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Research Article

Evaluation of Acute and Subacute Toxicity of Fruit Methanolic Extract from *Citrullus colocynthis* in male Albino rats

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ABSTRACT

In this study, we investigated the effects of acute and subacute administration of the methanolic fruit extract of *Citrullus colocynthis* (CCT) in male Albino Wistar rats. The acute toxicity study was undertaken firstly to determine the acute median lethal dose (LD_{50}) of the fruit extract. Then, the second step aimed to evaluate its toxic effects when administered at single daily oral dose 440mg/kg (1/3 of the LD_{50}) after one, three, five, seven, ten and fourteen days on liver function indices (AST, ALT, ALP, total proteins), kidney function parameters (uric acid, urea and creatinine), haematological parameters and electrolyte concentrations (Na^+, K^+, P^-, Ca^{+2}) using standard methods. The sub-chronic toxicity was undertaken to evaluate the toxic effects of the same extract and the target organs after its oral administration for six weeks (265 mg/kg) to male rats. The median lethal dose (LD_{50}) of the extract was found to be 1311.45 mg/kg. After acute oral administration of the methanolic extract a dose of 440mg/kg (1/3 of the LD_{50}), there were remarked a loss in body weight and a significant reduction in the relative weights of liver, kidney, lungs, spleen and testis of treated rats. The RBC, WBC count, HCT and HGB concentration were also significantly lower in treated groups than control one. The liver enzymes ALT and AST activities were higher for the main treated groups and significantly elevated in the group sacrificed after three days of the treatment. The ALP has enregistered a significant elevation for the majority of the tested groups. The analysis of kidney function parameters revealed a significantly high creatinine levels for all treatment group (exceptely for the group sacrificed after 14 days), a significant increase in urea level for the group sacrificed after three days of treatment with no remarkable changes in uric acid concentrations. Electrolyte concentrations (Na^+, K^+, Ca^{+2}) were not significantly affected by CCT alcoholic extract, but the levels of PO_4^- have shown a significant reduction for the main treatment groups. Histo-pathological examination revealed a discreetly congestion in hepatic and renal parenchyma in groups sacrificed after one and five days of treatment. The present study showed that the intake of extract of ripe *Citrullus colocynthis* fruit induced a characteristic signs of poisoning: diarrhea, ruffled hair, loss in organ weight and feed intake. The effects on the functions of the liver, kidney and bone marrow in rats resulted in alterations in serum AST, ALT and ALP activities, in the levels of total proteins, urea, uric acid, creatinine and electrolytes as well as in organ lesions.

Keywords: Acute toxicity, *Citrullus colocynthis*, LD_{50}, Methanolic extract, subacute toxicity.

INTRODUCTION

Medicinal plants are widely used for the treatment and prevention of various diseases in Africa and other developing countries of the world[1]. One of these traditional medicines is the cucurbitaceous plant, *Citrullus colocynthis* or Colocynth very common in many places from the north to the arid habitats of Algeria. This plant, is locally known as «Handhal» «Hdejj» «Tifersit» «Taferzizt» «Tatoor» «Alqam» «Mararet Essahra» and usually matures between September and October.

In folklore, the fruits and, in particular, the pulp of this plant are wellknown natural cathartics since ancient times. The leaves of this herb are used to treat asthma and jaundice, whereas the root is a traditional treatment for amenorrhea, breast inflammation, arthralgias and ophthalmic diseases. Other medicinal uses include the treatment of seizures, tuberculosis, syphilis, and parasitic infections[2]. *C.colocynthis* is mostly used by many diabetics in developing countries[3,4]. In the south of Algeria, the fruit of *Citrullus colocynthis* in its dried or fresh forms is widely used singly or in combination with other plants by rural communities of several regions for its antidiabetic, antijaundice, antirheumatic, antiheamorrhoids activities without considering its safety. Colocynth (CCT) contains active substances such as saponins, alkaloids and glycosides[5]. The main constituents of the plant are highly oxygenated tetracyclic triterpene compounds called cucurbitacins[6]. There are a variety of cucurbitacin compounds including cucurbitacin A, B, C, D, E, F, I, L and glucosides[7]. Fruit contains α-glucosides, colocynthin, its aglycone -α-elaterin, citrullin, citrullene and citrullic acid. Unripe fruits contain p-hydroxy benzyl ester. Roots contain α-elaterin and hentriacontane[8].

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Case reports associate the use of extracts of this plant with the development of bloody diarrhea, vomiting, colicky abdominal pain, and dehydration[9]. Pathologic lesions primarily involve edema, erythema, superficial erosions and inflammatory exudates of the mucosa in the sigmoid and descending colon. Ulcerations and pseudopolyps are unusual features of this toxic colitis. Symptoms typically resolve within 3-6 days, and the pathological lesions resolve within 14 days without sequelae. The older medical literature suggests that 0.6- 1 g of colocynth extract can produce bloody diarrhea[10].

Despite the fact that the fruit of *Citrullus colocynthi*s is well reputed for its therapeutic activity in folklore, there are few data on the dose response of the toxin in colocynth, in part because of the lack of identification of the specific compound associated with the toxic effects. Furthermore, in order to develop and establish the safety and efficacy level of a new drug, toxicological studies are very essential in animals under various conditions. No drugs is used clinically without its clinical trial as well as toxicity studies. Toxicological data help to make decision whether a new drug is adopted for clinical use or not[11,1]. This led to the present study where we investigated the acute and the subacute of *C. colocynthis* methanolic extract on haemogram, biochemical functional biomarkers of liver and kidney and on histo-pathological changes in the visceral organs of male Wistar Albino rats.

MATERIAL AND METHODS

Plant material

Citrullus colocynthis (CCT) plant were collected during August from the desert area of Maârif (35,5°W and 5,25°N) in province of Boussaâda, Wilaya of M'sila (South east of Algeria). The plant has been identified based on morphological aspects. CCT Fruits were washed and shade dried (Fig. 1). Seeds were separated manually from the pulp of the fruits and then minced with electrical grinder (Muleinex) into a powder and finally stored in airtight containers prior to use.

Plant extraction

One hundred grammes of air dried powdered fruits were extracted to exhaustion with 600ml methanol, using a soxhlet apparatus for 18 h. The resulting extracts were evaporated at reduced pressure to obtain crude extract. The yield of this extract was approximately 13.73± 0.02% (w/w).

Animals

Male Albino-Wistar rats clinically healthy (180- 210 g) were purchased from animal center of Pasteur's Institute (Algiers – Algeria). Rats were housed in hanging transparent polypropylene plastic cages (55 × 33× 19 cm) lined with husk and maintained in Animal house of Faculty of natural sciences and life, University Ferhat Abbas Setif-1, Algeria. They were let to accommodate to standard environmental laboratory conditions for 3 weeks prior to experiment. The litter was renewed every 3 days. They were fed with a standard pellet and tap water *ad libitum* throughout the experimental period. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and in accordance with the scientific council of the Faculty of Natural Sciences and Life of the University Ferhat Abbas, Sétif -1, Algeria.

Determination of oral median lethal dose (LD_{50})

The oral median lethal dose (LD_{50}) of *C. colocynthis* and its range was determined according to the graphic method of Litchfield and Wilcoxon[12].

The fruit extract of *C. colocynthis* to be tested is dissolved in 600µl of ethanol, diluted by normal saline solution (0.9% v/v) and administered at different doses, via intragastric intubation at a dose per group. Male rats were weighed (186.25 ±2.80 g), identified by labeling with an aqueous solution of picric acid and divided into groups of 10 animals each and fasting overnight before oral administration of single dose of the fruit *C. colocynthis* extract.

