Subchronic haemotoxicity and histotoxicity of *Citrullus colocynthis*.

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Abstract: This study aimed at examination of the toxicity of *Citrullus colocynthis* extract in rats after oral administration of ¼ of LD_{50} every week for 10 weeks. The oral LD_{50} was determined for the extract of *Citrullus colocynthis* plants obtained from three different localities in Libya including Tarhona, Alzawia and Suq-Alkhamis, which were calculated as 100, 101.7 and 162.4 mg/kg. b.wt., respectively. Rats used in the present experiment were classified into four groups; the first, the second and the third groups were given the extract of the plant collected from Tarhona, Alzawia and Suq-Alkhamis, respectively, while the fourth one was kept as a control. Blood samples were collected for haematological and biochemical examination. Specimens from lung, liver, kidney, intestine, stomach and spleen were taken from the treated and control rats for histopathological examination. The treated rats suffered from severe yellow diarrhea, dyspnea, depression and weakness of hind limbs. The blood picture of treated rats showed marked changes in total RBC count, PCV %, Hb concentration, WBC count and differential leukocytic count. Serum GPT, GOT, ALP, glucose, total protein, urea and creatinine and serum electrolytes were severely affected. Pathological changes in lung, liver, kidney, spleen, stomach and intestine were recorded in the treated rats.
[A. Elgerwi, Z. Benzekri, S. Awaidat, A. El-Magdoub, A. Abusnina, A. El-Mahmoudy. **Subchronic haemotoxicity and histotoxicity of *Citrullus colocynthis*.**. *J Am Sci* 2013;9(5):79-87]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 12

Key Words: *Citrullus colocynthis*, Rats, Hematological, Biochemical, Histopathological.

1. Introduction

Citrullus colocynthis plant belongs to the *Cucurbitaceae* or Squash Family which produces seeds rich in oil (50%) and protein (29%) [1]. Oil composed of myristic, palmitic, stearic, oleic, linoleic, and linolenic fatty acids [2]; while protein composed of lysine, leucine, and methionine amino acids [3]. It is believed that the plant is native in Africa and Middle East and is probably ancestral type of the water melon. It is a long-lived perennial and grows wild in deserts under extreme xerophitic conditions. Young fruits are freshly, mottled with dark green and usually turn yellow when ripe [4]. They are extremely bitter in taste and are full of smooth and shiny seeds which are thought to be rich in oil and protein. Several cucurbit oils are currently used for cooking in countries other than the United States [5-6].

Controversial data were reported on *C. colocynthis,* including either medical or toxic studies. Along medical ones, *C. colocynthis* was proved to have antimicrobial [7], anti-inflammatory [7], antihistaminic [8], antihyperlipidemic [9], antioxidant [10-11], anti-ulcer [12] and antidiabetic [13-14] effects.

On the other hand, toxic effects have been recorded for *C. colocynthis*. Haemogram and biogram effects were recorded in goats upon feeding on fresh fruits and leaves for 10 days [15]. Signs of *C. colocynthis* poisoning were recorded in sheep given as a daily drench of 0.25 g./kg.b.wt. of minced *C. colocynthis* fruits [16]; the marked signs were depression, diarrhoea, dyspnea, inappetence, froth at the mouth, loss of condition, weakness of the hind limbs and eventually recumbency. Detectable pathologic lesions were recorded in goats and calves experimentally received minced fruits (5 g./kg.b.wt. for 9 days and 0.5 g./kg.b.wt. for 13 days) and dried leaves (0.25 g./kg.b.wt. for 9 days) of *C. colocynthis*. They were almost similar and were in the form of echymotic haemorrhage in the endocardium, kidney, spleen and gall bladder; congestion of the lungs and liver in addition to catarrhal enteritis and pulmonary emphysema [17]. Another study was performed by Elawad and others [18] who recorded the effect of *C. colocynthis* on sheep upon giving fresh fruits and leaves to ten sheep at daily doses ranging from 0.25 to 10 g./kg.b.wt. The study included toxic clinical signs within 4 hours and died 25 days after dosing, toxic histopathological effects and toxic biochemical and haematological effects. Shab and others [19] mentioned that *C. colocynthis* has significant haematological and spermatogenic dysfunction effects. The toxicity of *C. colocynthis* was estimated in chicks by Bakhiet & El Adam [20]. In addition, Dehghani & Panjehshahin [21] recorded a toxic

effect for the alcoholic extract of *C. colocynthis* on the liver of rats.

