

## Histopathological changes on Splenomegaly induced in Experimental rats *Wistar albino*

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### الخلاصة

تهدف الدراسة الحالية الى تقييم التغيرات النسيجية المرضية في الجرذان المختبرية ذات تضخم الطحال، وتليف الكبد المستحث عن طريق تجريع مركب الثياواستاميد (Thioacetamide (TAA). لوحظ التليف الكبدي في جميع الجرذان المخبرية بعد الحقن تحت البريتون بجرعة مقدارها (200ملغم/كغم) من وزن الجسم ولمدة (8) اسابيع متتالية وجميع النتائج قورنت مع جرذان السيطرة التي حقنت بالمحلول المنظم. تم تسجيل وزن الجسم والطحال والكبد في جرذان السيطرة والمجموعة المعاملة وكذلك حسب النسبة لكل من الكبد والطحال الى وزن الجسم. تضمنت الدراسة التغيرات العيانية والتي تشمل اللون والحجم والاحتقان وعلامات التليف وعدم الانتظام وطبيعة السطح لكل من الكبد والطحال. اظهرت الاكباد في الجرذان المعاملة سطح محبب وخشن مع مناطق من التليف بشكل بقع بيضاء ولون شاحب واحتقان مع زيادة في الحجم كذلك كان الطحال متضخم، احمر غامق مع مناطق تليف وتخصر ويبدو هش سهل التحطم والنزف.

اشارت النتائج البايوكيميائية الى نقصان وبفارق معنوي ( $P < 0.05$ ) في نسب الالبومين وتركيز البروتين الكلي في الجرذان ذات الطحال المتضخم مقارنة مع جرذان السيطرة وكذلك تثبيط الفعالية الانزيمية لانزيمي الكبد (GOT والـGPT) في مجموعة التليف الكبدي المستحث والطحال المتضخم مقارنة مع السيطرة إذ اظهرت النتائج زيادة معنوية في تركيز هذين الانزيمين في مصل الدم للجرذان ذات التليف. كما تم تقدير تركيز البيلروبين الكلي في مصل الدم للجرذان ذات التليف الكبدي المستحث و اشارت النتائج الى فرق معنوي احصائي عالي مقارنة مع تركيز البيلروبين في جرذان السيطرة. اظهرت نتائج الفحص المجهرى لمقاطع في اكباد الجرذان ذات التليف الكبدي والطحال المتضخم الى اتساع واحتقان الاوردة البوابية مع زيادة في قطر الوريد البوابي وبفارق معنوي احصائي مقارنة مع السيطرة وكذلك هناك زيادة في اقطار العقيدات اللمفية للطحال المتضخم عند مستوى  $P < 0.05$  مقارنة مع معدل اقطارها في جرذان السيطرة. اكدت الدراسة الحالية على تاثير المركب (TAA) في حث التغيرات النسيجية المرضية في اكباد وطحال المجموعة المعاملة وقورنت مع مجموعة السيطرة و اشارت نتائج الفحص المجهرى الضوئي للمقاطع النسيجية في الكبد والتي صبغت باستخدام كاشف (PAS) الى ترسيب حبيبات الكلايكوجين بشكل حبيبات خشنة غامقة لونها احمر تترسب في سايتوبلازم الخلايا الكبدية، كذلك لوحظ التليف، فرط التنسج، تليف الحواجز الكبدية، ترسيب كميات كبيرة من الالياف الغراوية مع تحطم التركيب الطبيعي للفصيحات الكبدية. اشارت النتائج الى خلايا كبدية منتفخة تشبه البالون وتنكس الانوية مع وجود فجوات تتجمع فيها الدهون مما تعطي اشارة الى التنكس الدهني في معظم المقاطع النسيجية للاكباد ذات التليف، فضلاً عن فرط التنسج واتساع الاقنية الصفراوية مع ارتشاح خلايا التهابية ومناطق من التليف ضمن النسيج الحشوي للكبد. احتقان اللب الاحمر مع بعض العقيدات اللمفية الفعالة وبعض منها اكثر من عقيدة لمفية تتجمع مع بعضها. زيادة في سمك الحواجز مع وجود عدد كبير من الخلايا الملتزمة التي يترسب فيها صبغة الـHemosidrin. لوحظ النزف مع اعداد من كريات الدم الحمر والصفائح الدموية التي تترسب في الاوعية الدموية الكبيرة، وهناك زيادة في سمك المحفظة مع نسيج متليف في مناطق مختلفة من النسيج الحشوي مقارنة مع المقاطع في طحال جرذان السيطرة.

### Abstract

This study was aimed to evaluate histopathological changes in experimental rats with induced splenomegaly and liver fibrosis by administering thioacetamid (TAA).

Liver fibrosis was clearly noticed in all experimental rats after intraperitoneally injected with a dose (200mg/kg) body weight for 8 weeks ,while control rats injected with buffered saline. The body , spleen and liver weight were recorded , also ratio of liver and spleen per body weight was estimated. Macroscopical changes including (colour, size, congested, signs of fibrosis, irregularity, nature of surface) regarded to liver and spleen were clarified.

The livers showed coarse granulation surface, fibrous (white) spots, pale color and congested with large size, while spleen appeared enlarged, reddish, with fibrous regions, twister and fragile easy to destruct. Biochemical results revealed to statistical difference ( $p < 0.05$ ) in the rate of albumin and total protein concentration in splenomegaly rats compared to control. Inhibition of both (Gpt and Got) enzymes was estimated in rats with splenomegaly up compared to control rats. There was statistical difference in the mean concentration of total bilirubin in splenomegaly rats in comparison to control rats.

