ORIGINAL RESEARCH PAPER

Pharmacology

DICLOFENAC-INDUCED LIVER TOXICITY IN ALBINO RATS: DOSE-DEPENDENT STUDY.

KEY WORDS: Diclofenac, drug-induced hepatotoxicity, Serum markers, liver injury, NSAIDs

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ABSTRACT

Liver is the major organ which helps in detoxifying the drugs; during the process the long standing use of some may cause hepatotoxicity. The NSAIDs are major class, known to cause hepatotoxicity ex; Diclofenac, Sulindac and Aspirin. Hence, Diclofenac sodium was evaluated for its hepatotoxic effect. Albino rats were administered with Diclofenac sodium (72, 96 and 240 mg/kg) respectively as a single oral dose & 24-hours of post-treatment, serum levels of the liver enzymes were evaluated to demonstrate its hepatotoxic effect. Further, the liver was subjected for histopathological study. On statistical analysis Diclofenac had shown significant rise in the levels of serum SGOT & serum SGPT (< 0.05), when compared with the control, which was evident for the hepatotoxic effect of the Diclofenac sodium. It is concluded that, Diclofenac in above mentioned doses has hepatotoxic effect in rats.

INTRODUCTION

Diclofenac sodium was introduced in late 70's as a potent anti-inflammatory and analgesic preparation, which on long term use has shown hepatotoxic effects, which presented in the form of hepatic injury ranging from mild to fatal liver injury [1,2].

Liver being a principle organ for maintenance and regulation of the internal milieu is involved for the structural alterations of the administered drugs. It is the target organ, which gets exposed to the drugs in higher concentration, than other organs of the body, when they are orally administered[3]. Hence, it is the most vulnerable organ to be injured by the chemicals and the drugs, which leads to hepatic dysfunction. Generally, any drug in excess could be a burden on the liver causing toxic effects, but, sometimes, even the drugs introduced in therapeutic ranges may also injure the liver. Some of the commonly hepatotoxic drugs, include, antitubercular drugs such as Isoniazid, Rifampicin, Pyrazinamide, which contribute to hepatotoxicity, with the toxicity ranging between 2% to 28% (Girling 1978, D. Hong 1986)[4,5]. More than 900 drugs are known to be hepatotoxic[6,7,8] for example, Non-Steroidal Anti-inflammatory Drugs (NSAIDs) such as Acetaminophen, Nimesulide, Diclofenac, Ibuprofen, which are very commonly used in the treatment of rheumatological conditions and apart from which they are the most commonly used analgesics and antipyretics [9]. They also make the most important group of drugs used over-the-counter as OTC preparations and contribute to the adverse drug reactions (ADRs) [10]. Concurrent administration of Acetaminophen along with other hepatotoxic drugs is usually seen in many clinical situations[11]. Drug-induced liver injury (DILI) according to the recent estimates show the incidence of 14-19 cases per 100,000[6,7].

Hence, the study was taken up to evaluate the most commonly used preparations in today's practice, i.e. Diclofenac sodium, for its hepatotoxic effect.

MATERIALS AND METHODS

The study was conducted after obtaining the approval from the Institutional Animal Ethics Committee (IAEC), of S.B.K.S.M.I. & R.C., Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, considering the rules and regulations of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Albino rats of either sex weighing between 100 - 400g were used. Each animal was used only once. The animals were housed separately in poly-propylene rat-cages under controlled environmental conditions temperature 24° ± 2°C and 55% ± 5%, relative humidity, in a 12-hour light-/dark cycle throughout the experiment, which were kept fasting for 24 hours, before administering the drug.

The drugs and chemicals used were Diclofenac sodium from Aatur Instru Chem, Vadodara. The chemicals included 10% Formalin, Xylene, Hemotoxylin and Eosin stains.

To evaluate the levels of liver enzymes, serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Aminotransferases (SGOT), Serum Alkaline Phosphatase, Serum bilirubin – Direct and Indirect Bilirubin, Total Bilirubin ; Serum Gamma-Glutamyl Transpeptidase (GGTP) the diagnostic kit reagents (Erba Diagnostics, Manheim) was used.

