (Calviño-Cancela et *al*., 2014; Gaspar et *al*., 2018).

Several statistically significant changes in biochemical parameters were not considered to be of toxicological relevance since the changes were all small in nature and fell within the historical control range of the testing laboratory. A few statistically significant differences in organ weight values were recorded, but all again were within the historical control ranges, and there were no histological or clinical pathology correlates (Li et *al*., 2018).

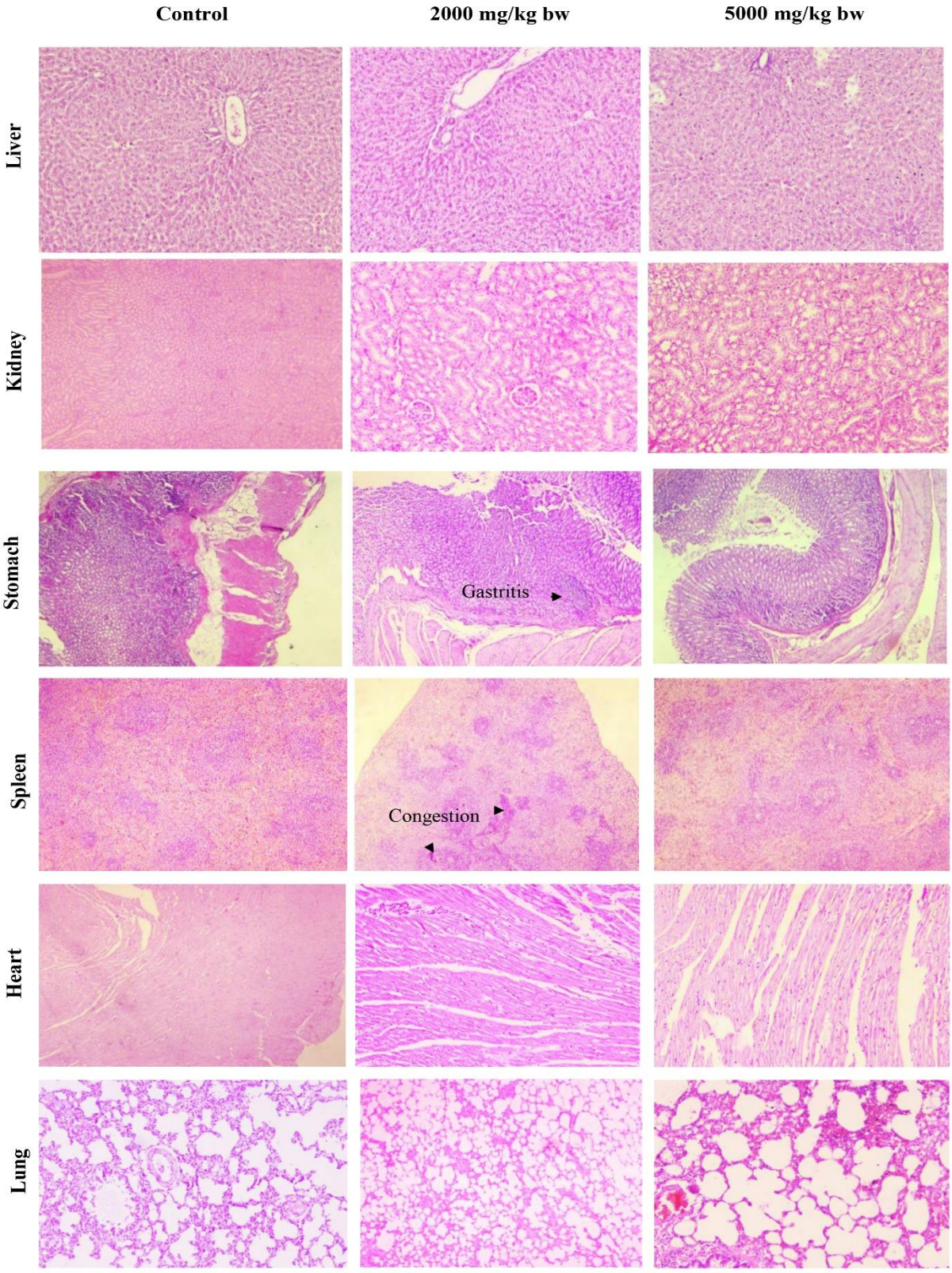


Figure 24: Photomicrographs of liver, kidney, spleen, heart, lung and stomach sections stained with hematoxylin and eosin of treated groups with *Oxalis cernua* AME and control in acute toxicity. Liver, kidney, spleen, heart and lung sections (X100), and stomach (X40).

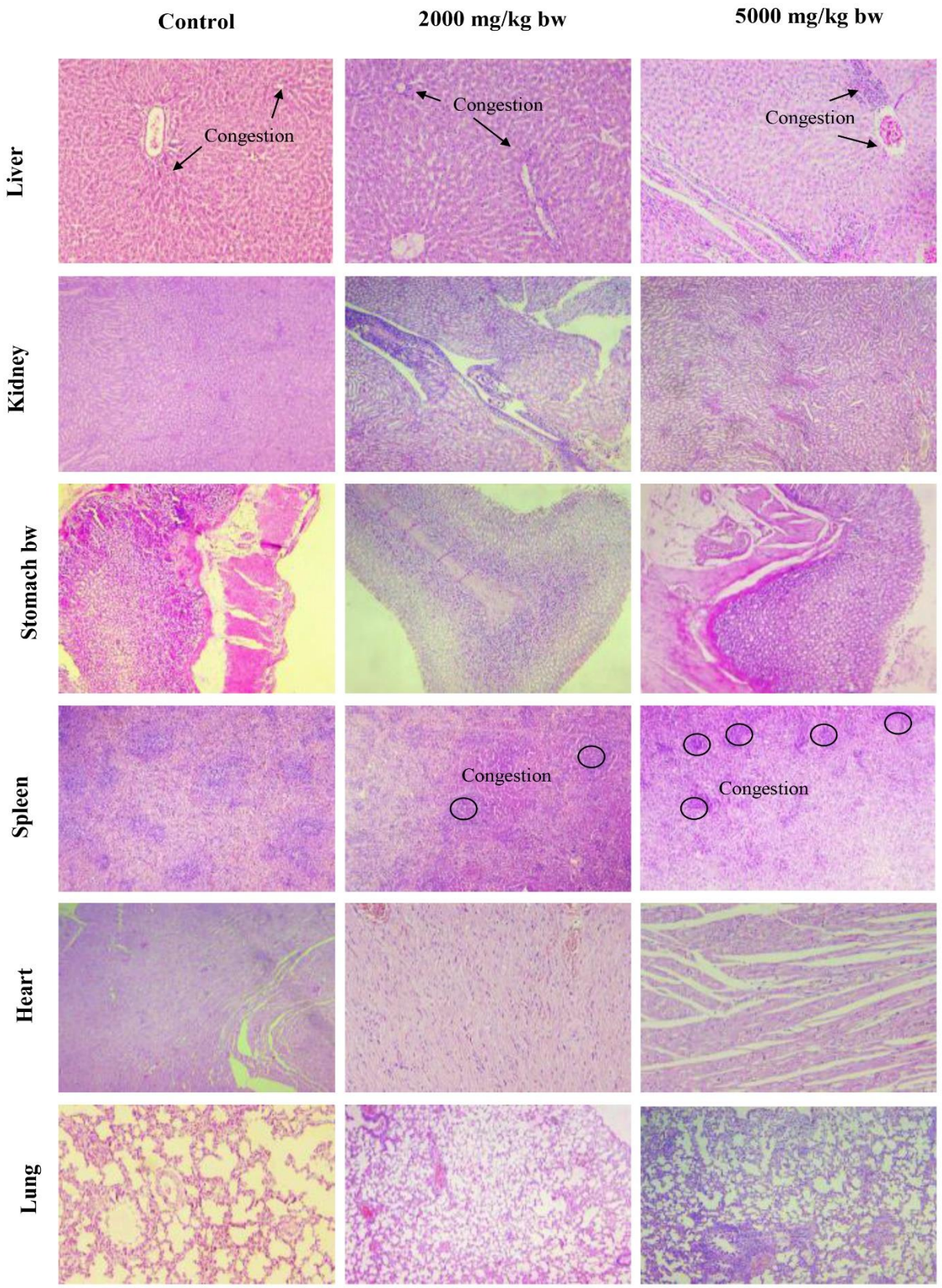


Figure 25: Photomicrographs of liver, kidney, spleen, heart, lung and stomach sections stained with hematoxylin and eosin of treated groups with *Oxalis cernua* RME and control in acute toxicity. Liver, kidney, spleen, heart and lung sections (X100), and stomach (X40).

6. Subacute toxicity and *in vivo* antioxidant activity

Studies have reported that the long history of folk remedies promote a false feeling of their safety, an assumption of minimal side effects, and a belief in their usefulness in disease prevention and control (Ekar and Kreft, 2018). In order to better understand *Oxalis cernua* areal part methanolic extract properties, safety and efficiency on normal metabolic conditions, *Swiss albino* mice were used. The treatment was performed *per os* for 21 successive days in order to observe the subacute effects and the *in vivo* antioxidant activity.

6.1. Behavioural observations and mortality patterns

In the subacute toxicity experiment, oral administration of *Oxalis cernua* methanolic extracts (100, 200, and 400 mg/kg bw) and Vitamin C (100 mg/kg bw) for 21 days to female mice did not showed any behavioural changes or discomfort compared to the control group. There were no symptoms of increase in the rate of respiration or breathing trouble. Eyes were normal in colour and no lacrimation was caused. Skin and fur colour were normal and no inflammatory or other abnormal effects were observed. There was no case of coma or mortality in animals during the entire period.

6.2. Body weights

Throughout three weeks of *per os* treatment, an improvement was noticed in weight gain with all groups which received *Oxalis cernua* AME, and no statistical differences were noticed comparing treated groups to the control (Figure 26). Similar results were found with mice treated with *Oxalis cernua* RME, except animals received dose (200, and 400 mg/kg bw), which displayed a decrease, however, it was insignificant. Moreover, the same doses presented significant differences were noticed in weight gain over the first and the second week, comparing treated groups to the control (Figure 27).

All the animals exhibited a steady increase in body weight indicating that the *Oxalis cernua* methanolic extracts did not alter food intake or appetite stability which validates the oral route traditional usage of the plant. However, the decrease noted with *Oxalis cernua* RME, may be due to the disturbances in metabolism of macronutrients, hydro-mineral deregulation or intra, and extra-cellular dehydration, and also to the inhibition of adipocytes proliferation (Dongmo et *al*., 2019).

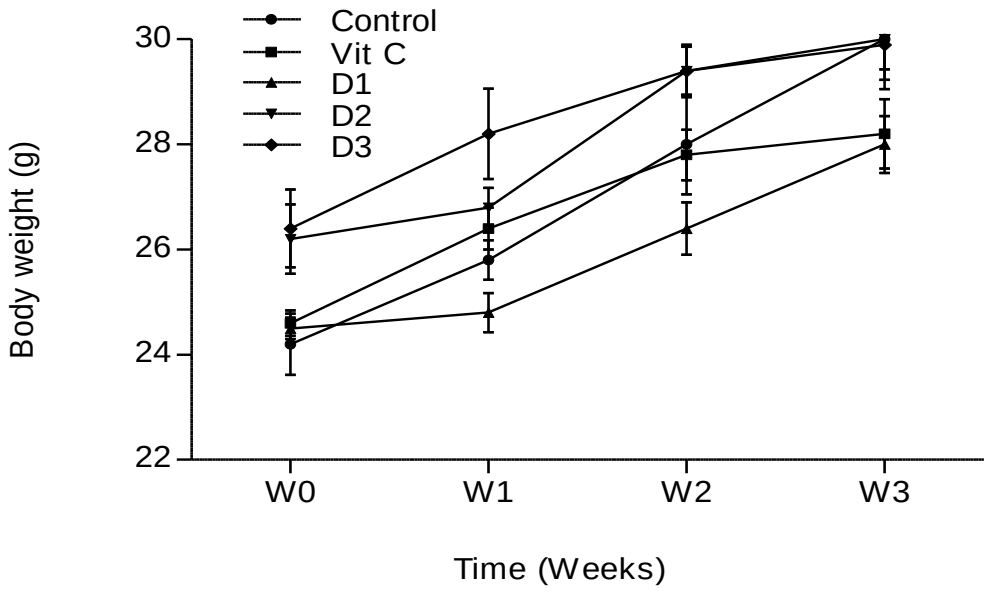


Figure 26: Evaluation of body weights of mice treated orally with AME. Values expressed as mean ± SEM; n=5.

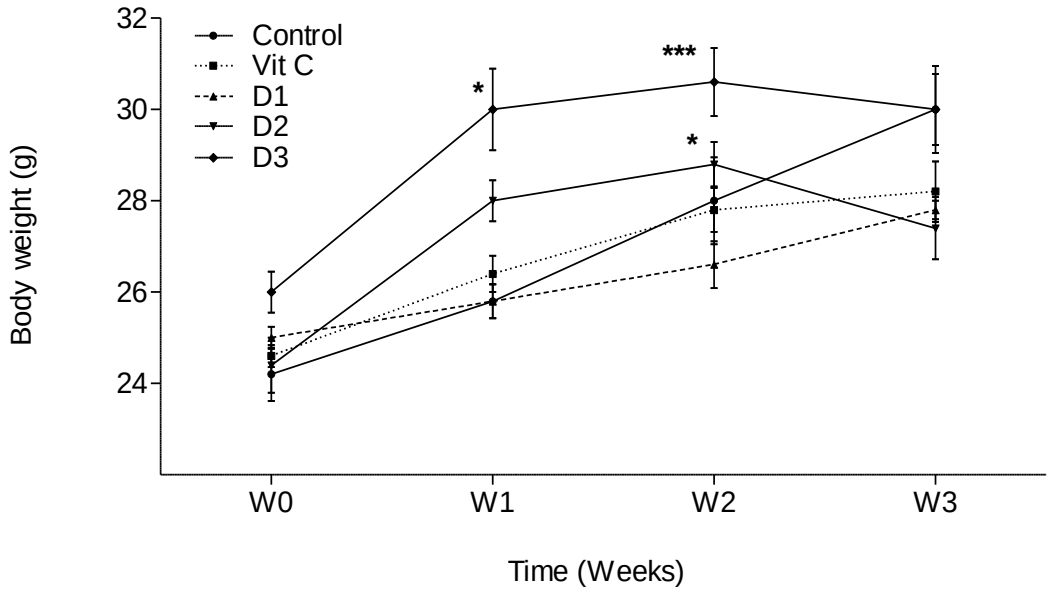


Figure 27: Evaluation of body weights of mice treated orally with RME. Values expressed as mean ± SEM; n=5.

6.3. Relative organ weights

The organ weights are markers of pathological and physiological wellness status of animals. Changes in organ weights are hallmarks of toxicity in experimental animals, which are determined by toxicity tests (Unuofin et *al*., 2018). As shown in table 9 and 10, excepting the stomach of mice treated with *Oxalis cernua* RME (200 mg/kg bw), there were no significant changes in organ weights of all the treated animals (100, 200 and 400 mg/kg bw), Vitamin C and control groups in subacute toxicity during the 21 days of oral administration. Organ weights indicating no toxic effect in both control and treated groups and the differences were statistically non-significant ($p > 0.05$) suggesting that the plant extracts were not toxic to the animals at the tested doses. However, the increment in stomach relative weight, which revealed a significant difference ($p<0.01$), could be due to the gastritis.

Table 9: Relative organs weight of mice treated with *Oxalis cernua* AME, Vitamin C and control group in subacute toxicity.

		Doses (mg/kg bw)			
Organs	**Control**	**Vit C**	**100**	**200**	**400**
Liver	53.20±3.75	48.01±3.12^{ns}	50.93±4.79^{ns}	45.93±3.65^{ns}	50.13±2.02^{ns}
Kidney	11.8±0.92	11.41±1.03^{ns}	14.07±2.36^{ns}	11.11±0.30^{ns}	12.44±0.40^{ns}
Stomach	8.73±0.77	9.29±0.36^{ns}	6.71±0.80^{ns}	10.18±0.60^{ns}	10.40±0.92^{ns}
Spleen	7.93±2.69	5.46±0.64^{ns}	6.42±0.75^{ns}	4.87±0.20^{ns}	6.39±0.87^{ns}

Values were expressed as mean ± SEM; n=5. Comparisons are made with respect to the control group, ns: non-significant ($p>0.05$).

Table 10: Organs weight of mice treated with *Oxalis cernua* RME, Vitamin C and control group in subacute toxicity.

		Doses (mg/kg bw)			
Organs	**Control**	**Vit C**	**100**	**200**	**400**
Liver	53.20±3.75	48.01±3.12^{ns}	49.64 ±5.26^{ns}	50.43±3.71^{ns}	50.66±2.12^{ns}
Kidney	11.8±0.92	11.41±1.03^{ns}	12.08±0.90^{ns}	11.31±0.40^{ns}	11.2±0.41^{ns}
Stomach	8.73±0.77	9.29±0.36^{ns}	9.85±0.30^{ns}	12.11±1.07**	8.60±0.64^{ns}
Spleen	7.93±2.69	5.46±0.64^{ns}	5.89±0.30^{ns}	6.27±0.60^{ns}	5.46±0.38^{ns}

Values were expressed as mean ± SEM; n=5. Comparisons are made with respect to the control group, ns: non-significant ($p>0.05$).

6.4. Histological Examination

At the end of the experiment, the anatomy revealed that the organs, liver, kidneys, stomach and spleen, were all within the normal range, and no pathological signs were observed in these organs (Figure 28 and 29). Liver histological evaluation of animals treated *with Oxalis cernua* AME displayed vascular congestion with inflammatory infiltrates within (100, 200 mg/kg bw) and control group. The kidneys showed adequate glomeruli and normal tubules; the spleen has a normal architecture and no evidence of lesions. However, histological cutting of stomach revealed gastritis and inflammatory infiltrates within the treated groups of *Oxalis cernua* AME (100, 200 and 400 mg/kg bw). In addition, mice treated with *Oxalis cernua* RME revealed that livers were conjested, while stomach sections exhibited gastrite with inflammatory infiltrat.

