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Research Paper

Oxidative Stress in Mouse Brain Associated With Intramuscular Administration of Diclofenac Sodium

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ABSTRACT: *In the present study, the chronic administration of diclofenac (10 mg/kg body weight; 30 days) resulted in various changes in brain antioxidant status and lipid peroxidation levels of mice. An increase in the formation of thiobarbituric acid reactive species (TBARS) is observed in brain of mice. Maximum increase is seen in drug treated mice at 30 days stage of investigation. Diclofenac sodium treatment increases the superoxide dismutase activity in brain that protects the mice from free radical attack. Increase in SOD activity is an indicator of the oxidative stress. This antioxidant enzyme acts directly or indirectly to remove reactive oxygen species and thus a dose dependent elevation in lipid peroxidation levels and SOD activity is observed.*

KEYWORDS*: Diclofenac Sodium, Brain, Lipid Peroxidation, Superoxide Dismutase.*

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I. INTRODUCTION

Diclofenac sodium (DS) is among the most widely prescribed and used drug in the community for rheumatologic as well as nonrheumatologic conditions, which include acute and chronic pain, like dysmenorrhoea, fever, menorrhagia and other application that derive from the suppression of prostaglandin synthesis [1] and [2]. There is a considerable interest in the toxicity of diclofenac because of its clinical uses. This drug is a dichlorinated diphenylamine containing an acetic acid group as a substituent, which is known to be extremely toxic to some vulture species and to readily undergo phototransformation reactions in the environment[3]. Diclofenac works by interfering with the action of cyclooxygenases (COX), and leads to prostaglandin (PG) deficiency. The drug possess high affinity for COX-1 than COX-2 and have a greater tendency to promote ulcers and bleeding [4]. This drug is absorbed systemically and is transported throughout the body by blood stream and reaches unintended targetes where it can have adverse effects. Diclofenac exposure results in the generation of stereospecific endoperoxides and hydroperoxides by enzymatic and non-enzymatic involvement of "reactive oxygen species" [5]. These species initiate peroxidation of membrane lipids leading to the accumulation of lipid peroxides and free radicals, which is a primary factor in various untoward effects of diclofenac. An increased rate of free radical production may exceed the capacity of the cellular defense systems. These radicals are capable of damaging virtually any biomolecule, sugars, fatty acids and nucleic acids. SOD is an endogenously produced intracellular enzyme and being a key cellular antioxidant, is highly responsible for the elimination of O_2^-. Antioxidant enzymes such as superoxide dismutase (SOD), catalase, peroxidase convert reactive oxygen species to less harmful species.

II. MATERIALS & METHODS

Healthy male mice of Swiss Balb-C strain weighing about 25-30g were procured from Central Research Institute, Kasauli, India. Animals were maintained in the animal house of the department of Biosciences of HP University, Shimla under suitable hygienic conditions of temperature and light (16 hr day light; 24 ± 2^0C). All experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee (IAEC/Bio/8-2009). Mice were divided into two groups, first group of animals were treated as control and received sterile distilled water. Diclofenac sodium (Sigma Chemical Co.) was administered intramuscularly to the second group animals (10mg/kg/body weight) for 30 days. Mice of second group were sacrificed by cervical dislocation at day 10, 20, 30 along with control animals and brain was excised at each

stage of investigation. The excised brain was weighed and employed for biochemical studies. Brain homogenate was prepared in Tris buffer and supernatant was collected. The assay mixture contained distilled water, 1.5 ml of 0.1 M carbonate bicarbonate buffer and 50 µl of supernatant. The reaction was started by adding 5 µl of 0.3 M epinephrine solution prepared in acidic water. Change in absorbance of reduced epinephrine per minute was immediately measured at 490 nm. The protein concentration of the samples was determined using bovine serum albumin as a standard [6]. SOD specific activity was finally calculated in unit/ml/min. Levels of malondialdehyde index of lipid peroxidation were estimated according to the method of Dhindsa *et al* [7]. Tissue was homogenized in 2 ml of 0.1% TCA and centrifuged at 6000 rpm for 15 minutes. To the supernatant, 2 ml of 0.5% TBA prepared in 10% TCA was added. The test tubes were then cooled in ice-cold water bath and then centrifuged again. Absorbance of the supernatant was measured in a HITACHI Spectrophotometer (VSU-2 model 150). MDA contents formed were calculated in n moles/ml.

