International Journal of Medicinal Plants and Alternative Medicine Vol. 2(3), pp. 032-046, October 2014
Available online at http://academeresearchjournals.org/journal/ijmpam
ISSN 2327-560X ©2013 Academe Research Journals

Full Length Research Paper

Amelioration of indomethacin systemic toxicity by gum arabic administration in adult albino rats

Said Said Elshama[1]*, Ayman El-Meghawry El-Kenawy[2], Hosam-Eldin Hussein Osman[3] and Hamdi Mohamed Youseef[4]

[1]Department of Forensic Medicine and Clinical Toxicology, College of Medicine, Taif University Suez Canal University,
[2]Department of Pathology, College of Medicine, Taif University, Department of molecular biology, GEBRI. University of Sadat City,
[3]Department of Anatomy, College of Medicine, Taif University, Al-Azhar University.
[4]Microbiology Department, College of Medicine, Taif University.

Accepted 22 September, 2014

Indomethacin is the most widely used drugs in the recent years for treatment of rheumatoid arthritis, ankylosing spondylitis and gout. Overdose of indomethacin releases the highly toxic free radical inducing oxidative tissue damage and systemic toxicity. Gum arabic has anticarcinogenic effect and anti-oxidant effect with a protective role against hepatic and cardiac toxicities. This study investigates the protective role of gum arabic on modulation of indomethacin systemic toxicity. Eighty albino rats were used in this study, and were divided into four groups, with each group consisting of twenty rats. The Control group received water, the second group received indomethacin, the third group received gum arabic, whereas the fourth group received indomethacin with gum arabic for seven days. Evaluation of indomethacin systemic toxicity and protective role of gum arabic were done by assessment of the liver and renal function tests, coagulation profile, complete blood picture, oxidants and antioxidants parameters, and morphological changes of liver, kidney and retina. Overdose of indomethacin induced systemic toxicity was manifested by hepatic, renal and retinal histopathological changes. It led to statistical significant abnormalities of liver and renal function tests, complete blood picture and coagulation profile associated with significant disturbance of antioxidant mechanism. Administration of gum arabic with high dose of indomethacin induced significant improvement of its systemic toxicity manifestations in the rats.

Key words: Indomethacin, gum arabic, toxicity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs are the most widely used drugs in the recent years. It is used by over thirty million people per day as class of medications for pain and inflammation treatment. Indomethacin is one of the most commonly used anti-inflammatory and analgesic drugs. Therefore, it was used to relieve the inflammation, swelling, stiffness and joint pain in cases of rheumatoid arthritis, degenerative joint diseases, ankylosing spondilitis, gout and acute musculoskeletal disorders (Musu et al., 2011).

Indomethacin may increase the risk of developing serious systemic toxicity without warning signs especially with long term use. It produces lipid peroxidation which may occur in the course giving rise to free radical which is highly toxic to tissue inducing oxidative tissue damage. Recent studies detected hepatic toxicity, nephrotoxicity and intestinal toxicity depending on the dose and long term use (Vijayalakshmi et al., 2011).

Gum arabic (GA) is used as an emulsifier, thickening agent and flavor stabilizer in both pharmaceutical and

*Corresponding author. E-mail: saidelshama@yahoo.com.

food industries. It is extracted from exudates of Acacia Senegal or Acacia seyal trees in Sudan and many African countries. It consists of a mixture of polysaccharides, oligosaccharides and glycoproteins. Its composition can vary with its source, climate and soil. It readily dissolves in water to form low viscosity solutions. Thus it is used in various industries such as textile, pottery and cosmetics (Goodrum et al., 2000).

Gum arabic is non-toxic and used extensively in pharmaceutical preparations, in the folk medicine and in most categories of processed foods such as candy products. It is indigestible to both humans and animals, not degraded in the intestine, but fermented in the colon to give short-chain fatty acids, leading to a large range of possible health benefits. It has prebiotic effect because it causes significant increases in Bifidobacteria, Lactobacteria and Bacteriodes. It has anti-carcinogenic and anti-oxidant effect with a protective role against hepatic and cardiac toxicities. Acceptable daily intake of gum arabic was evaluated for man by the Joint FAO/WHO Expert Committee on Food Additives since 1969 (Calame et al., 2008).

So, the present study aims to investigate the protective effect of gum arabic for modulation of the systemic toxicity of indomethacin by assessment of the hepatic and renal functions, complete blood picture, coagulation system, oxidants and antioxidants parameters, and morphological changes of liver, kidney and retina.

MATERIALS AND METHODS

Eighty healthy adult albino rats weighing 200-300 g were obtained from the animal house of king Abdel Aziz University-Jeddah. Rats were exposed to 12 h day-night cycles. It had free access to water and food during the experimental period. Animals were divided into four groups, with each group consisting of twenty rats. The first group (control) received distilled water only, while the second group received 20 mg/kg /day of indomethacin. The third group received 10 gm/kg/day of gum arabic, while the fourth group received 20 mg/kg /day of indomethacin (Silva et al., 2012; Vijayalakshmi et al., 2011) with 10 gm/kg/day of gum arabic (Al-Kenanny et al., 2012). Administration of indomethacin and gum arabic were done after dissolving in distilled water, once daily by gastric gavage for seven days. Indomethacin drug (Indocid®- 25 mg) was obtained from Kahira Pharm and Chem. IND. CO. Cairo - Egypt in capsule form.