The histological examination revealed that the *Oxalis cernua* methanolic extracts did not have any major effects in the tissue morphology (Figure 28 and 29). Although a slight degree of organ inflammation, and congestions was noted, which could be due to the vasoconstriction action of the extract on the walls of blood vessels (Fonseca et *al*., 2019). It is suggested that *Oxalis cernua* sour taste derives from its high content of oxalic acid (up to 16%) of the dry weight may be the cause of gastritis seen in treated groups (Calviño-Cancela et *al*., 2014; Gaspar et *al*., 2018). In addition, the intervening factors including stress because of multiple volume oral administration and handling probably caused them (Vakili et *al*., 2017). Furthermore, this extracts could contain some substances capable of acting like non-steroidal anti-inflammatory drugs by provoking a hypersensitivity reaction that led to the inflammation observed (Aouachria et *al*., 2017).

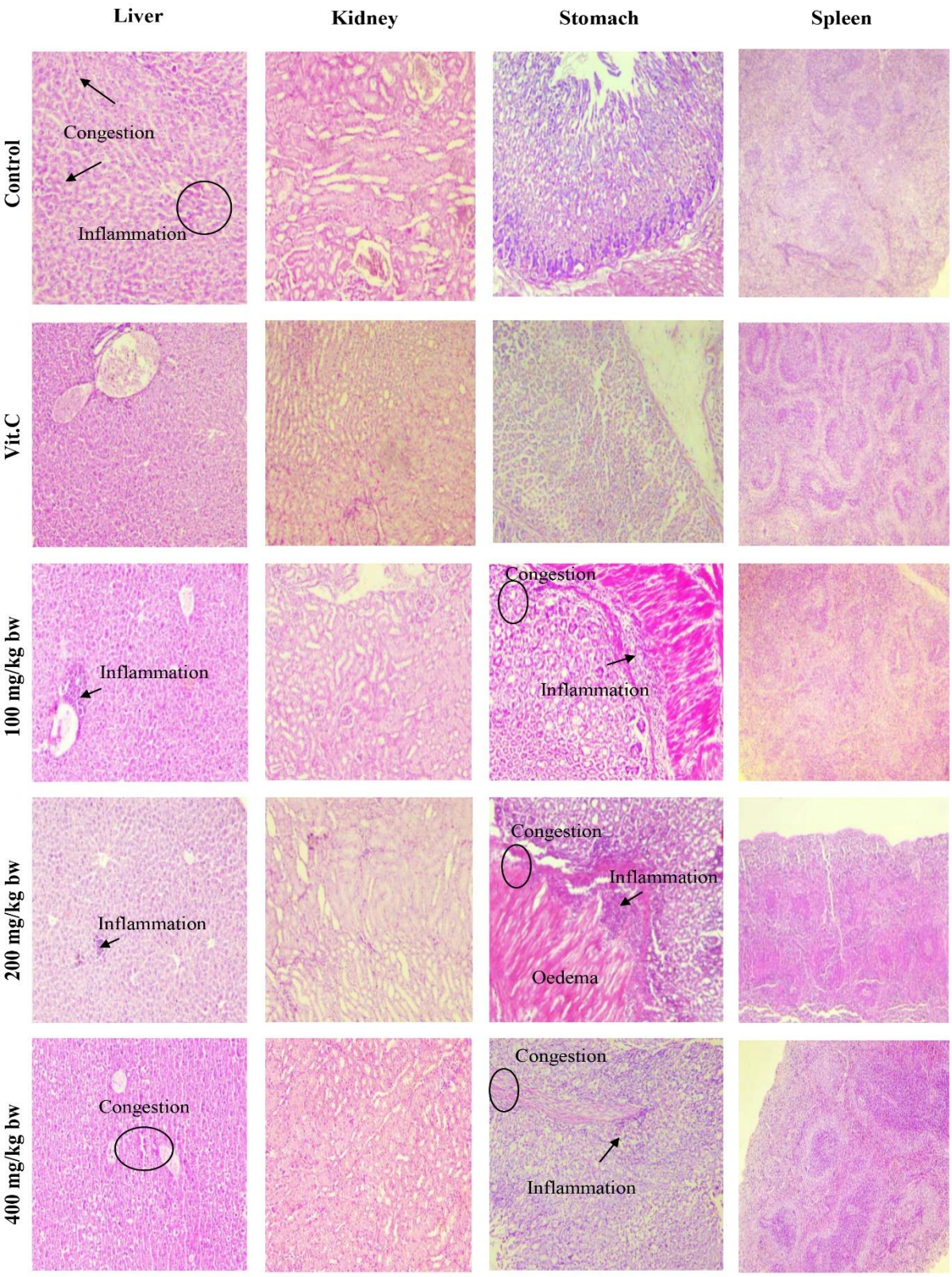


Figure 28: Photomicrographs of liver, kidney, stomach, and spleen sections stained with haematoxylin and eosin of treated groups with *Oxalis cernua* AME, Vitamin C and control group in subacute toxicity. Spleen (X40), liver, kidney, stomach (X100).

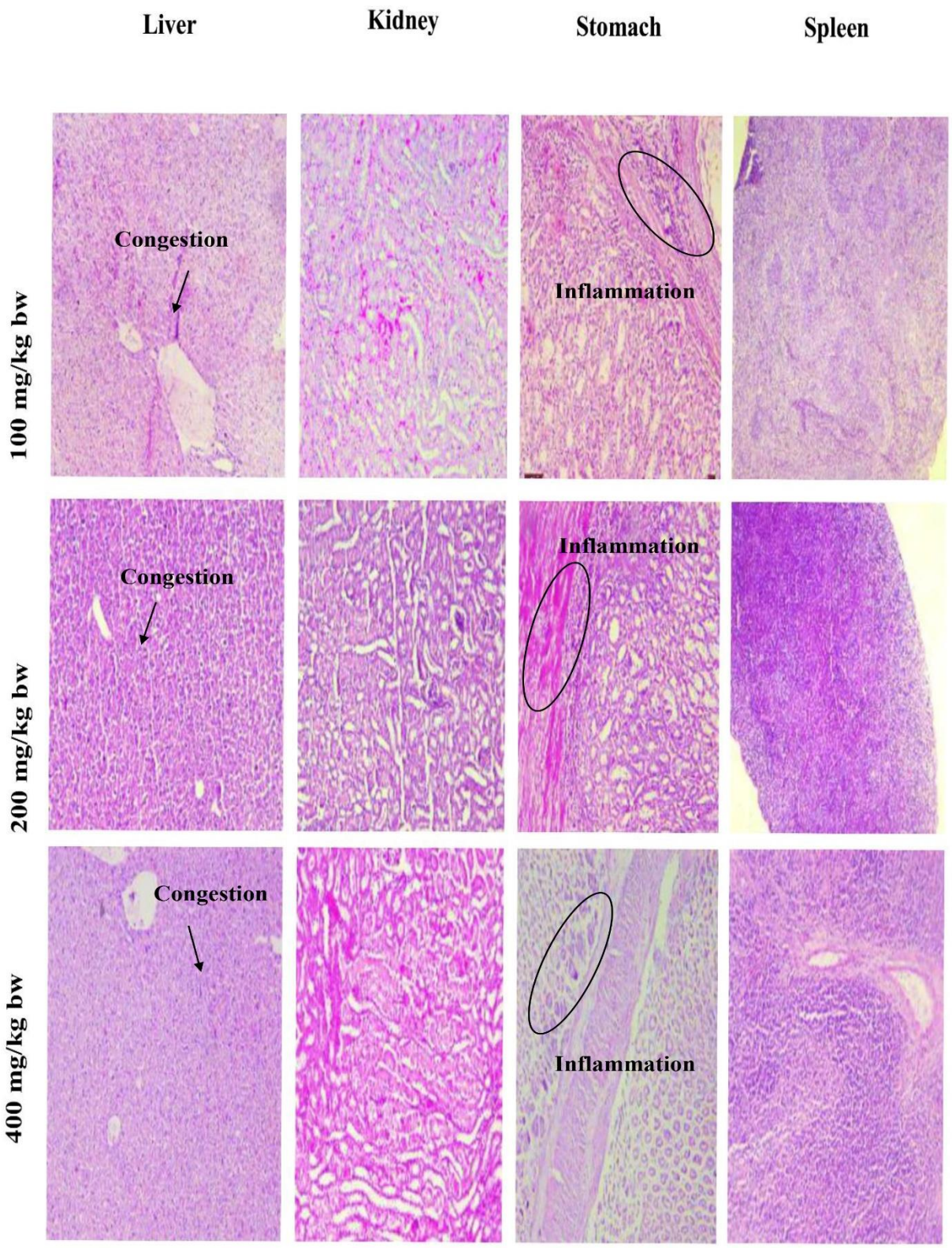


Figure 29: Photomicrographs of liver, kidney, stomach, and spleen sections stained with haematoxylin and eosin of treated groups with *Oxalis cernua* RME, Vitamin C and control group in subacute toxicity. Spleen (X40), liver, kidney, stomach (X100).

7. *In vivo* antioxidant activity of plant extract

Oxalis cernua antioxidant potential was evaluated *in vivo* through DPPH scavenging assay and reducing power test in plasma, and also CAT activity, GSH content and MDA levels in both liver and kidney homogenates. The correlation between *in vitro* and *in vivo* data is a fundamental issue for the antioxidant ability of polyphenols to reduce oxidative stress, however, the obtained results revealed that the *Oxalis cernua* methanolic extracts exhibited free radical scavenging activities in *in vivo*.

7.1. DPPH radical-scavenging activity of plasma

The effectiveness of the antioxidant treatment in plasma over three weeks was assessed using DPPH radical and expressed as an inhibition percentage. The obtained findings (Figure 30) showed that *per os* administration of AME at doses of (100, 200 and 400 mg/kg bw) and Vitamin C (100 mg/kg bw) in mice increased significantly the plasma antioxidant capacity ($p < 0.05$). This increment was not relative to the concentration. The values were (26.96 ± 3.51%), (29.88±3.32%), (30.31±3.32%) and (29.23±2.51%) respectively compared to the control group (16.85±1.93%).

Furthermore, the antioxidant activity in the plasma of animals treated *per os* with *Oxalis cernua* RME was dose dependent, however, the obtained value of (100 mg/kg bw) (24.93±3.55%) was statistically insignificant ($p>0.05$). The values of dose (200 and 400 mg/kg bw) of *Oxalis cernua* RME were, (29.96±3.42%), (33.12±2.20%) respectively (Figure 31).

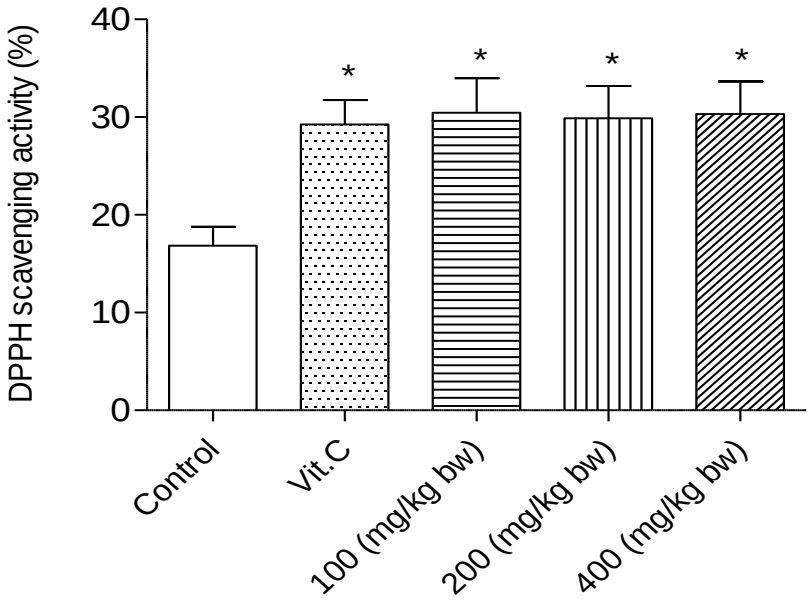


Figure 30: Antioxidant potential of plasma in mice treated by *Oxalis cernua* AME using DPPH assay. Values presented as mean ± SEM; n = 5. Comparisons are made with respect to the control group; *: $p < 0.05$.

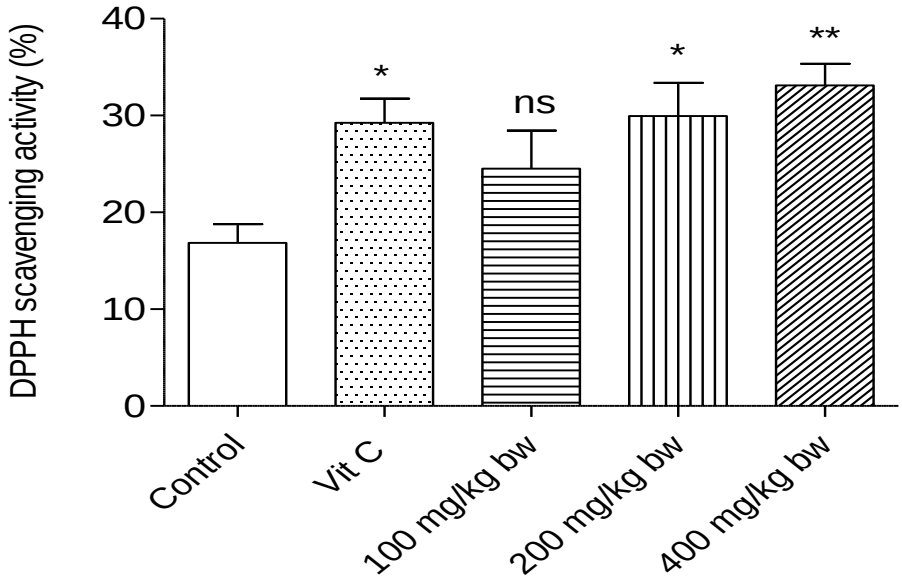


Figure 31: Antioxidant potential of plasma in mice treated by *Oxalis cernua* RME using DPPH assay. Values presented as mean ± SEM; n = 5. Comparisons are made with respect to the control group; ns: non-significant $p>0.5$, *: $p < 0.05$, **: $p<0.01$.

7.2. Reducing power activity of plasma

Plasma antioxidant capacity was evaluated by reducing power scavenging assay. It appeared that plasma scavenging ability was not significantly improved following the oral administration of AME and this with all doses (100, 200 and 400 mg/kg bw) and Vit C (100 mg/kg bw), the values were (14.09 ±1.04%), (15.00±1.50%) and (17.02±1.70%) and 17.64±1.00% respectively compared to the control group (15.39±1.30%) during 21 days. Concerning *Oxalis cernua* RME, only the high dose exhibited a difference statistically significant. while the values of doses (100, 200, and 400 mg/kg bw) were (0.15±0.01%), (0.16±0.01%), and (0.28±0.02%) respectively.

The antioxidant capacity assessment in *in vivo* is difficult due to their complex mechanisms of action and pleiotropic activities. The function of hydrolysed polyphenols and flavonoids in the intestine depend on their bioavailability which is influenced by their stability, their interaction with other components, their metabolism and uptake from the intestine where 15% of polyphenols' aglycones (nuclei) are absorbed after oral ingestion (Kim et *al*., 2014; Bjørklund et *al*., 2017; Oyenihi and Smith, 2018). However, the short half-life of the polyphenols in the organism and their poor water solubility are likely explanations of their low toxicity. The long-term toxicity of flavonoids is very low as consumed in low doses like constituents of the normal foodstuffs, therefore, these substances do not accumulate in the organism (Bjørklund et *al*., 2017).

7.3. Estimation of catalase activity, glutathione content and lipid peroxidation level

The results obtained from the assessment of catalase (CAT) activity, glutathione (GSH) content and lipid peroxidation (MDA) level in both livers and kidneys of animals treated with *Oxalis cernua* AME were showed in (Table 11). The CAT activity in liver homogenate has not revealed any significant changes during the treatment with AME (100, 200 and 400 mg/kg bw),

while a significant change was observed with Vitamin C. Furthermore, in kidneys homogenate the CAT activity indicate a significant decrease at all doses including Vitamin C (100 mg/kg bw).

From the analysis of the table 12, it was observed that the administration of *Oxalis cernua* RME (100, 200 and 400 mg/kg bw) caused a significant increase in GSH content in liver. However, in kidneys a significant decrease of GSH level was noted including that of Vitamin C. The obtained results clearly indicated a significant decrease in MDA level and lipid peroxidation inhibition in liver at dose (100 mg/kg bw), the other doses and Vitamin C recognised a significant increase compared to the control. Kidneys homogenate of treated animals increased significantly with doses (100 and 400 mg/kg bw). In addition, no significant changes were recorded with dose (200 mg/kg bw), and a significant decrease was noted with Vitamin C dose.

Table 11: Estimation of CAT activity (μmole/min/mg of protein), GSH (nmole/g of tissue) and MDA (nmole/g of tissue) in liver and kidney of mice treated with *Oxalis cernua* AME.

