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Gastropotective and antidiabetic effects of *Phlomis crinita* and *Phlomis herba venti* extracts

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JURY

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الملخص

تهدف هذه الدراسة إلى تقييم المحتوي الفينولي و النشاطية المضادة للأكسدة والمضادة لداء السكري وحماية المخاطية المعدية من التقرح للمستخلصات الميثانولية (PC ME وPVME) والمائية (PC AQE, PV AQE) للجزء الهوائي لنبتتي *P. crinita* و *P. herba venti* تنتمي هذه النبتتان الي عائلة النعناعيات. أظهرت النتائج أن المستخلص الميثانولي لنبتة *P. herba venti*يحتوي اكبر كمية من عديدات الفينول والفلافونويدات في حين ان اعلى نسبة للدباغ سجلت في المستخلص الميثانولي ل *P.crinita* . تم تحديد الخواص المضادة للأكسدة لمستخلصات باستخدام إزالة جذر DPPH، ABTS، وتبييض البيتا كاروتين، القدرة الإرجاعية والنشاط المخلبي للمعادن. اظهرت مستخلصات *P. herba venti* قدرة كبيرة على استخلاب جذري ABTS و DPPH وكذا القدرة الارجاعية للايونات في حين ان مستخلصات *P. crinita* اظهرت قوة معتبرة في تثبيط تبييض البيتا كاروتين والنشاط المخلبي للمعادن. بعد تحريض السكري باستعمال الالوكسان، شوهد إرتفاع في تركيز السكر في الدم لدى الحيوانات غير المعالجة مقارنة مع الحيوانات الطبيعية، كما لوحظ انخفاض كبير في وزن الجسم للحيوانات. أدت معالجة الحيوانات بمستخلصي PC ME و PV ME بجرعات 250 و 500 ملغ/كغ إلى زيادة في نشاط الكاتلاز، كميةGSH وانخفاض مستوى MDA في أنسجة الكبد والكلى ومستويات الكرياتنين ،حمض اليوريك و الكوليسترول والدهون الثلاثية في البلازما. من جهة اخرى اظهر مستخلص PV ME قدرة كبيرة في تثبيط انزيم الفا غليكوزيداز مقارنة بالشاهد الموجب. أدت معاملة الحيوانات باستخدام 250 و 500 مغ/كغ من مختلف المستخلصات الى حماية مخاطية المعدة (68 إلى 95٪) ضد القرحة التي يسببها الإيثانول والتي كانت أعلى مقارنة بالرانيتيدين كمرجع ايجابي .يمكن ان ترتبط هذه الحماية بالارتفاع في مستويات البروتينات الكلية، الغليثاثيون و/أو تثبيط أكسدة الدهون.

كلمات مفتاحية: مضادات للأكسدة، مضادات السكري, الفا غليكوزيداز، *P. herba venti* ، *P.crinita* نشاطية ضد قرحة المعدة المحدثة بواسطة الإيثانول.

Abstract

The present study was aimed to evaluate phenolic content, antioxidant and antidiabetic activity, and gastroprotective effect of methanolic (PC ME, PV ME) and aqueous extracts (PC AQE, PV AQE) of aerial parts of *P. crinita and P. herba venti* which are plants species of the Lamiaceae family. This study, showed that methanolic extract of *P. herba venti* contained high polyphenol and flavonoid contents, while the high level of tannins was registered in *P. crinita* methanolic extract. The antioxidant properties of plants extracts were determined using DPPH, ABTS, β-carotene bleaching, reducing power and metal chelating activity assays. *P. herba venti* extracts showed strong DPPH, ABTS radical scavenging activity and reducing power; while *P. crinita* extracts exhibited a good activity in β-carotene bleaching and metal chelating activity assays. After Induction of diabetes, the concentration of blood glucose increased in the alloxan group in comparison with normal ones, but the body weight decreased in the alloxan animals. The administration of PC ME and PV ME at doses of 250 and 500 mg/kg increased catalase activity, GSH level and decreased lipid peroxidation in the tissues of liver and kidneys, serum creatinin, uric acid, total cholesterol and triglycerides levels. *In vitro* inhibitory effect on diabetic enzyme showed that the methanolic extract of *P. herba venti* was inhibited (IC_{50} = 0.16±0.01 mg/mL) more than positive control on alpha-glycosidase. Treatment of rats with 250 and 500 mg/kg of PC ME ,PC AQE, PVME and PV AQE protected the stomach (68 to 95%) against ethanol (70%) induced ulcer and higher protection in comparison with ranitidine (positive control). This protection may be related to the augmentation of levels of total protein, GSH levels and/or inhibition of lipid peroxidation.

Keywords: anti-oxidant activity, anti-diabetic activity, alpha glycosidase, *P. herba venti*, *P. crinita*, ethanol induced gastric ulcer.

Resumé

La présente étude a été menée pour évaluer le contenu phénolique l'activité antioxydante, antidiabétique et l'effet gastro protecteur des extraits méthanoliques (PC ME, PV ME) et aqueux (PC AQE, PV AQE) des parties aériennes de *P. crinita* et de *P. herba venti* qui sont des espèces de la famille des Lamiacées. Dans cette étude, les résultats ont montré que l'extrait méthanolique de *P. herba venti* contenait des teneurs élevées en polyphénols et en flavonoïdes, alors que la grande quantité des tanins a été enregistrée dans l'extrait méthanolique de *P. crinita*. Les propriétés antioxydantes des extraits des plantes ont été déterminées en utilisant le piégeage des radicaux DPPH, ABTS, le blanchiment du β-carotène, le pouvoir réducteur et les tests d'activité de chélation des métaux. *P. herba venti* extracts ont montrè une forte activité de piégeage des radicaux ABTS et DPPH et bonne activité dans les tests : pouvoir réducteur, tandis que les extraits de *P. crinira* ont montré une bonne activité dans les essais d'activité de blanchiment β-carotène et de chélation des métaux. Après l'induction du diabète, la concentration de glucose sérique a augmenté chez les animaux non traités par rapport aux animaux normaux, mais le poids corporel a diminué chez les animaux non traités. L'administration de PC ME et de PV ME à des doses de 250 et 500 mg/kg a augmenté l'activité de la catalase, le niveau de GSH et diminué la peroxydation lipidique dans les tissus du foie et des reins, la créatinine sérique, l'acide urique, le cholestérol total et les triglycérides. L'effet inhibiteur *in vitro* sur l'enzyme diabétique a montré que l'extrait méthanolique de *P. herba venti* était plus inhibé (CI_{50} = 0.16 0.01 mg/mL) que le contrôle positif de l'alpha-glycosidase. Le traitement des rats avec 250 et 500 mg/kg de PC ME PC AQE PVME et PV E a protégé l'estomac (68 à 95%) contre l'éthanol (70%) induit un ulcère et une protection plus élevée par rapport à la ranitidine (témoin positif). Cette protection peut être liée à l'augmentation des niveaux de protéines totales, des niveaux de GSH et/ou à l'inhibition de la peroxydation lipidique.

Mots clés: anti-oxydant, anti-diabétique, alpha glucosidase, *P. herba venti*, *P. crinita*, ulcère gastrique induit par l'éthanol.

List of abbreviations

ABTS: 2,2'-azino-bis (3-ethylbenzenothiazoline acid).

ALcl3: aluminium trichloride.

ALT: alanine aminotransferase.

AST: aspartate aminotransferase.

BHT: butylated hydroxytoluene.

CAT: catalase.

DPPH: 2, 2-diphenyl-1-picryl-hydrazyl.

DM: diabetes mellitus.

EDTA: ethylenediamine tetraacetic acid.

FTC: ferric thiocyanate.

GDM : gestational diabetes.

GLUT2 : Glucose Transporter 2.

GPx: glutathione peroxidase.

GSH: reduced glutathione.

HDL: high density lipoprotein.

HPLC: high performance liquid chromatography.

H_2O_2: hydrogen peroxide.

HO2: perhydroxyl radical.

I%: Inhibition percentage.

IC50%: Inhibitory concentration for 50% of activity.

LDL: Low density lipoprotein.

MeOH: Methanol.

MDA : malondialdehyde.

NIDDM : non-insulino dependent diabetes mellitus.

NO-: nitric oxide.

NSAIDs: None steroidal anti-inflammatory drugs.

OH^-: hydroxil radical.

$OONO^-$: peroxynitrite anion.

PC AQE: aqueous extract of *P. crinita*.

PC ME: methanolic extract of *P. crinita*.

PV AQE: aqeoous extract of *P. herba venti*.

PV ME: methanolic extract of *P. herba venti*.

PLT: Blood platelets.

RBC: red blood cells.

ROS: reactive oxygen speices.

SEM: Standard error of the mean.

SOD : superoxide dismutase.

T1D : type 1 diabetes mellitus.

T2D: type2 diabetes mellitus.

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Introduction

Oxidative stress is a process that occurs at excessive production/generation of reactive oxygen species (ROS) in cells and tissues. The ROS at low concentrations in the cell contribute to various metabolic pathways or regulate various physiological functions as a response to biotic and environmental stresses but over production of ROS leading consequently to oxidation of the main cell macromolecules, abnormal gene expression, and cell death (Pham-Huy *et al*., 2008; Del Río, 2015). Oxidative stress and ROS play a major role in several disorders such as Alzheimer's disease, diabetes, cancer and gastrointestinal disorders (Liu *et al*., 2018).

Diabetes mellitus is the collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycaemia. The cause is a disturbed insulin secretion or a disturbed insulin effect or usually both (Petersmann *et al.,* 2019). Diabetes is one of the largest global public health concerns, imposing a heavy global burden on public health as well as socio-economic development. Although incidence has started to decrease in some countries, the prevalence of diabetes has increased in recent decades in most other developed and developing countries (Dwyer-Lindgren *et al*., 2016; Patterson *et al*., 2019).

Increasing evidences have suggested that oxidative stress plays a major role in the pathogenesis of diabetes mellitus (DM). Oxidative stress also appears to be the pathogenic factor in underlying diabetic complications (Asmat *et al*., 2016). ROS can attack lipids, proteins, nucleic acids simultaneously in living cells. Most of the studies reveal the inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, impaired Glutathione metabolism and decreased Vitamin C levels (Yang *et al*., 2011). This alteration causes defective insulin gene expression and insulin secretion as well as increased β cells damage and apoptosis by activating a variety of stress-sensitive intracellular signaling pathways (Maiese, 2015; Yaribeygi *et al*., 2020). Oxidative stress induced complications of diabetes may include stroke, neuropathy, retinopathy and nephropathy. There are some drugs that works to lower abnormally high glucose (sugar) levels in the blood, which are characteristic of the endocrine system disorder known as antidiabetic drug, but synthetic drugs like acarbose which is a carbohydrate enzymes inhibitor can induce several undesirable symptoms such as excessive flatulence or soft stool, diarrhea and nausea and abdominal disorders

The stomach is a sensitive digestive organ that is susceptible and exposed to exogenous pathogens from the diet. In response to such pathogens, the stomach induces oxidative stress, which might be related to the development of gastric organic disorders such as gastritis,

gastric ulcers, and gastric cancer, as well as functional disorders such as functional dyspepsia (Suzuki *et al;* 2011). The peptic ulcer has been considered as one of the most common digestive desorders in the present century. Peptic ulcers are chronic and often single lesions that may occur in any part of the digestive tract (Kumar *et al*; 2017). The pathophysiology of this disease has a multifactorial process that is caused by the imbalance between aggressive factors, in particular, acid and pepsin on one hand, and mucosal defense factors, especially blood flow and prostaglandins, on the other (Bafna and Balaraman; 2004). Factors that may increase the incidence of peptic ulcer disease (PUD) include stress, alcohol consumption, smoking, Helicobacter pylori, and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) (Vonkeman *et al*., 2007). Although using antibiotics, proton pump inhibitors (omeprazole), prostaglandin analogs, and H2 receptor blockers (cimetidine, e, and famotidine) reduce the mortality of stomach ulcers, attempts to discover new drugs with lower cost and fewer side effects are necessary (Sistani *et al*.,2019).

Phlomis crinita and *P. herba venti* belong to the Lamiaceae family; in this family include more than 100 perennial herbs, shrubs, and sub-shrubs species native to the Mediterranean, Central Asia, and India (Sarikurkcu *et al*., 2015). Like many natural herbs, *Phlomis crinita* and *P. herba venti* also known to be is good natural source of various secondary metabolites and contain some of the most powerful known antioxidant's compounds (Sarkhail *et al*., 2014; Kabouche *et al*., 2005; Sarikurkcu *et al*., 2015). Thus, the main objectives of the present study were to put forward the scientific basis for the evaluation of these two plants in the management of some diseases by:

-Determination of polyphenols and flavonoids in plant extracts.

- Evaluation of the in vitro antioxidant properties using different methods.

- Studying the antidiabetic activity of extracts using alloxan-induced diabetes in rats.

- Studying the antioxidant properties of plant extracts *in vivo* (MDA, GSH and catalase activity).

- Determination of biochemical and hematobiochemical parameters.

- Assessment the gastric mucosal protection against ethanol-induced ulceration in rats.

Review of Literature

1. Oxidative stress

1.1. Definition of oxidative stress

Oxidative stress as a concept in redox biology and medicine has witnessed fulminant development of the past 30-odd years. It is a global concept, defined as "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage (Sies, 2018). Excessive or uncontrolled production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can cause damage to nucleic acids, proteins and lipids and this is closely associated with human disease pathogenesis such as cancer, autoimmune disorders, rheumatoid arthritis, aging, cardiovascular, and neurodegenerative diseases (Liu *et al.*, 2018).

1.2. Reactive oxygen species' (ROS)

Reactive oxygen species' (ROS) is a generic term that defines a wide variety of oxidant molecules with vastly different properties and biological functions that range from signalling to cause cell damage (Sies *et al.*, 2022). Free radicals are atoms or groups of atoms containing at least one unpaired electron in their orbital and can be formed when oxygen interacts with certain molecules (Phaniendra *et al.*, 2015). Free radical is any chemical species capable of independent existence and possessing one or more unpaired electron, an unpaired electron being one that is alone in an orbital. Radicals, often denoted by the insertion of the superscript dot (.), are generally less stable than non-radicals, although their reactivities vary (Hey and Waters, 1937; Moad and Solomon, 2006).

Over the years, the terms ROS (reactive oxygen species), ROI (reactive oxygen intermediates) and RNS (reactive nitrogen species) have been coined to define an emerging class of endogenous, highly reactive, oxygen (and also nitrogen) bearing molecules. According to some definitions the term ROI describes the chemical species formed upon incomplete reduction of molecular oxygen, namely superoxide radical anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot}$), while ROS includes both ROI and ozone (O_3) and singlet oxygen ($^1O_2$) (Nathan and Ding, 2010). A somewhat more encompassing definition also includes within ROS compounds such as hypochlorous (HOCl), hypobromous (HOBr), and hypoiodous acids (HOI). Incorporation of peroxyl ($ROO^{\cdot}$), alkoxyl ($RO^{\cdot}$), semiquinone ($SQ^{\cdot -}$) and carobonate ($CO_3^{\cdot -}$) radicals and organic hydroperoxides (ROOH) is also frequently encountered within the definition of ROS (Liou and Storz, 2010). ROS may also be classified as free radicals and nonradical species (Winterbourn, 2008). RNS that bear oxygen

atoms include nitric oxide radical (NO or $NO^{\cdot}$), nitrogen dioxide radical ($NO_2^{\cdot}$), nitrite (NO_2^-), and peroxynitrite ($ONOO^-$) (Nathan and Ding, 2010).

1.3. Main sources of ROS and RNS

ROS can either be generated exogenously or intracellularly from numerous sources. They are produced in a wide range of biochemical and physiological processes (figure 01).

1.3.1. Endogenous Sources of ROS

Several different enzymes have been implicated in the generation of ROS.

- **NADPH oxidases (NOXs)**

Cytosolic enzyme systems contributing to the generation of ROS, among others, are the seven isoforms of the expanding family of transmembrane NADPH oxidases (NOXs), a superoxide-generating system (Lambeth, 2004; Nathan and Cunningham-Bussel, 2013). The cytosolic domains of NOX transfer an electron from NADPH to a FAD cofactor. From there, the electron is passed to a haem group, which donates it to O_2 on the extracellular side of the membrane, generating $O_2^{\cdot -}$(Nathan and Cunningham-Bussel, 2013). Depending on the specific NADPH oxidase expressed in different cells, they can trigger different cellular transformations with widely differing biological outcomes. The NADPH oxidase family of enzymes illustrates the specificity in ROS generation and its impact on normal cellular signaling and homeostasis (Nathan and Cunningham-Bussel, 2013).

$$2O_2 + NADPH \longrightarrow 2O_2^{\bullet -} + NADP^+ + H^+$$

- **Mitochondrial respiratory chain enzymes**

Mitochondria represent another major source for intracellular ROS production. The production of mitochondrial superoxide radicals occurs primarily at two discrete points in the electron-transport chain, namely at Complex I (NADH dehydrogenase) and at Complex III (ubiquinone–cytochrome c reductase) upon one electron transfer to oxygen (Turrens, 2003; Brown and Griendling, 2015) In vitro, these two sites in mitochondria convert 1–2% of the consumed oxygen molecules into superoxide anions both under normobaric or hyperbaric conditions (Dröse and Brandt 2012) These initial estimates were made on isolated mitochondria and it may be concluded that the in vivo rate of mitochondrial superoxide production is considerably less (Finkel and Holbrook, 2000). Although one-electron reactions

predominate, two-electron reactions that allow the direct reduction of molecular oxygen to hydrogen peroxide do exist within the mitochondria (Zhao *et al*., 2019) Superoxide produced at Complex I is thought to form only within the matrix, whereas at Complex III superoxide is released both into the matrix and the inner mitochondrial space (IMS). A nonenzymatic source of ROS in mitochondria is the formation of the free radical semiquinone anion species ($Q^{\cdot -}$) that occurs as an intermediate in the redox cycling of coenzyme Q10 (Finkel and Holbrook, 2000). Once formed, $Q^{\cdot -}$ can readily transfer electrons to molecular oxygen with the subsequent generation of a superoxide radical. The generation of ROS therefore becomes predominantly a function of metabolic rate.

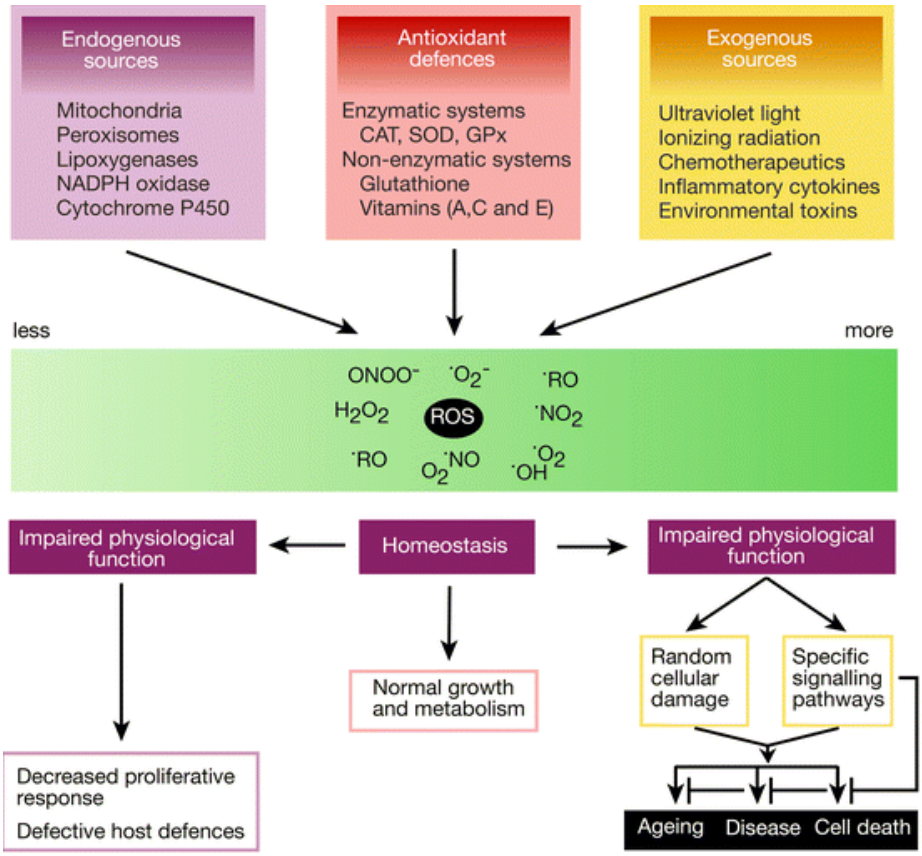


Figure 01: Sources of ROS, antioxidant defenses, and subsequent biological effects depending on the level of ROS production (Krumova and Cosa, 2016).

- **Other cellular sources of ROS**

In addition to the mitochondria and NADPH oxidases, other cellular sources of ROS production include a number of intracellular enzymes such as the flavoenzyme ERO1 in the endoplasmic reticulum, xanthine oxidase, cyclo-oxygenases, cytochrome p450 enzymes,

lipoxygenases, flavin-dependent demethylase, oxidases for polyamines and amino acids, and nitric oxide synthases that produce oxidants as part of their normal enzymatic function (Nathan and Cunningham-Bussel, 2013) Free copper ions or iron ions that are released from iron–sulfur clusters, haem groups or metal-storage proteins can convert $O_2^{\cdot -}$ and/or H_2O_2 to $OH^{\cdot}$ in what is known as the Fenton reaction. A similar reaction but involving lipid hydroperoxides accounts for the formation of lipid alkoxyl ($LO^{\cdot}$) and peroxyl radicals ($LOO^{\cdot}$) in the lipid membrane (Xie *et al*., 2019, Godoy-Gallardo *et al.,* 2012).

$$Fe^{3+} + O_2^{\bullet -} \longrightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + OH^-$$

1.3.2. Exogenous sources

Free radicals naturally occur in the body as a result of chemical reactions during normal cellular processes. The formation of ROS can be stimulated by a variety of agents such as pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, certain drugs like halothane, paracetamol, and radiation (Phaniendra *et al.,* 2015)

Environmental agents including non-DNA reactive carcinogens can generate ROS in cells by metabolism to primary radical intermediates or by activating endogenous sources of reactive oxygen species. The induction of oxidative stress and damage has been observed following exposure to xenobiotics of varied structures and activities (Caliri *et al*., 2021)

The mechanism of action of many chemotherapeutic cancer drugs involves ROS-mediated apoptosis. For example, the classic antitumor drugs cisplatin and adriamycin appear to produce ROS at excessive levels, resulting in DNA damage and cell death (He *et al*., 2012) Some classes of antibiotics rely on a similar mechanism for their bactericidal activity. For example, it was recently shown that bactericidal antibiotics, regardless of drug–target interaction, induce a breakdown in iron regulatory dynamics, stimulating the production of highly deleterious hydroxyl radicals through Fenton reaction in gram-negative and gram-positive bacteria, which ultimately contribute to cell death (Van Acker and Coenye, 2017).

1.4. ROSs and cellular damage

Biological macromolecules are constantly exposed to oxidants and oxidative damage to cellular components has been increasingly recognized as a significant pathophysiological event leading to disease and aging processes. Free radicals and other oxidizing species are

derived from both endogenous sources such as mitochondria, peroxisomes, and phagocytic cells, but also exogenous sources such as tobacco smoke, pollution, alcohol, and certain drugs

1.4.1. Effects of oxidative stress on lipids

Autoxidation of polyunsaturated fatty acid residues is initiated by a free radical such as the hydroxyl radical, which upon reaction with fatty acids generate lipid carbon centered radicals. Lipid carbon centered radicals in turn readily trap molecular oxygen under physiological conditions to form lipid peroxyl radicals, effective chain carriers in the lipid chain auto-oxidation (Andreyev *et al*., 2015). In the oxidation process, fatty acyl chains mostly in their cis configuration are either converted to the trans configuration, or form corresponding hydroperoxides and alcohols, or may fragment into electrophilic αβ-unsaturated aldehydes, among others (Porter 1986; Zeng *et al*., 2020). Peroxidation is a complex process that includes three phases: initiation, propagation, end-decomposition, which interpose, so that only end products can be determined chemically: aldehydes (malondialdehyde), polymerized carbonyl compounds (lipofuscin) (Birben *et al*., 2012; Lavie, 2015). Which are capable of inactivating many cellular proteins by forming protein cross- linkages. Peroxidation lead to a reduction in the membrane fluidity (Borst *et al*., 2000) and appearance of liquid-order domains and thus changes the functions of many receptors and transmitters and the process of transmitting signals (Catala, 2006).

1.4.2. Effects of oxidative stress on Proteins

A large number of proteins are affected by ROS, where following ROS attack conformational changes take place that regulate protein activity. This is best exemplified by the ever-increasing (Nishii *et al*., 2015) list of proteins where Cys residues act as redox switches. Here, disulfide bond formation following oxidation of Cys residues may result in structural and associated activity changes. Oxidative damage to proteins results in modifications of site-specific amino acid, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis (Cross *et al*., 2004; Nishii *et al*., 2015).

1.4.3. Effects of oxidative stress on DNA

DNA: mitochondrial DNA is a major target of ROS given that mitochondria are the prevalent source of ROS within cells, ROS can interact with it and cause several types of damage: modification of DNA bases, single- and double-DNA breaks, loss of purines (apurinic sites), damage to the deoxyribose sugar, DNA-protein cross-linkage, and damage to the DNA repair system (Kohen and Nyska, 2002). Free radicals attack also causes the activation of the poly (ADP-ribose) synthetase enzyme which can lead to fragmentation of DNA and programmed cell death. This process depletes the cellular level of NAD^+ levels thereby disrupting electron transport chain function (Birben *et al*., 2012; Lavie, 2015).

1.5. Antioxidant defense system

1.5.1. Classification of Antioxidant

There are several ways to define an antioxidant substance. An antioxidant molecule can be identified by its chemical structure that allows a free radical scavenging reaction and/or the chelation of redox-active metals. However, antioxidants are often defined as molecules that experimentally prevent the oxidation of a biological or chemical system (Azzi *et al.*, 2004). The antioxidant defenses are based on enzymatic and non-enzymatic systems. The non-enzymatic system consists of low molecular weight compounds that can be either endogenous or exogenous. Also, the maintenance of these enzymatic systems requires the presence of a number of trace elements such as copper, manganese, zinc and selenium in particular (Kurcutas, 2015).

1.5.1.1. Enzymatic antioxidant

The most important antioxidant enzymes are:

- **Superoxide dismutase (SODEc 1.15.1.1):**

These metalloproteins ensure the elimination of $O_2^{\bullet-}$ by a dismutation reaction by transforming it into H_2O_2 and O_2. The SODs are classified by their metal cofactors into known types: extracellular and cytosolic Cu/ZnSOD and mitochondrial enzyme MnSOD (Fukai and Ushio-Fukai 2011).

- **Catalase (CAT Ec 1.11.1.6):**

is a common enzyme found in nearly all living organisms exposed to oxygen. It catalyzes the decomposition of hydrogen peroxide to water and oxygen Asmat *et al*., (2015). The CAT also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme by H_2O_2 as it is reduced to water (Birben *et al*., 2012). .

- **Glutathion peroxydase (GPx EC 1.11.1.19)**

A tetrameric selenium enzyme that can reduce H_2O_2 to water using glutathione (GSH) as reductant. They limit the propagation of chain radical reactions by reducing unstable peroxides to hydroxylated fatty acids Brigelius-Flohé and Maiorino (2013).

1.5.1.2. Non-enzymatic antioxidants

Non-enzymatic antioxidants include different chemical compounds such as tocopherol (vitamin E), ascorbic acid (vitamin C, Vit C), caretinoids, GSH, phenolic compounds, ubiquinol (coenzyme Q10), phospholipids (proteoglycans and hyaluronic acid), lipoic acid, proteins binding free iron and copper (ceruloplasmin, transferrin, taurine, albumin), protein hydrolysates, bilirubin, melatonin, uric acid, mucin, surfactant, amino acids and peptides (Moussa *et al.*, 2019).

- **Glutathione (GSH)**

The GSH, a tripeptide (γ-L-glutamyl-L-cysteinylglycine), is an endogenous antioxidant and an important cellular defence agent against oxidative damage. Under normal physiological conditions, GSH is mainly reduced. However, under pathological conditions, the GSH/GSSG ratio can decrease significantly. The pentose phosphate pathway regulates the GSH/GSSG ratio by providing NADPH which is required for the reduction of GSSG to GSH-by-GSH reductase (Aquilano *et al*., 2014). The GSH can directly scavenge ROS such as H_2O_2 and •OH or indirectly through the reaction catalyzed by GPx. Moreover, GSH prevents the oxidization of sulfhydryl groups in the protein structure. It is especially important for the activity of GPx, GSH reductase and GSH-S-transferase (Kıvrak *et al.*, 2017). The GSH can regenerate other antioxidants such as Vit C and vitamin E to their active forms (Lü *et al.*, 2010).

- **Carotenoids**

Carotenoids, also known as tetraterpenoids, are a group of phytonutrients produced by plants and algae, as well as some bacteria and fungi (Stahl *et al.,* 2003) The long unsaturated hydrocarbon alkyl chain renders carotenoids highly liposoluble. Hence, they play a key role in the protection of lipoproteins and cellular membranes from lipid peroxidation and exhibit particularly efficient scavenging capacity against peroxyl radicals as compared to any other ROS and they are known to be the most common lipid-soluble antioxidants. β-carotene is considered as the most efficient scavenger of O_2^1 (Mortensen *et al*., 2001; Muller *et al*., 2011).

- **Vitamin C**

Vitamin C (L-ascorbic acid) is an optically-active hydrosoluble free radical scavenger. The role of Vit C as an antioxidant is indicated by its known free radical-scavenging action. As a reducing and antioxidant agent, it directly reacts with $O_2^{\bullet-}$, $^{\bullet}OH$ and various lipid hydroperoxides. Another main function of Vitamin C as an antioxidant is to regenerate vitamin E (HO-tocopherol) from its oxidized form (.O-tocopherol) back to its active state by reducing vitamin E radicals formed when vitamin E scavenges oxygen radicals (Moussa et al., 2019).

- **Vitamin E**

Vitamin E is a collection of optically active methylated phenolic compounds comprising four tocopherols and four tocotrienols (Bohm, 2018) where α-tocopherol is the most common and biologically active species. Vitamin E is a potent chain-breaking antioxidant that inhibits the production of ROS when fat undergoes oxidation and during the propagation of free radical reactions. It acts as the first line of defence against lipid peroxidation, protecting the cell membranes from free radical attack (Rivzi *et al*., 2014).

- **Polyphenols**

Polyphenols are a large group of compounds found and one of the most important groups of secondary metabolites of plants. In the plant, polyphenols protect from UV radiation, pathogens, oxidative stress, and harsh climatic conditions. In the human body, polyphenols are considered the most abundant antioxidant in the diet although the diversity of their structures makes them different from other antioxidants and have diverse biological properties (Pandey *et al*., 2009).