Nevertheless, Ickert [22] noticed that *C. colocynthis* has no toxicological effect in Wister rats treated subcutaneously by dried fruit extract for four weeks and advised to use the extract as cathartic purgative.

The present study aimed at investigating the subchronic toxicological effects of *C. colocynthis* poisonous plant on haemogram, biochemical markers of liver and kidney function in addition to the histopathological changes in the visceral organs of albino rats.

2. Material and Methods

1- *Citrullus colocynthis* plant: *Citrullus colocynthis*-L is distributed in different localities in Libya. Plant samples used in this study were collected from their natural habitat in three different Libyan localities, namely Tarhona, Alzawia and Suq-Alkhamis.

2- Animals: Two hundred and fifty albino rats of both sexes were obtained from Experimental Animals Unit, College of Veterinary Medicine, University of Tripoli. Animals were clinically healthy, sexually mature and weighing 180 - 250 g. Animals were let to accommodate to the laboratory conditions for two weeks before being experimented. They were maintained on balanced diet and water *ad. libitum* throughout the experimental period.

3- Plant extraction: Extraction of glucosidal principles was carried out according to the method described by Rehm and others [23], where the minced fruit pulp was covered with an equal amount (w/v) of a mixture of 96 % alcohol and saturated basic lead acetate solution. Under these conditions all the hydrolytic enzymes in the mixture were destroyed. Filtration gave a clear liquid from which the excess of lead acetate was removed by precipitation with a saturated aqueous solution of potassium dihydrogen phosphate. Chloroform extraction removed the bitter principles completely from the aqueous phase.

4- Determination of oral LD_{50}: The oral median lethal dose of *C. colocynthis* extract obtained from Tarhona, Alzawia and Suq-Alkhamis was determined according to the method described by Behrens &Karber [24]. In order to determine the zero and 100% mortality, actual trials were carried out on 6 groups of animals each of four rats for each locality. Rats dosed orally *via* intra-gastric intubation using rat stomach tube with 24, 20, 16, 12, 8 and 4 g./kg.b.wt. for each locality. In each trial a group of 6 control animals administered distilled water only was included. The actual determination of LD_{50} was carried out. All animals were kept under close observation for clinical signs. Dead animals were subjected to post-mortem examination.

5- Subchronic toxicological study:

Forty eight apparently healthy albino rats were used to study the subchronic toxicological effects of *C. colocynthis* extract. Rats were equally classified into four groups. The first three groups were given orally (¼ of the LD_{50}) of the extract of *C. colocynthis* fruit of Tarhona, Alzawia andSuq-Alkhamis, respectively, while the fourth group was kept as control and received only distilled water. The extract was given every week till 10 weeks, then rats were sacrificed and EDTA-blood was collected for haematological examination. Additional blood samples were collected without anticoagulant for biochemical examination of serum.

5-1- Haematological examination: Erythrocytic (RBC) count was determined according to the method described by Natt & Henrick [25] using haemocytometer; Haemoglobin content (Hb) was estimated using the acid hematin method by Sahli's haematometer according to Lynch and others [26]; Total leukocytic (WBC) count was performed using the haemocytometer counting chamber and Turk's solution as reported by Schalm [27]; Differential leukocytic cell count was adopted by preparing thin blood films, leaving them to dry and staining by Leishman's stain, the differential leukocytic count was carried out according to Mac-Gregorand others [28].

5-2- Analysis of serum biochemical parameters: The activities of serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxalacetic transaminase (SGOT) were measured according to the method described by Reitmann and Frankel [29] using serum transaminase kits from Bio-merieux®. Alkaline phosphatase (ALP) was estimated according to the method of Roy [30] using serum alkaline phosphatase kit from Bio-Merieux®. Total protein was determined using Biuret method as cited by Wootton [31]. Glucose was determined colorimetrically according to Torlotin [32] using glucose kit from Bio-Merieux®. Urea was estimated in serum of treated rats according to the method of Patton and Crouch [33] using urea kit Bio-Merieux®. Serum creatinine was estimated according to the method of Husdan & Rapoport [34] using creatinine kit fromBio-Merieux®. Sodium and potassium were estimated using flame photometer according to Oser [35].

5-3- Histopathological examination: Specimens from lungs, liver, kidneys, spleen, stomach and intestine were collected from the examined animals and immediately fixed in 10% formalin for histopathological examination according to Harris [36].

6- Statistical analysis:

Experimental results were statistically analyzed using analysis of variance and *t*-test after Snedecor and Cochran [37].