Five groups of rats are treated with simple application and successively with the following doses: 500, 1000, 1800, 2000 and 3000 mg/kg. The control group received physiological saline with 600µl of ethanol. After administration of the fruits extract, animals were kept under close observation every hour during the first day and every day for 14 days. Behavior and clinical symptoms of animals are noted throughout the duration of the experiment. Dead animals were subjected to post-mortem examination.

Acute oral toxicity

The male rats were allotted randomly to six groups of 10 animals and were given orally a single dose of 440 mg/kg (1/3 DL_{50}) body weight of the fruit of *C. colocynthis*, but not lethal dose to try to investigate the target organs. The control group (10 rats) received saline water. Animals were observed and recorded systematically 1, 2, 3, 4, 5 and 6 h and daily after test substance administration. The visual observations included changes in skin and fur (hair), eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system. The treatment groups were sacrificed after 24 h, 3, 5, 7, 10 and 14 days of the treatment. In humans, after acute poisoning by *C. colocynthis*, hospitalization and recovery takes between 3 to 6 days[10].

Subacute toxicity

Thirty healthy albino male rats were used to study the sub-acute toxicity of *C. colocynthis* fruit extract (divided into three groups of 10 rats each). The extract was administered daily *per os* at dose of 265 mg/kg (1/5 LD_{50}) body weight for six weeks (first group) followed by one week of recovery (second group). The control group (10 rats) was given saline water. The animals were observed daily for abnormalities. Average feed intake and body weight were determined weekly for each group.

Hematological examination and clinical chemistry analysis

At the end of all experimental periods, after overnight fasting, rats were anaesthetized with urethane at the dose 760 mg/kg. Blood samples for haematological and biochemical analysis were collected from the retro-orbital vein into tubes with or without EDTA, respectively. Red blood cells count (RBC) and white blood cells (WBC) count, haemoglobin concentration (HGB), hematocrit

Figure 1: *Citrullus colocynthis* ripe fruit in its dried and fresh forms with cut section.

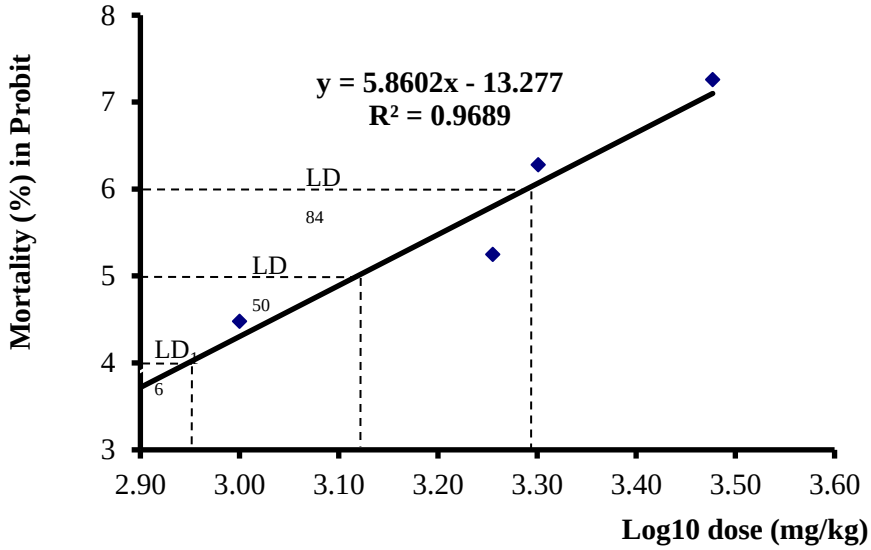

Figure 2: Median lethal dose (LD_{50}) of the fruit *C. colocynthis* methanolic extract administered per os to male albino rats.

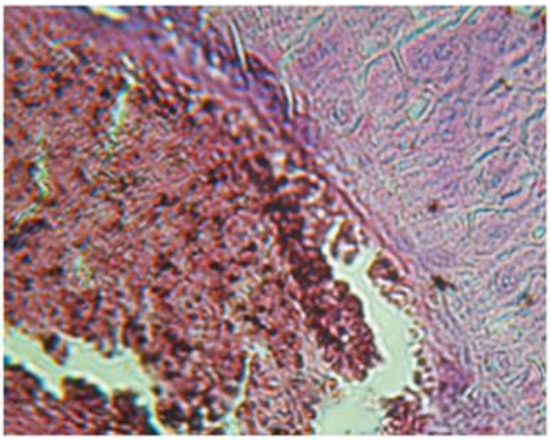

Figure 3a: Liver parenchyma traversed by congestive blood vessels in male rats treated with fruit CCT extract at a dose of 3000 mg/kg. H&E (× 400).

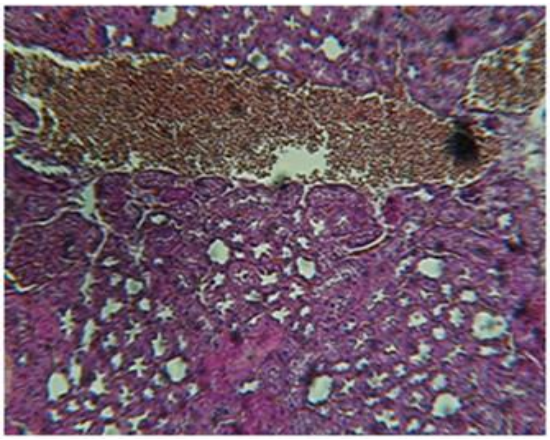

Figure 3b: Renal parenchyma traversed by blood vessels with distended lumen engorged with blood in male rats treated with fruit CCT extract at a dose of 3000 mg/kg. H&E (× 100).

(HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count (PLT) were determined using automatic counter MEDONIC (Beckman Coulter – USA). Thebiochemicalparameters: Glutamic-oxaloacetictransaminase (GOT), Glutamic-Pyruvic Transaminase (GPT) (using commercial Kits – SGM Rome-Italy), alkaline phosphatase (ALP) (using

Table 1: Effect of acute per os administration of CCT fruit methanolic extract (440 mg/kg) on body weight of male rats. Values are mean ± SEM.

Group	1st day	3rd day	5th day	7th day	10th day	14een day
Control	244.82±6.72					258.24±9.96
1st day	238±14.30					
3rd day	244.40±12.04	237.86±9.10				
5th day	255.40±5.65		247.34±6.05			
7th day	237.60±8.32			231.20±9.30		
10th day	242.54±10.48				237.40±9.34	
14een day	242.88±10.15					240.46±9.39

Table 2: Relative organ's weight of male AlbinoWistar rats treated orally with 440 mg/kg of methanolic CCT fruit extrac Values are mean ± SEM.