All the drug solutions were freshly prepared before use and were administered orally with the volume of 10 ml/kg. The animals were divided in four groups, with each group containing 6 rats. The dose of Diclofenac sodium was selected based on the LD_{50} dose in rats, when administered orally[12].

The 24-hour fasted rats were administered orally with Distilled Water for the control group while the remaining three groups were administered with Diclofenac sodium in the doses of 72, 96 and 240 mg/kg respectively and were re-housed in the individual polypropylene cages

Following 24 hours of post-treatment, under light ether anaesthesia, upto 3 ml of blood sample was collected from retro-orbital plexus by capillary method technique in the test tube, which was centrifuged at 3000 rpm for 10 minutes to separate serum that was subjected to analyse the Liver Function Tests (LFTs) so as to evaluate its hepatotoxic effect.

Liver from each animal was immediately dissected out and cleaned with normal saline and was preserved into the specimen collection jars that contained 10% formalin. The liver samples were quickly fixed in 10% formalin and embedded in paraffin. Sections of about 4-6 µm were stained with haematoxylin for 5 minutes at room temperature; 15 minutes later was counterstained with eosin for 2 minutes; washed with xylene and blocked by eosin for histopathological studies and were observed under photomicroscope.

RESULTS

Statistical analysis:

All the observed data were subjected for statistical analysis and the results were expressed as Mean± SEM. All calculations were

performed using statistical software SPSS version 21.0 computer-based. Values were considered to be significant when P values were less than or equal to 0.05 ($p \leq 0.05$).

Observations and results:

Albino rats (n=6) that were administered Distilled Water (10 ml/kg) was considered as control (Group- I). Diclofenac sodium administered as a single oral dose in each group (n=6) Group II (Diclofenac sodium 72 mg/kg), Group III (Diclofenac sodium 96 mg/kg) and Group IV (Diclofenac sodium 240 mg/kg), respectively; when compared to the control group showed statistical significant rise (**p < 0.0001**) in the serum SGPT and serum SGOT levels as indicated in the **table number 1** below, and as depicted in the figure 1 and figure 2, respectively.

Table - 1: Shows changes in the levels of liver enzymes, following administration of Diclofenac sodium (72 mg/kg, 96 mg/kg and 240 mg/kg).

Sr. no.	Liver Function Tests	Group (n=6)	Mean± SEM
1.	SGPT (IU/L)	Control DW 10 ml/kg	32.83 ± 2.91
		Diclofenac 72 mg/kg	85.67 ± 7.33***
		Diclofenac 96 mg/kg	147.67 ± 13.72***
		Diclofenac 240 mg/kg	236.50 ± 24.01***
2.	SGOT (IU/L)	Control DW 10 ml/kg	126.00 ± 15.07
		Diclofenac 72 mg/kg	528.33 ± 86.50***
		Diclofenac 96 mg/kg	1220.83 ± 130.50***
		Diclofenac 240 mg/kg	1490.00 ± 168.88***
3.	Total Serum Bilirubin (µmol\L)	Control DW 10 ml/kg	0.70 ± 0.08
		Diclofenac 72 mg/kg	01.13 ± 0.12
		Diclofenac 96 mg/kg	1.07 ± 0.12
		Diclofenac 240 mg/kg	1.25 ± 0.11
4.	Serum ALP (IU/L)	Control DW 10 ml/kg	106.17 ± 23.15
		Diclofenac 72 mg/kg	120.17 ± 23.551
		Diclofenac 96 mg/kg	153.83 ± 32.01
		Diclofenac 240 mg/kg	229.00 ± 32.06
5.	Serum GGTP (IU/L)	Control DW 10 ml/kg	2.33 ± 0.56
		Diclofenac 72 mg/kg	04.95 ± 1.45
		Diclofenac 96 mg/kg	3.03 ± 1.40
		Diclofenac 240 mg/kg	1.60 ± 0.28

Note:

* p value < 0.05 = significant, values are presented as Mean± SEM

Serum Glutamic-Pyruvic Transaminase (SGPT), Serum Glutamic-Oxaloacetic Aminotransferases (SGOT), Total serum bilirubin, Alkaline Phosphatase (ALP) and Gamma Glutamyl Transpeptidase (GGTP) or γ-Glutamyl Transferase (GGT), DW = Distilled Water.

