ORIGINAL ARTICLE

Biochemical and Histological Evaluation of Indomethacin-induced Hepatotoxicity in Rats

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ABSTRACT

Background: It is becoming progressively more understandable that overdose of indomethacin in both humans and animals causes severe hepatotoxicity.
Aim: To determine the biochemical and histological changes in liver due to of indomethacin administration in adult male albino rats.
Methods: Drug-treated rats were given food for the next 24 h until they were sacrificed. Measurement of gamma glutamylransferase (ɣ-GT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin , and 5`-nucleotidase(5-NT) in serum were performed , and the histopathology of the liver in all groups were investigated. Statistical evaluation of the results at a $p < 0.05$ elucidated significantly elevated values of these enzymes when compared with control.
Results: Indomethacin induced rats showed a significant increase in serum levels of AST and ALT, ALP, GGTP,5-NT**and** 5-NT,whereas the serum total protein, albumin was significantly decreased in treated group compared with group 1.
The histological examination shows, heavy lymphocytes infiltration, degenerative changes in the hepatocytes, dilated and congestedportal vein.These findings support the need for caution in administration of indomethacin particularly for long term administration.
Conclusion: Indomethacin treatment in wistar albino male rats has a toxic effect on the structure and function of hepatic tissue.
Keyword: Indomethacin; Rat; Histological examination; Hepatotoxicity

INTRODUCTION

Over 50 years ago, inflammation is treated by nonsteroidal anti-inflammatory drugs (NSAIDs) such as salicylates, naproxen, ibuprofen, and indomethacin that represent one of the most common classes of medications used world-wide, with an estimated usage of >30 million per day , which are differ in their structure but all have similar anti-inflammatory, analgesic and anti-pyretic effects through the suppression of prostaglandin (PG) synthesis, by inhibiting the enzyme cyclooxygenase (COX)[1].

Indomethacin is 1-(p-chlorobenzoyl)-5-methoxy-2-methyllindole-3-acetic acid that emerged as one of the most extremely potent -antipyretic ,analgesic, and anti-inflammatory properties through non-selective potent inhibitor of the cyclooxygenases (COX) 1 and 2 enzyme which responsible for the conversion of arachidonic acid to prostaglandins[2,3].

Liver is an important organplays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body, its major functions are, carbohydrate, protein and fat metabolism, immunity, exogenous (drug) and endogenous substances detoxification, secretion of bile and storage of vitamin[4,5,6].

Hence the present study was designed to highlight the impact of indomethacin on the biochemicaland histopathological profiles in hepatic functions in adult male albino rats.

MATERIALS AND METHODS

Twenty four male 8-week-old Wistar rats (190 to 220 grams). They were kept in wire meshed cages and fed with commercial rat pellets and allowed water ad libitum. The animals were housed under standard conditions of temperatureof 19 °C, relative humidity of 55±10% and 12-h light/dark cycle. They were classified into 3 equal groups each comprises 8 rats and treated daily for 21 days, as follow:
G1 served as normal control and received normal saline.
G2 and G3 received indomethacin (5, and 10 mg/kg/day) orally given once daily.

The blood samples were taken from the heart; then it centrifuged for five minutes at 3000 rpm to prepare the serum for biochemical analysis of nitric oxide(Miranda,2001).

ALT, AST, ALP, 5' NT, bilirubin, protein, andalbuminwere determined by routine colorimetric methods(Wiener lab- Rosario, Argentina). The fibrinogen measured by the coagulation method using a Sysmex CA7000 System.

Histological finding: After sacrifice, livers tissues were obtained from the rats. The tissues excised and covered with physiological normal salineand cleaned from attached fat and connective tissue. Blocks of tissues were fixed in 10% neutral buffered formalin, dehydrated with graded series of ethyl alcohol and embedded in paraffin.Serial sections of five µm thickness were cut and stained with

hematoxylin and eosin (H&E). Two examiners unaware of experimental details independently determined the histomorphological changes in the liver using a light microscope (Leica Microsystems)[7].

