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Physiological and Histopathological Effects of Abetacept (Orencia) Drug on Liver of Albino Male Mice

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Abstract

A study investigated the effects of abetacept (Orencia) drug on the level of liver enzyme and on liver histology in albino male mice. Fourty five adult male mice of 8 weeks age and weighting 25-35g divided into three groups (15 mice each). The second & third groups were treated with abetacept drug while the first group was used as a control.

Abetacept was intraperitoneally given twice every week at 125 mg/kg B.W. and 250 mg/kg B.W. to the second and third groups respectively for 6 weeks, whereas the control group was intraperitoneally injected with normal saline. The results showed a significant ($p< 0.05$) increase in the levels of liver enzymes [serum alanine aminotransaminase (ALT), serum aspartate aminotransaminase (AST) & alkaline phosphatase (ALP)] compared to control group. Also there was a significant ($p< 0.05$) increase in the level of these enzymes in the third group compared to the second group. Some histopathological changes were found in liver of treated mice with this drug represented in ; hyperplasia of bile ducts epithelial lining cells and fibrosis in portal area of bile ducts, infiltration of neutrophile and mononuclear cell in the lumen of dilated sinusoids and central vein, necrotic area of hepatocytes and hyper atrophy of proliferated kupffer cells.

The results of this study concluded that the treatment with abetacept caused increased in the level of liver enzymes and damage in its tissues.

Keywords: Abetacept , liver functions , hepatotoxicity.

التأثيرات الفسيولوجية والنسجية المرضية لعقار البيتاسبت (اورنشيا) على كبد ذكور الفئران البيض

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الخلاصة

اختبرت الدراسة عن تأثير عقار البيتاسبت (اورنشيا) على مستويات إنزيمات الكبد وأنسجته في ذكور الفئران البيض. قسمت خمسة واربعون ذكر بالغ من الفئران البيض إلى ثلاث مجاميع (١٥ لكل مجموعة). عوملت المجموعة الثانية والثالثة بعقار البيتاسبت بينما عدت المجموعة الأولى كمجموعة سيطرة. أعطي البيتاسبت داخل الغشاء البريتوني مرتين أسبوعيا بتركيز ١٢٥ملغ/كغم من وزن الجسم و ٢٥٠ ملغ/كغم من وزن الجسم للمجموعة الثانية والثالثة على التوالي لفترة ستة أسابيع بينما مجموعة السيطرة حقنت داخل الغشاء البريتوني بالمحلول الملحي الفسلجي . أظهرت النتائج وجود ارتفاع معنوي ($P< 0.05$) بمستوى إنزيمات الكبد (ALT, AST& ALP) مقارنة بمجموعة السيطرة.كذلك هناك ارتفاع معنوي ($P< 0.05$) بمستوى هذه الإنزيمات للمجموعة الثالثة مقارنة بالمجموعة الثانية. كما وجدت بعض المتغيرات النسجية المرضية في كبد

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الفئران المعاملة بهذا العقار مثل تضخم الخلايا الطلائية المبطنة للقنوات الصفراوية وتليف في المنطقة البوابية للقنوات الصفراوية ،ارتشاح كريات الدم البيضاء المتعادلة والخلايا وحيدة النواة في تجويف الجيبانيات المتوسعة والوريد المركزي، تنخر في الخلايا الكبدية وتضخم في خلايا كوفر المتنامية. نستنتج من نتائج هذه الدراسة بأن المعاملة بعقار البيتاسبت تسبب ارتفاع بمستوى إنزيمات الكبد وضرر في انسجته.

Introduction

Many studies have focused on the impact of chemical compounds and drugs to follow up on the level of liver enzymes as a key member of the body which is the metabolism of different compounds and eliminate toxicity [**1**]. Rheumatoid arthritis (RA) is a chronic disease that leads to inflammation and progressive joint damage. Current therapies target the inflammatory consequences of autoimmune activation with the use of disease-modifying antirheumatic drugs (DMARDs) such as methotrexate and biologic DMARDs, which inhibit inflammatory cytokines such as tumor necrosis factor α (TNF-α) [2].

