Original Article

Philosophical an histological assessment of diclofenac sodium on the liver of local rabbits

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DOI: 10.47750/pnr.2022.13.S06.336

Abstract

The current Study was done to show the physiological and histological defect at different doses of diclofenac sodium on livers of Rabbits. Twenty rabbits was divided into four groups the therapiutic group (T) , over does group1 (T1), over dose group2 (T2) and control group each group was injected intraperitoneally by (2 , 20 , 50 mg/km) respectively and Distilled water, respectively for 14 days, at the fourteen day of experiment all animals were sacrificed, blood sample collected and liver was fixed immediately in 10% formalin for histological preparation and obtained serum for physiological test. There was no significant difference at the level of (0.05 <P) in the effectiveness of the Alanine aminotranseferase (ALT) and Aspartate aminotranseferase and gamma-glutamate transferase (GGT) in the serum of male rabbits in the T group and T1 group compared to the control group. Microscopic examination of the liver tissue in T group showed the presence of degeneration of hepatocytes and swelling of hepatocytes. showed edematous cells, infiltration of inflammatory cells in sinusoids in T1 group. In T2 group it showed colloid appearance in blood vessel, disappear of sinusoid, cell degeneration, degeneration of tunic cells of blood vessels.

Keywords: Diclofenac sodium, diclofenac sodium.

INTRODUCTION

There are a number of mechanisms in which pain is treated, especially chronic and persistent, through which pain becomes chronic and an ongoing problem. Multimodal and specialized treatment models are used to treat it, including multiple categories of medications known as Analgesics (1).

Diclofenac continents are a non-steroidal anti-inflammatory drugs that are prepared by mixing it with sodium, potassium, or ipuramin salts. Diclofenac sodium can be given orally in tablet or suspension, intramuscularly in the form of a solution, also intravenously as a solution, through the skin in a gel, or by rectum as a suppository, while potassium diclofenac is given orally in tablet or suspension. Diclofenac Ipuramin is available in the form of transdermal adhesive pieces (2).

Diclofenac sodium (DS) is one of the most common non-steroidal anti-inflammatory drugs that was synthesized in 1973 and is an effective anti-inflammatory drug and one of the most prescribed drugs worldwide (2,3). It is approved by the US Food and Drug Administration (FDA) and is used in the treatment and control of acute and chronic pain associated with inflammatory conditions, especially those involving the musculoskeletal system such as osteoporosis and rheumatoid arthritis, but it cannot prevent the chronic joint damage that appears with these diseases (4).

Diclofenac sodium has antipyretic and analgesic effects at the same time and is found mainly in the form of the sodium salt which is derived from Benzeneacetic acid with anti-inflammatory properties. By inhibiting cyclooxygenase (COX), which binds to both forms of these enzymes represented by (COX-2-1) (COX), it prevents the conversion of arachidonic acid to prostaglandins that promote inflammation through the chelation mechanism (5,6), meaning that the main role of diclofenac sodium is to inhibit these cyclic enzymes (7).

The mechanisms of diclofenac-induced toxicity involve mitochondrial dysfunction and production of pro-oxidant radicals when metabolized by peroxidase (8,9). This study aims to studying the effect of drug overdose on liver function and estimating the level of ALT, ALP, and GGT. Doing tissue sections of the liver and in order to reveal the effect of treatment on the histological structure of these organs.

Materials and Methods:

Experiment Animals

This study was done in medical laboratory department of biology/ Education college/ university of Samarra. Twenty-three male white rabbits,. were employed, weighing (1.086 – 1.500)g obtained from college of medicine, Tikrit university. They were leaved for preparation before experiments, maintained on 12:12 light: dark bases, and 24 ±2°C with mouse pelleted food and water ad libitum. rabbits were housed in group not bigger than five animals (all from the same experimental group) in placed in wooden cages with metal covers, with wood, Twenty male albino mice were randomly divided into control (n =5) and experimental (n =15) groups. The experimental groups are subdivides into three groups of rabbits, each once is injected Intra Peritoneum. with different doses of Diclofenac sodium (DS) once daily for 14 days

Drug administration

Diclofenac sodium (DS) ample 200 mg/2ml. Female were injected daily Intra Peritoneum (I.P.) administrated in three doses: Therapeutic dose, over dose T1 and over dose T2 (2, 20 and 50) mg/kg for 14 days respectively, and Control group were injected with normal saline 0.9 mg/ L.