3. Results

1- Determination of LD_{50}: The LD_{50} of the extract of *C. colocynthis* plant obtained from Tarhona, Alzawia and Suq-Alkhamis localities were calculated as 100, 101.7 and 162.4 mg/kg.b.wt., respectively.

2- Clinical symptoms and post-mortem findings: Rats in the three treated groups suffered from severe yellow diarrhea, dyspnea, depression, loss of condition and weakness of hind limbs. The post-mortem findings were in the form of congestion and petechial haemorrhage in the liver as well as congestion of the intestine and spleen.

3- Haematological effects: The blood picture of *C. colocynthis*-treated albino rats is shown in tables (1 & 2). The total RBC count was significantly higher in Suq-Alkhamis group (7.86 ± 0.5 x 10^6 / mm^3) followed by Alzawia group (7.33 ± 0.42 x 10^6 / mm^3) and then Tarhona group (5.26 ± 0.42 x 10^6 / mm^3) and those were significantly higher than that of the control one (5.0 ± 0.35 x 10^6 / mm^3). The packed cell volume was higher in Suq-Alkhamis group (52.5 ± 1.75 %) followed by Alzawia group (50.63 ± 0.56 %) and then Tarhona group (45.86 ± 1.1 %) compared with that of the control group (35.92 ± 1.83). The haemoglobin content was higher in Suq-Alkhamis group (8.66 ± 0.19 g./dl) followed by Alzawia group (7.93 ± 0.38 g./dl) then Tarhona group (7.15 ± 0.41 g./dl) compared with that of the control group (5.93 ± 0.32 g./dl).

Total and differential leukocytic counts were variable among the treated groups. Total WBC count was higher in Suq-Alkhamis group (10.026 ± 1.22 x 10^3 / mm^3) followed by Alzawia group (8.843 ± 0.95 x 10^3 / mm^3) and then Tarhona group (7.237 ± 0.75 x 10^3 / mm^3) compared to the control group (5.145 ± 0.47 x 10^3 / mm^3). Regarding differential leucocytic count, monocytes, lymphocytes, neutrophils, band cells, eosinophiles and basophiles showed highly significant increase in their numbers in treated groups compared with those in the control group.

3- Serum biochemical effects: The effect of *C. colocynthis* extract on liver function biomarkers in serum of albino rats after oral administration of ¼of the LD_{50} of *C. colocynthis* extract for 10 weeks was shown in table (3). SGPT and SGOT values showed significant increase in all treated rats groups than the control group. The values of SGPT in Suq-Alkhamis, Alzawia and Tarhona were 124 ± 30.2, 91.88 ± 10.5 and 74.75 ± 1.27 u/l, respectively, whereas in the control group, it was (46.3 ± 3.15 u/l). Similarly, the SGOT level was also increased significantly in all treated albino rat groups compared to the control one. The mean values of SGOT in Suq-Alkhamis, Alzawia and Tarhona groups were 275.63 ± 10.19, 225.88 ± 2.35 and 211.25 ± 27.9 u/l, respectively, while it was (128.8 ± 3.39 u/l) in the control one. The mean levels of ALP in the treated albino groups were significantly higher (122.25 ± 14.3; 197.0 ± 19.96 and 234.38 ± 18.99 ul/100 ml in Tarhona, Alzawia and Suq-Alkhamis, respectively) than that of the control rats (32.12 ± 1.26 ul/100 ml).

The influence of *C. colocynthis* extract on blood glucose level indicated that blood glucose level was reduced significantly in treated groups compared to the control one. The mean blood glucose levels were 11.63 ± 0.85; 8.24 ± 1.2 and 5.31 ± 0.86 mg /100 ml in Tarhona, Alzawia and Suq-Alkhamis, respectively, while it was 15.2 ± 0.92 mg / 100ml in the control group. In addition, the total serum protein was also decreased significantly in all treated groups (6.72 ± 0.36; 5.7 ± 0.32 and 5.68 ± 0.38 g/dl in Tarhona, Alzawia and Suq-Alkhamis groups, respectively) than that of the control group (7.28 ± 0.24 g/dl).

Kidney function parameters in serum of albino rats after oral administration of ¼ of the LD_{50} of *C. colocynthis* extract for 10 weeks were shown in table (4). Serum urea of treated groups increased significantly compared with that of the control one where they were 49.25 ± 1.22; 60.6 ± 1.64 and 78.25 ± 1.13 mg / 100 ml in Tarhona, Alzawia and Suq-Alkhamis groups, respectively, and 37.1 ± 4.1 mg / 100 ml in the control one. Serum creatinine was higher in the treated groups (13.85 ± 0.25; 15.04 ± 0.25 and 18.99 ± 0.19 mg / 100 ml in Tarhona, Alzawia and Suq-Alkhamis, respectively) than that of the control one (10.12 ± 0.38 mg / 100 ml).