Approximately 10 000 naturally occurring compounds belong to the category of "phenolics", all of which possess a common structural feature: an aromatic ring bearing at least one hydroxyl substituent. From this basic structure, several thousand of naturally occurring compounds have been described, from simple phenolic acids to highly polymerized compounds such as tannins

- **Phenolic acids**

Phenolic acids or phenolcarboxylic acids are types of aromatic acid compounds. Included in that class are substances containing a phenolic ring and an organic carboxylic acid function (C6-C1 skeleton). Two important naturally occurring types of phenolic acids are hydroxybenzoic acids and hydroxycinnamic acids, which are derived from non-phenolic molecules of benzoic and cinnamic acid, respectively (Heleno *et al*., 2015). The majority of phenolic acids are linked through ester, ether, or acetal bonds either to structural components of the plant (cellulose, proteins, lignin) or to larger polyphenols (flavonoids), or smaller organic molecules (glucose, quinic, maleic, or tartaric acids) or other natural products (terpenes) (Goleniowski *et al*., 2013).

- **Flavonoids**

Flavonoids are secondary metabolites synthesized mainly by plants. The general structure of flavonoids is a 15-carbon skeleton, containing 2 benzene rings (A and B) and a heterocyclic ring (C, the ring containing the embedded oxygen) connected by a 3-carbon linking chain (Bravo, 1998). Therefore, they are depicted as C6-C3-C6 compounds. Depending on the chemical structure, degree of oxidation, and unsaturation of the linking chain (C3), flavonoids can be classified into different groups, such as anthocyanidins, chalcones, flavonols, flavanones, flavan-3-ols, flavanonols, flavones, and isoflavonoids (Jucá *et al*., 2020) Furthermore, flavonoids can be found in plants in glycoside-bound and free aglycone forms. The glycoside-bound form is the most common flavone and flavonol form consumed in the diet (Ma *et al*., 2014)

- **Tannins**

Tannins (or tannoids) are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. they are widely distributed in many species of plants, where they play a role in

protection from predation (including as pesticides) and might help in regulating plant growth (Ferrell *et al*., 2006).

Hydrolyzable tannins, phlorotannins and condensed tannins are three major classes and base unit or monomer of the tannin. Particularly in the flavone-derived tannins, the base shown must be (additionally) heavily hydroxylated and polymerized in order to give the high molecular weight polyphenol motif that characterizes tannins. Typically, tannin molecules require at least 12 hydroxyl groups and at least five phenyl groups to function as protein binders (Das *et al*., 2020).

2. Diabetes mellitus (DM)

2.1. Definition

Diabetes mellitus is the collective term for heterogeneous metabolic disorders characterized by a chronic hyperglycemia, accompanied by disruption of carbohydrate, lipid and protein metabolisms (Ighodaro *et al.,* 2017). The clinical symptoms of diabetes mellitus are presented as weight loss, polyuria, thirst, blurring of vision and complication of renal failure, neuropathy, foot ulcers and prolong illness will lead to microvascular and macrovascular diseases (WHO, 1999). The cause is either a disturbed insulin secretion or a disturbed insulin effect or usually both (Ighodaro *et al*., 2017). The insulin is secreted by the β-cell of the pancreas. The insulin levels in the portal vein and systemic circulation are changed by the meals and blood glucose levels. After meal the blood glucose level is high so insulin is produced by β-cell in the pancreas to normalize the glucose level (Röder *et al*.,2016). It increases plasma membrane glucose transporters of glucose from bloodstream into the muscle, liver and adipose tissue. In addition, it converts glucose to glycogen in the muscle and the liver for storage of the nutrients. Finally, the level of glucose in the blood will come down (Wilcox, 2005).

DM corresponds to chronic hyperglycemia random plasma glucose value of ≥ 200mg/dl (≥ 11.1mmol/l), Fasting plasma glucose value of ≥ 126mg/dl (≥ 7.0mmol/l) or 2- h oral glucose tolerance test (OGTT) value in venous plasma ≥ 200mg/dl (≥ 11.1mmol/l) (Petersmann *et al*., 2019).

2.2. Epidimiology

Diabetes mellitus is a major health problem worldwide. Epidemiological studies have shown that it affects all populations and all age groups indiscriminately. Globally, the number of diabetic patients has increased dramatically in recent years. It is projected that, by 2045, at least 700 million people worldwide will have diabetes. Despite the heterogeneous etiological and clinical nature of the disease (Mekala and Berton, 2020) The chronic disease has hurt more than 171 million persons globally in 2000, One person dies of diabetes every 6 seconds in the world, its more than AIDS, tuberculosis and malaria (Alam *et al*., 2019; IDF, 2015) Currently, approximately 15.5 million of people (20-79 years) have diabetes (3.3%); 40.1 million others (8.6%) have diabetes of glucose intolerance, present a high risk of contracting the disease one, alarming value which is expected to increase further to 102.0 million by 2045 (IDF, 2017).

2.3. Types of Diabetes Mellitus: There are mainly three types of diabetes, type 1 diabetes, type 2 diabetes and gestational diabetes.

2.3.1. Type 1 Diabetes Mellitus (T1D)

Also known as juvenile-onset T1D is caused by an autoimmune disease where antigen-specific T cells selectively destroy insulin producing pancreatic β-cells. As a result, no or very little insulin is produced by the body, leading to hyperglycemia. Although 5%-10% of people with diabetes have T1D, it remains a serious, life-threatening disease. It develops more frequently in children and adolescents and is considered the third most common disorder during childhood. The cause of T1D is not fully understood but multiple environmental and genetic risk factors (eg. HLA haplotypes) have been implicated (Nyaga *et al.*, 2018). People with T1D need daily insulin injection to survive, which is why this type is also known as an insulin-dependent diabetes mellitus.

2.3.2. Type 2 Diabetes Mellitus (T2D)

This was previously known as non-insulin dependent diabetes mellitus (NDDM), or adult-onset diabetes mellitus which was mostly onset in adult due to the rising prevalence of obesity, physical inactivity and poor diet (Henning, 2018). Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, and it represents more than 90% of all cases (Cefalu, 2006). T2D a chronic metabolic disorder characterized by hyperglycemia in which the body becomes resistant to the normal effects of insulin and slowly loses the capacity to produce

insulin in the pancreas. As a result, the β cells in the pancreas respond by producing more amount of insulin, in order to achieve some degree of management of the blood glucose levels. Thus, this overproduction of insulin causes β cells to wear themselves out (Cerf, 2013; Alam *et al.,* 2019). The cause of T2D is not completely understood but overweight and obesity, old age as well as genetics or family history have been linked with it (Matboli *et al.,* 2019).

2.3.3. Gestational Diabetes Mellitus (GDM)

One of the most common complications in pregnancy is GDM, which affects 14% of pregnancies worldwide. Mild hyperglycemia that is first detected during pregnancy is classified as GDM. Women with considerably higher blood glucose during pregnancy are classified as women with hyperglycemia in pregnancy (Radenković, 2011) GDM usually exists as a transient disorder affecting pregnant women typically around the 2nd and 3rd trimesters of their pregnancy and resolves after delivery. However, pregnant women with hyperglycemia have at least a seven-fold higher risk of developing GDM in subsequent pregnancies and half of them later develop T2D. Offspring born to mothers with GDM develop increased lifelong risks of developing obesity, T2D and metabolic syndrome (Landon *et al*., 2009; IDF, 2017).

Maternal overweight and obesity, later age at childbearing, previous history of GDM, family history of type 2 diabetes mellitus and ethnicity are major GDM risk factors Dietary modification and increased physical activity are the primary treatments for GDM, but pharmacotherapy, usually insulin, is used when normoglycaemia is not achieved (McIntyre *et al.*, 2019).

2.4. Structure of pancreas

The pancreas is an organ of the digestive system and endocrine system of vertebrates. In humans, it is located in the abdomen behind the stomach and functions as a gland. The pancreas is a mixed or heterocrine gland, it has both an endocrine and a digestive exocrine function (Longnecker, 2014). Anatomically, the pancreas is divided into a head, neck, body, and tail, there are no clear-cut borders between these parts (Dolenšek *et al.,* 2015). The head abuts the C-shaped second portion of the duodenum in the right upper quadrant of the abdomen (figure 02). The tail emerges into the peritoneal cavity (covered by peritoneal

serosa) and extends to the hilum of the spleen in the left upper quadrant. The pancreas weighs about 100 g and is 14–25 cm long (Hruban *et al.*, 2007).

The pancreatic islets (islets of Langerhans) collectively comprise the endocrine pancreas that synthesizes and secretes insulin, glucagon, pancreatic polypeptide, and somatostatin. Most islets are too small to be seen by gross examination, Islets (numbering approximately one million in an adult human pancreas) comprise 1-2% of pancreatic mass and are present throughout the organ. Each islet is composed of about 2000 cells (Friesen, 1982; Longnecker and Morgan, 2018).

As a part of the digestive system, it functions as an exocrine gland secreting pancreatic juice into the duodenum through the pancreatic duct and cells, this juice contains bicarbonate, which neutralizes acid entering the duodenum from the stomach; and digestive enzymes, which break down carbohydrates, proteins, and fats in food entering the duodenum from the stomach (Longnecker, 2014).

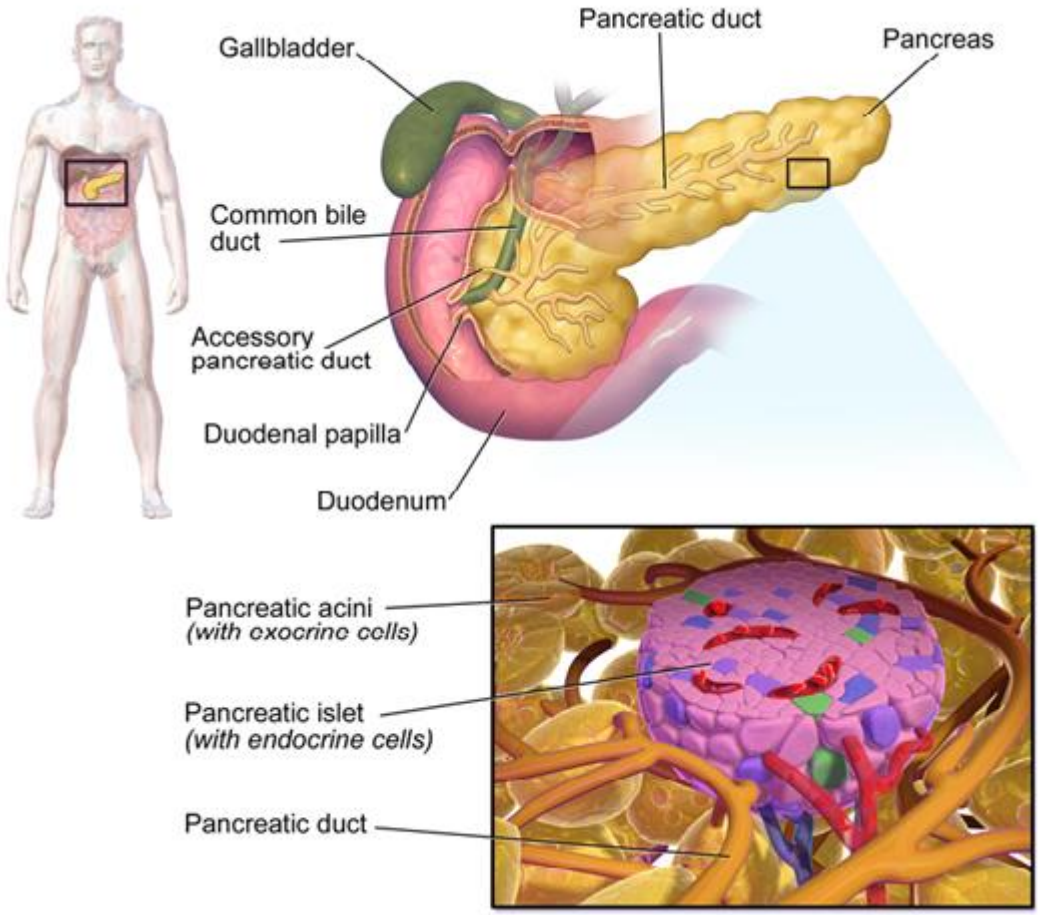


Figure 02: Structure of pancrea (http://health.howstuffworks.com/diabetes1.htm).

2.4.1. Pancreatic β-cells

Beta cells (β-cells) are a type of cell found in pancreatic islets that synthesize and secrete insulin and amylin. Beta cells make up 50–70% of the cells in human islets (Dolenšek *et al.,* 2015) In patients with Type 1 diabetes, beta-cell mass and function are diminished, leading to insufficient insulin secretion and hyperglycemia (Chen *et al.,* 2017). The primary function of a beta cell is to produce and release insulin and amylin. Both are hormones which reduce blood glucose levels by different mechanisms. Beta cells can respond quickly to spikes in blood glucose concentrations by secreting some of their stored insulin and amylin while simultaneously producing more (Boland *et al.,* 2017) Primary cilia on beta cells regulate their function and energy metabolism. Cilia deletion can lead to islet dysfunction and type 2 diabetes (Hegyi and Petersen, 2013).

4.2.2. Insulin synthesis

Beta cells are the only site of insulin synthesis in mammals (Boland *et al.,* 2018). As glucose stimulates insulin secretion, it simultaneously increases proinsulin biosynthesis, mainly through translational control (Boland *et al.,* 2017).

The insulin gene is first transcribed into mRNA and translated into preproinsulin (Boland *et al.,* 2017). After translation, the preproinsulin precursor contains an N-terminal signal peptide that allows translocation into the rough endoplasmic reticulum (RER). Inside the RER, the signal peptide is cleaved to form proinsulin. Then, folding of proinsulin occurs forming three disulfide bonds. Subsequent to protein folding, proinsulin is transported to the Golgi apparatus and enters immature insulin granules where proinsulin is cleaved to form insulin and C-peptide (Fu *et al.,* 2013) After maturation, these secretory vesicles hold insulin, C-peptide, and amylin until calcium triggers exocytosis of the granule contents (Boland *et al.,* 2017). Through translational processing, insulin is encoded as a 110 amino acid precursor but is secreted as a 51 amino acid protein (Fu *et al.,* 2013).

2.2.3. Insulin secretion

In beta cells, insulin release is stimulated primarily by glucose present in the blood. As circulating glucose levels rise such as after ingesting a meal, insulin is secreted in a dose-dependent fashion (Boland *et al*., 2017) This system of release is commonly referred to as glucose-stimulated insulin secretion (GSIS) (Komatsu *et al.,* 2013) There are four key pieces to the triggering pathway of GSIS: GLUT2 dependent glucose uptake, glucose metabolism,

KATP channel closure, and the opening of voltage gated calcium channels causing insulin granule fusion and exocytosis

When the glucose concentration outside the cell is high, glucose molecules move into the cell by facilitated diffusion, down its concentration gradient through the GLUT2 transporter (De Vos *et al.,* 1995) Since beta cells use glucokinase to catalyze the first step of glycolysis, metabolism only occurs around physiological blood glucose levels and above (Boland *et al.,* 2017) Metabolism of the glucose produces ATP, which increases the ATP to ADP ratio (Santulli *et al*., 2015)

The ATP-sensitive potassium ion channels close when this ratio rises (Ashcroft *et al*., 1990). This means that potassium ions can no longer diffuse out of the cell (Keizer and Magnus, 1989; Marinelli *et al.,* 2021). As a result, the potential difference across the membrane becomes more positive (as potassium ions accumulate inside the cell). This change in potential difference opens the voltage-gated calcium channels, which allows calcium ions from outside the cell to diffuse in down their concentration gradient (MacDonald *et al*., 2005; Fridlyand and Philipson, 2010) When the calcium ions enter the cell, they cause vesicles containing insulin to move to, and fuse with, the cell surface membrane, releasing insulin by exocytosis into the hepatic portal vein (Lang and Light, 2010; Edgerton *et al.,* 2017).

In addition to the triggering pathway, the amplifying pathway can cause increased insulin secretion without a further increase in intracellular calcium levels. The amplifying pathway is modulated by byproducts of glucose metabolism along with various intracellular signaling pathways (Edgerton *et al*., 2017).

2.3. Oxidative stress and diabetes mellitus

Increasing evidences have suggested that oxidative stress plays a major role in the pathogenesis of diabetes mellitus (DM). Oxidative stress also appears to be the pathogenic factor in underlying diabetic complications (Asmat *et al.,* 2016). ROS produced either endogenously or exogenously can attack lipids, proteins, nucleic acids simultaneously in living cells. Most of the studies reveal the inference of oxidative stress in diabetes pathogenesis by the alteration in enzymatic systems, lipid peroxidation, impaired Glutathione metabolism and decreased Vitamin C levels (Yang *et al.,* 2011). This alteration cause defective insulin gene expression and insulin secretion as well as increased β cells damage and apoptosis by activating a variety of stress-sensitive intracellular signaling pathways such

as Nf-κb (nuclear factor kappa b), p38 MAPK (p38 mitogen-activated protein kinases), JNK/SAPK (stress-activated protein kinase/c-Jun NH(2)-terminal kinase), hexosamine pathways, PKC (protein kinase C), AGE/RAGE (advanced glycation end product/receptor for AGE) interactions, and sorbitol synthesis (Maiese, 2015; Yaribeygi *et al.*, 2020). Oxidative stress induced complications of diabetes may include stroke, neuropathy, retinopathy and nephropathy. The various biomarkers for oxidative stress in patients with diabetes include malondialdehyde (MDA), total cholesterol, and reactive hydroperoxides (ROOH) (Rösen *et al.*, 2001; Yaribeygi *et al*., 2020). Various methods for determining biomarkers of cellular oxidative stress have been developed, and some have been proposed for sensitive assessment of antioxidant defense and oxidative damage in diabetes and its complications. However, their clinical utility is limited by less-than-optimal standardization techniques and the lack of sufficient large-sized, multi-marker prospective trials (Yang *et al.*, 2011; Ma *et al.*, 2018).

2.4. Experimental diabetes induced by alloxan

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) is a pyrimidine derivative that is synthesized by uric acid oxidation (Lenzen, 1988, Lenzen, 2008). It is a hydrophilic unstable compound with a structure similar to glucose (figure 03). These alloxan properties are essential for the development of diabetes. Namely, its hydrophilicity prevents alloxan from penetrating the lipid bilayer of the plasma membrane, while glucose-like structure enables an entrance of alloxan into beta cells. It has been successfully induced in a variety of animal species; rabbits, mice, rats, monkeys, cats and dogs (Battell, 2018). Alloxan has been administered in single or multiple doses, through different routes (intraperitoneal, intravenous and subcutaneous); with single intraperitoneal administration apparently the most employed mode. The dosage of the drug also varies across studies, ranging between 90 and 200 mg/kg of body weight (Chougale, 2007).

Alloxan is a hydrophilic compound and it is not able to freely pass the cellular membrane without additional action of specific protein transporters. Namely, it enters B cells via GLUT2 transporters and this mechanism is tightly connected to its glucose-like structure, its toxicity via ROS increases, leading to induced rupture of the secretory granules and cell membrane of the beta cells (Lenzen, 2008; Szkudelski, 2001). The attendant effect of this burst up is flooding of the circulation with insulin, a pathophysiological condition that results in a severe transitional hypoglycemic phase which is observable a couple of hours after alloxan injection. The hypoglycemic phase in alloxan diabetogenicity has also been associated with the ability

of alloxan to cause significant influx of free Ca^{2+} into the cytosol of pancreatic islet beta cells, thereby compromising the intracellular calcium homeostasis (Park *et al.*, 1995). The process involves the depolarization of the pancreatic beta cells, which facilitates further calcium entry into pancreatic cells via voltage dependent calcium channels. High intracellular level of Ca^{2+} has been noted to contribute significantly to super high level of insulin release (Szkudelski, 2001; Ighodaro *et al.*, 2017).

Alloxan exhibits a high affinity for the SH-containing cellular compounds such as glucokinase which is important for the process of glucose storage in form of glycogen, while in B cells it has a function of glucose sensor and it controls insulin secretion. As a potent glucokinase inhibitor, alloxan reduces glucose oxidation and ATP generation, which further suppresses insulin secretion (Wilson, 2003, Iynedjian, 2009; Radenković *et al.*, 2016). The effects of alloxan on different organs have been extensively studied. alloxan is diabetogenic, hepatotoxic, nephrotoxic (Chougale, 2007; Namjou *et al*., 2022) and also causes chronic cellular injury and mutagenesis (Lucchesi *et al*., 2015).

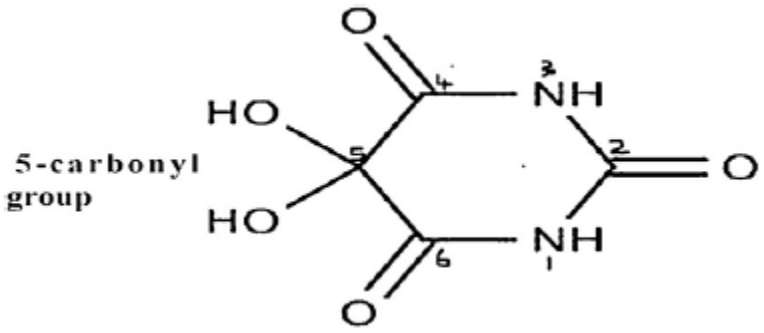


Figure 03: alloxan structure.

2.5. Antidiabetic drug

Antidiabetic drug, any drug that works to lower abnormally high glucose (sugar) levels in the blood, which are characteristic of the endocrine system disorder known as diabetes mellitus.

2.5.1. Insulin preparations

Insulin cannot be administered orally, because it is a polypeptide whose physiological properties are destroyed by proteolytic enzymes present in the stomach and gastrointestinal tract. Thus, in order to enter the bloodstream and reach the body's cells, insulin must be

injected parenterally (e.g., subcutaneously). In addition, insulin has strong hydrophilicity and large molecular weight and extremely low oral bioavailability (Wang *et al.,* 2022). Insulin preparations may be classified based on their duration of action and expediency of onset. In order to mimic the body's natural patterns of insulin release, which include post-meal secretion and between-meal background, or basal, secretion, treatment regimens typically involve the administration of two different types of preparations. For example, a rapid-acting insulin preparation, meant to mimic secretion following a meal, often is combined with intermediate-acting insulin, which mimics basal secretion. An overdose of insulin can produce hypoglycemia (low blood sugar), which may lead to convulsions.

2.5.2. Oral antidiabetic drugs

Type II diabetes often may be treated with oral hypoglycemic or antihyperglycemic drugs instead of with insulin.

- **The sulfonylureas** (Tolbutamide, Glibenclamide, Glimepiride and Glipizide) for example, stimulate the insulin-secreting beta cells of the pancreas to produce more insulin and decrease hepatic clearance of insulin. With long-term use, this effect appears to diminish, but plasma glucose levels remain low. (Sola *et al.,* 2015). Results from large, multicentre, randomised controlled trials such as the UK Prospective Diabetes Study and ADVANCE have confirmed the microvascular benefits of sulphonylureas, a reduction in the incidence or worsening of nephropathy and retinopathy, and no increase in all-cause mortality, although whether these benefits were due to sulphonylurea therapy (Khunti *et al.*, 2018).

- **Repaglinide and nateglinide**, which belong to a class of chemicals commonly called glinides, are other orally active compounds that stimulate insulin release from the pancreas. They are increasingly used either in monotherapy or in combination with other oral antihyperglycaemic agents for the treatment of type 2 diabetes. These agents work by closing potassium channels on the surface of beta cells, which causes an influx of calcium ions into the cells and a consequent outflow of insulin from cellular storage vesicles (Gromada *et al.*, 1995; Kawamori *et al*., 2012).
- **The thiazolidinediones** (e.g., pioglitazone, rosiglitazone) decrease insulin resistance. These oral hypoglycemic drugs exert their effects by activating so-called PPARγ (peroxisome proliferator-activated receptor-gamma) receptors, which are found primarily in adipose tissue; when activated, PPARγ prompts the transcription (synthesis of RNA from DNA) of genes that

regulate glucose and lipid metabolism. Because hepatotoxicity is a major concern with thiazolidinediones, regular monitoring of liver metabolism is performed in individuals taking these drugs (Tonelli *et al*., 2004; Soccio *et al.*, 2014).

- **Metformin**, an oral antihyperglycemic drug that belongs to the biguanide class of agents, lowers glucose levels by decreasing liver production of glucose and increasing the action of insulin in fat and muscle by activation of to AMPK (adenosine 5′- monophosphate—activated protein kinase). AMPK is a key regulator of numerous metabolic pathways, including glucose metabolism, lipid metabolism, and energy homeostasis (Hardie *et al*., 2012; Lin *et al.*, 2018). Besides, metformin plays important roles by inhibiting insulin and IGF (insulin-like growth factor) receptor signaling, resulting in changes in metabolic homeostasis (Martin and Marais, 2012).

- **Pramlintide, exenatide, and sitagliptin**

Other antidiabetic drugs include pramlintide and exenatide. Pramlintide is an injectable synthetic hormone (based on the human hormone amylin) that regulates blood glucose levels by slowing the absorption of food in the stomach and by lowering glucagon secretion which normally stimulates liver glucose production, without altering clearance in subjects with T1D (Ruchi *et al*., 2022).

- **Exenatide** is an injectable antihyperglycemic drug that works similarly to incretins, or gastrointestinal hormones, such as gastric inhibitory polypeptide, that stimulate insulin release from the pancreas. Exenatide has a longer duration of action than incretins produced by the body because it is less susceptible to degradation by an enzyme called dipeptidyl peptidase-4 (DPP-4).
- A drug called **sitagliptin** specifically inhibits DPP-4, thereby increasing levels of naturally produced incretins. Side effects associated with these drugs are often mild, although pramlintide can cause profound hypoglycemia in patients with type I diabetes.

2.6. Naturel Treatment of diabetes mellitus

Natural products are secondary metabolites widely distributed in plants, having a broad range of biological activities. The development of antidiabetic medication from natural products, especially those originating from plants with a traceable folk-usage history in treating diabetes, is receiving more attention (Chen *et al*., 2015). Substances and extracts isolated from

different natural resources especially plants have always been a rich arsenal for controlling and treating diabetes problem and complication arising due to it. (Verma *et al.,* 2018). A wide array of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of diabete mellitus. Among these are polyphenols, flavonoids, alkaloids , polysaccharides, hypoglycans, , steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions and phenolic acid (Li *et al.,* 2015; 2019; Meneguin *et al.,* 2021; Singh *et al.,* 2022) Studies on the antidiabetic mechanisms of medicinal plants have shown that most of them produce hypoglycemic activity by stimulating insulin secretion, augmenting peroxisome proliferator-activated receptors (PPARs), inhibiting α-amylase or α-glucosidase, glucagon-like peptide-1 (GLP-1) secretion, advanced glycation end product (AGE) formation, free radical scavenging plus antioxidant activity (against ROS/RNS), up-regulating or elevating translocation of glucose transporter type 4 (GLUT-4), and preventing development of insulin resistance (Nazarian-Samani, 2018). A target pathway to control diabetes is the 5′-adenosine monophosphate-activated protein kinase (AMPK) signaling pathway. AMPK is a heterotrimeric protein with α, β, and γ subunits. In several studies, AMPK activation enhanced glucose uptake into cells and inhibited intracellular glucose production. Impairment of AMPK activity is present in diabetes, according to some studies. Drugs used in the treatment of diabetes, such as metformin, are also known to act through regulation of AMPK. Thus, drugs that activate and regulate AMPK are potential candidates for the treatment of diabetes (Joshi *et al.,* 2019).

3. Human digestive system

The digestive tract begins at the lips and ends at the anus. It consists of the mouth, or oral cavity, with its teeth, for grinding the food, and its tongue, which serves to knead food and mix it with saliva; the throat, or pharynx; the esophagus; the stomach; the small intestine, consisting of the duodenum, the jejunum, and the ileum; and the large intestine, consisting of the cecum, a closed-end sac connecting with the ileum, the ascending colon, the transverse colon, the descending colon, and the sigmoid colon, which terminates in the rectum (Daniel *et al.,* 2001; Leung, 2014).

The primary function of the digestive system is to move nutrients, water, and electrolytes from the external environment into the body's internal environment. To accomplish this, the system uses four basic processes: digestion, absorption, secretion, and motility (Leung, 2014).

3.1. The anatomical structure of the stomach

Stomach, saclike expansion of the digestive system, between the esophagus and the small intestine; it is located in the anterior portion of the abdominal cavity in most vertebrates. The human stomach is subdivided into four regions (figure 04) the fundus, an expanded area curving up above the cardiac opening (the opening from the stomach into the esophagus); the body, or intermediate region, the central and largest portion; the antrum, the lowermost, somewhat funnel-shaped portion of the stomach; and the pylorus, a narrowing where the stomach joins the small intestine. Each of the openings, the cardiac and the pyloric, has a sphincter muscle that keeps the neighbouring region closed, except when food is passing through. In this manner, food is enclosed by the stomach until ready for digestion.

The stomach has the ability to expand or contract depending upon the amount of food contained within it. When contracted, the interior walls form numerous folds (rugae), which disappear when the walls are distended. The thick mucous-membrane lining of the walls is densely packed with small gastric glands; these secrete a mixture of enzymes and hydrochloric acid that partly digest proteins and fats (Keshav, 2009).

3.2. Histologie

The structure of the stomach wall consists of four layers: (1) an inner mucosa facing the lumen, (2) a layer known as the submucosa, (3) layers of smooth muscle known collectively as the muscularis externa, and (4) a covering of connective tissue called the serosa (Kent *et al.*, 2001) (figure 05).

3.2.1 Mucosa

The mucosa, the inner lining of the gastrointestinal tract, has three layers: a single layer of mucosal epithelium facing the lumen; the lamina propria, subepithelial connective tissue that holds the epithelium in place; and the muscularis mucosae, a thin layer of smooth muscle. Several structural modifications increase the amount of mucosal surface area to enhance absorption (Leung, 2014). **At the base of the gland are**

- **The zymogenic (chief) cells**, which are thought to produce the enzymes propepsin and rennin. (Pepsin digests proteins, and rennin curdles milk.)

- **Parietal, or oxyntic, cells:** occur throughout the length of the gland and are responsible for the production of hydrochloric acid, which is necessary to activate the other enzymes.
- **Mucous neck cells:** is to secrete mucus.
- **Gastric neuroendocrine cells (G cells):** which are found mainly in the pyloric cavity and secrete gastrin into the bloodstream under stimulation of the parasympathetic system and gastric peptides (Tortora and Derrickson 2007)

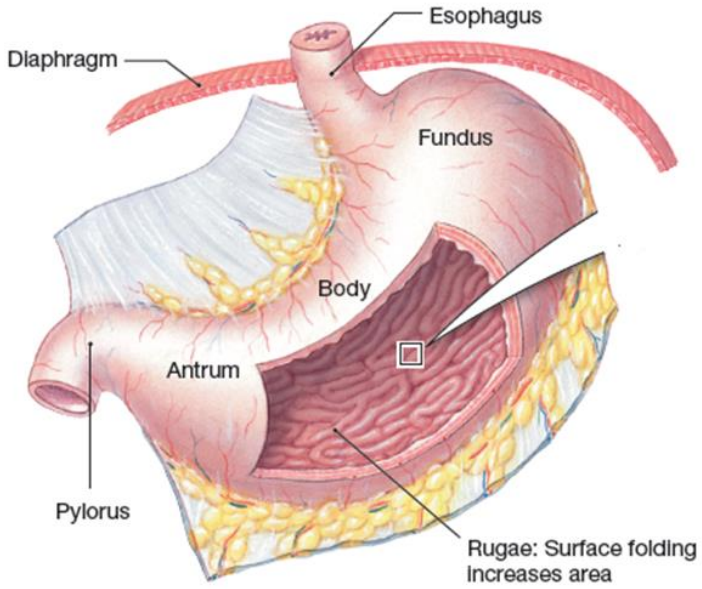


Figure 04: Internal macroscopic anatomy of the stomach
(https://www.britannica.com/science/human-digestive-system/Esophagus)

3.2.2. Submucosa

The submucosa is the middle layer of the gut wall. It is composed of connective tissue with larger blood and lymph vessels running through it (figure 05). The submucosa also contains the submucosal plexus (plexus, interwoven), one of the two major nerve networks of the enteric nervous system. The submucosal plexus (also called Meissner's plexus) innervates cells in the epithelial layer as well as smooth muscle of the muscularis mucosae (Whitcomb, 2015).

