HISTOLOGICAL STUDY OF THE EFFECT OF AQUEOUS EXTRACTION OF THE CASTOR SEEDS ON THE INTERNAL ORGANS IN MALE WHITE MICE

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ABSTRACT

Water extract of castor bean was prepared and injected in mice in order to study the effect of acute phase of toxicity (in different doses) on living tissue, for these reasons, 24 male white mice were divided equally into 4 groups. The 1st, 2nd, and 3rd groups were injected with the aqueous extraction of the castor seeds subcutaneously with 20, 30 and 60 mg/Kg of body weight, respectively, while the 4th group used as control group.

After 24 hrs. the internal organs showed degenerative changes and proteintious material in the spleen and kidney, also these organs in addition to liver and stomach showed inflammatory reaction in their parenchyma. The lesions in the examined internal organs were mild in a dose of 20 mg/Kg B.W. and more sever lesions seen in a dose of 60 mg/kg of body weight.

INTRODUCTION

Castor plant (*Ricinus communis*) from which castor beans and oil are subsequently derived grows naturally over a wide of geographical regions and may be activating under a variety of physical and climatic regimes. The plant is however essentially a tropical species, although it may grow in temperate regions (1). Castor beans contains about 30-35% oil (2) which can be extracted by variety of processes or combination of processes (3).

The toxicity of the castor bean was first discovered in 1889 by Stillmark who also stated that the castor bean contain a toxic protein which he named ricin (4)

Ricin intoxication is a fatal clinical condition in human (5). Animals showed variable responses to such toxic substances, for example, chickens and frogs are the least sensitive animals whereas horses are the most sensitive (6).

The ricin content of castor beans varies between 1% and 5%. The degree of toxicity depends on the dose and the route of exposure (7). Ricin intoxication can occur via ingestion, inhalation, or injection. Person-to-person transmission does not occur (8).

The present study was designed to examine the toxic effect aqueous extraction of the castor seeds on mice internal tissue.

MATERIALS AND METHODS

Castor plant seeds

Castro bean seeds were collected from Al-Mahmodia Baghdad, the water extract of the castor bean was prepared as in Mohamed and Mohamed (2006) (4) with few modifications: The seeds were washed and dried in room 25±3 ˚C for 48 hours, then the seeds were grounded for 10 minutes and the powder was kept in a clean, dry and tightly closed jar till used. Twenty gram of seed powder were then mixed with 100 ml of distilled water and stirred, then transferred into 250 ml conical flask, tightly closed and placed in Rortry shaker for 3 hours and left for 24 hours at room temperature, filtered and the filtrated solution was placed in a rotary evaporator for evaporation at 40 ˚C.

The extracted phase transferred into a separation funnel to which 10 ml of ether was added. The water phase was collected in a small conical flask (25 ml), tightly closed and stored at -20 ˚C.

Experimental animals

Twenty four (24) Swiss albino male mice, body weight (27.2±3), obtained from the Center of Biotechnology department / Al-Nahrin University. The mice kept in the animal house of the College of Veterinary Medicine / University of Baghdad at 25±3 ˚C and feed on pellet and water adlibtim. The mice were left for 3 weeks for adaptation.

Experimental design

The mice (n=24) were divided randomly into 4 equal groups (n=6), the first 3 groups were injected subcutaneous (S/C) with the water extract of the castor bean in a dose of (20, 30 and 60 mg/kg), respectively. The 4^{th} group (n=6) considered as control group which injected S/C with 0.1 ml of phosphate buffer saline (PBS).

All animals were sacrificed after 24 hours post treatment and samples from spleen, liver, kidney and stomach were collected and fixed in 10% buffer formaldehyde solution for 72 hr, and then used the routine tissue section preparation for histopathology examination. All sections stained with Hematoxylin and eosin stain (9).