Organ/ Group	Liver	Kidney	Brain	Testes	Lungs	Heart	Spleen
Control	0.0391 ±0.0003	0.0075±0.0002	0.0075±0.0002	0.0103±0.0006	0.0074±0.0004	0.0031±0.00003	0.0038±0.0002
1st day	0.0341±0.0015*	0.0056±0.0005*	0.0073±0.0005ns	0.0083±0.0003*	0.0056±0.0007*	0.0026±0.0002*	0.0033±0.0005*
3rd day	0.0354±0.0018*	0.0049±0.0007*	0.0073±0.0002ns	0.0069±0.0002*	0.0057±0.0003*	0.0027±0.0002 ns	0.0033±0.0002*
5th day	0.0356± 0.0022*	0.0053± 0.0002*	0.0075± 0.0003ns	0.0084± 0.0011ns	0.0063± 0.0002*	0.0028± ns	0.00020.0031± 0.0002*
7th day	0.0357± 0.0025*	0.0054± 0.0002*	0.0073± 0.0003ns	0.0093± ns	0.00060.0065± 0.0006*	0.0029± ns	0.00010.0034± ns 0.0003
10th day	0.0359± 0.0014*	0.0061± 0.0002*	0.0075± 0.0003ns	0.0093± ns	0.00080.0065± 0.0003*	0.0029± ns	0.00020.0035± ns 0.0005
14een day	0.0378± 0.0005ns	0.0067± 0.0007ns	0.0077± 0.00001ns	0.0099± ns	0.00120.0068± ns	0.00050.0030± ns	0.00010.0035± ns 0.0001

Table 3: Effect of oral administration of CCT fruit methanolic extract (440 mg/kg) on some haematological parameters in male rats. Values are mean ± SEM.

Parameter	Control	1st day	3rd day	5th day	7th day	10th day	14een day
RBC (10^6/mm^3)	8.703±0.112	7.881±0.348*	7.619±0.069*	7.665±0.095*	7,.993±0.172*	8.301±0.132*	8.416±0.075*
HGB (g/dl)	14.818±0.344	12.863± 0.240*	11.250± 0.378*	12.171± 0.261*	12.535± 0.197*	13.038± 0.309*	13.825± 0.207ns
HCT (%)	47.700±0.803	43.313±1.711*	41.975± 0.515*	42.271± 0.662*	42.513± 1.009*	43.388± 0.436*	45.713± 0.505*
MCV (µm^3)	56.138±1.045	51.438± 0.713*	41.963± 0.851*	52.125± 0.578*	52.475± 0.678*	54.125± 0.767ns	55.1± 0.798ns
MCH(pg)	17.450±0.276	17.275± 0.264*	17.163± 0.115*	17.343± 0.251*	16.674± 0.356*	17.775± 0.281*	18.888± 0.418*
MCHC(g/dl)	36.038±0.480	33.563± 0.668*	30.55± 0.351*	31.375± 0.730*	31.00± 0.477*	33.391± 0.408*	35.20± 0.140ns
WBC (10^6/mm^3)	11.315±0.588	9.363± 0.729*	8.258± 0.060*	8.50± 0.274*	8.604± 0.242*	9.729± 0.244*	9.893± 0.243*
PLT (10^6/mm^3)	818.00±11.759	669.00± 66.311*	593.50± 41.386*	697.875± 17.898*	745.625± 17.274*	762.125±13.571*	780.909± 14.780*

commercial Kits – CYPRESS DIAGNOSTIC Langdrop – Belgium),concentrations of total protein, urea, glucose, creatinine, sodium, potassium, calcium and phosphorus were determined using apparatus TECHNICON RA-1000-USA. The biochemical parameters were determined in serum obtained after centrifugation of total blood without anticoagulant, at 2500 rpm for 15 min.

Histo-pathological examinations

After blood collection, the animals were sacrificed by cervical dislocation. After autopsy, all tissues were examined grossly in situ and major's organs (liver, brain, heart, kidneys, spleen, testicles, and lung) were weighted. The relative organ weight was calculated and compared with the value of the control. Tissues from liver and kidney of all animals were fixed in 10% neutral buffered formalin solutions then embedded in paraffin and cut with a microtome set at 5 µm, stained with hematoxylin and eosin

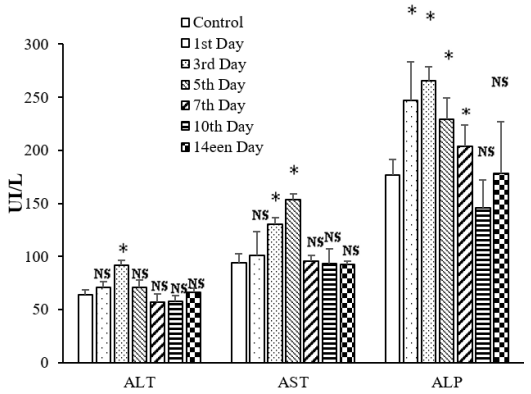

Figure 4 : Effect of acute administration of CCT fruit methanolic extract of CCT (440mg/kg) on some biochemical parameters in male rats. Values are mean±SEM.

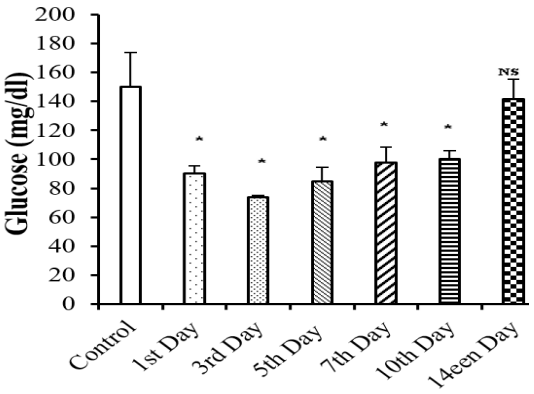

Figure 5 : Effect of acute administration of fruit CCT methanolic extract (440mg/kg) on glucose level in male rats. Values are mean ± SEM.

and examined by light microscopy for histopathological evaluation.

Statistical analysis

All data were expressed mean ± standard error of mean (SEM). Statistical significance of the differences between means was assessed using *t*-test and analysis of variance followed by Tukey's test for multiple comparison test. A probability level of $p< 0.05$ was chosen as the criteria for statistical significance. All statistical analysis were carried out using Sigma Stat software (version 2.01).

RESULTS

The median lethal dose determination

The animals were kept under close observation for 72 hours after dosing to check for symptoms, behavioral changes and death. The doses tested produced, early within the first hour of treatment the following signs, which worsens with the passing of the time: loss of locomotion activity, ataxia and at the highest doses administered, inappetence, weakness of the hind limbs, lethargy, hypothermia, cyanosis and eventually death. Severe diarrhea was the most serious symptoms; after developing it, the experimental animals died. The animals that survived had some symptoms, including mild diarrhea, but were able to recover. The intensity of the toxic effects was dose-dependent. Rat's mortality after different doses of CCT methanol extract was plotted against probability values[12].

The mortality rate in male rats was maximum (100%) in groups treated with 3000 mg/kg. The acute median lethal doses (LD_{50}) of the extract were found to be 1311.45 mg/kg at 95% confidence limit: [1037.80 to 1657.27 mg/kg] for rats males. The DL_{16} and DL_{84} were respectively 825.61 mg/kg and 2083.19 mg/kg (Fig. 2). The post-mortem findings were in the form of congestion in the liver as well as in renal parenchyma (Fig. 3a, b).

Acute toxicity study

The main signs of toxicity observed after oral administration of single dose tested (440 mg/kg ≈ 1/3 DL_{50}) were: diarrhea, ruffled hair, acceleration of heart rate breathing difficulty, soft feces and huddling together at one end of the cages. None of the rats in all treated groups died during the course of the experiment.

There were no statistically significant differences in average body weight of the control group and *Citrullus colocynthis* fruit-extract treated groups during the acute toxicity. Nevertheless, a slight loss in body weight was noted in CCT fruit extract treated rats as compared to control group (Table. 1).

Macroscopic examination of various organs *in situ* did not show any morphological changes in organs of treated animals compared with those of control rats. The effects of CCT fruit extract on relative organ weights (liver, kidney, lung, heart, brain, testis and spleen) are presented in table 2. Significant statistical reductions were noted on the relative weights of liver, kidney, lungs, spleen and testes among the treated groups. After fourteen days of the treatment, all treated groups have recovered the normal relative organ weight.