			Doses (mg/kg bw)			
Groups		**Control**	**Vit.C**	**100**	**200**	**400**
Liver	**CAT**	5.76±0.69	12.88±0.69***	5.80±1.48^{ns}	5.70±0.49^{ns}	3.69±0.22^{ns}
	GSH	12.33±1.89	15.19±0.98^{ns}	20.89±1.63*	25.62±1.56**	21.58±2.35*
	MDA	51.69±2.69	15.16±3.64^{ns}	25.38±3.50***	62.46±2.3***	75.23±3.61**
Kidney						
	CAT	12.04±0.84	7.9±0.69***	5.53±0.44***	2.35±0.00***	6.73±0.26***
	GSH	45.38±2.13	28.67±1.63***	27.99±1.97***	24.05±1.25***	31.06±2.50**
	MDA	108.39±1.30	95.76±2.43*	122±2.01*	108.92±2.73^{ns}	147.69±3.21***

Values were expressed as mean ± SEM; n=5. ns: non-significant p> 0.5; * p < 0.05; ** p <0.01; *** p <0.001.

During the treatment with *Oxalis cernua* RME (100, mg/kg bw), the CAT activity in liver homogenate has revealed a significant change (p <0.05), while a significant change was also observed with Vitamin C (p <0.001). Furthermore, in kidney homogenate the CAT activity indicate a significant decrease at all doses (100, 200, and 400 mg/kg bw) including Vitamin C (100 mg/kg bw). From the analysis of the table 12, the administration of *Oxalis cernua* RME (100, 200 and 400 mg/kg bw) and Vitamin C caused a significant decrease (p <0.05; p < 0.01; p <0.001) in GSH content in both of liver and kidney. Accordingly, the results clearly indicated a significant increase in MDA level and lipid peroxidation inhibition in liver at dose (100, and 400 mg/kg bw), and Vitamin C. The other dose recognised a significant decrease compared to the control. Kidneys homogenate of treated animals increased significantly with doses (200 and

400 mg/kg bw), a significant decrease recorded with dose (100 mg/kg bw) and Vitamin C was noted.

Table 12: Estimation of CAT activity (µmole/min/mg of protein), GSH (nmole/g of tissue) and MDA (nmole/g of tissue) in liver and kidney of mice treated with *Oxalis cernua* RME.

			Doses (mg/kg bw)			
Groups		**Control**	**Vit.C**	**100**	**200**	**400**
Liver	**CAT**	5.76±0.69	12.88±0.69***	8.50±0.28*	3.75±0.14^{ns}	4.93±0.78^{ns}
	GSH	12.33±1.89	15.19±0.98^{ns}	6.95±0.52*	6.16±0.17**	5.64±0.27**
	MDA	51.69±2.69	15.16±3.64^{ns}	69.26±2.21***	59.53±2.58^{ns}	89.07±3.27***
Kidney						
	CAT	12.04±0.84	7.9±0.69***	4.58±0.44***	6.49±0.22***	4.74±0.26***
	GSH	45.38±2.13	28.67±1.63***	8.19±0.42***	7.50±0.89***	6.98±0.31***
	MDA	108.39±1.30	95.76±2.43*	71.76±2.01***	148.92±3.92***	136.38±3.60***

Values were expressed as mean ± SEM; n=5. (ns> 0.5; * p < 0.05; ** p <0.01; *** p <0.001).

Catalase is an oxidoreductase enzyme, acts as an antioxidant and protects the cell against oxidative stress quenching the reactive oxygen species. It hydrolyses 50% of hydrogen peroxide into oxygen and water (Kaushal et *al*., 2018; Sepasi Tehrani and Moosavi-Movahedi, 2018). Statistically, no significant changes were observed in the activity of this enzyme in liver homogenates compared to the control group ($p > 0.05$). This consequence was somehow expected since the consumption of the studied extract did not induce the synthesis of this enzyme in normal mice. Otherwise, this would be an intervention of the body homeostasis (Aouachria et *al*., 2017). Accordingly, it was found that the activity of catalase increase in mice

tissues during 21 days. This increase can act as an adaptive defence response mechanism to the excessive production of ROS during oxidative stress (Popova et *al*., 2017).

Glutathione (GSH) is the most abundant thiol in cells, which plays a vital role in the control of oxidative stress by trapping free radicals. In these processes, the oxidised and the reduced forms of glutathione act together to maintain the intracellular redox homeostasis. They can also be used to evaluate the intracellular oxidative stress, an increased ratio in GSH content is an indication of increased oxidative stress (Meng et *al*., 2017; Lee et *al*., 2018). Thus, Oral administration of *Oxalis cernua* methanolic extracts protects kidneys against oxidative damage by the decrease of reduced glutathione levels with different percent compared to control.

MDA levels in the biological system can be utilized as an imperative indicator of lipid peroxidation in *in vivo* analysis. Lipid peroxidation refers to the oxidative degeneration of lipids, which is characterized by the formation of a hydro-peroxide group in the lipid tails. The later forms in a chain of reactions, during which the intermediate peroxidation products propagate the radical damage to adjacent molecules. Hydroperoxide lipid derivatives can ultimately reorganize and decompose into secondary products such as the cytotoxic 4-hydroxynonenal and the mutagenic malondialdehyde (Amudha, P. and Vanitha, V., 2019; Rems et *al*., 2019). Hence, liver homogenate of the treated animals exhibited a significant decrease in MDA level indicating a potent lipid peroxidation inhibition by *Oxalis cernua* methanolic extracts.

There is growing evidence to promote a link between increased levels of ROS and deteriorated activities of enzymatic and non-enzymatic antioxidants. This study data showed that ROS induced by exposure to *Oxalis cernua* methanolic extracts compounds mediated lipid peroxidation inhibition by increasing MDA level and suppressing the activity of antioxidant enzymes such as GSH and CAT. The decline in catalase and glutathione can be accredited to

ineffective scavenging activity, then resulting in increasing levels of H_2O_2, which can react with oxygen to form the OH radicals which increase lipid peroxidation (Najimi et *al*., 2017; Amudha, P. and Vanitha, V., 2019)**.**

8. Effect of plant extracts on hepatotoxicity and nephrotoxicity induced by CCl_4

Carbon tetrachloride (CCl_4) is a toxin, present at low concentrations in air and water, widely used in various industries as a solvent and in medicine as an anthelmintic. Numerous studies have reported that CCl_4 causes free radical generation in an array of tissues including the aggravation of inflammatory processes, acute and chronic hepatic injuries, as well as hepatotoxicity (Zhang et *al*., 2018; Hozzein et *al*., 2019). For instance, in mammalian hepatocytes, microsomal cytochrome P450 produce reactive metabolites like trichloromethyl radical ($CCl_3^{\bullet}$) which, in the presence of oxygen, converts to a highly reactive trichloromethylperoxyl radical ($CCl_3OO^{\bullet}$). The latter cause the impairments of important cellular molecules, such as lipids, proteins, and nucleic acids, which heavily contribute to subsequent cell damage (Gazwi and Mahmoud, 2019).

Inadequate performance of these molecules activates some secondary paths which incited morphological and pathophysiological features. $CCl_3OO^{\bullet}$ could initiate the chain reaction of lipid peroxidation and destroy polyunsaturated fatty acids, in particular, those associated with phospholipids. In addition, it may affect the permeability of cells membranes and transmembrane transport mechanisms resulting in the loss of cellular calcium and homeostasis. Consequently, the antioxidant activity of the body and the inhibition of free radicals' production become the most important means in preventing CCl_4-induced hepatopathies and nephropaties (Ritesh et *al*., 2015; Hashemzadeh et *al*., 2018).

8.1. Relative organ weights

Organs weight was summarized in table 13. Some significant differences were recorded when comparing pre-treated animals to control and CCl_4 groups. The comparison of liver relative weights of CCl_4 treated rats with the control group showed a significant increase in the relative weight of the latter. At the concentration (100 mg/kg bw), rats treated with *Oxalis cernua* AME and RME revealed no significant difference compared to the control group, however, a significant difference ($p<0.001$) was noted with respect to the CCl_4 group. At the dose (200 mg/kg bw), rats treated with *Oxalis cernua* AME reported a significant difference in liver weights compared to control group, but not with those treated with *Oxalis cernua* RME, while a significant difference was noted compared to CCl_4 group. Rats treated with *Oxalis cernua* RME (400 mg/kg bw) presented a significant deference in liver weights compared to CCl_4 intoxicated group, and no changes were noted with respect to the control group, suggesting the presence of toxicity (Gazwi and Mahmoud, 2019). The Olive oil group did not reveal any significant changes compared to the control group.

Although the subsequent treatment with both extracts, they could overcome CCl_4-induced symptoms like diarrhoea and sedation in these animals. Nevertheless, the lack of such behavioural changes in animals treated may indicated that they offer better protection against acute toxicity induced by CCl_4 (Marslin et *al*., 2018). In addition, the remarkable increase in the weight of liver indicates a hepatomegaly, which is a liver abnormality being stated by many authors as a result of CCl_4 impairment. It was reported that the increasing weights of animal organs is an indicator of toxicity induced by the toxic substances used (AlSaid et *al*., 2015).

Regarding the weight of kidneys, as indicate in table 13, whether for *Oxalis cernua* AME or RME with the concentration (100, 200, 400 mg/kg bw) revealed that no significant changes were observed ($p>0.05$) compared to both of control and the CCl_4 group, excepting animals

treated with *Oxalis cernua* RME (400 mg/kg bw), which exhibited a significant decrease ($p<0.01$) with respect to CCl_4 group.

Table 13: Relative organ weight of rats treated with *Oxalis cernua* AME and RME, Vitamin C, CCl_4, Olive oil and control group.

Group	Liver (g)	Kidney (g)
Control	37.94±1.62	7.31±0.40
Olive oil	35.43±1.45 ^{ns}	7.07±1.09 ^{ns}
CCL_4	55.60±3.95 ***	7.66±1.05 ^{ns}
Vit C	43.92±1.83 $^{ns, d}$	7.14±0.05 $^{ns, a}$
***Oxalis cernua* AME**		
100 mg/kg bw	39.53±2.56 $^{ns, d}$	7.24±0.31 $^{ns, a}$
200 mg/kg bw	41.60±1.88 $^{ns, d}$	7.25±0.16 $^{ns, a}$
400 mg/kg bw	42.54±1.25 $^{ns, d}$	6.90±0.10 $^{ns, a}$
***Oxalis cernua* RME**		
100 mg/kg bw	36.79±3.81 $^{ns, d}$	6.94±0.30 $^{ns, a}$
200 mg/kg bw	40.64±0.73 $^{ns, d}$	6.89±0.08 $^{ns, a}$
400 mg/kg bw	45.06±0.58 $^{ns, c}$	6.60±0.20 $^{ns, c}$

All data are reported as mean ± SEM, n=5. (ns: non-significant $p> 0.05$, *: $p< 0.05$, ***: $p <0.001$) significant differences compared to the control group. (a: $p> 0.05$, c: $p<0.01$, d: $p < 0.001$) significant differences with respect to CCl_4 group.

8.2. Biochemical parameters in plasma of rats treated with plant extract and CCl_4

The effects of *Oxalis cernua* AME and RME on hepatic and nephrotic biomarkers in CCl_4 intoxicated rates are summarized in table 14. The results displayed a statistically significant increase in AST, ALT levels and also a significant decrease in urea and creatinine values of the animals which received only CCl_4 compared to the control group, which suggested that CCl_4

could induce liver and kidneys injury. However, (100 mg/kg bw) of Vit. C, as observed in table 14, reduced AST and ALT activity and asserted its high protective effect against the induced damage. Olive oil group did not show any significant changes compared to the control group.

Treated rats with *Oxalis cernua* AME and RME recorded a significant increase in the AST and ALT values compared to the CCl_4 group. Furthermore, the activity of AST and ALT was restored by *Oxalis cernua* AME to normal levels that observed in control group, except ALT value of the dose (100 mg/kg bw) which presented a significant decline. The animals received *Oxalis cernua* RME, when compared to the control group, revealed almost similar AST and ALT serum levels, which may suggest the protective effect of the *Oxalis cernua* extracts.

Both extracts compared to the CCl_4 group revealed a significant increase in urea serum levels. Moreover, this deference reminded under that of the control group except the dose (100 mg/kg bw) which conserved normal levels. All the doses in both extract compared to CCl_4 group showed a statistically significant increase in creatinine serum levels. Furthermore, with the same biochemical parameter, a significant decrease was noted with *Oxalis cernua* AME (200 mg/kg bw) and *Oxalis cernua* RME (100 and 400 mg/kg bw).

Liver is very vulnerable to poisonous chemical, toxic as well as non-toxic drugs, besides viral penetrations which can severely induce hepatotoxicity. A single CCl_4 exposure can generate trichloromethyle (CCl_3) free radicals and ROS after being metabolized by cytochrome P450 (this enzyme is usually used to transform or catalyse the poisonous drugs, compounds or chemicals in the interconnected network of tubular membranes). These metabolites initiate a lipid peroxidation chain reaction that leads to hepatocytes membrane permeability and transport function disorder (Marslin et *al.*, 2018).

Inside rodent organism, enzymes like aminotransferase AST (a mitochondrial one that has iso-enzyme in other cells beside hepatocytes such as myocardiocytes, myocytes, kidney) and ALT (a cytoplasmic enzyme) are useful and frequently used to evaluate amount or severity of CCl_4 toxicity. The alteration of hepatic parameters in this model of toxicity, evoked by CCl_4, are very similar to the symptoms of acute hepatitis in humans. An acceptable level of nephrotoxicity and hepatotoxicity made this model obsolete faithful and trusted at least at histological level (Hashemzadeh et *al*., 2018; Thiesen et *al*., 2018).

Consequently, hepatotoxicity induced by CCl_4 in normal rats increased the levels of AST and ALT biochemical framework prominently, which could indicate their extent alteration. Liver cells change their functional transition, cause membrane permeability, and lead to the leakage of enzymes into extracellular space. However, the reduction of the enzyme level in the animals treated with the extract asserted the plant potential to prevent liver injury (Mahmoodzadeh et *al*., 2017; Marslin et *al*., 2018; Ali et *al*., 2018).

Table 14: Biochemical parameters in the plasma of rats treated with *Oxalis cernua* AME and RME, Vitamin C, CCl_4, Olive oil and control group.

Group	AST (U/l)	ALT (U/l)	Urea (g/L)	Creatinine (mg/L)
Control	132.27±5.50	22.06±3.29	0.62±0.04	5.99±0.27
Olive oil	130.20±1.23 ^{ns}	20.85±2.01 ^{ns}	0.61±0.02 ^{ns}	5.80±0.32 ^{ns}
CCL_4	281.93±2.94***	62.76±1.80***	0.14±0.01***	1.02±0.17***
Vit. C	100.76±7.22$^{**, d}$	24.72±4.59 $^{ns, d}$	0.50±0.06 $^{ns, b}$	6.01±0.13 $^{ns, d}$
AME				
100 mg/kg bw	118.76±14.03 $^{ns, d}$	16.44±2.44$^{*, d}$	0.54±0.03 $^{ns, d}$	5.82±0.23 $^{ns, d}$
200 mg/kg bw	113±16.07 $^{ns, d}$	21.20±2.46 $^{ns, d}$	0.43±0.05$^{***, d}$	5.2±0.41$^{***, d}$
400 mg/kg bw	123.63±14.56 $^{ns, d}$	24.44±2.90 $^{ns, d}$	0.41±0.10$^{***, d}$	5.67±0.45 $^{ns, d}$
RME				
100 mg/kg bw	124.4±13.07 $^{ns, d}$	26.38±3.5 $^{ns, d}$	0.5±0.01$^{*, d}$	5.25±0.29$^{**, d}$
200 mg/kg bw	154.16±27.45 $^{ns, d}$	25.84±3.77 $^{ns, d}$	0.49±0.04$^{**, d}$	6.33±0.30 $^{ns, d}$
400 mg/kg bw	116.23±10.40 $^{ns, d}$	25.22±2.93 $^{ns, d}$	0.35±0.14$^{***, d}$	5.27±0.20$^{**, d}$

Values were expressed as mean ± SEM, n=5. (ns: non-significant $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$) significant differences with respect to control group. (b: $p < 0.05$, d: $p < 0.001$) significant differences compared to CCL_4 group.

The findings of the present investigation demonstrated that no significant differences were noted between biomarkers in control and pre-treated rats. It was interesting that AST and ALT serum level were significantly restored to near-normal levels. The higher reduction reaching the same level to control animals was observed. Additionally, the hepato-protection effect of both extracts was not dose dependent. All this results may enhance that *Oxalis cernua* AME and RME could protect liver damage. However, the effect of *Oxalis cernua* AME (100 mg/kg bw) on ALT value may be related to the timing of exposure to the toxic agent (Thiesen et *al*., 2018).

The treatment with medicinal plant largely modulated the severity of CCl_4 induced liver damage. Pre-treated rats showed that *Oxalis cernua* extracts can stabilize liver cell membranes and prevent the leakage of enzymes. Preventing the production of free radicals and neutralizing them. The protecting potential of this plant against hepatotoxins can be other probable reasons for the healing effect and use of *Oxalis cernua* (Ali et *al*., 2018; Mahmoodzadeh et *al*., 2017).

Regarding kidney, although CCl_4 exhibited a high affinity to the kidney, the mechanism of CCl_4 induced kidney damage is similar to that of liver. The activities of creatinine and urea as kidney function indices were significantly decreased in treated animals with CCl_4 compared to the control group ($p < 0.001$). However, treatment with both extracts of *Oxalis cernua* caused a significant increase in kidney function parameters, which may indicate their protective effect against nephrotoxicity induced by CCl_4.

Indeed, the efficacy of any bioactive compound could be responsible for a normal or near normal physiology function which has been perturbed by the toxic agent. The offered protection might be possibly attributed to the anti-inflammatory and antioxidant activity of the plant (Ali et *al*., 2018; Khalid et *al*., 2018; Tir et *al*., 2019).

8.3. Reducing power and DPPH radical-scavenging activity of plasma

Plasma antioxidant capacity was evaluated by reducing power scavenging assay (Table 15). It appeared that olive oil group have the same effect as the control group as no significance differences were distinguished. Animals treated with CCl_4 showed a difference statistically significant ($p<0.001$), which reflects the influence of the toxic product. However, those treated with Vit. C (100 mg/Kg bw) did not mention any significant changes ($p>0.05$) compared to normal rats, yet, it was statistically significant compared to CCl_4 group.