The Orencia drug example of chemicals that is widely used in the treatment of RA in people who have had an inadequate response to one or more DMARDs [3]. One of commonly DMARDs is abetacept which is TNF-α [4].

The term DMARDs indicates a wide group of drugs potentially able to inhibit the occurrence /progression of particular damage in RA patients [5]. Abetacept is useful in delaying the progression of structural damage and reducing symptoms of rheumatoid arthritis. [6]. The targets of a large number of non-anti-TNF biological drugs are very wide; abetacept is a selective co-stimulation modulator as it inhibits the co-stimulation of T cells and acts through preventing antigen-presenting cells (APCs) from delivering the co-stimulatory signal to T cells to fully activate them [7].

Liver is an important organ as it performs multiple functions like organic metabolism, cholesterol metabolism, digestive functions via bile production and secretion, clotting functions, endocrine functions, excretory and degradative functions. It biotransforms many endogenous and foreign organic molecules [8].

Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation . In addition serum levels of many biochemical markers like alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), triglycerides, cholesterol, bilirubin, alkaline phosphatase, are elevated [9].

Serum aspartate aminotransaminase (AST) is a pyridoxal phosphate dependent transaminase enzyme .AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells, and it is commonly measured clinically as a marker for liver health [10].

Serum alanine aminotransaminase (ALT) an enzyme that is normally presents in liver and heart cells [11]. It is released into blood when the liver or heart is damaged [12]. Another enzyme called Alkaline Phosphate (ALP) found in the liver, the level of this enzyme increased in diabetes mellitus and hepatic diseases, for example, hepatic obstructions. Also this enzyme found in the body tissues at different levels and concentrations, it exists highly in the cells of the intestinal walls and renal tubes. Its function in the intestinal cells to help the absorption and transport of inorganic phosphorus [13].

Materials and methods

Experimental Design

Fourty five male albino mice (***Mus musculus***) of 8 weeks age and weighing 25-35 g were used throughout the study. All mice were kept under constant environmental conditions (24 to 26°C) with a 12-hour light/dark cycle. They were housed in cages with wood dust and given free access to food and tap water *adlibitum.*. In this part, the biochemical effects of two doses of abetacept were investigated. Animals were divided into three groups designated as 1, 2 and 3. Each group consisted of 15 animals and treated as follows:

Group 1: The animals were treated with normal saline.

Group 2 The animal were treated with abatacept at dose of 125 mg / kg body weight

Group 3: The animals were treated with abetacept at a dose of 250 mg / kg/body weight

Abetacept was injected intraperitonially as double dose (0.1 ml) per week for six weeks. At the end of the experiment , the blood was obtained by heart puncture as in [14] in the small test tube and placed in a water bath at 37 ° C for 30 min for the purpose of the clot (or left vertically on the table for 30 min) and followed by centrifugation at 2000 rpm at 37 ° C for 10 minutes. The serum was

collected in the tubes and kept it in deep freeze (-20 C) . Diagnostic Kits which were purchased from Randox, were used to determine the serum activity of the Aspartat transaminas (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) [15, 16].

Histopathological examination

Livers were excised and subsequently fixed in 10% formalin overnight then dehydrated through increasing concentrations of ethanol and washed , the livers were processed, wax block cut into section 4-5 µm thickness and slides were prepared then stained in haematoxylin and eosin for histological examination. Histological sections were examined by light microscope and photographed at a magnification of 40X [17].

Statistical Analysis

The results are expressed as mean ± standard deviation (SD). Differences between control and treated groups were tested for significance using a one-way analysis of variance (ANOVA), and P value of $p< 0.05$ was considered significant [18].