RESULTS

Histopathological examination of the first group (20 mg/kg aqueous extraction of the castor seeds) showed the following changes, in the spleen there is congestion of the blood vessels of the red pulp and deposition of proteintious material especially around blood vessels with mild infiltration of inflammatory cells mainly neutrophils (Fig. 1), while the liver showed normal section and there is no lesion in the liver parenchyma (Fig. 2). The kidney showed proteintious material in the lumen of the dilated renal tubules with degenerative changes in epithelial (Fig. 3), also histopathological section in the stomach after 24 hr post treatment with 20 mg showed normal section

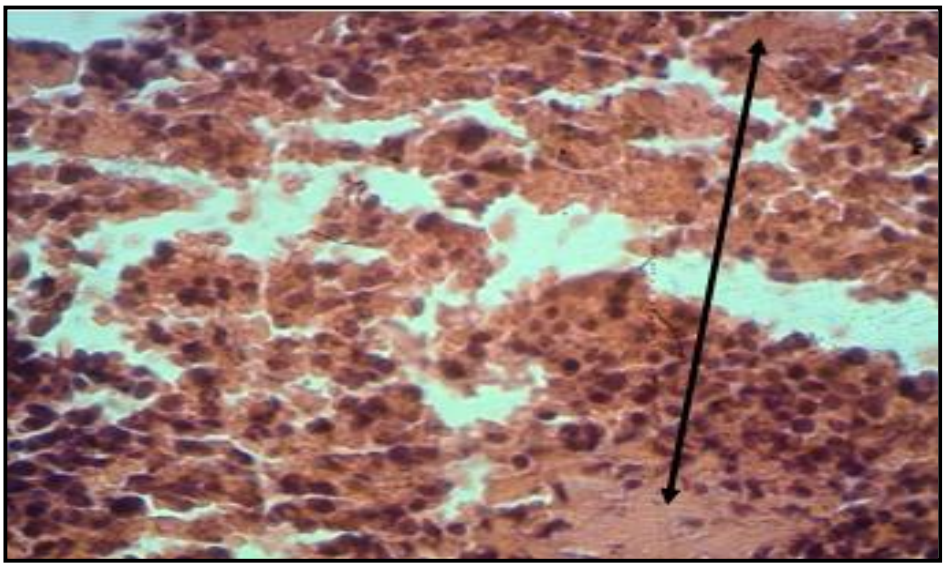

Fig. 1: Histopathological section in the spleen of the 1st group (24 hr post treatment with 20 mg/kg aqueous extraction of the castor seeds), shows congestion of the red pulp with proteintious material (⟷) and infiltration of inflammatory cells mainly neutrophils (H & E; 40X).

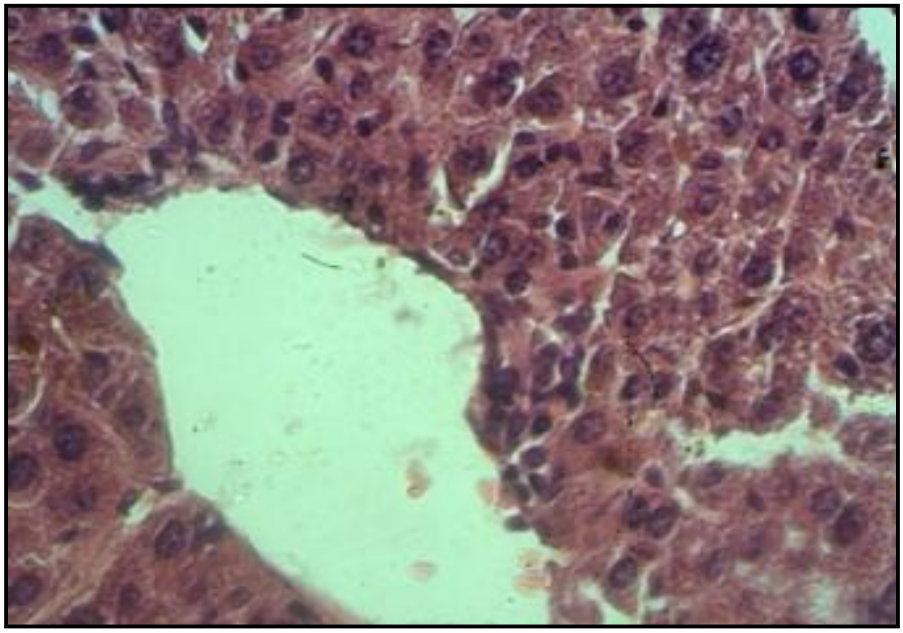

Fig. 2: Histopathological section in the liver of the 1st group, showed normal liver section (H and E; 40X).

