Histological Study on the Effect of Ampiroxicam Drug on liver of Females Mice

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Abstract
Thirty females' albino mice with average body weight of 25-30gm and 10-14 weeks old were used to investigate the toxicity of the oral administration of ampiroxicam on liver. The animals were given (single dose) of drug and were divided into three groups (10mice / group), control group were given distilled water, the two remaining groups (treated group2 were administered by 20 mg/kg of ampiroxicam for one month and treated group3 were administrated by20 mg/kg of ampiroxicam for tow month). The results showed that the changes in of tissue of liver in treated animals include: degeneration of hepatocyte and hepatic sinusoidal dilation, also lymphocyte infiltration, necrosis, dead cell, detachment of basement membranes and hypertrophy while there are no significant microscopic changes in control group1 (untreated animals). The increase in duration of the oral administration of ampiroxicam has increasing significant effect on liver of mice.

Keywords: Ampiroxicam, liver, hepatocytes, central vein , hepatic sinusoid, mice.

دراسة نسجية لتأثير عقار امبيروكسيكام على كبد اناث الفئران

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الخلاصة:
استخدمت (30) من إناث الفئران بمعدل وزن (25−30) غرام وعمر (10−14) أسبوع لتحري عن سمية عقار امبيروكسيكام. لدراسة تأثير العقار على الكبد, جرعت الاناث عن طريق الفم بواقع جرعة واحدة من العقار. قسمت الإناث إلى ثلاثة مجاميع (عشرة لكل مجموعة) وشملت مجموعة السيطرة اعطيت ماء مقطر, والمجاميع الاخرى (مجموعة معاملة 2 اعطيت 20 ملغم/كغم من عقار الامبيروكسيكام لمدة شهر واحد والمجموعة المعاملة 3 اعطيت20 ملغم/كغم من عقار الامبيروكسيكام لمدة شهرين). أظهرت النتائج تغيرات في انسجة الكبد في الحيوانات المعاملة وتتضمن تحلل خلايا الكبد , توسع الجيوب الكبدية كذلك نضوح الخلايا الكبدية, تنخر الخلايا وانفصال الغشاء القاعدي بينما لم تظهر عليها تغيرات مجهرية معنوية في مجموعة السيطرة 1 (الحيوانات غير المعاملة). الزيادة في فترة التجريع لعقار الامبيروكسيكام له زيادة معنوية في التأثير على الكبد الفئران

Introduction

Ampiroxicam, a prodrug of piroxicam, has been in use as a non-steroidal anti- inflammatory drug. In active ampiroxicam is hydrolyzed to active piroxicam by an intestinal carboxyesterase during absorption through the intestinal wall [1]. The chemical name of ampiroxicam is carbonic acid ethyl 1-[[2-methyl-3-[(2-pyridinyl) aminocarbonyl]-2H-1,2-benzothiazin-4-yl]oxy]ethylester,s.s,dioxide [2]. When administered to human, ampiroxicam will completely and rapidly convert to piroxicam, probably in the intestinal wall during the absorption process. Presumably because of the lower solubility of ampiroxicam compared to piroxicam resulted in slower dissolution in gastrointestinal tract before absorption [3].

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Ampiroxicam, droxicam and pivoxicam are prodrug of piroxicam that have been synthesized to reduce piroxicam related gastrointestinal irritation [4]. It adverse effects profile is expected to be similar to that of piroxicam [5]. Common side effects are heartburn, nausea, anorexia but it is less toxic than many other non-steroidal anti-inflammatory drugs (NSAIDs). It is useful as an analgesic and anti-inflammatory drug for rheumatoid, osteoarthritis, acute gout, dentistry and muscle injury [6]. Ampiroxicam may causes skin to become more sensitive to sunlight [7]. The aim of present study is to study of side effects and toxicity of ampiroxicam on liver

Materials and methods

Animal breeding:

Adult females mice approximately were (10-14) weeks old and their weights were around 25-30 gm, the animals were housed in standard plastic cage measuring 40×25×15 cm in animal house in Biology Department of College of Science in University of Baghdad under 12 hours light /12 hours dark at 21 ± 4 ^{o}c, were placed in each cage with wood shaving bedding, free access to food and water ad libitum was allowed all the time [8].

