RESEARCH ARTICLE Indian Journal of Animal Research, Volume 60 Issue 6: 1030-1037 (June 2026)

Hepatorenal Toxicity with Hematological and Biochemical Changes in Wistar Rats Fed the Ethanolic Extract of the Pulp of *Citrullus colocynthis* from Two Regions in Saudi Arabia

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10.18805/IJAR.BF-2006

ABSTRACT

Background: Fruits of *Citrullus colocynthis* were collected from the central (Riyadh) and the northern (Hail) regions of Saudi Arabia. The total phenolic and flavonoid concentrations of the pulp extract from both regions were determined. The hepatorenal toxicity and the associated hematologic and biochemical changes in male Wistar rats were evaluated in experimental animals.

Methods: Forty male Wistar rats were assigned to five equal groups. Group 1 as control and fed normal saline. Group 2 and 4 were fed 100 and 300 mg/kg bodyweight of *C. colocynthis* pulp extract from the central region respectively. Group 3 and 5 were fed 100 and 300 mg/kg bodyweight of *C. colocynthis* pulp extract from the northern region respectively.

Result: The total phenolic and flavonoids contents of the pulp extract from the central and the northern regions were determined. There was a significant increase ($p<0.05$) in the total leucocytes counts in animals fed high doses of *C. colocynthis* pulp extract from both regions. The total platelets count was significantly increased ($p<0.05$) in groups 4 and 5. There was a significant increase in the serum concentrations of alkaline phosphatase and alanine aminotransferase. No significant increase in the levels of blood urea nitrogen and creatinine. Histologically, livers of the experimental animals showed dilatation of central veins, congestion of sinusoids, cytoplasmic degeneration of hepatocytes and infiltration of inflammatory cells. Kidneys showed dilatation of the glomeruli, severe congestion of kidney tissue with infiltration of inflammatory cells. Histological changes were dependent on the dose.

Key words: *Citrullus coclcynthis*, Hematology, Hepatorenal, Toxicity.

INTRODUCTION

Interest in traditional remedies has grown as people have become more aware of health issues and the negative effects of synthetic medications. Many ailments can be cured by medicinal plants, raising living standards. As a result, interest in ethnomedical research has grown and demand for herbal remedies has surged (Petrovska, 2012). The Cucurbitaceae family contains the plant "*Citrullus colocynthis*," which is referred to as "bitter apple" in English, as "hindal or hanzal" in Arabic and as "hendewane abujahl" in Persian. *C. colocynthis* is an herbaceous plant that is rich in nutrients and is essential for enhancing wellbeing. *C. colocynthis* is grown in various world's desert regions such as the Mediterranean, Arabia, west Asia including India and tropical Africa (Kumar *et al.*, 2021). The plant bears bitter fruit and tiny yellow flowers. It grows fast in the sandy soils and widely distributed in Saudi Arabia's various regions (Al-Zahrani and Al-Amer, 2006). *C. colocynthis* fruit and leaves are particularly rich in chemicals that are both bioactive and frequently poisonous. The pulp's bitter flavor and inedibility to animals are caused by cucurbitacins, which can also be dangerous or even lethal in some circumstances (Li *et al.,* 2022; Shafaei *et al.,* 2012). Polyphenols, which function as antioxidants and scavengers of various reactive oxygen species (ROS) like hydroxyl radicals and peroxy-free radicals, are abundant in *C. colocynthis* extracts (Al-Nablsi *et al.,* 2022; Sagar *et al.*, 2020). The therapeutic benefits of *C. colocynthis* are attributed to a variety of bioactive substances, including cucurbitacin, flavonoids and polyphenols. Plants' natural components and chemicals are what give them their therapeutic qualities (Li *et al.,* 2022).

C. colocynthis fruits are traditionally used for their antidiabetic effect because of the phenolics, flavonoids and cucurbitacins present in their extracts (Drissi *et al.,* 2021; Sansri *et al.,* 2022; Afzal *et al.,* 2023). The plant significantly

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How to cite this article: Al-Ghamdi, N.A., Omer, S.A., Alterary, S.S., El-Tohamy, M.F., Virk, P., El-Nagar, D.M. and Mohammed, O.B. (2026). Hepatorenal Toxicity with Hematological and Biochemical Changes in Wistar Rats Fed the Ethanolic Extract of the Pulp of *Citrullus colocynthis* from Two Regions in Saudi Arabia. *Indian Journal of Animal Research*. **60(6):** 1030-1037. doi: 10.18805/IJAR.BF-2006.