Heamatological changes

The blood picture of *C. colocynthis* treated rats after acute administration of fruit alcoholic extract is shown in table 3. The total RBC count, WBC, HCT and HGB content were significantly lower ($P < 0.05$) in the treated groups than the control one.

Changes in serum constituents

The effects of CCT extract on liver function biomarkers in serum of albino rats after oral administration of (1/3 DL_{50}): AST, ALT and ALP are given in figure 4. It was observed that the values of AST (GOT) and ALT (GPT) were higher but not significantly different in the main treatment groups compared with those of control group. However, the elevation of the levels of GOT (AST) and GPT (ALT) were significant ($P < 0.05$) after 3 days of the treatment. In addition, the alkaline phosphatase values were significantly higher ($P < 0.05$) almost in the majority of the tested groups (1st, 3rdand 5th day), and start to return to the normal values after 10 to 14 days of the treatment. However, total proteins levels were no significantly different in comparison to control group (Fig. 5). Finally, almost all treatment groups (except the group sacrificed

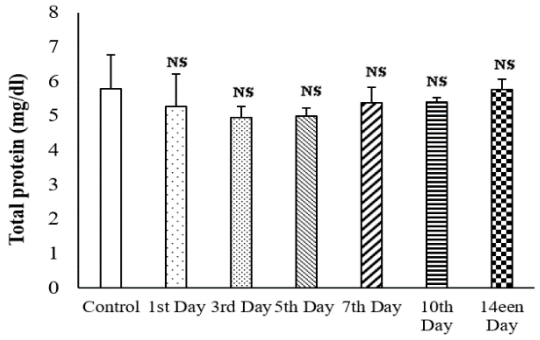

Figure 6 : Effect of acute administration of fruit CCT methanolic extract (440mg/kg) on total protein level in male rats. Values are mean ± SEM.

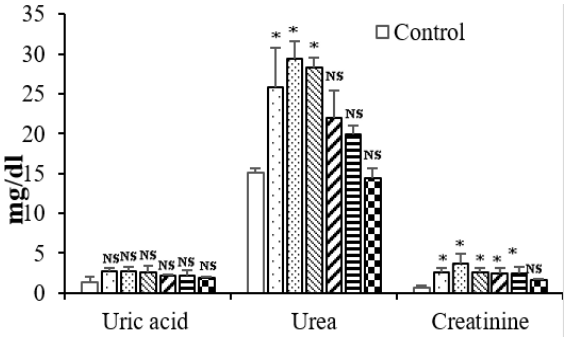

Figure 7 : Effect of acute administration of fruit CCT methanolic extract on some renal function parameter in male rats. values are mean ± SEM.

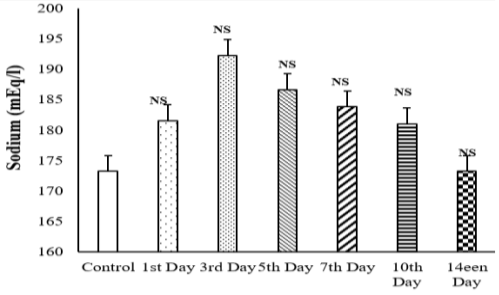

Figure 8a: Effect of acute administration of fruit CCT methanolic extract (440mg/kg) on sodium level in male rats. Values are mean ± SEM.

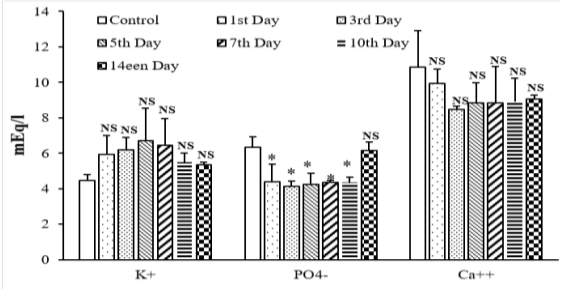

Figure 8b: Effect of acute administration of fruit CCT methanolic extract (440mg/kg) on some electrolyte's level in male rats. Values are mean ± SEM.

after 14 days) have enregistered a significant reduction in blood glucose level when compared with control (Fig. 6).

The results of the effects of CCT-fruit-extract on the main kidney function parameters are shown in figure 7. From this figure, creatinine level was significantly higher ($P < 0.05$) in treatment groups compared with the control. The urea level has enregistered a significant elevation ($P < 0.05$) in the three first groups and showed normal values for the other treatment groups. However, the uric acid has demonstrated a no significant variation for all treatment groups.

It appears from the figure 8a and b that CCT methanolic extract did not affect significantly the electrolyte concentrations, but it induced a non-significant elevation in sodium and potassium levels and a reduction in calcium level. Phosphorus was the single electrolyte which has presented a significant reduction ($P < 0.05$) in its level comparatively to control values.

Pathological changes

The histological examination of liver from male rats was performed in both control and treated groups. Hepatic parenchyma traversed by congestive blood vessels and a congestive renal parenchyma were observed in the group sacrificed after 24 hours (Fig.9a, b). The renal parenchyma becomes discreetly congestive in the group sacrificed after 5days (Fig.9c). Congestion disappears completely after 14 days of treatment in both the liver and kidneys (Fig.9d, e). Control group presented a liver parenchyma with preserved architecture made of Remack's radial spans converging towards a central vein and renal parenchyma with a preserved architecture (Fig.9f, g). None of the rats had cardiac or splenic lesions.

Sub-acute toxicity study

For the result of the subacute toxicity test; in clinical evaluation, some changes in general behaviour and physiological activities of the animals such as: diarrhea, hypoactivity, ruffled hair, inappetence, huddling together at one end of the cage were observed after oral treatment with fruit CCT extract at a dose of 265 mg/kg (1/5 LD_{50}) for six weeks. However, the treatment of rats with saline or CCT extract did not induce mortality during the whole study period.

Furthermore, the administration of CCT extract resulted in a significant reduction in the final body weight of the animals when compared with the control (Figure10) especially on the 4^{th}, 5^{th} and 6^{th}week of treatment. In parallel, a reduction in feed intake was also observed in the treated animals compared to control rats (Fig. 11).

A non-significant reduction were noted on the relative weights of liver, brain, kidney, testes, heart and spleen of the second treatment group, but it was significantly different for lungs. However, the first group has presented a significant decrease in relative weight of the liver, kidneys, lungs and heart in comparison with the control group (Table 4).

The haematological values of treated rats for six weeks were significantly different from those of control group (Table 5). RBC, HGB, HCT and PLT values of the control group were significantly higher than those of the treated group.

Biochemical observation

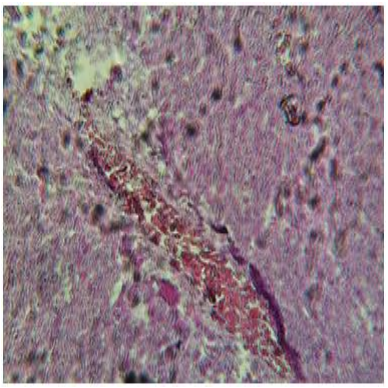

Figure 9a: Liver parenchyma traversed by congestive blood vessels after one day of acute treatment with CCT extract (440 mg/kg). H&E (×100).