3.2.3. Muscularis Externa

The outer wall of the gastrointestinal tract, the muscularis externa, consists primarily of two layers of smooth muscle: an inner circular layer and an outer longitudinal layer (figure 05). Contraction of the circular layer decreases the diameter of the lumen. Contraction of the longitudinal layer shortens the tube. The stomach has an incomplete third layer of oblique muscle between the circular muscles and the submucosa (figure 05). The second nerve network of the enteric nervous system, the myenteric plexus {myo-, muscle + enteron, intestine}, lies between the longitudinal and circular muscle layers. The myenteric plexus (also called Auerbach's plexus) controls and coordinates the motor activity of the muscularis externa (Furness, 2006).

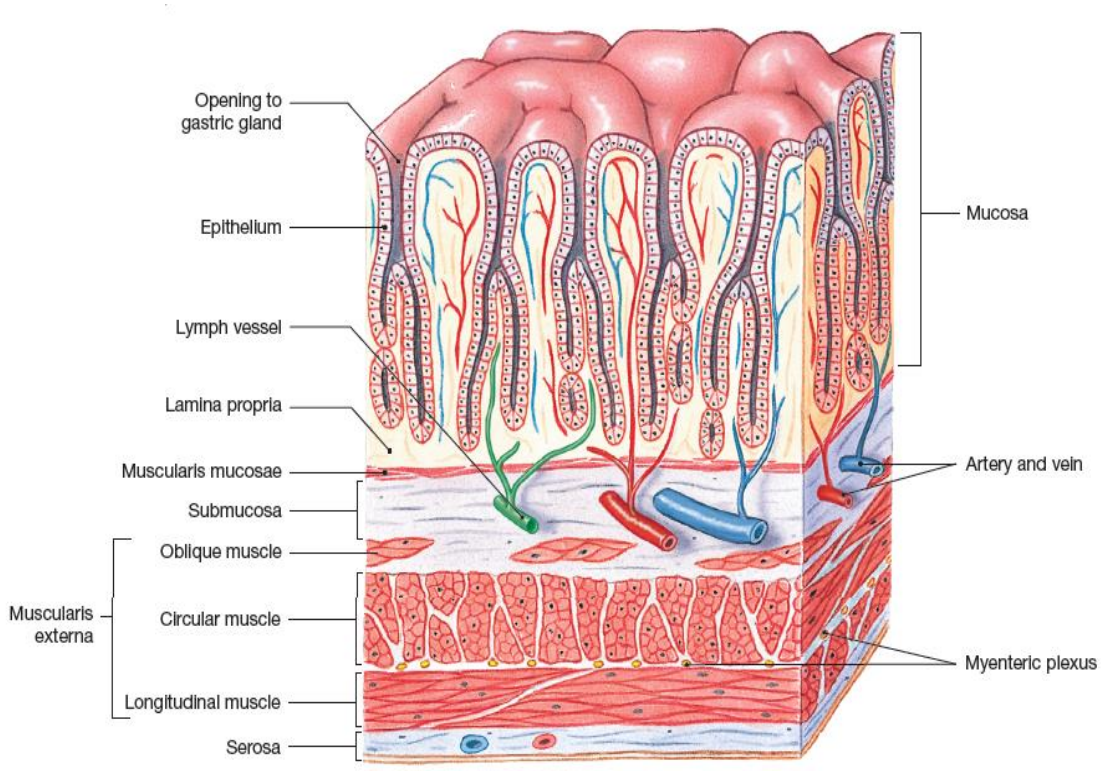


Figure 05: Histology of the stomach (Tortora and Derrickson, 2010).

3.2.4. Serosa

The outer covering of the entire digestive tract, the serosa, is a connective tissue membrane that is a continuation of the peritoneal membrane (peritoneum) lining the abdominal cavity. The peritoneum also forms sheets of mesentery that hold the intestines in place so that they do not become tangled as they move. The next section is a brief look at the four processes of secretion, digestion, absorption, and motility. Gastrointestinal physiology is a rapidly

expanding field, and this textbook does not attempt to be all inclusive. Instead, it focuses on selected broad aspects of digestive physiology (Marieb, 2008; Daniel *et al.*, 2001; Furness, 2012).

3.3. Gastric secretion

The gastric mucosa secretes 1.2 to 1.5 litres of gastric juice per day. Gastric juice renders food particles soluble, initiates digestion (particularly of proteins), and converts the gastric contents to a semiliquid mass called chyme, thus preparing it for further digestion in the small intestine. Gastric juice is a variable mixture of water, hydrochloric acid, electrolytes (sodium, potassium, calcium, phosphate, sulfate, and bicarbonate), and organic substances (mucus, pepsins, and protein) (Al-Howiriny *et al.*, 2005). This juice is highly acidic because of its hydrochloric acid content, and it is rich in enzymes. As noted above, the stomach walls are protected from digestive juices by the membrane on the surface of the epithelial cells bordering the lumen of the stomach; this membrane is rich in lipoproteins, which are resistant to attack by acid.

3.3.1. Acid and bicarbonate secretion

Gastric acid secretion is produced in several steps. Chloride and hydrogen ions are secreted separately from the cytoplasm of parietal cells and mixed in the canaliculi. Gastric acid is then secreted into the lumen of the gastric gland and gradually reaches the main stomach lumen. Gastric acid secretion is regulated by several molecules such as Acetylcholine released by parasympathetic neurons, Histamine released by mast cells and gastrine produced in G cells of the gastric antrum (Schubert, 2017). Proton secretion occurs in the parietal cell by exchanging H for K via the HK-ATPase. This is coupled with extrusion of Clvia an apical chloride channel and K via an apical potassium channel (Schubert and Peura 2008; Schubert, 2017).

The enzyme carbonic anhydrase catalyses the reaction between carbon dioxide and water to form carbonic acid. This acid immediately dissociates into hydrogen and bicarbonate ions. The hydrogen ions leave the cell through H+/K+ ATPase antiporter pumps (Tortora and Derrickson, 2007). Bicarbonate secretion in the stomach is stimulated by many catalytic factors such as acetylcholine, prostaglendins and gastric acidity. in the other hand HCO3- ions secretion can be inhibited by noradrenaline (Sun *et al*., 2004).

3.3.2 Mucus secretion

The gastrointestinal mucus is a hydrogel that lines the luminal side of the gastrointestinal epithelium, offering barrier protection from pathogens and lubrication of the intraluminal contents, it is synthesized and secreted by specialized goblet and mucous cells in the columnar epithelia that line the lumen of all of the organs and glands that are exposed to and communicate with the external environment (Barmpatsalou *et al.*, 2021). Mucus is a complex aqueous fluid (74–95% water) that owes its viscoelastic, lubricating and hydration properties to the glycoprotein mucin (1–5%) combined with electrolytes, lipids (1–2%) and other smaller proteins (Dubbelboer *et al*., 2022). cAMP and Ca^{2+} are the internal correspondence on which both secretine, prostaglandines and acetylcholine are dependent in stimulating the release of mucous (Hamada *et al.*, 1997).

3.4 Peptic ulcer: Pathophysiology and Etiologies

Peptic ulcer is defined as disruption of the mucosal integrity of the stomach and/or duodenum due to imbalance between aggressive and protective factors. It is characterized by high acidity resulting in mucosal erosion causing extreme pain and discomfort (Siddique, 2014). Aggressive factors can be exogenous like *Helicobacter pylori (H. pylori)*, None steroidal anti-inflammatory drugs (NSAIDs), alcohol, fatty foods and smoking and endogenous factors like acid pepsin hyper secretion, stress, free radical generation (ROS and LPOs) (Onasanwo *et al.*, 2010) bile and reduced local blood flow are the major factors that disrupt this balance (Repetto and Llesuy, 2002; Srivani and Dhanraju, 2013; Siddique, 2014,). Endogenous gastro protective factors include alkali mucus secretion, mucosal microcirculation, PGE PGI, NO (7, 8), gastric mucosal glycoproteins HSPs and GSH (Repetto and Lesuy, 2002)

Patients with perforated peptic ulcers typically present with symptoms including abdominal pain, nausea, bloating and feeling of fullness. The classic triad of signs and symptoms is described as sudden onset abdominal pain, tachycardia and abdominal rigidity

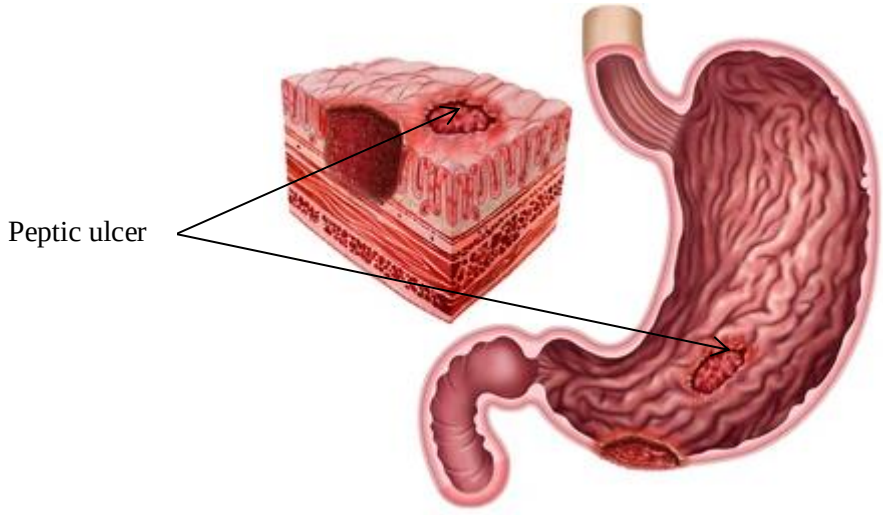


Figure 06: Representation of peptic ulcer

3.4.1. Epidemiology of Peptic Ulcer Disease

Peptic ulcer disease (PUD) represents a worldwide health problem because of its high morbidity, mortality and economic loss. PUD affects 4 million people worldwide annually. The incidence of PUD has been estimated at around 1.5% to 3%. Perforated peptic ulcer (PPU) is a serious complication of PUD and patients with PPU often present with acute abdomen that carries high risk for morbidity and mortality. The lifetime prevalence of perforation in patients with PUD is about 5%. PPU carries a mortality ranging from 1.3% to 20%. Thirty-day mortality rate reaching 20% and 90-d mortality rate of up to 30% have been reported (Chung and Shelat, 2017).

3.4.2. Models of Peptic Ulcer

Animal models have played a significant role in research that aims to understand peptic ulceration. They have helped define basic mechanisms of gastric mucosal defense and repair (Lee, 2000). A wide number of models for inducing gastric lesions are used to screen for the ability of new therapeutics to protect the gastric mucosa. The choice of a particular model is sometimes influenced by local resources, the objectives of the study, the hypothesis being tested, or research questions being answered by the researcher. The choice of model may also depend on the relevance to the type of peptic ulcer disease under investigation (Adinortey *et al.*, 2013). Gastric mucosal lesions can be induced by hypothermic restraint stress, pylorus ligation, or agents that damage of necrotize the mucosa including ethanol, HCl, NaOH, and non-steroidal anti-inflammatory drugs (like aspirin, indomethacin, and phenylbutazone) (Simões *et al.*, 2019)

3.4.2.1. Ethanol induced ulcer

It has long been known that alcohol consumption can trigger inflammation of the gastric mucosa. Once administered, ethanol rapidly penetrates the gastrointestinal mucosa causing membrane damage, cell exfoliation, and erosion. Increased mucosal permeability to gastric acid resulting from these effects, together with the release of vasoactive products from mast cells, macrophages, and blood cells can lead to necrosis and subsequently to ulcer formation (Simões *et al*., 2019). Ethanol directly affects the gastric mucosa by reducing bicarbonate and mucus secretions and exposing it to the damaging effect of acid and pepsin or by acting on neurohormonal factors that stimulate acid secretion (Bode and Bode, 1997). Ethanol may also decrease blood flow (Adinortey *et al*., 2013).

A number of studies have suggested that ethanol-induced gastric damage is associated with generation of oxygen-derived radicals independent of the xanthine oxidase system. Reduction of prostaglandin synthesis and increased synthesis of leukotrienes are other processes may also contribute to alcohol-induced gastric lesions. Thus, the use of the ethanol-induced ulcer model allows the induction of gastric ulcers by direct action on the mucosa (Cheng *et al.*, 2000; Chang *et al.*, 2017; Liang *et al.*, 2018).

3.4.3. Management of Peptic Ulcer Disease

Peptic ulcer disease once was a condition with great morbidity and mortality and surgery was the only curative treatment option. This changed dramatically since the introduction of medical therapy with H2-receptor antagonists, and later on with proton pump inhibitors. The most important step forwards in the treatment of this chronic recurrent disease was the discovery of *H. pylori* and the acceptance of its pathogenetic properties (Loffeld *et al*., 2016). The treatment goal of peptic ulcer disease include; *H. pylori* eradication, healing of ulcers, prevention of recurrences and complications of ulcer. The management principle of peptic ulcer depends on the etiologic factor of ulcer.

3.4.3.1. Management of Non-Helicobacter related peptic ulcers

It is the treatment of ulcer with different classes of drugs that reduce the aggressive factors and or increase the endogenous defensive mechanism. These agents include anti-secretary drugs, acid neutralizing agents and mucosal protective barriers (Townsend, 2013).

- **Antacids**

Antacids reduce gastric acidity by neutralizing HCl, forming a salt and thereby raising the gastric PH. Antacids differ greatly in their buffering ability, absorption, and side effects. They are most effective with meal because they can be retained in the stomach and exert their buffering action for longer periods (Townsend, 2013).

- **H2 Receptor Antagonists**

The H2 receptor antagonists (Ranitidine, Famotidine and Cimetidine) are structurally similar to histamine and selectively blocking the action of histamine at the histamine H2 receptors of parietal cells and suppress basal and meal-stimulated acid secretion (Katzung, 2009).

- **Proton Pump Inhibitors**

Proton pump inhibitors (Omeprazole, Esomeprazole, Lansoprazole, Pantoprazole, and Rabeprazole) are the most potent anti-secretory agents and negate all types of acid secretion from all types of secretagogues. Drugs under this group forms a covalent disulfide bond with the H+/K+ ATPase, irreversibly inactivating the enzyme (Mössner, 2016; Maes *et al.*, 2017).

- **The mucosal Protective Agents**

Sucralfate is an aluminum salt of sulfated sucrose that dissociates under the acidic conditions in the stomach. It is hypothesized that the sucrose polymerizes and binds to protein in the ulcer crater to produce a protective coating that can last for up to 6 hours. It has also been suggested that it may bind and concentrate endogenous basic fibroblast growth factor, which appears to be important for mucosal healing (Townsend, 2013).

Misoprostol is a methyl analog of PGE1. It has both acid inhibitory and mucosal protective properties. It is believed to stimulate mucus and bicarbonate secretion and enhance mucosal blood flow. In addition, it binds to a prostaglandin receptor on parietal cells, reducing histamine-stimulated cAMP production and causing modest acid inhibition (Townsend, 2013).

3.4.3.2. Management of peptic ulcers associated with *Helicobacter pylori*

In clinical practice, the initial course of eradication therapy, heretofore referred to ‘‘first-line’’ therapy generally offers the greatest likelihood treatment success. There is no treatment regimen which guarantees cure of *H. pylori* infection in 100% of patients. *Helicobacter pylori*

eradication regimen includes use of antibiotics and antacids (Alliance; 2002; Fasher and Gitu, 2015). Triple drug regimen of proton pump inhibitors, amoxicillin and clarithromycin with an eradication rate of 80% to 90% has long been the recommended first-line therapy to eradicate *H. pylori* (Loffeld *et al.,* 2016; Chey *et al.,* 2017). Substitution of metronidazole for amoxicillin showed eradication rate of 70% to 85% (Alliance; 2002; Fasher and Gitu, 2015). However, increasing resistance to clarithromycin is associated with declining eradication rates.

3.4.4. Herbal therapy of Peptic ulcer

Herbal medicines are now commonly used all over the world and this has increased global demand. Quality, safety, and efficacy of these drugs have become a serious concern. (Kumadoh *et al.,* 2021).

Studies have demonstrated that the efficacy of herbal medicines is comparable or superior to that of drugs such as omeprazole or cimetidine in humans and animal models, and herbal medicines display fewer adverse effects. The mechanisms by which herbal medicines benefit gastric ulcer include stimulation of mucous cell proliferation, anti-oxidation, and inhibition of gastric acid secretion and H (+)/K (+)-ATPase activity. Some herbal medicines also exhibit antimicrobial properties. Utilization of herbal medicines could be a valuable alternative to treat gastric ulcer in humans effectively, with few adverse effects (Bi *et al.,* 2014).

Chloroform and ethanol extract of *Arctium lappa* Lowering gastric acid secretion via gastric H+, K+ -ATPase, renewing of the damaged gastric mucosa (Da Silva *et al.,* 2013). Data published by Zhang and his collaborator (2021) demonstrated that The flower extracts of *Jasminum grandiflorum* alleviated gastric mucosal ulceration by increasing PGE2 production and the activity of antioxidant enzymes, along with the suppression of reactive oxygen species (ROS) generation, lipid peroxidation, apoptosis-related proteins, pro-inflammatory cytokines and nitric oxide (NO) production. Ngobidi *et al*., (2018) suggest that *camellia sinensis* (green tea) possess antiulcer activity, which could be due to its cytoprotective actions or strengthening of gastric mucosa, with the enhancement of mucosal defense. Oxyresveratrol, an active ingredient of *Artocarpus lakoocha*. oxyresveratrol possess significant anti-ulcer property which might be attributed to attenuated expression levels of IL-6, TNF-α, NF-κB, and COX-2 and elevated expression levels of TFF-2 (Aziz *et al.,* 2019).

4. Selected Lamiaceae plants

4.1. *Phlomis crinita Cav*

4.1.1. Botanical description

Phlomis crinita Cav. is a rare and endemic Algerian species. It is one of nine endemic plants recorded in the National Report on Biological Diversity in Algeria (UNDP, 2014), and its disturbed in the western Mediterranean area, in Algeria, Tunisia, Morocco and Spain.The plant is 75 cm tall, its basal leaves are 6-18 mm long, they are lanceolate or oval, the floral leaves are rhombic of 2-4 x1-2.5 cm and sessile. Bracts less than 1 mm wide, linear 10-18 mm with soft hairs exceeding 3 mm, emanating from a stellar base. The calyx is covered with stellate, silky linear-toothed hairs; the corole is brown to yellow intensely of gold. the stems of 50-100 cm, rowing and have a gray color. The popular name of *P. crinita* in Algeria is El Khayatta or Khayatt adjarah (Quézel and Santa, 1963; Pottier-Alapetite, 1976; Adjoudj and Latreche, 2017).

4.1.2. Botany systetematics of the plant

Kingdom: Plantae

Phylum: Spermaphytes

Subphylum: Angiosperms

Class: Dicotyledons

Subclass: Asteridae

Order: Lamiales

Family: Lamiaceae

Genus: Phlomis

Species: *Phlomis crinita Cav*

Figure 07: *Phlomis crinita* (www.monjardin.org).

4.1.3. Traditional uses of *P. crinita* in folk medecine

Flowers and leaves of the *P. crinita* have been used in folk medicine since the old. in Spain, Tunisia and Algeria a chopped leaves preparation used as plaster to treat burns and for cicatrizant, skin lesions and infections as well as certain allergies (Quézel and Santa, 1963; Pottier-Alapetite, 1976; Limem-Ben Amor *et al*., 2009). It is also used to relieve pain and/or inflammation of muscles and joints (sprains, bruises, pain and for its gastroprotective properties (Gürbüz *et al*., 2003; Limem-Ben Amor *et al*., 2009).

4.1.4. Chemical composition and biological activities

More than 151 compounds have been isolated and identified from *Phlomis* genus so it's very rich in several classes of natural constituents (polyphenols flavonoids, tannins aromatic oils …. extr). The major constituents of flower oil of *P. crinita* were β-caryophyllene (58.1%) and germacrene D (35.1%), the essential oil obtained from the leaves was mainly composed of trans-caryophyllene (40.8%) and germacrene D (39.1%) (Limem- Ben Amor *et al.*, 2008). Kabouche *et al.*, 2005 demonstrated that *P. crinita* extacts contain a phenylethanoid glycoside (verbascoside = aceoside) and three known flavonoids namely chrysoeriol 7-(3''-p-E-coumaroyl)-b-D-glucopyranoside, lutéoléine and lutéoléine-7-O-β-lucopyranoside, 6"-

syringylsesamoside in addition polar extracts from *Phlomis crinita* characterized by the presence of tannins, flavonoids, iridoids, sterols, cardiac glycosides, and anthraquinones (Dellai *et al.,* 2009; Limem- Ben Amor *et al.,* 2008; Tamert *et al.*,2016)

Due to the widespread uses of the *Phlomis crinita* in folk medicine and its richness with numerous secondary metabolites, there are many experimental studies to confirm the scientific activity and the relationship between the latter and chemical composition of this plant. Limem- Ben Amor and his collaborator (2009) demonstrated that the aqueous extract of *Phlomis crinita* reduced alcohol-induced ulcerogenesis in mice. It reduces ulcerogenic by 91%, It demonstrates that these species possess high gastroprotective activity by protecting the stomach epithelia from alcohol-induced lesions. This finding proves the ethnopharmacological uses of *Phlomis* as a gastroprotective plant.

Antimicrobial activity of *P. crinita* was investigated against *Porphyromonas gingivalis* and *Prevotella intermedia* which are Gram-negative strains implicated in periodontal diseases. These plant aqueous extract showed powerful bacterial activity against these bacterial strains Arbia *et al.,* 2017). The essential oil extracted from the leaves and flowers of *Phlomis crinita* inhibited the growth of *Staphylococcus aureus, Enterococcus faecalis,* and *Salmonella typhimurium* (Limem- Ben Amor *et al*., 2008).

Several literatures demonstrated that *P. crinita* extracts exhibited a strong antioxidant activity which estemated by numerous methods, one of them is radical scavenging activity using DPPH (Merouane *et al*., 2018, Tamert and latrech, 2016) and ABTS (Dellai *et al.,* 2009) as a free radical. Merouane *et al.,* (2018, 2020) showed that *P. crinita* extract can scavenge ROS in the lipid milieu.

Aqueous and methanolic leaf extracts from *Phlomis crinita* subs. mauritanica Munby were improved their potential immunomodulatory activity on mouse lymphocytes and macrophages in vitro (Limem *et al.,* 2011) and its antimutagenicity (Dellai *et al.,* 2009).

4.2. *Phlomis heba venti*

4.2.1. Botanical description

Phlomis can be herbaceous perennials or evergreen shrubs, with sage-like leaves and whorls of tubular, hooded flowers over a long period in summer. *Phlomis herba venti* L., is an annual plant characterised by a raised stem very ramified and pyramidal (20 – 50 cm), lower leaves

are lanceolate and petiolate, but the upper ones are lanceolate and linear, quickly sessile. Calyx with a long prickly serration, longer than the tube (Caroline *et al*., 2003). It's disturbed in the western Mediterranean area, in Algeria, Tunisia, Morocco, Spain (Fernández-Oca˜na *et al.*,1996) and Iran (Morteza-Semnani *et al.*, 2004). Its vernacular name "Djeda"

4.2.2 Botany systetematics of the plant

Kingdom: Plantae

Sub- Kingdom: tracheobionta

Class: magnoliophyta

Subclass: Asteridae

Order: Lamiales

Family: Lamiaceae

Genus: Phlomis

Species: *Phlomis herba venti* (Caroline *et al*., 2003).

4.2.3. Traditional uses of *P. herba venti* in folk medicine

P. *herba-venti* is specie belonging to the Lamiaceae family, which have been traditionally used to prepare tonic and digestive drinks. Calyx of *P. herba venti* are used as Veterinary antidiarrheic and soothe muscle pains. the *Phlomis herba-venti* has been used as anti inflammation and anti infection in traditional medicine in Iran (Jafari Footami *et al*., 2017).

4.2.4. Chemical composition and biological activities

Twenty-six compounds were identified in the flower oil: germacrene D (33.9%), hexadecanoic acid (12.9%) and α-pinene (9.4%), 2-pentadecanone (7.6%) and α-copaene (3.2%) are the major constituents. In the flower, the major components among the 25 constituents identified were hexadecanoic acid (33.1%), 6,10,14-trimethylpentadecan-2-one (16.2%), 3-methyltetradecane (6.7%), terpinolene (9.1%) and germacrene D (6.7%) and β-bourbonene (5.9%). All oils consisted of monoterpenes, sesquiterpenes and aliphatic compounds. (Mortez-Semnani *et al*., 2004; Khalilzadah *et al*., 2008; Delnavazi *et al*., 2014).

Figure 08 : *Phlomis herba venti* (www.monjardin.org).

Ferrante *et al.,* 2019 demonstrated that *P.herba venti* extracts are rich in benzoic acid and tow known flavonoids namely epicatechin and rutin and this finding is in line with several data which improved the richness in *P.herba venti* extacts with flavonoids and polyphenols (Azad *et al.*, 2016; Gaamoune, 2017) in addition polar extracts from *Phlomis herba* venti characterized by the presence of iridoids such as lamiid (Alipieva *et al*.; 2000) and type of stéroïde which is Stigmastérole-3β-D-glucopyranoside (Bouzergoune, 2017).

Antimicrobial effects of essential oils and other extracts are taking the most attention in the investigations of the pharmacological potency of these species. Ferrante and his collaborator (2019) showed that alcoholic extract of *P. herba venti* exhibited strong inhibitory effects on specific bacterial fungal strains (*Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, Candida albicans* and *Candida tropicalis.*). In addition, the flavonoids extracted from *P. herba venti* w*as* improved their antimicrobial activity against others bacterial and fungal strains such as *Bacillus subtilis, Klebsiella pneumonia, Salmonella typhimurium, Aspergillus niger and Aspergillus flavius* (Mortez-Semnani *et al.,* 2006; Gaamoune, 2017).

There are several studies demonstrated that *P. herba venti* extracts exhibited a strong antioxidant activity which evaluated by numerous methods, one of them is radical scavenging activity using DPPH (Delvarasi et *al*; 2014; Azad *et al*., 2016; Gaamoune, 2017) as a free

radicals and reduction of transformed metal (Gaamone, 2017). Mortez-Semnani *et al*., (2006) showed that *P. herba venti* extract can scavenge ROS in the lipid milieu. Analgesic properties have been reported for some Phlomis species like *Phlomis caucasica, Phlomis fruticosa, Phlomis herba-venti, Phlomis lychnitis* (Sarkhail *et al*., 2003).

Materials and Methods

1. Materials

1.1. Plants materials

The flowering aerial part of *P. crinita* and *Plomis herba venti* were collected from Jijel city (North-East of Algeria). After the botanical identification of the plant, the areal part was washed by tap water to get rid of impurities and dust, dried in the shade at room temperature, and then ground into a mortar to form a fine powder. Each plant powder was then used for the extraction.

1.2. Animals

Healthy male and female adult albino rats weighing 150–250 g were purchased from Pasteur Institute, Algiers. Animals were kept for one week and housed in an air-conditioned animal room, with light/dark cycle naturele photoperiod, and given free access to water and feeding ad libitum for 20 days.

1.3. Chemicals

Methanol (MeOH), Tannic acid, aluminium trichloride ($AlCl_3$), Butylated hydroxytoluene (BHT), Tween 40, and Carbonate, Sodium phosohate monohydrate, Sodium phosphate dibasique, Ammonium thiocyanate, Ascorbic acid, Iron (II)chloride tetrahydrate. The various products used were purchased from Merck and Sigma.

β-carotene,Linoleic acid, Ammonium thiocyanate, butylated hydroxytoluene (BHT) were purchased from Fluka Chemical Co. (Buchs, Switzerland). The chemicals such as 2,2-diphenyl- 1picryl-hydrazyl (DPPH), ethylenediamine tetraacetic acid (EDTA), Gallic acid, 6-hydroxy- 2,5,7,8-tetramethyl chroman-2-carboxylic acid (trolox), 2.2´-Azino-bis(3-ethylbenzenothiazoline-6-sulfonic acid) (ABTS), Folin-Ciocalteu reagent were obtained from Sigma Chemical Co. (St. Louis, Missouri, USA). Potassium persulphate, potassium ferricyanide, trichloroacetic acid, thiobarbituric acid (TBA), ferrozine, ferrous and ferric chloride were obtained from Merck. Alloxan from Sigma, St. Louis, MO, USA. All other reagents were of analytical grade.

2. Methods

2.1. Extraction Procedure

The PC ME and PV ME of the plants were prepared as described elsewhere by Marrkham (1982) with slight modification. Briefly, the dried powder of *P. crinita* and *P.herba venti* was extracted at room temperature with methanol (85%) for 72 hours. The resulted mixtures were filtered and evaporated at 45 C° to form methanolic extracts. Whilst, the aqueous extract was prepared according to the method previously described by Kandil and collaborators (1994). Here a 100 g of plants material powder was boiled in 1 L of distilled water for 4 hours, and then filtered and dried at 45°C to form an aqueous extract. The dried ME and AQE extracts were screened for their pharmacological properties.

2.2. Quantitative determination of phytochemicals

2.2.1. Determination of total polyphenols

The total phenolic compounds in plants extracts were determined by the Folin-Ciocalteu (FC) method with slight modifications (Sánchez-Rangel *et al*., 2013). Briefly, 100 µL of each extract or standard (gallic acid) was mixed with a 500 µL folin-ciocalteu reagent (diluted 10 times). After 4 minutes, 400 µl of 7.5% sodium carbonate solution (Na_2Co_3) was added, and then the resulted mixture was incubated for 1 hour and a half at the laboratory temperature. The mixture absorbance was measured against blank at 765 nm, and the total polyphenols content was expressed as gallic acid equivalents (mg GA/g DW). All measurements are repeated 3 times (figure 09).

2.2.2 Determination of total flavonoids contents

The total flavonoid contents in plant extracts were quantified by $AlCl_3$ method (Bahorun *et al*., 1996). Briefly, 1 mL of $AlCl_3$ solution (2% dissolved in methanol) was mixed with 1 mL of the extract samples and incubated at room temperature for 10 min prior to the absorbance reading at 430 nm. The results were expressed as mg of quercetin or rutin equivalent per gram of dry plant extract weight (mg QE/g extract); (mg RE/g extract).