The results of microscopic investigation of tissue sections related to rats liver with enlarged spleen showed dilation in portal veins with an increased in (PV) diameter in relation to control rats, also significant increased with splenic lymph nodules diameter in splenomegaly rats compared to control rats. Results on liver sections stained with (PAS) reagent indicated to dense, coarse and red glycogen granules were deposit on hepatocytes also fibrosis, hyperplasia, fibrous septa, large amount of collagen fibers and destruction of normal lobules were observed. The results referred to swollen hepatocytes, pyknotic nuclei, vacuoles with lipid accumulation and moderate steatosis was obvious in all sections of livers with cirrhosis. Hyperplasia, dilated bile ducts and infiltration of inflammatory cells. Spleen sections of splenomegaly rats showed congested red pulp, some lymph nodules appeared active and more than one aggregated together, thickening in fibrous trabeculae and numerous macrophages with hemosiderin deposits. Hemorrhage with large numbers of erythrocytes and platelets deposit in large blood vessels, thickening in capsule with more fibrous tissue compared with control rats.

## Introduction

The spleen is an organ has a couple of important function involving blood cells within the body, it filters blood and removes old and damaged red blood cells, bacteria and other particles as they pass through blood vessels within the spleen, also it produces assists immune system and is considered the draining site for compounds that are administered intravenously (1,2). Weight of spleen is highly variable, in human adults, the spleen generally weights (150-250g) and the elderly spleen is often smaller, even when there is no apparent hypofunction (3). Splenomegaly is often detected in patients with liver cirrhosis and portal hypertension with a prevalence of 60-65%, some cases can be very prominent and cause symptoms like abdominal pain, splenic infarction and cytopenia (4,5). (6) explained why portal hypertension produce congestive splenomegaly and regarded it to the blood reaches the spleen via the splenic artery, a large branch of the celiac artery,

and enters the spleen through four to six branches while venous outflow via four to six venous branches, called the splenic vein which drain into the portal vein. Influence of portal hypertension on the weight of spleen and splenic tissues has been studied and histological changes in rats were similar to the histological findings of splenomegaly accompanying portal hypertension in humans (7).

Splenic enlargement had been found to correlated with increase in the white blood cells but not with that of circulating (CD<sub>34</sub>) positive cells (8). Study on effect of serious environmental pollutant which toxic effects are associated with oxidative stress showed histological changes on spleen and the spleen white pulp was decreased significantly in treated group compared with control (9). Splenomegaly in liver cirrhosis was not only congestive but there was also significant increase of reticular fibers, red pulp area and angiogenesis, moreover, nitric oxide (No)

reduction resulting from suppression of e-NOS and TNF- $\alpha$  contributed to the increased volume of spleen (10).(11) referred that chronic hepatitis is a well recognized problem in canine practice and affect dogs of many breeds, however knowledge of its etiology, remains limited .The pathways to splenomegaly was determined in rats with fully developed portal hypertension and the pathogenic mechanisms was

characterized (12,13). (14) suggested that splenectomy may be of a preventive role against CCl<sub>4</sub> induced rat liver fibrosis to a certain degree .Since splenomegaly a common cause of liver cirrhosis, the aim of this study was to evaluate histopathological changes occur with splenomegaly in relation to acute liver damaged and hyperplasia induced in an experimental model.

## Materials and Methods

### Experimental animals and mode of treatment

Total number of wister rats (*Rattus norvegicus*) (30) rats aged 12 weeks weighing (200-250)g were used in this experiments. Animals were housed in the metal cages under controlled temperature (25 $\pm$ 2C $^{\circ}$ ) and constant dark-light cycle (12hr light, 12hr dark). The rats received the same food and water *ad libitum* through the study ,animals divided into two groups, (10)rats as control received only buffered saline (2ml) intraperitoneally. While (20) rats as experimental group treated with (200mg/kg) of body weight intraperitoneally of thioacetamid (TAA) to iniduced liver fibrosis and splenomegaly, the dose gives three times a week for (8 weeks). (TAA) powder dissolved in 0.9% normal saline according to (7,15).After 8 weeks, all the animals were fasted overnight, anaesthetized, body weight was recorded and then the rats were sacrificed, the abdomen cut to excised both spleen and liver, both organs weighing to estimated ratio of wet liver and spleen per animal body weight (16,10).Both liver and spleen from each rat fixed with formalin (10%) and some pieces of liver fixed with Bouin's fixative for glycogen fibrillar matrix deposition. Macroscopical observations of organs included (colour, appearance, shape, nature of liver and spleen surface, regularity, congestion) were recorded. The blood was

immediately collected with sterile tubes, let for 10 minutes to clot, then serum was separated by centrifuging at 2500rpm/min for 15min., stored at (-25C $^{\circ}$ ) for biochemical estimations. All the results expressed as (mean  $\pm$  SD).

### Biochemical analysis

Liver parameters like (GPT and GOT) enzymes were estimated in serum, the enzymes activity was determined by kit from Bio-labo-SA company (France) according to(17),total protein was determined by (17) respectively. Albumin concentration was estimated according to(18) and the result compared with standard curve accompanying the kit. Total bilirubin was determined by a method of (19).

### Tissue processing for light microscopic examination

Samples from spleens and livers related to control and experimental animals were cut to suitable pieces and fixed with formalin (10%) and some pieces of livers fixed with Bouin's fixative for (12hr) to clarified changes with glycogen .Specimens prepared by paraffin-wax method according to(20) . After fixation specimens fixed with formalin washed with distilled water while these fixed with Bouin's fixative washed with 70% alcohol, dehydrated with series of alcohol, cleared with xyelen, infiltrated for (3hr) with mixed of xyelen and paraffin, embedded with paraffine then sectioned to (5 $\mu$ ) thickness

sections ,stained with haematoxylin and eosin .