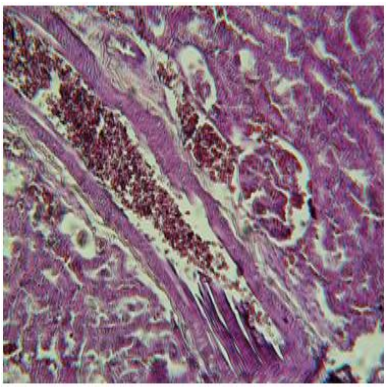

Figure 9b: Congestive renal parenchyma after one day of acute treatment with CCT extract (440 mg/kg). H&E (×100).

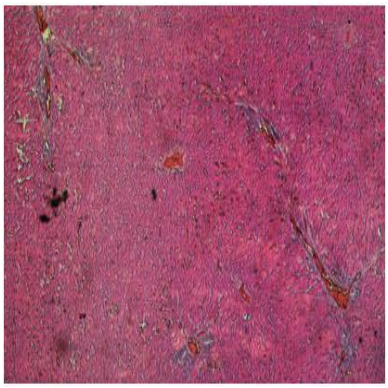

Figure 9c: Liver parenchyma with discreetly congestive architecture after 5 days of acute treatment with CCT fruit extract(440 mg/kg). H&E (×40).

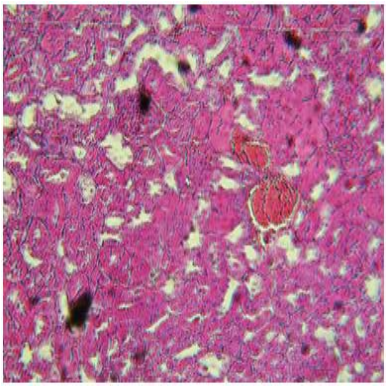

Figure 9d**:** Renal parenchyma with discreetly congestive architecture after 5 days of acute treatment with CCT fruit extract(440 mg/kg). H&E (×100).

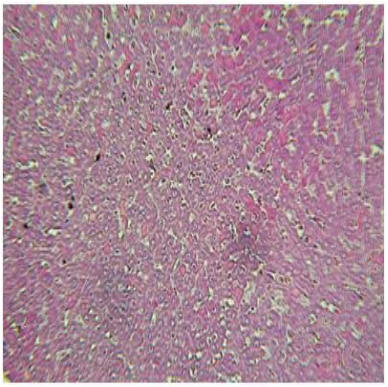

Figure 9e**:** Hepatic parenchyma without any particularity after 14 days of the administration of CCT fruit extract(440 mg/kg). H&E (×100).

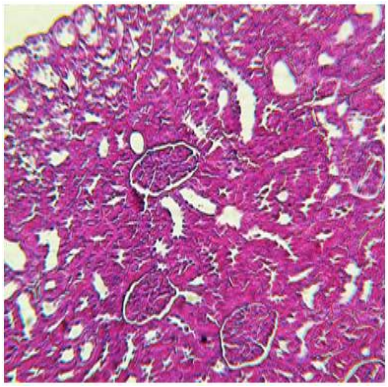

Figure 9f: Normal or discreetly congestive kidney parenchyma after 14 days of the administration of CCT fruit extract(440 mg/kg). H&E (×100).

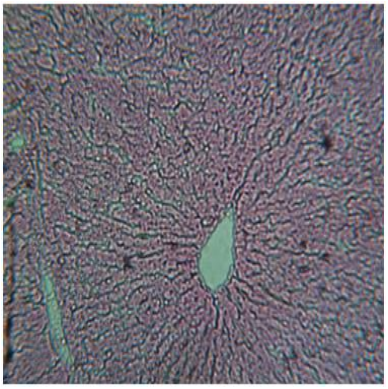

Figure 9g: Preserved morphology of control rat liver without any congestion. H&E (×100).

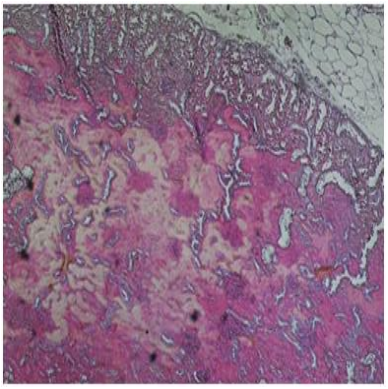

Figure 9h: Normal kidneys without any congestion in control rats. H&E (×100).

The evaluation of various enzymes activities in the sub-acute toxicity revealed significant differences. The GOT, GPT and ALP exhibited a significant elevation, when compared with saline control (Figure 12). However, the same enzymes did not enregistered a significant difference after one week recovery period following six weeks of treatment with CCT fruit extract. These changes were accompanied with a significant decrease in glucose and

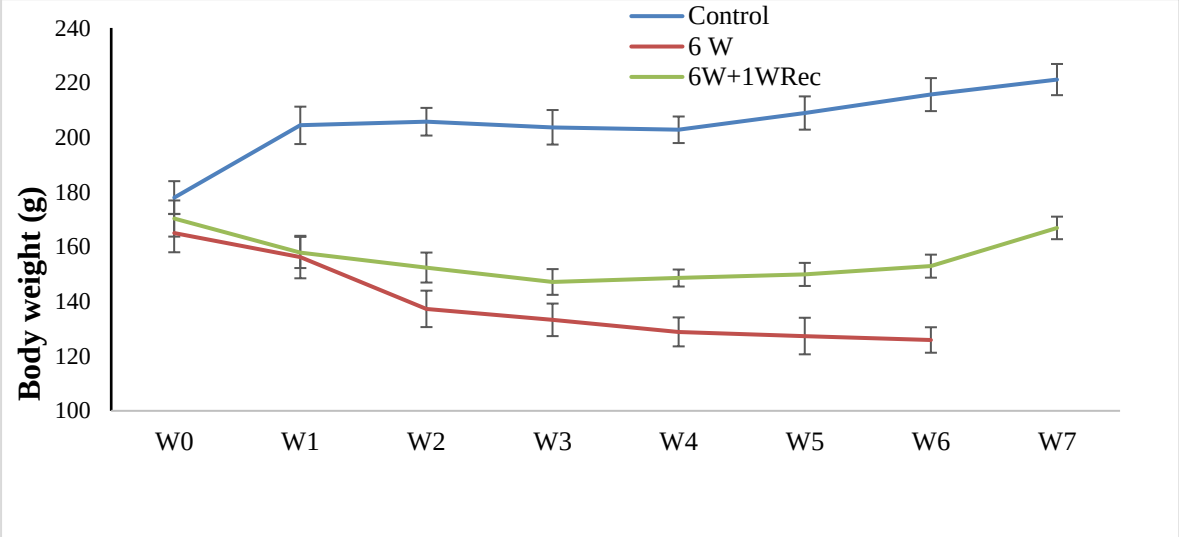

Figure 10: Effect of sub-acute administration of CCT fruit methanolic extract (265 mg/kg) for six weeks on body weight of male rats.