Values are expressed as the mean ± SE. A paired t test was used in the statistical analyses; p values less than 0.05 were considered to be statistically significant.

RESULTS

Biochemical finding: Concerning the liver biochemical parameters in [Table 1 and 2], AST and ALT, ALP, GGTP,and 5-NT, NOwere highly significantly increased and the serum total protein, albumin was significantly decreased in treated group compared with group 1.

Histopathological examination:

G1 [Control]: The parenchyma of the liver was formed by hepatic lobules,each lobule was consist of Central vein, surrounded by hepatic cellular cords or columns which are present honey-comb like with a spherical nuclei inside each cell, these cells are surrounded by sinusoid, which was containing Kupffer cell. The portal area were containing branch of portal vein, branch of hepatic artery, Bile duct and lymph vessels(Fig.1).

G2 [received indomethacin 5mg/kg/day]: The parenchyma of the liver appeared normal with very slight narrowing of the sinusoid, the presence of WBC (inflammatory cells) to be forming sheath –like around the bile duct of the portal area. The blood vessels of this area of the sections had homogenous blood (hemolysis) (Fig 2 & 3).

G3 [received indomethacin 10mg/kg/day]: The parenchyma of the liver was contains irregular shape of hepatocytes, which tend to be round instead of polygoral –shape,and the cells in this group were hypertrophied, the nuclei of other cells were karyopyknosis (irreversible condensation of chromatin)karyorrhexic (fragmentation of the nucleus of a dying cell) or karyolytic(complete dissolution of the chromatin of a dying cell).The sinusoids were narrow compared with control group and kupffer cells inside it were demonstrated well (Fig . 4).

The portal vein widening and enlargement was seen with congested blood, in some area the portal vein occupied most of the portal tried area. The results revealed also heavy infiltration of lymphocyte cells around the bile ducts (Fig. 5).

Table 1: Effects of indomethacin on the serum total protein, albumin, total bilirubin, NO, and 5-NTin adult rats.

Parameters	TP(g/dL)	Albumin(g/dL)	TB(µmol /L)	NO(Umol/L)	5-NT(IU/L)
+ve Control	7.45±0.02	4.11±0.14	0.85±0.02	17.50±2.09	11.2±3.42
Indomethacin(5 mg/kg)	6.97±0.04	3.68±0.12*	5.93±0.241***	21.77±2.75*	24.07±4.33
Indomethacin (10mg/kg)	6.96±0.09*	3.55±0.20*	6.27±0.281***	27.6±2.85**	29.87±3.94***

Table 2: Effects of Indomethacin on the serum AST and ALT, ALP, GGTP, TB, and Fibrinogen in adult rats.

Parameters	AST(U/L)	ALP(U/L)	ALT(U/L)	TB(µmol /L)	GGTP(IU/L)	Fibrinogen (mg/dL)
+ve Control	52.29±1.26	37.63±1.93	33.82±0.54	0.85±0.02	2.02±0.09*	259.20±11.54
Indomethacin (5 mg/kg)	92.27±0.89*	55.07±2.34*	80.4± 0.6146****	5.93±0.241***	2.43±0.45**	392.91±66.55
Indomethacin (10mg/kg)	98.43±1.32***	58.15±3.68*	73.30±0.847****	6.27±0.281***	0 .82±1.10*	420.6±40.7**

Fig. 1: A photomicrograph of transverse section in the control rat liver shows hepatic lobule ,Central vein (A) hepatocyte with well-shaped nucleus (B),Blood sinusoid (C),Kupffer cells (D). (H &E ×20).