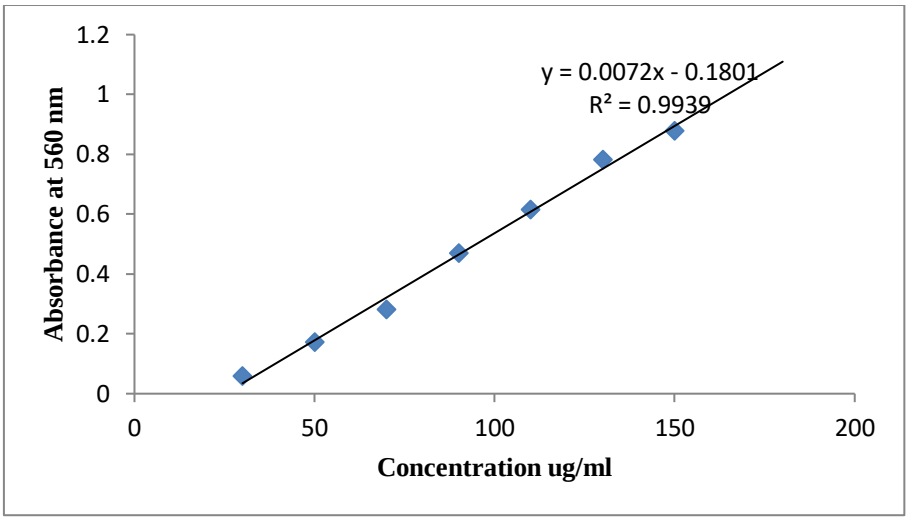


Figure 09: Standard curve of gallic acid for the determination of total polyphenols. (mean± SD).

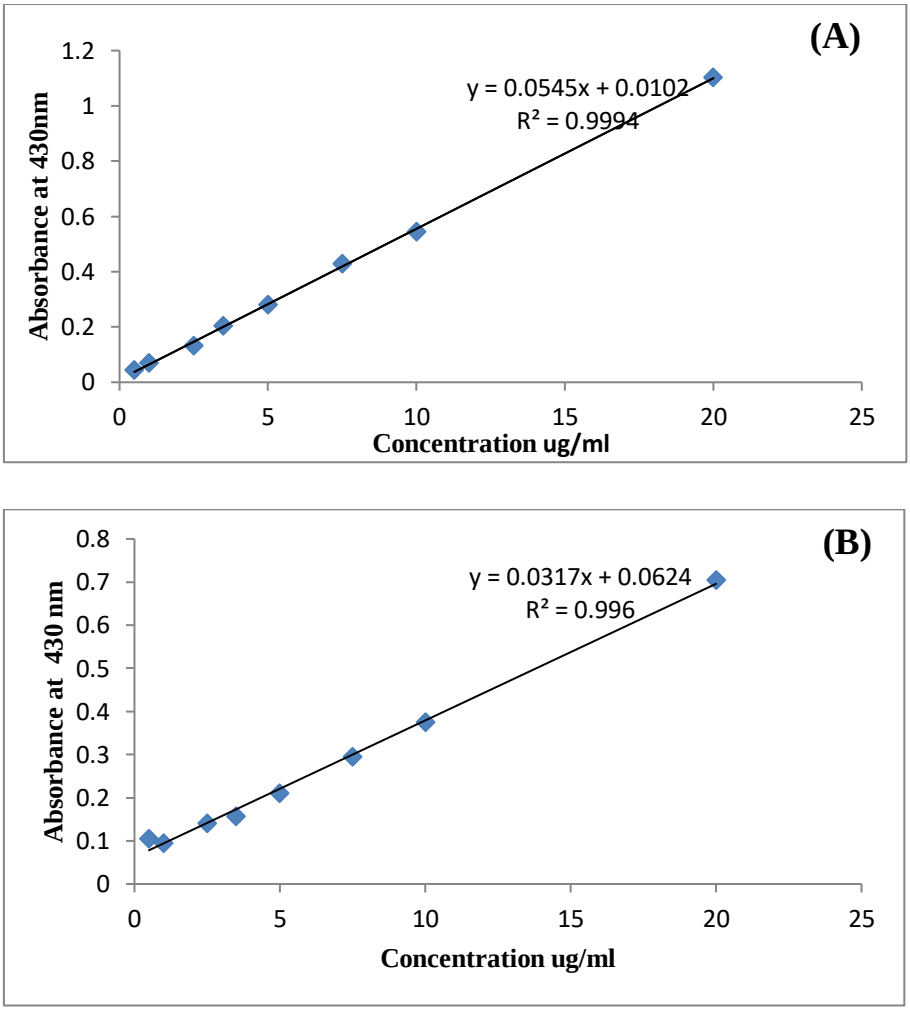


Figure 10: Standard curve of quercetin (A) and rutin (B) for the determination of total flavonoids. (mean± SD of three measurements).

2.2.3. Tannins quantification

The total tannins content in plants extracts was quantified by the method of Bate smith (1973) based on the precipitation of hemoglobin from fresh bovine blood by tannins. In brief, a volume of diluted blood solution (optical density = 1.6) with the same volume of plants extracts was mixed and dissolved in distilled water. After 20 min of incubation, the mixture was centrifuged (400 rpm for 10 min) and then subjected to spectrophotometric measurement at 576 nm. The amount of tannins was expressed as tannic acid equivalents (mg tannic acid/g dried extract).

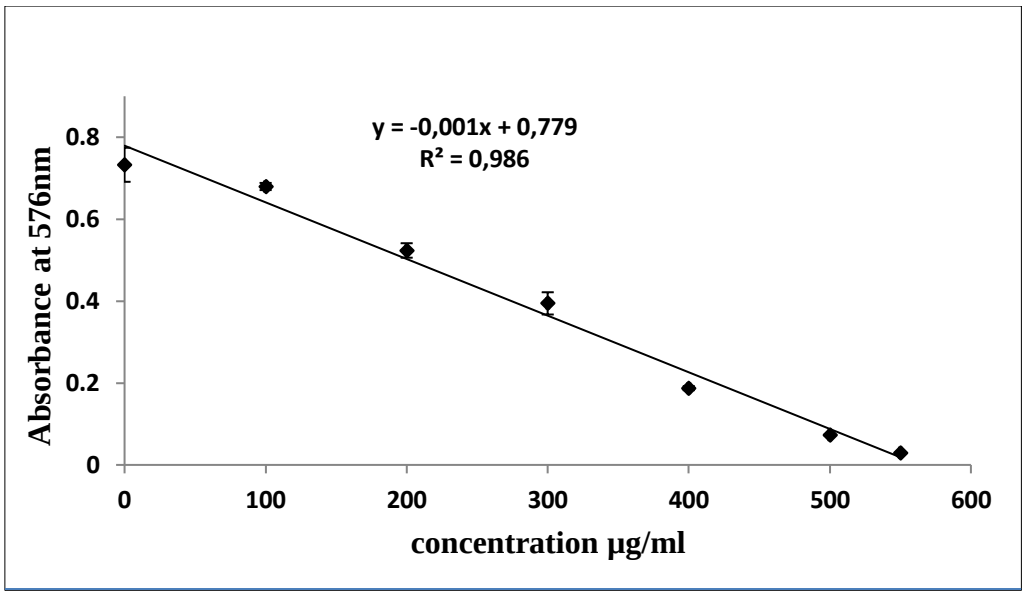


Figure 11: Standard curve of tannic acid for the determination of total tanins. (mean± SD of three measurements).

2.4. *In vitro* pharmacological effects

2.4.1. Antioxidant activities

2.4.1.1. DPPH free radical-scavenging assay

The scavenging effect of the plants extracts on the DPPH free radical scavenging ability was spectrophotometrically determined as described elsewhere (Burits and Bucar, 2000). In this method, a volume of 50 µL was taken from plants extracts of various concentrations, and mixed with 1250 µL of methanol solution of DPPH (0.004%). Of note, the butylated hydroxytoluene (BHT) was used as a positive control. All mixtures were incubated for 30 minutes in darkness at room temperature prior to spectrophotometric measurement at 517 nm

in comparison with the negative control. The free radical scavenging activity of the extracts is expressed in percent % and calculated as follow:

Scavenging activity (%) = (AC – AS) * 100 / AC

Where Ac and As are the absorbances of the DPPH solution without extract and the test sample respectively. The antioxidant activity is expressed in terms of IC_{50} (mg/mL).

The IC_{50} value is the sample concentration required to reduce the absorbance of the solution controls by 50% in order to form methanol and DPPH. The IC_{50} values were determined using linear regression, with the X-coordinate representing the concentration of the substances tested and the amount of inhibition ordered by (I %).

2.4.1.2. ABTS radical scavenging assay

The antioxidant activity of the plant's extracts assayed by ABTS method was according to procedures described by Re *et al*. (1999) with slight modifications. The working solution was prepared by mixing two stock solutions (mM of ABTS solution and 2.4 mM of potassium persulfate solution) in equal quantities and allowing them to react for 24 h at room temperature in the dark. The solution was mixed and diluted in 1 mL cuvette containing ABTS solution and methanol to obtain a spectrophotometric absorbance of 0.706 ± 0.01 at 734 nm. The Fresh ABTS solution was prepared for each assay. Plant extracts (50 µl) were afterward mixed with 1 mL of the ABTS solution and the absorbance was read at 734 nm after 30 min. The ABTS scavenging capacity of the extracts was compared to that obtained with BHT. ABTS radical scavenging activity is calculated as follows:

ABTS radical scavenging activity (%) =Abs control−Abs sample /Abs control

Where Abs control is the absorbance of ABTS radical in methanol; Abs sample is the absorbance of ABTS radical solution mixed with sample extract/standard.

All determinations were performed in triplicate (n = 3).

2.4.1.3. β-Carotène-linoleic acid bleaching assay

The antioxidant activity in this test was determined by the ability to inhibit the formation of diene conjugated hydroperoxide bonds and volatile organic compounds resulting from the oxidation of linoleic acid (Dapkevicius *et al*., 1998). This method consists to dissolve 0.5 mg

of β-carotène in 1 mL of chloroform, to add 25 µL of linoleic acid and 200 mg of Tween 40. The chloroform was completely evaporated at 40 °C, and followed by addition of 100 mL of oxygen-saturated distilled water. After that, the emulsion system was prepared by adding 2.5 mL of the resulted mixture and placed in test tubes containing 350 µL (2 mg/mL) of the prepared samples and incubated at darkness and room temperature. BHA and Gallic acid (GA) were also used as a positive control. The absorbance was measured at 490 nm during 0, 1, 2, 3, 4, 6, 12, and 24 h. The antioxidant activity of the samples was compared to that of BHT and negative control. The activity of relative antioxidant extracts (AA%) is calculated according to the following equation:

$$\mathbf{A\% = (A_S / A_C) \times 100}$$

Where **Ac** and **As** are the absorbance of BHA at t=0 and test sample respectively.

2.4.1.4. Ferrous ion chelating activity assay

The Ferrous ion chelating activity of the plants extracts was determined by the method based on the capacity of extract samples to chelate ferrous ion and to inhibit the formation of ferrous ion-ferrozine complex, after long term incubation with $FeCl_2$ (Decker and Welch, 1990). 250 µL of the extracts (in different concentrations) was mixed with 50 µL of $FeCl_2$ (0.6mM in distilled water) and 450 µL of methanol. After 5min of incubation time, 50 µL of ferrozine (5mM) solution was added and the mixture was then stirred for 10 minutes up to the end of the reaction. The absorbance was measured at 562 nm, and the rate of chelation of iron ions is calculated according to the following equation:

$$\mathbf{Chelation\ (\%) = [(Ab_C - Ab_S) / Ab_C] \times 100}$$

Where **Abc** is the control absorbance, and Abs is the absorbance of the test sample

2.4.1.5. Reducing power assay

Then reducing power of the plant extracts was determined by the method previously described (Chung *et al.*, 2005). In brief, 0.1 mL of various methanol-diluted extracts were mixed with the same volume of phosphate buffer (0.2 M, pH=6.6) and 0.1 mL of K_3FeCN_6 (1%). The resulting mixture was incubated at 50° C for 20 min, and 0.25 mL (1%) of acid trichloroacetic was then added to stop the reaction of transformating ferricyanide to ferrocyanide. Thereafter, the mixture was centrifuged at 3000 /minutes for 10 minutes, and 0.25 mL of the supernatant was added to 0.25 mL of distilled water and 0.5 mL $FeCl_3$ (0.1%). The absorbance of the resulting solution was measured at 700 nm to quantify the formed ferric ferrocyanide.

2.4.2. Enzymatic inhibitory assay

2.4.2.1. α-Glucosidase inhibition assay

Alpha-glucosidase assay was performed according to the chromogenic method described by Schäfer and Högger (2007), with slight modifications. Briefly, 3mM of p-nitrophenyl α-D-glucopyranoside (pNPG) was prepared in 0.1M phosphate buffer, adjusted to pH 6.9, to simulate a model of intestinal fluid. Yeast α-glucosidase was dissolved in 0.1M phosphate buffer, pH 6.7, to yield a final stock-solution of 1 IU/ml. For each assay, 0.075 IU of enzyme solution was premixed with *P. crinita and P. herba venti* extracts at various concentrations and pre-incubated for 10 min at 37°C. The enzymatic reaction was initiated by adding 0.95mM pNPG and the reaction mixture was incubated for 10 min at 37°C. The activity of α-glucosidase was determined by measuring the product ρ-nitrophenol released from ρNPG at 405nm using a microplate reader and compared to that of the control which had buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as inhibition percent and was calculated as follows:

$$\textbf{\% inhibition} = [(\Delta \textbf{Abscontrol} - \Delta \textbf{abssample}) / \Delta \textbf{Abscontrol}] \times 100$$

The inhibitory results were expressed as the half maximal inhibitory concentration (IC_{50}), which is a measure of the effectiveness of a compound in inhibiting biological or biochemical function.

2.5. *In vivo* biological activities

2.5.1. Antidiabetic activity of plants extracts *in vivo*

Diabetes was induced in rats as described by Sharma *et al* (2010) with some modifications. A single intraperitoneal injection of a freshly prepared alloxan monohydrate solution (120 mg/kg) in cold normal saline (0.9%) was made to overnight fasted rats. Animals, which did not develop more than 200 mg/dl blood glucose levels, were rejected.

The rats were divided into 9 groups of 7 animals each, and the treatment was given every day via orogastric tube for 15 days as the following:

Group1: received normal saline (0.9%) and served as healthy group.

Group2: received ME of *P. herba venti* at the dose of 250 mg/kg.

Group 3: received ME of *P. herba venti* at the dose of 500 mg/kg.

Group 4: was treated with ME *P. crinita* at the dose of 250 mg/kg.

Group5: was treated with ME of *P. crinita* at the dose of 500 mg/kg.

Group 6: received glibinclamide (3 mg/kg) and served as drug control or reference control.

Group 7: diabetic but received only normal saline (0.9%) and served as alloxan group.

After 15 days of treatment, the rats were sacrificed. Blood was collected into 2 tubes: the first tubes, containing heparin and the second tubes containing EDTA, the blood in tubes containing heparin was used to obtain the plasma which was separated by centrifugation (15 min, 3000rpm) and was separated into two portions: one portion was analyzed for: Triglycerides, total cholesterol, LDL, HDL, Uric acid, TGO or AST, TGP or ALT and Creatinine levels using an automate apparatus. Whereas tubes containing EDTA were used to analyse the hematological parameters. The liver and kidney were removed from each animal for further studies.

2.5.2. Anti-ulcer activity

2.5.2. 1. Ulcer induction

The gastro protective action of *P. crinita and P. herba venti* extracts against the ulcer caused in animals by administration of 70% ethanol was followed the method of Gharzouli *et al.,* (2002). 10 groups of animals were fasted for 20h each group contained 6 males' rats each rat in each group was subsequently separately placed in a cage. The animals were divided and treated as follow:

Group1: ethanol group received NaCl 0.9%.

Group2: served as drug control or reference control received ranitidine 50mg /Kg.

Group 3: received ME of *P. herba venti* at the dose of 250 mg/kg.

Group 4: received ME of *P. herba venti* at the dose of 500 mg/kg.

Group 5: received AQ E of *P. herba* venti at the dose of 250 mg/kg.

Group 6: received AQ E of *P. herba* venti at the dose of 500 mg/kg.

Group 7: received ME of *P. crinita* at the dose of 250 mg/kg.

Group 8: received ME of *P. crinita* at the dose of 500 mg/kg.

Group 9: received AQ E of *P. crinita* at the dose of 250 mg/kg.

Group10: received AQ E of *P. crinita* at the dose of 500 mg/kg.

After 60 min all groups of animals were received 500 ul of 70% ethanol, 30 min later the animals were scarified and the stomachs were removed and opened a long great curvature cleaned with NaCl 0.9% and photographed for the determination of ulcer surface. The digital photos were used for determination of the area (mm^2) of the gastric lesions using image J

1.48d software. For each stomach, the sum of areas of all forms of gastric lesions was recorded including the multifocal erosions and the linear hemorrhagic lesions.
The stomach was used to measure parameters (MDA GSH and catalase).The percent of ulceration was calculated as follow:

% Ulceration = [UAs/ UAc] ×100.

Where: UAc: ulcer area of the control. UAs: ulcer area of the Sample.

2.5.3. *In vivo* antioxidant activity evaluation

2.5.3.1. Preparation homogenate

- **liver and kidney homogenate**

A volum 4.5 mL (1.15M KCl buffer) were added to 0.5 g of liver or kidney tissues, and homogenized using dounce homogenizer in ice-cold condition. The obtained homogenate was centrifuged at
4°C and 4000 rpm for 15 min and the supernatant collected was used for the determination of lipid peroxidation (MDA), catalase activity, reduced glutathione (GSH) and total protein. All experiment were conducted on ice.

- **Stomach homogenate**

Gastric tissue homogenate 10% (w/v) was prepared in ice-cold 50 mM tris HCl (pH 7.4) then centrifuged at 4.000 rpm for 10 minutes (4°C). the supernatant collected was used for the determination of lipid peroxidation (MDA), catalase activity, reduced glutathione (GSH) and total protein. All experiment was conducted on ice.

2.5.3.2. Determination of lipid peroxidation levels

Malondialdehyde (MDA) is one of the markers of lipid peroxidation it is considered as a terminal product formed during the decomposition of polyunsaturated fatty acids (PUFAs) by free radicals. The determination of MDA is based on the test of TBARS (reactive substances with thiobarbituric acid), in an acidic and hot medium. The condensation of MDA with two molecules of thiobarbituric acid, gives rise to a complex of pink colour which can therefore be measured by spectrophotometry at 530 nm. The method of Ohkawa *et al.*, (1979) was used to measure MDA levels. Briefly, 125 µL of each homogenate tissue were mixed with 125 µL of

20% TCA and 250 µL of 0.67 % TBA. The previous mixture was incubated in boiling water for 15 min. after that 1 mL of n-butanol was added to each sample and centrifuged at 3000 rpm for 15 min. 532 nm is the wavelength at which the absorbance of supernant was readed. The concentration of MDA was expressed as µmol/ g tissue.

2.5.3.3. Determination of reduced glutathione (GSH) level

The colorimetric determination of glutathione is based on the reaction of the reductive group (HS) contained in the sample and 5,5'-dithiobis-2-nitrobenzoïque acid (DTNB). This reaction releases an aromatic derivative, thionitrobenzoïque acid (TNB), whose DO measured at 412 nm actually expresses the amount of glutathione formed. Total reduced glutathione (GSH) content was measured as follow, 3 mL of 0.5 % homogenate was mixed with 20 µL of DTNB (0.01 M) after 5 min of incubation the absorbance was measured at 412 nm this method was described by Ellman (1959).

2.5.3.4. Determination of catalase activity

Catalase activity was measured following the method of Clairborne (1985) the principle is based on the degradation reaction of hydrogen peroxide (H_2O_2) to water (H_2O). This activity is measured at 240 nm using a UV/visible spectrophotometer by the variation in optical density following dismutation of H_2O_2. A solution of 19 mM H_2O_2 (2.9 mL) was prepared in 50 mM phosphate buffer pH 7.4 and mixed with 50 µL tissue homogenate. In the presence of CAT, the rate of H_2O_2 decomposition was calculated spectrophotometrically at 240 nm for 30 second at 5-second intervals; the enzymatic activity was represented as nmol/min/mg tissue.

2.5.3.5. Determination of total protein

Protein level was estimated by the method of Gornall *et al.*, (1949) using the bovine serum albumin as a standard Biuret reagent. Briefely, 25 µL sample or standard (albumin) was mixed with 1 mL biuret reagent, after 10 min of incubation at 37 °C the absorbance of mixture was measured at 540 nm. Total protein concentration was calculated as follows:

$$\textbf{Total protein (mg/mL)} = (\textbf{Abs of sample} / \textbf{Abs of standard}) \times \textbf{n}$$

Where n is standard concentration.

2.6. Statistical analysis

Statistical analysis was performed using GraphPad Prism8.00 software. *In vitro* results were expressed as mean ± standard deviation (SD). The *in vivo* pharmacological results were presented as mean ± standard error of mean (SEM). In all cases, the values were compared by using oneway analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The P-values less than 0.05 were considered statistically significant.

Results and Discussion

1. Preparation of extracts

Extraction process is widely used to obtain a crude extract of phytochemicals from the plant materials. Solvent types, solvent resistance, extraction time, agitation speed, sample-solvent ratio, PH of extraction, temperature and particle size are several factors influence the concentration of the desired components in the extract, However, solvent types are reported to be the most important factor. Extraction efficiency very according to process conditions (Azwanida, 2015).

In this study, two types of solvents (methanol and water) with different polarity were used to extract plant bioactive compounds. Our result showed that PV ME registered yield of 14% which was significantly higher than the finding of Azad and his collaborators (2016) (8.34 %) and Morteza-Semnani *et al*., in (2006) 10.6 %. While PC ME gave a yield of 9.8% it was approximately the same yield 9.1% published in data of (Dellai *et al*., 2009) 8.12% for leaves (Limem-Ben Amor *et al*., 2009), these finding is lower than extraction with hydromethanol solvent of flowers 25.46% and leaves 20.82% (Merouane *et al*., 2019). The difference between the yield percent may be due to the method of extraction as well as the collection season, because the collection period for plants affects the quantity and quality of its phenolic content as well as the location from which the plant was obtained (Alba *et al*., 2022).

Table 01: Appearance, color and yields of *P. crinita* and *P. herba* venti extracts.

Extracts	**Appearance**	**Color**	**Yield (relative to to 100 g of dry weight)**
PC ME	Powder	dark green	9.8%
PC AQE	Powder	Brownish	18.10%
PV ME	Pasty	dark green	14%
PV AQE	Powder	Brownish	17.7%

For the aqueous extracts the highest yield was registered with *P.crinita* (18%) which is more than yield of the same extract (14%, 10.70%) published by Merouane and his collaborator (2018) and Limem-Ben Amor *et al.,* (2009) respectively. Our result are in line with finding of Limem-Ben Amor *et al* (2009) which demonstrated that the yield of hydromethanolic extract of *P.crinita* is lower than aqueous extract.

2. Determintion of total phenolics, flavonoids and tannins contents

There are two types of plant metabolisme, the first one is primary metabolism (lipids, proteins, carbohydrates, and nucleic acids) witch are importante to cells maintenance (Vickery and Vickery, 1981) and secondary metabolism like Plant polyphenols that can be found in a variety of plant tissues, including roots, stems, leaves, flowers, and pulp. Polyphenols are essential for plant survival in the environment, but they are not directly responsible for plant developpment and growth. Polyphenols are originated from several biosynthetic pathways (Li *et al.*, 2018). They are a type of phytonutrient that can be found in abundance in fruits, cereals, and vegetables (Ma *et al.*, 2020; Rubab *et al.*, 2020).

Table 02. Total phenolics, flavonoids and tannins contents of. *P. herba venti* and *P. crinita* extracts.

Extracts	Total phenolics (mg GAE/g extract)	Total flavonoids (mg QE/g extract)	Total flavonoids (mg RE/g extract)	Total tannins (mg TAE/g extract)
PV ME	116.21±0.15	37,44± 0.9	46.31±0.6	87.06±0.45
PV AQE	98.14±0.44	25,86±0.6	39.45±0.33	69.66±0.23
PC ME	82.71±0.79	24.60±0.45	38.42±0.45	132.13±0.68
PC AQE	81.28± 0.13	26.32±0.88	39.9±0.69	54.86±0.4

The concentration of total phenols in plants extracts is determined by several methods, the most important of which is the Folin-Ciocalteu method. This method based on the transmission of an electron (in the basal medium of the reaction) from the hydroxyl groups of phenolic compounds to the phosphomolybdic/phosphotungtic acid complex from the Folin-Ciocalteu reagent, which changes the detector color from yellow to blue to measure the absorbance at wavelength 765 nm (Dai and Memper, 2010) The TPC value of extract samples was determined using formula obtained from standard solution of gallic acid with various concentrations, the results were expressed as µg gallic acid equivalent/ milligram of extract. As shown in table (02), the highest amount of total phenols is in PV ME, followed by PV AQE, this result are better than that found in the data of (Ferrante *et al.*, 2019) where the extraction was made from the dried leaves of *P.herba venti* applying an ethanol (80%m/m) and the same thing for the finding of (Azad *et al*., 2016). In this study, *P.crinita* of both extracts showed comparable polyphenols level, and this is similar to that found in the study of

Merouane and his collaborator (2018) for PC ME (81.80 ± 2.25 µg GAE/ DW) and higher for the aqueous extract (47.67±2.08 µg GAE/mg DW).

Flavonoids were estimated using the $AlCl_3$ method and using Quercetine and Rutine calibration curve, where it was found that there was a direct correlation between high concentration and increased absorption at the wavelength of 430 nm. It is clear from the results in table 02 that the amount of rutine-equivalent flavonoids is higher than the amount of quercetine-equivalent flavonoids in all extracts. The equivalent values of flavonoids for the rutine range from between (39.9 ± 0.69) and (46.31± 0.6) mg for the rutine/g DW, while for the amount of quercetine equivalent flavonoids, they are limited to (24.23) and (37.44) mg of dry weight of the extracts. The results of the present data revealed that the PV ME contain the highest levels of flavonoids (37.44 mg QE/g extract, 46.31 mg RE/g extract) These results were higher than to that found by Ferrante *et al.*, (2019) (7.17 ± 0.59 mg RE/g extract) who applying ethanol (80%) as a solvent for flavonoid extraction, the same observation for the PV AQE our value (25,86 mg QE/g extract, 39.45 mg QE/g extract) were very higher (8.95 ± 0.75 mg RE/g extract). The total flavonoids content from aerial part of *P.crinita* was estimated to be twice more than of Merouane *et al.*, (2018) who finds that the total flavonoids were (10.38±0.55 µg QE/mg DM) for the methanolic extract and tree time more for the aqueous extract (7.91±0.37 µg QE/ mg DM). Early study by Gaamoune (2017) had shown that the *P.crinita* ethyl acetate extract is rich in flavonoids (35.76±3.07 mg EQE/g extract) this result is similar to our finding.

The tannin was estimated based on its characteristic in the precipitation of proteins by forming complexes with it, using tannic acid as a reference to express the phenolic content per gram of g of the extract. Table 02 showed that the PC ME have the highest amount of tannins (132.13 mg TAE/g extract) this value is very higher than the result of Dellai and his collaborators in 2009 (21.5mg TAE/g extract) and followed by PV ME extract (87.06±0.03 mg and PV AQE (69.66±0.01 mg TAE/g extract), while the lowest amount appear in the PC AQE (54.86±0.02 TAE/g extract). Our results is in line with finding of Tamert *et al.*, (2015) which shown that *P. crinita* contain large amount of hydrolysable and condensed tannin; Plant extracts can have a variable polyphenolic profile depending on various factors, including variety, climate, geographical location, plant diseases, maturity, and solvent used for extraction (Skotti *et al.*, 2014).

3. The *in vitro* evaluation of antioxidant activity

3.1. DPPH scavenging assay

Antiradical activity of the free radicals was estimated by using a 2.2 'diphenylpicrylhydrazyl (DPPH) radical which is a very stable free radical, this radical is returned by receiving a proton from any hydrogen giver then it loses its violet color characteristic by turning yellow and thus becomes uncreative ones (Xie *et al.,* 2014). The antioxidant activity of scavenging free radicals is due to being able to give the hydrogen proton or electron, and this is why the DPPH method, which is one of the most popular methods of measuring this activity, has been used due to its ease and speed. IC_{50} was calculated for these extracts and it is the concentration corresponding to inhibition of 50% of DPPH (since the smallest value of IC_{50} reflects the best scavenging action of the compounds).

In this study, we observed that both PV extracts had the highest radical scavenging activity than PC extracts. As shown in figure (12) none of extracts showed activity as high as BHT their effect show significant difference ($P<0.0001$) and the scavenging activity increased in the order of PC AQE< PV AQE < PC ME < PV ME < BHT.

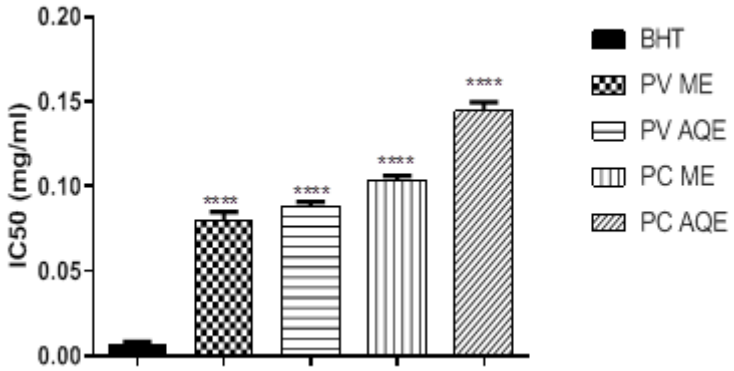


Figure 12: IC_{50} values of different plants extracts in DPPH assay. Results are expressed as mean± SD (n =3). (**** $p < 0.0001$) compared to BHT as standard. PV ME: *Phlomis herba venti* methanolic extract PV AQE: *Phlomis herba venti* aqueous extract, PC ME: *Phlomis crinita* methanolic extract, PC AQE: *Phlomis crinita* aqueous extract.

The results of this data confirm the correlation between the content of phenolic components and the radical scavenging activity which has been described in numerous studies (Bencheikh *et al*; 2016; Barhé *et al.,* 2016). *P. crinita* extracts showed their best activity with IC_{50} = 0.103 mg/mL) for ME and 0.144 mg/mL for AQ E. These results are greater than those obtained by

Merouane *et al*., (2018) (IC_{50}=1.04±0.02 mg/mL) and Tamert and latrech (2016)(1.78 mg/mL for aqueous extracts) and three times higher than those of limem-ben Amor *et al.*, (2009) (32 µg/mL). On the other hand, the *P. herba venti* extracts (ME and AQE) showed relatively higher activity, compared to *P. crinita* extracts (ME and AQE) and crossed through its IC_{50} value 0.08 mg/mL and 0.088 mg/mL respectively. In addition to the above, many studies confirm the effectiveness of *P. herba venti* extracts in scavenging of free radicals, among them the study of Gammoun (2017) and the data of Azad (2016). Several studies reported that the phenolic contents plant extracts are responsible for their radical scavenging activity, but other factors must be taken into account such as the nature and structure of these compounds, for example, Cho (2012) showed that the double bond Between carbon 2 and 3 atoms connected to group OH in cycle C increases the scavenging power of flavonoids in addition to that the caticols-ring (O-dihydroxybenzene) increases the scavenging power of flavonoids and this is shown by the flavonoids derived from hydroxy-luteolin which It is considered among the most important flavonoids separated from the extract of the PC ME (Kabouche *et al*; 2005). The phenolic compounds may act as free radical scavengers because of their hydrogen-donating ability and scavenging ability (Saha and Verma, 2016).