The plasma scavenging ability was not significantly improved following the oral administration of *Oxalis cernua* AME and RME except the animals treated with *Oxalis cernua*

RME (100 mg/Kg bw), which restored the value of normal rats compared to the control group. On the other hand, compared to the CCl_4 group both extracts demonstrated a significant difference ($p<0.001$), except those treated with *Oxalis cernua* RME (200 mg/Kg bw).

Table 15: DPPH radical scavenging activity and reducing power chelating effect in rats' plasma.

Group	DPPH (%)	Reducing power (%)
Control	18.56±2.09	0.73±0.03
Olive oil	18.51±1.20 ^{ns}	0.73±0.01^{ns}
CCL_4	2.64±1.54***	0.84±0.03***
Vit. C	36.61±2.90$^{***, d}$	0.69±0.00 $^{ns, d}$
***Oxalis cernua* AME**		
100 mg/kg bw	13.60±2.04$^{**, d}$	0.53±0.00$^{***, d}$
200 mg/kg bw	30.94±3.64$^{***, d}$	0.59±0.01$^{***, d}$
400 mg/kg bw	22.58±2.01 $^{ns, d}$	0.60±0.00$^{***, d}$
***Oxalis cernua* RME**		
100 mg/kg bw	27.35±2.54$^{***, d}$	0.76±0.03 $^{ns, d}$
200 mg/kg bw	17.68±1.88 $^{ns, d}$	0.81±0.03$^{***, a}$
400 mg/kg bw	24.59±3.63$^{**, d}$	0.42±0.00$^{***, d}$

Values were expressed as mean ± SEM, n=5. (ns: non-significant $p> 0.05$, **: $p < 0.01$, ***: $p <0.001$) significant differences compared with control group. (a: $p> 0.05$, d: $p < 0.001$) significant differences compared with CCL_4 group.

The effectiveness of the antioxidant treatment in plasma using DPPH radical was expressed as a percentage inhibition. In this study, the results obtained in table 15 exhibited that olive oil group did not reveal significant changes ($p>0.05$) compared to the control group. Accordingly, it had not improved the antioxidant activity in the plasma. Moreover, CCl_4 group presents a significant difference ($p<0.001$) with the lowest value of inhibition percentage,

confirming the harmful effect of CCl_4 in organism tissues. Nevertheless, Vit. C (100 mg/kg bw) group indicated a difference statistically significant endorsing its protective effect as an antioxidant.

The administration *per os* of *Oxalis cernua* AME and RME at doses of (100, 200 and 400 mg/Kg bw) in rats increased significantly the plasma antioxidant capacity except (200 and 400 mg/Kg bw) of the extracts respectively, whit respect to normal rats. Nevertheless, comparing the effect of the same extract to CCl_4 group demonstrate the presence of a significant difference ($p<0.001$), indicating an increase in the antioxidant activity of plasma.

It is believed that antioxidant activity plays an important role in preventing the oxidative stress and/or inhibiting free radical generation, this features are important in terms of protecting the liver from CCl_4 induced damage. The action mode for these second metabolites may differ depending upon the presence of synergists and antagonists, and obviously the matrix in which they were applied (Lukitaningsih, 2020). Furthermore, the possible beneficial effects of the *Oxalis cernua* extract against the toxicity of CCl_4 may be due to the effect of a single molecule or to a combined effect of several compounds, which suggested that some phenolic compounds react slowly and need a longer reaction time. In addition, the antioxidant activity of a mixture is not only dependent on concentration but also on the structure and synergy between antioxidants (El-haskoury et *al*., 2018; Marslin et *al*., 2018; Tir et *al*., 2019).

8.4. Estimation of catalase activity, glutathione content and lipid peroxidation level

Treated rats with CCl_4 only showed that CAT activity did not noted any changes while GSH and MDA levels increased significantly compared to normal rats. Meanwhile, those treated with olive oil did not indicate significant changes.

Comparing the levels of GSH and MDA in the tissues of animals pre-treated with Vit. C to those of CCl_4 damaged rats demonstrated their significant decrease after the induction of

damage and inflammation ($p<0.001$). The CAT antioxidant activity was maintained, which asserted Vit. C protective effect against CCl_4 alteration.

Table 16: Estimation of CAT activity (µmole/min/mg of protein), GSH (nmole/g of tissue) and MDA (nmole/g of tissue) in livers.

Group	CAT	GSH	MDA
Control	1.50±0.17	6.96±0.07	56.67±0.29
Olive oil	1.45±0.8 ^{ns}	6.88±0.01 ^{ns}	55.35± 0.45^{ns}
CCL_4	1.34±0.20 ^{ns}	8.32±1.23***	238.70±12.4***
Vit. C	1.49±0.21 $^{ns, a}$	8.57±0.07 $^{ns, d}$	38.86±6.34 $^{ns, d}$
***Oxalis cernua* AME**			
100 mg/kg bw	3.93±0.4$^{ns, a}$	10.68±0.22$^{***, d}$	82.89±2.17$^{*, d}$
200 mg/kg bw	19.61±5.20$^{***, d}$	8.47±0.20$^{ns, d}$	83.49±4.70$^{*, d}$
400 mg/kg bw	4.67±0.44$^{ns, a}$	7.48±0.57$^{ns, d}$	65.67±1.76$^{ns, d}$
***Oxalis cernua* RME**			
100 mg/kg bw	3.67±0.48$^{ns, a}$	9.18±0.11$^{*, d}$	118.98±2.52$^{***, d}$
200 mg/kg bw	4.06±0.71$^{ns, a}$	6.09±0.24$^{ns, d}$	28.47±0.92$^{*, d}$
400 mg/kg bw	2.93±0.31$^{ns, a}$	8.02±0.35$^{ns, d}$	55.70±2.92$^{ns, d}$

Values were expressed as mean ± SEM, n=5. (ns: $p> 0.05$, *: $p< 0.05$, ***: $p < 0.001$) significant differences compared to the control group. (a: $p> 0.05$, d: $p < 0.001$) significant differences compared to CCL_4 group.

Table 17: Estimation of CAT activity (µmole/min/mg of protein), GSH (nmole/g of tissue) and MDA (nmole/g of tissue) in kidneys.

Group	CAT	GSH	MDA
Control	1.28±0.29	14.51±1.03	64.56±2.71
Olive oil	3.20±0.08 ^{ns}	24.08±0.01 ^{ns}	95.0.98±1.02***
CCL_4	1.14±0.20 ^{ns}	15.30±0.13 ^{ns}	115.60±2.20***
Vit. C	15.26±2.13** ^{c}	56.44±1.03 $^{ns\ a}$	94.52±3.30*** ^{a}
***Oxalis cernua* AME**			
100 mg/kg bw	11.63±1.81* ^{b}	32.48±2.15 $^{ns\ a}$	131.63±2.50*** ^{a}
200 mg/kg bw	6.45±0.55$^{ns\ a}$	18.49±0.35 $^{ns\ a}$	182.76±3.78*** ^{d}
400 mg/kg bw	4.36±0.45$^{ns\ a}$	9.20±0.41 $^{ns\ a}$	95.90±2.77*** ^{d}
***Oxalis cernua* RME**			
100 mg/kg bw	19.15±1.25*** ^{d}	82.68±2.89** ^{c}	92.07±4.56*** ^{d}
200 mg/kg bw	13.33±1.27** ^{c}	26.44±0.91 $^{ns\ a}$	97.15±2.63*** ^{d}
400 mg/kg bw	03.62±1.25$^{ns\ a}$	17.11±0.64 $^{ns\ a}$	70.38±3.36$^{ns\ d}$

Values were expressed as mean ± SEM, n=5. (ns: non-significant p> 0.05, *: p< 0.05, **: p<0.01 ***: p <0.001), significant differences compared to the control group. (a: p> 0.05, b: p< 0.05, c: p <0.01 d: p < 0.001) significant differences compared to CCL_4 group.

The CAT activity of *Oxalis cernua* AME and RME (Table 16), in liver, did not indicated any significant changes except pre-treatment with *Oxalis cernua* AME at the dose (200 mg/kg bw) which revealed a significant increase (p<0.001). GSH level showed a significant increase

in dose (100 mg/kg bw) in both extract compared to the normal rats and a significant decrease of all doses of *Oxalis cernua* AME and RME with respect to the CCl_4 group. MDA activity showed a significant decrease in all doses of both extracts compared to the CCL_4 group. However, with respect to healthy rats, *Oxalis cernua* AME (100 and 200 mg/kg bw) and RME (100 mg/kg bw) revealed a significant increase. Pre-treatment with the *Oxalis cernua* RME (200 mg/kg bw) decreased significantly MDA level after the induction of oxidative stress ($p< 0.05$). Animals treated with *Oxalis cernua* (400 mg/kg bw) maintained MDA level. Therefore, it seems that the effect of *Oxalis cernua* AME and RME on plasma and homogenate tissues total antioxidant capacity is dose dependent.

Regarding kidneys (Table 17), the CAT activity of Vit. C, *Oxalis cernua* AME (100 mg/kg bw), and both of (100 and 200 mg/kg bw) of *Oxalis cernua* RME presented a significant increase compared to control and CCl_4 groups. In addition, GSH content of animals treated with (100 mg/kg bw) of *Oxalis cernua* RME exhibited a significant increase ($p<0.01$) compared to the same groups. However, all the other groups did not show any significant differences. Concerning lipid peroxidation, MDA level of all the groups presented a significant increase with respect to control group, except *Oxalis cernua* RME (400 mg/kg bw). Moreover, compared to CCl_4 group Vit. C and (100 mg/kg bw) of *Oxalis cernua* AME doses did not noted a difference statically significant. Yet, the dose (200 mg/kg bw) exhibited a significant increase, and the other groups displayed a significant decrease ($p < 0.001$).

The organism has effective defence mechanisms to prevent and neutralize free radicals. This action is finely accomplished through regulating endogenous antioxidants such as glutathione, MDA and catalase. They form a shared support system against ROS. In CCl_4 model, the balance between ROS production and antioxidant defence system of these enzymes is disturbed as a result of oxidative stress, which disrupts cellular functions and causes liver damage and necrosis. The return of biomarkers to the normal levels after being damaged by

CCl_4 in the groups administered with extracts might be due to the ability of this extracts to stimulate these antioxidant enzymes to neutralize the ROS produced by CCl_4 (Mahmoodzadeh et *al*., 2017).

Vitamin C have antioxidants properties such as delaying or inhibiting oxidation. Moreover, ascorbate is able to effectively scavenge superoxide in *in vivo*, and reduce amino acid radicals. It may control ROS formation and decrease their cytosolic levels. In this respect, it is more reactive than GSH. Furthermore, it can reduce toxicity, neutralizing some carcinogens and may prevents diseases, especially in relation to activation and inhibition of genes related to inflammation, apoptosis and necrosis. In addition, Vit. C significantly lowered antioxidant parameters which was in agreement with other studies (Smirnoff, 2018; de Carvalho Melo-Cavalcante et *al*., 2019).

Cellular antioxidant enzymes such as CAT offer protection against the toxicity produced by ROS. Macromolecules' oxidative injuries are the result of an excess of ROS production which induces inactivation of antioxidant enzymes. The results of the present study did not display a significant difference between control rats and groups pre-treated with CCl_4. Pre-treatment with extracts increased the activity of the studied enzyme and made it near normal as the control group, which could also be attributed to the antioxidant capacity of *Oxalis cernua* extracts. Previous reports have suggested that biomolecules can increase endogenous antioxidant enzymes activity by reducing protein and lipid oxidation (Tir et *al*., 2019).

Glutathione, a cytosolic tripeptide, ubiquitously present in all cell types at millimolar concentration, is also the major non-enzymatic regulator of intracellular redox homeostasis. The oxidative biomarkers GSH is significantly increased in the liver after exposure to CCl_4, which presumably caused an oxidative damage of protein and lipid. These oxidative markers were significantly reduced by the extracts at the dose (100 mg/kg bw). Furthermore, methanolic extracts maintained the GSH level which indicated their protective ability (200 and 400 mg/kg

bw). The use of *Oxalis cernua* extracts significantly attenuated the elevation of these markers, which could be attributed to the ability of extracts to scavenge excessive free radicals' generation during oxidative stress (Ritesh et *al*., 2015).

Malondialdehyde (MDA) is a reactive aldehyde and an indicator of the imbalance between free radicals and antioxidants in damaged cells. It is formed as one of the end products during the peroxidation of polyunsaturated fatty acids. This extra oxidant can bind with the double bonds of membrane and produce oxidative stress, which led to multiple acute and chronic tissue damage. Interestingly, the results did not show a significant difference between control and toxic-pre-treated animals. Treatment by *Oxalis cernua* extracts significantly decreased the MDA concentration in the liver and kidneys. The results reinforced the role of biomolecules from *Oxalis cernua* as a promising medicinal plant for treating hepatic and nephrotic tissue through their antioxidant properties (El-haskoury et *al*., 2018).

8.5. Histological examination

Anatomical examination of CCl_4 treated rats, at the end of the study, showed the visible pale, gross, and irregular surface of the liver suggesting severe hepatocellular injury compared to control group. However, histological examination showed extensive damage to the hepatocytes architecture, multi focal area necrosis, congested blood vessel and fatty degradation, infiltration of inflammatory cells, lymphocytes, and histiocytes. Histological sections of control animals, olive oil and Vit. C groups presented in figure 32 revealed a normal hepatic architecture and polyhedral hepatocytes, sinusoidal spaces, and a central vein was observed.

Liver sections of animals treated with *Oxalis cernua* AME (100 mg/kg bw) displayed a slight change in liver architecture and congested blood vessel with inflammatory infiltrate

(Figure 33). Although the liver tissues of animals treated with *Oxalis cernua* AME (200 mg/kg bw) showed important vascular congestion, and discrete inflammation accompanied hepatocyte cells in the parenchyma. However, an improvement has been observed in liver tissue of animals pre-treated with (400 mg/kg bw) which exhibited less liver congestion and inflammatory infiltrate too.

Rats received *Oxalis cernua* RME (100 and 400 mg/kg bw) indicated congested blood vessel, inflammatory infiltrate, while dose (200 mg/kg bw) demonstrated a worse inflammatory infiltrate, with vascular congestion. Histological results of kidneys (Figure 34) exhibited adequate glomeruli and normal tubules, congestion occurred in kidneys of both extracts with all doses of the studied groups.

Biotransformation of a single dose administration with CCl_4 leads to the production of highly reactive free radical metabolites, which causes lipid peroxidation and damage to hepatocytes membranes, manifested as centri-lobular necrosis and steatosis of hepatocytes accompanied by lipid degeneration. Neutrophils cumulate around the damaged hepatocytes, and induce inflammatory reaction through pro-inflammatory cytokines (Chalupová et *al*., 2015; Khalid et *al*., 2018). The beneficial effects of bioactive molecules from *Oxalis cernua* AME and RME in protecting the liver architecture against CCl_4-induced liver injury has already been reported and agreed with the results obtained with biochemical indicators. Herbal medicinal products are considered to present a lower risk of toxicity. However, natural products are not completely free of the possibility of inducing toxicity or other adverse effects (Thiesen et *al*., 2018; Tir et *al*., 2019).

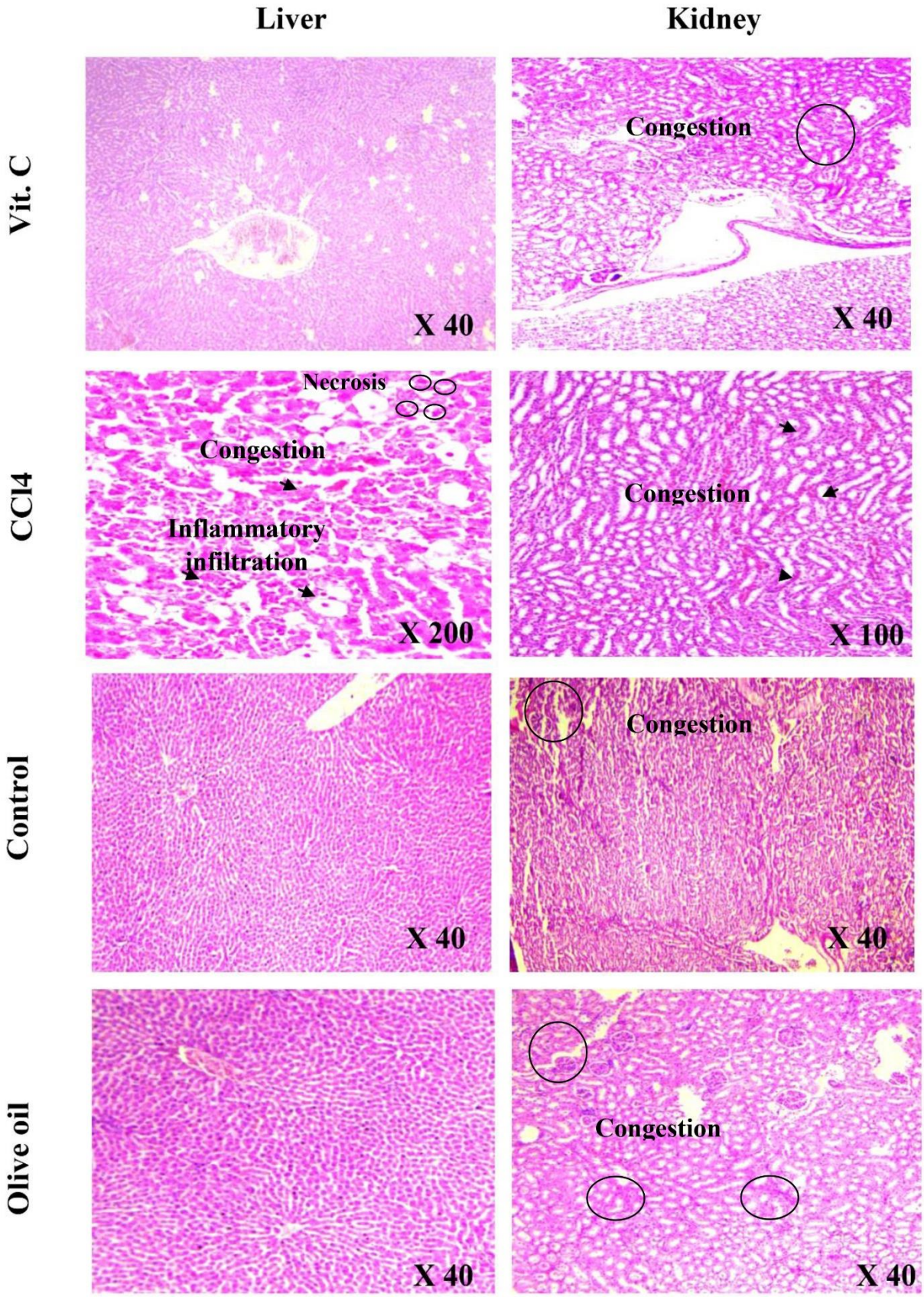


Figure 32: Photomicrographs of liver and kidney sections stained with haematoxylin and eosin of treated groups with Vit. C, CCl_4, Olive oil and control group.

