Effects of Indomethacin Administration on Some Biochemical and Brain Histological Changes in Male Rats

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Abstract

Reactive oxygen species and lipid peroxidation play a role in the pathogenesis induced by the non-steroidal anti-inflammatory drug indomethacin.

This study was designed in order to investigate and demonstrate the histopathological and biochemical changes in rat brain due to chronic usage of indomethacin.

Twenty one male albino rats were assigned to three groups. GA: served as normal control and received normal saline for 21days. G B:received indomethacin 10 mg/kg/day orally given once daily.**GC:** received indomethacin 10 mg/kg/dayorally given once daily.

Results: Treated rats with indomethacin with a concentration 5 and 10mg/kg·b·w, expressed a significant increase in several parameters includes, serum malondialdehyde (MDA), Tumor Necrosis Factor-alpha (TNF-α), C-reactive protein(C-RP), and nitric oxide (NO) (< 0.05). In addition, a significant reduction in serum superoxide dismutase (SOD), glutathione (GSH), catalase (CAT) in the in comparison with control group.Histological assessment of the haematoxylin and eosin stained sections of the brain of rats treated with indomethacin showed varying degrees of architectural distortions.It could be concluded that administration of indomethacin at high dose induced some adverse effects on biochemical, oxidative parameters as well as histology of brain. That could be attributed to oxidative stress induced by the drug. However, these effects were reversible.

Keywords: *Indomethacin ;brain; oxidative stress;male rats*

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely and extensively used for their analgesic, antipyretic, and anti-inflammatory properties[1]. Indomethacin1-(p-chlorobenzoyl)-5-methoxy-2-methyllindole-3-acetic acid is a NSAID and exerts anti-inflammatory effects by non-selectively interfering with cyclooxygenase (COX) 1 and COX-2, and accordingly reducing the production of prostaglandin (PG) E2[2(1)]. They are a class of drugs that reduce pain and decrease the inflammation[(2)].

Indomethacin is known to induce the reactive oxygen metabolites in animal models, which may contribute to tissuedamag[(3)].These free radicals also damage the cellular antioxidant enzymes such as CAT, SOD and others, acting as the first line of cellular defense against oxidative injury. This might lead to aggravated tissue damage.Body and organ weights are key indices for the toxicological assessments of drugs because they could be modified with the advent of drug-induced toxicity[(4)].

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The brain has increased oxygen and glucose depreciation, which makes them more vulnerable to reactive oxygen species (ROS) production because it metabolizes 20% of total body oxygen and has a limited amount of antioxidant capacity. Besides vasculature system, free radicals are constantly produced in the brain "in vivo." Because of its high ATP demand, the brain consumes oxygen rapidly, and is thus susceptible to interference with mitochondrial function, which can in turn lead to high production of superoxide radical[5,6]. Free radicals in central nervous system arise by the leakage of electrons from the mitochondrial electron transport chain to generate superoxide radical[7].

The present study was conducted to evaluate the effects of the indomethacin on brain tissues and biochemical parameter of albino rats.

Materials and Methods

Animals and experimental protocol:

The present study was performed on 21 albino male rats with weight ranging within 185 - 210g. They were 4-6 weeks old. They were divided into 3groups. Each group made of 7 Albino Rats.

The animals were kept in separate cages with average temperature (22-24 C˚) and humidity in an adequately ventilated room under a regular 12h light/12h dark cycle and were allowed free access to food and water ad libitum. The animals were obtained from the animal house of the Faculty of Veterinary Medicine, University of Tikrit.

The animals were randomly allocated into 3 groups (Each group made of 7 Albino Rats):

1. Group A: Control group received 1 ml of normal saline 0.9% orally three successive weeks.

2. Group B: it included seven rats that were orally given indomethacin in a dose of 5 mg/kg body weight dissolved in saline daily for 3successive weeks.

3. Group C: it included seven rats that were orallygiven indomethacinin a dose of 10 mg/kg body weight dissolved in saline daily for 3 successive weeks.

After three weeks, 5 ml of blood samples were collected from rats hearts, and 1 ml of the sera prepared through centrifuging at 3000 rpm for 10 minutes, then stored at freeze until assayed. Serum SOD, GSH, MDA, andG-Px levels were measured by spectrophotometric kit, tumor necrosis factor-α was measured by ELISA technique.