III. RESULTS AND DISCUSSION

Toxic effects of diclofenac treatment were observed in mice brain in terms of change in biochemical levels. Intramuscular dose of diclofenac sodium (10 mg/kg body wt.) lead to significant decrease in body weight of treated mice. A decrease of 14.68% is observed in body weight at 30 days stage (Figure 1). Figure 2 shows the effect of drug on mice brain weight. Decrease in brain weight is observed at all the stages after diclofenac administration. The activity of antioxidant enzyme SOD in brain homogenate of mice was increased by 22.8%, 48.69% and 84.06% respectively (Figure 3). So drug treatment enhanced the enzyme activity throughout the investigation. A significant (*p< 0.05) increase of lipid peroxidation was observed in mice brain after 10, 20, 30 days' stages. Brain from control mice maintained MDA level between 34.96 ± 0.87 to 35.96 ± 0.34 n moles/g of fresh tissue weight. A significant (*p< 0.05) percentage increase in the formation of thiobarbituric acid reactive species (TBARS) is observed in the mice brain. This percentage increase is maximum in drug treated mice at 30 days' stage of investigation.

Superoxide dismutase is a metalloenzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. SOD plays an extremely important role in the protection of all aerobic life systems, including man, against oxygen toxicity and the free radicals derived from oxygen. Increase in SOD activity is an indicator of the oxidative stress in tissues of drug treated mice and is required for protection against free radicals. This antioxidant enzyme acts directly or indirectly to remove reactive oxygen species and thus elevation in SOD level is observed [8]. MDA is a breakdown product of unsaturated fatty acids and a significant increase in levels of lipid peroxide indicates enhanced lipid peroxidation by free radicals. The end products of lipid peroxidation may be mutagenic and carcinogenic [9]. Many reports suggest that non steroidal anti-inflammatory drugs cause mitochondrial injury by dissipating the mitochondrial transmembrane potential and inducing mitochondrial permeability transition pore, which liberates cytochrome C. This enzyme generates reactive oxygen species which trigger the cellular lipid peroxidation, resulting in cellular apoptosis [10]. A significant increase in the MDA level after diclofenac treatment is observed by Ismail *et al* [11].

Table I: Body weight and brain weight of normal mice and treated mice with diclofenac sodium after 10 to 30 days period.

	Days		
	10	**20**	**30**
Body weight (g) Normal	24.33±0.71	24.66±0.54	25±0.46
Body weight (g) Treated	21.00±1.24	19.67±1.27*	21.33±0.72*
% decrease	-13.69	-20.24	-14.68
Brain weight (mg) Normal	492.76±3.77	481.20±3.26	486.86±2.96
Muscle weight(mg) Treated	482.66±5.16	469.00±3.12*	481.96±2.74*
% decrease	-2.05	-2.54	-1.01

Values are mean ± SEM; n = 3 (*p < 0.05).

Table II: Lipid peroxidation and SOD activity in normal mice and diclofenac sodium treated mice after 10 to 30 days period.

	Days		
	10	**20**	**30**
Lipid Peroxidation (n mole MDA /g of tissue) Normal	34.96±0.87	35.96±0.34	35.20±0.79
Lipid Peroxidation (n mole MDA /g of tissue) Treated	62.42±1.04*	92.07±1.5*	125.06±2.62*
% increase	78.55	156.03	255.28
SOD (units/mg tissues) Normal	8.68±0.11	8.81±0.47	8.72±0.04
SOD (units/mg tissues) Treated	10.66±0.18*	13.10±0.13*	16.05±0.18*
% increase	22.80	48.69	84.06

Values are mean ± SEM; n = 3 (*p < 0.05).