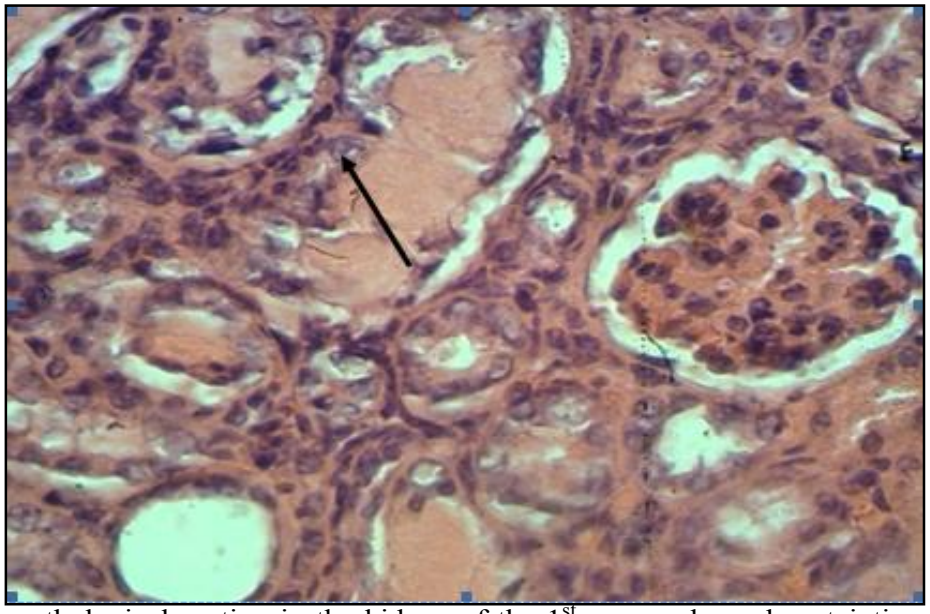

Fig.3: histopathological section in the kidney of the 1st group, showed proteintious material in the lumen of the dilated renal tubules (⟶) with degenerative changes in epithelial (H & E; 40X).

The lesions of the 2nd group (30 mg/kg aqueous extraction of the castor seeds) revealed proliferation of mononuclear cells around congested sinuses form cord like appearance (Fig. 4) in the spleen, while the liver showed infiltration of mononuclear cells in the liver parenchyma and in the dilated sinusoids (Fig. 5). The kidney showed mononuclear cells aggregation in the interstitial tissue and increased in the thickness of glomerular wall due to proliferation of fibrous connective tissue also there is acute cellular degeneration of the epithelial cells which lining the renal tubules (Fig.6).

The stomach showed few mononuclear cells mainly macrophages in the subepithelial region (Fig.7)

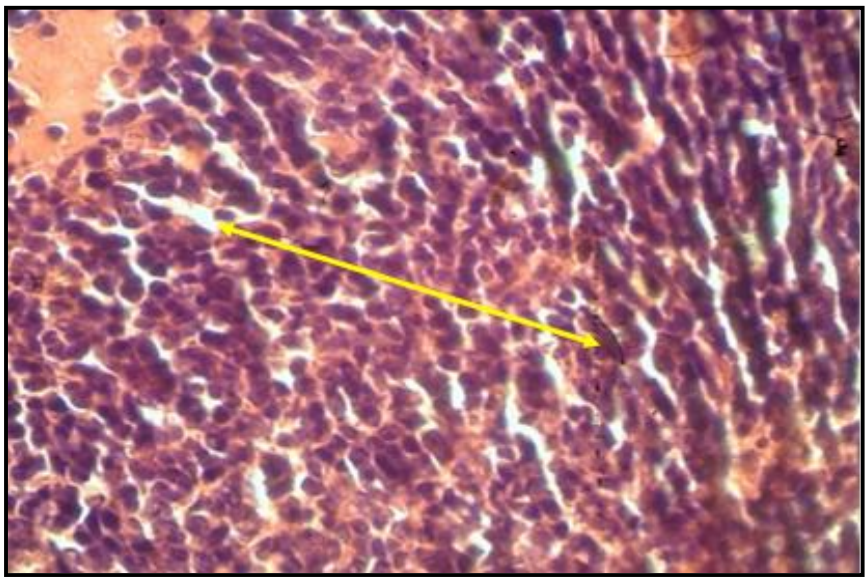

Fig.4: Histopathological section in the spleen of the 2nd group (24 hr post treatment with 30 mg/kg aqueous extraction of the castor seeds), shows proliferation of mononuclear cells around congested sinuses form cord like appearance (⟷) (H & E stain 40X).