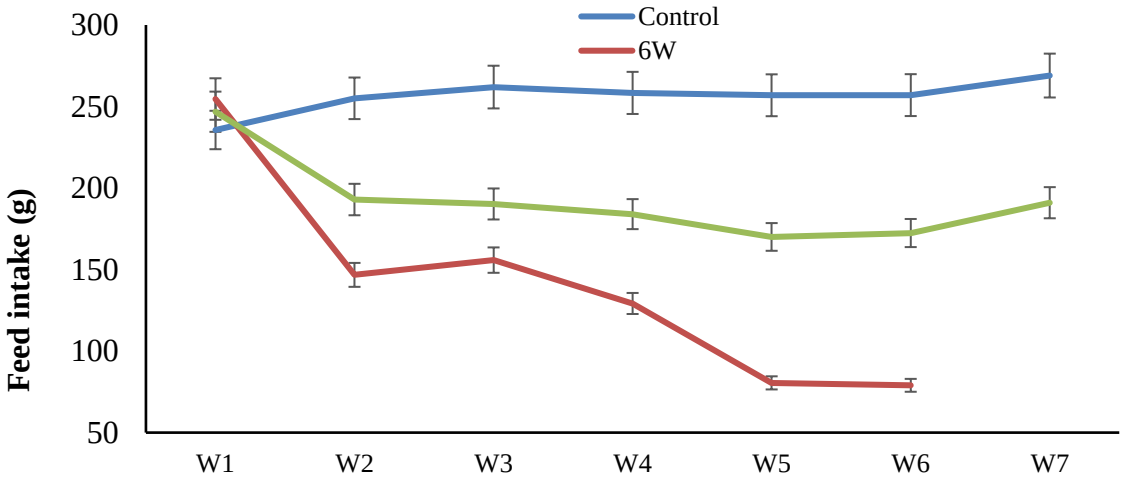

Figure 11: Effect of sub-acute administration of CCT fruit methanolic extract (265 mg/kg) for six weeks on feed intake of male rats.

total protein levels as compared to control group (Figure 13a, b).

A same picture is remarkable on the values of kidney function parameters where a significant elevation in urea, uric acid, creatinine, Na^+, K^+ were enregistered in the first group versus a significant reduction in PO_4^- and Ca^{+2} levels (Figure 14, 15 a and b).

Observation and examination of the organs for gross pathological lesion immediately after dissection has demonstrated that the extract did not cause any gross morphological abnormalities in both treatment groups when compared with those of control rats. However, under the microscopic examination, the liver of the group treated for six weeks without recovery showed a congestive liver and kidneys with preserved architecture (Fig. 17, 18).

DISCUSSION

Citrullus colocynthis find widespread use in folkloric medicine for treatment of various disorders. Many diabetics in developing countries mostly use *C. colocynthis*. Livestock owners have suspected theirs toxicoses in grazing animals especially at time of drought[4, 13, 14]. The fruits and leaves of this plant contain cucurbitacins A, B, C, and D and α-elaterin and probably other constituents[7, 15].

The present work was conducted to study the acute and subacute toxicological effects of methanolic ripe fruit extract prepared from *C. colocynthis* plant developing in M'sila region in Algeria.

The clinical picture of male rats treated with the *C. colocynthis* fruit extract was characterized by relatively rapid appearance of symptoms including: mild diarrhea, ruffled hair and hypoactivity, early in the first hour. Probably, this is could be due to the rapid absorption of the active principles present in the fruit extract of the plant. Such signs of toxicity are in accordance with those reported and described by Elgerwi et *al.*[16], Kingsbury[17], Barri et *al.*[18] and Elawad et *al.*[19]: diarrhea with yellowish

Table 4: Relative organ weights of male rats treated per os with 265 mg/kg of fruit methanolic extract of *Citrullus colocynthis* for six weeks.

Group	*Control*	*Group 6W*	*Group 6 W+1WRec.*
Liver	0.0546 ± 0.0044	0.0341±0.0021*	0.0445±0.0046^{ns}
Kidneys	0.0082±0.0002	0.0064±0.0003*	0.0072±0.0007 ^{ns}
Brain	0.0081±0.0005	0.0081±0.0004 ^{ns}	0.0083±0.0005 ^{ns}
Testis	0.0146±0.0006	0.0118±0.0012 ^{ns}	0.0136±0.0009 ^{ns}
Lungs	0.0083±0.0005	0.0065±0.0004*	0.065±0.0006*
Heart	0.0038±0.0002	0.0030±0.0002*	0.0031±0.0003 ^{ns}
Spleen	0.0029±0.0001	0.0024±0.0003 ^{ns}	0.0027±0.0005 ^{ns}

Table 5: Haematological changes in male Albino Wistar rats treated orally with 265 mg/kg of methanol CCT fruit extract for six weeks.

Group Parameter	Control	Groupe 6 W	Groupe 6W+ 1W Rec.
RBC (10^3/mm^3)	10.514±0.132	8.509±0.164*	9.926±0.225^{ns}
HBG (g/dl)	16.700±0.327	15.225±0.158*	15.975±0.173 ^{ns}
HCT (%)	56.521±0.691	45.65±0.909*	54.850±1.511 ^{ns}
MCV (µm^3)	56.317±1.079	52.925±0.212*	55.35±0.83 ^{ns}
MCH(pg)	15.500±0.152	18.686±0.237*	18.863±0.166*
MCHC(g/dl)	28.743±0.306	34.45±0.262*	29.550±0.620 ^{ns}
WBC (10^3/mm^3)	11.750±0.193	8.450±0.856*	11.688±0.462 ^{ns}
PLT (10^3/mm^3)	632.429±22.104	537.143±25.544*	666.375±52.832 ^{ns}

green feces, anorexia, dyspnea, decreased body weight, colic and tremors, followed by general depression, excitability followed by convulsions and death. However, according to Scott et *al*.[20] rats fed with the oil extracted from *C. colocynthis* seeds didn't presented any toxicological effect. This could be due to absence of the toxic principles the in seeds' oil and its accumulation in the mesocarp layer of the fruits. Similarly, Wistar rats injected subcutaneously with *C. colocynthis* extract up to four weeks hasn't presented any effect[21]. This disagreement between studies may come from the source of the C. colocynthis, its environment, percent of the active ingredients in the fruit extract, the period of administration and the difference in rat strains.

The oral LD_{50} in male rats in the present work was calculated as 1311.45mg/ kg, which is an indication that the extract is not completely safe. Given the results of the LD_{50} (500mg/kg <LD_{50}<5000mg/ kg), the present fruit extract of *C.colocynthis* can be classified as moderately toxic products as classified by Loomis and Hayes[22] and Pascoe[23]. These calculations seem different from those reported by Rahawi et Youkana[24], who have found that the oral LD_{50} of the methanolic extract of the whole fruit (including seeds) determined by the up-and-down method in Wistar rats was to be 383.8mg/kg. Furthermore, Marzouk et *al*.[25] have found that the LD_{50} of the aqueous extract of both immature and ripe fruits was to be 553.7mg/kg and 487.6mg/kg respectively when administered intraperitoneally to mice. Moreover, acoording to Marzouk et *al*.[26], the LD_{50} of the aqueous extract of the fresh immature fruits collected from seven states: Hammamet, Mehdia, Kasserine, Sbeitia, Sidi-Bouzid, Sfax and Medenine seem to be: 795.45 mg/kg, 749.97mg/kg, 750.03mg/kg, 799.64mg/kg, 795.49mg/kg, 385.54 mg/kg and 553.73 mg/kg respectively. These differences could be attributed to the use of the whole fruit with or without seeds, modes of extract preparation and administration, degree of fruit maturity (percentage of the active ingredients in the fruit) and the locality of the plant. The symptoms and pathoanatomical features observed in our results may be attributed to the local effect of *C. colocynthis* on gastrointestinal tract.

The component(s) of the fruit plant extract responsible for the toxic manifestations after the oral dose are not known. The toxicity and lethality of the fruit-extract may be due to any one or more of the phytochemicals present in the crude methanol extract (saponins, glycosides, alkaloids and cucurbitacins), some of which have been isolated and identified[5, 14, 27, 28, 29].