Brain tissues were collected from different groups and were fixed in 10% buffered formalin solution. The tissues were processed, embedded in paraffin and sections of 5µm thickness were obtained. The sections were stained with hematoxylin and eosin and examined using a light microscope[8].

Statistical Analysis

All the data submitted to statistical analysis by using Tukey tests and one-way analysis (ANOVA) at ($p<0.05$) wherever by application following formula (mean±SD) to evaluate the results.

Results

Results indicated that the serum MDA,TNF-α, C-RP, and NO levels, were significantly increased in rats treated with indomethacin when compared to the normal group, on contrary, indomethacin treatment significantly ($P < 0.01$) decreased the level of SOD, GSH, GPx when compared to the control group.

Table 1. MDA, nitric oxide, and GSH levels, and PON-1 activity in the serum of rats treated with

Treatment	Control	Indomethacin(5mg/kg)	Indomethacin(10mg/kg)
MDA (µmol/L)	3.76 ± 0.329	5 ± 0.35	6.34 ± 0.34a
NO (µmol/L)	32 ± 1.43	46.79 ± 1.67	60± 1.91
SOD (U/mL)	30.1 ± 1.31	17.03 ± 0.91*	0.6 ± 0.69*
TNF-α (Pg/ml)	26 ± 1.96*	70.1 ± 5.73*	37.2 ± 3.3*
GSH (mg/mg protein)	76.8 ± 6.7	57± 6.3	51± 5.21*
GPx (U/g protein)	33.6 ± 0.42	25.4 ± 0.57	22.37 ± 0.75
TNF-α (pg/dL)	26.9 ± 8.1	31.7 ± 7.8	39.6 ± 4.9
C-RP(mg/dL)	07.95 ± 1.45	1.60 ± 0.28	3.03 ± 1.40
Vit.E (µg/ml)	17.79±1.58	10.07± 0.46	12± 0.46

Histopathological Observations:

Control group

The brain tissue was sheathed with pia mater and the brain cortex was formed by molecular cells, granular cell (which are external and internal),there were small pyramidal cells, associated with glial cells (supporting cells) and minute blood vessels (Fig.1).

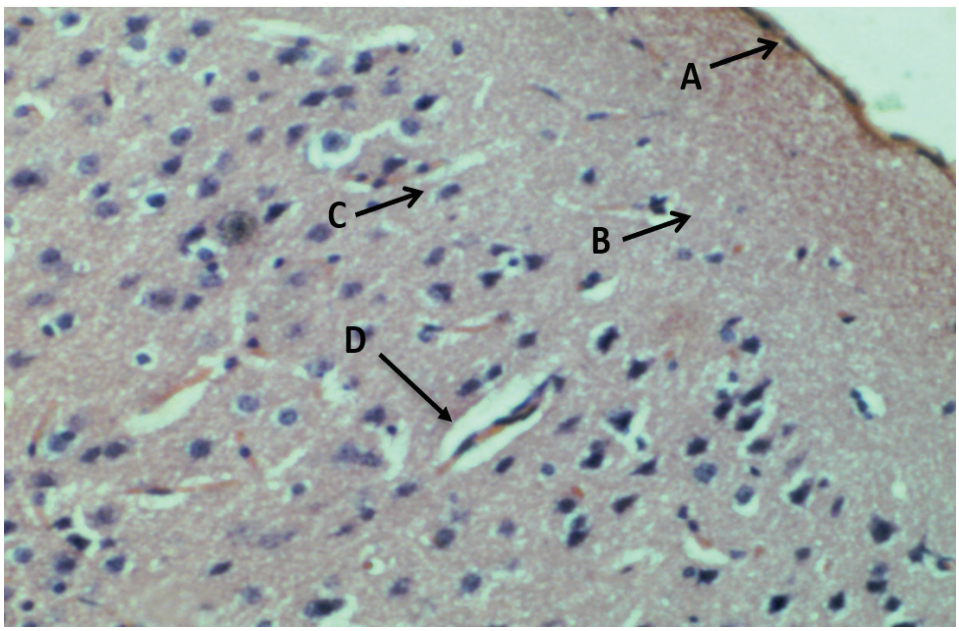

Fig.1:Brain tissue; Demonstrating pia mater (A), externalmolecular layer (B). external granular layer (C) and blood vessels (D). (H &E ×20).