The normal average level of serum Na^+ in control rats was 141.9 ± 2.1 mEq/L and increased significantly (144.4 ± 0.62, 145.6 ± 1.78 and 146.6 ± 2.86 mEq/L) in Tarhona, Alzawia and Suq-Alkhamis groups, respectively. The concentration of serum K^+ decreased significantly in the treated groups of rats. Its level was 7.41 ± 0.87; 7.05 ± 0.28 and 6.48 ± 0.31 mEq/L in Tarhona, Alzawia and Suq-Alkhamis groups, respectively, while it was 7.94 ± 0.22 mEq/L in the control rats.

4- Histopathological effects:

Lungs: In Alzawia treated group, the examined lungs were hepatized in texture, reddish in color with grayish-white spots on their surface and blood oozed from the cut-surface. Microscopically, diffuse thickening of inter-alveolar septa due to congestion of the alveolar capillaries and leukocytic infiltration particularly lymphocytes and a few eosinophils were detected (Fig.1). Bronchi and bronchioles revealed hyperplasia of the lining epithelial cells, desquamated cells with eosinophilic material in their lumen in

addition to peribronchial lymphocytic hyperplasia. Diffuse perivascular edema with leukocytic aggregation mainly lymphocytes and a few eosinophils had been observed. Some interstitial blood vessels revealed focal hyalinization of the *tunica media*. In Tarhona treated group, compensatory alveolar emphysema had also been seen while focal thickening of the inter-alveolar septa due to lymphocytic infiltration in addition to diffuse alveolar emphysema were seen in Suq-Alkhamis group.

Liver: In Alzawia treated group, the examined liver was friable in consistency and blood oozed from the cut-surface. The hepatic parenchyma contained grayish-white spots (½ -1 mm in diameter) scattered all over the surface. Microscopically, some hepatic lobules showed individualization of the hepatic cells. Some of these cells suffered from necrobiosis and the others showed coagulative necrosis. Multiple focal aggregation of lymphocytes and macrophages replaced the hepatic parenchyma were outstanding. Coagulative necrosis was evidenced in some hepatic cells by dark small stained nuclei (pyknosis) with cytoplasmosis. Other hepatocytes suffered necrobiosis in the form of cloudy swelling and vacuolar degeneration. Congestion of the central veins and sinusoids and slight aggregation of lymphocytes near to central veins with focal haemorrhage had been noticed. Hypertrophied Kupffer's cells were seen. Multiple focal interstitial haemorrhage (Fig. 2) and severe congestion of central veins and sinusoids had also been observed in Tarhona group. In Suq-Alkhamis group, the portal tract showed leukocytic infiltration mainly lymphocytes. Diffuse coagulative necrosis of all hepatic cells was evidenced by small dark stained nuclei (pyknotic nuclei) with cytoplasmosis in addition to histopathological alterations occurred in Alzawia treated group.

Kidneys: Macroscopically, the examined kidneys were enlarged in size, friable in consistency with grayish-white spots on the renal parenchyma in Alzawia treated group. Microscopically, leukocytic infiltration in between renal tubules mainly lymphocytes and few eosinophiles had been detected. The renal epithelium of the proximal and distal convoluted tubules suffered from coagulative necrosis evidenced by pyknotic nuclei with cytoplasmosis and cloudy swelling besides hydropic degeneration. Perivascular edema especially around the large inter-tubular blood vessels and albuminous material (eosinophilic material) in the lumen of some renal tubules were predominant. In Tarhona group, perivascular haemorrhage and hyperplasia of the *tunica intema* (endothelosis) with vacuoles in the tunica media in some blood vessels (Fig.3) were also observed while in Suq-Alkhamis group, perivascular edema and haemorrhage were prevalent.

Spleen: In all treated groups, the examined spleen was apparently normal while microscopically, predominant hyperplasia of the white pulp was seen (Fig. 4).

Stomach and intestine: Macroscopically, the examined stomach and intestine were apparently normal in all treated groups. Microscopically, desquamated epithelial cells with slight exudate in the lumen in addition to severe leukocytic infiltration in the *lamina propria* mainly lymphocytes were observed (Fig.5). The intestine showed complete desquamation of the intestinal villi, lymphocytic infiltration in the *lamina propria* and eosinophilic material in the sub-mucosal layer (Fig.6).