Submitted: 07-04-2025 **Accepted:** 30-08-2025 **Online:** 11-09-2025

lowered fasting blood glucose and glycated hemoglobin in a clinical experiment involving 50 patients with diabetes. However, there was no discernible modification in the cholesterol profile (Abdel-Hassan *et al.,* 2000; Al-Ghaithi *et al.,* 2004). *C. colocynthis* decreased triglyceride and total cholesterol in a different research experiment (Rahbar and Nabipour, 2010). Folane *et al.* (2020) studied the effects of oral polyherbal medication administration on blood glucose levels as well as the pathological alterations in the pancreas and other visceral organs of alloxan- induced diabetic rats. A dose of 100 mg/kg/day, *C. colocynthis* pulp extracts induced severe lesions in rabbit small intestine, kidney and liver (Dehghani and Panjehshahin, 2006). In contrast to pulp extract from *C. colocynthis*, animals fed with 100 or 200 mg/kg/day of seed extract only revealed minor digestive changes. Rats given *C. colocynthis* ripe fruit extract had worse negative effects on their liver and kidneys (Shafaei *et al.,* 2012).

The Kingdom of Saudi Arabia (KSA) flora has long been used as traditional medicines; however, these uses have not been well investigated and it is important to determine the safety of the Kingdom's flora. Chemo-taxonomic relevance for *C. Colocynthis* species gathered from different locations in Saudi Arabia. Large-scale gradients in the effects of environmental factors that cause growth variation and adaptation have long piqued the curiosity of ecologists. A vast range of climates and habitats, including plains, mountains, sand dunes, rocky deserts, valleys and salt pans, are found in the Kingdom of Saudi Arabia (Bukhari *et al.,* 2014).

The present study aims to determine the phenolic and flavonoid contents of the pulp extracts of *C. colocynthis* from two different localities in Saudi Arabia. It also aims to evaluate the toxic effect of ethanolic extract of the pulp of *C. colocynthis* on experimental Wistar rats through investigating the hematological, biochemical and histological changes.

MATERIALS AND METHODS

Written ethical assent for the study was reviewed and approved by Institutional Review Board (IRB) reference number (KSU-SE-23-121) *via* the Ethics Committee at King Saud University, Riyadh, Saudi Arabia.

Acquisition of plant material and extraction methods

Fruits of *C. colocynthis* were collected during January 2024 from two distinct geographical locations in Saudi Arabia, specifically from Hail representing the northern region and from Riyadh representing the central region. After harvest, the seeds were carefully removed and extraction was performed using the pulp to isolate the bioactive compounds. The identity of the plant was confirmed by a botanist from the Department of Botany and Microbiology at King Saud University.

The pulp of *C. colocynthis* was extracted using the ethanolic extraction method (70% ethanol) as described by Manikandan *et al.* (2008).

Phytochemical screening

Phytochemical screening of the *C. colocynthis* fruit pulp extract collected from Hail and Riyadh was conducted to assess total phenolic and flavonoid content using colorimetric assays. The total phenolic content was quantified with the Folin-Ciocalteu reagent, employing gallic acid as the standard (Siddiqui *et al.,* 2017). Meanwhile, total flavonoids were measured and expressed as catechin equivalents in milligrams per gram of sample (mg/g) (Fattahi *et al*., 2014). The method provided a reliable quantitative assessment of the phenolic and flavonoid concentrations in the extracts from both the central and northern regions, highlighting any potential regional variations in phytochemical composition.

Determination of the total phenolic and total flavonoids contents

The total phenolic contents of the ethanolic extracts of *Citrullus colocynthis* were estimated using the Folin Ciocalteau reagent as described by Singleton and Rossi (1965). The total flavonoid content of the ethanolic extract of *C. colocynthis* was estimated using the methodology described by Ordonez *et al.* (2006).

Experimental design

Forty male Wistar rats (90-120 g) were obtained from the Animal House, Zoology Department, King Saud University. Animals were fed on standard rat chow and water *ad libitum*. The animals were maintained in their respective groups for 7 days before the beginning of the experiment. They were housed at a controlled ambient temperature of 25±2°C with 50±10% relative humidity and with a 12 hr light/12 hr dark cycle. Animals were divided into 5 groups each containing 8 rats, they received the plant extract daily for 21 days, through gastric lavage and the groups were assigned as follows:

Group 1- Served as the control group and received normal saline (0.9% NaCl).