The present results suggest that at the oral dose, the C.C-fruit extract is not completely safe when administered at high concentration or in case of long term administration. In the acute toxicity, male rats given C.C-fruit extract orally at dose 440 mg/kg presented signs of toxicity: diarrhea, ruffled hair, huddling together, inappetence with a reduction in the body weight of the treated rats. The same signs of poisoning were observed when the CCT fruit extract was administered orally to rats for six weeks (subacute toxicity study). The changes in feces (soft stool, diarrhea) indicated disturbances of the gastrointestinal tract. According to Al-Yahia et *al*.[30], diarrhea was the prominent sign of phytotoxicity and could be due to the presence of cucurbitacins in *C. colocynthis* fruits. Piloerection of the fur can be indicative of disturbances of the autonomic nervous system. The body weight and food consumption data are considered gross indicators of general systemic toxicity. Reduction in body weight may be directly related to reductions in food consumption[31] or to damage to vital organs. It's speculated that the mechanism of side effects such as anorexia and weight loss

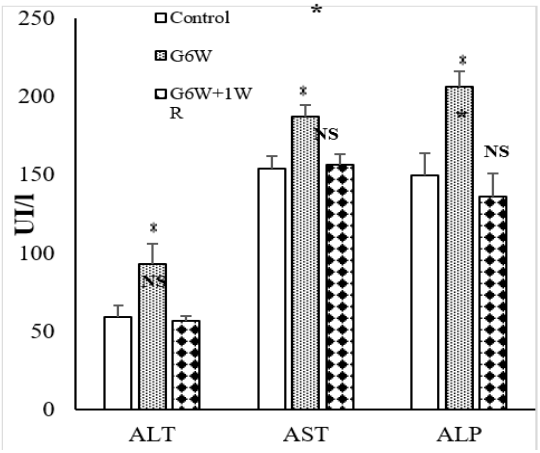

Figure 12: Effect of oral CCT fruit methanolic extract administration at a dose of 265 mg/kg for six weeks on some liver biochemical parameters in male rats.

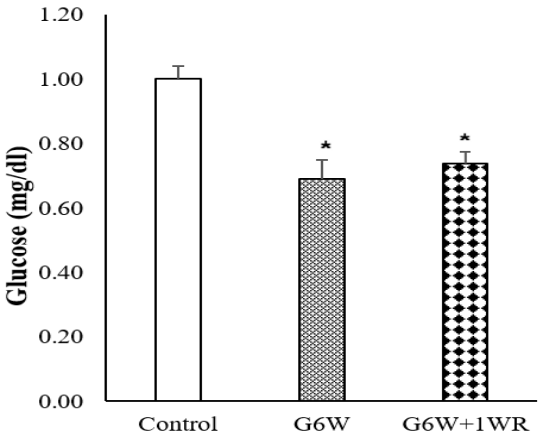

Figure 13a: Effect of oral CCT fruit methanolic extract administration at a dose of 265 mg/kg for six weeks on glucose level in male rats.

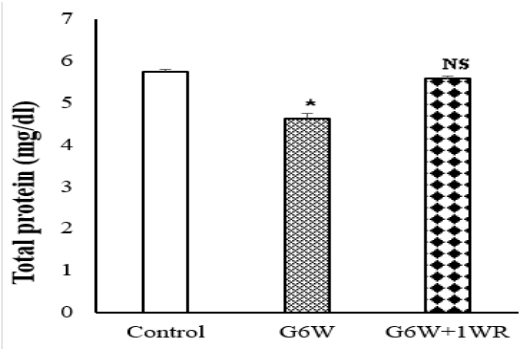

Figure 13b: Effect of oral CCT fruit methanolic extract administration at a dose of 265 mg/kg for six weeks on total protein level in male rats.

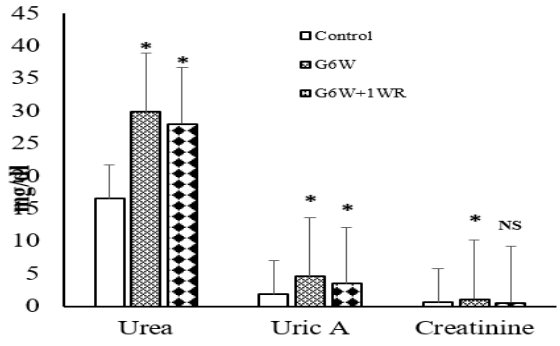

Figure 14: Effect of oral CCT fruit methanolic extract administration at a dose of 265 mg/kg for six weeks on some kidney indices in male rats

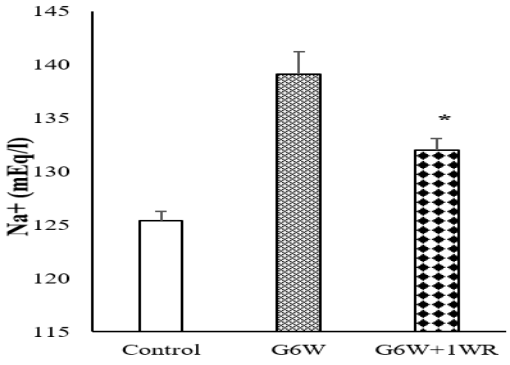

Figure 15a : Effect of oral CCT fruit methanolic extract administration at a dose of 265 mg/kg for six weeks on sodium level in male rats.

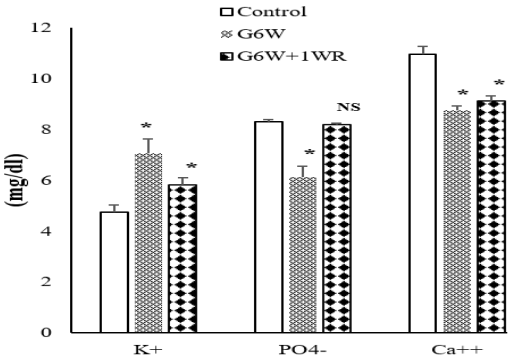

Figure 16 : Effect of oral CCT fruit methanolic extract administration at a dose of 265 mg/kg for six weeks on electrolytes level in male rats.

is related to an inhibitory effect of gastric acid secretion[32,33].

The present study was designed to evaluate the effects of methanol extract of *C.colocynthis*fruit on the liver and kidney of male rats after acute and subacute treatment .The liver and the kidney are the most common targets of chemical toxicity, due to their major metabolic and excretory functions.

The liver, known to be key organ in the metabolism and detoxification of xenobiotic, is vulnerable to damage induced by a huge variety of chemicals[34]. This is due to the fact that most chemicals are metabolized in the liver before being eliminated from the body, often through the bile. An

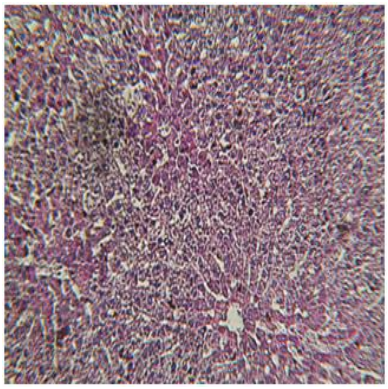

Figure 17: Hepatic parenchyma with preserved architecture made with radial span of Remack converging towards a central vein in control rats. H&E (×100).

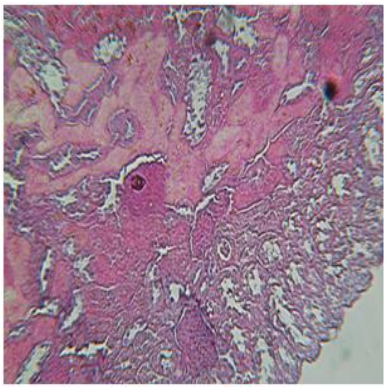

Figure 18: Renal parenchyma fact tubes and regular glomeruli in control rats. H&E (×100).