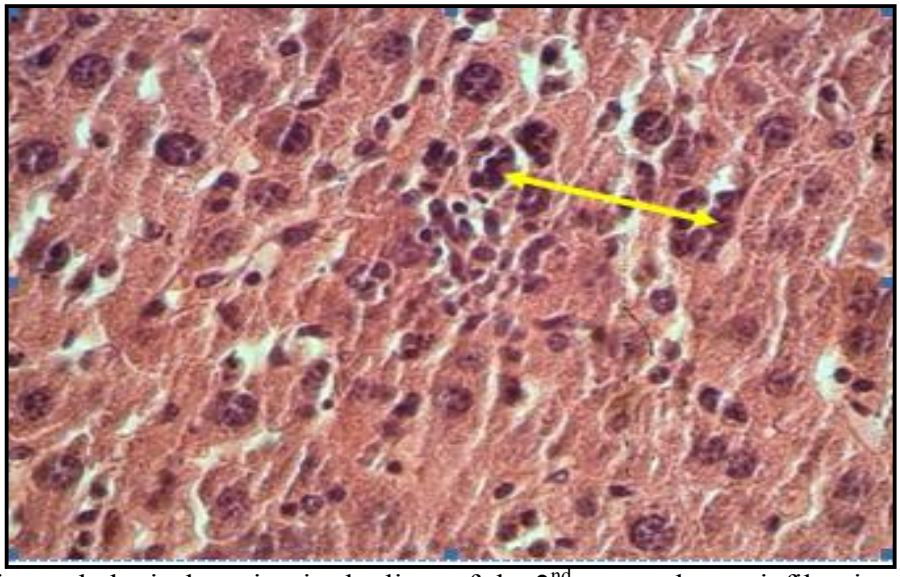

Fig.5: Histopathological section in the liver of the 2nd group shows, infiltration of mononuclear cells in the liver parenchyma and in dilated sinusoids (⟷) (H & E stain 40X).

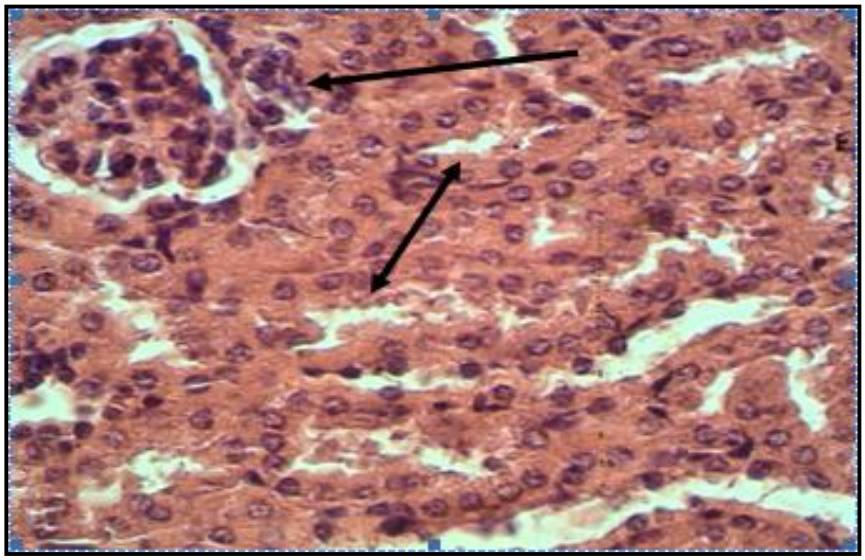

Fig 6: histopathological section in the kidney of the 2nd group, showed mononuclear cells aggregation in the wall of the glomeruli (⟶) and increased in the thickness of glomerular wall due to proliferation of fibrous connective tissue. Acute cellular degeneration of the epithelial cells which lining the renal tubules (⟷) (H & E; 40X).

