Histopathological Changes in Liver Induced by Piroxicam Administration in Adult Male Albino Mice *Mus musculus*

Intidhar Mohammed Mnati[1], Baydaa Hussain Mutlak[1]

[1] *Lecturer, Department of biology / College of Education for Pure Science (Ibn Al- Haitham) / Baghdad University, Iraq*

Abstract

Background: Piroxicam is one of non-steroidal anti-inflammatory drugs which is widely used in treating rheumatic disorder. **Aim of the study**: Studying the effects of piroxicam pertaining on the liver tissue to the chronic administration in male albino mice *Mus musculus* histologically and histochemically changes. **Material and methods**: A total number of (30) adult male albino mice were utilized in this study, they were randomly distributed into three groups, the first and second groups were orally treated with concentrations (75, 150) mg/kg of piroxicam respectively for one and two months, the third group considered as control group were orally treated with distilled water, liver samples were collected every one and two months for the histological and histochemically study. **Results**: This study recorded the presence of histopathological changes in liver tissue of the treated mice with concentration 75 mg/kg represented by degeneration of hepatocyte, sinusoidal dilation, inflammatory cellular infiltration, vascular congestion in the portal vein and beginning of calcium casts formation, while the group treated with 150 mg/kg of the drug showed portal vein congestion and dilation, blood sinusoidal were highly obliterated, proliferation of bile duct and nuclei appeared pyknotic, in addition there were a remarkable decrease in the glycogen controls of cells. **Conclusion**: It could be concluded that piroxicam drugs has drastic effects on liver tissue as represented by the observed histopathological changes.

Key words*: Piroxicam, Histopathological changes, Liver, Albino mice.*

Introduction

Non steroidal anti-inflammatory drugs(NSAIDs) are some of the most commonly prescribed and utilized medication. These agents have proven particularly effective in the treatment of a variety of inflammatory disease. Some disease like osteoarthritis required their use for long periods of time [1] . All NSAIDs are well absorbed when given orally. They are eliminated from the body by the liver and kidney [2] .Piroxicam is one of the most popular (NSAIDs) belong to the oxicam group and used for the treatment of inflammatory conditions and rheumatic disorders, it is useful in the management of ankylosing spondylitis, acute musculo_skeletal disorders and dysmenorrhea, they exert their theraquetic effect by reducing prostaglandin biosynthesis through inhibition of cyclooxygenease(cox) enzymes [3] .

The studies showed that piroxicam has many side effects on digestive system, represented by ulcers, gastritis [4] , furthermore there were side effects of piroxicam on the testes of male mice [5]. [6] also suggested that injecting piroxicam caused a remarkable histpathological symptoms in kidney of male albino. On the other hand, several NSAIDs drugs have been associated with liver damage [7] . Many NSAIDs have been withdrawn from the market because of adverse hepatic drug reaction. Some cases of liver disease have been reported in patients taking piroxicam [8]. Therefore, the present study was designed to investigated the histological effects of piroxicam on the liver of adult laboratory mice treated with the drug piroxicam.

Material and Method

-Experimental animals

A total number of (30) healthy adult albino mice *Mus musculus* and age approximately (8-10) weeks old ranging in weight from (25-30)gm, purchased from Iraqi

national center for drug control and research, Baghdad, Iraq. They were randomly distributed into three main groups of ten mice for each group, they were housed in cages and were kept in the laboratory under constant conditions for at least one week before use, they were fed a standard commercial diet.

-Experimental design

The drug used in this study was piroxicam in capsule form of (20)mg, concentrations (75,150)mg/kg were prepared daily and they were given orally using stomach cannula for the periods one and two months, two of the three groups were orally treated with concentrations (75,150)mg/kg respectively, the third group considered as control group were treated with 0.1ml of distilled water for two months.

-Histological and histochemical preparations

Animals from control and treated groups were scarified, dissected and small pieces of liver were quickly removed, then fixed in 10% formalin and bouin's fixatives for (20-22)hours followed by changing the fixative with 70% alcohol after washing the specimens for many times, following the washing specimens were dehydrated, embedded and then sectioned to 6 µ thickness, for the histological examination, sections were stained with heamatoxylin and eosin [9] then examined under light microscope (Olympus), photographs were taken by digital camera.

