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Biochemical changes in the testes of mice after treatment with different doses of diclofenac sodium

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Abstract : Diclofenac sodium (DS), a well-known member of acetic acid family of non-steroidal anti-inflammatory drugs (NSAIDs) is used to reduce inflammation and pain associated with arthritis, osteoarthritis and ankylosing spondylitis. The drug is one of the most common non-steroidal compounds which bind extensively to plasma albumin, having inhibitory effects on prostaglandin biosynthesis. The continuous use of diclofenac for short term relief from discomforts due to ailments paves way for the development of complications in the reproductive system. The present study is focussed on the effect of different doses of diclofenac sodium (4mg/kg/body weight and 14mg/kg/ body weight) on different biochemical parameters like acid and alkaline phosphatase, aminotransferases like GOT/GPT and lipid peroxidation on testes of mice. A Significant decrease in the specific activity of acid and alkaline phosphatase was observed in both low and high dose groups after different days of DS treatment. The present study also revealed a decrease in the specific activity in the mice testes after 28 days of treatment. The increased concentration of malondialdehyde in the mice testes observed in the present study indicated the toxicity of diclofenac resulting in enhanced lipid peroxidation.

Keywords: Diclofenac sodium, inflammation, aminotransferases, malondialdehyde.

INTRODUCTION

The study of reproductive system and its functioning forms an integral part of evaluation of infertility and impotency, which seems to be prevalent widely in recent years, among humans. The endocrine system being the major regulator of the reproduction, so anything that can cause an imbalance within the endocrine system affects the whole reproductive system. In the recent years, the intake of over the counter drugs has increased. The short term relief from discomforts due to ailments usually leads to the development of complications in the reproductive system and other organs mainly liver. One of the class of these drugs are NSAIDs commonly used for the treatment of pain, inflammation and fever, all common features of rheumatic conditions. Anti-inflammatory drugs are traditionally classified into steroidal and non-steroidal.[1,2] The non- steroidal anti-inflammatory drugs are the heterogeneous group of compounds, chemically unrelated, but mostly organic acids.[3]

Diclofenac sodium is one of the most common non-steroidal compounds of phenyl acetic acid class, binds extensively to plasma albumin, having inhibitory effects on prostaglandin biosynthesis. Inhibition of prostaglandin synthesis is considered to be fundamental to the mechanism of action of diclofenac sodium.4 Prostaglandins are secreted by male accessory reproductive structures in large quantities and are needed for normal male reproductive functions. These are produced via action of COX enzymes, which exists in two isoforms COX-1 and COX-2.5

Testes being chief organ concerned with spermatogenesis has been found to linked with diclofenac toxicity. Since the drug is known to enhance reactive oxygen species due to its toxicity, testes has developed defence mechanism to protect itself from toxic effects of ROS. This imbalance between production of free radicals

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and antioxidant defence in the body results in a condition of oxidative stress, which may alter the structural and functional architecture of testicular cells resulting in change in the specific activity of the enzymes.

MATERIALS AND METHODS

The protocol of the present investigation was approved by Institutional Animals Ethics Committee (IAEC approval no. IAEC/Bio/5/2011-H.P.U.) Himachal Pradesh University, Shimla. Healthy, pathogen free Swiss albino mice of Balb C strain weighing 22-25 g were procured from Central Research Institute (CRI) Kasauli, Himachal Pradesh. These were maintained in the animal house of the department of Biosciences, H.P. University, Shimla under suitable hygienic conditions with 16 hr. day light and temperature of 24± 20C.

Animals were caged in polypropylene cages (six mice/cage) on soft chip bedding. Animals were provided with commercial feed (Hindustan Lever Ltd. New Delhi, India) and were given water ad libitium. Each animal was assigned a unique identification number by individual marking on fur.

Diclofenac sodium was purchased from Sigma Aldrich Co., USA. Stock solution was made in distilled water. Dilutions were made according to the body weight of animals and mice were administered drug at the dose levels of 4mg/kg body weight and 14mg/kg body weight in the dose volume of 1ml/100g body weight. The control animals were given saline water only. Oral administration was selected as it is one of the proposed routes for toxicity and canular feeding was preferred for accuracy The animals were divided into three groups, as

1. The mice of the first group served as normal (control) animals.
2. The mice of the second group were administered a daily dose of diclofenac sodium (4mg/kg body weight) for 28 days.
3. The mice of the third group were given a daily dose of diclofenac sodium (14 mg/kg body weight) for 28 days.