Table (1): Erythrocytic parameters (mean ± S.E.) after oral administration of ¼ of the LD_{50} of *Citrullus colocynthis* extract for 10 weeks to albino rats.

	RBC's (10^6/mm^3)	P.C.V (%)	Hb (g/dl)
Control	5.0 ± 0.35	35.92 ± 1. 83	5.93 ± 0.32
***C. colocynthis* (Tarhona)**	5.26 ± 0.42	45.86 ± 1.1	7.15 ± 0.41
***C. colocynthis* (Alzawia)**	7.33 ± 0.42	50.63 ± 0.56	7.93 ± 0.38
***C. colocynthis* (Suq-Alkhamis)**	7.86 ± 0.5	52.50 ± 1.75	8.66 ± 0.19
***F* value**	12.45**	26.99**	9.97**

**Highly significant at $P \geq 0.001$ compared to the control.

Table (2): Leucocytic parameters (mean ± S.E.) after oral administration of ¼ of the LD_{50} of *Citrullus colocynthis* extract for 10 weeks to albino rats.

	Total leukocytic count (10^3/ mm^3)	Differential leukocytic count					
		Monocytes (10^3/ mm^3)	Lymphocytes (10^3/ mm^3)	Neutrophils (10^3/ mm^3)	Band cells (10^3/ mm^3)	Eosinophils (10^3/ mm^3)	Basophils (10^3/ mm^3)
Control	5.145 ± 0.47	0.204 ± 0.048	3.593 ± 0.195	0.997 ± 0.1	-	0.193 ± 0.031	0.158 ± 0.02
***C. colocynthis* (Tarhona)**	7.237 ± 0.78	0.474 ± 0.10	4.307 ± 0.63	1.427 ± 0.1	0.28 ± 0.061	0.568 ± 0.054	0.181 ± 0.017
***C. colocynthis* (Alzawia)**	8.843 ± 0.95	0.452 ± 0.11	5.493 ± 0.86	2.012 ± 0.44	0.309 ± 0.07	0.347 ± 0.085	0.062 ± 0.0
***C. colocynthis* (Suq-Alkhamis)**	10.026 ± 1.22	0.612 ± 0.15	6.093 ± 1. 06	2.049 ± 0.31	0.259 ± 0.055	0.499 ± 0.12	0.514 ± 0.09
***F* value**	6.64**	3.42*	4.19*	4.79*	5.22*	5.59**	26.38**

*Significant at $P \geq 0.05$ compared to the control.
** Highly significant at $P \geq 0.001$ compared to the control.

Table (3): Liver function parameters (mean ± S.E.) in serum of albino rats after oral administration of ¼ of the LD_{50} of *Citrullus colocynthis* extract for 10 weeks.

	Transaminases		ALP (u/100 ml)	Glucose (mg/100 ml)	Total protein (g/dl)
	GPT (u/L)	GOT (u/L)			
Control	46.3 ± 3.15	128.8 ± 3.39	32.12 ± 1. 26	15.2 ± 0.92	7.28 ± 0.24
***C. colocynthis* (Tarhona)**	74.75 ± 1.27	211. 25 ± 27.9	122.25 ± 14.3	11.63 ± 0.85	6.72 ± 0.36
***C. colocynthis* (Alzawia)**	91.88 ± 10.5	255.88 ± 2.35	197.0 ± 19.96	8.24 ± 1. 2	5.7 ± 0.32
***C. colocynthis* (Suq-Alkhamis)**	124 ± 30.2	275.63 ± 10.19	234.38 ± 18.99	5.31 ± 0.86	5.68 ± 0.38
***F* value**	30.0**	10.5**	30.0**	3.3**	4.66*

*Significant at $P \geq 0.05$ compared to the control.
** Highly significant at $P \geq 0.001$ compared to the control.

Table (4): Kidney function parameters (mean ± S.E.) in serum of albino rats after oral administration of ¼ of the LD_{50} of *Citrullus colocynthis* extract for 10 weeks.