Collection of blood sample

At the day fourteen the blood samples were collected directly from the heart by medical syringes and emptied into tubes containing gelatin with a yellow cover (Gel tube) used for one time, in order to separate the serum from the blood and left for about a quarter of an hour at room temperature until blood clotting, and then it was Serum was separated by centrifugation at 3000 rpm for five minutes, after which biochemical tests were performed.

Histological preparation

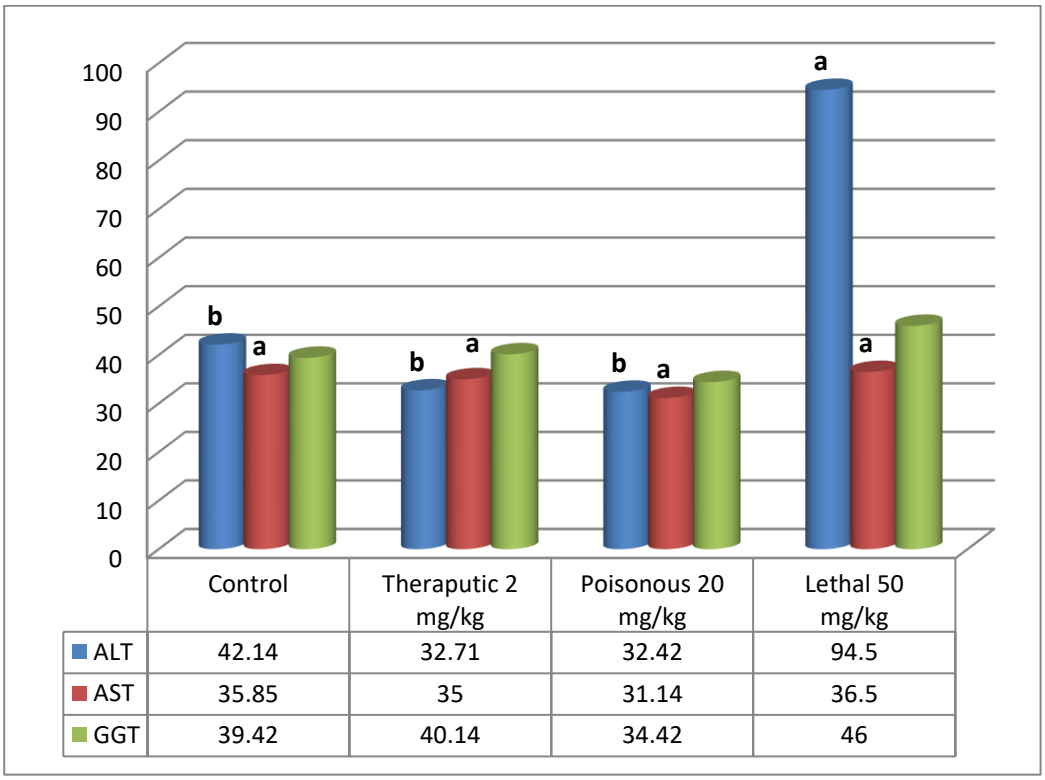
The collected tissues Each segments of skin was taken and immersed in 10 % formalin foe 24 hours followed by immersion in graded series of alcohol from 70, 80, 90 and 100 %, then clearing with xylene and embedded in paraffin wax at 60 c^o. Blocking of the samples were done and the sectioning were performed using a rotary microtome. The thickness of the sections were 6 micrometer. The tissue sections after application of staining with Hematoxylin and Eosin were mounted on the slides using D.P.X and covered by cover slides. The slides were examined using light microscope and photographed by manipulated camera prepared for this purpose.