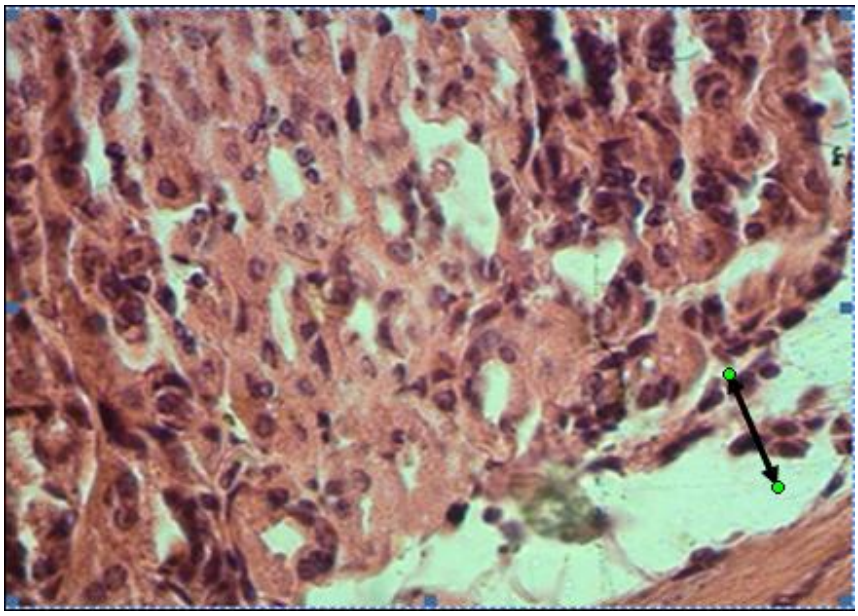

Fig.7: Histopathological section in the stomach of the 2nd group, showed few mononuclear cells mainly macrophages in the subepithelial region (⟷) (H & E stain 40X).

The histopathological section in the internal organs of the 3rd group (treated with 60 mg/kg aqueous extraction of the castor seeds) showed the following lesions, the spleen showed proteintious material deposition in the red pulp with atrophic white pulp (Fig. 8), while the histopathological section in the liver showed aggregation of mononuclear cells and neutrophils in the liver parenchyma and proliferation of kupffer cells (Fig.9).

The kidney showed acute cellular degeneration of the epithelial of renal tubules and hypercellularity of the glumerular tuft which lead to narrowing of Bowman's space (Fig. 10) while other sections showed infiltration of mononuclear cells.
histopathological section in the stomach showed edema, fibrin network and inflammatory cells mainly neutrophils in the submucosa of the stomach (Fig. 11).

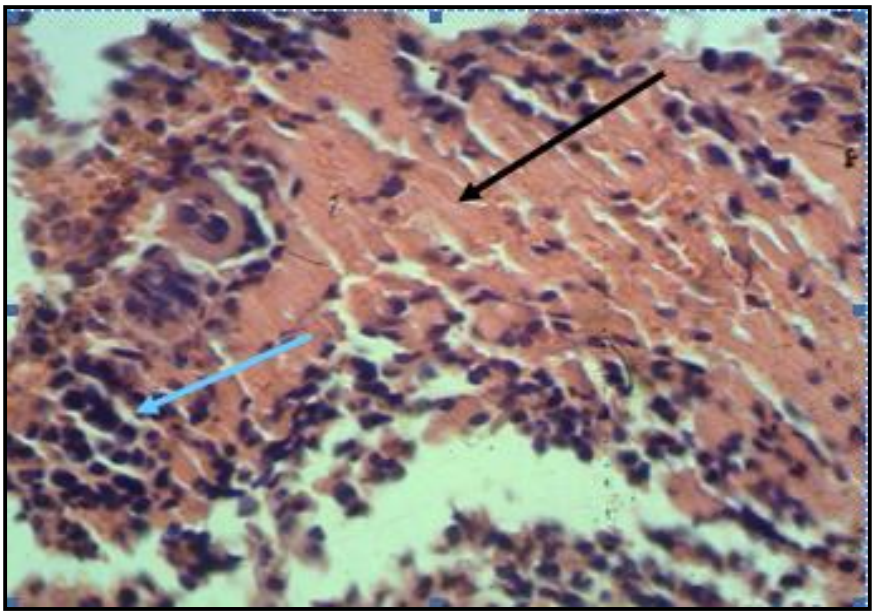

Fig. 8: histopathological section in the spleen of the 3rd group, showed proteintious material deposition in the red pulp (➞) with atrophic white pulp (⟶) (H & E; 40X).