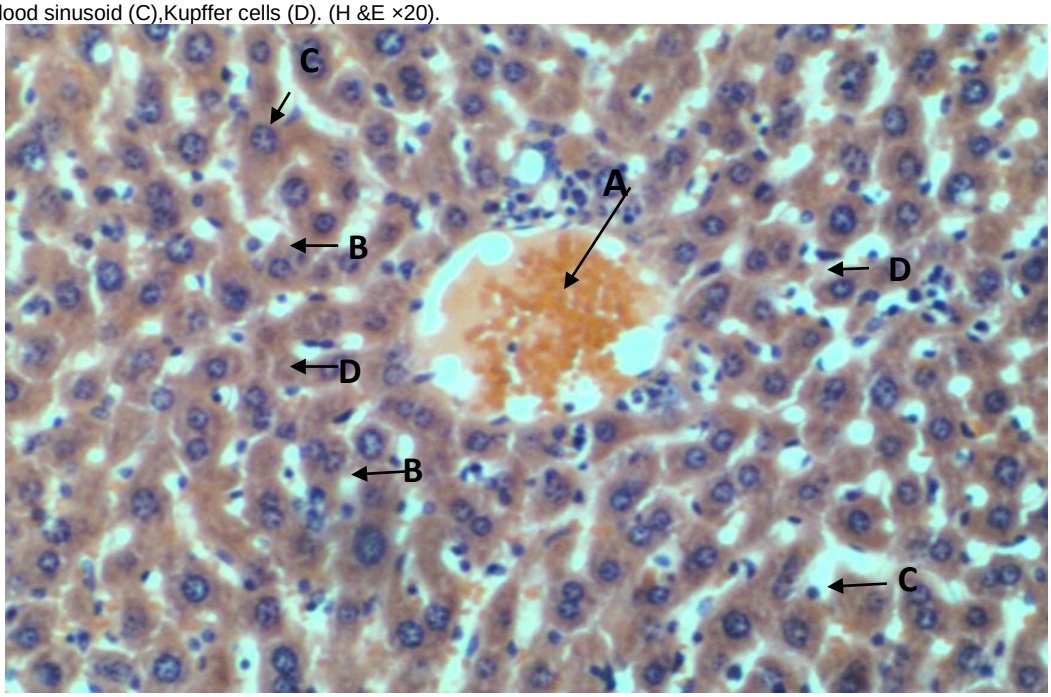


Fig. 2: Hemolysis of RBC(A)inside the portal vein in the,sheath of lymphocytic mass around the bile duct. Slight narrowing in the sinosoid (C).(H&E ×20).