Biochemical tests

The level of the Aspartate Aminotransferase (AST) enzyme in the blood serum was estimated using the Kit analysis kit equipped by the British company RANDOX (1957, Reitman and Frankel). The level of the enzyme Estimation of Alanin Aminotransferase (ALT) and Glutamine transferase (GGT) in the blood serum was estimated using the ready-made analysis kit (Kit) from the British company (RANDOX), (1957, Reitman and Frankel).

Results:

The results in Figure (1) showed no significant difference at the level of (0.05 <P) in the effectiveness of the Alanine aminotranseferase (ALT) and Aspartate aminotranseferase and gamma-glutamate transferase (GGT) in the serum of male rabbits in the therapeutic and toxic group compared to the control group.



	Control	Theraputic 2 mg/kg	Poisonous 20 mg/kg	Lethal 50 mg/kg
ALT	42.14	32.71	32.42	94.5
AST	35.85	35	31.14	36.5
GGT	39.42	40.14	34.42	46

Figure (1): Effect of diclofenac the concentrations of antioxidant enzymes (AST, ALT, GGT) in the serum of male rabbits compared to control group.

Microscopic examination of the liver tissue in Figure (2) showed the presence of degeneration of hepatocytes and swelling of hepatocytes as a result of the drug used in T group.

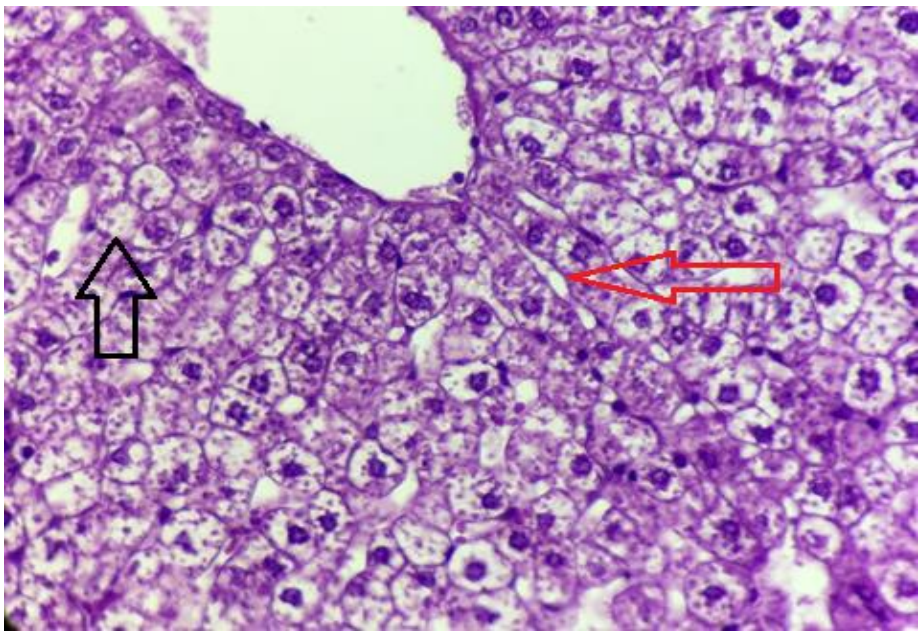


Figure (2) A cross-sectional image of the liver T group, swelling of hepatices (red arrow), degeneration of hepatocyte (black arrow) (H&E, X40)

Figure (3) showed edematous cells, infiltration of inflammatory cells in sinusoids in T1 group.