In the histochemical study , sections were stained with periodic acid Schiff's method (PAS) to demonstrate carbohydrates [9].

Results

Histological examination in liver tissue

- Control group

Section of this group appeared with normal histological structure the liver covered with thin capsule composed of loose connective tissue called Glisson's capsule.The liver of this group are normal in shape and size (Fig.1).

Histological examination of mice livers of this group showed normal liver histology with hepatic cords or plates are arranged around central vein (Fig.2). These hepatic cords separated from each other by blood sinusoids, the blood sinusoidal are lined with two types of cells, first type of cells represented by endothelial cell that appears flattened cell and second type of cells represented by kupffer cell that appear as a big cell with circular nuclei (Fig.1).

The portal area of liver composed of branch of portal vein, hepatic artery, bile ducts and lymphatic vessel (Fig.3).

-Groups treated with (75)mg/kg of piroxicam

The examination of liver sections of treated mice with(75)mg/kg of piroxicam for two periods revealed many pathological changes compared with control group. The changes are represented by degeneration of hepatocyte, hepatic sinusoidal dilation, also inflammatory cellular infiltration, few vacuoles in the cytoplasm of hepatocyte and congestion of central vein after one month of administration (Fig.4,5), while after two months of administration the cytoplasm appeared to be highly vacuolated and the nuclei of most of the hepatocytes appeared pyknotic and some nuclei appeared disappeared (Fig.6), as well as the beginning of calcium casts formation, vascular congestion in the portal vein, cellular infiltration was more intense around the portal area than the previous treatment and dilation of portal vein in the portal area (Fig.7,8 ,9).

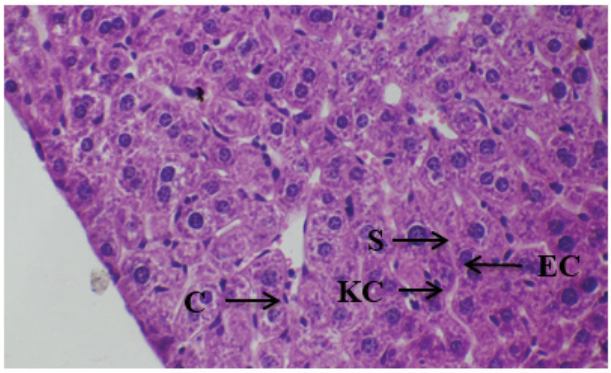
-Groups treated with (150)mg/kg of piroxicam

The results of microscopic examination showed severe changes that represented by cellular infiltration and portal vein dilation in the periportal area, the blood sinusoidal were highly obliterated than the previous group. In addition, the nuclei of most of the hepatocytes appeared with dense clumps of chromatin and some nuclei appeared Pyknotic after one month of administration (Fig.10,11), whereas after two month of administration the changes were proliferation of bile duct, congestion of portal vein, the cellular infiltration was more intense than the previous group, detachment of basement membrane of hepatocytes and hepatocytes were swollen (hypertrophy). In addition, the cytoplasmic vacuoles were less in number .(Fig. 12,13,14).