Treatment:

Ampiroxicam was obtained from pharmacy was used by oral administering of 0,01mg and rout of gavage with one concentration (20) mg/kg of ampiroxicam body weight of mice by aid of special needle [9].

Experimental design

Thirty female mice were divided into three groups:

group1: Ten female were given distilled water and served as control.

group2: Ten female mice were given a dosage (20) mg/kg of ampiroxicam for one month.

group3: Ten female mice were given a dosage (20) mg/kg of ampiroxicam for tow month.

Each female in these groups administered by ampiroxicam given once daily then liver of female examined.

Animals killing and specimens collection:

Females were euthanized with an overdose of ether an aesthesia, females placed on dissecting plate then the liver were removed and fixed in Bouins fluid for 12-24 hours then washed several times with ethanol 70% and kept until use [10]. For histopathology, processed routinely in histokinette, the thickness of slices was at seven micrometer [11] and stained with haematoxylin and eosin stain then examined under light microscope. Photographs were taken by digital camera.

Results and discussion

The results of ampiroxicam effect on the liver of female mice revealed in treated group2 showed that toxicity effect of ampiroxicam in more than in treated group1.

Control group1:

There are no significant microscopic changes in control (untreated animales). The liver of this group are normal in shap and size figure 1.

Treated group 2

It treated group of mice were given a dosage (20) mg/kg of ampiroxicam for one month, the results showed degeneration (loss of architectures), degeneration of hepatocyte and hepatic sinusoidal dilation figure 2, also lymphocyte infiltration and necrosis of the liver figure 3. In figure 4 and 5 showed hepatocyte degeneration, sinusoidal dilation and dead cell.

Treated group 3

It treated group of mice were given a dosage (20) mg/kg of ampiroxicam for two months, in this group, the results showed fatty change and congestion (figure6), also destroyed of hepatocyte, dead cell, detachment of basement membranes and hypertrophy figure 7-9.

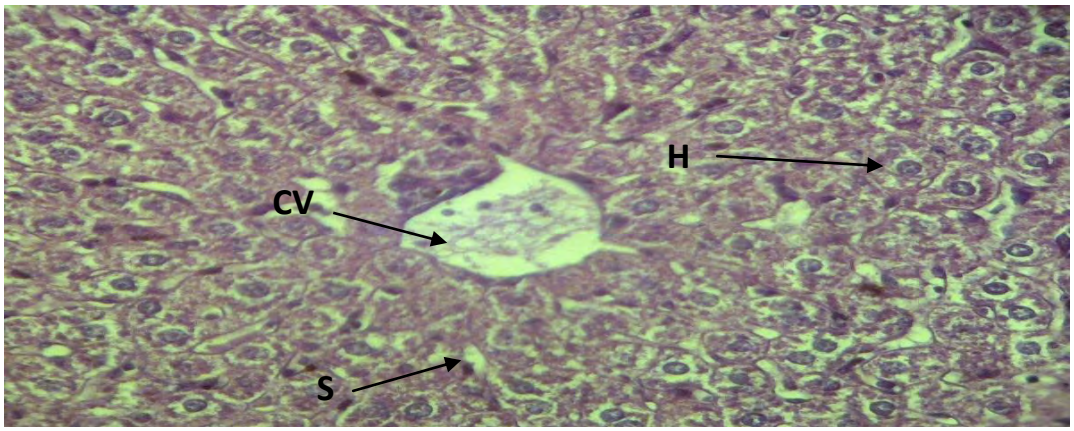

Figure 1- Cross section of liver (control group1) showing central vein (CV), hepatocyte(H) and hepatic sinusoid (S), (H&E) x40.