3.2. ABTS radical scavenging activity

As shown in figure (13) the PC ME and PV ME were found to significantly exhibit ($P< 0.001$, $P<0.0001$) a strong scavenger activity (IC_{50}=0.0130 mg/mL and 0.0121 mg/mL, respectively) which was surprisingly better than of BHA (IC_{50}=0.0181mg/mL). The ABTS scavenging activity decreased in the following order: PV AQE (IC_{50} = 0.0186 mg/mL) >PC AQE (IC_{50}=0.0187mg/mL). Interestingly, all the previous extracts showed significantly an important ABTS scavenging activity compared to BHA (IC_{50}=0.0181 mg/mL).

The ABTS (2,2'-azinobis acid (3-ethylbenzothiazoline-6-sulfonic acid) test is based on the ability of transformation and stabilization of dark blue cationic radical $ABTS^+$ into colorless ABTS by antioxidant . The preformed radical $ABTS^+$ is generated in the presence of potassium persulphate ions ($ABTS^+$ Persulphate ions $ABTS^+$). In the presence of an antioxidant, the transition from the $ABTS^+$ radical to the non-subject form is accompanied by the disappearance of the intense blue coloration which can be followed by the measurement of optical density at a wavelength of 734 nm (Schlesier *et al.*, 2002).

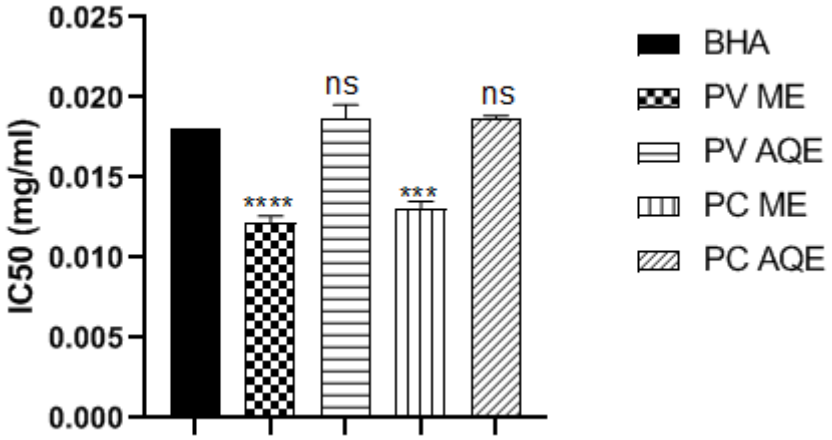


Figure 13: Antioxidant activity in ABTS free radical scavenging assay of plant extracts and the standard BHA. Data are presented as IC_{50} means ± SD (n = 3). ****P<0.0001; ***p<0.001 Statistically significant vs the standard BHA. PV ME: *Phlomis herba venti* methanolic extract PV AQE: *Phlomis herba venti* aqueous extract, PC ME: *Phlomis crinita* methanolic extract, PC AQE: *Phlomis crinita* aqueous extract.

P.crinita extracts revealed relatively strong antiradical activity toward the $ABTS^+$ free radical, This finding is in agreement with the study of Dellai *et al.* (2009), reporting high-dose-dependent $ABTS^+$ radical scavenging activities with an inhibition percentage of ABTS radical formation of 94% in methanolic flower's extracts of *P. crinita*. This activity may be due to the presence of tannins, flavonoids, iridoids, sterols, cardiac glycosides, and anthraquinones. Nam *et al.*, (2017) demonstrated in his data that anthraquinones had good ABTS radicalcation scavenging activity witch is higher than that positive control. *P. herba venti* extract also showed a strong antiradical activity in the plant extract. Interestingly, free radicals can be scavenged by antioxidant agents whether through hydrogen atom transfer (HAT) from O-H bond, or single electron transfer–proton transfer (SET–PT) (Košinová *et al.*, 2011). As reported Zhang and Wang (2009), the antioxidant radical scavenging activity of plant extracts is related to the contents in phenolic compounds which may act as free radical scavengers owed to their hydrogen-donating ability and scavenging ability.

3.3. β- carotene bleaching assay

The β -carotene / Linoleic acid test is used to measure the ability of extracts to inhibit lipids peroxidation, as this method depends on the color fading. Through the obtained results, it was found that all extracts have a strong inhibitory effect of oxidation of β-carotene molecules with rates ranging between 61.26% and 79.39% during 24 h. The inhibitory capacity of PV

ME is the highest (79.10%) followed by the PV AQE (75.91%) and then the PC ME (74.47%) This activity is much higher than that recorded with the gallic acid used as a positive control (64.37%) the weakest activity was observed with PC AQE with 61.26%.

The ability to inhibit lipids oxidation is among the indicators that are used in estimating the antioxidant activity and for that purpose the β-carotene test was used to estimate the antioxidant activity of lipids, since during the linoleic acid oxidation process the hydrogen atom is lost from the methylane group the resulting hydroperoxide radical attack the β-carotene molecules, Which changes his color from orange to yellow and this is accompanied by a decrease in absorbance at 490 nm , but in the presence of antioxidant agent this reaction was interrupted by the neutralization of linoleate free radical generated within the system.

The results showed that all extracts had a strong ability to inhibit lipid peroxidation which was better than gallic acide, the highest activity was observed with *P. herba venti* with 79.10% and 75.91% for the methnolic and aqueous extract respectively. *P. crinita* extracts showed their best activity at 61.26% and 74.47% for the aqueous and methnolic extract respectively results are quite similar to some previous result studies (Merouane *et al*., 2018, 2019), reporting the powerful antioxidant potency of *P. crinita* that can scavenge ROS in the lipid milieu.

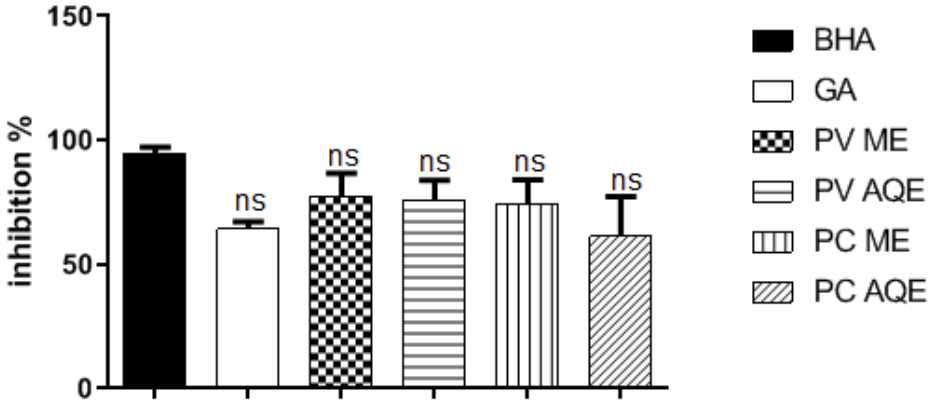


Figure 14: Inhibition percentage of different extracts of plants inβ-carotene/ linoleic acid assay after 24h using BHA as standard (ns: no significant). PV ME*: Phlomis herba* venti methanolic extract PV AQE: *Phlomis herba venti* aqueous extract, PC ME: *Phlomis crinita* methanolic extract, PC AQE: *Phlomis crinita* aqueous extract.

3.4. Reducing power assay

The reducing agent transfers electrons to another substance and is thus it oxidized. And, because it gives electrons, it is also called an electron donor. Electron donors can also form charge transfer complexes with electron acceptors. Reductants in biochemistry are very diverse. For example ferric ions (Fe^{3+}) are good reducing agents. Also, different bioanalytical reduction methods are available such as Fe3+-ferrous ions (Fe^{2+}) reduction method, ferric reducing antioxidant power reducing assay (Gülçin ; 2015).

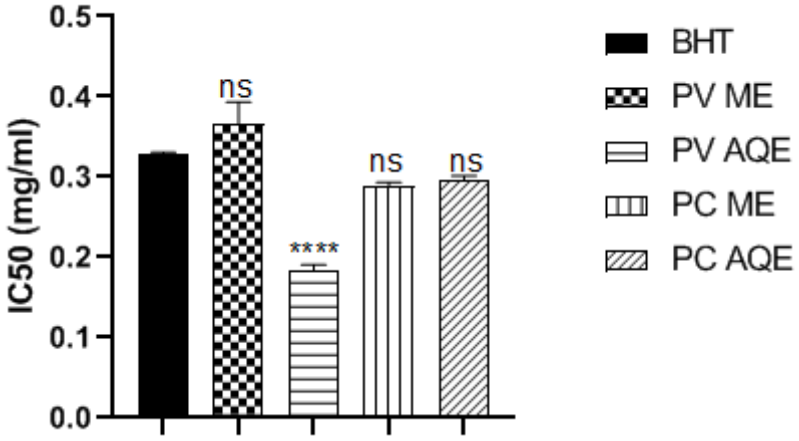


Figure 15 : IC_{50} value of different extracts in reducing power assay. (****: P<0.0001; ns: no significant) compared to BHT as standard. PV ME*: Phlomis herba* venti methanolic extract PV AQE: *Phlomis herba venti* aqueous extract, PC ME: *Phlomis crinita* methanolic extract, PC AQE: *Phlomis crinita* aqueous extract.

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric–ferrous complex that has an absorption maximum at 700 nm. Transformation of Fe^{3+} to Fe^{2+} is an important indicator of potential antioxidant activity (Firuzi *et al.,* 2005). The Fe^{3+} reducing capacities of the compounds indicate their electron donating abilities. Because of these properties, some compounds may play a role in terminating the free radical chain by converting reactive free radical species into more stable non-radical ones (Sudha *et al*., 2016). In some studies, it also reported that there was a strong correlation between the Fe^{3+} reducing capacity and the inhibition of lipid peroxidation.

P. crinita and *P. herba venti* examined extracts showed reducing power in a concentration-dependent manner. The results showed that the reducing capacity of the extracts is close to that of BHT. The PV AQE exhibited the highest reducing power (IC_{50} value=0.183 mg/mL) which was significantly ($p<0.0001$) better than BHT (IC_{50}=0.327 mg/mL), followed by PC ME and PC AQE with IC_{50} of 0.288 mg/mL and 0.296 mg/mL respectively then PV ME with an IC_{50}=0.366 mg/mL. Data of Gaamoun (2017) confirmed the good reducing power activity of *phlomis herba venti*, he reported that ethyl acetate extract had a strong reducing power (IC_{50}=9.20±0.56 µg/mL).this difference between ours and his data value may be explained by the difference in the extraction system used. Lamiide is iridoid isolated from *P.herba venti* (Alipieva *et al,*. 2000) this molecule showed moderate antioxidant activity using FRAP test (Zolfaghari *et al.,* 2020).

Our findings revealed that *P. critina* extract had potentiel reducing power activity. This activity may be due to its richness in phenolic compounds. Noteworthy, the antioxidant activity was reported to be directly correlated with the reduction power of some plant components, depending on multiple parameters such as the concentration of the metal ion and polyphenol, temperature, pH, and the presence of complexing agents (Ghedadba *et al.,* 2015).

3.5. Ion chelating assay

The metal chelating capacity is important since it reduces transition metal acting as catalysts to generate the first few radicals and initiate the radical mediated oxidative chain reactions in biological or food systems. Ion chelating agents also may inhibit the Fenton reaction and lipid peroxidation (Apak *et al.,* 2016). Thus, an antioxidant's ability to chelate metals is an important antioxidant property to measure. Ferrous ions are generally present in food systems and are considered as effective pro-oxidants. Ferrozine and Fe^{2+} form coloured complex (violet colour). In the presence of chelating agents, ferrozine-Fe^{2+} ion complex is disturbed, resulting in a decrease in color of the complex. Measurement of the color reduction allowed for estimate the metal chelating activity for the coexisting chelator (Kumar and Jain, 2015).

Therefore, the capacity of *P. herba venti* and *Pcrinita* extracts was measured In capturing minerals and thus measuring their ability to inhibit the formation of free radicals by relying on a chelation test where ferrozine was used as a picker for Fe^{2+} which after its interaction with ferrozine leads to the appearance of a violet color, in the presence of particles metals captured a decrease in the degree of red violet is observed, which results from a decrease in the ability to form Fe^{2+} -ferrozine complex (Symonowicz and Kolanek, 2012).

After reading the optical density of extracts with different concentrations in the wavelength of 562 nm, it was found that there is a large difference in the ability of the extracts to chelate the iron ions, where the results showed that both of the extracts of PC ME and PC AQE have the highest ability to chelate minerals, which was estimated at 0.020 mg/mL and 0.046 mg/ml Respectively, PV extracts' showed a lower chelating activity than PC extracts with IC_{50} values 0.153 mg/mL and 0.165 mg/mL for PV AQE and PV ME respectively.

When comparing the IC_{50} values of the various extracts among them, it is clear that the methanolic extract of In this study *P. crinita* showed higher chelating activity than that other other extracts, and this is likely due to the high tannins content in PC ME (Bencheikh *et al*., 2018). As previously reported, the effective metal chelating ability of Phlomis genus, including *P. viscosa P. platystegia* Post. and *P. brachyodon* Boiss (Al-Qudah *et al*., 2018) *P. pungens* (Keser *et al*., 2012) may be due to the presence of *Verbascoside (the common* phenylethanoid glycosides, *in* Phlomis *genus) in their extracts.*

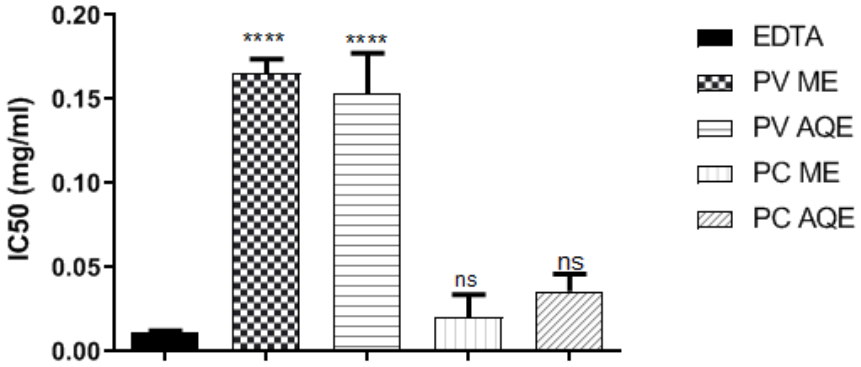


Figure 16: Metal chelating activity of different plants extracts. Data were presented as means±SD (n=3). (ns: no significant difference; ****: P<0.0001) compared to EDTA as standard .PV ME: *Phlomis herba venti* methanolic extract PV AQE: *Phlomis herba venti* aqueous extract , PC ME : *Phlomis crinita* methanolic extract , PC AQE: *Phlomis crinita* aqueous extract.

Also, it was reported (Fernandez *et al*., 2002) that flavonoids can chelate metals by two major phases; the loss of a hydrogen atom from the hydroxyl groups, and the formation of a stable complex between the oxygen atom and the iron ion. Recently, the polyphenols and flavonoids have been reported to be excellent chelators for iron and copper (Papuc *et al*., 2017), and noteworthy flavonoids can chelate metals by more than one possible process way. This depends on flavonoid structure, the type of metal ion and pH of the reaction (e.g., in vivo

conditions: acidic in the stomach and alkaline in intestine). In vitro previous studies (Chirug *et al*., 2021; Malešev and Kuntić, 2007) have revealed many different dependencies between pH and the flavonoid moieties responsible for metal chelation. As instance in quercetin, the ortho-dihydroxyl group chelates part in Fe^{3+}, Cu^{2+} and Al^{3+} in alkaline solutions, but can create complexes with Fe^{3+} (1:2) in acidic solutions with coordination via the 3–4 or 4–5 site and induce binding of Fe^{3+} to the catechol group in a 1:1 metal/ligand ratio at higher pH. Additionally, the strong correlation between the Fe^{3}+ reducing capacity and the inhibition of lipid peroxidation has been previously proved (Kasprzak *et al*., 2015) .

4. Antidiabetic and antioxidant activities

4.1. Effect of *P. crinita* and *P. herba venti* methanolic extracts on body weight

In addition to others parameters, the variation of body weight in alloxan-induced diabetes was recorded. As shown in table (03), the alloxan group and animals treated with glibanclamid had significantly ($P<0.0001$) lower body weights compared to their initial body weights. By contrast, the body weight of the healthy group significantly ($P<0.01$) increased, This is in agreement with the findings of Thomas *et al*., (2020). The observed weight loss may be attributed to the harmful effects of diabetes such as, the breakdown of tissue proteins, muscle wasting, dehydration and catabolism of fats (Chatterjee and Shinde, 2002). Administration of *P.crinita* and *P.herba venti* methanolic extract to rats for 15 days did not significantly alter their body weigth. This observation may be due to the protective action of these plants against the cytotoxic effects of alloxan. This is in line with data of Sarkhal and his collaborators (2010) who demonstrated that the decrease in body weight in *Phlomis persica* -treated groups was significantly lower than that of diabetic group in STZ induced diabetic rat model.

Table 03: Effect of *P.crinita* and *P.herba venti* methanolic extracts on rat's body weight.

Treatment / Body weight	PVME 250 mg/kg	PVME 500 mg/kg	PCME 250 mg/kg	PCME 500 mg/kg	glibinclamide	alloxan	healthy
Initial body weight (g)	171.4± 5.85	173.6± 5.89	168.8± 5.81	191.57± 5.99	151.8± 5.66	164.25± 5.74	176.75± 5.94
Final body weight (g)	156.25± 1.6^{ns} ***	167.6± 1.6^{ns} **ns**	152.8± 1.4^{ns} ***	184.14± $1.6^{ns\ ns}$	131.6± $0.82^{\lambda\lambda\lambda}$ ****	148.4± 0. $^{\lambda\lambda\lambda}96$ ****	191.5± $1.07^{\lambda\lambda}$

Values are mean ± SEM (n=5-6). PV ME: *P. herba venti* methanolic extract, PC ME: *P.crinita* methanolic extract (ns: no significant at P<0.05; $^{\lambda\lambda}$P<0.01; $^{\lambda\lambda\lambda}$P<0.001) compared to their initial body weight. (ns: not significant; *** P<0.001;;**** P<0.0001) *vs* healthy group.

The pancreas is the primary organ involved in the detection of the dietetic and energetic states of the body through the regulation of the concentration of glucose in the blood via the secretion of insulin. Alloxan is one of the common substances used for the induction of diabetes mellitus in a wide variety of species by damaging the insulin-secreting cells of the pancreas. This damages a large number of β cells, resulting in decreased endogenous insulin release, paving the way for decreased tissue glucose use (Sharma *et al.,* 2010). Alloxan-induced diabetes is characterized by severe weight loss which is in agreement with the present study. The decreased body weight in uncontrolled diabetic is probably due to the increased lipid and protein catabolism under the effect of the peripheral non-use of glucose by insulinosensible tissues and lack in the carbohydrate serving as an energy source, leading thus to atrophy of muscle and loss of tissue protein. It can be suggested that diabetic agent like streptozotocin and alloxan induced diabetes (hyperglycaemia) and hypoinsulinemia causes decrease in the body weight of diabetic animals. Zafar and Naeem (2010) found that the decrease in body weight was associated with increase in the kidney and liver relative weights, while the pancreas weight was unaffected. The decrease in the pancreas weight may be assigned to the disruption and disappearance of pancreatic islets and selective destruction of insulin-producing cells (Kim *et al,*. 2006; Heidari *et al*., 2008).

4.2. Effect of *P. crinita* and *P. herba* venti methanolic extracts on α-Glucosidase inhibitioy activity

To determine the inhibitory effects of *P crinita* and *P. herba venti* extracts on α-glucosidase activity, p-nitrophenyl α-D-glucopyranoside (pNPG) was used as the substrate to measure the yellow color of the enzyme's degradation product, p-nitrophenol. PC ME, PC AQE and PVAQE treatments exhibited an IC_{50} more than 1 mg/mL with values of 1.73±0.01 mg/mL; 1.35±0.02 mg/mL and 1.28±0.01mg/mL respectively. The results showed that the methanolic extract of *P. herba venti* was significantly ($P<0.0001$) more inhibited (IC_{50}=0.16±0.01 mg/mL) than acarbose (IC_{50}=0. 31±0.01 mg/mL) on alpha-glucosidase (figure 17). This effect is probably due to the presence of secondary metabolites such as polyphenols, flavonoids and tannins inherent in the composition of this extract, which was revealed during phytochemical screening. Indeed, Hanhineva and his colleague's (2010) demonstrated that flavonoids, phenolic acids and tannins inhibit the activity of enzymes such as α-amylase and α-glucosidase that are the main enzymes of carbohydrate metabolism. Boudjelthia *et al.*, (2017) suggested that this consequent inhibitory or anti-diabetic effect is associated with the richness of extracts by tannin.

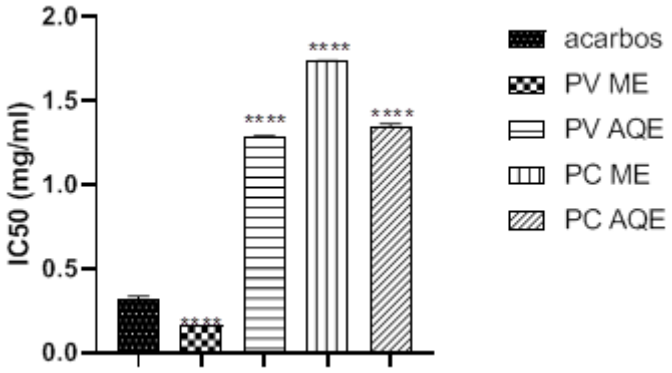


Figure 17: α-Glucosidase inhibitory activity of *P. crinita* and *P. herba venti* extracts. PV ME*: Phlomis herba* venti methanolic extract PV AQE: *Phlomis herba venti* aqueous extract, PC ME: *Phlomis crinita* methanolic extract Acarbose was used as standard drug. **** $P<0.0001$ *vs* acarbose.

Phlomis genus exhibited a remarkable enzyme inhibition profile in α-glycosidase test system. Sarikurkcu *et al* (2014 and 2015) confirmed the inhibitory effect of three Phlomis species (*Phlomis nissolii, Phlomis pungens var. pungens* and *Phlomis armeniaca)* on this enzyme, This can be explained by the richness of the plants belonging to the genus Phlomis in iridoid glucosides, flavonoid glycosides, phenylethanoid glycosides, polyphenols.

4.3. Effect of *P.crinita* and *P.herba venti* methanolic extracts on glucose loaded rats

Glycemic homeostasis refers to glucose balance within the circulatory system. However, it is largely compromised in diabetes, and thus lading to several complications including retinopathy, nephropathy and neuropathy, which are all collectively referred to as diabetic complications. They are the principal factors in morbidity and mortality often associated with diabetes (Ighodaro *et al*., 2017). Diabetes mellitus caused by alloxan is one of the widely used mechanisms animals for the experimental induction of Type I diabetes (Etuk, 2010). Alloxan, an important glucose analogue is accumulates in beta cells, where it exerts its cytotoxic effects. Moreover, Alloxan produces reactive oxygen species (ROS), which are responsible for its cytotoxic action. Reduction of alloxan to dialuric acid form a redox cycle in which superoxide radicals are formed and dismutate to hydrogen peroxide (H_2O_2). Highly reactive hydroxyl radicals are also formed through the Fenton reaction. Further, the rapid destruction of beta cells in pancreatic islets is caused by the extreme increase in calcium concentration in the cytosol (Rohilla and Shahjad, 2012).

As shown in table (04) and figure (18), both doses (250 and 500mg/kg) of PC ME were significantly ($P<0.01$, $P<0.001$) decreased blood glucose level compared to alloxan group after 15 days of treatment. The blood glucose level was found to be 2.79±0.41 and 1.25g/dL after 10 and 15 days, respectively in 250 mg/kg PC ME, and 3.17±0.48, 3.2±0.51 and 2.42 g/dL after 5, 10 and 15days respectively in 500 mg/kg PC ME treatment. 500 mg/kg of PV ME treated animals decreased ($P<0.01$) blood glucose level after 15 days of treatment (from 4.8±0.9 to 3.25±0.48 g/dL). this effect may be due to the presence of Stigmastérole-3β-D-glucopyranoside in *P.herba venti* extract (Bouzergoune, 2017) this compound was identified as antidiabetic molecule (Snelle *et al*., 2019; Nagappan *et al*., 2018) Whilst, the blood glucose level has not significantly changed in 250 mg/kg of *P.herba venti* extract, and this might be explained by the selected low dose of the extract.

Table 04: Glucose levels changes in the blood of treated and alloxan animals.

Days / Treatment	0day	5day	10day	15day
Healthy	0.79±.03$^{\&\&}$	0.79±.03$^{\&\&}$	0. 91±.03$^{\&\&\&}$	0.84±0.00$^{8\&\&\&\&}$
PV ME 250 mg/kg	2.94± 0.76^{**ns}	2.86±0.55^{*ns}	2.83±0.55^{*ns}	2.60±0.49$^{*\&}$
PV ME 500 mg/kg	4.8± 0.9$^{****\&}$	4.8±045^{****ns}	4.6±0.7^{***ns}	3.25±0.48^{**ns}
PC ME 250 mg/kg	3.74± 0.8^{****ns}	3.9±0.32^{****ns}	2.79±0.41^{**ns}	1.25±0.39$^{ns\&\&\&}$
PC ME 500 mg/kg	4.83±0.98^{****ns}	3.17±0.48^{**ns}	3.2±0.51^{**ns}	2.42±0.38$^{*\&\&}$
Glibenclamide 3g/kg	2.03± 0.63^{**ns}	1.95± 0.46^{nsns}	1.91±0.34$^{ns\&\&}$	1.90±0.30$^{ns\&\&}$
Alloxan 120 mg/kg	3.18±0.65**	3.81±0.65***	4.21±0.81****	4.80±0.46****

Blood glucose values (g/dL) are mean ± SEM (n=5-7), (ns: not significant, *P<0.05, **P<0.01, ***P<0.001; ****P<0.0001) *vs* healthy group, (ns: not significant, $^{\&}$P<0.05, $^{\&\&}$P<0.01, $^{\&\&\&}$P<0.001; $^{\&\&\&\&}$P<0.0001) *vs* alloxan group PV ME: *P. herba venti* methanolic extract, PC ME: *P. crinita* methanolic extract.

Rats treated with glibinclamide showed a slight decrease in blood glucose levels that was after 15 days of treatment to be 1.90±0.30 g/dL, unlike alloxan rats showed significant (P<0.0001) increase in their blood glucose level (from 3.18 ± 0.65 to 4.80 ± 0.46 g/dL) versus healty group during the experience period. The antidiabetic effect of the extracts was more effective than that observed with glibenclamide. The ability of therapeutic compounds including medicinal plants to restore glycemic balance or homeostasis in hyperglycemic condition has been an index of their antidiabetic function and relevance (Ighodaro *et al.*, 2017). *Phlomis* genus to be among the plants that had anti-diabetic effect. Sarkhail and his collaborators (2010) documented that *Phlomis persica* has positive effects on experimental diabetes. Anti-diabetic effect of *Phlomis persica* extrac was atributed to its potential to inhibit hepatocellular oxidative stress. Data of (Sarikurkcu *et al*., 2015) demonstrated that Ethyl acetate extract of *Phlomis armeniaca* exhibited considerable enzyme inhibition potential on α-glucosidase and α-amylase. These findings suggest that *P. armeniaca* may be useful in the development of an alternative agent for diabetes.

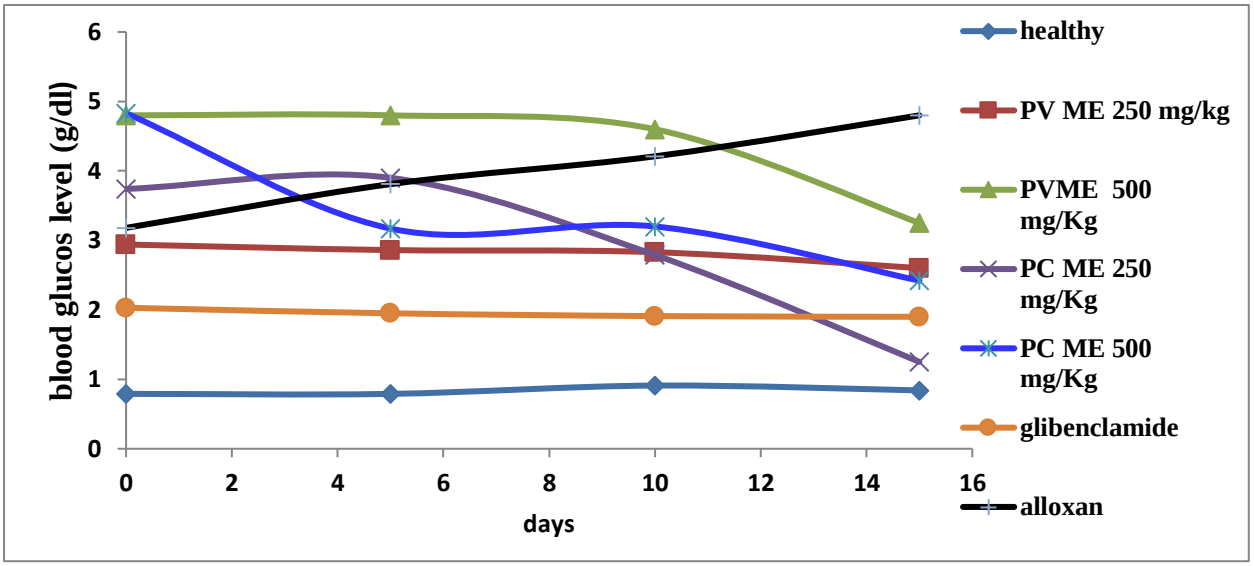


Figure 18: Effect of *P.crinita* and *P.herba venti methanolic* extract on blood glucose evolution. PV ME: *P. herba venti* methanolic extract, PC ME: *P.crinita* methanolic extract.

Phenylethanoid glycosides are type of secondary metabolite that are widely distributed in Phlomis genus. Tthes compounds achieve blood glucose regulation by inhibiting the activity of related enzymes (α-glucosidase and α-amylase). Morikawa *et al.*, (2014) found that verbascoside (phenylethanoid glycosides isolated from *Phlomis crinita*) from *Cistanche tubulosa* can inhibit the increase in postprandial blood glucose. Shimada *et al.*, (2017) demenstrated that verbascoside inhib sodium-glucose cotransporter 2 mediated glucose uptake and contributing to the inhibition of postprandial blood glucose levels. Boudjelal and *et al* (2012) found that the aqueous extract of *Marrubium vulgare* contain verbascoside have significant anti-diabetes effect in alloxan-inducing diabetes model. In Liu *et al* (2014) work, verbascoside had potent inhibitory effects on alpha glucosidase. El-Marasy *et al* (2020). Documented oral treatment with verbascoside (10, 20, and 40 mg/kg) caused a significant reduction of blood glucose in diabetic by elevation the serum insulin.