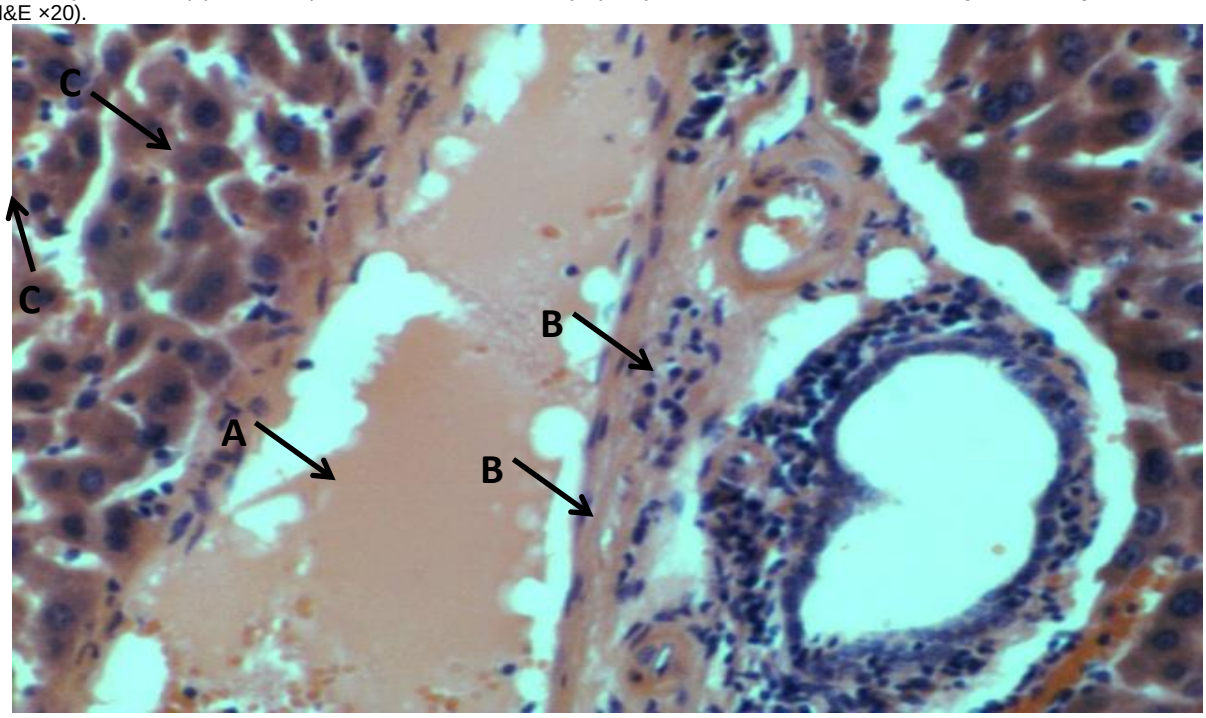


Fig 3: Liver parenchyma demonstrating a normal hepatocytes (A), with lymphocytic infiltration around portal vein and bile duct in the portal area (B) (H &E ×10).