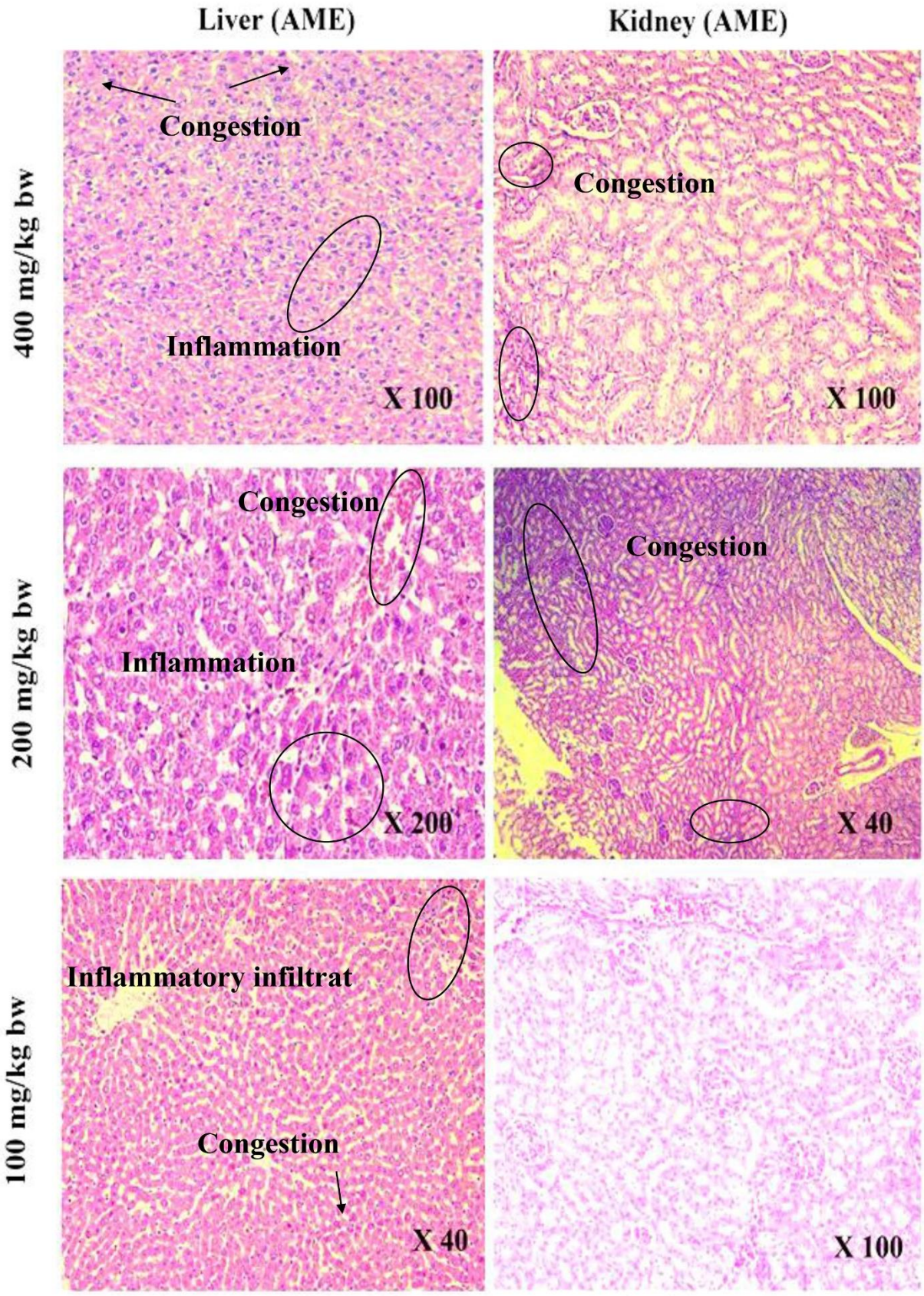


Figure 33: Photomicrographs of liver and kidney sections stained with haematoxylin and eosin of treated groups with *Oxalis cernua* AME.

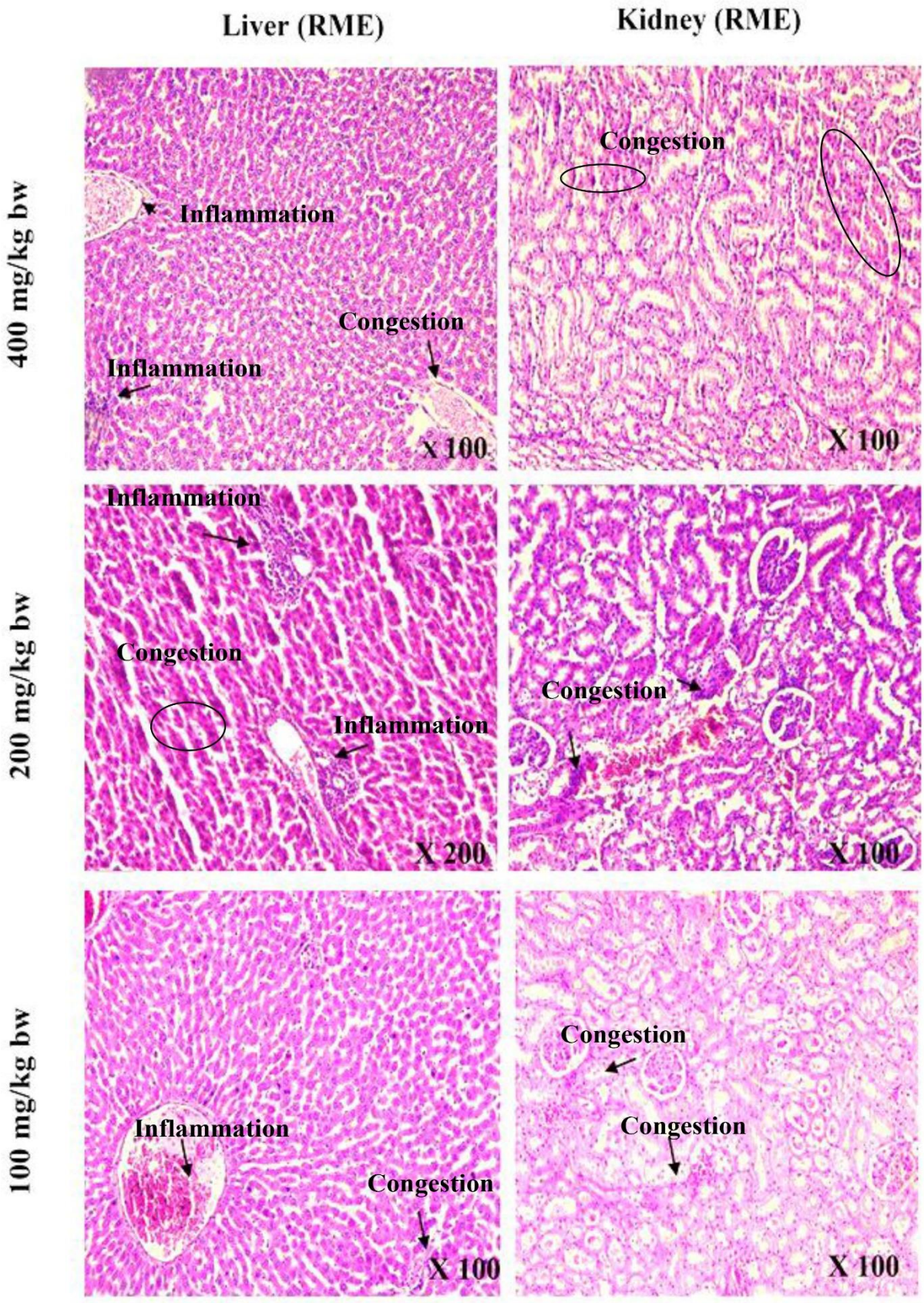


Figure 34: Photomicrographs of liver and kidney sections stained with haematoxylin and eosin of treated groups with *Oxalis cernua* RME.

9. *In vivo* anti-inflammatory activities of plant extracts

9.1. Xylene-induced ear oedema

Xylene is a volatile aromatic hydrocarbon, which at room temperature, has a liquid form. Often, it is a mixture of isomers containing variable proportions of ethylbenzene (Ogbu et *al*., 2019). This chemical substance, acting as a phlogistic agent, is topically applied to induce mice ear oedema. One of the classic models of acute inflammation. It is widely used to examine the *in vivo* anti-inflammatory effect of potential components (Akinloye et *al*., 2020). It causes immediate irritation, which leads to infiltration of inflammatory cells, vasodilation, accumulation of fluid, and oedema (Aham et *al*., 2019).

The application of xylene to the ear's surface, in this assay, stimulate neurogenic inflammation mediated through the release of a peptide related to the peripheral as well as central nervous system, which is substance P. The peripheral release of substance P and other neuropeptides from sensory neurons, then act on target cells including mast cells. Leukocytes as well as endothelial cell cause the release of inflammatory mediators such as histamine, serotonin, prostaglandins, thromboxanes, leukotrienes, cytokines, bradykinin, acetylcholine and nitric oxide. Together with vasodilatation and extravasation of plasma, they lead to the development of neurogenic inflammation and subsequent oedema formation of the ear (Babakura et *al*., 2019; Khan et *al*., 2019).

In case of neurogenic inflammation, substance P and calcitonin generated neuropeptides, and stimulate the secretion of histamine from the mast cells. On the other hand, histamine also causes the release of substance P. This shows that any substance which could inhibit histamine activity might also partially reduce xylene-induced inflammation (Aham et *al*., 2019).

Acute inflammation is characterized by classical symptoms, such as warmth, redness, swelling and acute nociceptive response. The latter was assessed by an increased amount of

time spent itching and licking the treated ear. In the current assay the mice's right ear of all the groups exhibited obvious inflammatory symptoms. The difference in the thickness of their ear before and after the application of xylene was used to evaluate the anti-oedema effect of the investigated extracts (Sarvaka et *al*., 2016; Erhirhie et *al*., 2019).

The obtained results from the effect of *Oxalis cernua* methanolic extracts on the mice ear oedema assay is illustrated in figure 35. Pretreated animals with *Oxalis cernua* AME (100, 200, and 400 mg/kg bw), and *Oxalis cernua* RME (400 mg/kg bw) revealed a week percentage of inhibition significantly different ($p < 0.001$) respectively (28.61%, 32.12%, 17.85%, and 18.66%), with respect to the Asp group (51.78%). Furthermore, comparing the inhibition percentage of *Oxalis cernua* RME (100 mg/kg bw) group (53.57%) to the control group, no significant differences were noted. Meanwhile, only *Oxalis cernua* RME (200 mg/kg bw) exhibited a high inhibition percentage (79.78 %).

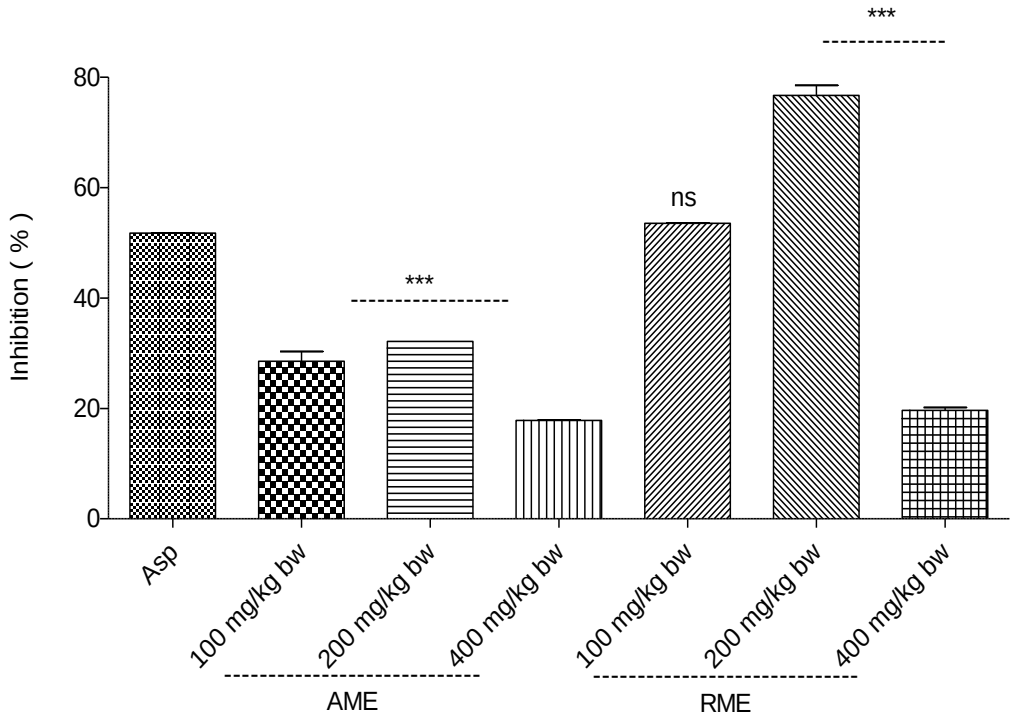


Figure 35: Anti-inflammatory effect of *Oxalis cernua* methanolic extracts on ear oedema induced by xylene. AME: Areal part methanolic extract; RME: root methanolic extract; Asp: aspirin. The values are expressed as mean ± SEM; n = 5; ns: non-significant $p>0.5$, *** $p < 0.001$.

Oxalis cernua RME (200 mg/kg bw) decreased ear oedema produced by xylene in mice, it was more effective than aspirin (100 mg/kg bw). This indicated that *Oxalis cernua* RME may have a potential anti-inflammatory effect on acute xylene-induced inflammation. The extract contained a significant amount of phenolic and flavonoid compounds, generally, considered as natural antioxidant, free radical scavenging and anti-inflammatory ingredients (Nagendra et *al*., 2019). Moreover, various studies reported that a wide range of phyto-constituents is responsible for the anti-inflammatory activity such as tannins, terpenoids, alkaloids, glycosides, phenols, and triterpenoids. It is also suggested that the extract's constituents may inhibit phospholipase A, or the release of substance P, which are involved in the pathogenesis of inflammation (Sarvaka et *al*., 2016; Alamgeer et *al*., 2019; Ketnawa and Ogawa, 2019).

9.2. Carrageenan-induced rat paw oedema

Paw swelling is a convenient method highly reproducible for assessing inflammatory responses to antigenic tests and irritants. The anti-inflammatory activity of test compounds by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling is one of the essential studies. Carrageenan is a sea weed sulphated polysaccharide, commonly used as a pro-inflammatory agent to induce acute inflammation in basic research (Rungqu et *al*., 2016).

When carrageenan is injected locally into the rat paw, it produced inflammation, which was occurred within 30 min as a result of sequential and integrated action of several inflammatory mediators (Osman and Idrees, 2017). The time course of oedema development in carrageenan-induced paw oedema model is generally represented by three distinct phases. The first phase (0 - 1.5 h) is partly attributed to trauma of injection and also to histamine, substance P, serotonin components. The second phase (1.5 - 2.5 h) is maintained with the production of bradykinin and protease. The third phase (after 2.5 h) is mediated by prostaglandin and lysosomes. Inflammatory mediation depends mainly on the production of cytokines, such as

TNF-α, IL-1α and IL-1β, IL-6. Maximal vascular response as determined with leukocyte migration to the inflamed area, also reaches its maximum level in the third phase (De et *al*., 2017; Modak et *al*., 2017).

After the injection of carrageenan to the sub planter of the treated rats, it was observed that they spent an increased amount of time in itching, touching and licking the treated paw. One hour later, oedema appeared in the paw, then the symptoms of acute inflammation in the site of injection became clear such as redness, warmth, swelling, nociceptive response, and also less mobility was noticed. The difference in the thickness of rats' paw before and after the application of carrageenan was used to evaluate the anti-oedema effect of the investigated extracts.

The *in vivo* anti-inflammatory activity of *Oxalis cernua* methanolic extracts was compared with control group in an hourly basis up to three hours after the induction of inflammation. The results of anti-inflammatory activity were expressed as percentage of oedema increase**.** Figure 36 and 37 illustrated the percentage of oedema increase of standard drug (diclofenac) and *Oxalis cernua* methanolic extracts with time and dose.

The results of this assay showed that, in control animals, the injection of carrageenan produced a local oedema, where the maximum was observed after the first hour (51.53%), then it decreases progressively. Paw oedema of animals treated with diclofenac (20 mg/kg bw), and *Oxalis cernua* (AME and RME) revealed a significance decrease statistically significant ($p < 0.001$) throughout 3 h. However, it was less than that noted with the control group. In the other hand, the dose (100 mg/kg bw) did not exhibited any significant differences compared to the control group (in 60 and 90 min), and also both doses (100 and 200 mg/kg bw) in 180 min after carrageenan injection.