However, there was no statistically significant rise in the serum levels of total serum bilirubin, alkaline phosphatase and γ-Glutamyl Transferase, as shown in figure 3, 4 and 5 as below.

Figure 1: Changes in the Serum Glutamic-Pyruvic Transaminase (SGPT) levels

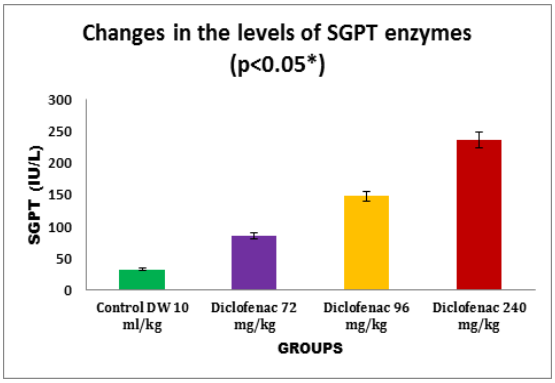


Figure 2: Changes in the Serum Glutamic-Oxaloacetic Aminotransferases (SGOT) levels.

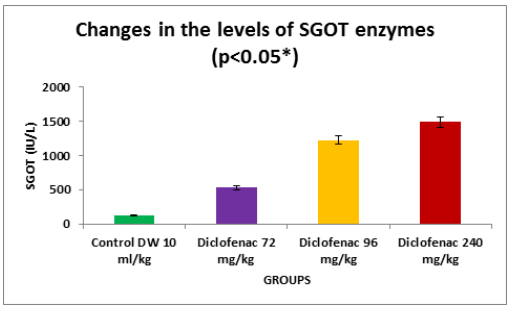


Figure 3: Changes in the Total Serum Bilirubin levels.

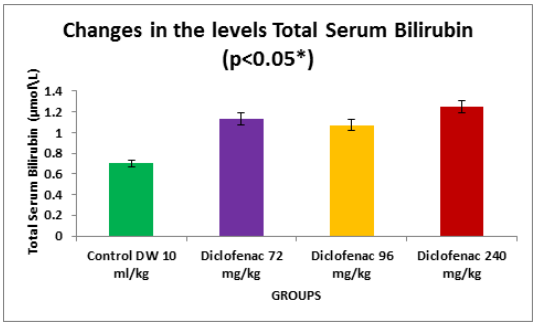


Figure 4: Changes in the serum Alkaline Phosphatase (ALP) levels.

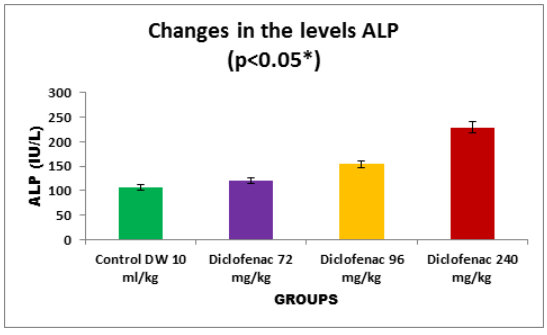
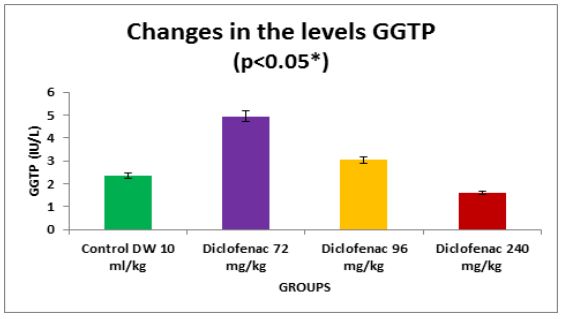


Figure 5: Changes in the Serum Gamma Glutamyl Transpeptidase (GGTP) levels.