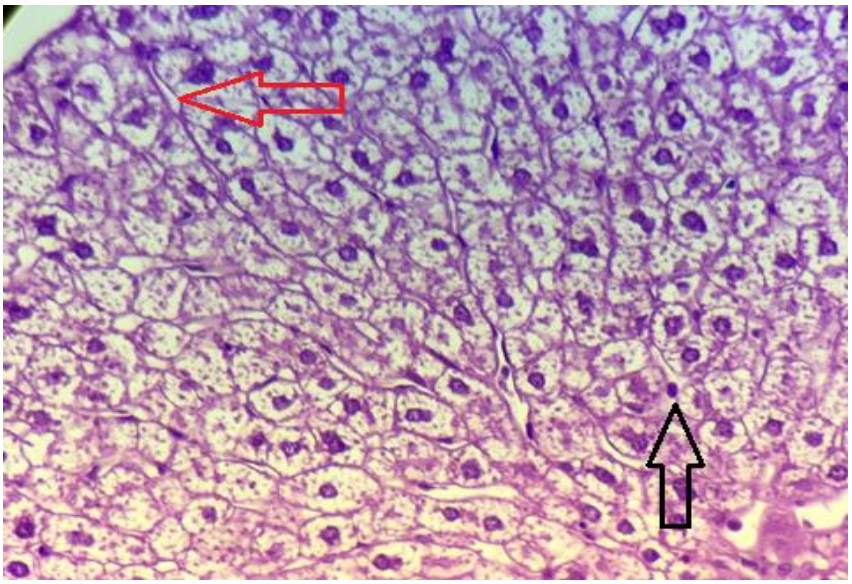


Figure (3) of T1 group of rabbit male liver, shows edematous cells (red arrow), infiltration of inflammatory cells in sinusoids (black arrow). (H&E, X40)

Microscopic examination in Figure (4) showed colloid appearance in blood vessel, disappear of sinusoid, cell degeneration, degeneration of tunic cells of blood vessels.

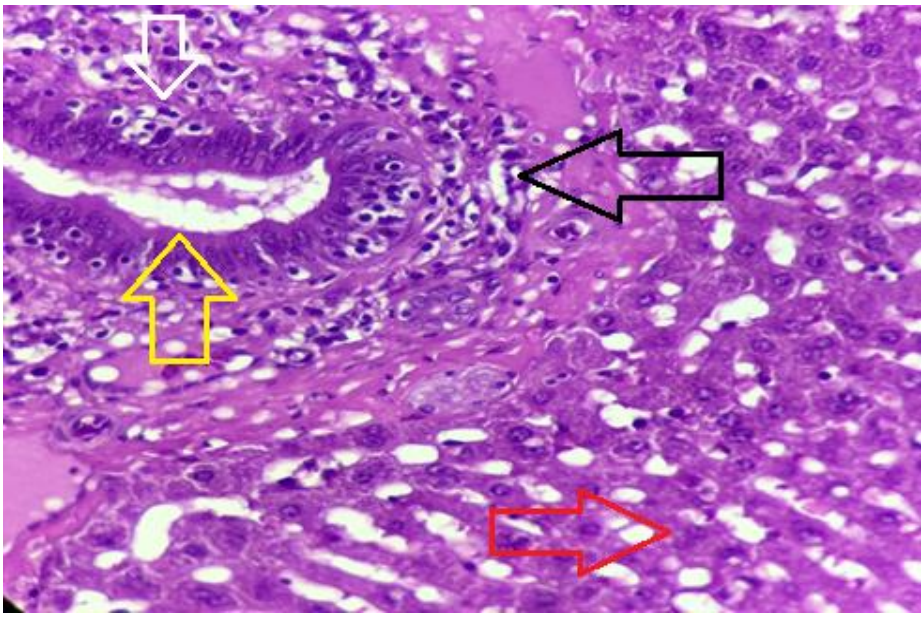


Figure (4) : liver of male rabbits T2 group shows colloid appearance in blood vessel (yellow arrow), disappear of sinusoid (red arrow), cell degeneration (white arrow), degeneration of tunic cells of blood vessels (black arrow) (H&E, X40)

Discussion:

It has been revealed that almost all NSAIDs cause elevated levels of aminotransferases that are not clinically relevant and return to normal when treatment is discontinued. By contrast, the dose caused a significant increase in ALT levels that remain high until the 10th day. This was consistent with (10,11) who found a high level of ALT with diclofenac in mice, rabbits, and cats respectively. This continuous increase in the level of ALT can be attributed to liver toxicity caused by diclofenac, where high ALT is directly related to liver cell cytoplasm or due to unstable diclofenac receptors that may bind to cell proteins leading to direct toxicity of liver cells (12). The results showed no significant difference in the level of the aspartate amine transport enzyme AST and gamma glutamyl GGT in dosed groups compared to the control group.