Group 2- Received 100 gm/kg bwt of *C. colocynthis* ethanolic extract collected from Riyadh region.

Group 3- Received 100 gm/kg bwt of *C. colocynthis* ethanolic extract collected from Hail region.

Group 4- Received 300 gm/kg bwt of *C. colocynthis* ethanolic extract collected from the Riyadh region.

Group 5- Received 300 gm/kg bwt of *C. colocynthis* ethanolic extract collected from Hail region.

Blood samples were collected at the end of the experiment for hematological and biochemical analyses. Blood was collected on both plain tubes and EDTA tubes. Unclotted blood was used to obtain hematological profiles of animals using the automatic hematologic analyzer VetScan HM5 (Abaxis, Tampa, FL 33615, USA). Parameters was including Leukocytes (WBC), differential WBC counts, RBC, hemoglobin (Hb), hematocrit (Hct) and erythrocytic indices and platelets (PLT) and associate parameters. Blood samples without anticoagulant was used for serum to investigate biochemical parameters including; alanine

aminotransferase (ALT) alkaline phosphatase (ALP), total bilirubin, creatinine, blood urea nitrogen, total protein, albumin, globulin, glucose, creatinine, amylase, as well as electrolytes (including, sodium, calcium, potassium, phosphorus) using an automated biochemistry analyzer VetScan VS2 (Abaxis, Tampa, FL 33615, USA).

Animals were sacrificed using overdose of isoflurane and tissues from the liver and kidney were preserved in 10% neutral buffered formalin for histopathological studies. Histological processing of tissues was performed, blocked into paraffin wax and sectioned using microtome and cut at 5 umm thickness. Then sections were stained using haematoxylin and eosin (H and E) and examined microscopically.

Statistical analysis

The data was analyzed using one-way ANOVA using SPSS 22.0 statistical software (Chicago, IL, USA). The student's t-test was used to analyze group differences. Statements of significance will be assumed on a *p*-value ≤0.05.

RESULTS AND DISCUSSION

The ethanolic pulp extract of *C. colocynthis* from the central and northern regions of Saudi Arabia were found to have different concentrations of phenolic and falvonoids compounds. With results expressed in terms of Gallic acid equivalents (GAE) in milligrams per gram of extract. The total phenolic content was determined as 255.74 mg/g in the Central region, while it was 263.84 mg/g in the Northern region. The total flavonoid contents in the extracts were calculated to be 8717.3 mg/g for the Central region and 6960.8 mg/g for the Northern region. Phenolic compounds are known for their therapeutic effects such as antioxidant, antimutagenic, anticarcinogenic as well as ability gene expression modification (Okpuzar *et al*., 2009; Priastomo *et al*., 2024). Phenolic compounds are considered as the largest group of chemicals from plants which account for antioxidant activity (Okpuzar *et al*., 2009). Among phenolic compounds, flavonoids are the largest and the widely characterized natural components in plant parts as free state or as glycosides (Osbourn and Lanzotti, 2009). Falvonoids are suggested as anti-oxidative stress and anti-heart diseases, they are considered to beneficial for both human and animal health (Wang, 2000; Clair and Anthony, 2005; Korkina, 2007; Patel *et al*., 2007).

The toxicity of *C. colocynthis* was evident in animals from group 5 which received 30°C of *C. colocynthis* from the northern region. All experimental animals showed diarrhea during the experimental period indicating intestinal involvement. Consuming *C. colocynthis* has been associated with diarrhea in human patients (Goldfain *et al*., 1989; AL-Farraj, 1995; Khan *et al*., 2003). The possible effect of diarrhea may be due to glycosides contained in the pulp of the plant which is known for its drastic hydragogue, cathartic and laxative affinity, where the same part of the plant has been used for extraction used in the present study (Dafni *et al*., 1984). Furthermore, the phenolic contents of the plant *obtained* from the northern region was higher than that from the central region, this may have affected the pronounced toxic effect. *C. colocythis* is known to contain cucurbitacin glycosides which may possibly be the cause of diarrhea here (Hatam *et al*., 1989). Furthermore, the effect of some *C. colocynthis* constituents which have a membranolytic may probably be involved in the intestinal damage which eventually resulted in diarrhea (Javadzadeh *et al*., 2013).