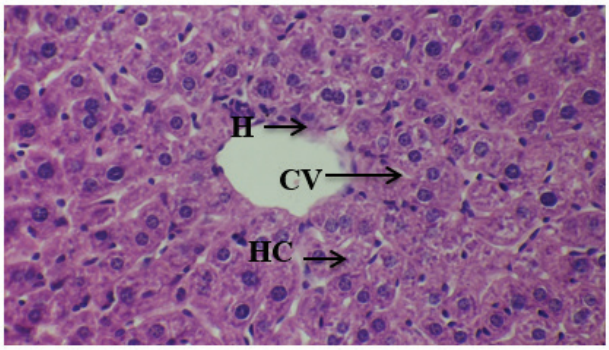
Histochemical changes in the liver

Control liver sections stained with PAS method

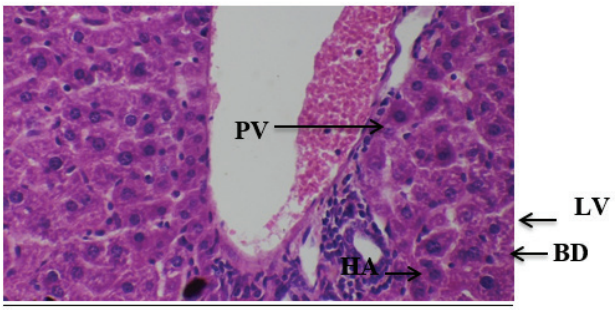
showed a positive reaction to the stain as an indication for the existence of carbohydrates in the basement membrane of hepatocyte glycogen appeared around the cell membrane (Fig.15).Liver tissue of treated mice with (75,150)mg/kg of piroxicam after two months of administration showed decreased of glycogen content. The reaction increase with the increase of concentration and administration period(Fig.16,17)



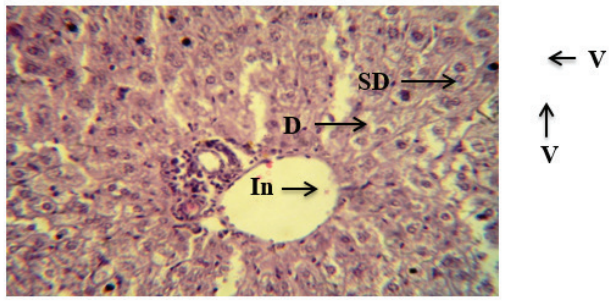
Figure(1): Cross section of liver showing capsule(C), sinusoid(S), endothelial cells(EC), kupffer cells(KC) (H&E10 X)



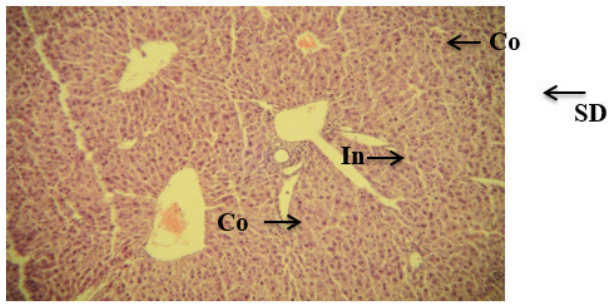
Figure(2): Cross section of liver showing central vein(CV), hepatic cords(HC), hepatocyte(H) (H&E 10X)



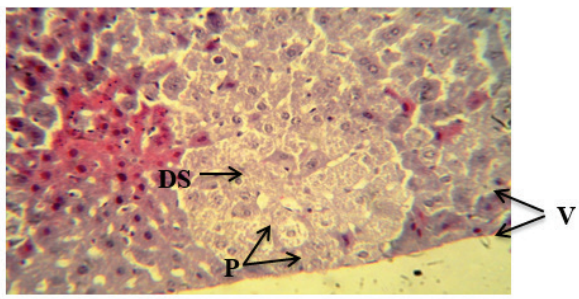
Figure(3): Cross section of liver showing portal vein(PV), hepatic artery (HA), bile duct(BD),lymphatic vessel(LV) (H&E 40X)



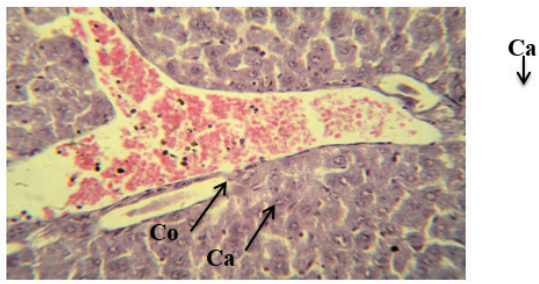
Figure(4): Cross section of liver (treated group with 75mg/ kg) for one month showing hepatocyte degeneration(D), hepatic sinusoidal dilation(SD), vacuole(V), infiltration of lymphocyte(In) (H&E 10X)