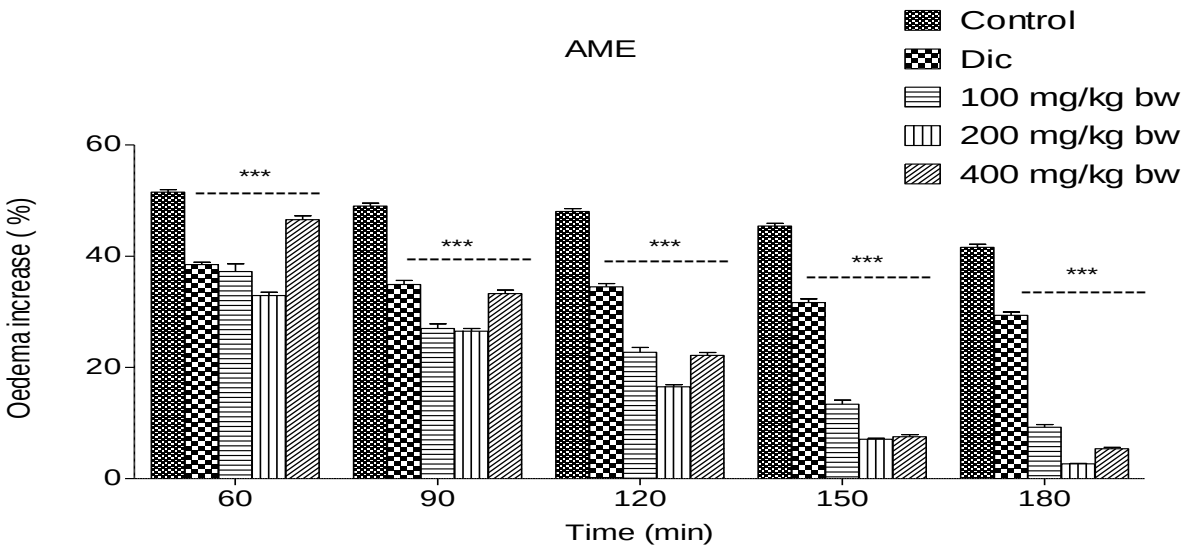


Figure 36: Anti-inflammatory effect of *Oxalis cernua* AME on paw oedema increase induced by carrageenan. AME: Areal part methanolic extract; Dic: Diclofenac. The values are expressed as mean ± SEM; n = 5; *** p <0.001.

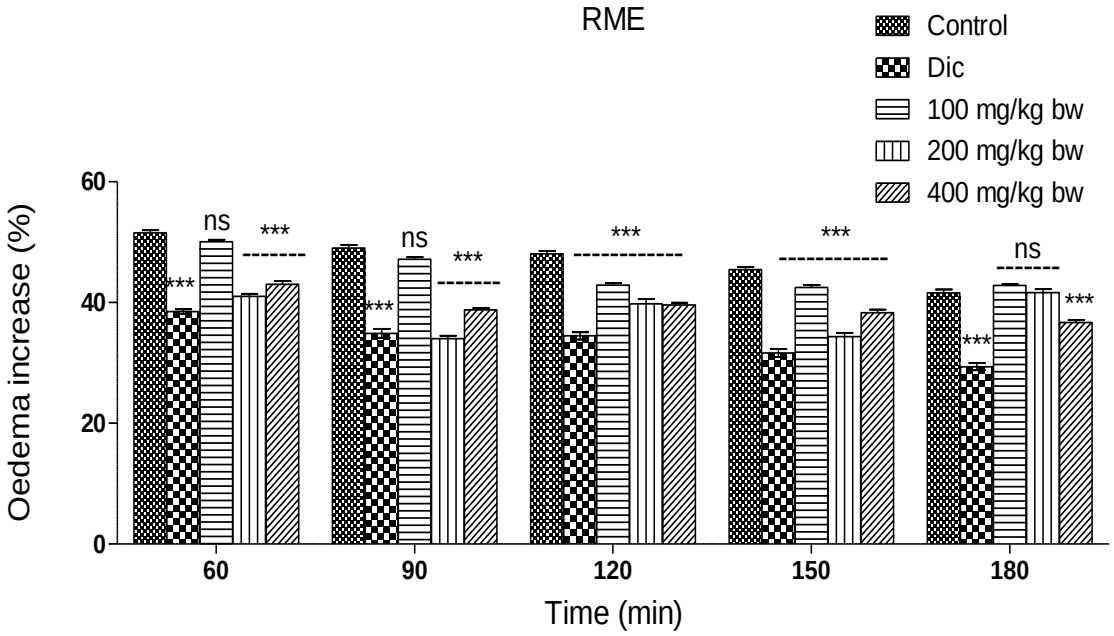


Figure 37: Anti-inflammatory effect of *Oxalis cernua* RME on paw oedema increase induced by carrageenan. RME: root methanolic extract; Dic: Diclofenac. The values are expressed as mean ± SEM; n = 5, ns: non-significant *p*>0.5; *** p <0.001.

Treatment with the doses (100, 200, and 400 mg/kg bw) has shown a significant anti-inflammatory effect ($p < 0.05$, and $p < 0.001$) with reduction in paw oedema at all-time points progressively in a non-concentration dependent manner, excluding the doses (100 and 400 mg/kg bw) at 60 min, with respect to standard diclofenac. Although, the dose (200 mg/kg bw) produced maximum reduction in oedema formation (93.61%) at 180 min (figure 38) after carrageenan injection.

In the contrary, treatment with *Oxalis cernua* RME did not only exhibit any reduction of the oedema (Figure 41), but also became a pro-inflammatory agent with the concentration (100, and 200 mg/kg bw) at 180 min, where the inhibition percentages were (-2.96% and -0.13%) respectively.

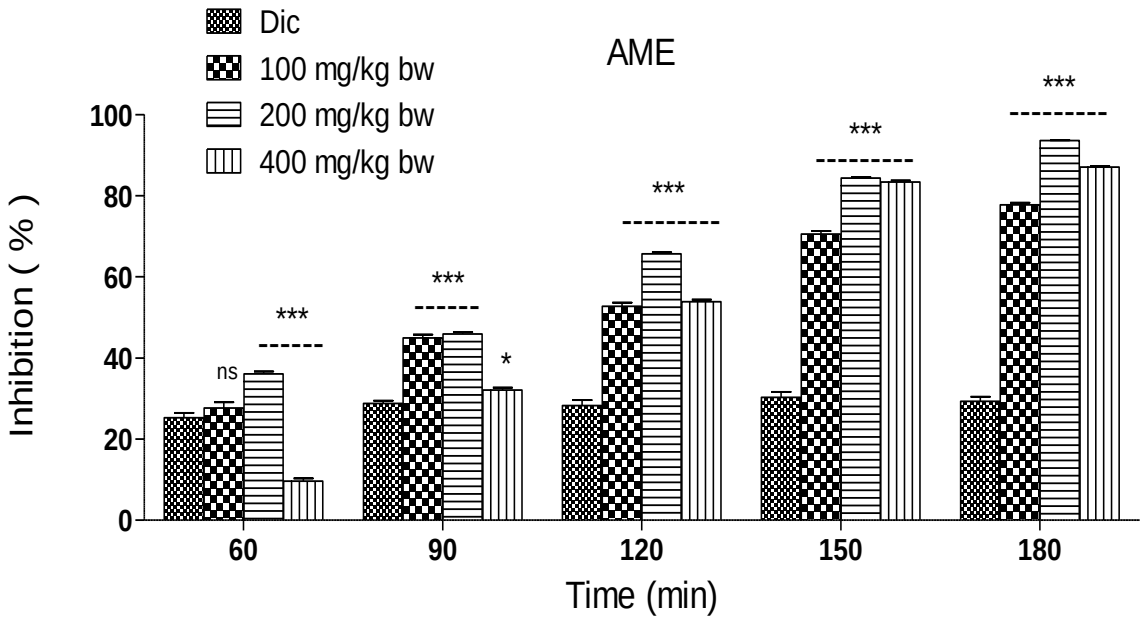


Figure 38: Anti-inflammatory effect of *Oxalis cernua* AME on paw oedema induced by carrageenan. AME: Areal part methanolic extract; Dic: Diclofenac. The values are expressed as mean ± SEM; n = 5; ns: non-significant and $p > 0.5$; * $p < 0.05$; *** $p < 0.001$.

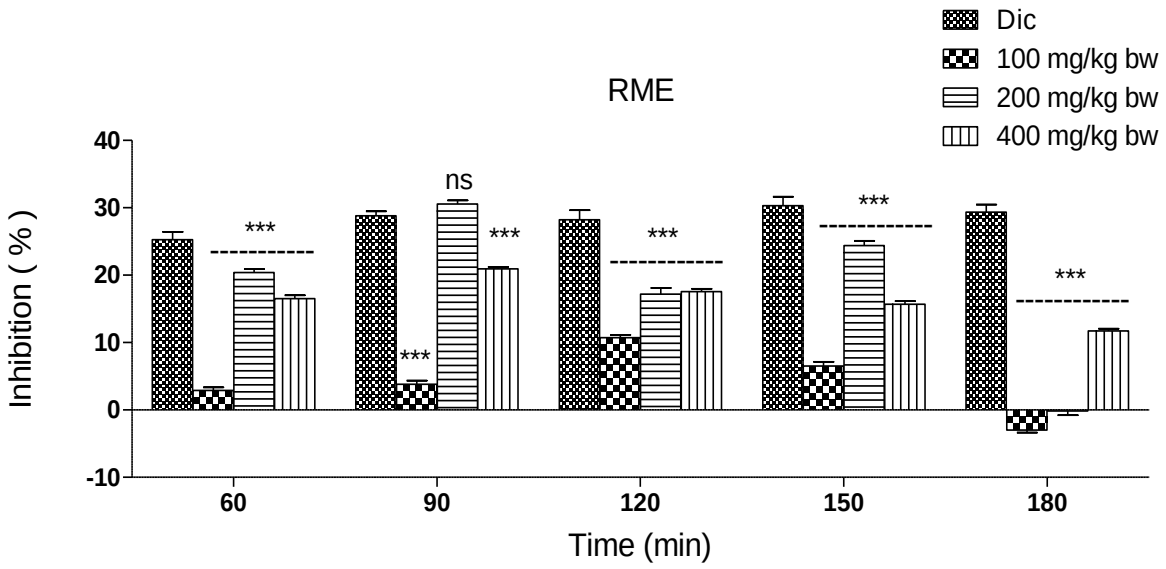


Figure 39: Anti-inflammatory effect of *Oxalis cernua* RME on paw oedema induced by carrageenan. RME: root methanolic extract; Dic: Diclofenac. The values are expressed as mean ± SEM; n = 5; ns: non-significant and p> 0.5; *** p < 0.001.

In this assay, inflammation was the initial response. It was characterized by the increased movement of plasma and innate immune system cells from the blood into the injured tissues. The sensation of heat caused by increased movement of blood through dilated vessels into the site of injection, also resulting in increased redness due to the additional number of erythrocytes in the area. Swelling is the results of increased passage of fluid from dilated and permeable blood vessels into the tissues, infiltration of cells into the damaged area, in prolonged inflammatory responses deposition of connective tissue. Pain is due to the direct effects of mediators, either from initial damage or that resulting from the inflammatory response and the stretching of sensory nerves due to oedema (Osman and Idrees, 2017; Djova et *al*., 2018). The loss of function refers to either simple loss of mobility in joints, due to the oedema and pain, or to the replacement of functional cells (Djova et *al*., 2019).

The reduction in the paw oedema, at both early and late phase, after pre-treatment with *Oxalis cernua* AME may be due to the suppression of increased vascular permeability caused by the secondary metabolites of the medicinal plant. Consequently, the anti-inflammatory

activity may be not only due to the inhibition of the synthesis and the release of pro-inflammatory mediators, but also to the presence of free radical scavengers, membrane stabilizing effects, or the regulation of gene expression (Kiranmayi, 2018; Mahdjar et *al*., 2020).

The bio-compounds of *Oxalis cernua* AME may interfere with the action or release of serotonin or histamine, as they are the predominant mediators in the initial phase. Also, this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract's compounds and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as diclofenac. However, *Oxalis cernua* AME was found more effective in reducing the Carrageenan induced paw oedema than the standard drug. Other mechanisms of action of the observed anti-inflammatory effect of the extract may be due to the presence of antioxidant compounds. It has been reported that ROS generated during inflammation can activate phospholipase A2, which releases more arachidonic acid from the phospholipid membrane, which is subsequently transformed into prostaglandins and leukotrienes (Djova et *al*., 2019).

According to previous studies, flavonoids which are a polyphenols' subclass possessing anti-inflammatory effects, both in *in vitro* and in *in vivo*. The anti-inflammatory activity of *Oxalis cernua* AME may also be proved due to the presence of flavonoids in a significant amount (43.69±0.61 mg Quercetin Equivalents/g of dry extract). Furthermore, the anti-inflammatory effect may be also due to the presence of saponins or tannic acid. Various studies have proved their effectiveness in animal models of inflammation. They were responsible for reducing the release of inflammatory mediators. Moreover, many saponins tested have displayed significant anti-nociceptive, anti-inflammatory and antipyretic activities possibly due to their non-glycosidic moiety, the sapogenin (Rungqu et *al*., 2016; Henneh et *al*., 2018; Saleem et *al*., 2020).

On the other hand, polyphenols are reported to exhibit antioxidant activity and have the capacity to alleviate oxidative stress which induce tissue damage. The anti-inflammatory

activity of *Oxalis cernua* AME may also be proved due to the presence of polyphenols in a significant amount (79.00±0.10 mg gallic acid Equivalents/g of dry extract). Numerous reports established the protective effects of inflammatory related diseases through inhibiting or quenching free radical reactions and also through delaying or inhibiting cellular damage by donating hydrogen atoms. Some antioxidants even have a chelating effect on free radical production (Djova et *al*., 2019).

Oxalis cernua RME exhibited a week reduction of the oedema, which may be explained by their moderate content on polyphenols and flavonoids, where the values were (26.20±0.45 mg gallic acid Equivalents/g of dry extract) and (9.95±0.10 mg Quercetin Equivalents/g of dry extract) respectively. Furthermore, the pro-inflammatory effect of *Oxalis cernua* RME was noted with the concentration (100, and 200 mg/kg bw) at 180 min, may be due to the uncontrolled production of pro-inflammatory mediators, which can maintain or amplify the inflammatory response leading to chronic inflammation. Despite the fact that pro-inflammatory mediators are the key regulators of physiological process (Nagendra et *al*., 2019).

9.2.1 Histological examination

The abnormal histology of control group section revealed capsule destruction, a severe inflammation, influx of inflammatory cells poly-nuclear and lymphocytes. However, Dic group reversed moderately the intention of this acute inflammation. *Oxalis cernua* AME diminished the inflammatory effect of carrageenan in a dose dependent manner, remarkable inhibition of all histological findings of inflammation was observed. Tissues treated with (100 mg/kg bw) showed congestion, and persistent of oedema without inflammation. While rats treated with (200 mg/kg bw) displayed a regression of the oedema with focal inflammation (Figure 40). Animals received (400 mg/kg bw) exhibited highly reduced inflammatory response, and no oedema was presented. Histological sections of *Oxalis cernua* RME (100, 200 and 400 mg/kg bw) presented persistence of oedema with focal inflammatory infiltrate. The analysis of

histological sections of rat paw tissue in Carrageenan-induced paw oedema endorses the previous results.

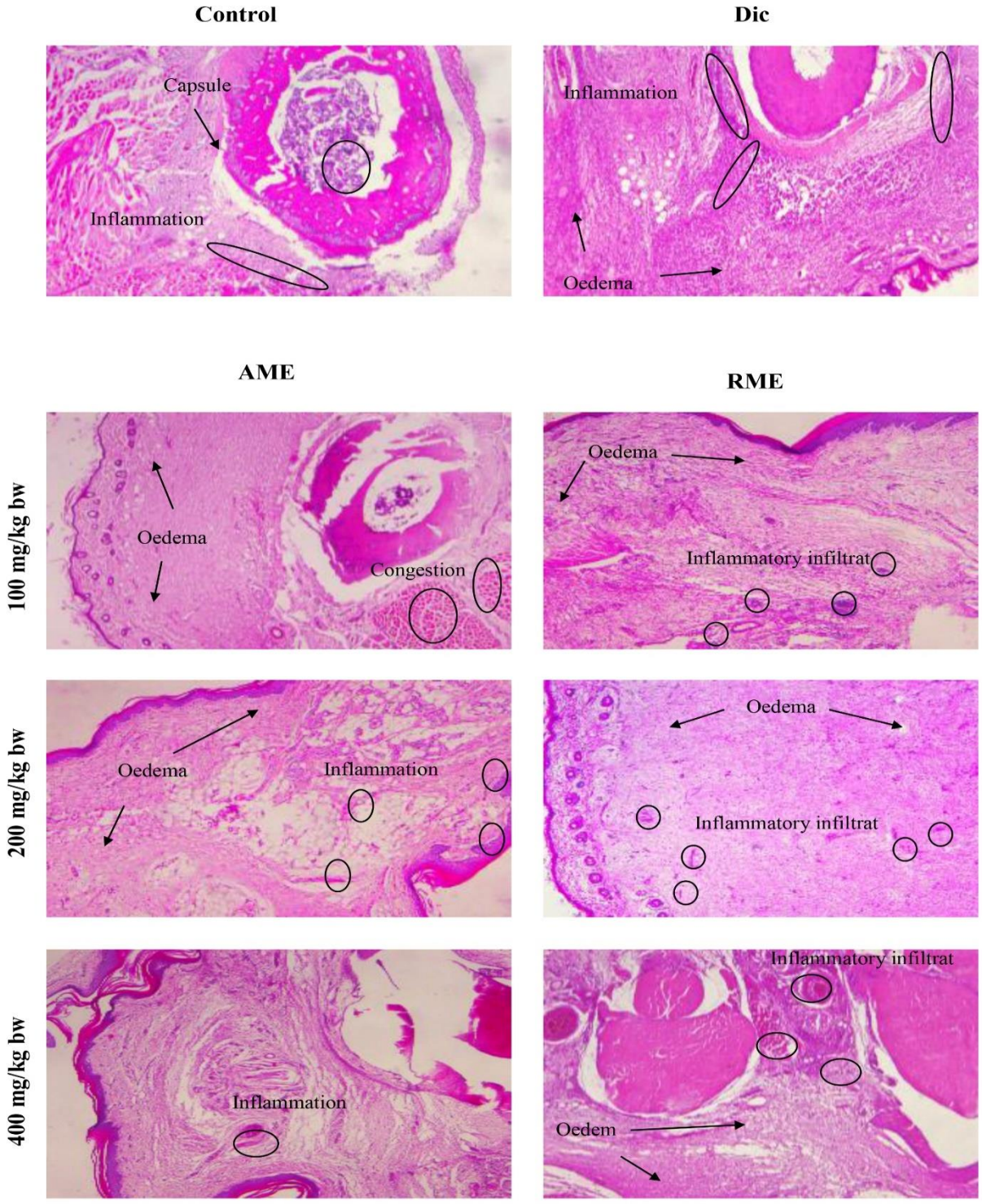


Figure 40: Photomicrographs of paws sections stained with haematoxylin and eosin of control group, diclofenac group (Dic), and treated groups with both extracts of *Oxalis cernua* in carrageenan induced acute-inflammation. X40.