Experimental animals which were sacr ificed on day 21 days after the start of the experiment, showed increase in the total leucocytic counts when compared with animals from the control group. The increase in leucocytes was significant ($p<0.05$) in animals in group 5 which received

Table 1: Hematological changes in male Wsitar rats treated with 100 ml and 300 ml of *Citrullus colocynthis* pulp extract from the Northern region (NR) and from the Central region (CR) of Saudi Arabia.

Group / Parameter	Units	Control	100 CC-CR	100 CC-NR	300 CC-CR	300 CC-NR
WBCs	10^9/l	10.5±0.2	11.2±1.5	12.5±1.5	13.8±1.7	15±0.1^{a}
Lymphocytes	10^9/l	7.1±0.3	7.3±1.2	7.5±0.9	10.9±0.9	5±0.1
Monocytes	10^9/l	0.43±0.06^{b}	1.04±0.47^{a,b}	0.52±0.05^{c}	0.08±0.01^{a,b}	0.06±0.01^{a,b,c,d}
Neutrophils	10^9/l	3.5±0.9	3.8±0.3	2.8±0.4	3.8±0.5	3±0.1^{b}
RBCs	10^{12}/l	8.4±0.5	7.8±0.6	7.8±0.1	7.9±0.2	7.9±0.3
HB	g/dl	14.6±0.5	14±0.4	14.1±0.1	14±0.1	13.9±0.5
HCT	%	37.2±2.3	36.3±3.2	35.4±1.5	36.3±2.7	35.7±1.1
MCV	fl	44.3±0.3	46±0	45.3±1.6	45.3±1.7	48±1
MCH	pg	17.4±0.6	17.5±0.3	18.1±0.2	17.2±1.1	17.7±0.1
MCHC	g/dl	39.4±1.1	37.9±0.6	37.1±1.9	37.9±0.9	37±0.5
PLT	10^9/l	439±130	928±25^{a,b}	920±109^{a}	968±72^{a}	960±36^{a,b}
PCT	%	0.82±0.1	0.45±0.1^{a,b}	0.41±0.1^{a,b}	0.40±0.1^{a,b}	0.40±0.1^{a,b}

WBC = White blood cells, RBC = Red blood corpuscles, HB = Haemoglobin, HCT = Haematocrit or packed cell volume, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, PLT = Platelet counts, PCT = Plateletcrit. *C. colocynthis* Northern Region 100 ml and 300 ml (CC-NR), *C. colocynthis* Central Region 100 ml and 300 ml (CC-CR). Data are mean ± SEM.

30°CC-NR (animals which received 300 mg/kg bwt of *C. colocynthis* pulp extracts from the northern region) as shown in Table 1. It was apparent in the form of leukocytosis resulted from treatment of experimental animals with *C. colocynthis* pulp extract coincided with results of Elawad *et al.* (1984) and Elgerwi *et al.* (2013). It contradicted with the results obtained by Al Qaraawi and Adam (2003), who reported decreased WBC in rats fed both who fed rats a diet containing 10% of *C. colocynthis* fruits and 10% of *Capsicum frutescens* for 6 weeks. This variation may probably be due to the variation of the *C. colocynthis* constituents from different parts of the world. Khan *et al*. (2023) reported that there were differences in the different extracts of *C. colocynthis* seeds between extracts from Riyadh compared to those from India as well as Tangier in Morocco. It appears, in the present study, there were differences in the contents of flavonoids and phenolics of the plant extract from the central region and the northern region.

There was no significant difference in the total RBCs count between different groups at the end of the experiment. The hematocrit (HCT) values from animals in group 5 (received 30°C of *C. colocynthis* extract from the northern region) were lower than other animals in different groups including those in the control group, however, the difference was not significant (p>0.05) Table 1. There were no significant differences in the MCV, MCH and MCHC values between experimental animals and the control group (p>0.05) Table 1. However, a decrease in the RBCs counts were observed in experimental animals. This may be explained by the fact that extracts of *C. colocynthis* may have some effect on the bone marrow. Similar observations were made by Soufane *et al*., (2013) who related the decrease in RBCs and related parameters as well as WBCs to detrimental effects on the bone marrow, liver and kidneys.