### Microscopic examination

All sections were examined and the histopathological changes were evaluated according to fibrosis area, portal vein dilation ,dense of inflammatory cells, glycogen deposition and cellularity of red pulp. Diameter of lymph nodules was determined in all sections of spleen

## Results

### Macroscopic observations

Results showed that liver has a coarse granulation surface with white fibrous regions, large congested area and large size also irregular margins was noticed, pale color compared with control liver which appeared normal size and red without any abnormal features, (Fig 1,2).Macroscopic observations on spleen treated with (TAA) to induced splenomegaly showed larger size than control with fibrous (white) regions on surface, twister sometimes with irregularity spots, the spleen looked fragile, dark reddish compared with control which it is relatively solid organ (Fig. 3a, b, fig.4).

The body weight of splenomegaly lower at ( $P<0.05$ ) than the control rats, the body weight mean ( $215 \pm 5g$ ) ( $235 \pm 15g$ ) respectively , weight of liver and spleen recorded after 8 weeks of treatment and both organs has weight higher than control, mean weight of spleen ( $3.01 \pm 0.2g$ ) and liver ( $14.5 \pm 0.16g$ ) compared with control which mean weight of liver and spleen ( $10.5 \pm 0.2g$ ) ( $1.88 \pm 0.15g$ ) respectively. (Table-1).We also determined the ratio of wet liver and spleen to the body weight and the results showed higher ratio on splenomegaly rats than of control (Table-1).

related to control and treated group .

Portal vein diameter was calculated and regarded abnormal if the mean diameter more than 2.1mm (21).

### Statistical analysis

All values were expressed as mean  $\pm$  standard deviation (SD). Means were compared in all results by analysis of variance (ANOVA).Significance levels were established at  $P<0.05$ .

### Biochemical analysis

Biochemical analysis revealed to significant difference and decreased with albumin and total protein concentrations in splenomegaly rats compared with control rats,mean concentration was ( $1.5 \pm 0.3$ )g/dl ( $0.85 \pm 0.02$ )g/dl at splenomegaly group compared to albumin concentration and protein content which reached to ( $2.1 \pm 0.3$ )g/dl and ( $1.9 \pm 0.03$ )g/dl respectively on control (Table-2).Total bilirubin was recorded and the mean concentration was ( $1.8 \pm 0.3$ )mg/dl of splenomegaly group compared to control ( $0.9 \pm 0.02$ )mg/dl (Table-2).

### Microscopic examination

#### Histomorphometric study

Data showed that portal vein diameter (PVD) was increased on livers sections regarded to an experimental rats,the mean diameter reached to ( $3.1 \pm 0.2$ )mm in comparison to control which reached to ( $1.5 \pm 0.01$ )mm (Table-3).Diameter of lymph nodules was determined in both groups, significant increase recorded spleen lymph nodules at splenomegaly rats ( $10.27 \pm 2.8$ ) $\mu$ m, while mean diameter of lymph nodules on control ( $6.21 \pm 1.4$ ) $\mu$ m (Table-3).

Table-1: Illustrate the body weight, liver weight and spleen weight, also ratio of liver/body weight and spleen/body weight in both control and splenomegaly rats. (the values expressed as mean  $\pm$  SD.)

Groups	Body weight (g)	Spleen weight (g)	Liver weight (g)	Ratio of spleen/body weight (%)	ratio of liver/body weight (%)
	Mean $\pm$ SD.	mean $\pm$ SD.	mean $\pm$ SD.		
Control rats	234 $\pm$ 13	1.88 $\pm$ 0.15	10.5 $\pm$ 0.2	0.8	0.46
Splenomegaly rats	215 $\pm$ 5	3.01 $\pm$ 0.2	14.5 $\pm$ 0.16	0.14	0.67

Table-2: Illustrate the concentration of albumin, total protein, (GPT and GOT) enzymes and total bilirubin in splenomegaly rats compared to control. All the values expressed as (mean  $\pm$  SD.)

Groups	Albumin g/dl	Total protein g/dl	GPT Iu/L	GOT Iu/L	Total bilirubin mg/dl
Control rats	2.1 $\pm$ 0.3	1.9 $\pm$ 0.03	46.2 $\pm$ 2.6	35.4 $\pm$ 1.53	0.9 $\pm$ 0.02
Splenomegaly	1.5 $\pm$ 0.3	0.85 $\pm$ 0.02	68.2 $\pm$ 2.4	49.3 $\pm$ 6.1	1.5 $\pm$ 0.3

Table-3: Illustrate portal vein diameter and lymph nodules diameter in both splenomegaly and control rats. The values expressed as (mean  $\pm$  SD.).

Groups	Portal vein ( $\mu$ m)	Lymph nodules diameter ( $\mu$ m)
Control rats	1.5 $\pm$ 0.01	6.2 $\pm$ 1.4
Splenomegaly	3.1 $\pm$ 0.2	10.7 $\pm$ 2.8

### Histological analysis

Examination of control sections related to livers in control showed paranchyma cells formed lobules around central vein, hepatocytes arranged in hepatic plates, with polyhedral shape, vesicular nuclei sometimes have binucleate. Vascular spaces referred as sinusoids separated between cells which lined with endothelial cells and kupffer cells (fig. 5, 6). Normal distribution of glycogen granules in hepatocytes cytoplasm as fine red dotes without any abnormal features (fig. 7,8).

