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Biochemical and histological evaluation of diclofenac sodium induced acute hepatotoxicity in rats

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Abstract:

This research aimed at evaluating the effect of diclofenac sodium in the live injury in rats. Hepatic injury induced by injection of diclofenac sodium (50, 100 and 150 mg/kg via i.p.) for 14 days. The results showed significant increase in the liver enzymes levels (aspartate transaminase AST, alanine transferase ALT and alkaline phosphatase ALP) in all the groups of rats treated with doses (50, 100 and 150 mg/kg via i.p.) and the histopathological change in the liver appear cellular degeneration, necrosis, dilation of blood vessels, congestion in the lobules, enlargement of portal areas and infiltration of inflammatory cells around the necrotic hepatocytes and the portal area. In conclusion, the present study proved that acute hepatic injury induced by diclofenac sodium which looked as increase in liver enzymes levels and histopathological changes in the liver tissues.

Key words: Diclofenac sodium, AST, ALT and ALP.

INTRODUCTION :

Hepatotoxicity is an antagonistic medication response related with nonsteroidal mitigating drug (NSAIDs) utilize. In spite of the fact that its event is less basic contrasted with other (NSAIDs) related complexities, hepatoxicity is recognized as the basic reason withdrawal of a few NSAIDs. (1)

Diclofenac is aphenlacetic corrosive subsidiary that was produced as anon-steroidal mitigating (NSAIDs) sedate. It contends with arachidonic corrosive for official to cyclo-oxygenase (COX), bringing about diminished arrangement of prostaglandins. This reduction impact in any event halfway clarifies the instrument of activity of the drug (2,3).

Diclofenac has power against COX-2 which is considerably more prominent than that of indomethacin and different NSAIDs. The selectivity of diclofenac for COX-2 looks like that of celecoxib. Likewise, it seems to lessen intra cell groupings of free amino corrosive in leucocytes, maybe by adjusting its discharge or take-up. (4).

Furthermore, diclofenac is effectively assimilated from the gastrointestinal tract, top plasma focus happen 1.5-2 hours after ingestion in fasting subjects. Diclofenac is metabolite in liver into 4-hydroxydiclofenac and other hydroxylated frames, after glucuronidation and sulfation the metabolites are discharged in the pee (65%) and bile (35%) (5).

Diclofenac is related with extreme gastrointestinal poisonous quality and a few unfriendly consequences for lung, hepatic and renal tissues (6,7). In this investigation the impact of diclofenac sodium (infusion) at various dosages on some biochemical and histopathological examinations of liver in pale skinned person rats were considered. Additionally, we examined the conceivable linkages between tranquilize instigated cell harm to layout the systems of hepato-danger of the medication. In addition, we distinguished whether the antagonistic impacts prompted by diclofenac sodium were reversible or not.

MATERIALS AND METHODS:

Drug: diclofenac sodium (voltaren) ampoules contain 75 mg diclofenac sodium is produced by Novartis Pharma company Switzerland.

Determination of liver enzymes

A-Determination of Serum Transaminase Activity Transaminases – Kit

ALT & AST activity were determine by colorimetric method according to the bio-labo kit,france (8).

B-Determination of Serum Alkaline Phosphatase Activity

Colorimetric determination of ALP according to biomerieux kit (9).

Experimental animals:

Using 20 adult male rats (*Rattus norvegicus*) weighting 200-250 gm were obtained from the animals house in Faculty of Science, University of Kufa. The rats were kept in animal house for acclimation to the laboratory conditions for one week before they were used for the experiment under standard environment conditions (temperature 25-28 C° and 12 h light-dark cycle) and allowed access to standard laboratory diet and water.

Experimental protocol

The rats were divided into four groups each group was formed five rats and the rats were treated as following for 14 days :

Group (1) rats were injection of normal saline.
Group (2) rats were injection of diclofenac sodium at dose 50 mg/kg.
Group (3) rats were injection of diclofenac sodium at dose 100 mg/kg.
Group (4) rats were injection of diclofenac sodium at dose 150 mg/kg.

Blood Collection

At the end of experiment. Each animal was anaesthetized by the mixture of xylazine 0.1 ml and ketamine 0.5 ml and they were scarified (10). Heart cut was finished with a 5 ml expendable syringe and 2-5 ml blood was drawn delicately and gradually. The blood was put in test tube containing gel and left for 30 minutes in room temperature and used to get serum through centrifugation at 3000 rpm for 15 minutes to separate serum and put in epindroff tubes which kept at (- 20) in a cooler for assurance biochemical examination and the abdomen was opened to get the liver and put immediately in the formalin 10% for tissue processing in compound microscope (11) for examination under the Light microscope.