Significant increase on the values of the platelets were recorded between animals received *C. colocynthis* extract from different regions compared with the control (P<0.05; Table 1). The PCT was significantly low in animals from group 5 compared to other experimental animals. The MPV values were higher in animals from group 5 compared with the control and the animals from group 4 which received 30°CC of the extract from the central region (Table 1). It has been reported that the hydro-alcoholic extracts of *C. colocynthis* has antiplatelets and profibrinolytic activity (Alhwaiti, 2018). Our finding contradicts with Alhwaiti (2018) findings which may probably be that the extract we used in the present study may not contain the ingredients responsible of the antiplatelet effect, furthermore, the effect of dehydration resulted from diarrhea may have resulted into hemoconcentration.

There was a significant difference in the values of albumin ingroups 4 and 5 compared with control group being lower (P<0.05) as shown in Table 2. The glucose level in group 5, however, was lower than glucose in group 3 and the control and the difference was not significant (P>0.05). The alanine aminotransferase values increased in all groups compared with the control group however, the increase was significant in group 2 (P<0.05). BUN and creatinine values were increased with the increase of the dose of the plant extract from both regions but the increase was not significant (P>0.05). Calcium and phosphorus levels were decreased in experimental animals; however, the decrease was significant in group 5 compared with control group and in groups 4 and 5 compared with control group in calcium and phosphorus respectively (P<0.05). Potassium level was significantly decreased (P<0.05) in group 2 compared to the control group which other groups did not show significant decrease in potassium level (Table 2).

There was a significant increase in the values of ALT and ALP in group 5 compared with the control group (P<0.05). This increase was accompanied by a decrease in the albumin which may suggest liver damage (Soufane *et al*., 2013; Ekudina *et al*., 2015). The increase in the ALP in animals fed 300 *C. colocynthis* extracts from the northern

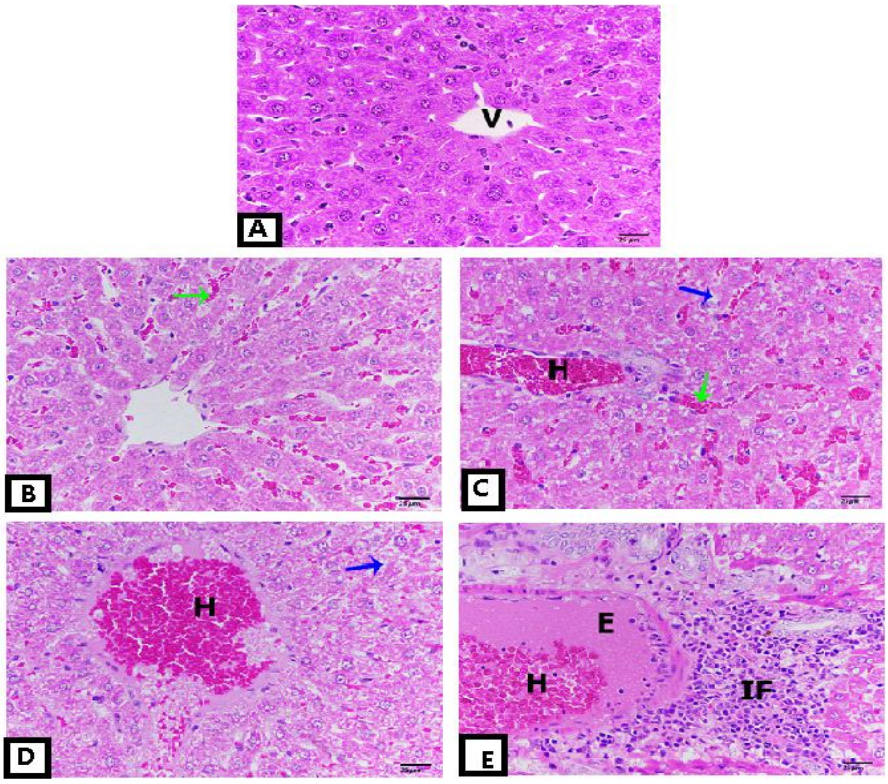
Table 2: Biochemical changes in male Wsitar rats treated with 100 ml and 300 ml of *Citrullus colocynthis* pulp extract from the Northern region (NR) and from the Central region (CR) of Saudi Arabia.