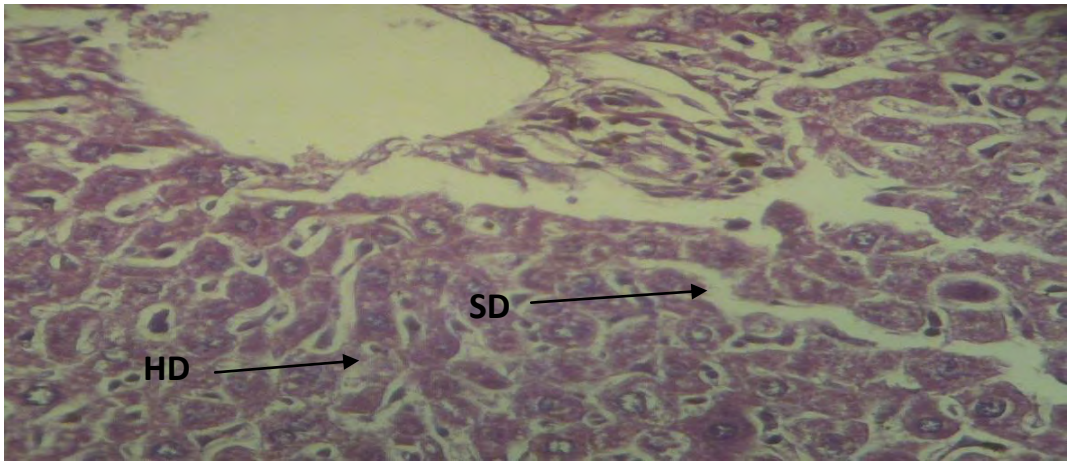

Figure 2- Cross section of liver (treated group2) showing hepatocyte degeneration (HD) and hepatic sinusoidal dilation (SD), (H&E) x40.

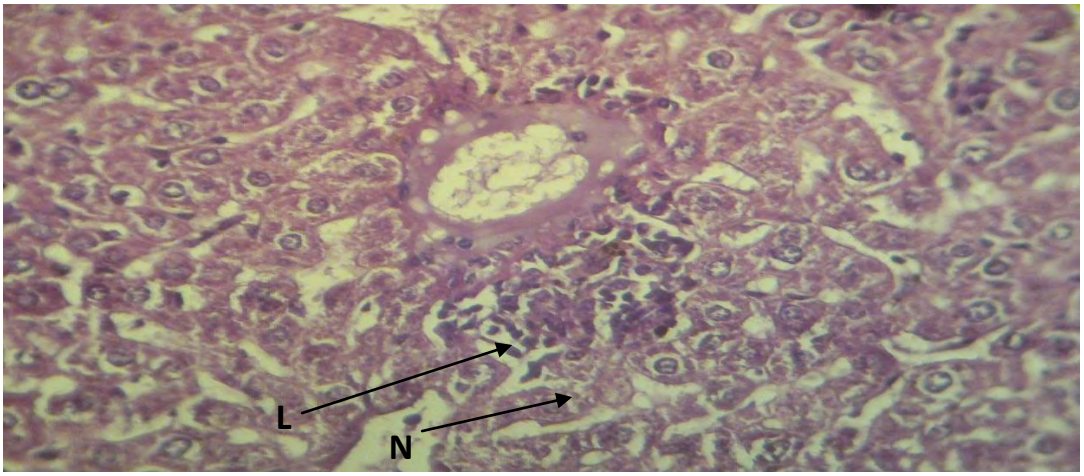

Figure 3- Cross section of liver (treated group2) showing lymphocyte infiltration (L) necrosis(N), (H&E) 40x.

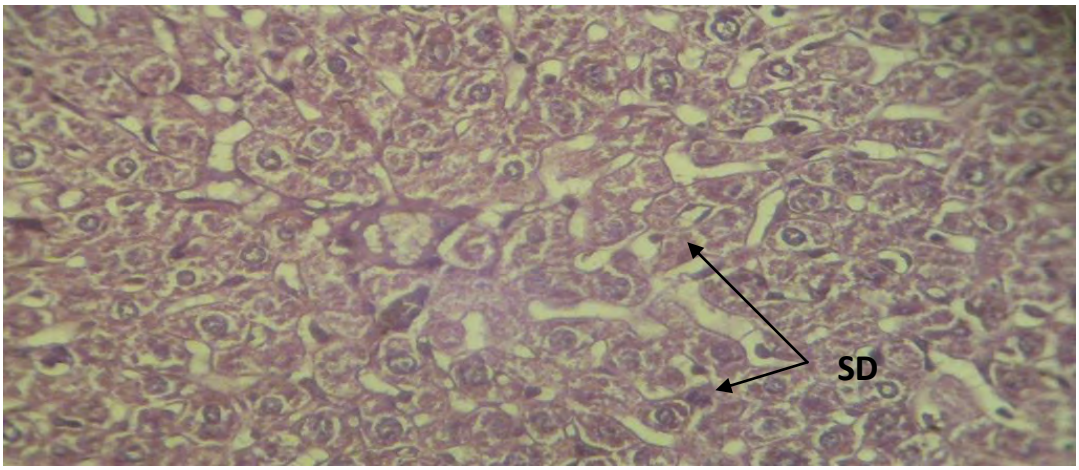

Figure 4- Cross section of liver (treated group2) showing disorganization and hepatic sinusoidal dilation (SD), (H&E) x40.