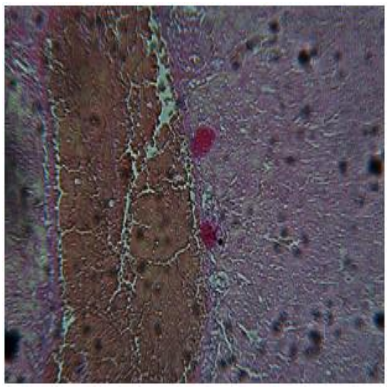

Figure 19: Congestive liver with architecture preservation of male rats after oral CCT fruit methanolic extract administration at a dose of 265 mg/kg for six weeks. H&E (×100).

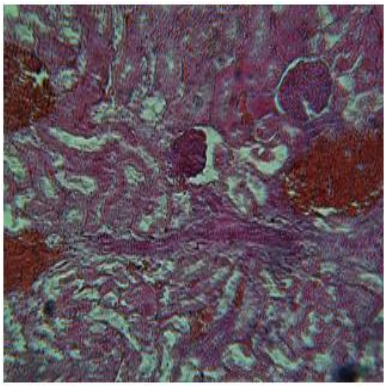

Figure 20: Congestive kidneys of male rats after oral CCT fruit methanolic extract administration at a dose of 265 mg/kg for six weeks. H&E (×400).

obvious sign of hepatocellular injury is leakage of cellular enzymes into plasma. When the liver cell membrane is damaged, a variety of enzymes normally located in the cytosol are released into blood stream. The estimation of the GPT (glutamic-pyruvic transaminase) and GOT (glutamic- oxaloacetic transaminase) in the serum is useful quantitative marker for the extent and type of hepatocellular damage[34,35]. An increase in the level of ALP (Alkaline phosphatase) is an indication of biliary obstruction[34, 36].

The male rats treated with 440 mg/kg of the methanol fruit extract of *C. colocynthis* showed changes in the level of these enzymes specially a significant increase in GOT and GPT activity after 3 days until the 5th day of the treatment. The level of ALP showed significant increase in groups sacrificed after 1, 3, 5, 7, 10 and 14^{een}days of the treatment. This result is in line with changes noted in of serum transaminases and ALP levels of the intoxicated rats in subacute test. The significant increase in serum GPT activity that was observed could be an evidence of hepatotoxicity caused by the extract. Moreover, the severity of the changes increased with increased of duration of the treatment, suggesting a cumulative effect of this extract. These findings seem to be in agreement with the work reported by Khatibi[37], Dehghani and Pananjehshahin[38] and Diwan *et al*.[39] in mice.

On the other hand, since the most plasma proteins can act as indicators of the synthetic capacity of the liver, the decrease in serum protein concentrations in rats treated with the CCT might be interpreted as a result of liver dysfunction. Indeed, following cellular damage, the capacity to synthesize protein is reduced, and as the extent of damage increases, the levels of these proteins inthe plasma will tend to decrease. Furthermore, the results obtained showed a significant reduction in glycaemia in treated rats, this result is in line with the work reported by Benmehdi et al.[40], who tested theeffect of saponosides crude extract isolated from *Citrulluscolocynthis* seeds on blood glucose level in rats. According to Shafaei et *al*.[41], the observed hypoglycemic effects of pulp extract may be due to wounded intestine and injured renal proximal tubules and their subsequent reduced ability to regulate

glucose transportation. In addition, impaired hepatic function and glucose metabolism may be a contributing mechanism for induced hypoglycemia.

The kidney is the second most common target of chemical injury[42, 43]. This is due to the fact that many chemicals are excreted through the urine. This vital organ exercises a major influence in regulating the composition of the blood to maintain the internal homeostatic mechanisms.

The kidneys possess the capacity to filter out, via the glomeruli, a whole range of endogenous substances from the plasma, e.g. creatinine, urea, electrolytes and proteins. Since creatinine and urea are normally filtered from the plasma and only reabsorbed or secreted by the proximal tubule to a minor extent, both have been used as indices of renal clearance. Renal diseases, which diminish the glomerular filtration, lead to urea retention. Additionally, creatinine retention in the blood is evidence of kidney impairment[44]. Plasma creatinine is generally thought to be a better marker than urea of glomerular function[45,46].

Regarding, the significant elevation in serum levels of some renal indices (uric acid, urea, creatinine) after acute and subacute treatment with colocynth fruit extract, this indicated the impairment in the renal function due to the alcoholic extract of this plant. These results are in correlation with the congestive appearance of renal parenchyma. Followed the same trends as renal parameters, CCT intoxication revealed significant elevation in Na^+ and K^+ values compared to those recorded from control rats.

Hematological analysis of plant extract in animals is one of the important methods of assessing the toxicity of plant extract in animals[47]. The hematopoietic system is often overlooked as a possible target for chemical injury[48]. Many chemicals can injure the system without damaging the marrow by such mechanisms as oxidative hemolysis within the circulation and immunotoxic reactions with blood components.

In this work, the effects on the hematopoietic system can be manifested as a significant reduction in RBC, HCT and HGB after the 1st, 3rd, 5th, 7th and 10th days of the treatment which indicated anaemia. The same picture is observed for these parameters after long-term treatment with CCT extract. It seems that this anaemia is microcytic hypochromic in treated rats as indicated by the low MCV and MCH values. In addition, the low levels of WBC indicated leucopenia which may be the result of overhelming inflammation, peripheral leukocyte destruction, bone marrow toxicity, loss of lymph, stress[49] or neutropenia[30]. Furthermore, the low HCT values can be due to decrease in the circulating RBC or to an increase in plasma volume. Moreover, a significant reduction in spleen relative weight was remarked until the 5th day of treatment. Changes in spleen weight in rodents can be a sensitive indicator of hypo- or hypercellularity of blood cells[31]. Then, CCT might be toxic to circulating red cells, interfered with their production and that of platelets. These findings are in disagreement with the significant increases in RBCs count, haemoglobin concentration and packed cell volume found by Elgerwi et *al*.[16] after administration of glucosidal principles CCT pulp fruit. However, the results obtained by Amamou et *al*.[50] who have shown a decreasing on erythrocytes and heamatocrit levels following colocynth oil treatment agrees with the present findings. These authors had explained such effects by the presence of phytosterols in this oil, which could replace cholesterol in cell membrane in erythrocytes and changes physics properties of erythrocytes membrane and reduced their flexibility. The reduction of flexibility of erythrocytes membrane can short cell life[51].

The histopathological findings in the liver revealed that oral administration of CCT fruit extract to rats in acute and subacute toxicity studies caused hepatic damage as blood congestion. The present data are in partial accordance with Khatibi[37] who observed in the liver sections after intraperitoneal administration of alcoholic extract of *C. colocynthis* at higher doses (200 and 400 mg/kg); small hemorrhages in many lobules and congestion of central veins and sinusoids accompanied mild nonspecific inflammation with hepatocellular necrosis. Mixed neutrophil and lymphocyte infiltrate involving the parenchyma was observed but no bile duct injury.

CONCLUSION

To sum up the above discussion, this study demonstrated that ripe fruit *C. colocynthis*extract produced a significant hepatorenal toxic effectin the case of long term administration or at high concentration evidenced by: low body weight and food consumption, aneamia, leucopenia, alterations in serum constituents, transaminases and ALP activities, tissue damage and death. Therefore, caution is needed in the use of this plant and effort must be exerted to identify plants utilized in folk medicine having narrowtherapeutic indices as their use is dangerous and should be carefully researched. Further studies are in progress in order to evaluate modes of action.

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