The group receive Indomethacin 5mg/kg b.wt .

The brain tissue was demonstrated adilated pia mater capillaries with presence of the inflammatory cells inside and around meningeal blood vessels (Fig.2).The blood congestion was progressed to the brain tissue its self.

The diffused vacuolization around and near neurons and among glial cells of the deepest layers of brain was detected (Fig.3).

The choroid plexus of the brain ventricles were detached from its roof which appeared as corrugated membrane (Fig.4).

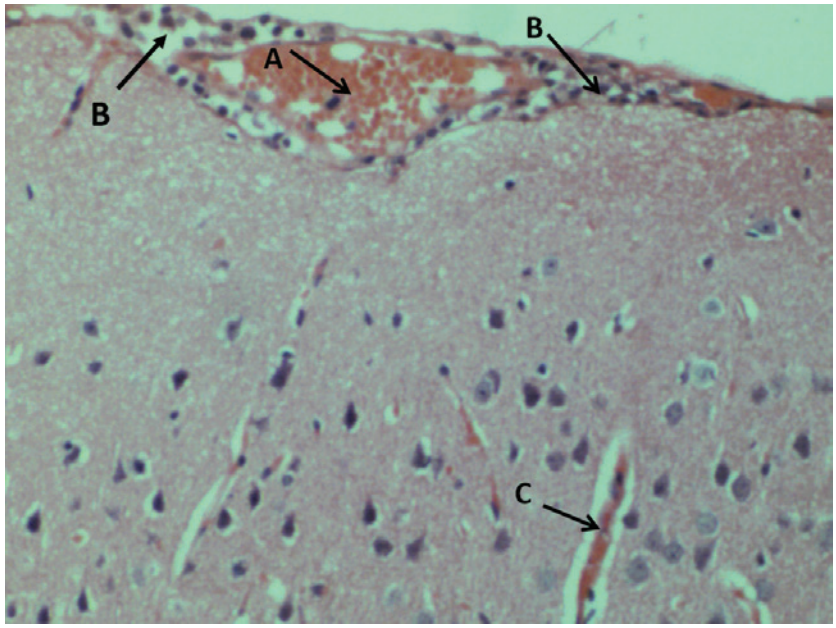

Fig.2: Congestion of blood inside pia mater capillary (A), inflammatory cellsaggregation of in and outside blood vessels (B) slight congestion of blood in brain tissue (H&E *20).

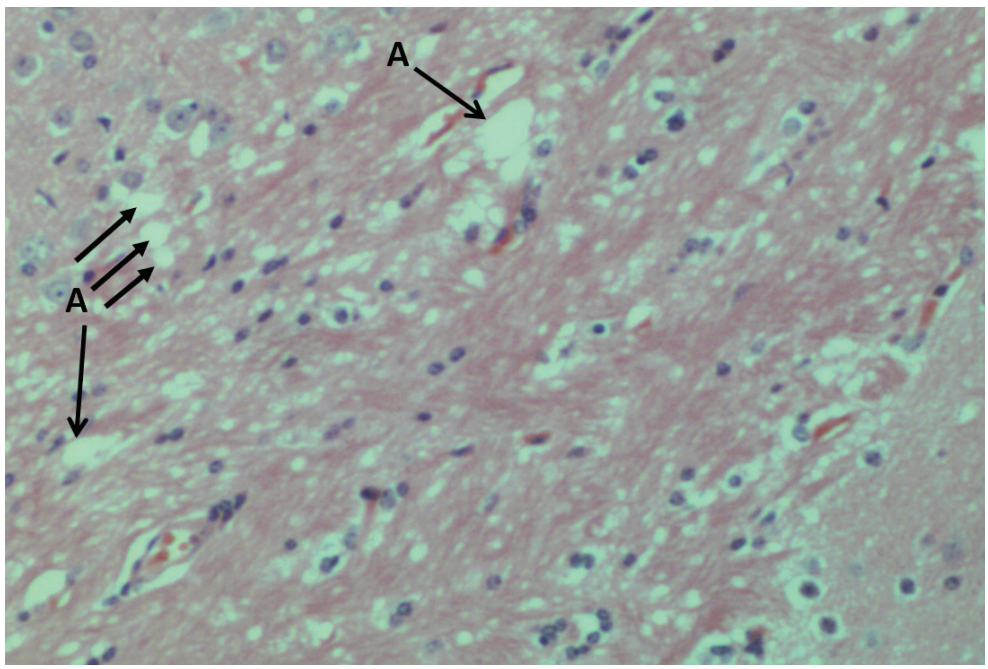

Fig.3:vacuolizationamong neurons and glial cells (A). (H&E *20).