The extent to which kidney and liver tissue is generated as a result of increased concentrations of MDA in renal and hepatic tissue, resulting in necrosis and back leakage of liver enzymes and kidney function into circulation.

Hussain (13) indicated in his results that compared to ALT values, AST values were lower so that the AST-ALT ratio was determined lower. Increased AST levels may be due to the oxidative load of drug metabolism in the liver (13,14). Hepatic toxicity associated with NSAIDs is caused by inhibition of metabolism, oxygen root toxicity, or immune damage leading to a predominant increase in aminotransferases. These results are in line with (15).

These results are consistent with what Somchit et al., (16) indicated when using low doses of indomethacin do not significantly affect liver tissue, and that increased effect is associated with increased dose. Histological effect in general consisted of the accumulation and leaching of lymphocytes, hepatic platelet hyperplasia, cell degeneration with spherical necrosis in cells and blood vessel walls, the appearance of cells with donor cytolysis, and erythrocyte decomposition.

That the proportion of these lesions increases with the increase in the dose given, consistent with what the researchers (17,18) indicated that therapeutic doses of the drug can cause liver damage when the drug is used for long periods. Hussam et al., (19) also agree with the appearance of tissue avas similar to the results of the current study when injecting white mice with Piroxicam. He also explained that the increase in the pathological effect of the drug increases with the length of time to exposure to the drug.

Hussam et al., (19) caused the explosion in the liver tissue to be caused by the drug's obstruction of mitochondria and thus the accumulation of many fatty vesicles, causing the phenomenon of phage. Hirak (20) also explained that non-steroidal drugs have the potential to disrupt the permeability of the plasma membrane to drugs, especially potassium infusion channels, and thus affect the movement of calcium ion and the production of ATP metabolic energy. Hepatitis caused by drugs is the most common. Inflammation arises from toxic chemicals produced by the body during its analysis of drugs.

That a high dose of diclofenac in mice causes the infiltration of mixed cells as well as the proliferation of the bile duct in the gate area and the degeneration of sperm cells in the liver. Nonsteroidal anti-inflammatory drugs (NSAID) are the cause of liver damage. Diclofenac is usually associated with hepatic toxicity (21). Oxidative stress has an important responsibility in the pathophysiology of tissue damage. Similarly, diclofenac has been shown to cause oxidizing injury to the liver (22). Diclofenac causes bio activation of reactive intermediate metabolites 4-OH and 5-OH diclofenac, after metabolized by CYP2C9 and CYP3A4 enzymes (23). Diclofenac metabolites can cause apoptosis of liver cells. Lar revealed that liver injury in mice treated with diclofenac was characterized by hepatic degeneration, necrosis, vasodilation, lobula congestion, enlarged gate areas, and access to mixed inflammatory cells in the dead liver cell region and gate area (24).

Mitochondria are essential organelles in cell balance. It is therefore a potential target for drug toxicity. Mitochondria dysfunction is the main event in the pathogenic chain, leading to cell death caused by ischemia from both necrosis and apoptosis (25). Ultra-brown morphological changes to the mitochondrial form represented by swelling, polymorphism, destruction of Christy, and condensation of its matrix caused by the treatment of diclofenac. Liver cell injury involves damage to the lysosome membrane and the release of protein-desed enzymes such as Cathepsins (B, D, and L), which promotes the opening of mitochondrial transmittance transmission (MPT) and the release of cytochrome C. This process then begins events that activate caspase-3 and apoptosis (26).

Conclusions:

There was no significant difference at the level of (0.05 <P) in the effectiveness of the Alanine aminotranseferase (ALT) and Aspartate aminotranseferase and gamma-glutamate transferase (GGT) in the serum of male rabbits in the therapeutic and toxic group compared to the control group. High dose of diclofinac in mice causes the infiltration of mixed cells as well as the proliferation of the bile duct in the gate area and the degeneration of sperm cells in the liver.

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