Original article:

Comparative adverse effects of aceclofenac and celecoxib on liver of wistar albino rats

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ABSTRACT:

Introduction: NSAIDS are most widely used analgesics with established gastrointestinal side effects which may even lead to death of the patient when used regularly. Many studies are suggestive of additional toxicities including cardiovascular and hepatocellular toxicities. The present study was planned to compare the hepatotoxic effects of Aceclofenac and Celecoxib on Wistar albino rats.

Methods: The animals were administered with the drugs intraperitoneally for a period of 30 days. After the study period, the rats were sacrificed and blood samples and liver were collected. The liver was stained with H/E stain and observed under light microscope for histopathological changes. The levels of serum AST and ALT were measured.

Observation: It was observed that the histological changes were more pronounced in case of animals treated with Celecoxib than those treated with Aceclofenac. There was increase in the AST and ALT levels in both the experimental animal groups when compared to control group but the increase is not significant.

Result: The histological changes are more pronounced in case of animals treated with Celecoxib than those treated with Aceclofenac.

Conclusion: It is inferred that the chronic intake or utilization of higher doses of Celecoxib and Aceclofenac will lead to fatty liver changes, hepatitis and other hepatic complications and Celecoxib is more hepatotoxic than Aceclofenac in therapeutic doses.

Keywords: Hepatotoxicity, Histopathological changes, NSAIDS

INTRODUCTION:

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly used over-the-counter drugs. They are mainly used for their analgesic, antipyretic and anti-inflammatory actions. They decrease the pain but do not cause the reversal of the disease process. They are used in various conditions like fever, rheumatoid and osteoarthritis, ankylosing spondylitis, toothache, dysmenorrhoea, etc. Their analgesic action is limited only to inflammatory pain. But these benefits come at a price.

The pain in inflammatory conditions is due to the synthesis of prostaglandins through the inflammatory pathway. Cyclooxygenase (COX) is the key enzyme which plays an important role in the production of prostaglandins. It was observed that NSAIDs decrease the pain by inhibiting this enzyme [1]. COX exists in two isoforms, COX-1 and COX-2. COX-1 is the constitutive form which is associated with housekeeping function. It was noted that COX-1 antagonism results in gastric ulceration and even caused number of deaths as a result of upper gastrointestinal damage [2]. Whereas COX-2 is the inducible form and its antagonism

does not cause marked gastric ulceration. Due to their mild gastrointestinal toxicity, the use of selective COX-2 inhibitors has been increased. This is due to the fact that they selectively inhibit only the COX-2 and does not interfere with COX-1.

However, both COX-1 & COX-2 inhibition leads to many unwanted effects on heart, blood vessels, lungs and kidney. Studies suggestive of considerable hepatic and cardiovascular injuries have been reported. Fatal hepatotoxicity secondary to Nimesulide was reported [2].Studies have suggested that the use of COX inhibitors relieve the pain and inflammation in rheumatoid arthritis [3], [4], [5]. But the use of non-selective COX inhibitors has produced gastrointestinal toxicity which is more severe when compared to that of selective COX-2 inhibitors [6]. The hepatic injury associated with NSAIDS observed in clinical trials was found to be quite variable ranging from mild cholestasis to severe hepatocellular injury along with biochemical changes [7], [8]. Selective COX-2 inhibitors have been associated with hepatotoxicity in some clinical trials [9], [10].

METHODS:

Study design: Experimental animal study

Sample size: 18 adult Wistar strain male albino rats weighing 180-200 gm.

Drugs

Aceclofenac and Celecoxib in pure form were obtained from A to Z Pharmaceuticals Pvt. Ltd, Chennai.

Dosage and Administration

The dosage of drugs to be administered to rats in this study was calculated according to the therapeutic dose as recommended for the treatment of arthritis in humans.

Aceclofenac: 10 mg/kg/day

Celecoxib : 2 mg/kg/day

The drugs were administered intraperitoneally to the experimental rats daily for 30 days. The drugs were freshly prepared daily by dissolving them in Dimethyl sulfoxide (DMSO) before administration.

Animals

18 Wistar strain male albino rats (180-200 gm) were obtained from the Experimental Animal House of Sri Manakula Vinayagar Medical College, Pondicherry. Permission was obtained prior to the study from the Institutional Animal Ethics Committee. The rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature, humidity and 12 hrs dark/light cycles were maintained. All animals were fed with rat pellet diet and water was allowed ad libitum under strict hygienic conditions. All the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for laboratory facility were strictly followed.

The rats were randomly divided into 3 groups of 6 rats each and drugs were administered intraperitoneally (i.p.) daily for 30 days as follows:

Group A: Control group received only Dimethyl sulfoxide, i.p. (isovolumetric amount).

Group B: Aceclofenac 10 mg/kg/day, i.p.

Group C: Celecoxib 2 mg/kg/day, i.p.

The rats were weighed weekly and at the end of the experiment, the ratio of body weight**:** liver weight of individual rats was calculated.

Sample collection

Blood sample and Enzyme Estimation

The blood samples were collected by cardiac puncture at the end of 30th day. The blood samples collected from the heart were allowed to stand for 45 min and clot. The serum was collected and centrifuged. The blood parameters indicative of liver function i.e. serum AST and ALT were measured.