	Urea (mg/dl)	Creatinine (mg/dl)	Serum Electrolytes	
			Na^+ (mEq/L)	K^+ (mEq/L)
Control	37.1 ± 4.1	10.12 ± 0.38	141.9 ± 2.1	7.94 ± 0.22
***C. colocynthis* (Tarhona)**	49.25 ± 1. 22	13.85 ± 0.25	144.4 ± 0.62	7.41 ± 0.87
***C. colocynthis* (Alzawia)**	60.6 ± 1. 64	15.04 ± 0.25	145.6 ± 1. 78	7.05 ± 0.28
***C. colocynthis* (Suq-Alkhamis)**	78.25 ± 1.13	18.99 ± 0.19	146.6 ± 2.86	6.48 ± 0.31
***F* value**	1. 52**	0.14**	0.98 N.S	1. 55 N.S

** Highly significant at $P \geq 0.001$ compared to the control.
N.S = Non significant compared to the control.

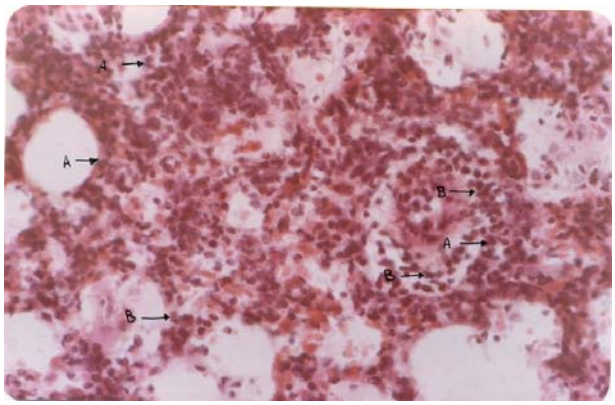

Fig. (1): Lungs of albino rats orally administered the extract of *Citrullus colocynthis* fruits of Alzawia group showing interalveolar leukocytic infiltration mainly lymphocytes (arrow A) and eosinophils (arrow B). H&E X 2500.

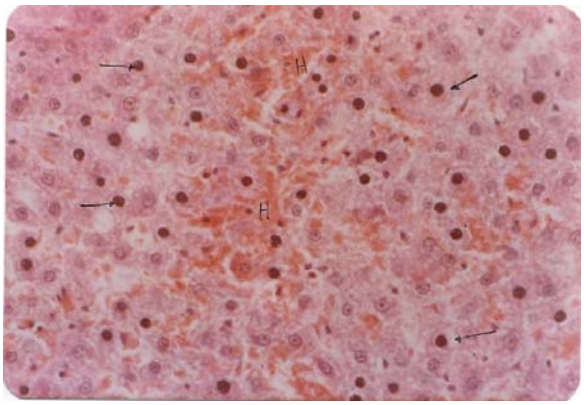

Fig. (2): Liver of albino rats orally administered the extract of *Citrullus colocynthis* fruits of Tarhona group showing coagulative necrosis in most hepatic cells (arrows) and interstitial haemorrhage (H). H&E X 300.

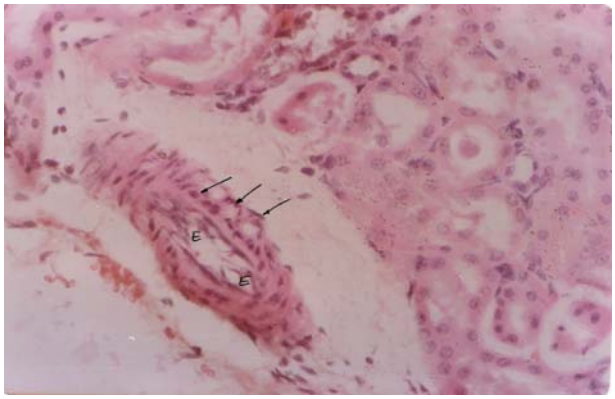

Fig. (3): Kidney of albino rats orally administered the extract of *Citrullus colocynthis* fruitsTarhona group showing endothelosis of the *tunica intema* (E) with vacuoles in the *tunica media* (arrows). H&E X 400.

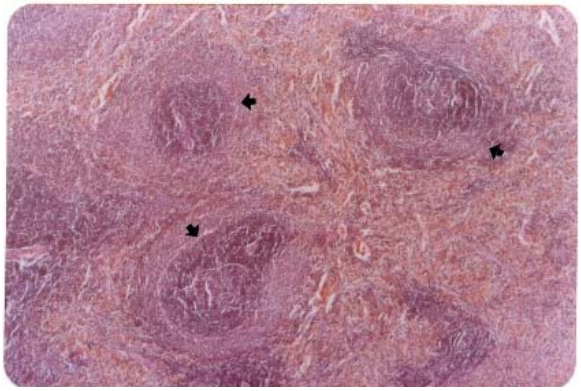

Fig. (4): Spleen of albino rats orally administered the extract of *Citrullus colocynthis* fruits of Alzawia group showing hyperplasia of the white pulp (arrows). H&E X 250.