Experimental rats with hpatomegaly showed changes in liver paranchymal tissue with induced hyperplasia and fibrosis, abnormal structure of liver lobules, fibrosis regions, fibrous septa, bundles of collagen fibers was observed while thin strand of collagen fibers deposit in control rats (fig. 9, 10). Most hepatocytes swollen, ballooned with pyknotic nuclei, vacuoles with lipid accumulation and absence of hepatocytes boundaries so mild to moderate steatosis was noticed, bile ducts hyperplasia, dilated canaliculi with portal vein dilation was obvious in sections of rats

livers treated with (TAA), infiltration of inflammatory cells on sinusoids and hepatic necrosis observed (fig. 11, 12, 13a,b, 14). Sections stained with (PAS) showed coarse granules of glycogen deposit on hepatocytes cytoplasm, and increase in fibrous material was confirmed as fibrille material (fig. 15, 16). Normal structure of spleen in control rats showed that spleen paranchyma tissue consist of white pulp composed of lymph nodules which associated with central arteriole and lymphatic tissue which surrounded each lymph nodule formed the periarterial lymphatic sheath (PALS), nodule consist of germinal centre and marginal zone separate white pulp from red pulp (fig. 17a, b, fig.18). The capsule surrounded the spleen and extend to send trabecullae to divided paranchymal tissue to white and red pulp, red pulp appeared as vascularized tissue composed of splenic cords and number of lymphocytes, macrophages and blood vessels (fig. 19, 20). Observations on spleen sections of splenomegaly rats were showed congested red pulp, irregular lymph

nodules masses, dilation on spleen venous sinuses and fibrous trabeculae (fig. 21a,b, 22a,b). Histological examination identified numerous macrophages with hemosiderin deposits, proliferation or hyperplasia with histiocytes and increase with myofibroblasts (fig. 23). Large number of

erythrocytes, platelets deposits in blood vessels, congested and splenic sinuses and more than one lymph nodes aggregated were also noticed (fig. 24). Hemorrhages noticed as yellow or brown pigments in fibrous paranchymal tissue or in the fibrous trabeculae. (fig. 25).

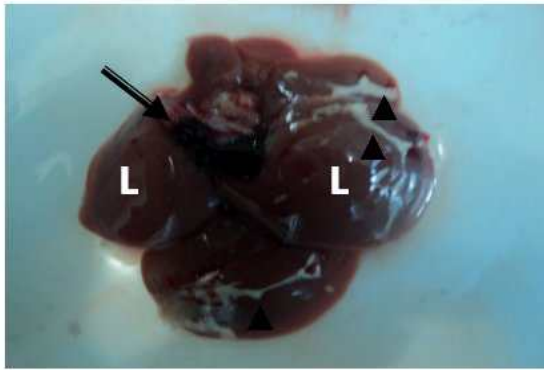


Fig.1: Anatomical picture on liver of hepatomegaly group showed congested area (⇒), fibrosis regions (▶) and irregular lobes (L).

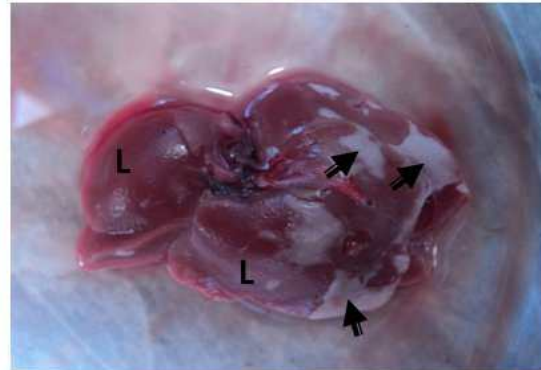


Fig.2: Macroscopic figure illustrated cirrhosis liver with fibrous regions (⇒), pale colour and hypertrophy lobes (L).



Fig.3a: Anatomical picture on spleen related to rats with induced splenomegaly showed coarse surface with extensive fibrosis (⇒) and congested area (▶).

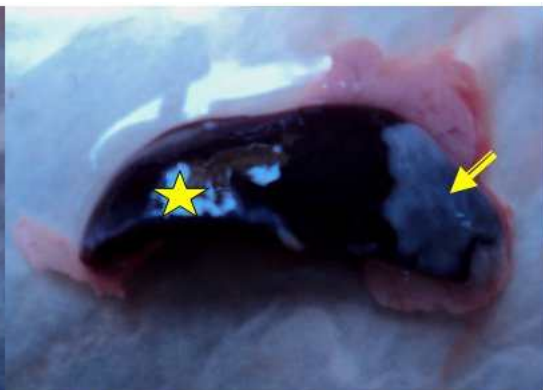


Fig.3b: Figure showed enlarged spleen with wide region of fibrosis (⇒) and erosion of external surface (★).



Fig.4: Picture showed irregular spots of fibrosis (⇒) on spleen and degenerated region (▶).

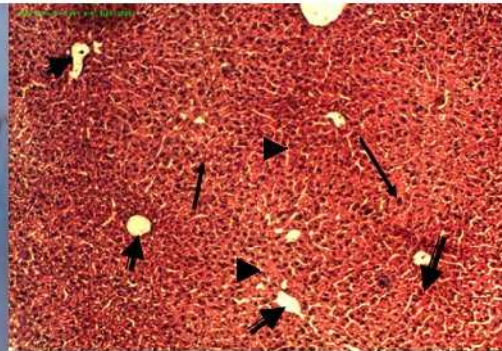


Fig. 5: Section in control liver showed the central vein (⇒) and hepatic plate (▶), hepatocytes with nucleus (→) (H & E). (125x)

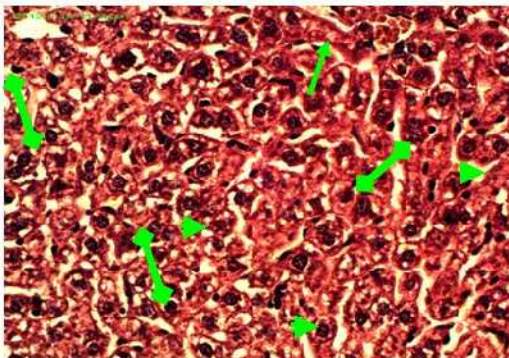


Fig. 6: Section on liver of control rat showed hepatic plates (→), sinusoids (▶), large hepatocyte (⇒) and kupffer cells (◀◀). (H & E). (500x)



Fig. 7: Cross section on control liver showed normal distribution of glycogen in hepatocytes also central vein (CV) and bile duct (↔). (PAS) stain (125x)