Results & discussion

Results are illustrated in table-1 represented data of the serum enzymes activity of (ALT, AST and ALP) in the studied group.In group 2 (34.86 ± 3.40, 74.14 ± 5.40 and 69.14 ± 3.32) which respectively and in group 3 which measure measure (48.71 ± 4.57, 99.48 ± 8.62 and 73.60 ± 7.45) respectively which shows significant $p< 0.05$ elevation in their activities in comparison with control group which measure (25.15 ± 3.12, 68.85 ± 3.13 and 61.28 ± 4.70 respectively). Also there was significant $p< 0.05$ increase in enzyme activity of ALT, AST and ALP in group 3compare with group 2. Because group 3 injected with highly dose of abetacept compare with group 2. So the level of its enzyme is higher than of group 2. This finding suggests that abetacept can cause inflammation in the liver and causing abnormal serum levels of liver enzymes.

The significante increasing activity of AST, ALT and ALP in the third group might be due to the cytotoxic effect of abetacept on liver cells. This may lead to damage of liver cell membrane which cause the release of high quantity of these enzyme to blood serum .This explains the increases of the enzyme level in blood serum after using these toxic agents [19]. Also this significant increase may be due to manifestations of hepatocellular necrosis [20] as in Fig 6.

Another reason may be the presence of the toxic material (abetacept) leading to autolysis of liver cell as a result to increasing the activity of lysosomes and this lead to necrosis which causing increase of liver enzymes in blood serum [21].

This finding is in agreement with Sokolove and co-workers who demonstrated that among anti-TNF users which have RA, there was a trend towards increased risk for elevations in liver enzymes compared with non users[22].

Li-Fern and coworkers demonstrated that a high level of ALP occur in case of hepatitis, obstructive liver disease [23].

Table 1-Levels of liver enzymes in all groups (ALT,AST,ALP) in the studied groups .

Liver enzyme activity / Groups	Mean±SD		
	ALT (U/L)	**AST (U/L)**	**ALP (U/L)**
Group 1 Normal saline	25.15 ± 3.12	68.85± 3.13	61.28 ± 4.70
Group 2 125mg/kg/B.W. of abetacept	34.86 ± 3.40 **A**	74.14 ± 5.40 **A**	69.14 ± 3.32 **A**
Group3 250 mg/kg/B.W. of abetacept	48.71 ± 4.57 **Aa**	99.48 ± 8.62 **Aa**	73.60 ± 7.45 **Aa**

The mean differences is significant at $p<0.05$ level.

A $p< 0.05$ group 2 & group 3 compared with control.

a $p< 0.05$ group 3 compared with group 2

The present study revealed some histopathological effects on liver, histological section of control mice shows normal central vein with hepatocytes arranged in to the plates separated by vascular channals (sinusoid) with Kupffer cell figure-1.

The histopathological changes seen in liver with the abetacept treated group dose (125 mg/ kg B.W.) include infiltration of neutrophile and mononuclear cell to the lumen of sinusoids and central vein in Figure-2, hyperplasia of epithelial lining cell of bile ducts Figure-3, fibrosis in the wall of bile ducts, sinusoidal dilation, mononuclear cell infiltration to the bile ducts, fibrosis in portal area of bile duct Figure-4.

The histopathological damages seen in liver with the abetacept treated group dose (250mg/ kg/bw) include mononuclear cell aggregation in portal area around bile duct Figure-5, necrosis in hepatocytes, aggregation of neutrophile and mononuclear cell around necrotic area of hepatocytes Figure-6 and hyperatrophy of proliverative kupffer cells Figure-7.

It has been known that ALT and AST had the greatest diagnostic accuracy for hepatocellular necrosis [24] .This result is in consistence with our study of increasing liver enzyme after treatment with abetacept.

Also hypertrophy or lipid accumulation are also often linked with transaminase changes. centrilobular hypertrophy is a common sequel to hepatic drug-metabolizing enzyme induction and concurrent ALT and ALP increases [25] .The liver damage after treatment with abetacept may be due to increasing in liver enzymes as aresult to histopathological changes which include hypertrophy and necrosis of hepatic cell.