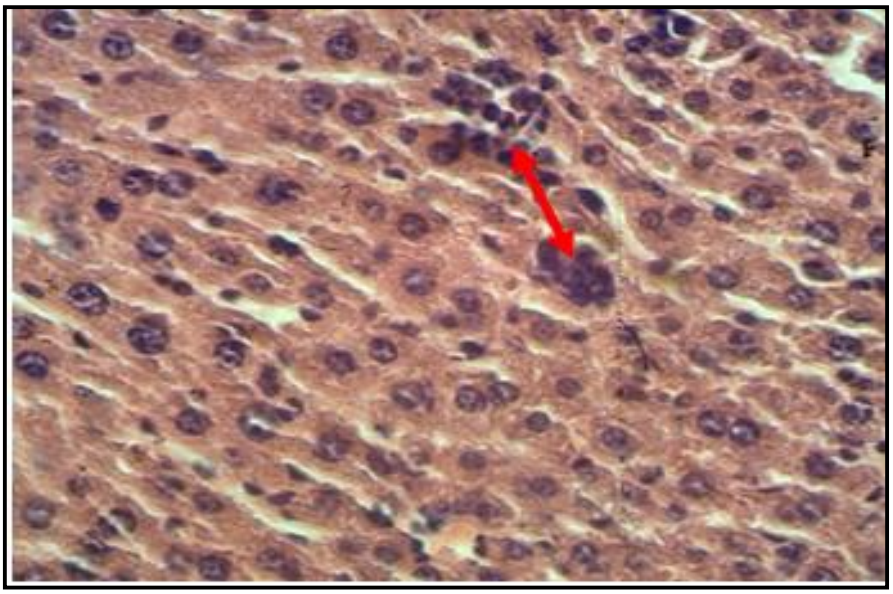

Fig. 9: Histopathological section in the liver of the 3rd group, showed aggregation of mononuclear cells and neutrophils in the liver parenchyma (⟷), and proliferation of kupffer cells (H & E; 40X).

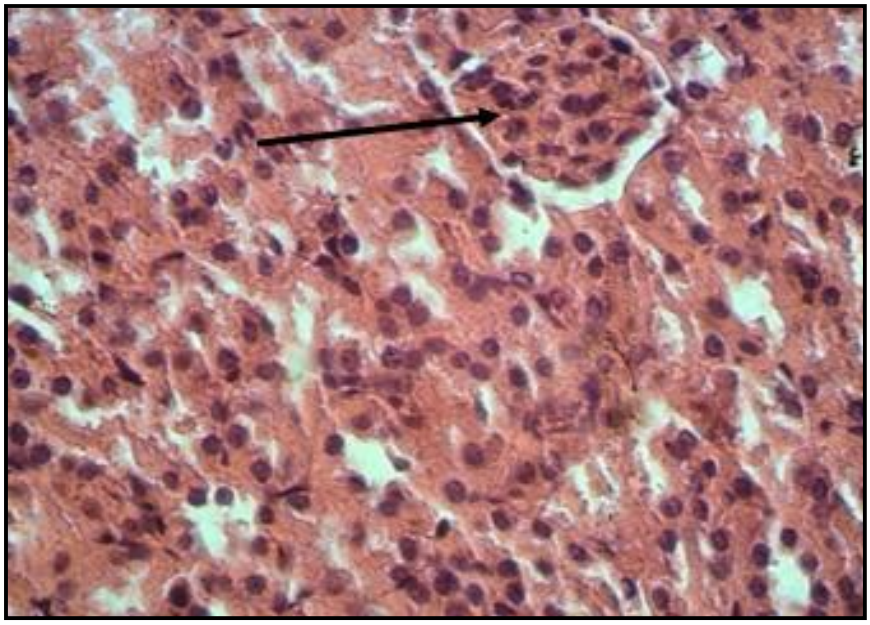

Fig. 10: Histopathological section in the kidney of the 3rd group, showed hypercellularity of the glumerular tuft which lead to narrowing of Bowman's space (⟶) (H & E; 40X).