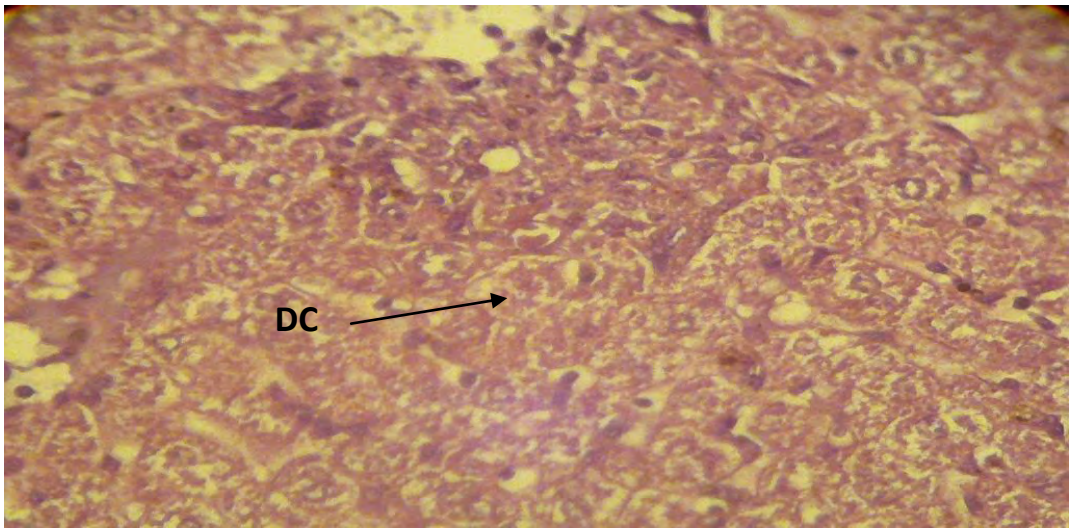

Figure 5- Cross section of liver (treated group2) showing degeneration (loss of architecture) dead cells (HD), (H&E) x40.

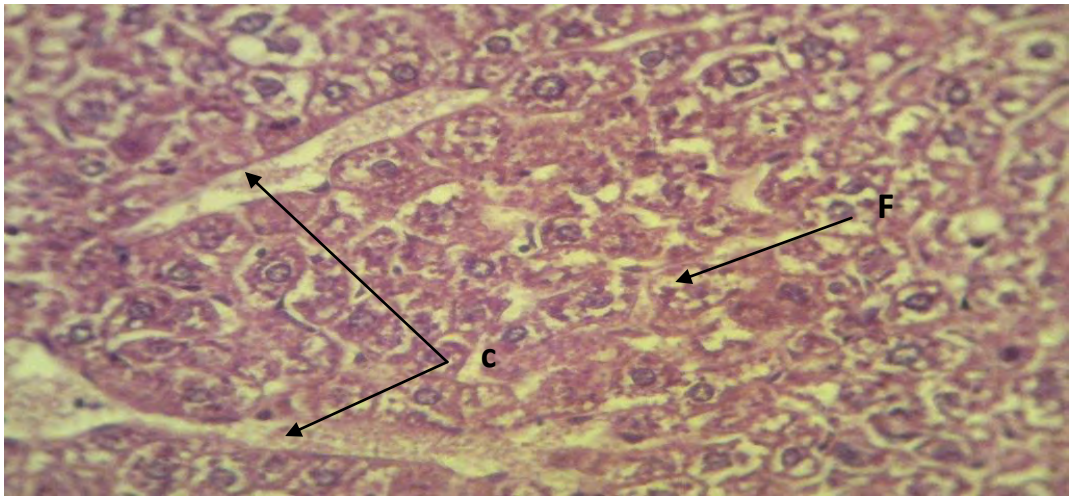

Figure 6- Cross section of liver (treated group3) showing fatty change (F) congestion (C), (H&E) x40.