FIGURES

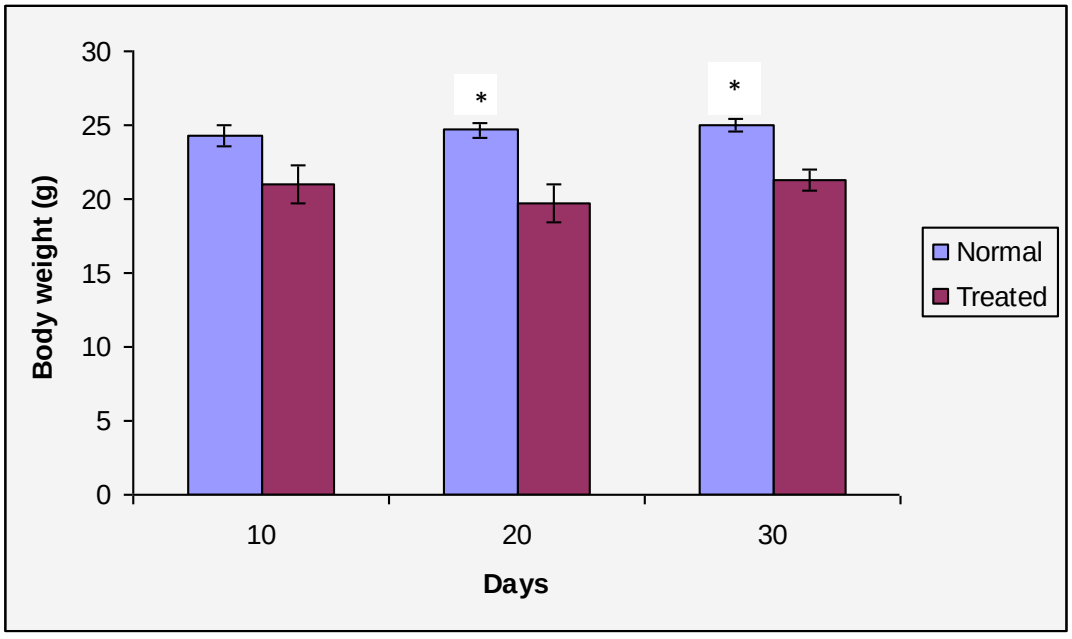

Fig. 1: Changes in body weight (g) of normal and diclofenac sodium treated mice after 10-30 days period .Values are mean ± SEM; n = 3 (*p < 0.05).

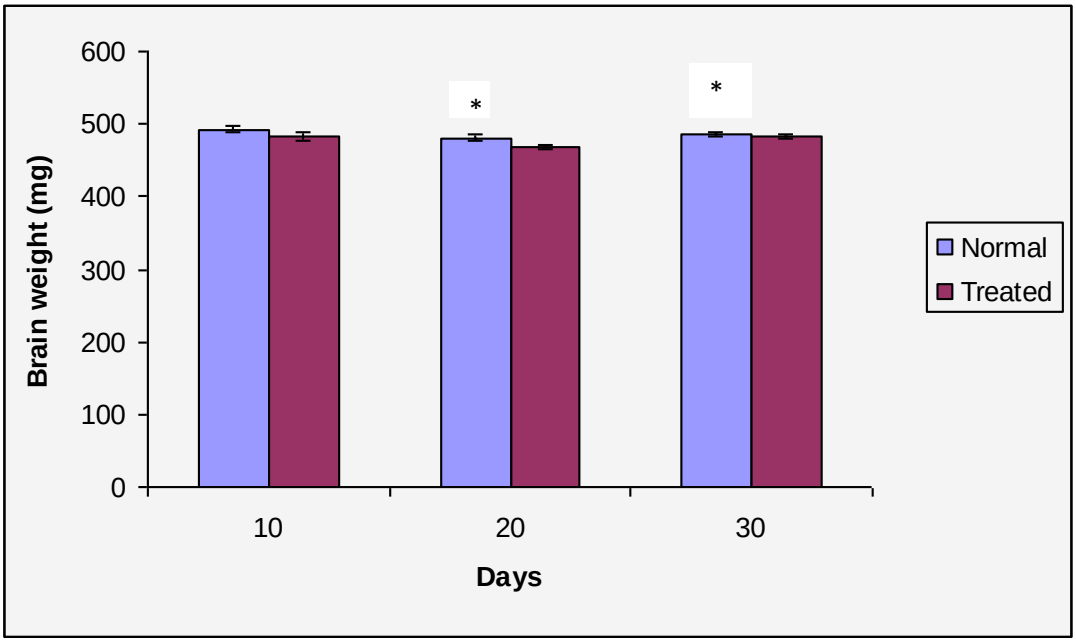

Fig. 2: Changes in brain weight (mg) of normal and diclofenac sodium treated mice after 10-30 days period .Values are mean ± SEM; n = 3 (*p < 0.05).