9.3. Egg albumin-induced rat paw oedema

Measuring the ability of medicinal plants to decrease local oedema induced by egg albumin (an irritant or phlogistic agent) is one of the well-known *in vivo* models used to evaluate the anti-inflammatory potentials of extracts against acute inflammation. Subcutaneous injection of the phlogistic agent into the rat paw produces paw oedema peaks within 30 minutes and maintains the same degree of oedema for about three hours before progressively declining with time. The early phase of oedema ranges from 0 to 40 min after the administration of irritant. It is due to the release of various inflammatory mediators such as histamine and serotonin, vasoactive amine/peptide and eicosanoids. Whereas, the later phase occurred from 60 to 120 min after the administration of egg albumin (Ghante et *al*., 2014; Ogbu et *al*., 2019; Akinloye et *al*., 2020; Hiralal).

In the present assay, subcutaneous injection of the phlogistic agent to the sub planter of the treated animals induced nociceptive response symptoms such as itching, touching and licking in the treated paw since the injection. Thirty minutes later, less mobility, redness, warmth, and swelling which are the signs of acute inflammation were noted (Figure 41 and 42). In this study, oedema was observed with treatments using aspirin (standard drug), *Oxalis cernua* AME and RME, within the more pronounced phase of inflammation. The difference in the thickness of rats' paw before and after the application of the irritant was used to evaluate the anti-oedema effect of the investigated extracts.

Figure 45 and 46 demonstrate the anti-inflammatory effect of *Oxalis cernua* methanolic extract over a period of 2 h, the comparison was made with respect to control group. Aspirin group as a drug standard showed a reduction in paw oedema increase percentage induced by egg-albumin (14.47%), where the differences were statistically significant through all time point ($p< 0.05$, and $p <0.001$). However, the dose (400 mg/kg bw) of the *Oxalis cernua* AME exhibited a significant reduction in the percentage of paw oedema thickness at 120 min

(32.37%). Concerning *Oxalis cernua* RME, at point time 60 min the three doses revealed a significant reduction in paw edema ($p<0.001$), However, only the effect of concentration (200, and 400 mg/kg bw) continue till 120 min.

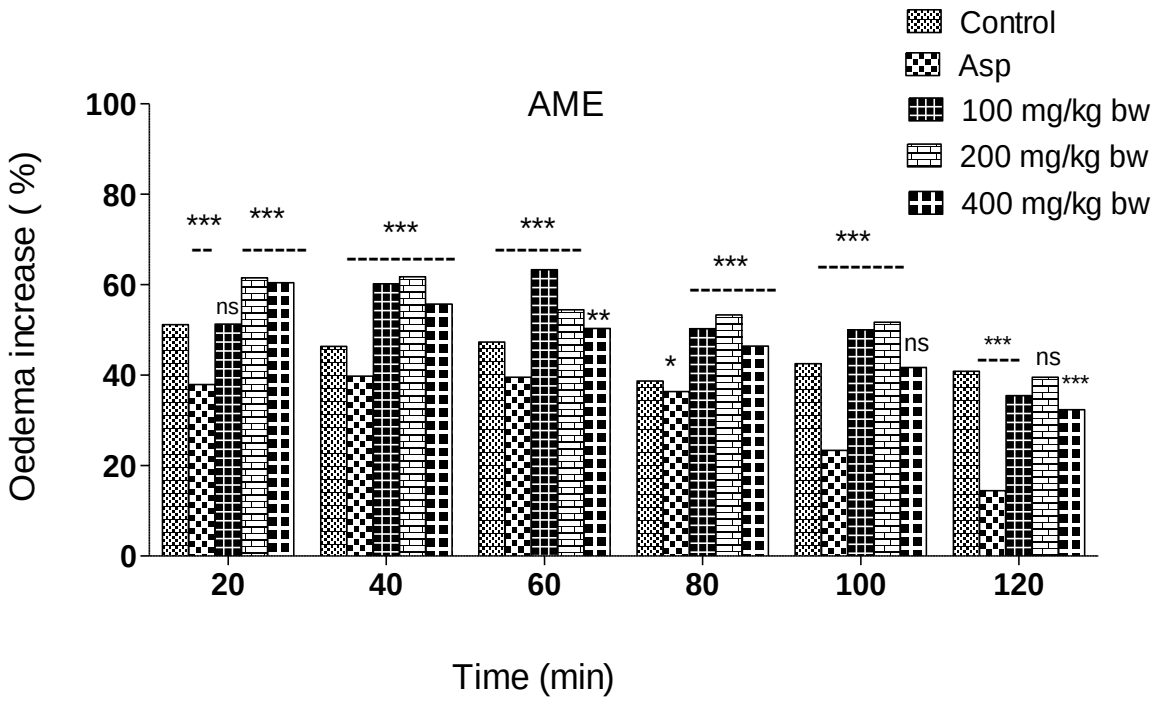


Figure 41: Anti-inflammatory effect of *Oxalis cernua* AME on paw oedema increase induced by egg-albumin. AME: Areal part methanolic extract; Dic: Diclofenac. The values are expressed as mean ± SEM; n = 5; ns: non-significant ($p>0.05$), * $p < 0.05$, ** $p<0.01$, *** $p <0.001$.

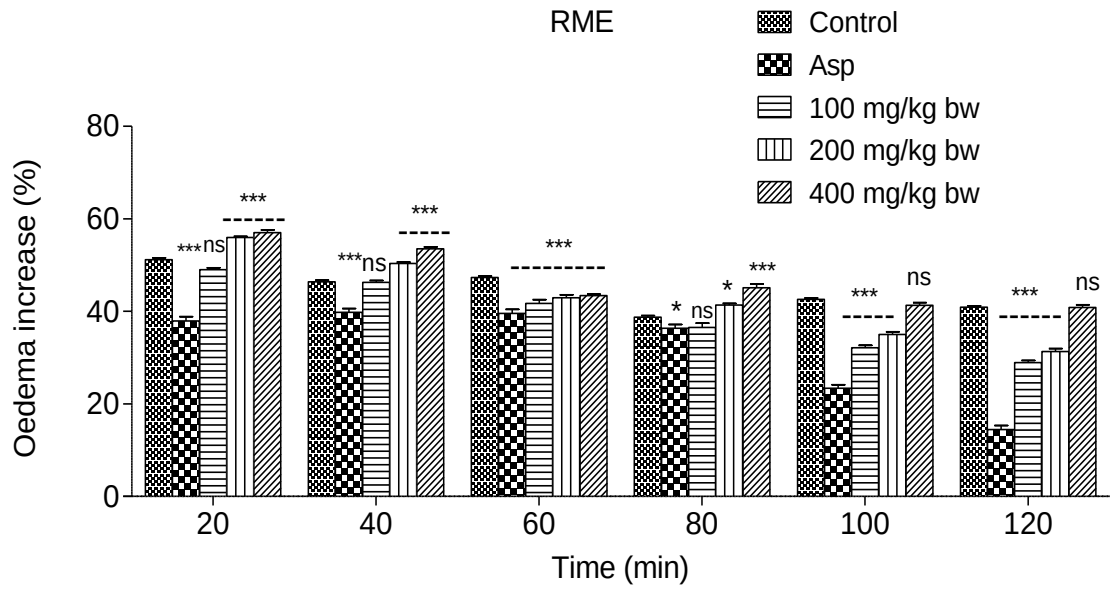


Figure 42: Anti-inflammatory effect of *Oxalis cernua* RME on paw oedema increase induced by egg-albumin. RME: root methanolic extract; Dic: Diclofenac. The values are expressed as mean ± SEM; n = 5; ns: non-significant ($p>0.05$), * $p<0.05$, *** $p<0.001$.

Figure 43 illustrated the anti-inflammatory effect of *Oxalis cernua* methanolic extract, in the first phase of inflammation post administration, which did not act dose dependently. The comparison was made with respect to aspirin group (20 mg/kg bw) as a drug standard, which had revealed a potent inhibition at 120 min (64.59%). *Oxalis cernua* AME (100 mg/kg bw) at 100 min, and the three doses at 120 min all indicated a weak anti-inflammatory effect. The inhibition was statistically significant ($p<0.001$), while the values were (2.05%), and (13.28%, 3.18%, 20.80%) respectively. *Oxalis cernua* AME at the others time point with all doses tested showed a negative inhibition percentage. Regarding, *Oxalis cernua* RME, dose (100 mg/kg bw) revealed a positive inhibition percentage at all-time point (4.18%, 0.22%, 11.84%, 5.66%, 24.44%, 29.20%) respectively. Moreover, the doses (200 and 400 mg/kg bw) presented a positive inhibition percentage only at point time 60, 100, and 120 min (Figure 44), their values were respectively (-9.44%, -8.49%, 9.18%, -6.76%, 17.71%, and 23.36%), and (-11.42%, -15.33%, 8.22%, -16.43%, 2.89%, and 0.07%). However, their anti-inflammatory effect remains significantly different and less than that of the standard.

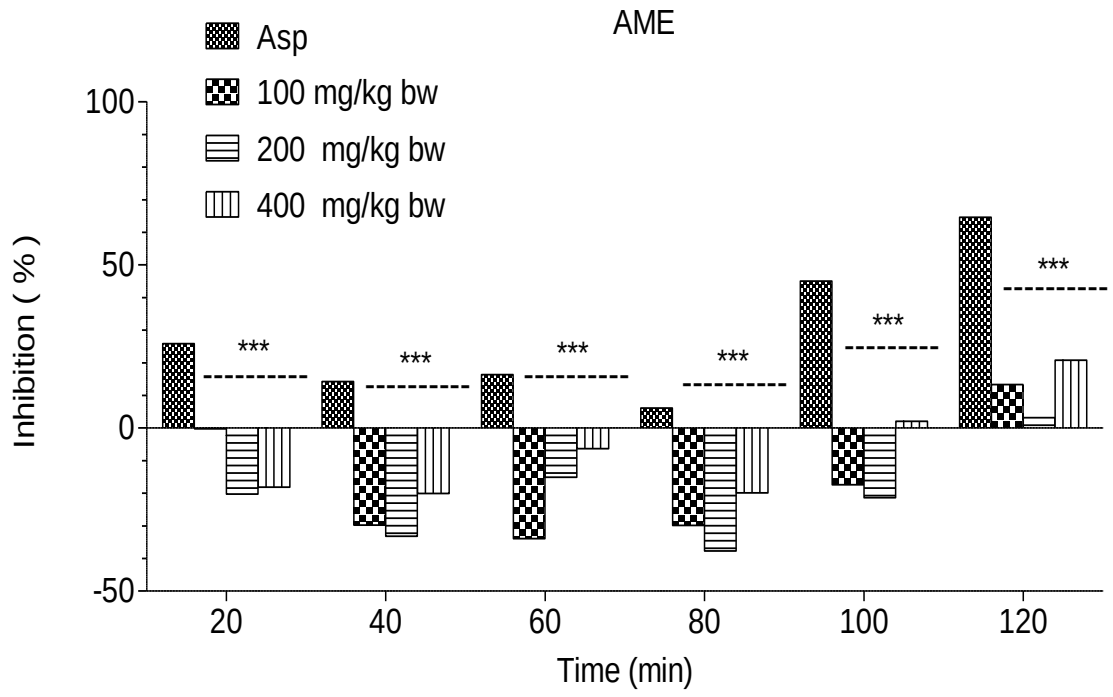


Figure 43: Anti-inflammatory effect of *Oxalis cernua* AME on paw oedema induced by egg-albumin. AME: Areal part methanolic extract; Dic: Diclofenac. The values are expressed as mean ± SEM; n = 5; *** $p < 0.001$.

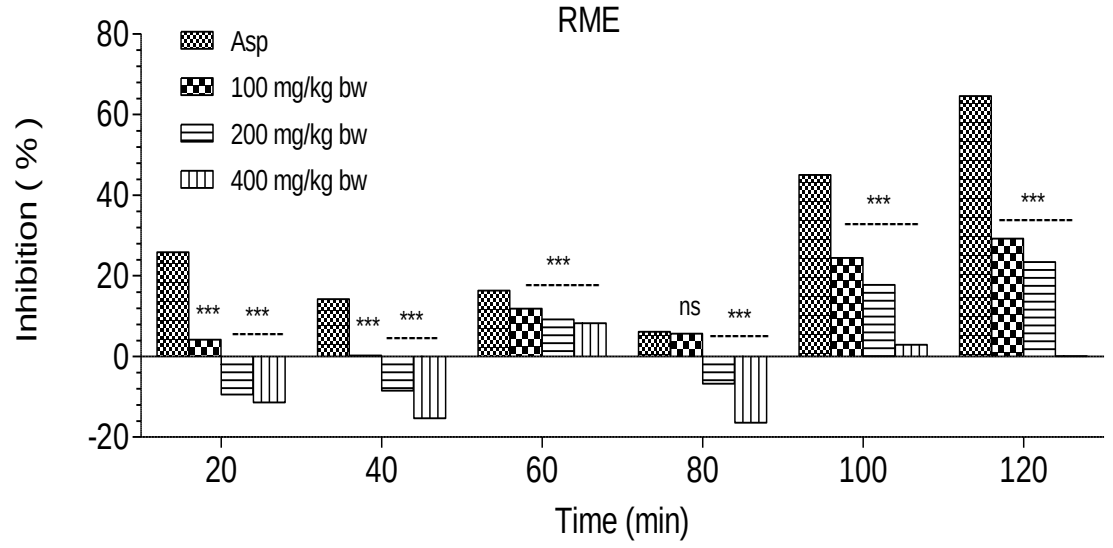


Figure 44: Anti-inflammatory effect of *Oxalis cernua* RME on paw oedema induced by egg-albumin. RME: root methanolic extract; Dic: Diclofenac. The values are expressed as mean ± SEM; n = 5; ns: non-significant and p> 0.5; *** $p < 0.001$.

The anti-inflammatory assay of *Oxalis cernua* AME, and RME displayed inhibition of inflammation. Meanwhile, at the site of injection, inflammation's mediators cause oedema by increasing vasodilatation and vascular permeability, the extract may reduce vascular permeability and fluid exudation, probably by preventing the contraction of endothelial cells and henceforth, reducing oedema through blocking the release of histamine and serotonin. Several studies reported that anti–inflammatory effect of medicinal plants extracts during the early phase of oedema could be attributed to their phyto-constituents (Aham et *al*., 2019; Babakura et *al*., 2019).

The second phase of the inflammation induced by egg albumin is mediated by prostaglandins, kinins, cyclooxygenases, protease, bradykinin and lysosome. The suppression of paw oedema suggests that the anti-inflammatory activity of the extract may also be due to the suppression of kinins and prostaglandins formation induced by egg albumin within this period (Tembeni et *al*., 2019).

Additionally, it has been reported that non-steroidal anti-inflammatory drugs do not effectively inhibit the first phase of inflammation. This explains the reason why the weak inhibition percentage observed for aspirin within the first hour was in single digits, whose mechanism of action is the inhibition of the cyclooxygenase enzyme. The reduced activity in the second phase for the extract also suggests that the extract may have little or no direct effect on cyclooxygenases site reaction (Tembeni et *al*., 2019).

Secondary metabolites such as phenols, alkaloids, flavonoids, tannins, steroids, glycosides, terpenoids and saponins in medicinal plants extracts have been reported to might have anti-inflammatory properties in paw oedema. Studies have shown that these phyto-constituents are present in several plants with anti-inflammatory properties like *Curcumma longa* (turmeric), *Zingiber officinale* (ginger) and *Rosmarinus offininalis* (rosemary) (Erhirhie et *al*., 2019; Ogbu et *al*., 2019).

In general, therapeutic actions of bioactive compounds are linked to their antioxidant effects. Bioactive compounds act as reducing agents, hydrogen donors and singlet oxygen scavengers, and hence, reduce inflammatory signals and key mediators of inflammation which activate signal transduction cascades. Activation of latter leads to changes in transcription factors that regulate immediate cellular stress responses and inflammation. Previously, they were found to exert free radical scavenging activities against radicals of nitric oxide and hydroxyl, and ferric-reducing antioxidant activities, suggesting a possible anti-inflammatory effect (Ogbu et *al*., 2019).