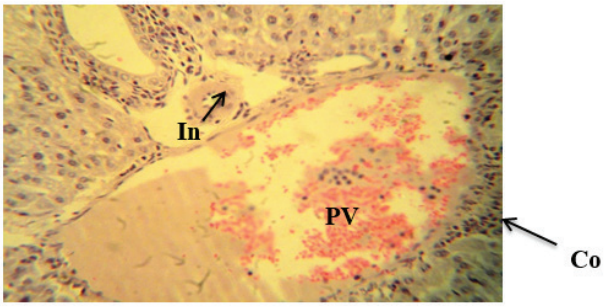
Figure(5): Cross section of liver (treated group with 75mg/ kg) for one month showing congestion of central vein (Co), infiltration of lymphocyte(In), hepatic sinusoidal dilation(SD) (H&E 10X



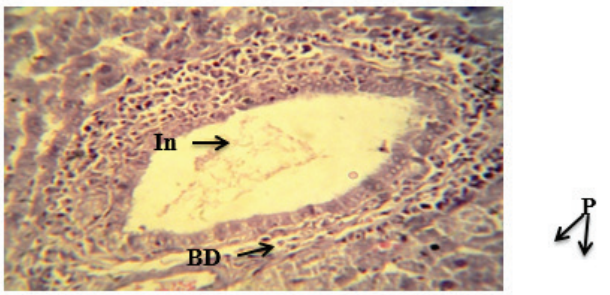
Figure(6): Cross section of liver (treated group with 75mg/ kg) for two months showing hepatocytic vacuolations(V), Pyknotic nuclei(P), cytoplasm appeared darkly stained(DS) (H&E 40X)



Figure(7): Cross section of liver (treated group with 75mg/ kg) for two months showing calcium casts formation(Ca), congestion of portal vein (Co) (H&E 40X)

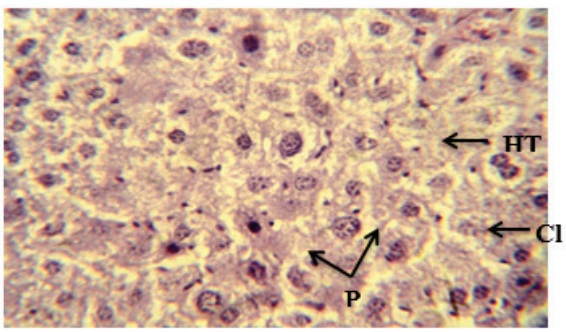


Figure(8): Cross section of liver (treated group with 75mg/kg) for two months showing vascular congestion of portal vein (Co), infiltration of lymphocyte(In), Portal vein dilation in the portal area(PV) (H&E 40X)

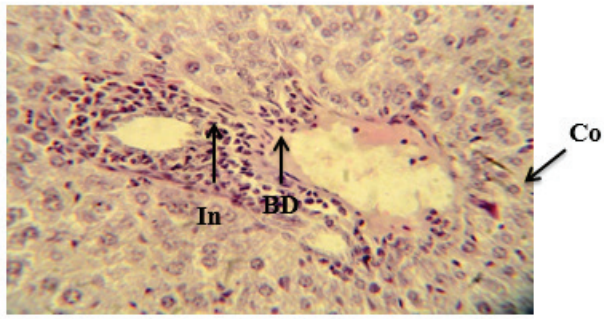


Figure(9): Cross section of liver (treated group with 75mg/kg) for two months showing increased cellular infiltration(In) around of bile duct(BD), pyknotic nuclei(P) (H&E 40X)