Statistical Analysis:

Data were presented as means ± S.E. and statistically analyzed using (ANOVA) test followed by least significant difference (L.S.D.) analyses at 0.05% probability of levels. Using computerized SPSS program (12).

RESULTS AND DISCUSSION:

Table (1) effect of intramuscular injection of diclofenac sodium for 14 days on serum AST, ALT and ALP levels of rats at different doses.

Doses (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)
Group (1) 50 mg/kg of DS	53.15 * ± 2.05	35.19 * ± 0.68	120.17 * ± 1.64
Group (2) 100 mg/kg of DS	74.10 * ± 0.21	55.21 * ± 1.27	135.15 * ± 1.95
Group (3) 150 mg/kg of DS	95.05 * ± 1.11	78.31 * ± 1.28	154.05 * ± 1.60
Control	35.32 ± 3.12	28.1 ± 0.98	90.25 ± 1.63
L.S.D.	0.17	0.13	0.10

Number of animals = 5 for each group Each value represents mean ± S.E.
*(mean significant difference (0.05) between the diclofenac sodium groups and control group.)

Histopathological changes :

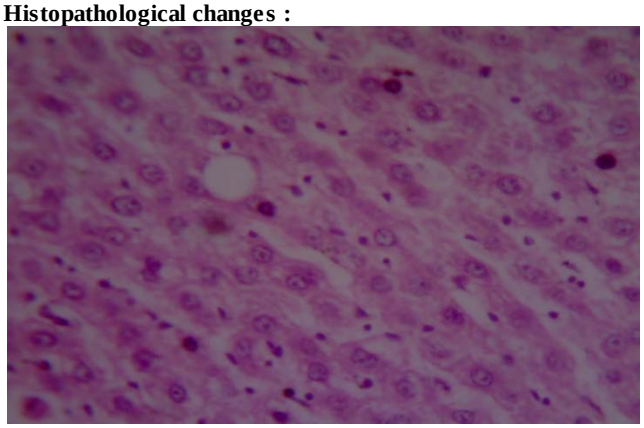

Figure (1) Liver section of male rats in control group show normal central vein and normal hepatocytes architecture (H&E 400X).

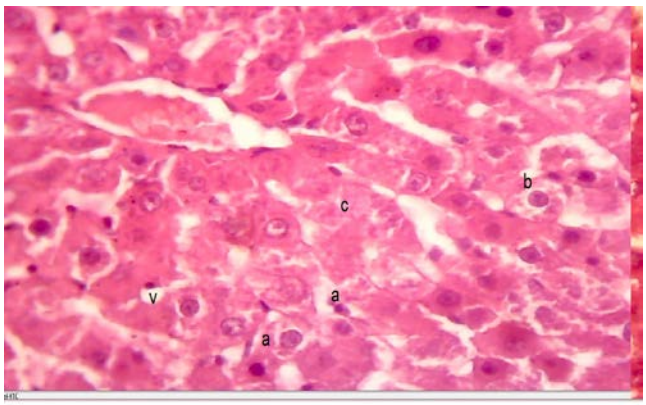

Figure (2) liver section of rats treated with 50mg/kg for 14 days which appear Degenerating hepatocytes (a), hyperhepatocytes with disintegrated cytoplasm (b), fatty changes and vacuoles (v) while apoptotic cells were numerous and shrunken (a) with pyknotic nuclei (H&E 400X).

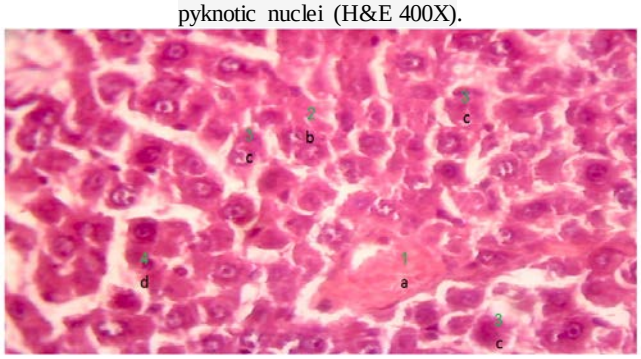

Figure (3) liver section of rats treated with 100mg/kg for 14 days which appear haemorrhage (a), highly degenerative cell with (b), karyolysis (c) with pyknosis. (H&E 400X).