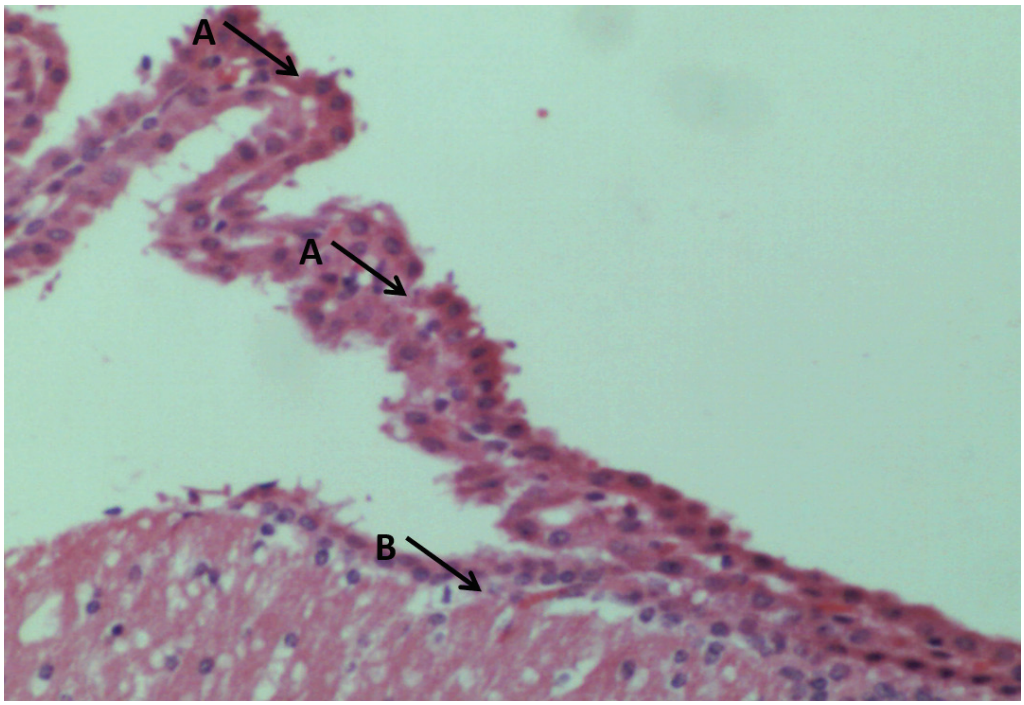

Fig.4: Choroid plexus of brain ventricles (A) showing detachment from its roof (B) (H&E *20).

The group receive Indomethacin 10mg/kg b.wt .

The brain tissue revealed that extensive vacuolization was present in whole layers of brain of white matter and there was disturbance in the arrangement of the molecular and granular layer of external and internal cells. The blood congestion in the brain tissue was easily detected and the blood had WBC also (Fig.5) and (Fig.6).