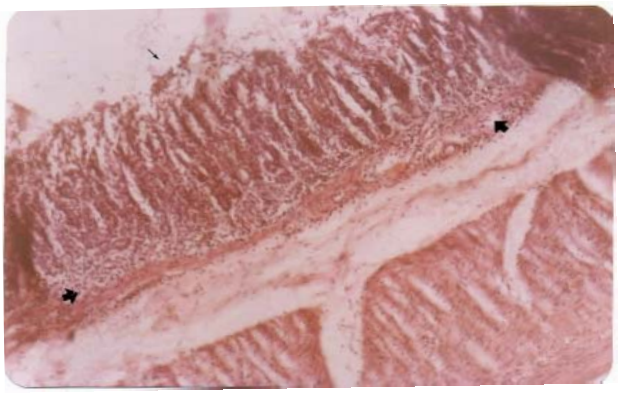

Fig. (5): Stomach of albino rats orally administered the extract of *Citrullus colocynthis* fruits of Suq-Alkhamis group showing desquamation of the epithelial cells with exudatein the lumen (thin arrow) and leukocytic infiltration in the *lamina propria* (thickarrow). H&E X 250.

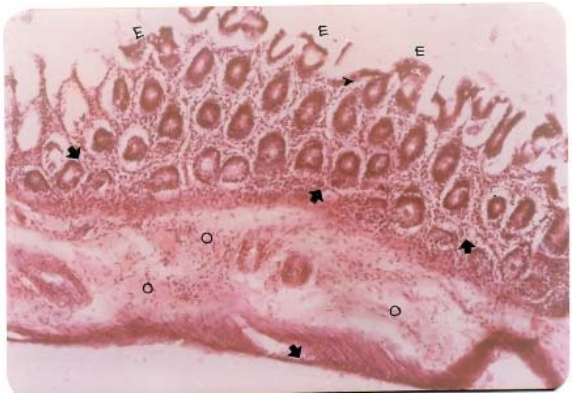

Fig. (6): Intestine of albino rats orally administered the extract of *Citrulluscolocynthis* fruits of Suq-Alkhamis group showing complete desquamation of intestinal villi (E), lymphocytic infiltration in *lamina propria* (arrows) and edema in submucosal layer (O). H&E X 250.

4.Discussion

Toxicosis due to plants is common in man, livestock and pets. Poisoning due to a variety of poisonous plants is a significant part of veterinary toxicology [38]. *C. colocynthis*, a native plant in Africa and the Middle East, has been known for its acute toxicity for a long time.

C. colocynthis is considered as a mixed blessing plant agent as the plant was reported to have some medical effects as well as it was reported to have toxic and even lethal effects as mentioned before in the introduction section. This wide range of pharmacodynamics and toxicodynamics seems to be mainly dependent on the dosage of the plant extract, the part of the plant administered, the locality of the plant and the species of the animal under investigation.

The present work was conducted to study the subchronic toxicological effects of three extracts prepared from *C. colocynthis* plant developing in three different localities in Libya in albino rats. Oral LD_{50} of the *C. colocynthis* fruit extract in albino rats were calculated as 100, 101.7 and 162.4 mg/Kg.b.wt. of the active ingredients of the plants collected from Tarhona, Alzawia and Suq-Alkhamis, respectively. These calculations seem different from those of Barri and others [17] and Elawad and others [18] who reported that the lethal doses of *C. colocynthis*were 5 and 10 g./Kg.b.wt., respectively, for goats, sheep and calves; this difference could be attributed to the species variation and vast anatomical variation in the gastrointestinal tracts of rats from one hand, and sheep, goats and calves from the other hand.

All rats treated with LD_{50} of crude minced fruits developed symptoms of toxicity shortly after administration including loss of appetite, decreased body weight, diarrhea with yellowish green feces, colic and tremors, followed by general depression. The post-mortem lesions revealed pinkish brown colored liver, dark colored kidneys and congested spleen and lungs. These findings correlate well with those described by Kingsbury [16], Barri and others [17] and Elawad and others [18] who found that the symptoms of *C.colocynthis* toxicity were diarrhea, anorexia, dyspnea, excitability followed by convulsions and death. On the other hand, Scott and others [39] didn't find any toxicological effect on rats fed with the oil extracted from *C. colocynthis* seeds. 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, Ickert [22] also hasn't found any effect on Wister rats injected subcutaneously with *C. colocynthis* extract up to four weeks. However, this disagreement with our results 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 symptoms and patho-anatomical features observed in our results may be attributed to the local effect of *C. colocynthis* on gastrointestinal tract, in addition to its effect on the central nervous and respiratory systems after absorption.