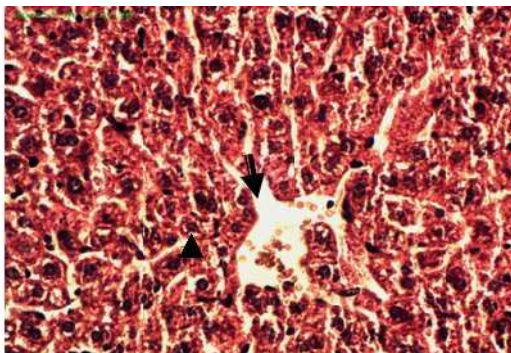


Fig. 8: High power magnification on liver of control rats showed part of central vein (⇒), fine glycogen granules deposit in hepatocytes (▶). (PAS) stain (500x)



Fig. 9: Section on cirrhosis liver showed congested paranchymal tissue (→), fibrosis around bile ducts (⇒), central vein (V). (H & E) stain (125x)

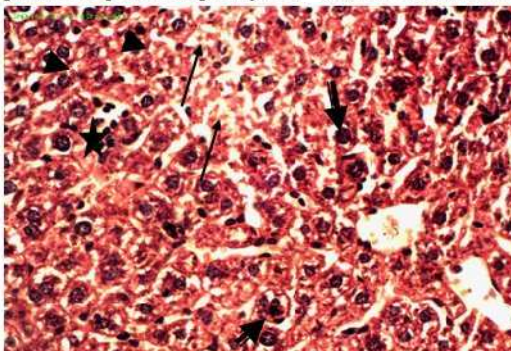


Fig. 10: Cross section on liver of rats with hepatomegaly showed destruction of hepatocytes (→), ballooned hepatocytes (⇒), some cells binucleate (▶), and inflammatory cells (★). (H & E) stain (500x)

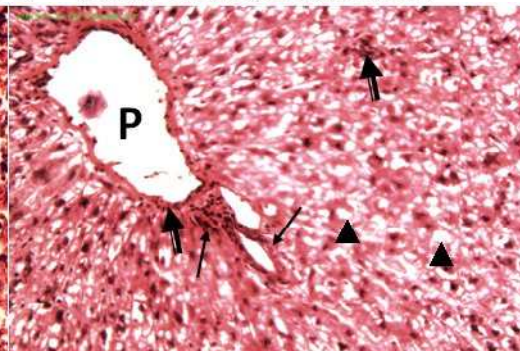


Fig. 11: Section on liver fibrosis showed bile duct hyperplasia (→), dilation of portal vein (P), and increase with inflammatory cells (⇒) and degeneration of hepatocytes (▶) with vacuoles formed. (H & E) stain (125x)

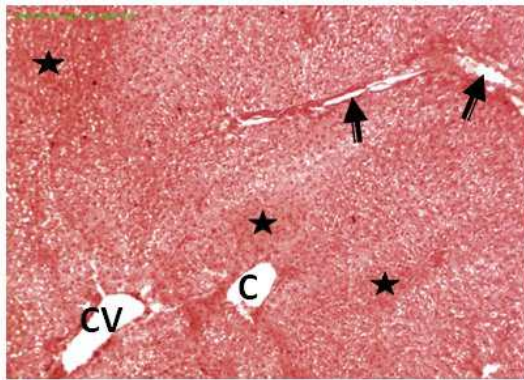


Fig. 12: Section on liver fibrosis showed septa fibrosis (⇒), absence of liver lobule around central vein (CV) and deposition of collagen (★). (H & E) stain (125x).

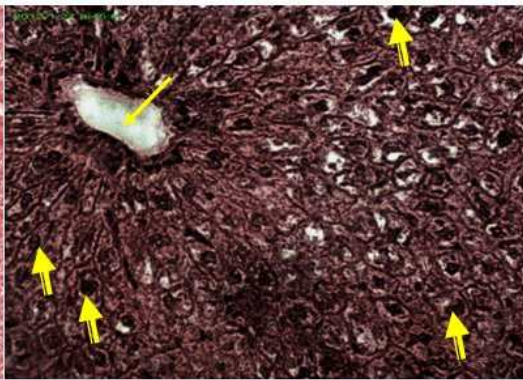


Fig. 13a: Section on liver cirrhosis illustrate increase with central vein wall thickness (⇒), degenerated hepatocytes mild steatosis and pyknotic nuclei (⇒). (H&E). (125x).

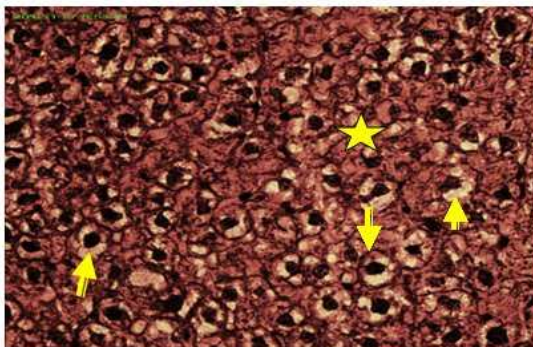


Fig. 13b: Transverse section in liver cirrhosis showed severe steatosis, swollen hepatocytes(⇒) with pyknotic nuclei and vacuoles formed (★). (H&E) (500x)

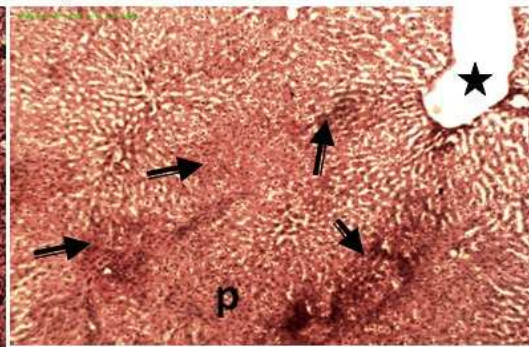


Fig. 14: Section in liver fibrosis clarified dilation portal vein (★), deposition of fibrillar material (⇒) and congested paranchyma tissue (P). (H&E). (125x).