Group / Parameter	Units	Control	100 CC-CR	100 CC-NR	300 CC-CR	300 CC-NR
Total protein	g/dl	6.6±0.38	6.4±0.67	7.2±0.41	7±0.50	7.7±0.20
Albumin	g/dl	4.2±0.2	3.7±0.2	4.3±0.1	4.6±0.1	5±0.1
Globulin	g/dl	2.3±0.1	2.7±0.3	2.8±0.3	2.3±0.1	2.7±0.2
Glucose	mg/dl	86±1	87±1	86±2	87±2	88±2
Amylase	U/l	835±57[a]	874±88[a]	877±19[a]	900±50	947±47
Alkaline phosphatase	U/l	534±71[a,b,d]	646±55[a,c]	507±40[a,b]	648±9[a]	373±38
Alanine aminotransferase	U/l	154±14	146±5	139±7	136±60	124±13
Blood urea nitrogen	mg/dl	20±2.00	20±0.88	19±0.67	18±0.5	17±0.5
Creatinine	mg/dl	0.39±0.06	0.40±0.03	0.37±0.03	0.40±0.10	0.35±0.06
Calcium	mmol/l	10±0.8	12.9±1.1	13.5±0.4	13.4±0.1	14.3±0.1
Phosphorus	mmol/l	12.8±0.4	14.2±1.01	16.7±1.3	17±0	18±0.4
Sodium	mmol/l	152±2	160±4.9	153±0.8	159±2.3	154±0.2
Potassium	mmol/l	7±0.2	6.2±0.2	7.6±0.4	6±0.5	8.5±0

C. colocynthis Northern Region 100 ml and 300 ml (CC-NR), *C. colocynthis* Central Region 100 ml and 300 ml (CC-CR). Data are mean±SEM.

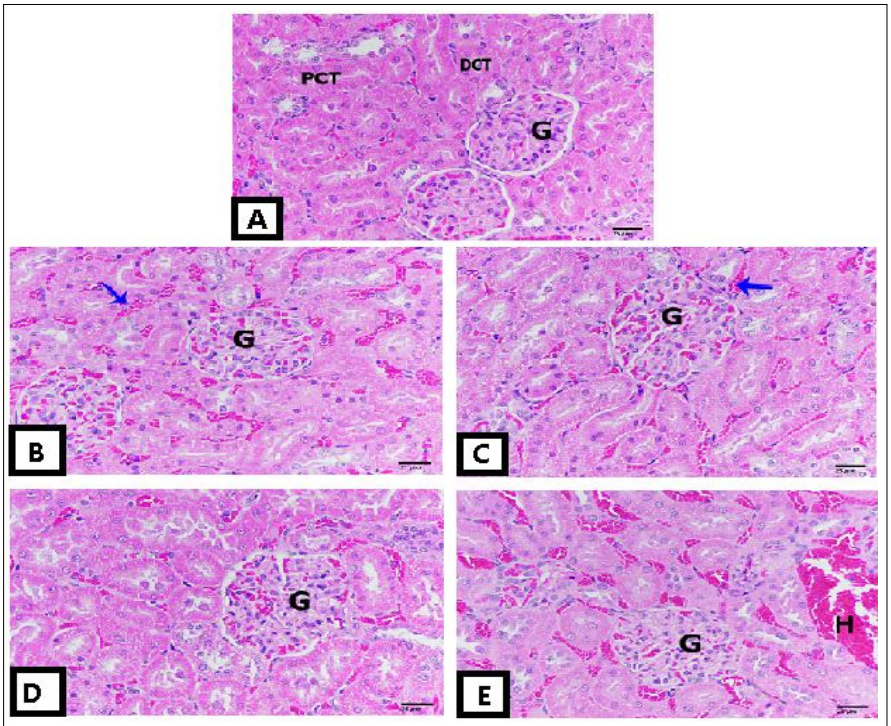
region was higher than other experimental animals including animals in the control group. Alkaline Phosphatase is concentrated in the liver, bile duct, kidney, bone and the placenta, therefore, the increase in ALP in this case may possibly be resulting from liver or kidney damage (Ekudina *et al*., 2015). However, the increase in ALT and albumin in experimental animals may be related to live damage. The results of the histopathology coincided with this finding. The levels of creatinine and blood urea nitrogen were increased in experimental animals particularly those who received a higher dose from both regions compared with the control group, an indication of kidney involvement. Results are associated with coagulative necrosis and hydropic degeneration in the renal epithelium in histopathology. These differences were not associated with significant differences in the levels of sodium and potassium as well as calcium and phosphorus. Renal profile was in accordance with earlier result obtained from Al-Ghaithi *et al*. (2004) and Atole *et al*. (2009). However, they are unlike what has been reported by Elgerwi *et al*. (2013) who reported significant differences in the levels of creatinine, BUN in experimental animals treated with the extract of the minced fruit pulp of *C. colocynthis* from Libya. They used plants from different three localities. The increase in the serum constituents related to liver and kidney functions could be as a result of the phenolic glycosides of the plant extract used in the present study. It has been reported before that feeding rats seed extracts of *C. lanatus* resulted into increase in the APL values and that was attributed to the increase in the ALP activity in of human osteoblast-like cells (Adedeji *et al*., 2017).