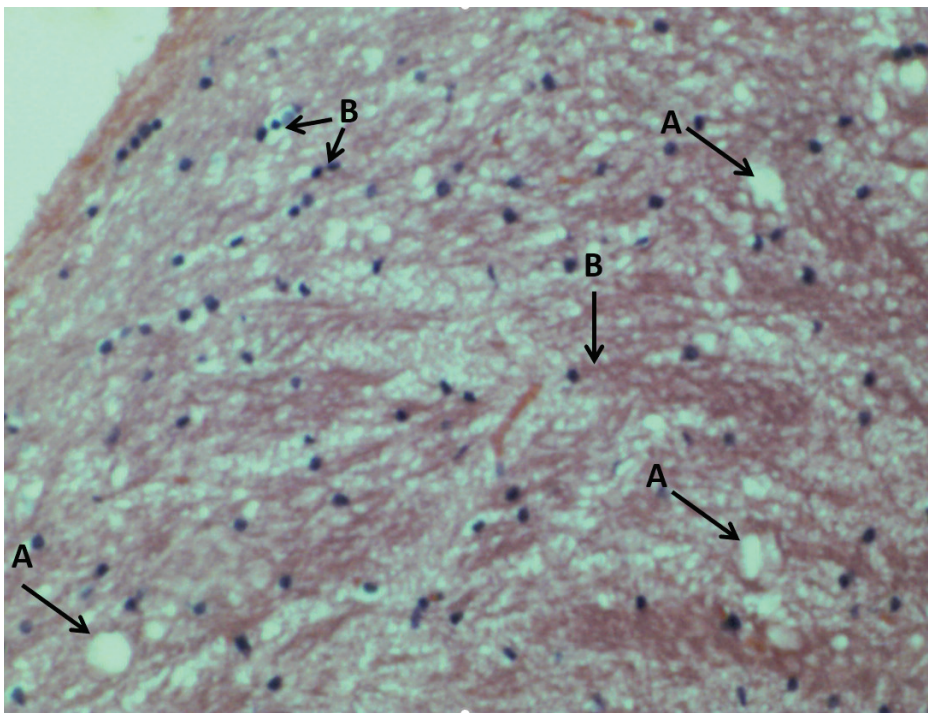

Fig.5: diffused vacuolizationwithin brain tissue (A) disturbance of molecular and granular layer cells arrangement (B) (H&E *20).

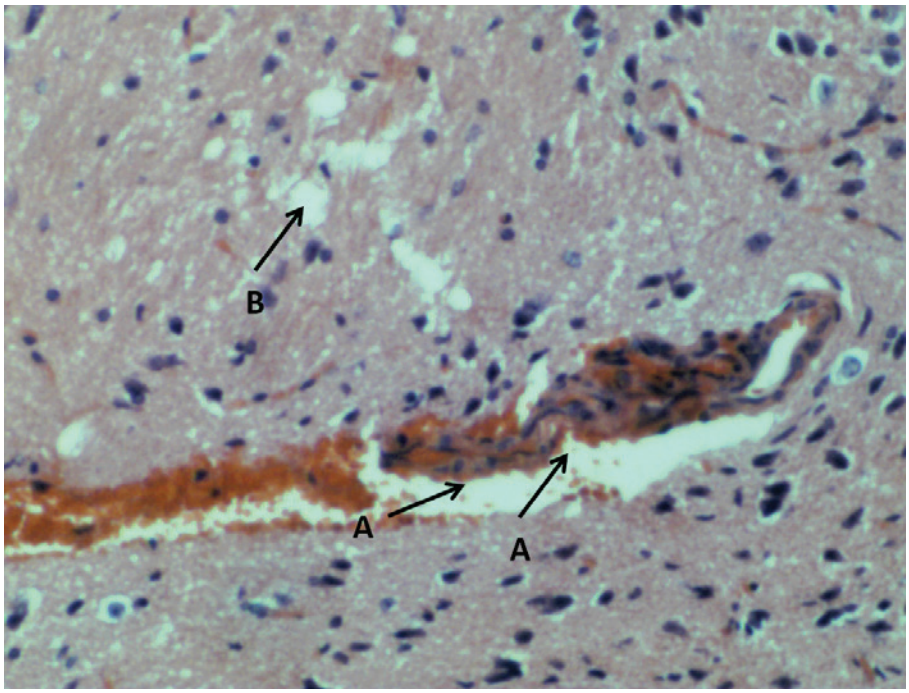

Fig.6:blood congestion of the brain capillary (A) vacuolation of brain blood tissue (B) (H&E *20).

Discussion

Inflammation and oxidative stress are closely associated events. Inflammation is considered as one of the consequences of oxidative stress, as the pathways that instigate the production of inflammatory mediators are all triggered by oxidative stress[9,10].

Indomethacin leads to mitochondrial oxidative stress associated with the generation of intra-mitochondrial reactive oxygen species (ROS), which induces imbalance of oxidants and antioxidants status in living system[11].

Oxidative stress which is formed by the breakdown of the balance between free radicals and antioxidants, due to the excessive production of ROS and the reduction in the rate of its removal by the antioxidant defense system.These ROS can damage proteins, lipids and DNA, which in turn change their structure and function causing cell damage and even death. So, most of the intertubular and intratubular changes that were reported in this study can be explained by lipid peroxidation of the cell and organelles, membranes, with consequent ROS generation[12-14].