Body weight of animals was recorded every week for 28 days. The animals were

sacrificed at 7,14,21,28, days of experiment by cervical dislocation.

BIOCHEMICAL ESTIMATIONS

Lipid peroxidation

Levels of malondialdehyde (index of lipid peroxidation) were estimated according to method of Dhindsa6 using thiobarbituric acid.

Glutamate pyruvate transaminase (EC 2.6.1.2) and glutamate oxaloacetate transaminase (EC 2.6.1.1)

The enzymes (GPT and GOT) were estimated by the method of Bergmeyer and Bernt.[7]

Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1).

The quantitative estimation of acid and alkaline phosphatase activity was made by the method of Weil and Russel.[8]

Groups	7	14	21	28
Control	11.84 ± 0.166	12.54± 0.094	13.61±0.106	11.22±1.149
4mg/kg b.w.	16.72 ± 0.188**	20.58±0.160**	20.71±0.106**	23.74 ± 0.153**
14 mg/kg b.w	18.75 ± 0.116**	20.93±0.105**	19.17±0.153**	24.50 ± 0.112**

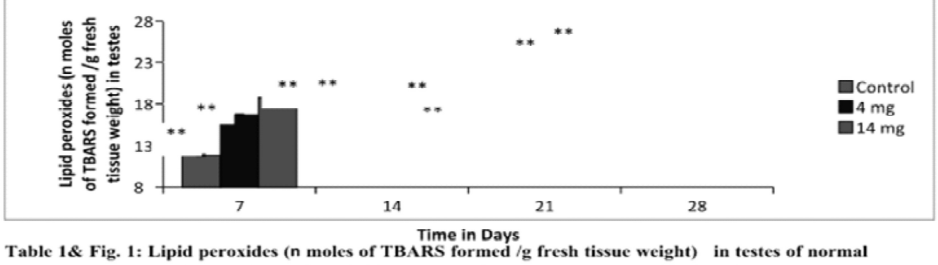

Table 1& Fig. 1: Lipid peroxides (n moles of TBARS formed /g fresh tissue weight) in testes of normal and treated mice from 7-28 days period. Values are mean ± SEM; n=5 in each group. [¨p < 0.001]

Groups	Days			
	7	14	21	28
Control	0.300±0.003	0.296±0.003	0.302±0.002	0.292±0.001
4mg/kg b.w.	0.256±0.0033*	0.306±0.003	0.326±0.002*	0.278±0.002*
14 mg/kg b.w	0.248±0.003*	0.327±0.001*	0.336±0.003*	0.272±0.003*

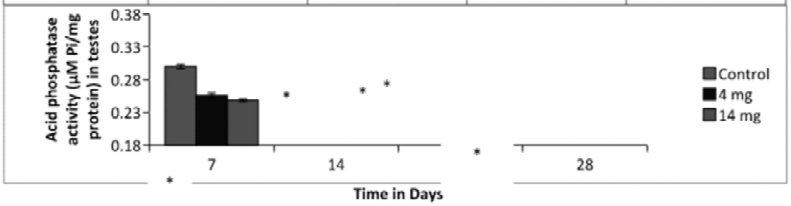

Table 2; Fig. II: Acid phosphatase activity (µM Pi/mg protein) in testes of normal and treated mice from 7-28 days period. Values are mean ± SEM; n=5 in each group. (*p<0.05)

Groups	Days			
	7	14	21	28
Control	0.384±0.002	0.391±0.002	0.397±0.002	0.385±0.002
4mg/kg b.w.	0.345±0.002*	0.422±0.002*	0.330±0.007	0.320±0.002**
14 mg/kg b.w	0.348±0.002*	0.427±0.002*	0.340±0.002**	0.336±0.003**

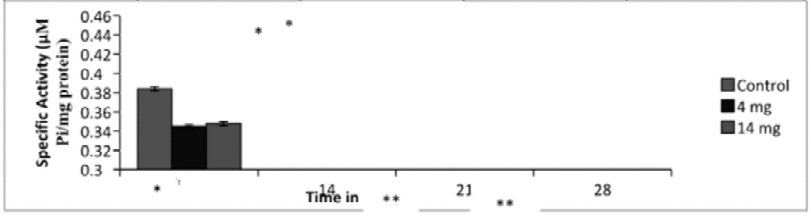

Table 3; Fig. III: Alkaline phosphatase activity (µM Pi/mg protein) in testes of normal and treated mice from 7-28 days period. Values are mean ± SEM; n=5 in each group. (*p<0.05, **p<0.001)

