Philosophical anhistological assessment of diclofenac sodium on the heart of local rabbits

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Abstract

The current Study was done to show the physiological and histological defect at different doses of diclofenac sodium on heart of Rabbits. Twenty rabbits was divided into four groups the therapeutic group (T), over does group1(T1), over dose group2 (T2) and control group each group was injected intraperitoniali by ( T,T1 ,T2 ) mg/kg and Distiled water, respectively for 14 days, at the fourteen day of experimental animals were sacrificed, blood sample collected and heart was fixed immediately in 10% formalin for histological preparation and obtained serum for physiological test the result was revealed T group significant decreases in lipoproteins so as to significant of low density-lipoproteins on ( p < 0.05 ) (VLDL-C). on the other hands the results showed significant differences on level of creatine kinase, troponin and lactate dehydrogenase, and in T2 group significant decreases of creatine kinase, troponin and lactate dehydrogenase on (P < 0.05 ) compared with control group. The histological examination of T groups was revealed degeneration in muscles fibers, zonal vacuolation around the nucleus of muscle fiber the result of T2 groups showed, atrophy in muscle fibers degeneration was appear of whole section spread out at inflammatory cells in between muscle fibers and zonal vacuolation around nucleus of muscle fibers. The results obtained from T2 groups was show atherosclerosis on marginal blood vessels of heart, hemolysis of RBCs with spread out of hemocidrin out 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 specialised 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 ipulamine 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 Ipolamine 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 (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).

High doses of diclofenac cause marked histological changes in body organs. The mechanisms (5) of diclofenac-induced toxicity involve mitochondrial dysfunction and production of pro-oxidant radicals when metabolized by peroxidase (6). This study aims to studying the effect of drug overdose on cardiac function by estimating the enzymes LDH and troponin, as well as Estimation of troponin I, Creatine Kinase MB level in serum was also done. Estimation of lactate dehydrogenase (LDH), Triglycerides, High density lipoprotein (HDL), Cholesterol, Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) levels in
serum was performed. Making tissue sections for each of the heart, liver and kidneys in order to reveal the effect of treatment on the histological structure of these organs.

Materials and Methods:

Experiment Animals

The study was done at Department of Biology \ College of Education \ Samarra University. Twenty-three male white rabbits, weighing (1,086 - 1,500) g. in animal house in the under temperature conditions, and were fed on the ration (35% wheat, 35% maize, 20% soybean, 10% animal protein concentrate, in addition to vitamins, preservatives and anti-fungal materials) and were given water and food continuously throughout the duration of the experiment.

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. 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 and50) mg/kg for 14 days respectively [11], 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

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°. 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 [9]. The slides were examined using light microscope and photographed by manipulated camera prepared for this purpose.

Biochemical tests

Estimation of troponin I in serum is carried out using a cassette for the determination of cardiac troponin I, supplied by Med Net GmbH (Germany). Estimation Of Creatine Kinase MB Level In Serum was also done. Estimation of lactate dehydrogenase (LDH), Triglycerides, High density lipoprotein (HDL), Cholesterol, Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) levels in serum was performed.
Results:

The results of the current study in Figure (4-1) showed a significant increase at the level of \((0.05 < P)\) in the concentration of total cholesterol in male serum compared to other groups. Figure (4-1) also showed the absence of a significant difference at the level of \((0.05 < P)\) in the concentration of triglycerides in the serum of male rabbit. While there was a moral decrease in the concentration of high lipoproteins in male rabbits dosed with the drug compared to the control group.

The results in Figure (1) also showed no significant difference at the level of \((0.05 < P)\) in the concentration of very low-density lipoproteins of VLDL-C cholesterol in the serum of male rabbits dosed compared to the control group.

![Graph showing fat standards in male rabbits](image)

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>Therapeutic 2 mg/kg</th>
<th>Poisonous 20 mg/kg</th>
<th>Lethal 50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>113.28</td>
<td>39</td>
<td>68.71</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>104.42</td>
<td>91</td>
<td>102.28</td>
</tr>
<tr>
<td>HDL-C</td>
<td>23.14</td>
<td>18</td>
<td>25.42</td>
</tr>
<tr>
<td>LDL-C</td>
<td>93.71</td>
<td>9.14</td>
<td>24.14</td>
</tr>
<tr>
<td>VLDL</td>
<td>20.71</td>
<td>18.14</td>
<td>20.28</td>
</tr>
</tbody>
</table>

Figure (1): Effect of treatment with diclofenac (2, 20 and 50 mg/kg bw) on the level of fat standards in the body of male rabbits.

The results in Figure (2) showed a significant increase in the levels of creatine kinase, troponin and lactate dehydrogenase in the groups treated with the therapeutic dose and the toxic dose of the drug compared to the control group. While the results of the third group treated with the lethal dose showed a significant decrease in the concentration of creatine kinase, troponin and lactate dehydrogenase compared to the control group, as shown in Figure (2).
Figure (2): Effect of diclofenac on the concentrations of (CKK, LDH, TROP) in the serum of male rabbits compared to the control group.

The histological examination of therapeutic group T shows, degeneration in muscle fibers, zonal vacuolation around cardiac around nucleus, vacuolation cytoplasmic, and pyknotic nuclei figure of muscle fibers (3).