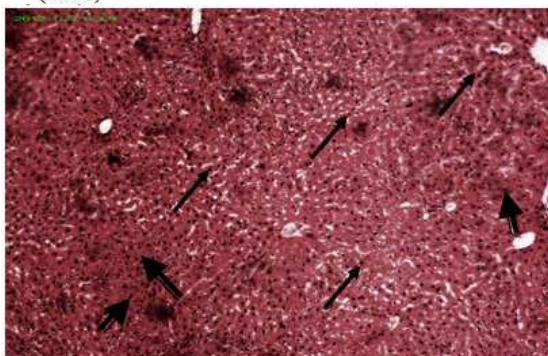


Fig. 15: Section on liver fibrosis stained with (PAS) showed heavy glycogen deposition (⇒), area of fibrosis (⇒) and destruction of liver lobule. (125x).

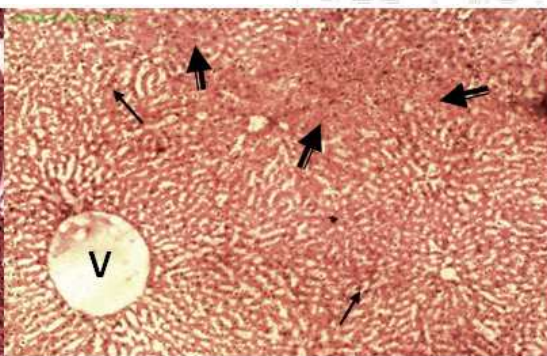


Fig. 16: Section on liver cirrhosis showed dilation on central vein (V), coarse glycogen granules in hepatocytes (⇒) and fibrous material deposit (⇒). (PAS) (375x)

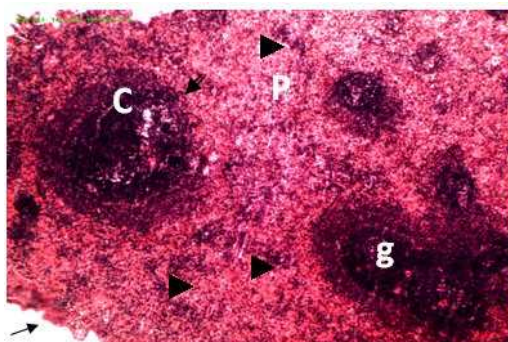


Fig. 17a: photomicrograph on control spleen illustrate normal lymph nodules (⇒) formed by germinal centre (g) and cortex (C) and vascular red pulp (P) filled the space (▶) the section surround with thin capsule (↔) (H & E) stain (125x).

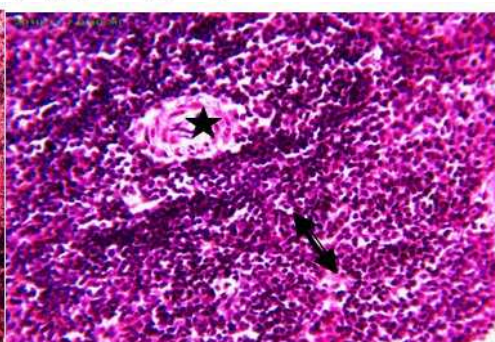


Fig. 17b: High power magnification control splenic section showed lymph nodule formed from active perifollicular zone (⇐⇒), and central artery (★) (H & E) (125x).



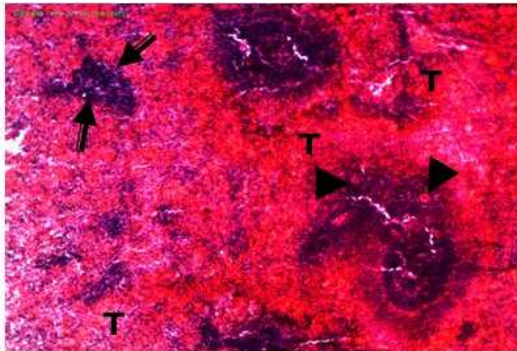


Fig. 18: Section on control splenic showing lymph node (→) and perivascular trabeculae (T), central artery (▶) also primary lymph node (⇨). (H & E) (125x).

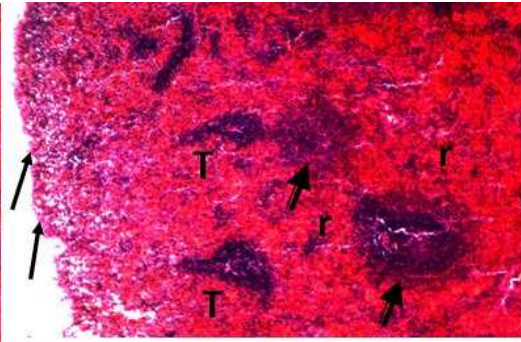


Fig. 19: Transvers section on control spleen showing thin capsule (→), trabeculae (T) and red pulp (rp) but with primary lymph node (⇨). (H & E) (125x).

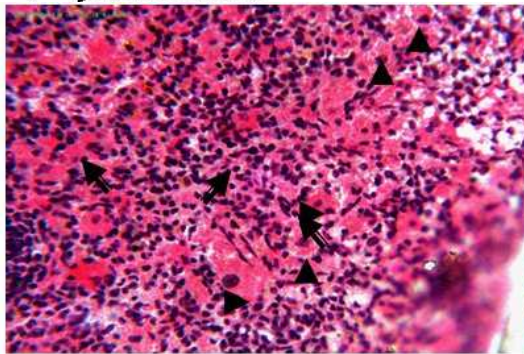


Fig. 20: High power magnification showing red pulp on control spleen which appeared vascularized with lymphocytes (⇨) and macrophages (▶). (H & E) stain (500x).