Groups	Days			
	7	14	21	28
Control	0.112±0.003	0.118±0.002	0.120±0.004	0.116±0.005
4mg/kg b.w.	0.151±0.007	0.145±0.005	0.177±0.006*	0.091±0.003*
14 mg/kg b.w	0.155±0.006	0.157±0.011	0.183±0.003*	0.089±0.002*

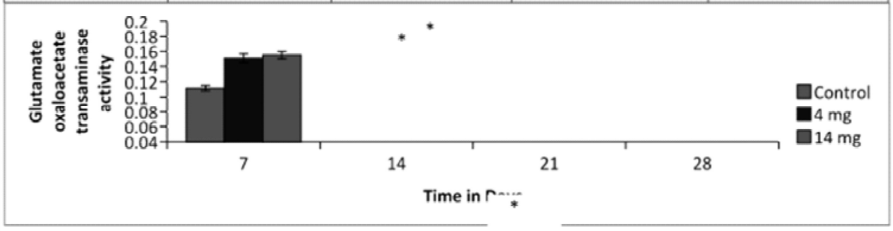

Table 4; Fig. IV: Glutamate oxaloacetate transaminase activity (µ moles of sodium pyruvate formed/ g protein/min at 37°C) in testes of normal and treated mice from 7-28 days period. Values are mean ± SEM; n=5 in each group. (*p<0.05, **p<0.001)

Groups	Days			
	7	14	21	28
Control	0.167±0.002	0.160±0.002	0.162±0.003	0.167±0.002
4mg/kg b.w.	0.190±0.004	0.244±0.001**	0.210±0.001	0.138±0.002*
14 mg/kg b.w	0.202±0.003	0.256±0.003**	0.238±0.004*	0.132±0.002*

Fig. XXV

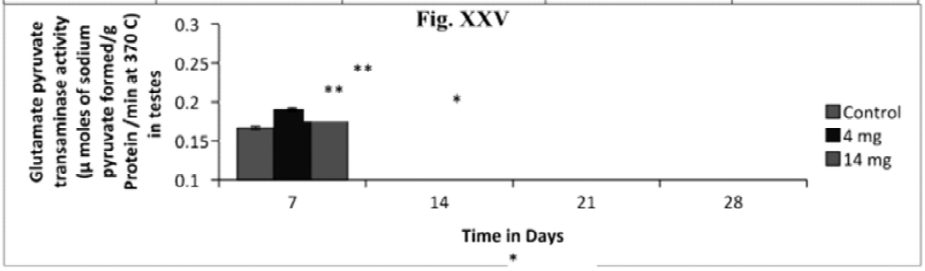

Table 5; Fig. V: Glutamate pyruvate transaminase activity (µ moles of sodium pyruvate formed/g Protein /min at 370 C) in testes of normal and treated mice from 7-28 days period. Values are mean ± SEM; n=5 in each group.(*p<0.05, **p<0.001).

RESULTS AND DISCUSSION

The therapeutic and many of the toxic effects of the NSAIDs result from reversible inhibition of the enzymes in the cyclooxygenase (COX) group, which results in decrease in the synthesis of prostaglandins and thromboxane from the precursor arachidonic acid.9 The prostaglandins have been shown to have wide variety of effects within the body. The use of diclofenac has been associated with both elevation of transaminases and hepatitis.[10,11,12,13] The drug, diclofenac sodium was administered orally at the dose rate of 4mg/kg body weight and 14mg/kg body weight daily for different durations of 7, 14, 21 and 28 days. The doses selected were moderate dose i.e. 4mg/kg body weight and higher dose i.e. 14 mg /kg body weight, which was higher than the therapeutic dose (8mg/kg) for mice.[14]

Oxidative stress is defined as the inability of the organ or cell to defend itself against the oxygen derived species resulting in oxidative injury.15 There are enough evidences to suggest that oxidative stress and resulting lipid peroxidation are involved in numerous pathological states including inflammation, atherosclerosis, neurodegenerative diseases and cancer. Such free radicals produced as a result of lipid peroxidation have some very local effects, because of their short life. The breakdown products of lipid peroxides may serve as "oxidative stress secondary messenger" due to their prolonged half-life and their ability to diffuse from the site of formation, compared to free radicals. One of these breakdown products, malondialdehyde, a non-specific marker of oxidative stress, has received a lot of attention because of its reactivity.16