Dietary polyphenols are one group of natural products that have shown promise as potential antidiabetic agents. Domínguez Avila *et al.*, (2017) proposed that polyphenols increase glucagon-like peptide1 half-life by inhibiting dipeptidyl peptidase-4 (DPP4), act as a stimulant on β-cells for insulin secretion, and stimulate peripheral response to insulin, increasing the overall effects of the GLP1-insulin axis. Polyphenols preventing and managing type 2 diabetes mellitus involve a preventive mechanism of pancreatic islet β-cell by reduction β-cell apoptosis, and a promotion of β-cell proliferation (Sun *et al.*, 2020)

Studies using *in vitro* assay and animal models demonstrated that flavonoids can prevent diabetes and its complications (AL-Ishaq *et al.,* 2019). Luteolin and luteolin-7-O-glucoside are flavonoids that have been identifided in *P.crinita* extract *(*kaabouche *et al*., 2005). Ding *et al*., (2010) showed that luteolin treatment for 24 h increased the response of glucose uptake to insulin stimulation in 3T3-L1 adipocytes and also demonstrated that luteolin enhanced Akt2 (phosphoinositide-dependent serinethreonine protein kinas) phosphorylation in an insulin-stimulated state. Akt2 prevents the dephosphorylation of the insulin receptor and thus prevents attenuation of the insulin-signaling process. Akt2 is also responsible for the regulation of uptake of glucose and this effect is mediated by the translocation of (GLUT-4) glucose transporter to the surface of the cell (Mackenzie and Elliott, 2014). Flavonoids are effectively involved in the regeneration process of pancreatic beta cells by versus reactive oxygen species (ROS) generation (Ghorbani, 2017), increase insulin release, and promote the calcium uptake by the cell tissues, which is very efficient in insulin deficiency condition.

Tannins are known to inhibit the loss of glucose transport which produced insulin. It is also suspected to induce phosphorylation from the insulin receptor by forming glucose 4 transporter (GLUT-4). The reduced blood glucose level can be referred to the direct action of steroids on insulin work in cells, distal insulin receptor, and glucose synthesis in the liver (Chikhi *et al*., 2014; Sobia *et al*., 2016; Solikhah *et al*., 2020). Moreover, the phytochemical analysis of the phenolic compunds in both phlomis species has revealed the presence of chlorogenic acid as a major constituent in these two plants (unpblished results). The administration of this constituent has been found to decrease glucose level in experimental diabetic animals (Naveed *et al.,* 2018; Pimpley *et al*., 2020; Mansour *et al.,* 2021).

4.4. Blood parameters

Blood a connective tissue, with cellular elements suspended in plasma. Elevated blood glucose level in diabetes contributes to disturbance of blood cells and its indices, Good glycemic control is the main recommendation in the prevention of the development of diabetic complications (Ziaee *et al*., 2017). It has been reported that the administration of plants extract can positively change in hematological parameters of experimental diabetic animals (Fagbohun *et al.,* 2020; Sekiou *et al.,* 2021). The evaluation of blood profiles can be used to determine the extent of the hematological parameter alterations, and also to explain blood relating functions of chemical compounds/plant extract (Muhammad *et al.,* 2012).

Red blood cells (RBC) were measured to check anemia and to evaluate normal erythropoiesis. Iron levels in cells are indicated by hemoglobin levels, while anemia or polycythemia are determined by the hematocrit, which represents the volume of RBC in 100 mL of blood (Edet *et al.,* 2013) The results in table (05) show that there is significant decrease ($P<0.05$) in RBC levels of alloxan group (4.292±0.77 $10^6/mm^3$) when compared to healthy group (7.29±0.23 $10^6/mm^3$) which is in agreement with the findings of (Muhammad *et al*., 2012). Treatment with the plants extracts produced no significant change of the RBC; the values being (6.43±0.49 $x10^6$/mm3; 7.05±0.509 $x10^6/mm^3$) for *P.herba venti* 250 and 500 mg/kg respectively and (7.34±0.326 $x10^6/mm^3$; 7. 12±0.288 $x10^6/mm^3$) for *P.crinita* 250 and 500 mg/kg respectively. The cytotoxic action of diabetogenic agent such as alloxan is mediated by reactive oxygen species (Rohilla and Shahjad, 2012). Oxidation of glycosylated RBC membrane proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipidperoxides that lead to haemolysis of RBC (Arun and Ramesh; 2002 Oyedemi *et al*., 2011) Hyperglycemia results in glycosylated haemoglobin, thus total haemoglobin level is decreased in alloxan induced diabetic rats (Sheela and Augusti, 1992). In our data the RBC level is improved after 15 days of treatment with *P.crinita* and *P.herba venti*. This suggests that polyphenols found in the extracts may influence erythropoietin production and secretion, which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2014).

In table (5), the red blood cell counts are directly correlated with the hemoglobin level (HGB), and hence the red blood cell counts were proportionally increased with increase in the HGB level and vice versa. There is no significant difference in HGB level in all groups compared to healty group. diabetic patients with complications are characterized with a decrease in hemoglobin concentration and these changes can cause rapid initiation of apoptosis in damaged erythrocytes, decreasing the oxygen-carrying capacity due to increase in hemolysis rate and thus decreasing their lifespan.

Table 05: Mean values of blood parameters in treated and alloxan rats.

Parameter / Treatment	RBC 10^6/mm^3	PLT 10^3/mm^3	WBC 10^6/mm^3	HGB g/dL
Healthy	7.29±0 ,23	515.7±1.37	5.517±0.237	18.06± 1.74
PV ME 250 mg/kg	6.43±0,49^{ns}	515.1±2.42^{ns}	4.767±0,757^{ns}	15.57±1.458^{ns}
PV ME 500 mg/kg	7.05±0,509^{ns}	440±1.9^{ns}	4.64±0,731^{ns}	16.27±1.046^{ns}
PC ME 250 mg/kg	7. 34±0,326^{ns}	395.8±1.17*	6.35±0.842^{ns}	16.26±0.95^{ns}
PC ME 500 mg/kg	7. 12±0,288^{ns}	473± 2.81^{ns}	5.05±0.743^{ns}	14.14±0.983^{ns}
Glibinclamide 3mg/kg	6.32± 0.2^{ns}	448.25±0.21^{ns}	7.9±0 .4^{ns}	15.01±0.33^{ns}
Alloxan 120mg/kg	4.292±0 .77*	321±1.78**	3.68±0.568*	14.00±0.116^{ns}

Value are mean ± SEM (n=5- 7) (ns: no significant difference,*$p < 0.05$, ** $p < 0.01$) compared to healthy group.

Concerning white blood cell (WBC) count the present data showed a notable significant decrease ($P<0.01$) in alloxan group as compared with healthy group (table 05), our results are in agreement with finding of Benchikh (2018) and Joy and Kuttan (1999) who showed that diabetic agent like streptozotocine and alloxan cause a decrease in WBC level. Joy and Kuttan (1999) suggested that administration of plants extracts considerably prevented alloxan-induced WBC damage. In the same context, Limem ben Amor and his collaborator (2011) demonstrated that methanolic and aqueous extracts of *P.crinita* induce splenocytes proliferation in the absence of mitogen stimulation and they suggest that leaf extracts of *P.crinita* may contain some mitogenic substrates capable of stimulating cell proliferation.

Blood platelets are small particles of cells that contribute to blood clotting. They initiate the recovery of blood vessel walls, and also are believed to be involved in the infection or inflammation acute phase. When compared to healthy group, Blood platelets value showed a slight change in all groups except the alloxan group which registered the lower values (321±1.78 x10^3/mm^3) with significant deference ($P<0.01$). glibenclamid treated group were

reported to have measurable effect on blood PLT ($448.25\pm0.21x10^3/mm^3$), whereas the PLT value of extract treated groups are so close to the healthy group (table 05). Piekarska *et al*., (2018) reported that iridoid (compounds were identified in the studied plant) administered orally for 5 and 7 consecutive days significantly increased the blood cell counts, and platelet counts.

4.5. Biochemical parameters

4.5.1. Effect of *P. crinita* and *P. herba venti* methanolic extracts on the aminotransferases (AST and ALT) and phosphatase alkaline (ALP) activities

The aminotransferases are the most frequently utilized and specific indicators of hepatocellular necrosis. Serum AST, ALT and ALP are the most sensitive biomarkers in the diagnosis of liver diseases (Olaniyan *et al.*, 2019). During hepatocellular damage, varieties of enzymes normally located in the cytosol are released into the circulation. Their quantification in plasma is useful biomarkers of the extent and type of hepatocellular damage. Serum ALT and AST are liver enzymes, that leak out into general circulation when there is damage to the liver cells (Pari and Murugan, 2004).

In this study, alloxan causes increase in ALT activity in all groups exept animals treated by PC 500 mg/kg. A significant ($P<0.01$) increase in ALT activity was observed in alloxan group (60.25 ± 1.52 UI/L) when compared with healthy group (24.77 ± 0.8 UI/L) Whilst, *P.crinita* and *P. herba venti* revealed a decrease in the levels of the identified metabolite, where the best effect was noticed in 250 mg/Kg PV ME (41.74 ± 0.5 UI/L), and 500 mg/Kg PC ME (28.74 ± 0.4 UI/L) treated animals (figure 19).

Aspartate amino transferase (AST) activity in glibinclamide (161.75 ± 0.99 UI/L) and alloxan group (131.82 ± 0.4 UI/L) was significantly ($P<0.0001$) increased when compared with healthy group (105.26 ± 0.24 UI/L). An insignificant decrease in AST activity was observed in animal treated with PV ME 250 and 500 mg/kg (98.13 ± 0.22 UI/L; 117.09 ± 0.95 UI/L respectively) and PC ME 250 mg/kg (116.74 ± 0.25 UI/L) while a significant ($P<0.01$) decrease was observed in PC ME 500 mg/kg (81.76 ± 0.86 UI/L) compared with healthy (figure 19).

Phosphatase alkaline activity was significantly ($P<0.0001$) increased in alloxan group (1236.75 ± 7.46 UI/L) compared to healthy (333.92 ± 2.52 UI/L) and other groups. PC ME 250 mg/kg (670.75 ± 3.97 UI/L) and PV ME 250 and500mg/kg (553.13 ± 2.95 UI/L;

722.25±3.97 UI/L respectively) showed an insignificant increase in concentration of ALP compared to healty group, while animal treated with *P.crinita* extract at dose of 500 mg/kg showed decrease in ALP activity (220.75±1.47 UI/L) but it's not significant at (P<0.05) when compared with healthy group.

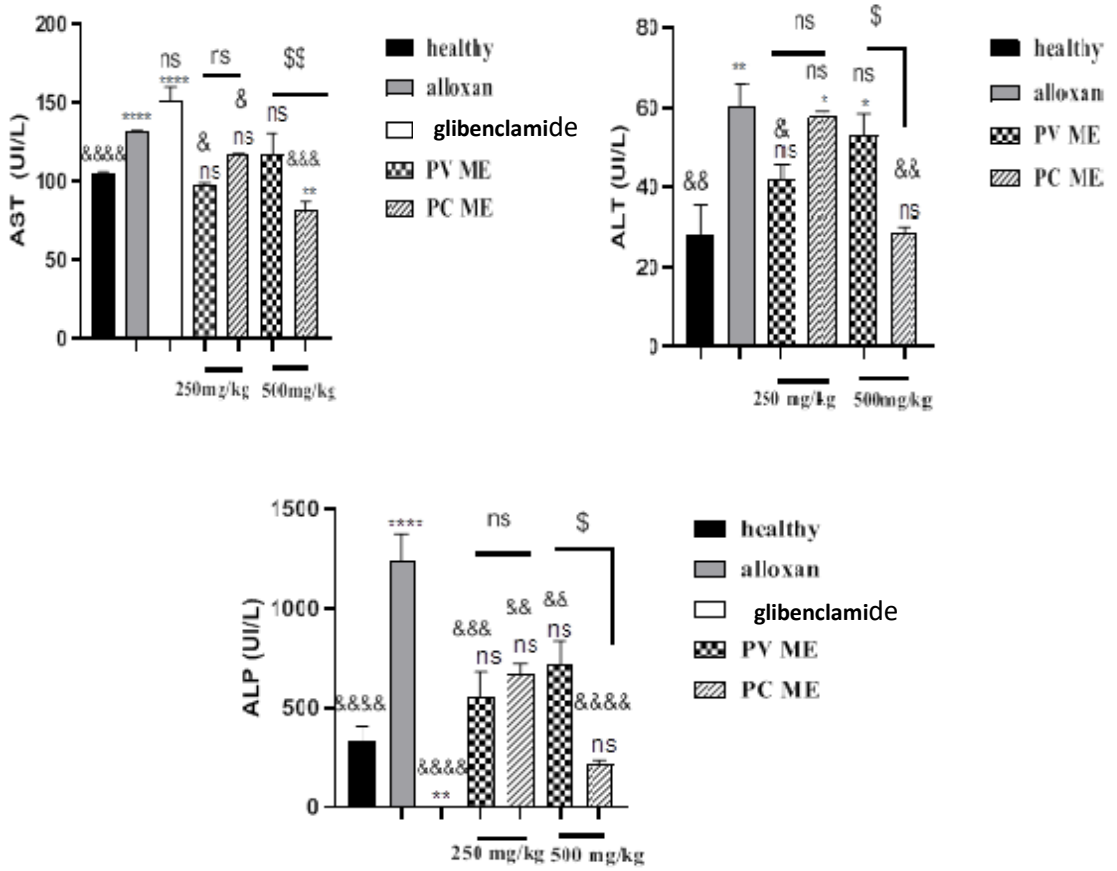


Figure 19: Variation in the enzymatic activity of transaminases and alkaline phosphatase after 15 days of treatment in the experimental groups. PV ME: *P. herba venti* methanolic extract, PC ME: *P.crinita* methanolic extract. Values are mean ± SEM (n=5-7) (ns: not significant; *P<0.05; **P<0.01;**** P<0.0001) *vs* healthy group. (ns :not significant; $^{\&}$P<0.05; $^{\&\&}$P<0.01; ;$^{\&\&\&}$ P<0.001 ;$^{\&\&\&\&}$ P<0.0001) *vs* alloxan group. (ns :not significant; $^{\$}$P<0.05 ; $^{\$\$}$P<0.01) comparison between extracts.

Results demonstrated that administration of *P.crinita* and *P. hrba venti* to diabetic rats protect their liver from hepatotoxicity of alloxan. The positive of PC ME and PV ME effect on enzymatic activity of transaminases and alkaline phosphatase may be related to the presence of secondary metabolites like flavonoids iridoids and anthraquinones. Luteolin and luteolin-7-O-glucoside are flavonoids can ameliorate serum AST and ALT activities in GalN/LPS-intoxicated mice (Park and Song, 2019), whil anthraquinones pretreatment could significantly, decreased levels of ALT and AST in the serum mice treated CCl4 Chen *et al.,* (2020).

The hepatoprotective effect of iridoids is mainly attributed to their antioxidant activity. This action is both indirect, through the stimulation of the antioxidant defense system, and direct, through the removal of reactive oxygen species ROS (Madrigal-Santillán *et al*., 2014). Tan *et al*., (2017) conducted assessed the hepatoprotective ability of iridoids isolated from *Veronica ciliata* against acetaminophen-induced liver injury in mice. The serum levels of liver functional markers, including transaminases aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were significantly decreased in animals that received iridoid glycosides isolated from *Veronica ciliate*, similar resuls were observed by Hua *et al* (2021).

4.5.2. Effect of *P.crinita* and *P.herba venti* methanolic extracts on uric acid and creatinine

Urine contains metabolic wastes such as uric acid, urea and creatinine, which are excreted by the kidney, it is also important to maintain a balance between water and electrolytes in the body (Musso *et al*., 2012). Abnormal levels of serum uric acid causes major health problem due to its pivotal role in the etiology of many systemic diseases. Many research works in recent past have shown elevated uric acid levels in diabetic subjects. Some reports indicate that uric acid elevation is related to diabetic complications, whereas a few claimed that uric acid elevation is also seen in pre-diabetic condition (Gowda *et al*., 2021) Uric acid (2,6,8 trihydroxypurine,$C_5H_4N_4O_3$) a heterocyclic weak organic acid, formed during the oxidation of purine nucleotides in humans. The purine nucleosides and purine nucleotides are catabolised to uric acid through adenosine deaminase enzymes (ADA) and 5'nucleotidase (Madianov *et al*., 2000; Palsamy and Subramanian, 2008).

The elevated plasma uric acid levels observed in the present study in alloxan rats (25.13±1.05 mg/L) is in agreement with previous reports which have showed an increment in the level of uric acid in diabetes mellitus (Gowda *et al.,* 2021). The uric acid level in alloxan group significantly ($P<0.0001$) very higher than healthy group (14.36±1.43 mg/L) The results showed that treatment with *P. crinita* and *P. herba venti* exhibited an important decrease in the level of serum uric acid (figure 20) were the best effect and the significant decrease ($P<0.05$; $P<0.0001$) appeared with PC ME 500mg/kg (3.41±0.49 mg/L) followed by PC ME 250mg/kg (11.72±0.22 mg/L), which is better than healthy group. Our result demonstrated that administration of *P. crinita* and *P. herba venti* to diabetic rats reduce uric acid concentration in dose-dependent ($P<0.0001$ and $P<0.001$ respectively) manner.

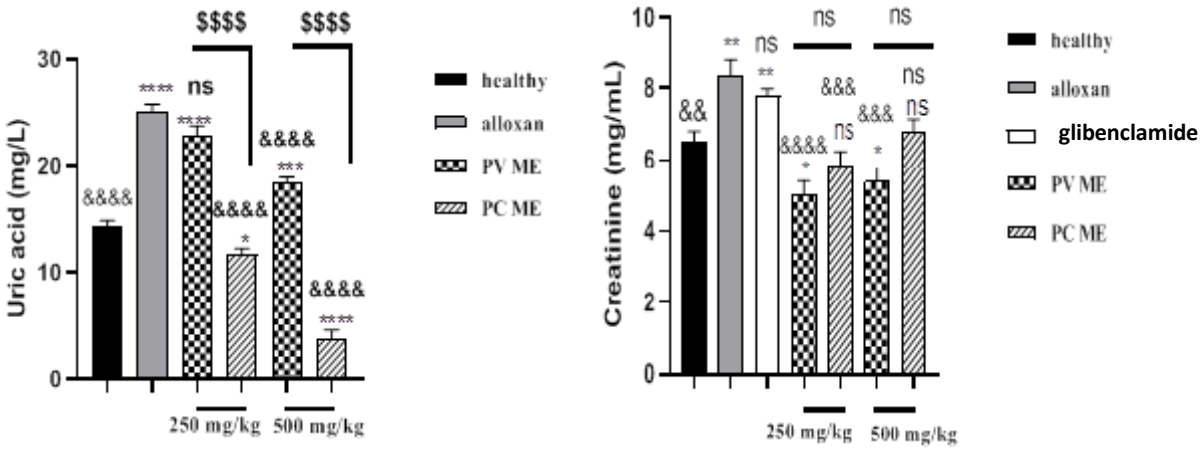


Figure 20: Changes in renal profile parameters (uric acid and creatinine) after 15 days of treatment in the experimental groups. PV ME: *P. herba venti* methanolic extract, PC ME: *P.crinita* methanolic extract. Values are mean±SEM (n=5-7) (ns: not significant; *P<0.05; **P<0.01;**** P<0.0001) *vs* healthy group(ns :not significant; $^{\&}$P<0.05; $^{\&\&}$P<0.01; ;$^{\&\&\&}$ P<0.001 ;$^{\&\&\&\&}$ P<0.0001) *vs* alloxan group. (ns:not significant; $^{\$}$P<0.05 ; $^{\$\$\$\$}$P<0.0001) comparison between extracts.

Serum creatinine is an important indicator of renal function. It is synthesized in the kidney from amino acids Arginine, glycine and methionine. An elevated blood creatinine level is observed with marked damaged to functioning nephrons (Gross *et al.*, 2005). Schrier *et al.*, (2004) proposed that plasma creatinine is a better indicator in the first phase of the toxicity of the kidney. Sodipo *et al*. (2012) reported higher creatinine levels in renal dysfunction and muscle injury. In figure (20), alloxan caused a significant (P<0.01) increase in serum creatinine in alloxan group (8.36±0.4 mg/mL) when compared with healthy group (6.53±0.15 mg/mL). Animals that were treated with the extract of PC ME 250 mg/kg (5.86± 0.15 mg/mL), PC ME 500 mg/kg (6.49±0.3 mg/mL) showed normal levels of plasma creatinine. These results suggest that *P. crinita* has a nephroprotective potential. The alloxan renal damage inducement is a familiar and well-replicated experiment model for the study of the effects of potential nephroprotective drugs and agents (Delfita *et al.*, 2021). The animals in the group administered PV ME 250 mg/kg (4.84±0.25 mg/mL) and PV ME 500 mg/kg (5.86±0.15 mg/mL) showed significant decrease (P<0.01; P<0.001) in the plasma creatinine level when compared with the alloxan group. These improvements are a likely indication or signs that the extracts was possibly reversing damages to kidney cells resulting from alloxan, but no significant effect was regestred the between doses used (250 and 500mg/kg).

Phytochemical study of *P.crinita* and *P.herba venti* has shown that they contain iridoids (kaabouche *et al*., 2005; Bouzergoune, 2017), These components may thus be responsible for diminution of serum uric acid and creatinine levels. Data published by Hou and his collaborators (2014) confirmed that treatment with iridoids (70 and 140mg/kg) significantly prevented the increases of uric acid in rat serum the same effect was registered with creatinine (Sundaram *et al*., 2012).

4.5.3. Effect of *P. crinita* and *P. herba venti* methanolic extracts on lipid profile parameters

Diabetes mellitus is often accompanied by a change in lipid profile resulting in hyperlipidemia, which is characterized by an increase in LDL-cholesterol levels, triglycerides and a decrease in HDL-cholesterol, which may suggest their participation in the development of cardiovascular diseases (Mohammed and Kakey, 2020). In this study, there was significant ($P<0.01$) increase in total cholesterol (0.72 ± 0.09 g/L) and LDL-cholesterol (0.33 ± 0.1 g/L) in alloxan group compared to healthy group (0.55 ± 0.1; 0.11 ± 0.09 g/L respectively). In this study there is significan decreased ($P<0.05$; $P<0.01$; $P<0.0001$) in LDL-cholesterol level in animals treated with PC ME and PV ME extracts compared to alloxan group (figure 21).

It was also found that the triglyceride levels in alloxan group (0.66±0.01g/L) increased significantly ($P<0.001$) compared to the healthy group (0.39±0.06 g/L) This increase in lipidemia may be due to insulin deficiency, leading to lipids accumulation and decrease in insulin release by cell tissues, since *P. crinita* treated diabetic animals decreased triglyceride level; 0.21 ± 0.06 g/L ($P<0.01$ *vs* alloxan group) and 0.49 ± 0.08 g/L respectively in 250 and 500mg/Kg treated groups, and similarly *P. herba venti* treated diabetic rats revealed triglyceride values as; 0.43 ± 0.06 g/L, and 0.49 ± 0.08 g/L in 250 and 500mg/Kg PV ME treated groups respectivly.

No significant ($P\leq0.05$) difference was regestred in HDL-cholesterol levels in two studied doses of PV ME (0.42 ± 0.07 mg/L and 0.56 ± 0.05 mg/L for 250 and 500 mg/kg respectively) compared to healthy group (0.57 ± 0.02 g/L). While it has been recorded a significant decrease ($P<0.01$; $P<0.001$; $P<0.0001$) in alloxan (0.38 ± 0.09 g/L), glibinclamide (0.34 ± 0.08 g/L), PC ME 250 (0.37 ± 0.05 g/L) and PC ME 500 mg/kg (0.40 ± 0.04 g/L) compared to healthy rats.

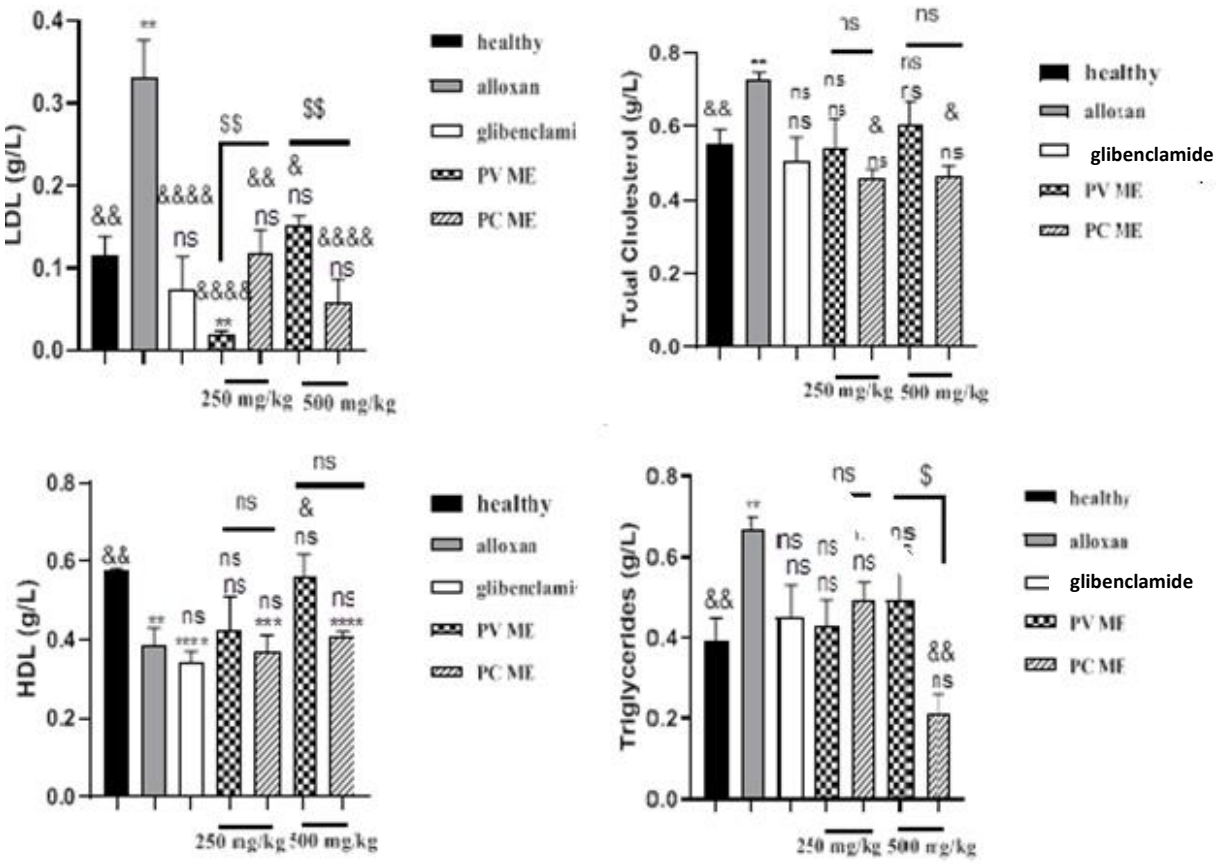


Figure 21: Changes in lipid profile parameters (total-Cholesterol, C-HDL, C-LDL and triglycerides), after 15 days of treatment in the experimental groups. PV ME: *P. herba venti* methanolic extract, PC ME: *P.crinita* methanolic extract. Values are mean±SEM (n=5-7) (ns: no significant; **P<0.01 ;*** <0.001 ; **** P<0.0001) *vs* healthy group(ns :no significant; $^{\&}$P<0.05; $^{\&\&}$P<0.01; $^{\&\&\&\&}$P<0.0001) *vs* alloxan group. (ns :no significant; $^{\$}$P<0.05 ; $^{\$\$}$P<0.01) comparison between extracts.

Kang and his collaborator (2018) demonstrated that administration of iridoid glycosides (75, 150, 300 mg/kg) could ameliorate the lipid profiles of diabetic mice in streptozotocin model. Jayaraman *et al.*, (2018) and Panahi *et al.*, (2017) depicted in their data the crucial role of flavonoids in amelioration of lipid profile in in type 2 diabetes mellitus. These metabolites present in the studied plant have been revealed as antidiabetic agents would also play a very important role in protective functions against the incidence of lipid peroxidation and cardiovascular disease (AL-Ishaq *et al.*, 2019). As previously reported (Ighodaro and Omole, 2012), the treatment with plant extracts promotes the transport of cholesterol and triglycerides from the blood stream to the tissues. These antilipidemic and anticholesterolemic activities observed in the treated rats may be resulted from the inhibition or suppression of some essential lipid metabolizing enzymes.

4.6. Effect of *P. crinita* and *P. herba venti* methanolic extracts on antioxidant parameters in liver and kidney

Intrarenal oxidative stress plays a critical role in the initiation and progression of diabetic kidney disease (DKD). Enhanced oxidative stress results from overproduction of ROS in the context of concomitant, insufficient antioxidant pathways. Renal ROS production in diabetes is predominantly mediated by various NADPH oxidases (NOXs), a defective antioxidant system as well as mitochondrial dysfunction may also contribute (Jha *et al*., 2016) However, under pathological situations, including in diabetes, the overproduction of ROS in the kidney is implicated in renal inflammation, affecting renal structure and function and subsequently leading to end-stage renal disease. Hyperglycemia-induced ROS production stimulates the recruitment of numerous inflammatory cells and production of inflammatory cytokines, growth factors, and transcription factors like upstream stimulatory factors 1 and 2 (USF1 and 2), activator protein 1 (AP-1), nuclear factor (NF)-kappaB implicated in the pathological processes of diabetic nephropathy (Chow *et al*., 2004).

Liver also is a major organ attacked by ROS, hyperglycaemia, mainly caused by insulin resistance, affects the metabolism of lipids, carbohydrates and proteins and can lead to non-alcoholic fatty liver disease, which can further progress to non-alcoholic steatohepatitis, cirrhosis and, finally, hepatocellular carcinomas. The underlying mechanism of diabetes that contributes to liver damage is the combination of increased oxidative stress and an aberrant inflammatory response; this activates the transcription of pro-apoptotic genes and damages hepatocytes (Mohamed *et al*., 2016).