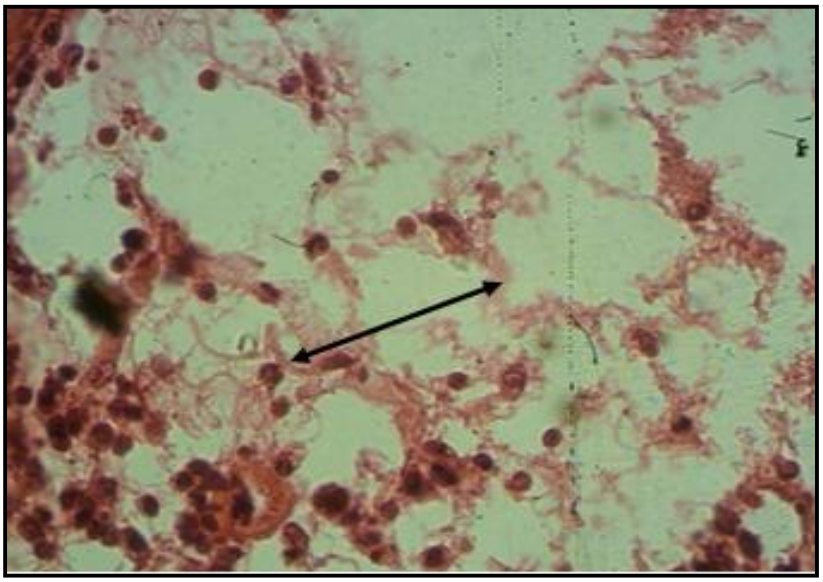

Fig. 11: histopathological section in the stomach of the 3rd group, showed edema, fibrin network and inflammatory cells mainly neutrophils (⟷) in the subemucosa of the stomach (H & E; 40X).

DISCUSSION

The present finding revealed different pathological lesions (after 24 hrs) in the internal organs, the severity of the lesions increased in a ejective manner with the dose. This indicate the potent toxicity of *Ricinus communis* seeds water extract, since the reported LD50 of the seeds water extract in female Swiss mice was 0.153 mg/Kg body weight and even lower LD50 (0.0702 mg/Kg) of the seeds extract in male mice (10).

The presence of proteinaceous material in the spleen and in the kidney tubules, as well as cellular infiltration mostly composed of neutrophils into these tissue were all obvious and according to (11) this is indicative of damage caused by ricin as they explain the acute lesion of ricin after 24 hrs of intranasal ricin challenge.

The deposition of proteintious material in the spleen and kidney may be explained by that, mammalian cells in particular posses numerous glycoproteins and glyolipids with galactose residues and the toxic action of ricin was stated by (12) in which the B-subunit of ricin molecule bind to galactose containing proteins of cell surface aiding the entrance of the toxin A-subunit.

The degenerative changes in the kidney revealed pathological stress on the renal tubules, (13) reported that inhalated ricin will elicit necrosis of liver, spleen and kidney, while they injected (as in this experiment) ricin caused gastro intestinal signs and gastro intestinal hemorrhage and this may explain the lesion in the stomach in this study specially in a dose of 30 and 60 mg/Kg b.w..

Other researchers (14, 15) have postulated that ricin has detectable hepatotic effects, where it causes severe liver cell damage and early degenerative signs of mitochondria, while the current results indicate an inflammatory reaction without necrosis.

Also the (16) have postulated that ricin decreases calcium uptake by the sarcoplasmic reticulum and increases sodium-calcium exchange. This deregulation of calcium homeostasis causes myocardial necrosis and cardiac hemorrhage. The above results supported the current results of degeneration.

دراسة نسيجية لمعرفة تاثير المستخلص المائي لبذور الخروع على الاعضاء الداخلية للفئران البيضاء

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الخلاصة

تم تحضير مستخلص مائي لبذور الخروع وحقنه في الفئران وذلك لغرض دراسة تاثير السمية الحاد بتراكيز مختلفة وتاثيرها على الانسجة الحية، لهذا السبب استخدم 24 من ذكور الفئران البيضاء قسمت بالتساوي الى 4 مجاميع. حقنت المجاميع الثلاثة الاولى بالمستخلص المائي لبذور الخروع تحت الجلد وبتراكيز 20، 30 و60 ملغم\كغم من وزن الجسم على التوالي، بينما استخدمت المجموعة الرابعة كمجموعة سيطرة سالبة.

بعد 24 ساعة من الحقن اظهرت الاعضاء الداخلية تغيرات تنكسية وترسب مواد بروتينية في الطحال والكلى، كذلك اظهرت هذه الاعضاء بالاضافة للكبد والمعدة تفاعلات التهابية في متنها. ان الافات المرضية في الاعضاء الداخلية المفحوصة كانت معتدلة عند استخدام جرعة 20 ملغم\كغم من وزن الجسم وازدادت شدت الافات بازدياد الجرعة اذ كانت اكثر شدة بجرعة 60 ملغم\كغم من وزن الجسم.

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