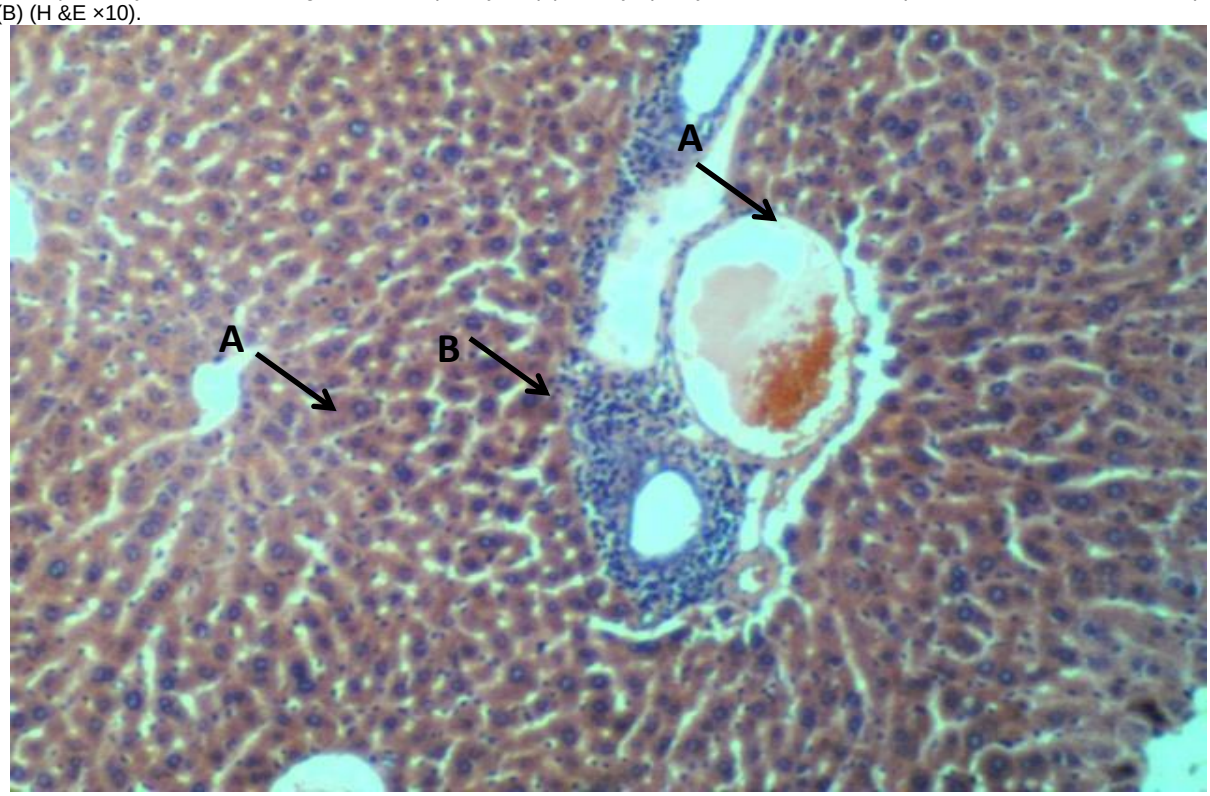


Figure 4: Aphotomicrograph of transverse section in group 2 rat liver showing round hypertrophiedhepatocytes (A),Kupffer cells (B) within narrow sinusoid, karyopyknosis (C), karyorrhexis(D) and karyolysis (E) (H &E ×20).

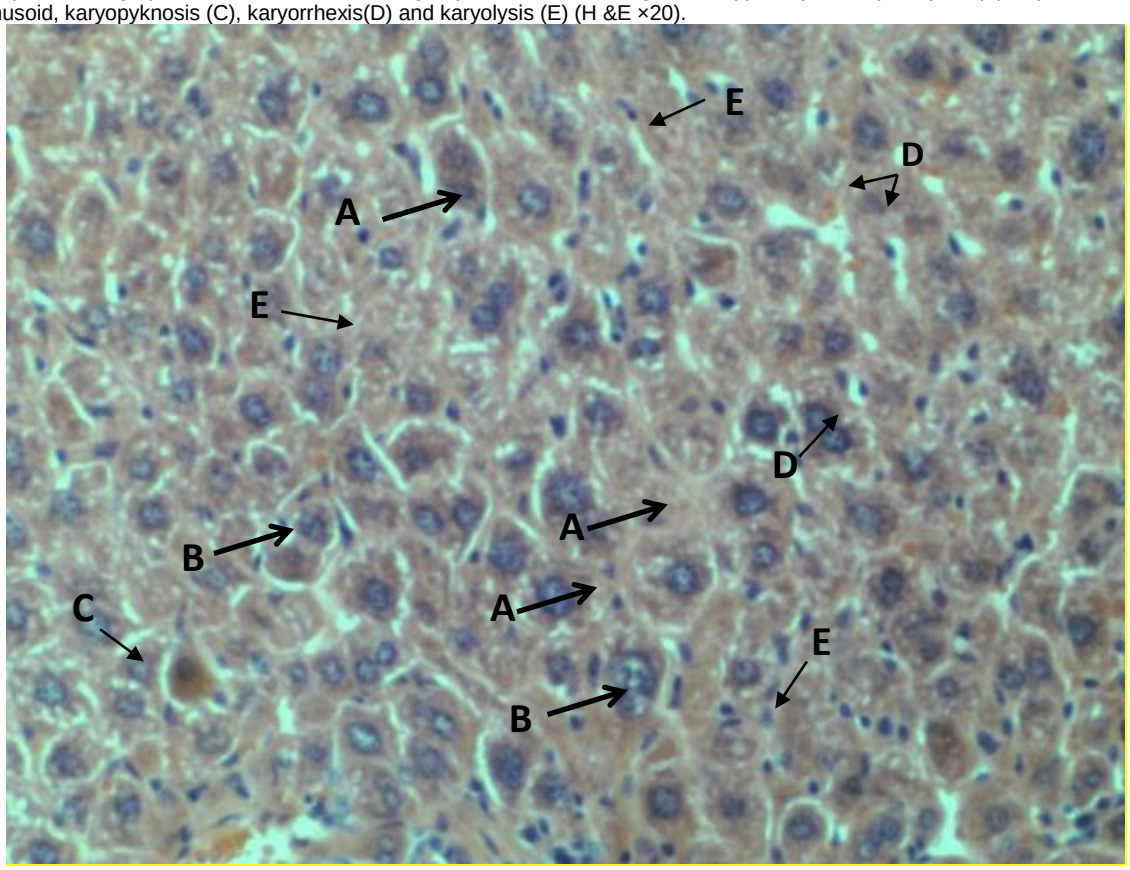
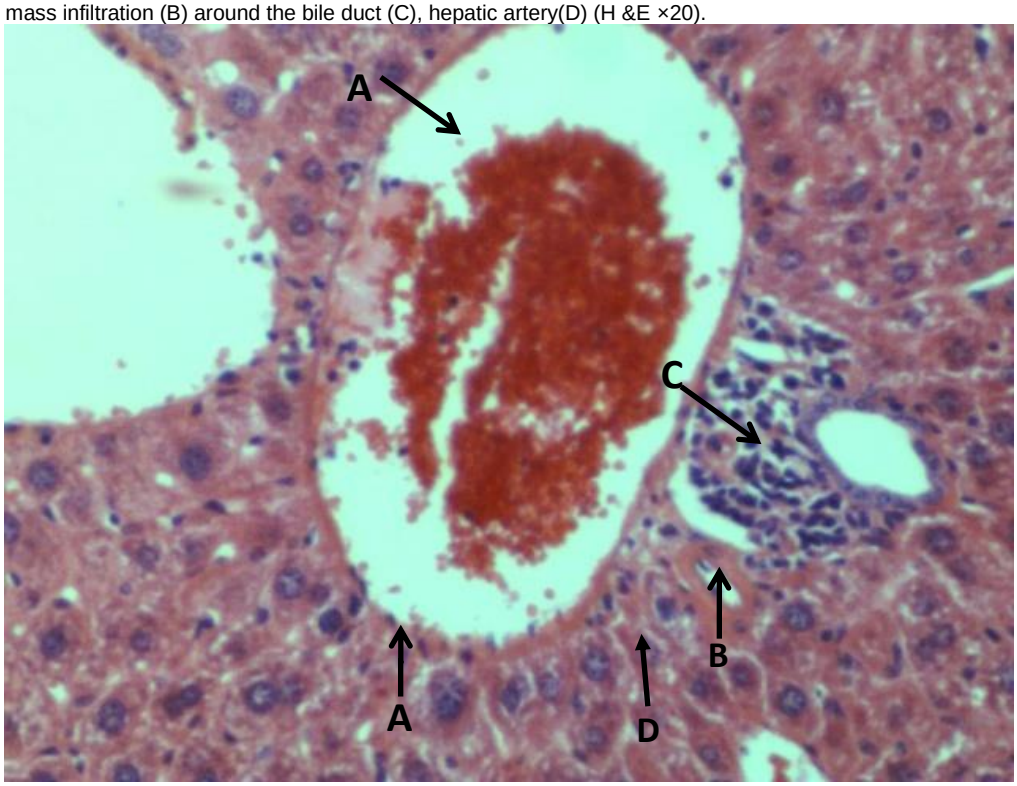