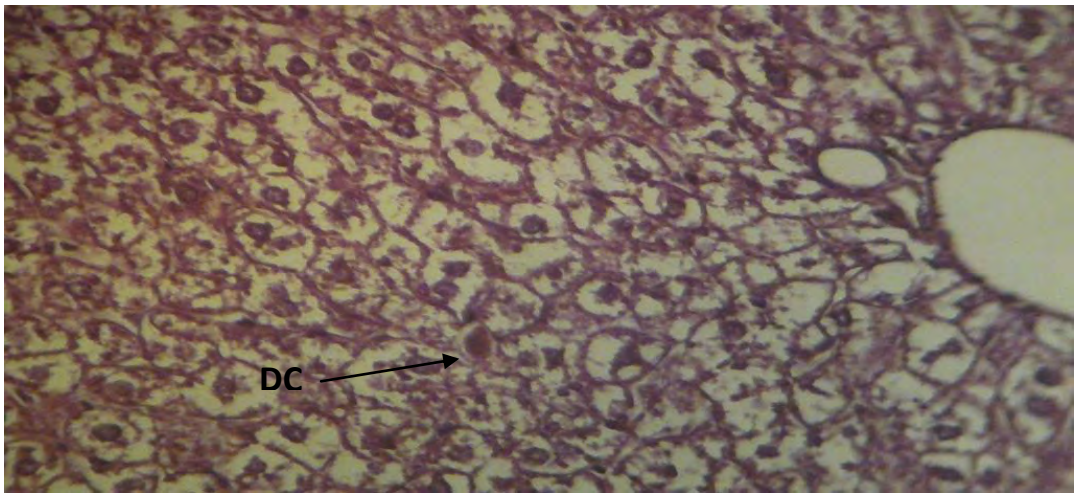

Figure 7- Cross section of liver (treated group3) showing destroyed hepatocytes and hepatic dead cell (DC), (H&E) x40.

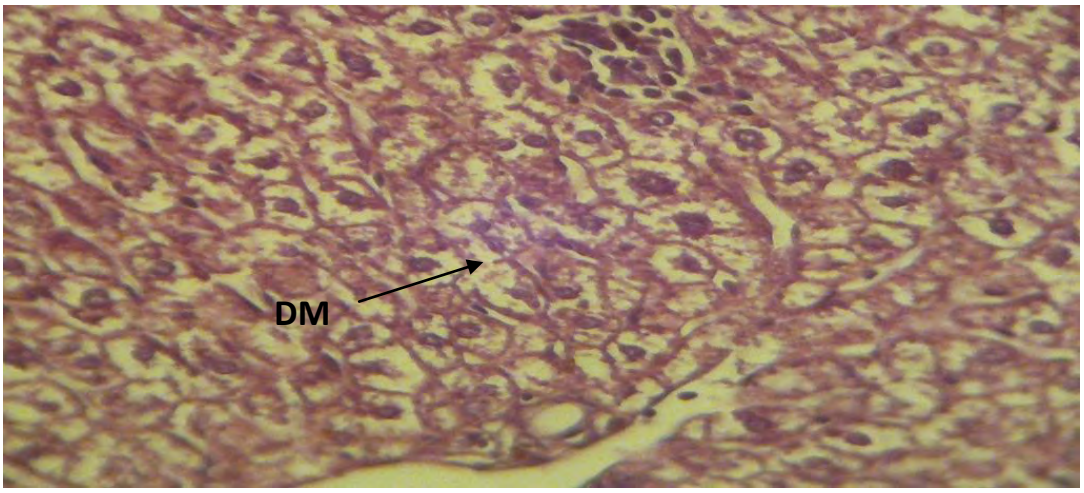

Figure 8-Cross section of liver (treated group3) showing degenerative and detachment of basement membrane of hepatocyte (DM), (H&E) x40.