Histopathological Observations:

i. Gross appearance of the liver sample:

The gross appearance of liver of albino rats administered with Distilled Water, did not show any abnormal changes in texture, shape, size or colour. It was reddish brown and showed typical lobular architecture; whereas, those treated with Diclofenac sodium were pale yellow to pale brown colour.

ii. Microscopic examination of liver:

The evidence of changes in the liver cells from the liver sections of Diclofenac sodium at 72 mg/kg, 96 mg/kg & 240 mg/kg. p.o. showed dose-dependent changes in the liver section.

The histopathological changes observed in the liver tissue sections shows mainly hepatocellular changes along with the changes in the portal area, which also revealed microvesicular vacoulation, that is diffuse hepatic vacoulation (degeneration) seen which was dose-dependent and marked congestion. Whereas, irreversible changes in the tissue such as severe hepatocellular degeneration or centrilobular focal necrosis was not seen.

Figure number 6: Liver sections from control rats showing central vein low power

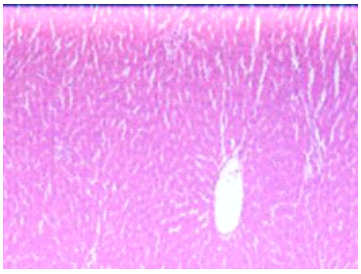


Figure number 7a: – Liver section from diclofenac sodium 72 mg/kg

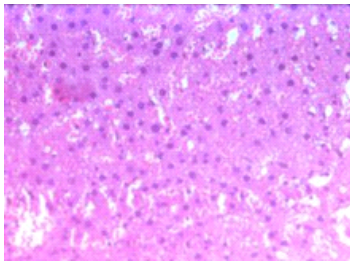


Figure number 7b – Liver section from diclofenac sodium 96 mg/kg

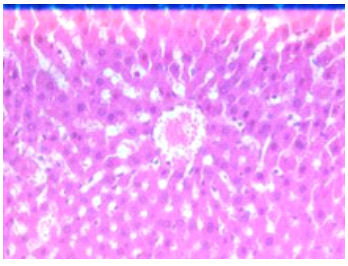
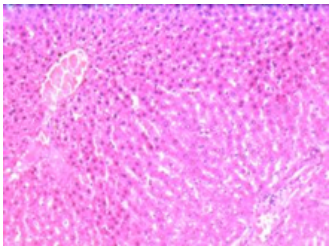


Figure number 7c - Liver section from diclofenac sodium 240 mg/kg



DISCUSSION

The NSAIDs are considered as the major groups to cause hepatotoxicity, since they are used as both prescriptive and OTC preparations [10]. Of the currently used NSAIDs, the most common drugs associated with liver disease include; Diclofenac, Sulindac and Aspirin [12].

Diclofenac sodium being widely used Non steroidal anti-inflammatory and analgesic compound, we have observed for the hepatotoxic effect of single dose of Diclofenac sodium in dose-dependent manner, in the albino rats.

Diclofenac had shown statistically significant rise (**p < 0.0001**) in the levels of serum SGOT and serum SGPT, when compared with the control group, which was evident for the hepatotoxic effect of the diclofenac sodium in all the three doses 72 mg/kg, 96 mg/kg & 240 mg/kg as a single oral dose. This observation of ours concurs with the observations made by D. Schapira et al [13].

Along with the rise in the serum levels, the histopathological studies have demonstrated the toxic effects of diclofenac sodium in the form of sinusoidal dilatation, cytoplasmic vacoulation and mild portal congestion.

CONCLUSION

With observations made by the authors, Diclofenac in the doses of 72 mg/kg, 96 mg/kg & 240 mg/kg as a single oral dose has shown to be hepatotoxic in albino rats.

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DECLARATIONS

Funding: NIL

Conflict of interest: NIL

Ethical approval: The present research study was accepted & approved by the Institutional Animal Ethics Committee (IAEC), which is registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of S.B.K.S.M.I. & R.C., Sumandeep Vidyapeeth Deemed to be University, Piparia.

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