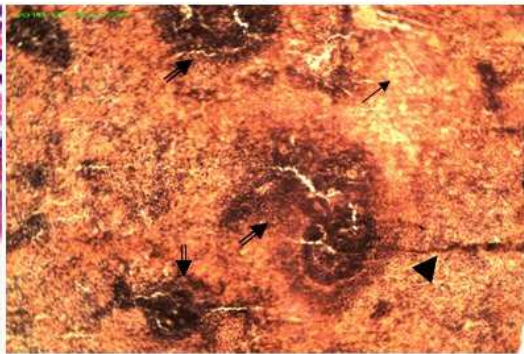


Fig. 21a: Section in splenomegaly group showing irregular lymph nodules (⇨), fibrous trabeculae (▶) and dilation splenic sinuse with yellowish pigment deposite (→). (H & E) stain (125x).

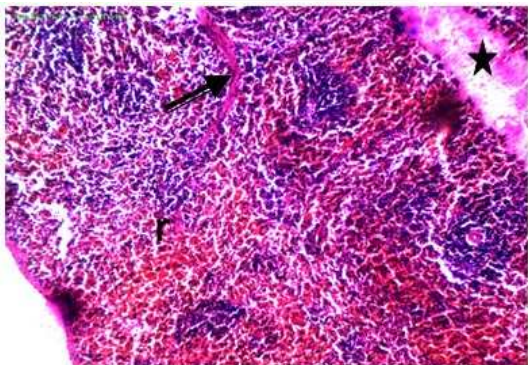


Fig. 21b: Transverse section in splenomegaly rats illustrated fibrosis trabeculae (⇨), congested red pulp (rp) and congested blood vessels (★). (H & E) stain (500x).

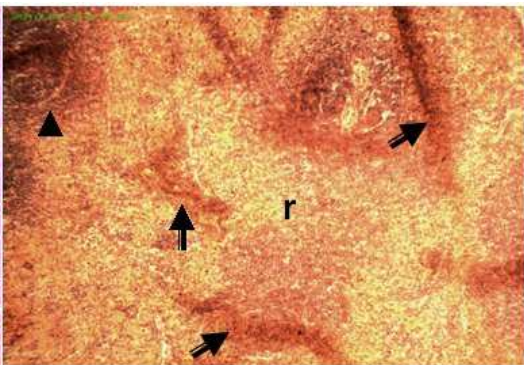


Fig. 22a: Photomicrograph in splenomegaly section illustrated fibrosis trabeculae (⇨), primary lymph node (▶) and degeneration red pulp (rp). (H & E) stain (125x).

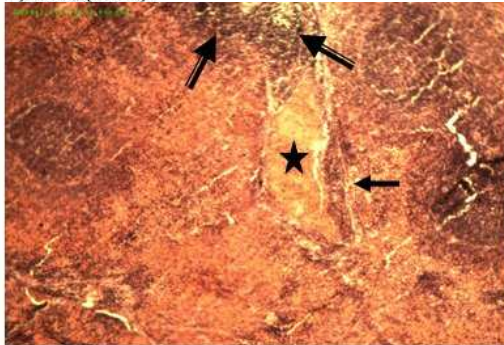


Fig. 22b: Section in splenomegaly group showing hemorrhage in large blood vessels (★) surrounding by fibrous trabeculae (⇨) also number of macrophages noticed. (H & E) stain (125x).

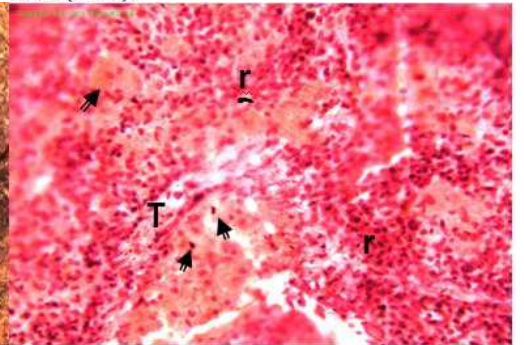


Fig. 23: Section on spleen tissue related to splenomegaly rat showing congested red pulp (rp) with macrophages (Hemiosidrin-laden) (⇨) supporting trabeculae (T) and large number of erythrocytes. (H & E) stain (350x).

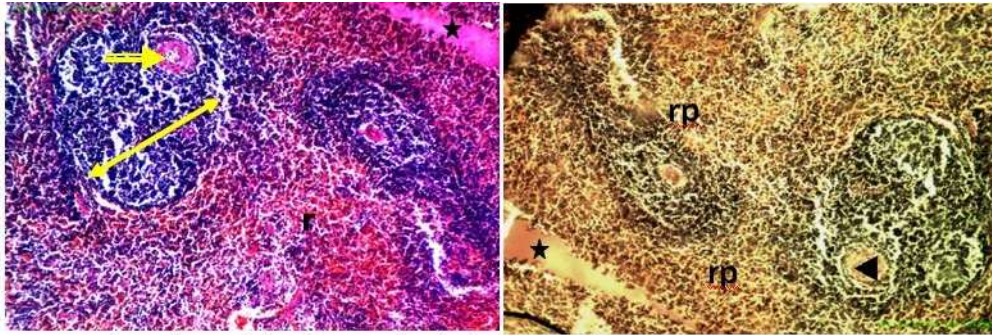


Fig. 24: Photomicrograph in splenomegaly splenic section clarified congested splenic sinuses (★), central artery fibrosis (⇒⇒), more than lymph nodules aggregated (⇔⇔) and congest red pulp (rp). (H & E) stain (350x).

Fig. 25: Photomicrograph on splenomegaly rat showed hemorrhage (★), periarterial fibrosis (▶) and degenerated red pulp (rp). (H & E) stain (350x).