Lipid peroxidation is known as a common mechanism of toxic manifestations of many drugs including most of the NSAIDs in liver and testes. Malondialdehyde (MDA) is a low molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids. Significant increase in MDA concentration observed in the present study reflected cellular damage in testes induced by diclofenac. The significant increase in the activity of TBARS in liver in diclofenac treated groups (4mg and 14mg/kg body weight) indicated on going peroxidative stress and compromised antioxidant defence mechanism. The sharp increase in the MDA concentration till 28th day of the experiment demonstrated the toxic effects of diclofenac on the tissues. An increase in liver transaminases, MDA, serum urea and creatinine was also reported after injecting diclofenac sodium intramuscularly.[17]

The generation of reactive oxygen species in the male reproductive tract due to diclofenac administration is potentially dangerous due to their toxic effects at high levels on sperm quality and function.[18] The increased concentration of malondialdehyde in mice testes observed in the present studies indicated the toxicity of diclofenac resulting in enhanced lipid peroxidation during all stages of the investigation. Increased lipid peroxidation may also contribute to the suggested vulnerability of this organ to oxidative stress. Appropriate production of reactive oxygen species has been suggested to play a physiological role in sperm-zona interaction.[19] Moreover, there is evidence of physiological role of reactive oxygen species in cellular differentiation.[20] The increase in lipid peroxidation in both dose groups (4mg/ kg body weight and 14mg/kg body weight) demonstrated the susceptibility of the testes to oxidative stress. The findings are in conformity with works on cadmium toxicity, where increase in lipid peroxidation and suppression of the antioxidant defence mechanisms in testicular tissues with significant reduction in testicular function and androgen secretion has been demonstrated.[21,22,23]

Male germ cells are highly susceptible to reactive oxygen species attack produced by any toxic agent or xenobiotic; hence, they are equipped with an effective defence system to combat the damaging effect.25,26 Alteration in the activities of testicular and hepatic acid phosphatase reflected the oxidative stress in the tissues caused by the diclofenac toxicity. Acid phosphatase is localized in cellular lysosomes and change in the activity of lysosomal enzymes can be attributed to the deleterious effects of the drug. The occurrence of acid phosphatase activity in normal animal tissues, including those of monkey, dog, pig, cow rabbit, rat and mouse has been examined.27 The fact that diclofenac toxicity in the present study on male mice caused the degeneration of testicular structures, delayed the onset of spermatogenesis and reduced the spermatogonial cell count seem to suggest a possible role of drug in inducing changes in the marker enzymes of testes under the control of adrenal cortical hormones. Further, the degenerative changes may increase the harmful effects of oxyradicals generated in the testes which may be toxic to spermatozoa. The initial decline in the activity of ACPase after 7 days of treatment may be attributed to the toxic effect of the drug resulting in the inhibition of protein synthesis. A significant increase in ACPase activity was noticed after 21 days of treatment in mice treated with low and high dose. Macrophages like cells that exhibit strong ACPase activity has been shown among the epithelial cells and have been associated with the removal of old and dead cells by phagocytosis and lysosomal digestion.

Hence, it may be postulated that increased ACPase activity in testes may be due to lysosomal activity of epithelial cells and increased number of ACPase positive macrophage like cells needed for phagocytosis and lysosomal digestion of dead and old epithelial cells and resorption of non-ejaculated surpulus spermatozoa. Aminotransferases, glutamate oxaloacetate transaminase (GOT) or aspartate aminotransferase (AST) and glutamate pyruvate transaminase (GPT) or alanine aminotransferase(ALT) are the first enzymes to be used in diagnostic enzymology. The general increase in the activity of AST in testes following diclofenac administration for 7-14 days in liver and 7-18 days in testes could be due to de novo synthesis of the enzyme molecule or an adaptation by the organs to the assault from the drug leading to the activity higher than the control. Similar increase in the activity of ALT was reported in fish kidney after Bisphenol-A treatment.28 The aminotransferases occupy a central position in the amino acid metabolism as they help in retaining amino groups during the degradation of amino acid and are also involved in the biochemical regulation of intracellular amino acid pool.[29] Significant decrease in the activity of AST and ALT observed after 28 days may be a consequence of cellular damage arising from diclofenac administration resulting in leakage of the marker enzyme to the extracellular fluid and labialization of the membranes, further strengthening the hypothesis.

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