Adle *et al*. [26] reported that abetacept induces several cellular changes .TNF-α inhibitors include infliximab, adalimumab, etanercept, golimumab and certolizumab pegol, rituximab, abatacept and tocilizumab[27]. Treatment with adalimumab which is one of anti TNF-α inhibitors caused liver damage represented by marked portal lymphoplasmacytic inflammation with periportal interface hepatitis and scattered lobular necroinflammatory changes [28]. And this finding is in agreement with our study.

Another study for women's liver biopsy treated from RA using anti TNF -α adalimumab and swich to abetacept) revealed portal fibrosis and periportal lymphatic infiltrate [29].

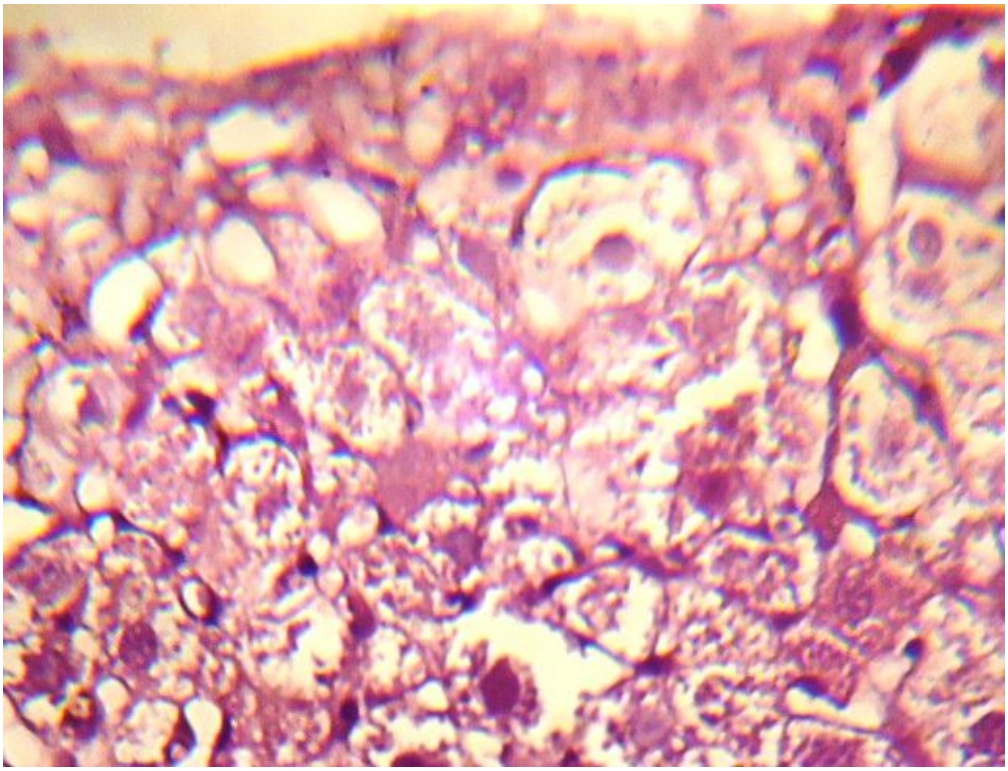

Figure 1-Section in liver of control group showing normal histology of liver (H&E 40X).