Figure 5: A photomicrograph of transverse section in the second group rat liver showing Congestion of blood and widening of the portal vein (A).Lymphocytic mass infiltration (B) around the bile duct (C), hepatic artery(D) (H &E ×20).



DISCUSSION

Indomethacin is considered to be safe at therapeutic doses and is a widely used antipyretic and analgesic drug in clinical practice. However, overdose of indomethacin in both humans and animals causes severe hepatotoxicity and necrosis.

Liver is responsible for the metabolism and excretion of indomethacin. Most of the toxic compounds in the body are metabolized in liver[8]. The mechanism of indomethacin overdose induced hepatic injury have not been fully illustrated, butChougule suggests that, inhibition of protective prostaglandins PGE1, PGE2 and prostacyclin (PG12) may be one of the mechanism by which indomethacin induces injury[9].

Fibrinogen, as well as several other homeostasis factors, belongs to the group of interleukin-6 (IL-6)-stimulated positive acute-phase proteins[9,10]. The current study observed that serum fibrinogen in the second and third groups which received indomethacin alone showed statistical significant increase in comparison to those of the control group, these increase may be a result of its increased synthesis in liver, after suffering the stimulatory action of interleukins TNF-α, IL-1 and IL-6, released by the inflammatory process. Additionally, fibrinogen degradation products (FDP) also enhance fibrinogen synthesis in the liver, which seems to play a regulatory role. The FDP stimulates IL-6 production by prostaglandin in dependent way, besides it also cause metabolic changes in the liver. It has been suggested that this metabolic effect of LPS is mediated by eicosanoids (PGD2) and (PGE2) produced by Kupffer cells[10].