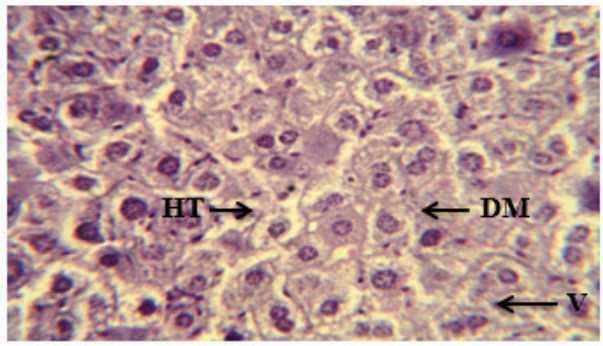
Figure(10): Cross section of liver (treated group with 150mg/kg) for one month showing dilation of portal vein (PV), hepatic sinusoidal dilated (SD), cellular infiltration of lymphocyte (In) (H&E 40X)



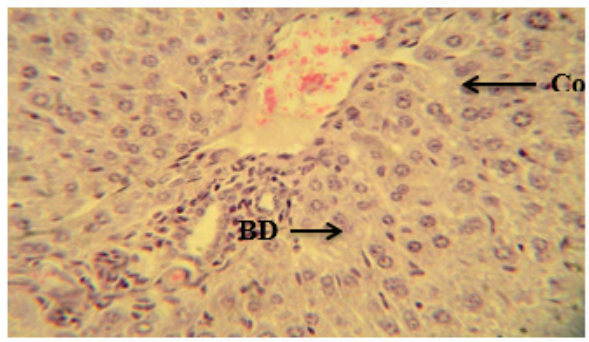
Figure(11): Cross section of liver (treated group with 150mg/kg) for one month showing pyknotic nuclei(P), hypertrophy of hepatocyte (HT), clumped chromatin (Cl) (H&E 100X)



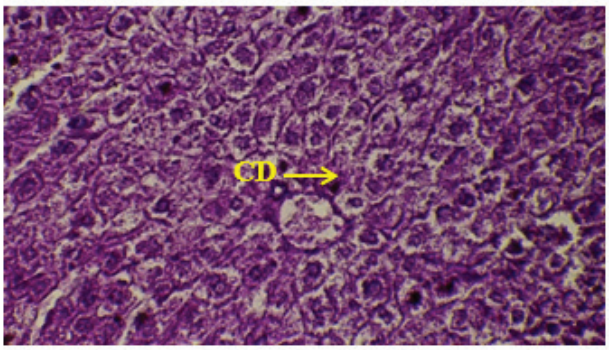
Figure(12): Cross section of liver (treated group with 150mg/kg) for two months showing infltratiom of lymphocyte (In), proliferation of bile duct (BD), congestion in the portal vein (Co) (H&E 40X)



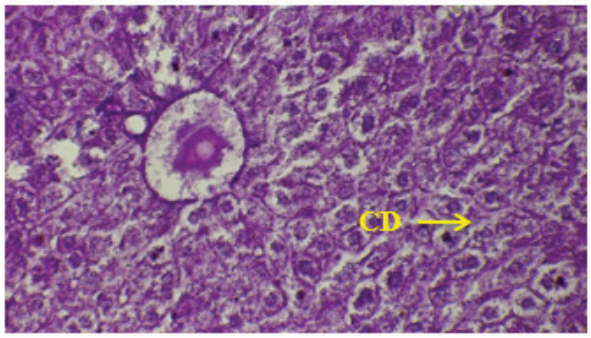
Figure(13): Cross section of liver (treated group with 150mg/kg) for two month showing detachment of basement membrane of hepatocyte (DM), hypertrophy of hepatic cells(HT), hepatocytic vaculations (V)(H&E 40X)



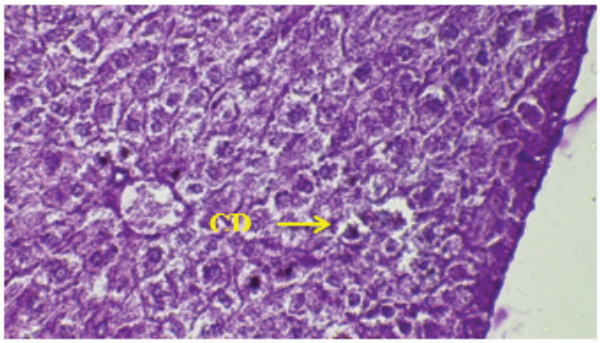
Figure(14): Cross section of liver (treated group with 150mg/kg) for two months showing proliferation of bile duct (BD), congestion in the portal vein (Co) (H&E 40X)