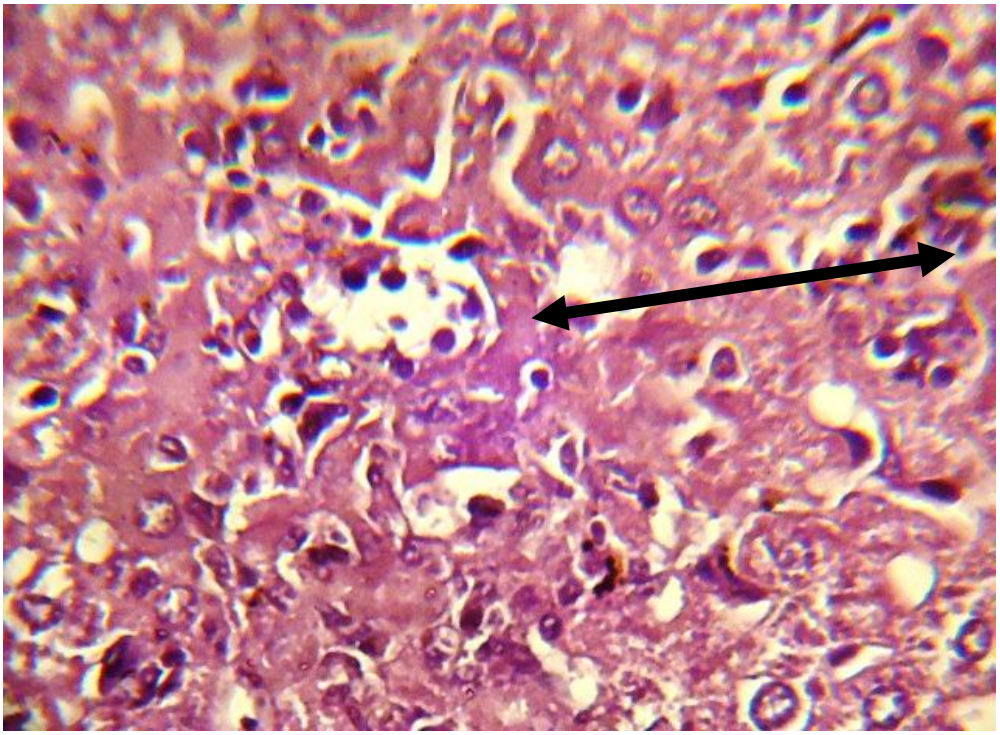

Figure 2-Section in the liver of treated mice with 125 mg/kg B.W. of abetacept shows neutrophile and mononuclear cell in the lumen of dialated sinusoids and central vein (40X.6.3) .