Regarding the effect of *C. colocynthis* on the blood picture, there were significant increases in RBCs count, haemoglobin concentration and packed cell volume which are likely due to hemoconcentration condition resulted from the dehydrating effect of *C. colocynthis*. There was also a significant increase in the total number of WBCs and differential leucocytic count. Such results are consistent with those reported by Elawad and others [18]. However, the increase in the number of neutrophils and lymphocytes could be due to the toxicodynamic effect of *C. colocynthis* on different tissues. Such results were also proved in our histopathological examination. The slight increase in the monocyte percentage observed in this study may be a defensive mechanism of the body towards the toxically active ingredients of the *C. colocynthis*. The results are not in accordance with Atole and others [40] who reported that the aqueous extract of *C. colocynthis* at doses of 50 and 100 mg/kg.b.wt of rats were safe and haven't cause any significant effects on haemoglobin concentration and packed cell volume.

Levels of serum transaminases (SGPT and SGOT) of the intoxicated rats showed significant increases compared to the control rats. The change in serum transaminases' levels as determined by their biochemical activity occur essentially as a result of some processes involving the body tissues [41]. Consequently, the higher concentration of such serum enzymes may be used diagnostically in order to assess the level of destruction of the body organs. Our results are in agreement with those of Barri and others [17]. This increase in the levels of serum transaminases is quite indication for the impairment of hepatic function which was associated with the lesions observed in the liver cells in the experimented rats. Although these results are in accordance with those recorded by Elawad and others [18] in sheep, yet they are inconsistent with Atole and others [40] who stated that *C. colcynthis* extract was without effect on serum transaminases of rats at doses of 50 and 100 mg/kg.b.wt. Also, our data are inconsistent with what was reported by Al-Ghaithi and others [42] that *C. colocynthis* extract didn't alter the level of transaminases in normal rats but significantly decreased their elevated levels in diabetic rats. The data also is partially inconsistent with Mukerjee and others [43] who reported that both aqueous and alcoholic extracts of *C. colocynthis* roots have

hepatoprotective effects against CCL_4-induced hepatotoxicity in albino rats. This hepatoprotective effect was at the dose level of 100 mg/kg.b.wt orally and was in the form of decreasing the levels of elevated serum transaminases in CCL_4-injected rats. This difference may come from using a different part of the plant that is the root in a hepatotoxic model that is CCL_4.

Regarding the effect of *C. colocynthis* intoxication on serum levels of urea and creatinine, the significant increase in their levels is an indicator for the impairment in the renal function due to *C. colocynthis* toxicity in our experiment. These results are associated with coagulative necrosis and hydropic degeneration in the renal epithelium. *C. colocynthis* intoxication revealed insignificant difference in Na^+ and K^+ values compared to those recorded from the control rats. Renal biochemical data are not in accordance with Atole and others [40] who didn't find any significant differences between control rats and those treated with 50 and 100 mg/kg.b.wt of *C. colocynthis* extract. Also the data is not in accordance with Al-Ghaithiand others [42] who didn't record significant differences in creatinine and blood urea nitrogen between normal and *C. colocynthis* extract-treated rats.

The histopathological findings in the liver, kidneys, lungs, intestine, spleen and stomach in this study were corresponding to those obtained by Barri and others [17], Elawadand others [18] and Golfain and others [44]. Moreover, the coagulative necrosis and necrobiotic changes, which were seen in the liver and kidneys, could be attributed to toxopathological dynamic action of *C. colocynthis* extract on the parenchymatous cells in different concentrations. Although the leucokytic infiltration and aggregation, particularly lymphocytes and eosinophils, in all examined organs declares the direct relationship of these cells with *C. colocynthis*. The detected lesions of the stomach and intestine point out the direct local irritant action of *C. colocynthis* extract on their mucosae. The present data are in partial accordance with Dehghani and Panjehshahin [21] who reported that the alcoholic extract of *C. colocynthis* didn't affect rat liver at lower doses (50 and 100 mg/kg.b.wt), however, it caused mild to moderate necrotic reaction at higher doses (200 and 400 mg/kg.b.wt); this necrotic reaction was in the form of karyorrehexis and chromatolysis and granulation of the cytoplasm after staining with H&E.

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3/4/2013