5'-Nucleotidase (5'-ribonucleotide phosphohydrolase; EC 3.1.3.5; 5'-NT), an intrinsic membrane glycoprotein present as an ectoenzyme disseminated throughout the tissues of the body confined in cytoplasmic membrane, catalyzes hydrolysis of 5-nucleotides to their corresponding nucleosides[11,12] .Significant increase in 5'Nucleotidase levels treated suggest that the extent of damage to the liver[13].

Serum aminotransferase assays are the most common laboratory tests for the detection of liver diseases. Commonly available tests include serum ALT is relatively specific, affected early by Hepatotoxicity and is considered an excellent marker of cellular necrosis, as it is a cytoplasmic enzyme.On the other hand, AST is mainly a mitochondrial enzyme. Although its elevated level in the serum is not specific of the hepatic disorder, AST is used mainly to diagnose and to verify persistent cellular injury with other enzymes like ALT[14,15]..

The present study showed significant increase in serum concentrations of AST, ALT, GGT and ALP in the groups administered indomethacin because liver marker enzymes (ALT, AST and GGT) might reflect cell rupture, a major permeability, cellular leakage , loss of functional integrity of the cell membrane and the release of these cytosolic enzymes from the damaged liver parenchymal cells. In addition, significant increase in the ALP which may be attributing to elevated biliary pressure, and acute cell necrosis, releasing of ALP from its membranes bound site and entry into blood is facilitated due to amphillic nature of bile salts[16,17,18].

The serum levels of total protein and bilirubin may indicate the state of the liver and the type of damage[19]. Serum protein level decreased in the indomethacin-treated rats which may reflect decreased protein synthesis or increase protein loss, nearly all proteins are synthesized in the liver; hence, hepatic failure is a cause of decreased serum protein[20].

Nitric oxide is extremely reactive signaling molecule and it is remarkable regulator for cellular functions including vasodilatation, inhibition of platelet aggregation,neutrophil adhesion, scavenging superoxide (O^{-2}) radical and inhibition of xanthine oxidase activity.Nitrative stress also plays a main role in inflammation. NO modifies DNA directly and inhibits the DNA repair enzymes.The increase in NO level may be due to the up-regulation of TNF-α and other cytokines[21,22].

In addition, the observed increased bilirubin level could be due to inhibition of hepatic glucuronidation of bilirubin and the nonselective vasoconstrictive effect of the drug causing reduction of blood flow through various organs[23,24].This agrees with the earlier reports by[25,26].

Histopathological examination of the liver showed normal liver tissue in the control group (Fig.1). In the indomethacin treated rat groups especially in G3 (Fig.1-4), the histopathological examination showed necrotic area with inflammatory cellular infiltrations, congested portal vein, degenerative changes [karyopaknosis, karyorrhexis andkaryotolysis]. These results could be explained by the increase in these liver parameters and is clear indications of cellular leakage and loss of functional integrity of the membrane resulting from liver damage, and due to mitochondrial dysfunction may be generated by the disruption of β-oxidation of lipids and oxidative energy production within the hepatocytes. Mitochondrial membrane permeabilization can lead to apoptosis, a rupture in mitochondrial membrane can lead to ATP depletion and subsequent necrosis[27].

Moreover, indomethacin treatment reduced the glutathione level and antioxidant enzyme activities, increased lipid peroxidation reduced cell viability, enhanced ROS generation and caused hepatic DNA fragmentation which ultimately leads to cellular necrosis[28].

CONCLUSION

In conclusion, Indomethacin treatment in wistar albino male rats has a toxic effect on the structure and function of hepatic tissue. There is a need for further experimental studies to detect useful and appropriate indomethacin dose in hepatotoxicity.

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

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