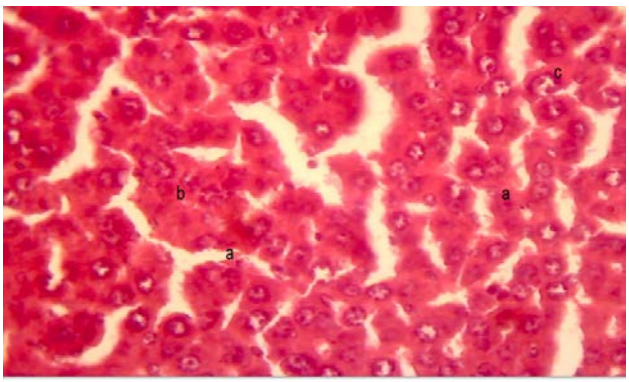

Figure (4) liver section of rats treated with 150mg/kg for 14 days which appear 150mg/kg-treated liver sections showed necrosis ,degenerating hepatocytes b, Apoptotic cells were shrunken and numerous (a) with pyknosis and karyorrhexis of nuclei ,necrotic hepatocytes(c) (H&E 400X).

DISCUSSION :

The liver is an organ engaged with numerous metabolic capacities and is inclined to xenobiotic damage in view of its focal part in xenobiotic digestion. Hepatotoxic medications like paracetamol can make harm the liver (13,14).

Non-steroidal calming drugs are the most every now and again endorsed helpful specialists utilized for treated rheumatic infections in light of the fact that have pain relieving, antipyretic and mitigating activities. There is impressive enthusiasm for the lethality of diclofenac in light of clinical utilize. Diclofenac sodium was utilized as a part of the present investigation to initiate liver harm as it has been accounted for to be hepatotoxic (15).

The hepatotoxic impacts of diclofenac in both human and exploratory creatures have been very much recorded (16).

Diclofenac sodium has been seemed to make liver mischief inciting change in biomarkers of liver limit. One of the more sensitive and enthusiastic markers of hepatocyte harm is the entry of intracellular impetuses, for instance, transaminases and serum acid neutralizer phosphatase into the course (17).

Enlistment of hepatic damage with diclofenac in rats reseated in extreme hepatotoxicity as reflected by an expansion in the serum levels of AST, ALT and ALP (18). It was watched that diclofenac lifted every one of these chemicals fundamentally showing extreme hepatic cell putrefaction . the ascent in serum levels of AST, ALT and ALP could be ascribed to the harm basic uprightness of the liver as affirmed by histopathological comes about which showed cell degeneration and loss of trademark setup.

The instrument of diclofenac incited hepatic peculiar unfavorable medication responses remain to a great extent unknown (19). There was prove that poisonous quality was identified with the medication digestion and was diminished by expansion of cytochrome p-450 inhibitor to the way of life medium (20).three

metabolites of diclofenac sodium are accounted for to be in charge of diclofenac sodium danger in the liver, to be specific 4O hydroxy 3 diclofenac, 5O hydroxy 4 diclofenac and 5O hydroxy 6 diclofenac (21). Both the development of a lethal metabolites and covalent authoritative of the medication to hepatic proteins have been summoned to clarify its danger. Mitochondrial harm and NADPH lack are likewise, thought to be in charge of diclofenac sodium hepatotoxicity (22).

In any case, both hepatocellular cytotoxicity from diclofenac or its metabolites is just seen in vivo with vast centralizations of diclofenac that are not chronicled in vivo.(23). Hindrance of prostaglandins is the most imperative reason for the antagonistic impacts of diclofenac on liver tissues in touchy people or creature species and conceivably amid long haul utilize (24).

Our histopathological finding in liver tissues run next to each other with the got biochemical adjustments. Histopathological examinations demonstrated seriousness of sores expanded with expanded the dosage of medication, direct diffuse degeneration and vacuolation and peri-acinar rot with mellow to direct invasion of gateway zones with mononuclear cells in the analyzed hepatic tissues of rats treated with diclofenac sodium at measurements 100 and 150 mg/kg for 14 days (25).

In conclusion, diclofenac sodium at high dose causes alterations in biochemical and histological changes in liver of male rats. However, the toxic effects of diclofenac sodium could be acute or reversible.

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