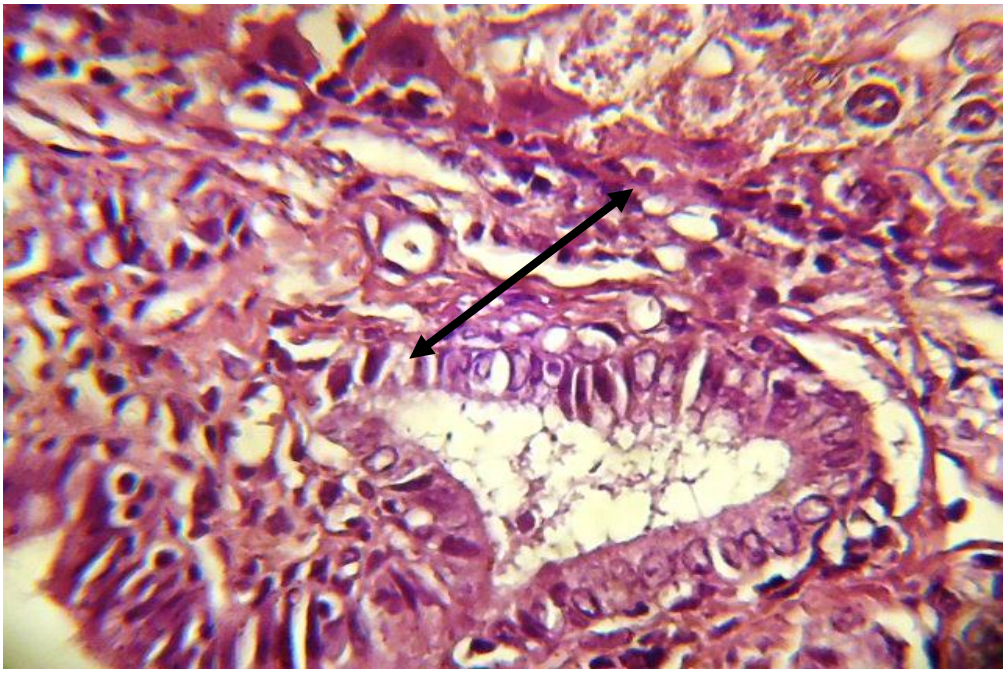

Figure 3-Section in the liver of treated mice with 125 mg/kg/bw shows mononuclear cell in fibrotic wall of bile ducts with hyperplasia of their epithelial lining cell (40X.4.1) .

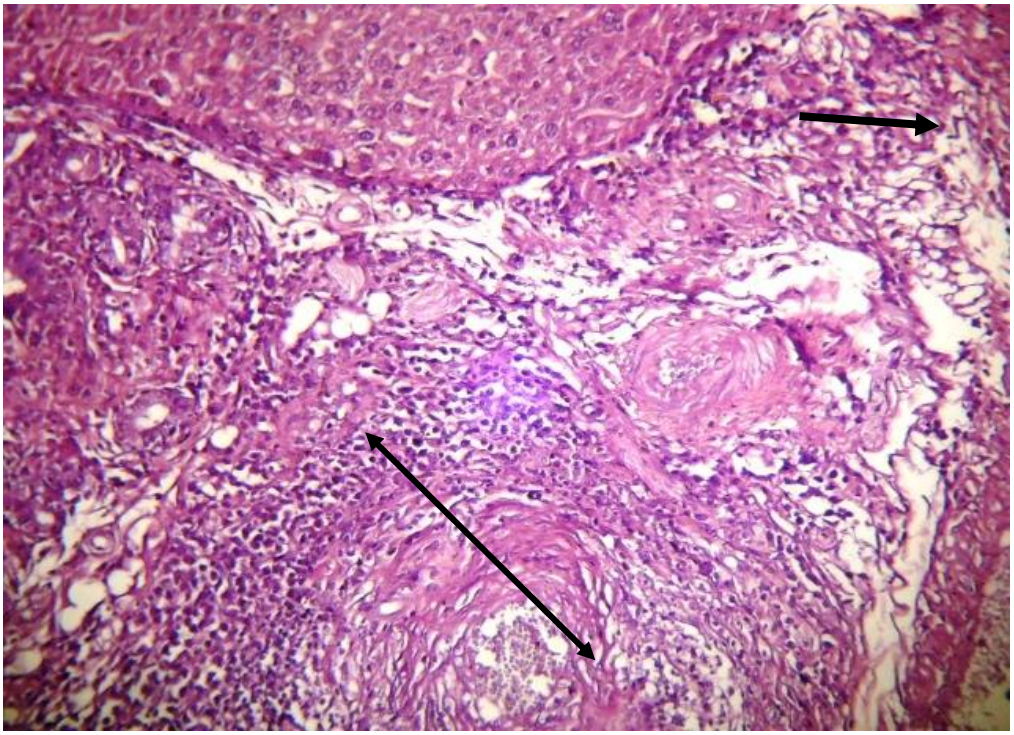

Figure 4-Section in the liver of treated mice with 125 mg/kg/bw shows mononuclear cells in fibrosis wall of bile ducts with hyperplasia of epithelial lining cells ⟷ And fibrosis in portal area ⟶(10X.4.6).