The liver of the control rats showed normal structure of the central veins and hepatocytes and normal sinusoids (Fig 1A). Liver from animals treated with 100 mg/kg of *C. colocynthis* extract from central and northern regions after 21 days revealed dilatation of central veins and congestion of sinusoids besides presence of intense inflammation (Fig 1B and C). Moreover, livers from animals



(A): Liver treated with 100 mg of CC extract of central region after 21 days of treatment revealing dilatation and congestion of blood sinusoids (green arrow); (B): Liver treated with 100 mg of CC extract of northern region after 21 days of treatment showing dilatation and congestion of blood sinusoids (green arrow), cytoplasmic degeneration of hepatocytes (blue arrow), dilatation of vein congested with hemorrhage {H}; (C): Liver treated with 300 mg of CC extract of central region after 21 days of treatment revealing intense cytoplasmic degeneration of hepatocytes (blue arrow), congestion of vein with hemorrhage {H}; (D): Liver treated with 300 mg of CC extract of northern region after 21 days of treatment displaying aggregation of inflammatory cells (IF), dilatation and congestion of vein with hemorrhage {H} and edema {E}; (E): Scalebar = 25 µm.

Fig 1: Histopathological changes of liver tissues of normal control rats.



(A): Kidney treated with 100 mg/kg of CC extract from the central region after 21 days of treatment revealing abundant glomeruli [G], congestion with hemorrhage in between tubules (blue arrow); (B): Kidney treated with 100 mg/kg of CC extract from the northern region after 21 days of treatment revealing abundant glomeruli [G], congestion with hemorrhage in between tubules (blue arrow); (C): Photomicrograph of animal kidney treated with 300 mg/kg of CC extract from the central region after 21 days of treatment revealing abundant glomeruli [G], congestion with hemorrhage [H]: (D): Kidney treated with 300 mg/kg of CC extract from the northern region after 21 days of treatment revealing abundant glomeruli [G]; (E): Scalebar = 25 µm.

Fig 2: Histopathological examination of kidney tissues of normal control rats normal glomeruli [G], proximal convoluted tubule [PCT], distal convoluted tubule [DCT].

treated with 300 mg/kg of *C. colocynthis* from central and norther regions after 21 days represented cytoplasmic degeneration of hepatocytes and congestion of sinusoids together with infiltration of inflammatory cells (Fig 1, D and E). The kidney of the control animals showed renal tissue with normal histological picture with abundant glomeruli and kidney tubules (Fig 2, A). Moreover, treatment with 100 mg/kg of *C. colocynthis* extract from central and northern regions for 21 days resulted in congestion of kidney tissue with hemorrhage in between tubules and in renal blood vessels (Fig 2, B and C). Additionally, increasing of *C. colocynthis* extract treatment as 300 mg/kg for 21 days revealed severe congestion of kidney tissue with infiltration of inflammatory cells (Fig 2, D and E). Histological changes in the liver and kidney tissues coincided with the biochemical alterations indicating hepato-renal damage as a result of feeding *C. colocynthis* pulp extract.

CONCLUSION

From the present study it was concluded that pulp extracts of *C. colocynthis* from the northern and the central regions of Saudi Arabia possess different concentrations of the total phenolic and flavonoids. Extracts showed varying degrees of toxicity to rats with increased does. Extracts of the pulp were found to be toxic to rats as the toxicity reflected on the total erythrocytic and total leucocytic counts as well as other parameters related to both RBCs and WBCs. Extracts from both regions have shown increased platelets counts. Liver and kidney function tests were affected as aa results of examination of some metabolites reflecting these organs functions. The histopathology results were in accordance with the biochemical findings confirming liver and kidney involvement.

ACKNOWLEDGEMENT

The work was supported by the Ongoing Research Funding Program (ORF-2025-94), King Saud University, Riyadh, Saudi Arabia.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the

information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare no conflicts of interest.

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