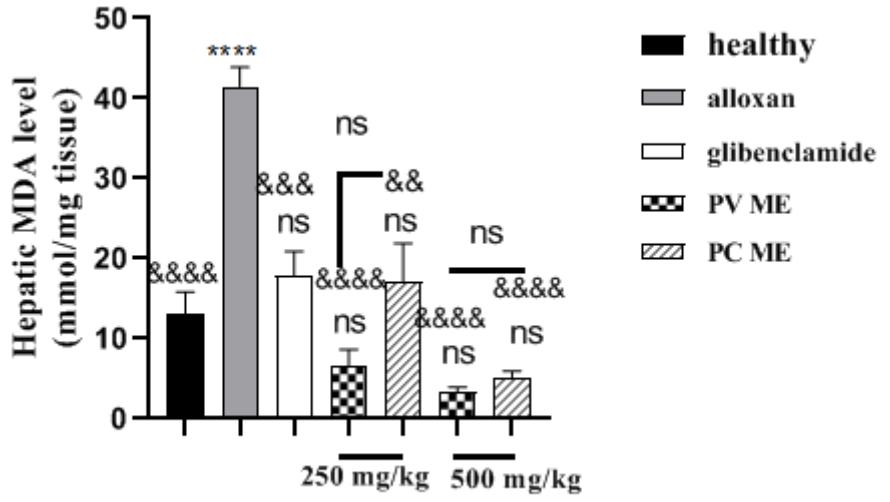
4.6.1. Effect of *P. crinita* and *P. herba venti* methanolic extracts on MDA level

The constituents of cell biomembranes are liable to free radical and active oxygen damage. The actions of these free radicals trigger cell damage by covalently binding with cellular macromolecules and formation of lipid peroxids (Ajay *et al*., 2010) which are implicated in the processes such as carcinogenesis, inflammation and aging . The peroxidation of polyunsaturated fatty acids (PUFAs) in the cell membrane produces malondialdehyde (MDA), a high concentration of this molecule in the hepatic and renal tissues indicates that induced lipid peroxidation has taken place, which has been cited as a contributing factor to the development of diabetes mellitus (Rukkumani *et al*., 2004; Akinosun, Bolajoko, 2007). Results obtained in figure (22) show a high significant ($P<0.01$; $P<0.001$; $P>0.0001$; $P<0.0001$) reduction in the production of malondialdehyde (MDA), which is considered as an

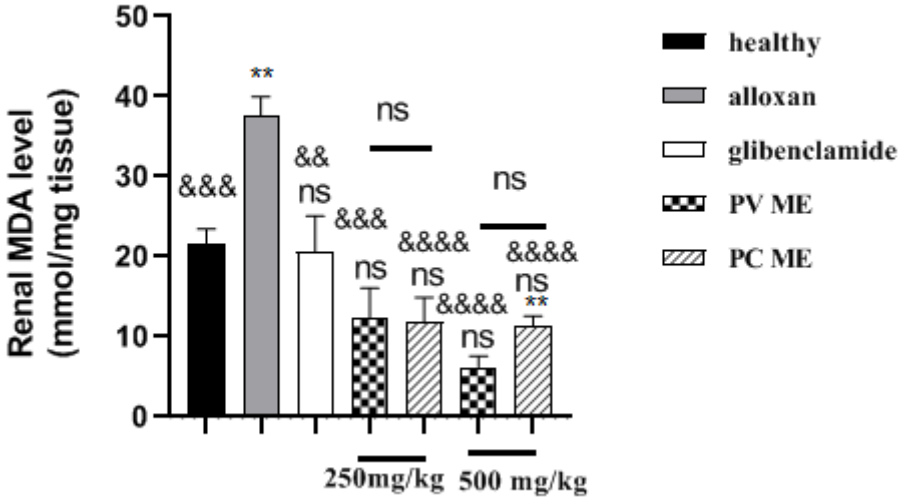
index lipid peroxidation. These results may ascertain the preventive effect of the extracts (PCME, PVME) on the lipid peroxidation by their anti-radical property and capture of free radicals.

In the liver, the administration of doses 250mg/kg and 500mg/kg of both *P.rinita and P.herba venti* reduced MDA formation (6.68±0.25 mmol/mg tissue) and (3.30±0.5 mmol/mg tissue) respectively for PV ME and (17.10±1.46 mmol/mg tissue) and (5.15±0.57 mmol/mg tissue) respectively for PC ME compared to alloxan group (41.47±1.05 mmol/mg tissue) wish signefecantly ($P<0.05$) reduced MDA formation at the higher dose (500 mg/kg). Whereas, healthy group has 13.03±0.5 mmol/mg tissue and glibinclamide has 17.90 ±0.18 mmol/ mg tissue. These finding is in accordance with results of Ferrante and his collaborators (2019) who showed a decrease MDA levels in isolated rat colon treated *P. herba venti* with and challenged with LPS. Sarkhail *et al.,* (2010) found that *P. persica* can reduces MDA production in diabetic rat liver after 10 days of treatments. Similarly Gu *et al.,* (2014) reported that the administration of *Phlomis Maximowiczii* protects the liver from lipid peroxidation in CCL4 inducing hepatictoxicity.

In this study renal MDA levels were significantly ($P<0.01$; $P<0.001$; $P<0.0001$) lower in all groups compared with alloxan group (37.51±1.03 mmol/mg tissue) figure (22). The treatment with *P.crinita* at doses of 250 and 500 mg/kg decrease MDA level to the values of: 14.22±1.8 mmol/mg tissue and 10.12±0.54 mmol/mg tissue, respectively. Similar effects were registered in animals treated with *P. herba venti* at doses of 250 and 500 mg/kg, The recorded values were 15.65±1.2 mmol/mg tissue and 6.21±0.75 mmol/mg tissue respectively , this dimunitionn reached statistically not significant difference ($P\leq0.05$) between doses (250 and500 mg/kg) of the two plants used. Glibinclamide and healthy groups had approximately an equal level of MDA (20.63±1.2 mmol/mg tissue and 21.64±0.85 mmol/mg tissue). It was previously shown that administration of iridoids (molecules identified in *P.crinita and P. herba venti* extracts) to diabetic animals led to a decrease in the lipoperoxidation products accumulation (Dzydzan *et al.,* 2020). A similar effect was noticed whit anthraquinone (AEMA exhibited significant suppression of the elevated hepatic levels of malondialdehyde (MDA) (Chen *et al*., 2020).



(A)



(B)

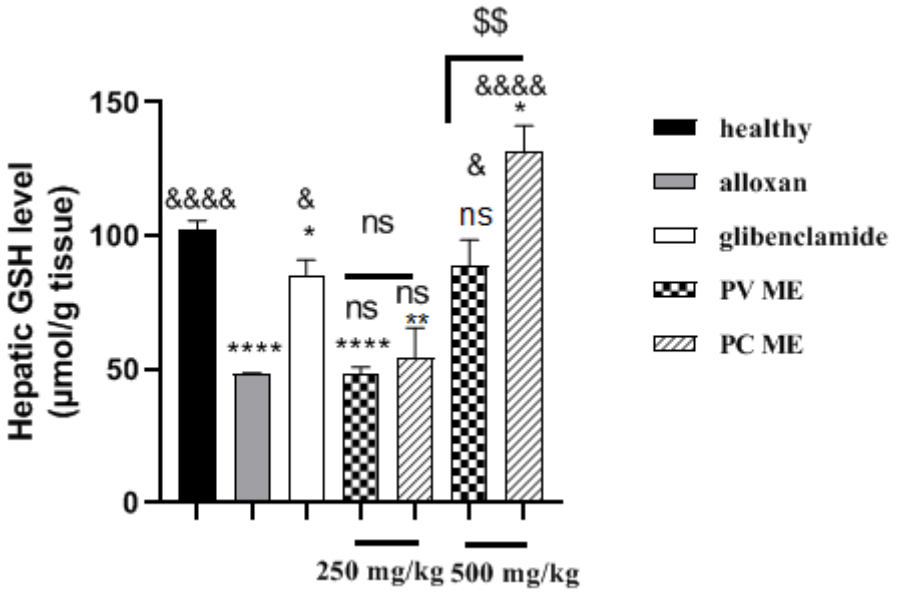
Figure 22. : Effect of methanolic extract of *P. herba venti*, *P. crinita* and glibinclamide on MDA level in liver (A) and kidney (B) of rats. PV ME: *P. herba venti* methanolic extract, PC ME: *P.crinita* methanolic extract. Values are mean±SEM (n=5-7) (ns: not significant; **P<0.01 ; **** P<0.0001) *vs* healthy group(ns :not significant $^{\&\&}$P<0.01; $^{\&\&\&}$P<0.001; $^{\&\&\&\&}$ P<0.0001) *vs* alloxan group. (ns :no significant) comparison between extracts.

4.6.2. Effect of *P. crinita* and *P. herba venti* methanolic extracts on GSH level

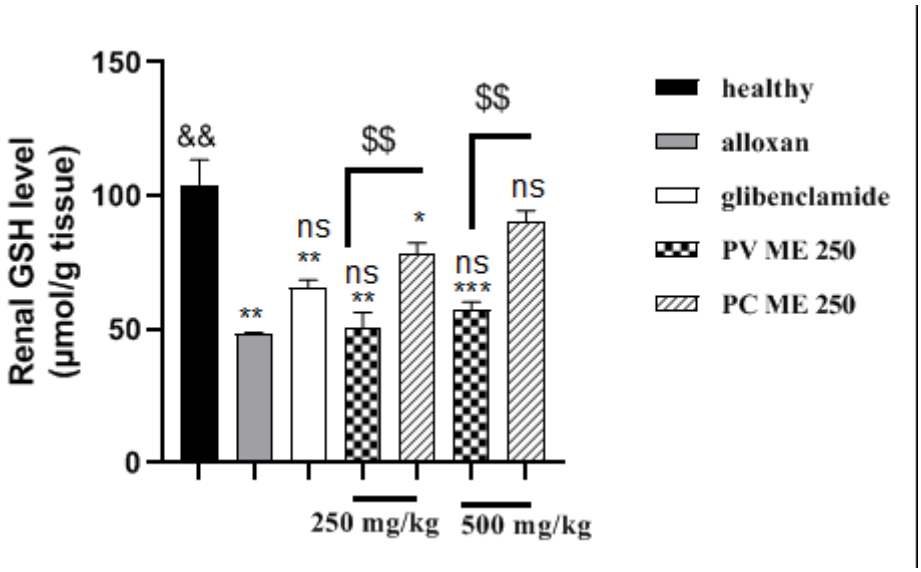
Reduced glutathione (GSH) serves as non-enzymatic antioxidant biological molecule tissue of the body. It functions to remove non radical compounds like hydrogen peroxide, Alkoxyl radical, and superoxide anion from tissue (Townsend *et al.*, 2003). The decrease in GSH levels in liver during diabetes may be due to its increased utilization by the hepatic cells as attempt to counteract the increased formation of lipid peroxides. Reduced activities of SOD, CAT and GSH-Px in the liver and kidneys have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide (Pari and Umamaheswari, 2000).

Figure (23) presents hepatic GSH levels in rats, the levels of hepatic GSH in alloxan group were significantly ($P<0.0001$) lower than those of healthy group. Animals treated with 500 mg/kg of PV ME showed no significant ($P<0.05$) difference in the hepatic GSH level (88.59±4.91µmoL/g tissue) compared with healthy group (103.4 ±1.03 µmoL/g tissue) and ($P<0.01$) for the hepatic GSH level of PC ME (131.7±1.90 µmoL/g tissue) Which is better than healthy group (103.4 ±1.03 µmoL/g tissue). Furthermore, reduced hepatic glutathione (GSH) depletion caused by alloxan was restored by administration of the glibenclamide for 15 days at the daily dose of 3mg/kg where we found an increase in the GSH level (85.39±1.5 µmoL/g tissue) compared to alloxan animals (48.27 ±0.18 µmol/g tissue), and PC ME (49.15±2.12 µmol/g tissue) and PV ME (48.18±1.03 µmol/g tissue) at dose of 250 mg/kg. the effect of PC ME and PV ME on hepatic GSH level was dose dependant ($P<0.0001$).

The level of GSH in renal tissues was significantly ($P<0.05$; $P<0.01$; $P<0.001$) deacreased in animals treated by 250 and 500 mg/kg of *P. herba venti* (50.63± 0.59 µmoL/g tissue; 57.42±1.60 µmoL/g tissue respectively) and *P. crinita* (78.56±1.24 µmoL/g tissue; 90.21±1.31 µmoL/g tissue respectively), glibinclamide 3 mg/kg (65.73±1.08 µmoL/g tissue) and alloxan group (48.94±0.35 µmoL/g tissue) compared to helthy group (101.06±0.92 µmoL/g tissue), In contrast, renal reduced glutathione (GSH) depletion caused by alloxan was significantly ($P<0.5$) restored by administration of 500 mg/kg of PC ME and PV ME compared to alloxan group (figure 23).



(A)



(B)

Figure 23: Effect of daily administration of *P. crinita*, P. *herba venti* methanolic extract and glibinclamide on liver (A) and kidney (B) GSH level. PV ME: *P. herba venti* methanolic extract, PC ME: *P.crinita* methanolic extract. Values are mean ± SEM (n=5-7) (ns: not significant; *P<0.05; **P<0.01 ; *** P<0.001 **** P<0.0001) *vs* healthy group (ns :not significant; $^{\&}$P<0.05; $^{\&\&}$P<0.01; $^{\&\&\&\&}$ P<0.0001) *vs* alloxan group. (ns :not significant; $^{\$\$}$P<0.01 ; $^{\$\$\$}$ P<0.001) comparison between extracts.

Sarkhail and his collaborators (2010) and Gu *et al*., (2014) showed that Phlomis genus can improved hepatic GSH level in diabetic rat and CCL4 inducing hepatictoxicity. This effect

may be due to the presence of secondary metabolites such as flavonoids iridoids and phenylethanoids.

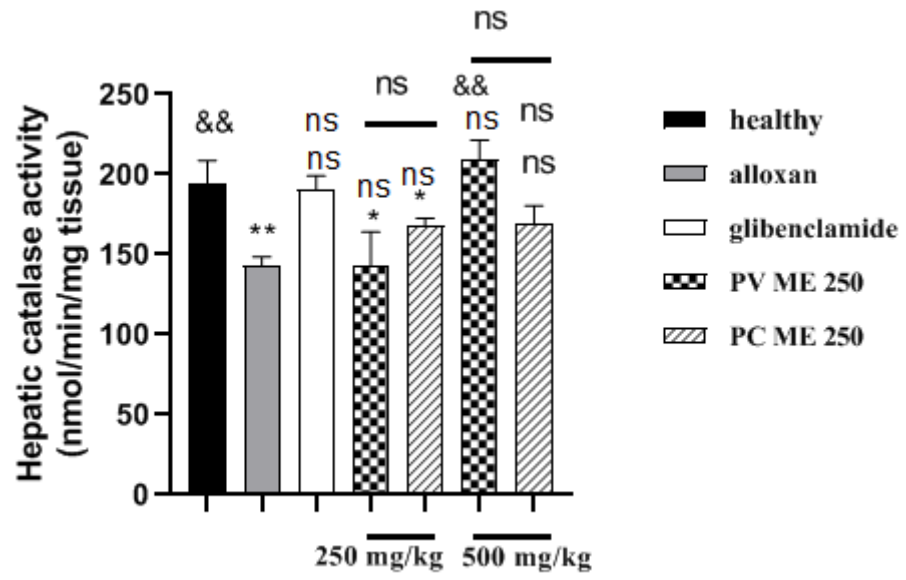
Iridoids are one of the major compounds wish isolated from *P. crinita* (Dellai *et al*., 2009) and *P. herba venti* (Alipieva *et al.,* 2000). Data of Dzydzan and his collaborator (2020) improved that administration of iridoids caused a 21% increase in the plasma GSH level compared to the no treated diabetic group.

4.6.3. Effect of *P. crinita* and *P. herba venti* methanolic extracts on catalase activity

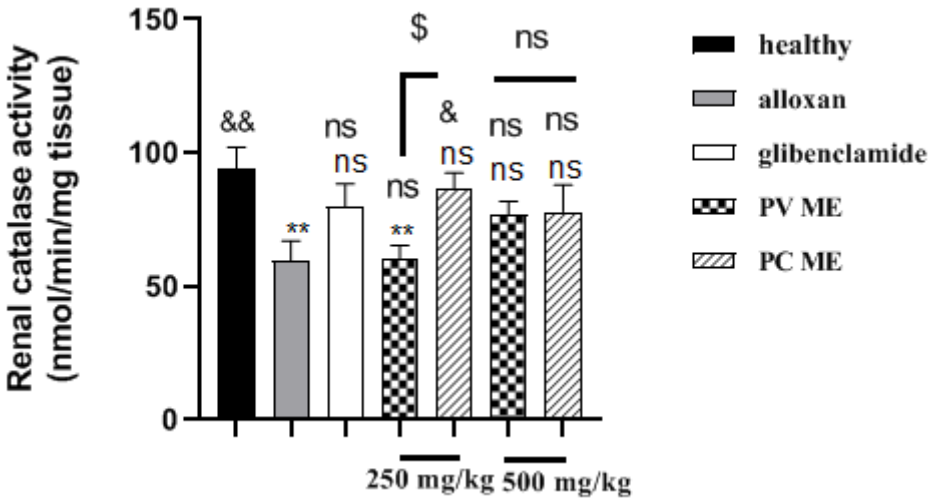
Impaired glucose metabolism due to diabetes and oversaturation of the electron transport chain of cells leads to the overproduction of superoxide anion and its derivatives. It is known that oxidative stress plays a significant role in the pathogenesis of diabetes and causes protein modification and subsequent complications. Antioxidant enzymes have shown an important role in maintaining physiological levels of superoxide anion and hydrogen peroxide by dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides (Gomes *et al*., 2012). Our results are in agrement with data of Dzydzan *et al* (2020) which showed a reduction in antioxidant enzyme activity in rats treated with diabetes drugs. The activity of enzymes may be affected by the processes of direct or indirect oxidation the excess of ROS, and ROS-mediated modification of proteins under diabetes, such as nitration (Bila *et al.,* 2019).

In this study, we found that administration of *P. crinita* and *P. herba venti* extracts have maintained catalase activity close to the values of healthy group (194.34± 0.72 nmoL/min/mg in the liver and 93.94± 0. 27 nmoL/min/mg in the kidney), while alloxan group showed a significant decrease ($P<0.01$) in catalase activity (147.61±0.69 nmoL/min/mg in the liver and (59.48± 0.8 nmoL/min/mg in the kidney) (figure 24).

In addition, the enzymatic activity of hepatic catalase was (143.32±0.53 nmoL/min/mg; 209.59±0.85 nmoL/min/mg) dose-dependently for PV ME 250 and 500 mg/kg respectively and (168.33±1.15 nmoL/min/mg; 169.40±1.15 nmoL/min/mg) in PC ME at 250 and 500 mg/kg respectively, since the catalase activity in glibinclamide group was similar (190.73± 0.72 nmoL/min/mg) to that of healthy group (figure 24).



(A)



(B)

Figure 24: Effect of methanolic extract of *P.crinita* and *P. herba venti* on catalase activity in liver (A) and kidney (B) of rats. PV ME: *P. herba venti* methanolicic extract, PC ME: *P.crinita* methanolicic extract. Values are mean ± SEM (n=5-7) (ns: not significant; *P<0.05; **P<0.01) *vs* healthy group.(ns :not significant; $^{\&}$P<0.05; $^{\&\&}$P<0.01) *vs* alloxan group. (ns :not significant; $^{\$}$P<0.05) comparison between extracts.

In the kidney the treatment with 250 mg/kg and 500 mg/kg dose of *P. crinita*, *P herba venti* and glibinclamide (3mg/kg) increased (not significantly P<0.05) the enzymatic activity of catalase with the value of (86.42±1.3 nmoL/min/mg; 77.49±0.85 nmoL/min/mg) in PC ME

treated group, and (76.93±1.03 nmoL/min/mg) in 500 mg/kg PV ME treated groups compared to alloxan group (59.42 nmoL/min/mg) (figure 24)

These results are in agreement with other findings on the antihyperglycemic effect of Phlomis extracts. It was found that the decreased activities of hepatic antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) in diabetic rats were restored by the use of *Phlomis persica extracts*, thereby attenuating the oxidative stress associated with diabetes (Sarkhail *et al.*, 2010). The catalase level in the treated groups were close to the healthy group and highier than alloxan group this may be due to the presence of phenolic compounds in the plants extracts which help to raise the antioxidant activities of such enzymes (Verma *et al.*, 2009). The positive effect of PCME and PVME on this antioxidant enzyme is most probably due to the existence of iridoid glycosides, flavonoids and other phenolic compounds in PCME and PVME as well-known antioxidants that scavenge the free radicals generated during diabetes (Sarkhail *et al.*, 2010).

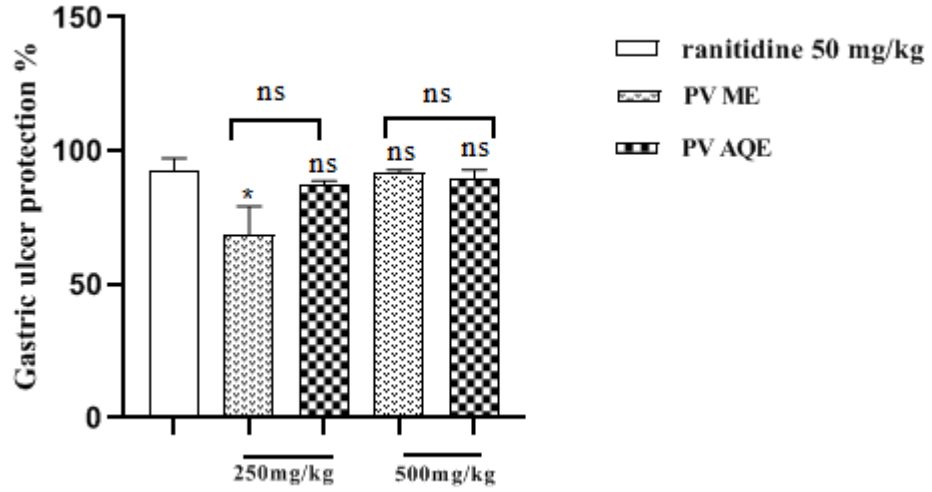
5. Gastroprotective effect

5.1. Effect of *P. crinita* and *P. herba venti* extracts on ethanol-induced gastric ulcer in rats

Ethanol-induced gastric ulcer is a common animal model used to assess the new anti-ulcer drugs (Arab *et al.*, 2015). Administration of ethanol causes gastric necrotic damage and subsequent inflammatory cell infiltration and reduces the secretion of bicarbonate, gastric mucus, and nitric oxide. In addition, ethanol reduces the gastric blood flow and induces oxidative stress by increasing the production of malondialdehyde and reducing glutathione production (El-Maraghy, 2015). Ethanol induces longitudinal ulcer lesions in the glandular part of the stomach (figure 25) and stimulates leukotrienes, the 5-lipoxygenase pathway, mast cell secretion, and the release of reactive oxygen species resulting in damage to the gastric mucosa .The administration of the *Phlomis* species (*crinita and herba venti*) methanolicic and aqueous extracts at doses 250 and 500 mg/kg significantly reduced the number and size of gastric ulcers induced by ethanol in the rat with a percentage that reached 95 % (figure 25 and 26).

It is evident from the results recorded in figure (25and 26) that all the extracts used showed very significant activity ($P<0.05$) in protecting the gastric mucosa from ulceration at rates ranging between 68% and 95%. the 250 mg/kg dose of PC ME as the highest protective effect

(95%) which was better than the ranitidine 50 mg/kg (92%), meanwhile, the treatments with *P.crinita* extracts revealed close protective ratios found between 88% and 87%, and 84% respectively in 500 and 250 mg/kg PC AQE, and 500mg/kg of PC ME groups. there is no significant difference is recorded in comparison with the different types of extracts.



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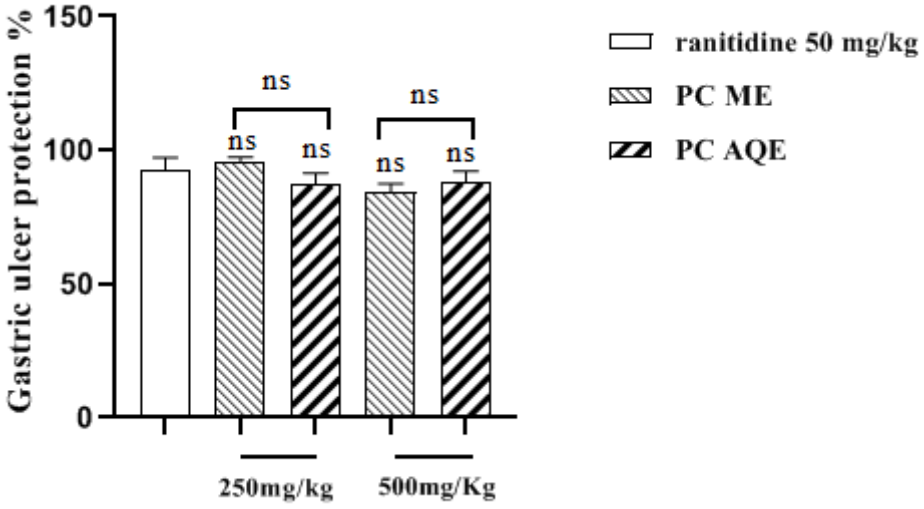


(B)

Figure 25: Effects of *P. herba venti* (A) and *P. crinita* (B) extracts on gastric ulcer protection in rats. PV ME: *P. herba venti* methanolicic extract, PV AQE: *P. herba venti* aqueous extract, PC ME: *P.crinita* methanolicic extract, PC AQE: *P.crinita* aqueous extract. Values are mean ± SEM (n=5-6).(ns: not significant , * P<0.05) *vs* ranitidine. (ns ; not significant in comparison between the effects of PV ME *vs* PVAQE and PC ME *vs* PC AQE at doses of 250and 500 mg/kg.

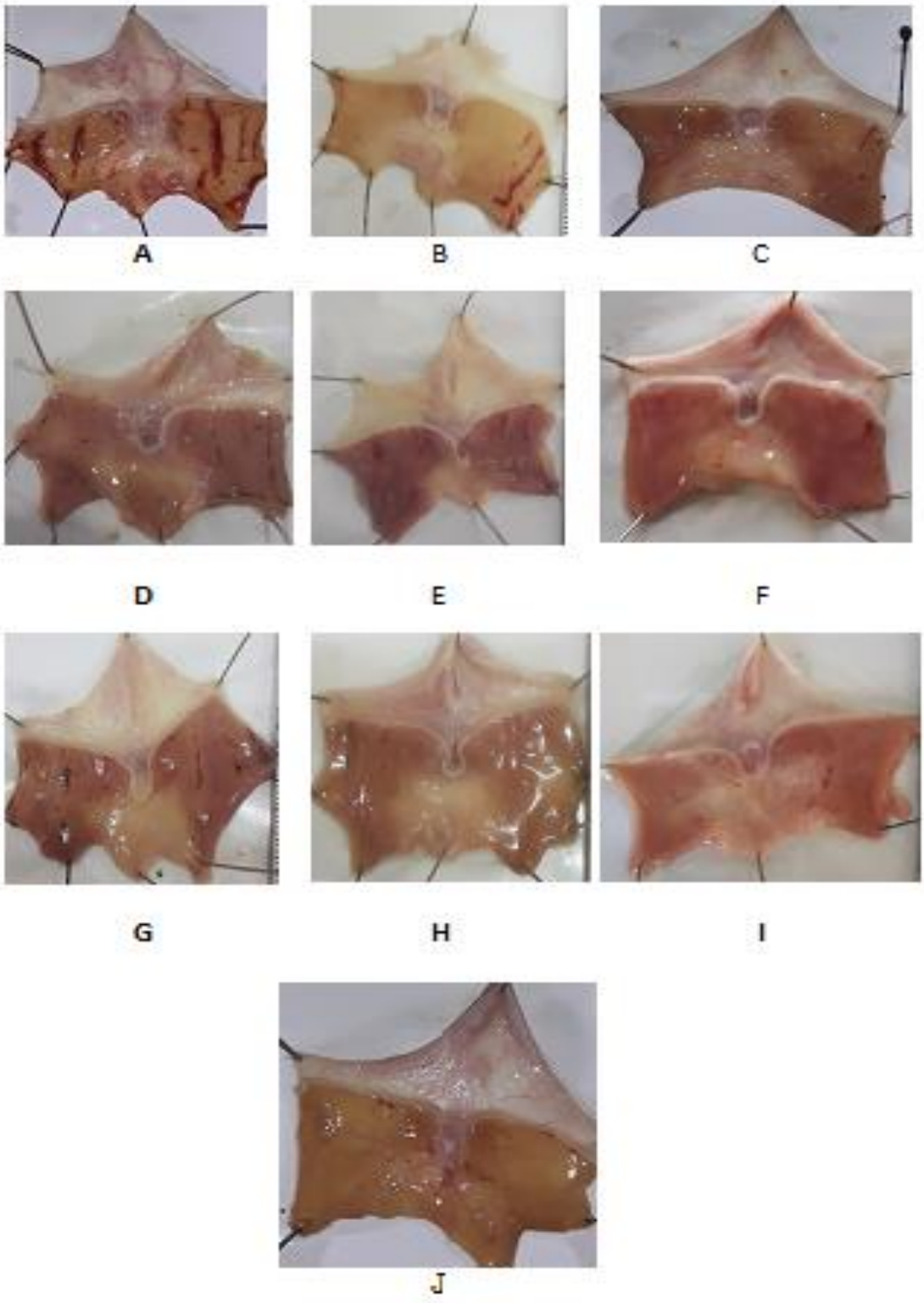


Figure 26: Effect of *P.crinita* and *P. herba vent* extracts on the macroscopic appearance of the gastric mucosa in ethanol-induced gastric mucosal lesions in rats. A: ethanol group, B: ranitidine 50 mg/kg,C: PC ME 250 mg/kg , D: PC ME 500 mg/kg, E: PC AQE 250 mg/kg. F: PC AQE 500 mg/kg. G: PV ME 250 mg/kg, H: PV ME 500 mg/kg, I: PV AQE 250 mg/kg, J: PV AQE 500 mg/kg.

The present findings are in line with the results of Gurbuz *et al* (2003) and Limem-Ben Amor *et al* (2009) who studied the antiulcergenic effect of *Phlomis grandiflora* and *P. crinita* aqueous extracts, respectively. These activities are most likely due to the different classes of phytochmicals such as, glycosides, diterpenoids, irridoids, phenylpropanoids, phenylethanoids, alkaloids, and flavonoids that have been identified in the genus Phlomis (Kumar *et al.,* 1992, Yalcin *et al.,* 2003, Kabouche *et al*., 2005; Aghakhani *et al*., 2018).

The qualitative chemical investigation of *P. crinita* reported that this plant contains luteolin 7-O-b-D-glucopyranoside and verbascoside (Kabouche *et al*., 2005). Luteolin 7-O-b-D-glucopyranoside has remarcable gastroprotective effect in indomethacin and ethanol-inducing-ulcer models in rats (Antonisamy *et al.,* 2016). The protective mechanism of verbascoside is apparently mediated via the inhibition of H+ K+-ATPase with corresponding decrease in plasma gastrin level (Singh *et al*., 2010). Moreover, the phytochemical analysis of the phenolic compunds in both phlomis species has revealed the presence of chlorogenic acid as a major constituent in these two plants (unpblished results). This compund has been linked to gastroprotective activity in different gastric ulcer models (Shimoyama *et al*., 2013; Ahmed *et al*., 2021).

Prostaglantdins are present throughout the gastrointestinal tract and participate in the regulation of various gastric functions that protect the gastric mucosa from aggressive agents, stress and NSAIDs through the control of acid secretion, mucus production and increased mucous blood flow (Takeuchi and Amagase, 2018). Antonisamy *et al*., (2016) reported that luteolin-7-O-glucoside increase the production of beneficial PGE2, a mechanism that may be involved in protecting the gastric ulcer (Antonisamy *et al.,* 2016). Another important mechanisms involved in protecting the gastric ulcer is the inhibition of inflammation. Luteolin is known as an inflammation inhibitor in gastric mucosal cells through the inhibition of COX-1 and COX-2 activity and pro-inflammatory cytokine production such as IL1, IL6, IL10 and TNFα (Antonisamy *et al.,* 2016).