9.3.1. Histological examination

Histological section of control rats expressed intact morphology of synovium and synovial lining, no inflammation or influx of inflammatory cells, while a significant oedema was observed (figure 45). Microscopic observations of aspirin group revealed no notable changes in the epidermis or regression of the oedema with respect to control group. Animals treated with *Oxalis cernua* AME (100, 200 and 400 mg/ kg bw) presented an increase of oedema and an intensification of the inflammation dependent of the concentration: a remarkable oedema and inflammation, then a more significant oedema, and an acute inflammation respectively. Animals treated with *Oxalis cernua* RME (100 and 200 mg/ kg bw) revealed the persistence of oedema. Moreover (400 mg/kg bw) displayed a significant oedema with inflammation, microscopic findings were similar to those of the control.

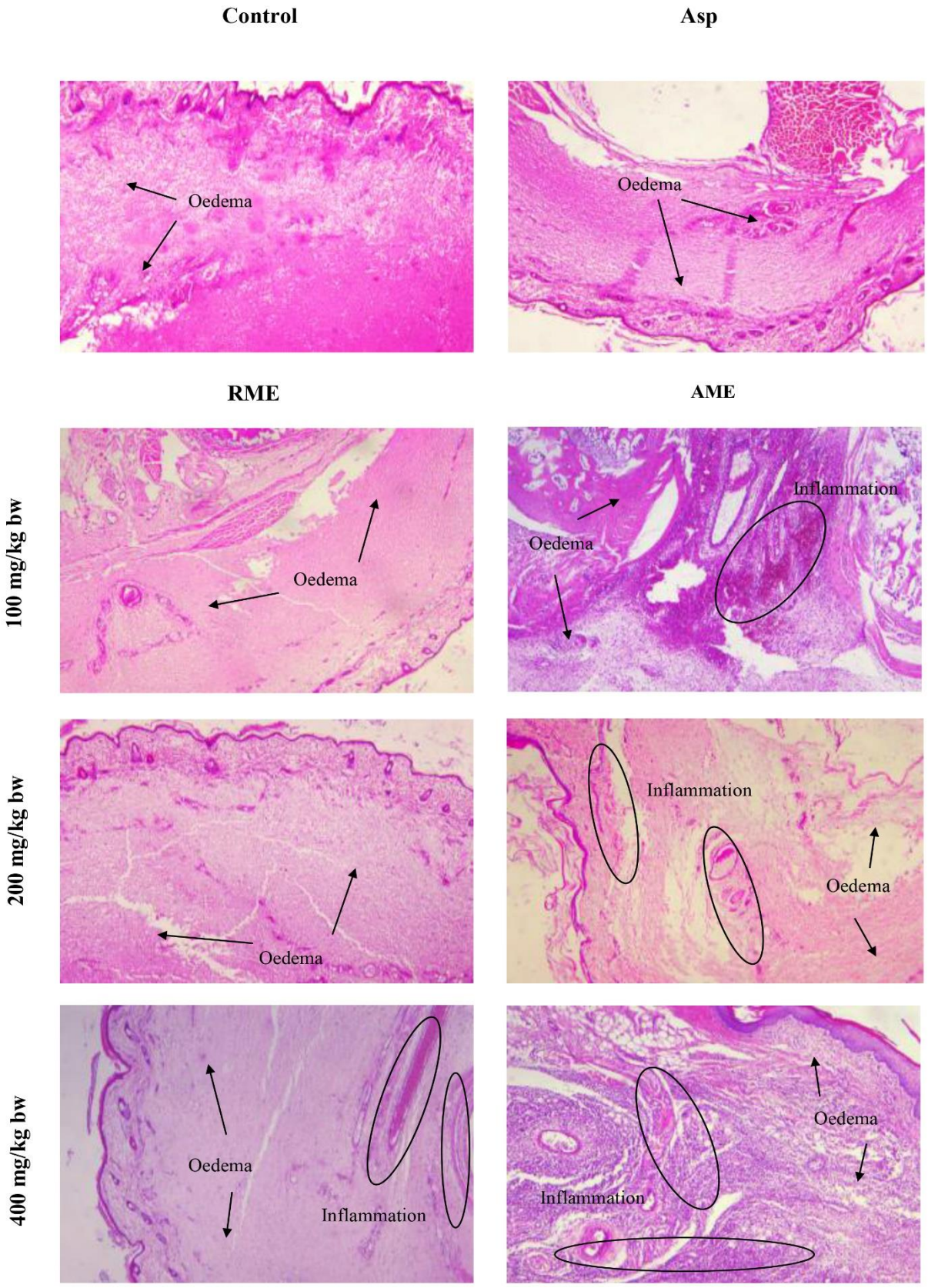


Figure 45: Photomicrographs of paws sections stained with hematoxylin and eosin of control group, aspirin group (Asp), treated groups with both extracts of *Oxalis cernua* in egg-albumin induced acute-inflammation. X40.

10. Conclusion and perspectives

Since ancient time, a wide range of world population depends on traditional medicine, which include plants and plants products, for their health care. Currently, there is a renewed interest in natural herbal plants as external source of antioxidants and anti-inflammatory compounds. The latter reduce incidence and severity of pathologies and prevent oxidative damage. Consequently, the therapeutic properties and multi-targeted efficacy of polyphenols and flavonoids, as secondary metabolites, get more scientific concern. However, unintentional poisoning by medicinal plants could occur not only due to the lack of quality control during collection and preparation but also due to the long history of treatment use, the false feeling of safety and the assumption of minimal side effects.

This study displays that *Oxalis cernua* methanolic extracts yield was relative to the weight of plant powder. The extracts were a rich source of polyphenolic and flavonoid compounds, where the most important amounts were found in CHE, and AME respectively. The extracts' content of polyphenols and flavonoids had potential anti-oxidant activity in *in vivo* as in *in vitro*, they present a very strong ability to scavenge hydroxyl radicals; to prevent haemolysis, lipids peroxidation; and also to inhibit protein denaturation.

Furthermore, *Oxalis cernua* methanolic extracts did not bring any changes in: behaviour, body weight, biochemical parameters, anatomical and histological signs of toxicity, and also did not present significant toxicity when administered in a single highest dose of both 2000 and 5000 mg/kg bw, being considered safe by the OECD which could give insight into its safety in humans' folk medicine. Additionally, the results obtained from the subacute toxicity study revealed some changes in biochemical and histological parameters suggesting that precautions should be taken when it is given over longer periods. However, it seems that *Oxalis cernua* may have a high margin of safety as it did not induce any toxicological effects.

Moreover, the findings of the present investigation demonstrated that *Oxalis cernua* methanolic extracts modulated the severity of CCl_4 induced liver damage. Biochemical parameters were significantly restored to near-normal levels. The higher reduction reaching the same level to control animals was also observed. The anatomical and histological examination endorsed the results of the biochemical study. The antioxidants properties were not dose dependant, therefore, the protective potential against hepatotoxins and nephrotoxins can be other probable reasons for the healing effect of *oxalis cernua* areal part and roots methanolic *extracts*.

In *in vivo* anti-inflammatory activity, *per os* administration of *oxalis cernua* methanolic extracts revealed a potent anti-inflammatory effect reducing ear oedema induced by xylene. However, in carrageenan model, only *Oxalis cernua* AME exhibited strong anti-inflammatory effect, while *Oxalis cernua* RME revealed the inverse effect, and became a pro-inflammatory agent. Besides, both extracts displayed a pro-inflammatory effect in egg albumin model.

Based on this scientific appraisal taken together the suggested *in vitro* and *in vivo* data, *Oxalis cernua* traditional use was reinforced showing promising health effects. Moreover it has innumerable biological properties, including those evaluated in this thesis. Therefore, its phyto-constituents could be of a great contribution for pharmaceutical industry.

Henceforth, these preliminary findings need to be followed by further in-depth investigations. Subsequently, many perspectives can therefore be envisaged like: isolate, purify and identify the secondary metabolites possessing pharmacological activities. Then study their mechanisms of action on inflammatory models, as well as expanding the research to compromise other physio-pathological conditions at cellular and molecular level, which could be useful before considering further therapeutic studies. The plant should be exploited as natural

antioxidants in real food systems. Hence, the preservation of *Oxalis cernua* is a fundamental component for health, and can provide quality remedies and reasonable cost, if cultivated.

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الملخص

تهدف هذه الدراسة إلى تقييم السمية، النشاطية المضادة الأكسدة والخواص المضادة للإلتهابات للمستخلص الميثانولي لكل من الجزء الهوائي وجذور النبتة *Oxalis cernua*. تم استخلاص عديدات الفينول متبوعا بفصل اولي لإعطاء المستخلص الخام للجزء الهوائي AME و مستخلص الكلوروفورم CHE مستخلص الايثيل اسيتات EAE و المستخلص المائي AqE و بالإضافة الى المستخلص الخام للجزء الترابي RME. لسيقان وأوراق هذه النبتة على قيمة علاجية عالية، ولها أيضًا وظائف مضادة للإلتهابات وإزالة السمية، في حين يتم استخدام الجذور بشكل خاص بسبب خصائصها المدرة للبول. أظهر القياس الكمي لعديدات الفينول والفلافونويدات أن المستخلص الميثانولي للجزء الهوائي (AME) يحتوي على أعلى كمية منهما. تم تقييم نشاطها المضاد للأسدة مخبريا باستخدام عدة اختبارات مختلفة :مثل DPPH، ABTS، جذور الهيدروكسيل، قدرة الإرجاع، واستخلاب أيونات الحديد، وكذلك فحوصات تبييض البيتاكاروتين؛ والإختبار المضاد للإنحلال الدموي. علاوة على ذلك، تم تقييم النشاطية المضادة للإلتهابات في المختبر باستخدام اختبار تثبيط تحلل البروتينBSA وألبومين البيض. بينت نتائج هذه الدراسة أن المستخلصات التالية: EAE، AME، CHE، AqE ؛ CHE، و AME أظهرت أعلى نشاطية مضادة للأكسدة على التوالي. بالإضافة إلى ذلك، كان كل من مستخلصي AME وRME أكثر تثبيطا لتحلل البروتين. في السمية الحادة، لم تظهر المستخلصات الميثانولية لـــ *Oxalis cernua* عند استعمال الجرعتين (2000 و5000 ملغم/كغم من وزن الجسم) مرة واحدة أي علامات سمية بيوكيميائية، ونسيجية طوال فترة 14 يومًا. عند استعمال الجرعات (100، 200، 400 ملغم/كغم من وزن الجسم) أثناء دراسة السمية الحادة خلال 21 يومًا على التوالي، لوحظ أن النشاطية المضادة للأكسدة التي تم تقييمها باستخدام اختبارDPPH وقدرة الإرجاع لم تعمل على تحسين حالة الأكسدة والإرجاع للبلازما. كما لوحظت بعض التغييرات للمؤشرات البيوكيميائية والنسيجية، إلا أنها كانت ضمن النطاق الفيزيولوجي. بينت نتائج سمية الكبد والتسمم الكلوي الناجم عن CCl_4، النشاطية المضادة للأكسدة لـــ *Oxalis cernua* عملت على إرجاع المعايير البيوكيميائية بشكل كبير إلى مستوياتها الطبيعية، كما أيد الفحص النسيجي نتائج الدراسة البيوكيميائية. بينت النشاطية المضادة للإلتهابات في الجسم الحي، أن مستخلص RME *Oxalis cernua* بجرعة (200 ملغم/كغم من وزن الجسم)، له تأثير قوي كمضاد للإلتهابات يقلل من وذمة الأذن التي يسببها Xylen. في نموذج carageennan، أظهر مستخلص AME *Oxalis ceernua* بجرعة (200 ملغم/كغم من وزن الجسم) تأثير قوي كمضاد للإلتهابات (80٪). إلى جانب ذلك، أظهر كلى المستخلصين تأثيرًا مؤيدًا للإلتهابات في نموذج ألبومين البيض. النتائج المتحصل عليها تعزز استخدام النبتة في الطب التقليدي.

الكلمات المفتاحية: *Oxalis cernua*، حمايضة، عديدات اليفينول، الفلافونويدات، السمية، الإجهاد التأكسدي، النشاطية المضادة للالتهابات.

Abstract

The aim of this study is to evaluate the toxicity, antioxidant and anti-inflammatory properties of *Oxalis cernua* areal part and root methanolic extracts. In folk therapy, *Oxalis cornea*'s stems and leaves have high medicinal values, and also have the functions of anti-inflammation and removing toxicity, whereas, the roots are used particularly due to their diuretic properties. Polyphenols extraction was followed by fractionation to give the crude extract of the aerial part AME, chloroform extract CHE, ethyl acetate extract EAE and aqueous extract AqE, in addition to the crude extract of the roots RME. Quantification of polyphenols and flavonoids revealed that AME contained the highest amounts. The evaluation of *in vitro* antioxidant activity was performed using several different methods: DPPH, ABTS, hydroxyl radicals; reducing power, ferrous ion chelating, and also β- carotene bleaching assays; and anti-haemolytic test. Furthermore, *in vitro* anti-inflammatory activity was assessed using BSA and egg albumin anti-denaturation assays. The findings of this study exhibited that EAE, AME, CHE, CHE, AqE; and CHE, AME noted the highest antioxidant activities respectively. Additionally, in *in vitro* ant-inflammatory both of AME and RME were the most potent anti-protein denaturation extracts. In acute toxicity, *Oxalis cernua* methanolic extracts were administered in a single highest dose of (2000 and 5000 mg/kg bw) did not cause any behavioural, biochemical, and histological signs of toxicity throughout a period of 14 days. In subacute toxicity study doses (100, 200, 400 mg/kg bw) were administered *per os* during 21 days successively. Antioxidant activity assessed with DPPH and reducing power tests did not improve plasma redox status. Biochemical and histological parameters showed some changes which remain inside physiological range. In hepatotoxicity and nephrotoxicity induced by CCl_4, *Oxalis cernua* antioxidant and biochemical parameters were significantly restored to near-normal levels. The histological examination endorsed the results of the biochemical study. In *in vivo* anti-inflammatory activity, *oxalis cernua* RME at dose (200 mg/kg bw), administered *per os*, revealed a potent anti-inflammatory effect reducing ear oedema induced wby xylene. In carrageenan model, *Oxalis cernua* AME at dose (200 mg/kg bw) displayed potential anti-inflammatory effect (80%). Besides, both extracts exhibited a pro-inflammatory effect in egg albumin model. The results obtained endorse the use of the plant in traditional medicine

Key words: *Oxalis cernua*, Hamaydha, polyphenols, flavonoids, toxicity, oxidative stress, anti-inflammatory activity.

Résumé

L'objectif de cette étude est d'évaluer la toxicité, les propriétés antioxydantes et anti-inflammatoires des extraits methanoliques de la partie aérienne et des racines de la plante *Oxalis cernua*. En thérapie traditionnelle, les tiges et les feuilles d'*Oxalis cernua* ont des valeurs médicinales cruciales et ont également des fonctions anti-inflammatoires en éliminant la toxicité, tandis que les racines sont utilisées en particulier en raison de leurs propriétés diurétiques. L'extraction des polyphénols a été suivi par fractionnement pour donner l'extrait brut de la partie aérienne AME, l'extrait chloroformique CHE, l'extrait d'acétate d'éthyle EAE et l'extrait aqueux AqE, en plus de l'extrait brut des racines RME. La quantification des polyphénols et des flavonoïdes a révélé que l'AME en contenait la plus grande quantité. L'évaluation de l'activité antioxydante, *in vitro*, a été réalisée en utilisant plusieurs méthodes différentes : DPPH, ABTS, radicaux hydroxyles ; pouvoir réducteur, chélation des ions ferreux, et également les tests de blanchissement de β-carotène ; et de l'activité anti-hémolytique. De plus, l'activité anti-inflammatoire *in vitro* a été évaluée en utilisant des tests de l'anti-dénaturation protéique de la BSA et de l'albumine d'œuf. Les résultats de cette étude ont montré que EAE, AME, CHE, CHE, AqE; et CHE, AME ont eu respectivement l'activité antioxydante la plus élevée. De plus, l'activité anti-inflammatoires, *in vitro*, des extraits AME et de RME était puissante. Concernant la toxicité aiguë, les extraits méthanoliques d'*Oxalis cernua*, lorsqu'ils sont administrés en une seule dose maximale de (2000 et 5000 mg/kg pc), n'ont présenté aucun signe de toxicité comportementale, biochimique et histologique durant une période de 14 jours. Au cours de la toxicité subaiguë les doses (100, 200, 400 mg/kg pc) ont été administrées *per os* pendant 21 jours successifs. L'activité antioxydante évaluée par le test DPPH et le pouvoir réducteur n'a pas amélioré le statut redox plasmatique. Les paramètres biochimiques et histologiques ont noté quelques changements qui restent dans l'intervalle physiologique. Dans l'hépatotoxicité et la néphrotoxicité induites par CCl_4, les paramètres antioxydants et biochimiques *d'Oxalis cernua* ont été significativement restaurés à des niveaux presque normaux. L'examen anatomique et histologique a confirmé les résultats de l'étude biochimique. Dans l'activité anti-inflammatoire *in vivo*, *Oxalis cernua* RME à la dose (200 mg/kg pc) administré *per os*, a révélé un puissant effet anti-inflammatoire réduisant l'œdème de l'oreille induit par le xylène. Dans le modèle de carageennan, *Oxalis cernua* AME à la dose (200 mg/kg pc) a présenté un effet anti-inflammatoire potentiel (80%). En outre, les deux extraits ont montré un effet pro-inflammatoire dans le modèle d'albumine d'œuf. Les resultats obtenus support l'utilisation de cette plante en médecine traditionnelle.

Mots clés : *Oxalis cernua*, Hamaydha, polyphénols, flavonoïdes, toxicité, stress oxydatif, activité anti-inflammatoire.