The obtained results revealed that a significant increase in serum MDA which is a useful biomarker for oxidative stress concentrations in indomethacin overdose groups, which indicate enhanced LPO leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals[12].

Catalase is a haemeprotein in all aerobic cells that metabolize H_2O_2 to oxygen and water. These antioxidant enzymes are inactivated by lipid peroxides or ROS[15]. The activity of serum CAT increased significantly, due to the absence of appropriate SOD activity, superoxide anions are not dismuted into H_2O_2, which is the substrate for CAT enzyme, leading to decreasing in CAT activity[16].which was attributed to the stimulation of antioxidant defense system, and this is in agreement with the findings of Mansour *et al*[17].

The results show that SOD level was significantly decreased in the treated rats, which might be because of an excessive formation of superoxide anions. Because of the production of superoxide radicals during the oxidative phosphorylation chain in the mitochondria, there is a superoxide dismutase enzyme (SOD) that catalyzes superoxide anion into oxygen and hydrogen peroxide[18].

Glutathione peroxidase (GPx), plays a primary role in minimizing oxidative damage. GPx an enzyme

with selenium and Glutathione-s-transferase (GST) works together with glutathione in the decomposition of H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione[19].

The GSH is one of the major endogenous antioxidant produced by cells participating directly in the neutralization of free radicals and reactive oxygen species[20].It is used as a cofactor in the removal of hydrogen peroxide and lipoperoxides by the GSH-Px family during which it is converted into the oxidized form of glutathione (GSSG).Hussien and Gobba[21]. suggested that the decrease in GSH could be the result of decreased synthesis or increased degradation of GSH by oxidative stress[22].In the present study, the elevation of serum GSH levels in were observed in the treated rats.

In the present study, decreased serum GST and GSH-Px activities in alone indomethacin-intoxicated rat can be explained by the consumption of these enzymes during the detoxification of reactive oxygen metabolites generated due to indomethacin, as well as consumption of erythrocytes GSH store. Decrease in GSH content due to indomethacin intoxication can simultaneously decrease the activities of GST as well as GSH-Px[23]. The results of the present study positively indicate the possibility of indomethacin mediated oxidative stress in erythrocytes.

Histological examination of the brain tissue reveals that indomethacin caused abnormal structural changes in the brain tissue, including spongiform necrosis, nuclear vacuolization, pyknosis and inflammatory changes.

Nitric oxide acts as a signaling messenger and under physiological conditions, the constitutively produced low levels of nitric oxide are important in intracellular signaling and in the regulation of basal vascular tone. In contrast, excess production of iNOS-derived nitric oxide by cells such as activated macrophages can cause tissue damage and organ dysfunction[24].This occurs through the formation of more ROS, including nitrogen dioxide (formed by the reaction of nitric oxide with molecular oxygen) or peroxynitrite (formed by the reaction of NO and superoxide). These species are capable of oxidizing free thiols in the cytosol and amine, and causing thiol nitrosation and lipid peroxidation, leading to inhibition of mitochondrial enzymes, mitochondrial impairment, and cellular energy failure[25].

In the present study, indomethacin significantly reduced serum NO level compared to control group. The possible mechanism of the increased NO level may be due to reduced production of NO by NOS and inactivation of NO by ROS produced by glycosylated proteinsthat was associated with an increase in the extent of damage. The results of this study agree with results of other studies[26,27].

Tumor necrosis factor-α(TNF-α) is produced by adipocytes ,neutrophils, activated lymphocytes, macrophages, and null killer cells but may be expressed by many non-immune cell types, has been implicated to play an important role in the cascade of inflammation[28]. TNF-α have been also determined as markers of the inflammatory response[29].

The TNF-α. levels significantly elevated when rats were administered with Indomethacin indicating thatNSAID usage is very important because elevated levels of TNF-alpha may contribute to the deterioration of cardiovascular function through various mechanisms. TNF-α also has been shown to exert pro-inflammatory vascular effects (e.g., induction of oxidative stress, endothelial apoptosis, up-regulation of adhesion molecules and chemokines[30].

In conclusion, indomethacin at high dose causes alterations in hematological, biochemical parameters and histopathological changes in brain of male rats. These adverse effects may be contributedto oxidative stress induced by the drug. However, thetoxic effects of indomethacin could be acute or reversible.

Conflict of Interests : The authors of this paper declare that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

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Ethical Approve : We declare that the study does not need ethical approval.

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