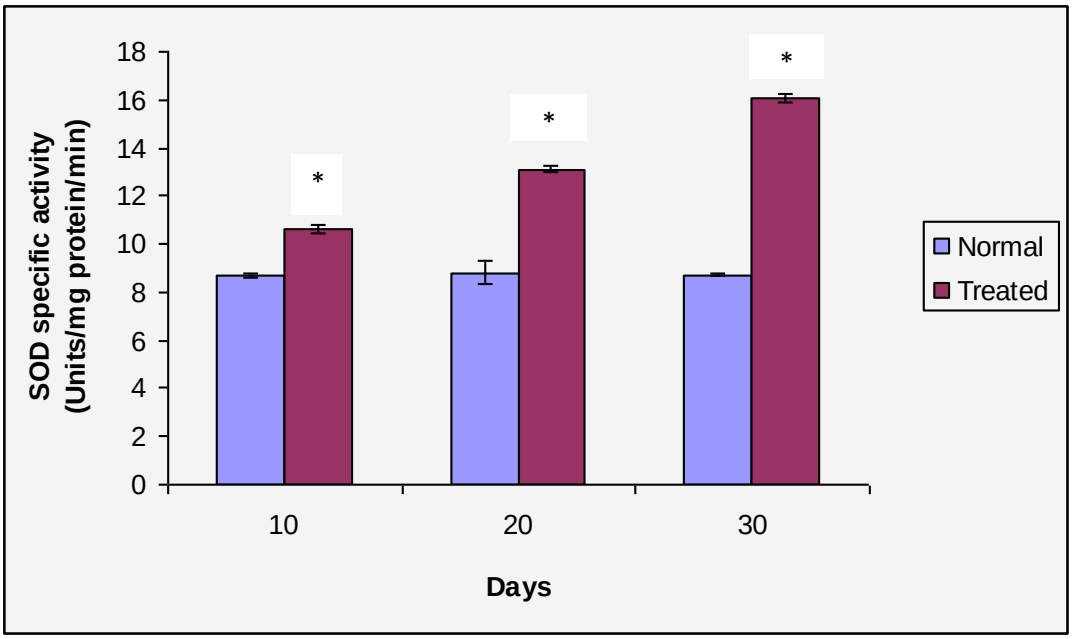

Fig. 3: Superoxide dismutase specific activity (units/mg protein/min) of normal and diclofenac sodium treated brain after 10-30 days period. Values are mean ± SEM; n = 3 (*p < 0.05).

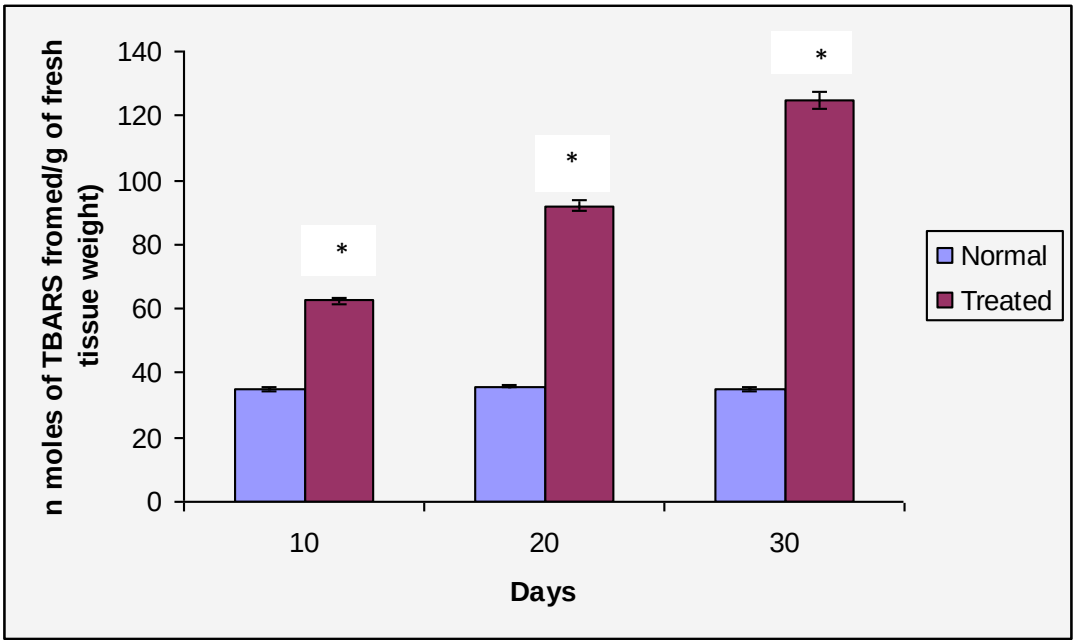

Fig. 4: Lipid peroxide (n moles of TBARS formed/g of fresh tissue weight) in brain of normal and diclofenac treated mice after 10-30 days period. Values are mean ± SEM; n = 3 (*p < 0.05).

IV. CONCLUSION

It is concluded from present study that there is altered SOD activity in brain in order to protect the organ from free radical attack. This enzyme shows scavenging action. A large increase in the formation of thiobarbituric acid reactive species (TBARS) is observed in brain of mice. So further investigations are necessary to provide additional information regarding unwanted effects of diclofenac sodium.

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