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Tissue sample

The sacrificed animals were perfused with 10% formalin and 0.9% normal saline and liver was collected and weighed. The sections of the liver were stained using H/E staining and examined under light microscope for presence of any histopathological changes.

RESULTS:

Histological changes

The histopathological sections of liver of Group A, the control group, showed no abnormal changes in the hepatic architecture. (Fig 1- A)

Sections belonging to Group B, rats treated with Aceclofenac, showed dilatation and distension of sinusoids (Fig 1- B). They also showed hydropic changes of hepatocytes around the central vein. But there were no inflammatory or fatty changes in the hepatocytes.

Sections belonging to Group C showed marked dilatation of sinusoids. Hydropic changes of hepatocytes around central vein were seen as compared to sections of Group B. Marked dilatation of the central vein with fibrin deposition was seen. Significant fatty changes were also seen around the periportal area (Fig: 1- C).

The liver weight of individual rats of each group was measured and the ratio of rat body weight: liver weight was calculated. There was significant decrease in the body weight of rats treated with Celecoxib. There was no significant difference in the liver weight of rats belonging to the other two groups.

Biochemical changes:

Serum AST levels were increased in both Group B and C as compared to the control group A, but the increase was not significant ($p>0.05$).

Serum ALT levels did not show any significant increase in both Groups B and C when compared to Group A (Table: 1).

Results are considered to be significant only if the value of $p<0.05$. Here $p=0.450$ for AST and $p=0.319$ for ALT.

DISCUSSION:

Previous studies have established the association between hepatic dysfunction and clinical use of NSAIDS. It was recorded that this class of drugs cause borderline increase in levels of liver enzymes in patients taking drugs regularly [11].

Present study shows a borderline increase in the level of liver enzymes. The histopathological changes are significant in case of experimental group treated with Celecoxib. Similar observation is made in previous studies performed with Celecoxib in clinical trials [12], [13]. Whereas a study conducted on pregnant and non-pregnant rats showed contradictory results to the present study [14]. None of the changes, histopathological or biochemical, of liver was observed with Nimesulide in clinical trials and studies on birds.

A previous study done with therapeutic and sub-therapeutic doses of Diclofenac and Valdecoxib on rats showed that the sub-therapeutic and therapeutic doses of Valdecoxib, on long term exposure can lead to hepatic dysfunction or acute hepatitis. Therapeutic doses of Diclofenac, on the other hand, can lead to pathological changes while its sub-therapeutic dose seems to be safer than Valdecoxib in hepatic outcome [15]. The present study was done with therapeutic dose of the drugs alone, not taking into consideration their sub-therapeutic effects.

The present study was conducted based only on the therapeutic doses of the drugs Aceclofenac and Celecoxib; both the drugs produced similar histological changes in the liver. But Celecoxib is seen to produce fatty change in liver sections which is absent in the groups treated with Aceclofenac.

Therefore, it can be concluded that Aceclofenac is safer than Celecoxib in relation to hepatic toxicity. Therefore, these drugs need to be taken with care in

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patients suffering from hepatic insufficiency. Also their chronic intake and usage of higher doses should be avoided.

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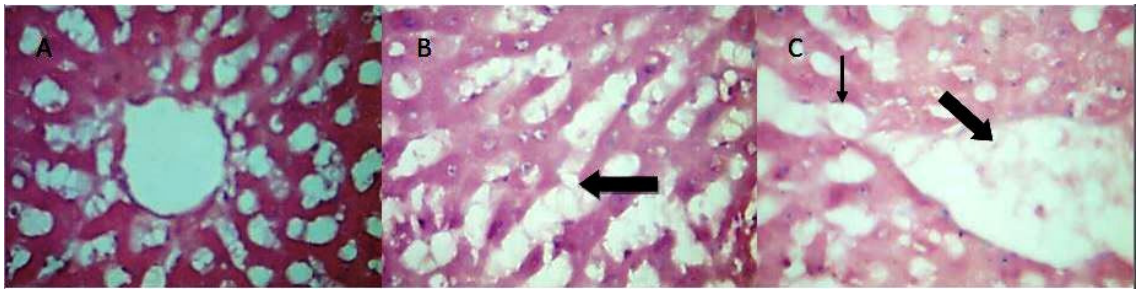
Table 1: Results of hepatic biochemical parameters in the three groups

Group (Drug/Dose i.p.)	Mean values	
	Serum AST	**Serum ALT**
Group A (Control-DMSO)	212.6	80.1
Group-B (Aceclofenac,10 mg/kg/day)	170	86.5
Group C (Celecoxib,2 mg/kg/day)	181.5	74.5

(i.p. - Intraperitoneally, DMSO- Dimethyl sulfoxide, AST- Aspartate aminotransferase, ALT- alanine aminotransferase)

Fig 1: A- Liver of group A (Control group) showing normal liver histology; B- Liver of Group B (Aceclofenac, 10 mg/kg), arrow showing dilated sinusoids (40X); C:- Liver of Group C (Celecoxib, 2 mg/kg) large arrow showing dilated central vein and small arrow showing fatty changes in hepatocytes (40X).



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