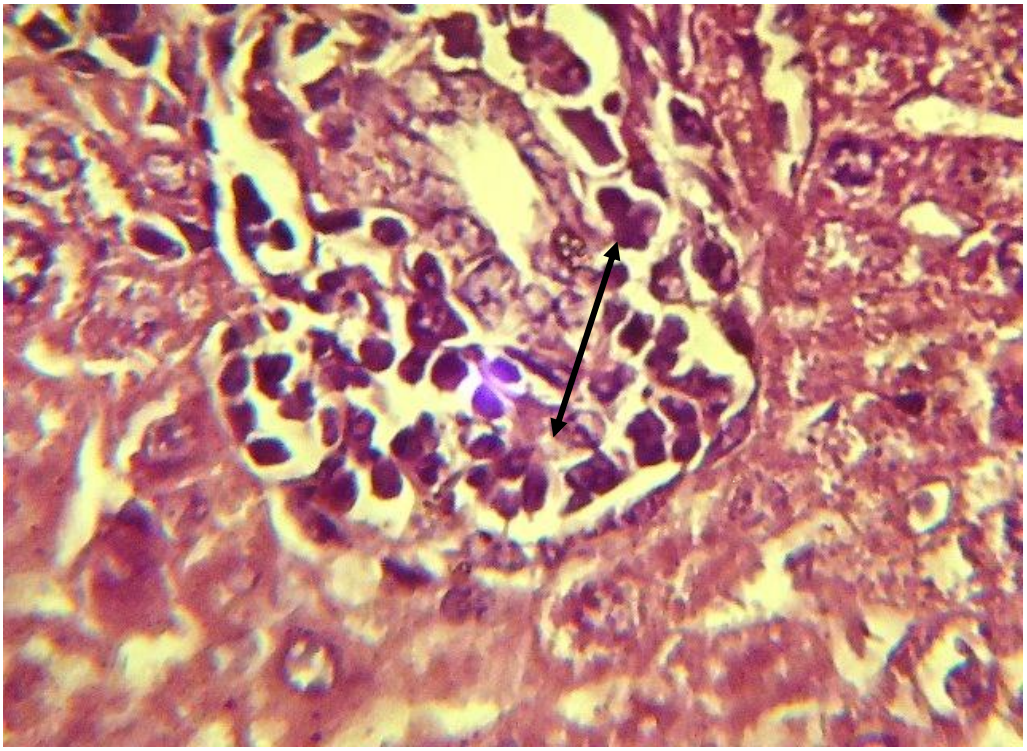

Figure 5-Section in the liver of treated mice with 250 mg/kg/bw shows mononuclear cell aggregation in portal area around bile duct (40X.4.4). ⟷