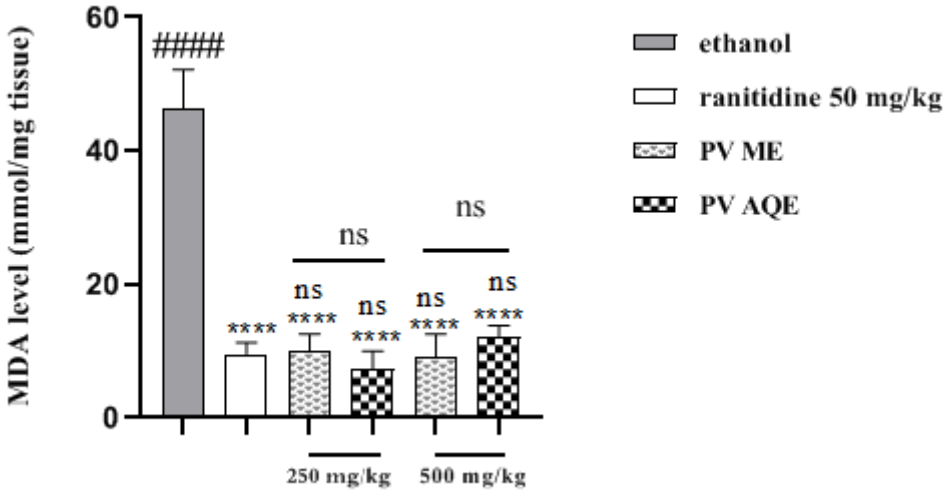
As we've already mentioned pretreatment of rats with *Phlomis herba venti* methanolicic and aqueaus extracts increased the ulcer gastric protection and did not show any significant difference versus the ranitidine (50 mg/kg) treated animals. The best gastric protection was observed at dose of (500 mg/kg) of PV ME (91%) followed by PV AQE at dose of 500 mg/kg and 250 mg/kg with percentage of protection 89% and 87%, respectively. However the PV ME At dose of 250 mg/kg showed the lowest gastric protection (68%). Ferrante and his

collaborator (2019) found that *P. herba venti* extract had a strong anti-inflammatory activity in a model of ulcerative colitis, which may explain its anti ulcerogenic effect in this study. Another possible mecanisme of this activity may be attributed to the antioxidant activity of the plant, since the ethanol-induceding gastric ulcer model is known to causes the overproduction of reactive oxygen species, such as superoxide anion, peroxide hydrogena and hydroxyl radical, which promote lipid peroxidation and the formation of hemorrhagic ulcerations (Sandhar *et al.,* 2011). Previous (Azad *et al.*, 2015; Merouane *et al.,* 2018) and present results showed that *P. herba venti and P. crinita* had a strong antioxidant activity.

5.2. *In vivo* antioxidant activities of *P. crinita* and *P. herba venti* extracts in the stomach of the rat

5.2. 1. Effect of of *P. herba venti and P. crinita* on lipid peroxidation

Our data revealed a significant decrease ($P<0.0001$) in stomach MDA level in *P.crinita and P.herba venti* treated rats (table 06 and figure 27) compared to the ethanol group (46.71±3.43 mmoL/mg tissue). Hence, both plant extracts are able to reduce the MDA level by more than 70%, and noteworthy, 500 mg/kg PC AQE results in 89% of MDA reduction (3.4±1.65 mmoL/mg tissue). These result concords with in vitro findings showing a strong lipid peroxidation inhibiting ability of plant extracts. As reported by Merouane and his collaborator (2018, 2019), *P. crinita* extracts has a potent antioxidant activity against ROS generation in the lipid environment. Our results are in line with those reported by Ferrante *et al.,* (2019) reporting the efficiency of *P. herba venti* extract in reducing MDA level in rat colon challenged with lipopolysaccharides (LPS). In this context, Sarkhail *et al.,* (2010) reported a reduced MDA level in Phlomis genus of the Lamiaceae family treated diabetic rat liver. Accordingly, several result studies reported high levels of secondary metabolites, including falvonoids, polyphenols, iredoids and antraquinons found in *P. crinita and P. herba venti* (Azad *et al*., 2016; Ferrante *et al*., 2019). These compounds have effective antioxidant activity able to protect the stomach from ulceration (Zakaria *et al*., 2014, 2016). Recently, drinking alcohol was reported to be harmful to the stomach tissues. Ethanol-induced gastric tissue injury and ulcerogenesis is one of the essential pathogenesis pathways involve oxidative stress mediated the generation of reactive oxygen species (ROS) (Yao, 2015; El-Naga, 2015). As a suggestion, the gastroprotective effects of these plants extracts are owed to their antioxidant activity by activating some enzymatic antioxidant mechanisms (CAT, and GSH) and reducing lipid peroxidation.



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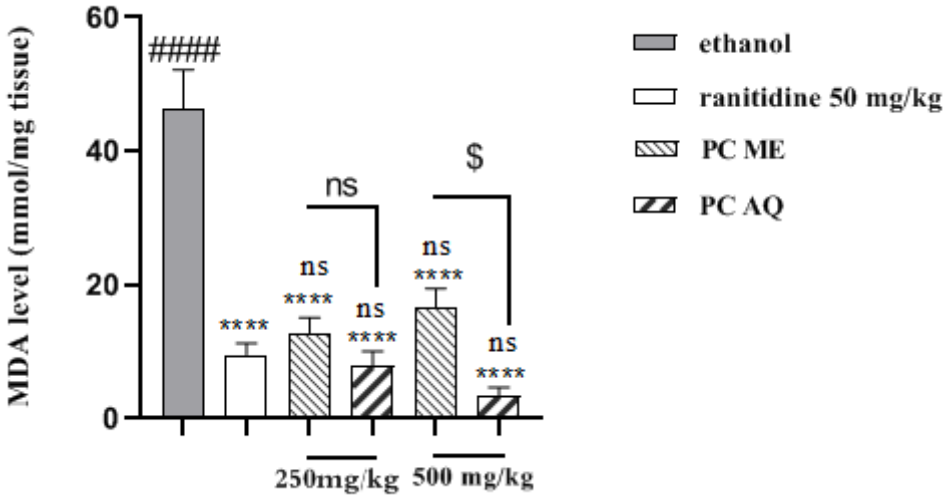


(B)

Figure 27: Effect of *P.herba venti* (A) and *P.crinita (B)* extracts on MDA level in gastric tissues of rats. PV ME: *P. herba venti* methanolicic extract, PV AQE: *P. herba venti* aqueous extract, PC ME: *P. crinita* methanolicic extract, PC AQE: *P. crinita* aqueous extract. Value are means ± SEM (n=5-6) (****$P<0.0001$) *vs* ethanol, (ns: not significant; #### $P<0.0001$) *vs* ranitidine. (ns: not significant; $^{\$}P<0.05$) comparison between extrcts.

5.2. 2. Effect of of *P. herba venti and P. crinita* on (GSH) level and catalase (CAT) activity

Our results revealed induction of ulcer following ethanol treatment disrupting, subsequently the antioxidant enzymatic activity in stomach (CAT and GSH), where their decreased activities in glandular part of stomach can be resulted from the over generation of reactive oxygen free radicals. These antioxidant enzymes are overall responsible for protecting cells from harmful ROS. Further, Superoxide dismutases (SODs) are the first defense line against free radicals , as well as they catalyze the conversion of O_2 to oxygen and H_2O_2, which then converted to H_2O by CAT.

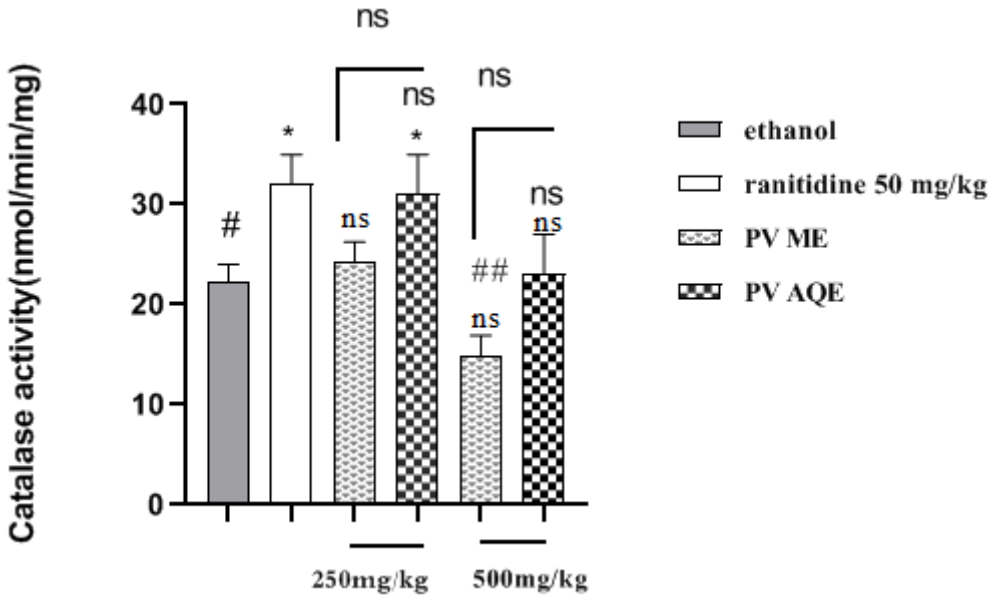
Figure (28) and table (06) depicted that there is reduction in catalase activity in ethanol group (22.2±0.32 nmoL/min/mg tissue). On the other hand, it was found that the administration of the PC AQE extracts at dose of 250 and 500 mg/kg (36.7±0.34 nmoL/min/mg tissue; 36.0±0.9 nmoL/min/mg tissue) increased significantly ($P<0.05$) the activity of reduced catalase compared with ethanol group, this increase is in order of 25%. The same effect was registered with PVAQE at dose of 250 mg/kg (30.29±0.32 nmoL/min/mg tissue) and ranitidine (32.09±2.1 nmoL/min/mg tissue). Al extracts doses showed no significant different ($P<0.05$) than ranitidine as a positive control (table 06).

Moreover, GSH level was significantly increased ($P< 0.0001$) in the pre-treatment groups, where the best effect was noticed in 250 and 500 mg/kg of PV AQE, 250 and500 mg/kg of PC ME and 500mg/kg of PC AQE with the values of 28.49±2.31 µmoL/g tissue; 38.60±2.31 µmoL/g tissue; 35.69±2.72 µmoL/g tissue and 32.00± 3.00 µmoL/g tissue than the ethanol control group (11.07±2.25 µmoL/g tissue). However, treatments with 50 mg/kg ranitidine, 250/500 mg/kg of PV ME, and 500mg/kg of PC AQE revealed no significant difference ($P<0.05$) in stomach GSH level compared with ethanol group (figure 29, table 06). significant ($P<0.01$; $P<0.001$) difference was noted when compared the effect of PV ME (250 and 500 mg/kg), and PC AQE at dose of 250 mg/kg and ranitidine 50 mg/kg as positive control .The current results and previously published data indicated an important relationship between gastric GSH levels and the ulcer severity. GSH and GSH-related enzymes are the main tissue protective agents owed to their antioxidant properties (Repetto and llesuy; 2002; Xue Wu *et al*., 2018).

Table 06: Effects of *P.crinita* and *P.herba venti* extracts on total protein level, CAT activity, GSH level and MDA level of stomach tissue in ethanoli nduced gastric mucosal lesions in rats**.**

parameters / Treatments	Proteins mg/g tissue	GSH µmoL/g tissue	Catalase nmoL/min/mg tissue	MDA µmoL/g tissue
Ethanol group	511.6±11.9	11.07±3.17	22.2±0.32	46.71±0.63
Ranitidine 50 mg/kg	537.82±7.44	17.15±2.49	34.0±0.8	9.55±0.41
PC ME 250mg/kg	597.42±8.91	38.60±2.31	24.4±0.21	12.8±0.39
PC ME 500 mg/kg	554.72±6.87	35.69±2.72	23 ± 0.32	13.69±0.46
PC AQE 250 mg/kg	625.76± 8.5	21.88 ± 2.31	36.7 ± 0.34	7.87±0.37
PC AQE 500 mg/kg	591.03±6.84	33.26 ± 3.04	36.0 ± 0.9	3.4±0.27
PV ME 250 mg/kg	565.1±6	11.20±1.93	20.54 ±0.86	9.96 ±0.39
PV ME 500 mg/kg	513.3±5	11.92± 1,95	14.94±0.85	9.11±0.24
PV AQE 250 mg/kg	611.5±8.5	28.49±2.71	34.40±0.52	7.3±0.48
PV AQE 500 mg/kg	579.3±8	32±3.00	23.16±0.66	6.67±0.43

Value are mean ± SEM (n=5-6).



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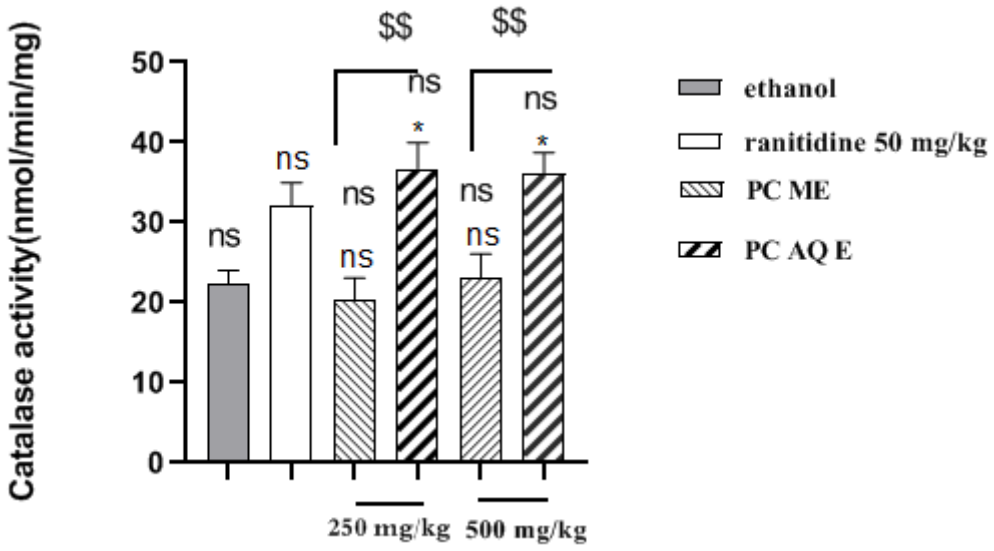
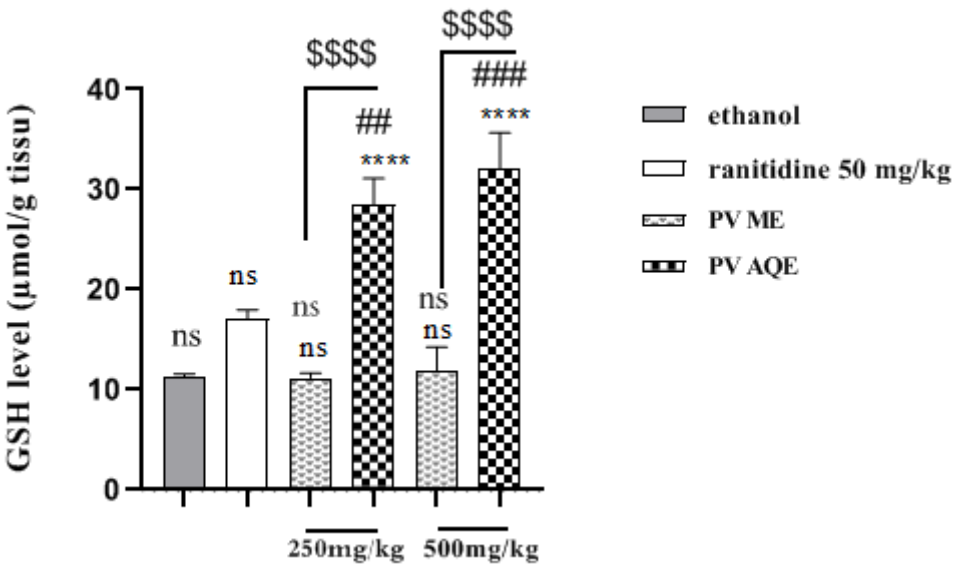
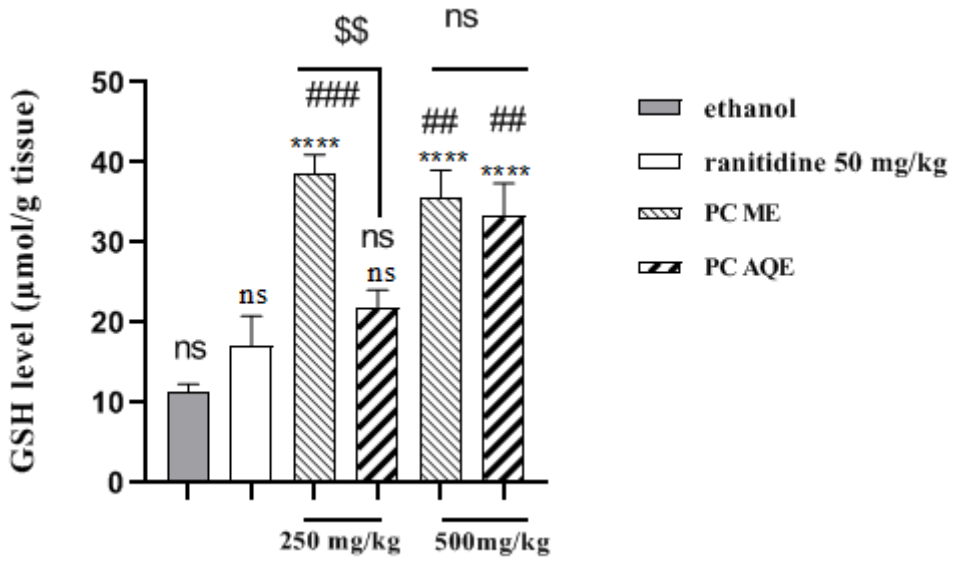


(B)

Figure 28: Effect of *P. herba venti* (A) and *P. crinita* (B) extracts on catalase activity in rats' stomachs tissue. PV ME: *P. herba venti* methanolicic extract, PV AQE: *P. herba venti* aqueous extract, PC ME: *P. crinita* methanolicic extract, PC AQE: *P. crinita* aqueous extract. Values are means ± SEM (n=5-6) (ns: not significant; *P<0.05) *vs* ethanol. (ns: not significant, ## P< 0.001) *vs* ranitidine. (ns: not significant; $^{\$\$}$P<0 .001) comparison between extracts.



(A)



(B)

Figure 29: Effect of *P. herba venti* (A) and *P.crinita* (B) extracts on GSH level in rats stomach tissue. The PV ME: *P. herba venti* methanolicic extract, PV AQE: P. *herba venti* aqueous extract, PC ME: *P.crinita* methanolicic extract, PC AQE: *P.crinita* aqueous extract. Values are means ± SEM (n=5-6) (ns: not significant; ****P<0.0001) *vs* ethanol. (ns: not significant: ## :P<0.01; ###: P<0.001) *vs* ranitidine. (ns; no significant ; $^{\$\$}$ P< 0.01; $^{\$\$\$\$}$ P< 0.0001) comparison between extracts

5.2. 3. Effect of of *P. herba venti and P. crinita* on total proteins estimation

As shown in figure (30) and table (06) treatment of rats with different doses of the extracts studied has no effect (P>0.05) on protein content compared to ethanol group and between extracts, this result is in line with that reported by Hajrezaie *et al*., (2015). Albumin is the protein itself and possibly also albumin-bound bilirubin, an important secondary antioxidant that binds iron, copper and many products, like hydro peroxides to prevent free radicals induced oxidation of the cell macromolecules (Halliwell, and Gutteridge, 1990). Due to the effective protective effects of gastric tissue

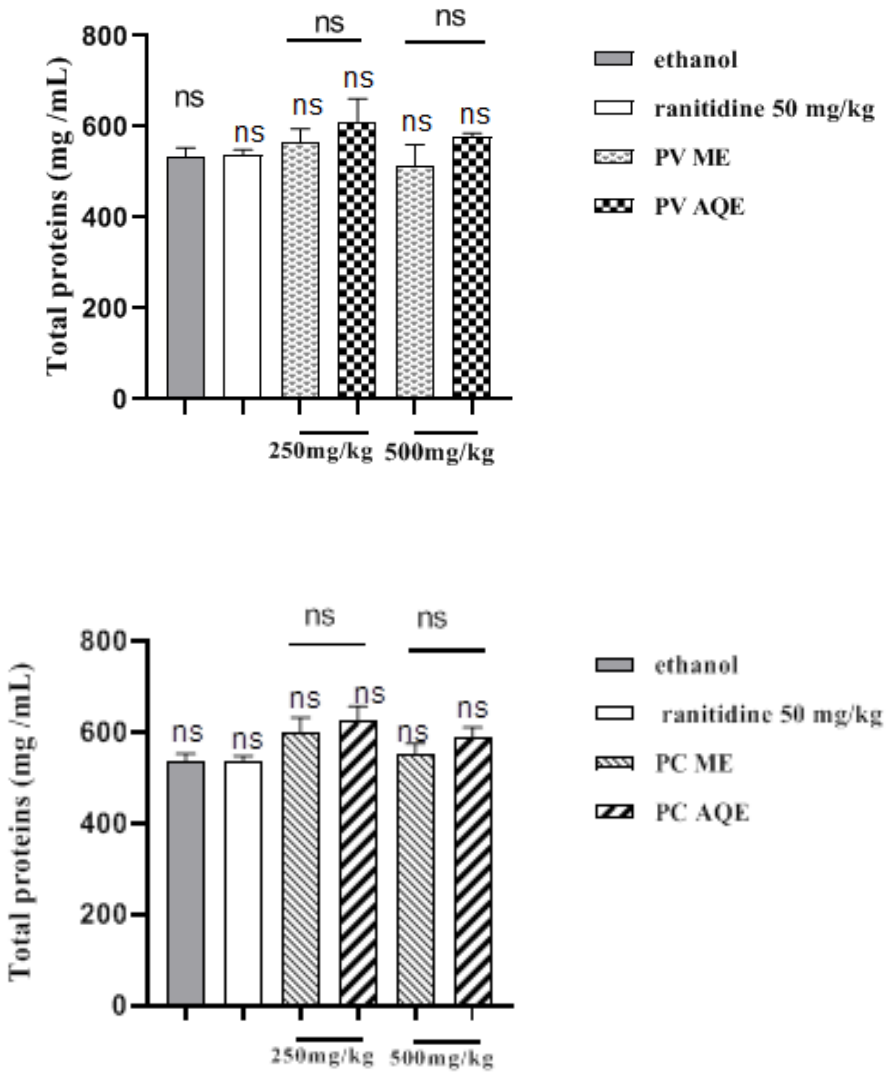


Figure 30: Effect of *P. herba venti* (A) and *P.crinita* (B) extracts on total proteins level in rat stomach tissue. The PV ME: *P. herba venti* methanolicic extract, PV AQE: *P. herba venti* aqueous extract, PC ME: *P. crinita* methanolicic extract, PC AQE: *P. crinita* aqueous extract. Values are ± SEM (n=5-6) (ns: not significant P<0.05) *vs* ethanol, ranitidine group and comparison between extracts.

Conclusion and future prospects

To conclused, *P. crinita* and *P. herba venti* are a rich source of phenolics. The *in vitro* experimental study revealed that the plant extracts have effective antioxidant activity. Here in, *P. herba venti* extracts revealed a strong reducing power, DPPH, ABTS radical scavenging activities. In addition, the β-carotene bleaching and metal chelating activity assays revealed an efficient antioxidant activity of *P. crinita* extracts.

A marked improvement in the hematological profiles, hepatic functional and oxidative markers was found in ME of *P. crinita* and *P. herba venti* extracts treated animals as evidenced by an increase in RBC and PLT counts, blood hemoglobin level, along with a decrease in the level of total cholesterol, AST, triglycerides, HDL, uric acid and creatinine. Furthermore, *P. crinita* methanolic extract was very effective in monitoring glucose in alloxan- induced diabetes in rats. The GSH level was increased while MDA level was deceased in methanolic plant extracts treated animals. In adition , *P. crinita* and *P. herba venti* extracts protect the rat stomach against ethanol-induced gastric ulcer.

In the future research plan it will be interesting to take in the profound

- Toxicological assessment of both plant extracts.
- The deep investigation of both plants of the defferent aspects of diabetes.
- The study of the effects of some isolated phytochemical compounds from thes two plants on intestine glucose transport and cell glucose uptake.
- The investigation of the phytochemicals and mechanisms of both plants that are involved in gastroprotection.
- Investigating the action mechanisms of the plant constituents in the gastric ulcer.

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الملخص

تهدف هذه الدراسة إلى تقييم المحتوي الفينولي و النشاطية المضادة للأكسدة والمضادة لداء السكري وحماية المخاطية المعدية من التقرح للمستخلصات الميثانولية (PC ME وPVME) والمائية (PC AQE, PV AQE) للجزء الهوائي لنبتتي *P. crinita* و *P. herba venti* اللتان تنتميان الي عائلة النعناعيات. في هذه الدراسة، أظهرت النتائج أن المستخلص الميثانولي لنبتة *P. herba venti* يحتوي اكبر كمية من عديدات الفينول والفلافونويدات في حين ان اعلى نسبة للدباغ سجلت في المستخلص الميثانولي ل *P.crinita* . تم تحديد الخواص المضادة للأكسدة لمستخلصات باستخدام إزالة جذر DPPH ، ABTS، وتبييض البيتا كاروتين، القدرة الإرجاعية والنشاط المخلبي للمعادن. اظهرت مستخلصات *P. herba venti* قدرة كبيرة في استخلاب جذري ABTS و DPPH وكذا القدرة الارجاعية للايونات في حين ان مستخلصات *P. crinita* اظهرت قوة معتبرة في تثبيط تبييض البيتا كاروتين والنشاط المخلبي للمعادن. بعد تحريض السكري باستعمال الالوكسان، شوهد إرتفاع في تركيز السكر في الدم لدى الحيوانات غير المعالجة مقارنة مع الحيوانات الطبيعية، ولكن لوحظ انخفاض كبير في وزن الجسم للحيوانات غير المعالجة. أدت معالجة الحيوانات بمستخلصي PC ME و PV ME بجرعات 250 و 500 ملغ/كغ إلى زيادة في نشاط الكاتلاز،GSH وانخفاض مستوى MDA في أنسجة الكبد والكلى، مستويات الكرياتنين ،حمض اليوريك, الكوليسترول والدهون الثلاثية في البلازما. من جهة اخرى اظهر مستخلص PV ME قدرة كبيرة في تثبيط انزيم الفا غليكوزيداز والتي تعتبر احسن من الشاهد الموجب. أدت معاملة الحيوانات باستخدام 250 و 500 مغ/كغ من مختلف المستخلصات الى حماية مخاطية المعدة (68 إلى 95٪) ضد القرحة التي يسببها الإيثانول والتي كانت أكبر مقارنة بالرانيتيدين كمرجع ايجابي .يمكن ان ترتبط هذه الحماية بالارتفاع في مستويات البروتينات الكلية، الغليثاثيون و/أو تثبيط أكسدة الدهون.

كلمات مفتاحية: مضادات للأكسدة، مضادات السكري, الفا غليكوزيداز، *P. herba venti P.crinita* ، نشاطية ضد قرحة المعدة المحدثة بواسطة الإيثانول.

Abstract

The present study was conducted to evaluate phenolic content, antioxidant, antidiabetic activity and gastroprotective effect of methanolic (PC ME, PV ME) and aqueous extracts (PC AQE, PV AQE) of aerial parts of *P. crinita and P. herba venti* which are plants species of the family Lamiaceae. In this study, the results showed that methanolic extract of *P. herba venti* contained high polyphenolic and flavonoid contents, while the high level of tannins was registered in *P. crinita* methanolic extract. The antioxidant properties of plants extracts were determined using DPPH, ABTS, β-carotene bleaching, reducing power and metal chelating activity assays. *P. herba venti* extracts showed strong DPPH, ABTS radical scavenging s activity and reducing power; while *P. crinita* extracts exhibited a good activity in β-carotene bleaching and metal chelating activity assays. After Induction of diabetes, the concentration of blood glucose increased in the alloxan group in comparison with normal ones, but the body weight decreased in the alloxan animals. The administration of PC ME and PV ME at doses of 250 and 500 mg/kg increased catalase activity, GSH level and decreased lipid peroxidation in the tissues of liver and kidneys, serum creatinin, uric acid, total cholesterol and triglycerides levels. *In vitro* inhibitory effect on diabetic enzyme showed that the methanolic extract of *P. herba venti* was inhibited (IC_{50} = 0.16±0.01 mg/mL) more than positive control on alpha-glycosidase. Treatment of rats with 250and 500 mg/kg of PC ME PC AQE PVME and PV E protected the stomach (68 to 95%) against ethanol (70%) induced ulcer and higher protection in comparison with ranitidine (positive control). This protection may be related to the augmentation of levels of total protein, GSH levels and/or inhibition of lipid peroxidation.

Keywords: anti-oxidant activity, anti-diabetic activity, alpha glycosidase, *P. herba venti, P. crinita*, ethanol induced gastric ulcer.

Resumé

La présente étude a été menée pour évaluer le contenu phénolique l'activité antioxydante, antidiabétique et l'effet gastro protecteur des extraits méthanoliques (PC ME, PV ME) et aqueux (PC AQE, PV AQE) des parties aériennes de *P. crinita* et de *P. herba venti* qui sont des espèces de la famille des Lamiacées. Dans cette étude, les résultats ont montré que l'extrait méthanolique de *P. herba venti* contenait des teneurs élevées en polyphénols et en flavonoïdes, alors que la grande quantité des tanins a été enregistrée dans l'extrait méthanolique de *P. crinita*. Les propriétés antioxydantes des extraits des plantes ont été déterminées en utilisant le piégeage des radicaux DPPH, ABTS, le blanchiment du β-carotène, le pouvoir réducteur et les tests d'activité de chélation des métaux. *P. herba venti* extracts ont montrè une forte activité de piégeage des radicaux ABTS et DPPH et bonne activité dans les tests : pouvoir réducteur, tandis que les extraits de *P. crinira* ont montré une bonne activité dans les essais d'activité de blanchiment β-carotène et de chélation des métaux. Après l'induction du diabète, la concentration de glucose sérique a augmenté chez les animaux non traités par rapport aux animaux normaux, mais le poids corporel a diminué chez les animaux non traités. L'administration de PC ME et de PV ME à des doses de 250 et 500 mg/kg a augmenté l'activité de la catalase, le niveau de GSH et diminué la peroxydation lipidique dans les tissus du foie et des reins, la créatinine sérique, l'acide urique, le cholestérol total et les triglycérides. L'effet inhibiteur *in vitro* sur l'enzyme diabétique a montré que l'extrait méthanolique de *P. herba venti* était plus inhibé (CI_{50} = 0.16 0.01 mg/mL) que le contrôle positif de l'alpha-glycosidase. Le traitement des rats avec 250 et 500 mg/kg de PC ME PC AQE PVME et PV E a protégé l'estomac (68 à 95%) contre l'éthanol (70%) induit un ulcère et une protection plus élevée par rapport à la ranitidine (témoin positif). Cette protection peut être liée à l'augmentation des niveaux de protéines totales, des niveaux de GSH et/ou à l'inhibition de la peroxydation lipidique.

Mots clés: anti-oxydant, anti-diabétique, alpha glucosidase, *P. herba venti, P. crinita*, ulcère gastrique induit par l'éthanol.