![Histological image](image1)

Figure (3) A transverse section of male rabbit heart of T group: shows the degeneration in muscle fibers (yellow arrow), zonal vacuolation around cardiac around nucleus (red arrow), vacuolation cytoplasmic (white arrow), and pyknotic of nuclei (black arrow).(H&E, X40)

Microscopic examination of T2 group showed the atrophy in muscle fibers, degeneration in cytoplasm of whole section, infiltrated of inflammatory cells in between muscle fibers, zonal vacuolation around the nucleus of muscle fiber, fig (4).
Figure (4) A cross-section of male rabbit heart treated with the T2 group shows: atrophy in muscle fibers (yellow arrow), degeneration in cytoplasm of whole section (red arrow), infiltrated of inflammatory cells in between muscle fibers (white arrow), zonal vacuolation around the nucleus of muscle fibers (black arrow), (H&E, X40).

Microscopic examination of the T2 Shows: atherosclerosis of the blood vessels (yellow Arrow), hemolysis RBCs spread out of hemosiderin, fig (5)

Figure (5) transverse Section of the heart of male rabbits T2 Shows: atherosclerosis of the blood vessels (yellow Arrow), hemolysis RBCs spread out of hemosiderin (red Arrow), (H&E, X40).

Discussion:

The high concentration of total cholesterol is caused either by the effect of oxidative stress and some disorders in the fat metabolism, leading to the occurrence of lipid oxidization and unsaturated fatty acids, which leads to some disturbances in digestion and absorption processes in the intestines due to inhibition of the secretion and excretion of steroids and bile salts (7).
While in the group of rabbits they are treated with diclofenac sodium and in doses (2, 20 and 50 mg/kg), respectively, which were classified as therapeutic, toxic and fatal, respectively. The results of male rabbits showed a significant decrease in the concentration of total cholesterol compared to the control group. The results in Figure (1) also showed no significant difference at the level of (0.05 <P) in the concentration of very low-density lipoproteins of VLDL-C cholesterol in the serum of male rabbits dosed compared to the control group. These results were similar to those of Al-Badrani and Nasser (8), and it was also noted that the drug reduced the concentration of total cholesterol and LDL.

The drug has beneficial effects, especially on harmful LDL. This effect can be attributed to the mechanism of action in reducing cholesterol production by reducing the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMGRs) in the liver (9). HDL levels and triglycerides were not affected by treatment using the drug in agreement with the study conducted by Gopi (10).

Diclofenac has a detrimental effect on the liver, leading to a significant change in total cholesterol, triglycerides, HDL, LDL, and VLDL. These results are consistent with those of Baravalia et al. (11), a marked increase in blood triglycerides and cholesterol was observed in dysclovinac-treated mice. This finding was consistent with Ahmed et al. (12), when hepatic toxicity caused by drugs was found to be associated with high levels of cholesterol and triglycerides. Features of blood lipids, triglycerides (TGs), total cholesterol (TCh), low-density lipoprotein (LDL-C) as well as low high-density lipoprotein (HDL-C) in response to diclofenac toxicity.

Study with findings from researchers Oda and Derbalah (13) in their study on the effect of diclofenac sodium on acute cardiotoxicity in male rats. The increase in the therapeutic and toxic groups may be due to the effect of diclofenac sodium on the myocardium, which leads to arterial thrombosis and myocardial infarction, and thus the activity of LDH enzyme increases several times more than the normal rate.

CK-MB is the subtype of creatine kinase that has a vital role in cell energy metabolism and is a specific sign of severe heart damage. CK stimulates the transfer of phosphate to adenosine diphosphate, and produces adenosine triphosphate, which acts as an energy source for many tissues, including muscles. Three different enzymes of CK have been identified: CK-MM, CK-BB, and CK-MB. CK-MB can be replanted as a sensitive and specific marker of myocardial infarction (14). While LDH and AST are adventurously undefined signs of heart damage, so they are usually estimated alongside specific signs such as CK-MB and Troponin I. The increase in LDH activity that plays an important role in mesenchymal metabolism refers to a link between amino acid metabolism and the citric acid cycle where lactate is converted into pyruvate due to the role of diclofenac in increasing the activity of the enzyme LDH (15,16).

regulation of the expression of bcl-2 and increased activities of caspase-3 in the heart muscle (17). Caspase-3 is an executioner involved in the process of apoptosis. Various apoptosis stimuli to caspase-3 lead to the cleavage of the protein analyzer in other caspases and internal protein substrates. The Caspase-3 estimate allows for a detailed investigation into the mechanisms of apoptosis (18). It is well known that mitochondrial dysfunction is associated with cell necrosis and apoptosis. Bel-2 proteins are closely related to the mitochondria membrane and are considered a gatekeeper for programmed response. Bel-2 proteins determine the result of substantial apoptosis that began with the release of cytochrome c and programmed agents such as caspase-9 and caspase-3 of mitochondrial. The onset of apoptosis can be attributed to excessive obstetrics of ROS (19). Similarly, Jane and others have documented that isopropyl-lartyrinol causes damage to the heart muscle through apoptosis of cardiac muscle cells (20).

The results of our study agreed with those of both (21). Where they proved that cardiovascular side effects can occur when diclofenac is used. Users of the drug may be at risk of producing congestive heart failure and oedema, as well as high blood pressure, arterial thrombosis, and myocardial infarction.

Conclusions:

Treatment with Diclofenac sodium led to an increase in both fat and enzymes of heart function. Cardiovascular side effects can occur when diclofenac is used. Users of the drug may be at risk of producing congestive heart failure and oedema, as well as high blood pressure, arterial thrombosis, and myocardial infarction.
REFERENCES