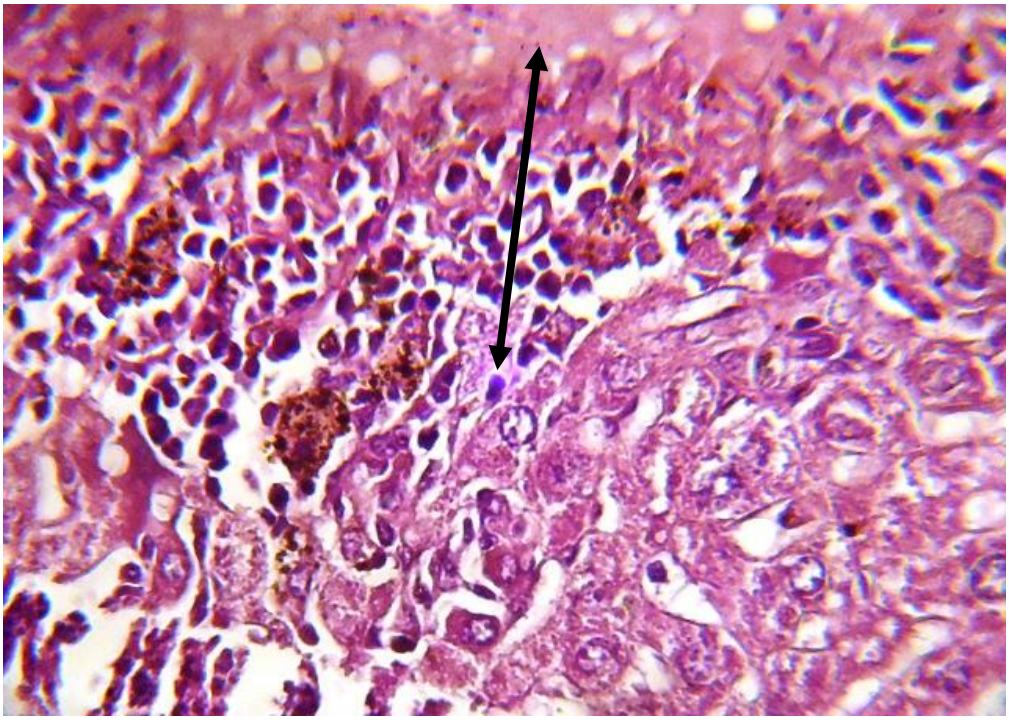

Figure 6-Section in the liver of treated mice with 250 mg/kg/bw shows neutrophile and mononuclear cell aggregation around necrotic area of hepatocytes (40X.4.3). ⟷

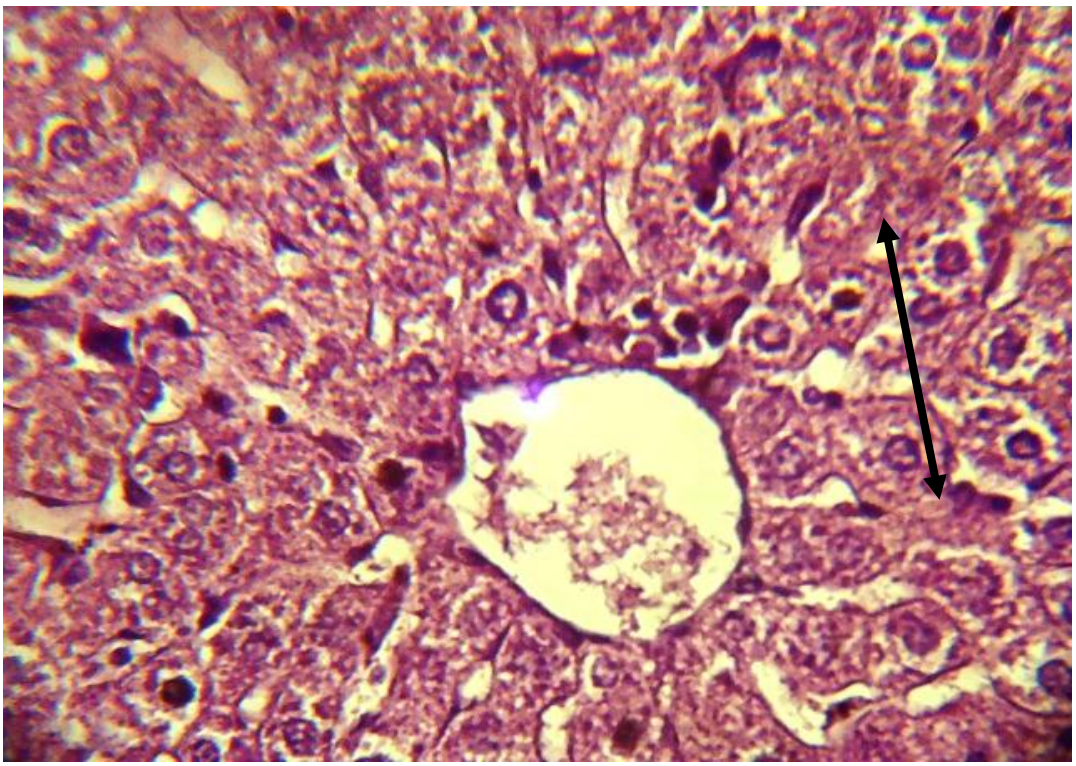

Figure 7-Section in the liver of treated mice with 250 mg/kg/bw shows hypertrophy of proliferation of kupffer cells (40X.4.5).

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