## Discussion

The study was carried out to assess the histopathological changes in experimental rats with induced liver fibrosis by TAA and its relationship to splenomegaly caused by .

The TAA model is more reliable and easy to induce liver cirrhosis than the  $\text{CCl}_4$  model (5). An increase in spleen and liver weight with significance difference in all rats related to splenomegaly group compared to control, this accompanied with decreased in body weight in rats with cirrhotic and splenomegaly compared to control rats, while spleen and liver indices (ratio) per body weight of cirrhotic rats were higher than these of control rats .These results agreed with other studies clarified the relationships between splenomegaly and portal hypertension in patients with liver cirrhosis, splenomegaly not only caused by portal congestion, but is mainly due to tissue hyperplasia and fibrosis, the spleen may enlarged as cause of cirrhosis, portal vein hypertension and other factors (22,23). Also an increase with liver size (hepatomegaly) caused by deposition of fibrillar material in interlobular space, portal vein dilation and hepatic cells hypertrophy which due to an increase on water permeability (24). Total protein and albumin levels on hepatomegaly rats indicated to significance decrease in their concentrations at ( $P < 0.05$ ) compared with control group, this may be related to the effect of TAA as hepatotoxic

compound caused hepatic cells damaged and abnormal synthesis of protein and albumin, results agreed with others studies showed that total protein and albumin levels depressed in hepatotoxic conditions due to disturbances in the carbohydrates, protein, lipid metabolism or abnormal protein biosynthesis in cirrhotic liver or may be regarded to decreased ribosomal RNA in TAA-treated rats (25).

Results referred to decrease with liver enzymes activity (GPT, GOT) in all splenomegaly rats in comparison to control rats, this may be illustrated that TAA induced lipid peroxidations caused damaged of hepatic cells, or necrosis which lead to an excretion of enzymes then elevate the level in serum. Results in agreement with (26) who showed that an increase in liver enzymes an indicator of cellular liver necrosis caused by TAA metabolism which mediated hepatocytes damaged. An increase with bilirubin concentration in serum of splenomegaly group compared to control rats, this may be regarded to the effect of TAA toxicity which lead to bile duct hyperplasia and obstruction, this result discussed by (25) who illustrated that high dose of bilirubin was indication of the increased erythrocytes degeneration rate, the liver excretes the breakdown products of hemoglobin, particularly as bilirubin or may be bile duct obstructed and caused effect on bile transport, the main

component of bile called bilirubin which accumulate in the blood. In this study we recorded the dilation and increase with portal vein diameter in all sections of rats with splenomegaly also there was an increase in lymph nodules diameter of spleen related to these rats compared with control this may be regarded to portal hypertension which induced by toxicity of TAA, also the splenic blood re drains into liver and caused dilation with central vein and portal vein.

These observations also determined by (27) who explained that splenic vein drain into the portal vein, and portal hypertension can produce congestive splenomegaly, the blood flow within the spleen is highly specialized and relates to the different functions of the spleen. Lymph nodules diameter showed an increase with significant difference in all spleen sections from splenomegaly group and this related to the immune response which take place on spleen. Evidence suggests that spleen is relatively insensitive indicator of immunotoxicity (28).

Observations also clarified an increase with glycogen granules deposition in hypertrophic hepatocytes while moderate quantity of glycogen evident in hepatocytes of control rats this caused by cytoplasm changes and disturbance of carbohydrates metabolism due to toxicity of TAA. (24) proved that hepatomegaly caused increase with water permeability inside hepatocytes and an increase with glycogen, fat content which deposite on hepatocytes. Histological investigations elaborate the effect of TAA on both liver and spleen of cirrhotic group compared to control and the results have shown increase with fibrosis appeared as nodular lesions, trabecullae thickening, capsular fibrosis and deposite of fibrous material in both liver and spleen paranchymal tissue, these results agreed with other studies indicated to liver fibrosis which was proven by nodular formation and collagen deposition on liver also appearance of wide spread

fibrosis and lesions on hepatic parenchyma are the main characteristic of liver cirrhosis (29,30). Results of histological observations have shown damaged of hepatocytes, some appeared hypertrophy with pyknotic nuclei and an increase with kupffer cells, inflammatory cells in most liver sections related to hepatomegaly rats also thickening of splenic trabecullae, congested splenic venous and increase with myofibroblast cells, these changes associated with the effect of TAA metabolism which caused necrosis and degenerated of liver and spleen paranchymal tissue. Several experimental studies have shown that the splenomegaly play an inhibitory role in hepatic liver regeneration, there is indicator that humoral factors produced by splenic tissue are carried to the liver through the portal circulation where they inhibit liver regeneration (14,31).

Also an immune cells are involved in mediation of fibrosis including hepatic macrophages (Kupffer cells), Natural Killer (NK) cells, cytotoxic T-cells. Activated macrophages produce pro-inflammatory mediators that further result in the activation of non-specific immunity which lead to the development of myofibroblasts responsible for the production of extra-cellular matrix (ECM) (31). Results identified active lymph nodes in spleen sections regarded to rats with splenomegaly and appeared as more than one lymph nodule aggregated together with large number of lymphocytes in marginal zone, this may be illustrated as immune role of spleen and the cytotoxic effect of TAA.

The marginal zone contain population of lymphocytes and macrophages that are functionally distinct from the cord histiocytes of the red pulp, which at least in animal models seem to be an important in maintaining the anatomic structure of the marginal zone by attracting newly differentiated B-lymphocytes (32,33). Findings from the present study concluded that splenomegaly caused as a result of liver

hyperplasia, cirrhosis and fibrosis induced by toxicity of TAA. The dependent dose very reliable to induced hepatomegaly and splenomegaly in

experimental rats, on the other hand hepatomegaly model very useful to understand the histopathological changes of splenomegaly.

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