Figure(15): Cross section of liver of control group showing distribution of carbohydrate deposition (CD) in the hepatocytes with intense red color(PAS 40X)



Figure(16): Cross section of liver (treated group with 75mg/kg) for two months showing slight decrease in carbohydrate deposition (CD) (PAS 40X)



Figure(17): Cross section of liver (treated group with 150mg/kg) for two months showing severe decrease in carbohydrate deposition (CD) (PAS 40X)

Discussion

In this study, we tried to identify the effect of piroxicam depended in the liver of adult male albino mice. It was clear that its effect was time depended, there were increased effects with prolonged time of dose administration this result is in agreement with [10] .

In general, NSAIDs are well know to induce hepatic injury [7 ,11]. Also the pathological changes may lead to impaired liver function witch interferes with the secretion of plasma proteins [8]. This lead to decreased blood osmotic pressure with subsequent decreased drainage of tissue fluids, which explains the congestion observed in the different tissue.

The histological examination revealed that there were histopathological changes in the liver of treated groups with the concentration (75 and 150)mg/kg, the histopathological changes treated with 75 mg/kg showed vascular congestion and beginning of calcium casts formation , these findings in agreement with foundation of [12].

There was also a remarkable histopathological changes in this groups treated with piroxicam that represented by vascular congestion that may be related to the use of drug that cause an acute inflammation this lead to change the blood flow inside blood vessels, which may cause a relaxation and an extension in these blood vessels, thus the blood will accumulate into the vessels[12,13].

Result showed formation of calcium casts , this may attributed to the elevation of hydrogen peroxidase H_2O_2 that cause the nephrotoxicity which is responsible for changing the permeability of mitochondrial cellular membrane, hence the amount of calcium taken by the mitochondria will increase and with the existence of oxygen , the mitochondria will break down and the calcium will be released in the liver tissue [13] .

Result also showed a remarkable cellular infiltration in the hepatic tissue. This supported [14]whose studies suggested that abundance of leucocyte, and lymphocytes, in particular, are a prominent response of body tissue facing any injurious impacts. Leukocyte elevations and adherence to the vascular endothelium have been suggested by [15] to play an important role in the pathologenesis of NSAIDs associated injury.

In this study, the vacuolation of the cytoplasm of the liver cells appeared at first in the hepatocytes of the peripheral zone of the hepatic lobules, extending gradually toward the center. This may be due to the direction of the lobular blood supply. Vaculation and damage of liver cells were noted by other investigators following treatment with different agents[16, 17].

The present study also showed the presence of histopathological symptoms in the group treated with (150)mg/kg of piroxicam that represented by blood sinusoidal dilation, detachment of basement membrane and hypertrophy of hepatocyte, there findings in agreement with[12, 18].

Also results showed degeneration of hepatocyte and damage of hepatic cells [19] suggested that the high exposure of drug and chemicals results in cell death or apoptosis. These two factors(drug and chemical) may show either functional or structural side effects as well as the loosing of the liver capacity to stress, but when reaching the threshold, the liver cell will reach the final irreversible stage which was death.

Also results showed hypertrophy which is characterized by enlargement of cells in comparison with control group. This enlargement of cell may be due to the enlargement of the component of these cells and this swelling may mostly due to accumulation of water inside the cell [20] .

Regarding the histochemical changes observed in this study under piroxicam administration, results showed a reduction in the polysaccharide in the liver tissue, the decrease in carbohydrate content was attributed by some investigators to be due to increased stress on organ, which lead to high energy consumption[10, 21], or may be due to the depletion of mucopolysaccharide in tissue which is attributed to the turbulence of Golgi apparatus [22] .

It is clear form this study that piroxicum has toxic effects on the liver tissue as represented by the observed histopathological damages.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Baghdad university, Iraq and all experiments were carried out in accordance with approved guidelines.

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