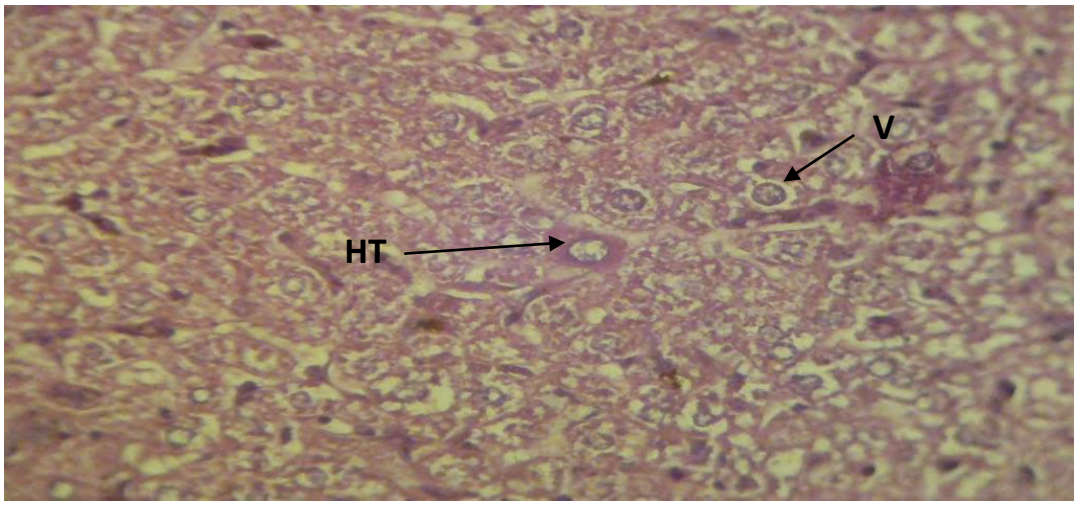

Figure 9- Cross section of liver (treated group3) showing disorganization, vacuolation and hypertrophy (HT) (H&E) x40.

Non-steroidal anti-inflammatory drugs (NSAIDs) are consumed massively worldwide and, along with antimicrobial agents, are the most frequent causes of drug-induced liver injury [12]. Indeed, roughly 10% of total drug-induced hepatotoxicity is NSAIDs related [13]**.** The mechanism of

oxicams-induced hepatotoxicity appears to be idiosyncratic and dose independent. Due to the absence of immunoallergic features in most of the reported cases, it is very difficult to support an immune-mediated mechanism of liver injury [14].

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit cyclooxygenase (COX)-dependent synthesis of prostaglandins that mediate inflammatory responses in multiple tissues [15]. NSAIDs also induce cell growth inhibition, apoptosis, and antiangiogenesis; activation of the pathways involved in these effects is critical for the reported anticarcinogenic activities of NSAIDs and related COX-2 inhibitors, such as celecoxib[16]**.** On the other hand, several non-steroidal anti-inflammatory drugs have been associated with liver damage [17]. Many NSAIDs have been withdrawn from the market because of adverse hepatic drug reactions. Some cases of liver disease have been reported in patients taking piroxicam [18]. Also the pathological changes may lead to impaired liver function which interferes with the secretion of plasma proteins [19]. This leads to decreased blood osmotic pressure, with subsequent decreased drainage of tissue fluids, which explains the oedema and congestion observed in the different tissue.

Also the Results showed a remarkable cellular infiltration in the hepatic tissue. This supports El-Banhawy *et al*. [20] whose studies suggested that abundance of leucocytes, in general, and lymphocytes, in particular, are a prominent response of body tissues facing any injurious impacts. Leukocyte elevations and adherence to the vascular endothelium have been suggested by Miura et al. [21] and McCafferty *et al*. [22] to play an important role in the pathogenesis of NSAID-associated injury. The results of the present study showed cytoplasmic vacuolation and liver damage, these result agreed with Zhang and Wang [23] suggested that the cytoplasmic vacuolation is mainly a consequence of considerable disturbance in lipid inclusions and fat metabolism occurring during pathological changes. The cross section in liver of the treated mice which were treated with 20 mg/kg of ampiroxicam showed degenerative, necrosis and sinusoidal dilation with certain loss of architecture of liver tissue, Paterson *et.al.*[24] mentioned that the piroxicam may induce submassive necrosis of the liver, probably as an idiosyncratic reaction.

Also results showed cell death and damage of plasma membrane, Butterworth *et al*., [25] 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 [26]. Rubbins, [27] reported that biotransformation of some drug and chemicals make them to be converted to reactive toxic metabolite which then act on the target cells; usually these target cells were mostly liver cells since the modification of drug and chemical is accomplish by cytochrome P 450 in smooth endoplasmic reticulum of liver than other organs, these toxic substances may stimulate the damage of plasma membrane. The 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 components of these cells and this swelling may mostly due to accumulation of water inside the cell [28].

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