Dose and time response course study

To assess the safety of protective gum arabic dose (10 gm/kg/day), we performed dose and time response course study. Thirty healthy adult albino rats weighing 200-300 g were used in this study, and were divided into three groups, with every group consisting of ten rats. The first group received 10 gm/kg/day of gum arabic and 20 mg/kg /day of indomethacin for seven days while the second group received 15 gm/kg/day of gum arabic and 20 mg/kg /day of indomethacin for five days. The third group received 20 gm/kg/day of gum arabic and indomethacin (20 mg/kg /day) for three days. We assessed maximum nephrotoxicity and hepatoxicity of gum arabic dose administration after seven days for the first group, five days for the second group, three days for the third group by assessment of the renal and liver functions tests. We chose the dose which caused less renal and hepatic adverse effects based on dose and time response course study.

Preparation of gum arabic

Crude gum arabic was obtained from the market as spheroidal tears which were milled and sieved to obtain fine pure powder. The concentration was prepared freshly in 10 g/ 100 ml. They were dissolved into warm water and given to the animals orally (Abd-Allah et al., 2002).

Blood sample collection

At the end of the experiment, the rats were anesthetized with diethyl ether. Blood samples were obtained from the orbital sinus using heparinized capillary tubes and collected into EDTA tubes for blood cell and platelet counts, hematocrit and hemoglobin values, while other samples were collected into 3.8% sodium citrate tubes for coagulation studies. The samples were centrifuged at 3000 × g at 4°C for 10 min to obtain plasma samples. Prothrombin time, partial thromboplastin time and fibrinogen were measured with a coagulometer (Sysmex® CA-1500-Siemens- Healthcare Diagnostics) (McKenzie, 2010). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP-Ph), albumin (Bain, 2003), urea and creatinine were determined by routine colorimetric methods using the commercial kits, after which they were quantified on clinical biochemistry autoanalyser (Alexander and Griffith, 1992).

Tissue preparation

A total of 500 mg of every organ tissues (liver and kidney) were homogenized in 4 ml of buffer solution of phosphate buffered saline at pH 7.4. Homogenates were centrifuged at 10.000 ×g for 15 min at 4°C. The resultant supernatant was used for antioxidant enzymes assay (catalase “CAT”, superoxide dismutase “SOD”, glutathione peroxidase “GPX”, glutathione “GSH”) and peroxidative stress assay (malondialdehyde “MDA”) (Davalos et al., 2003).

Histopathological studies

All animals were sacrificed under excess anesthesia after

Table 1. Effect of indomethacin used alone or with gum arabic on Mean ± SD of rats liver function tests.

Parameter \ Group	First M±S.D	Second M±S.D	Third M±S.D	Fourth M±S.D	F
AST	34.15±2.85	125.5±8.41*	21.9±2.04	29.2±2.87**	2082.22
ALT	34.15±2.85	85.45±3.13*	26.6±2.54	34.05±3.06**	1742.94
Alk. Ph.	74.9±3.19	211.4±3.66*	58.15±4.10	99.45±5.67**	5245.89
Albumin	4.53±0.27	6.53±0.25*	4.37±0.36	5.22±0.16**	254.79

Number per group = 20; SD = standard deviation; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALk.Ph = Alkaline phosphatase. First group (control) received distilled water; Second group received 20 mg/kg/day of indomethacin; Third group received 10 gm/kg/day of Gum arabic; Fourth group received 20 mg/kg/day of indomethacin with 10 gm/kg/day of Gum arabic. * = $p < 0.001$ (significant difference in comparison with the control group); ** = $p < 0.001$ (significant difference in comparison with the second group).

24 h from the last administration of drug and gum arabic. Incision was performed in the abdomen for kidney and liver excision, and in the eye for retina excision.

Tissue specimens (liver, kidney and retina) were collected from four groups and then fixed in 10% neutral buffered formalin. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6 µm thickness and stained by haematoxylen and eosin, Periodic Acid Schiff (PAS) and Mallory stain (Bancroft and Gamble, 2002).

Statistical analysis

Statistical analysis was performed using SPSS version 17. Variability of results was expressed as mean ± SD. Results were analyzed by using one-way ANOVA and post-hoc multiple comparison test (TUKEY) to investigate the difference among groups. *P* value of 0.05 was considered statistically significant.

Ethical considerations

The most appropriate animal species was chosen for this research. Promotion of a high standard of care and animal well-being at all times was done. Appropriate sample size was calculated by using the fewest number of animals to obtain statistically valid results. Painful procedures were performed under anesthesia to avoid distress and pain. Our standards of animal care and administration met those required by applicable international laws and regulations.

RESULTS

Hepatic biochemical findings

Table 1 represents mean ± SD values of liver function tests in the rats. Mean ± SD values of AST in the control group which received distilled water, the second group which received indomethacin, the third group which received gum arabic, and the fourth group which received indomethacin and gum arabic were 34.15±2.85, 125.5±8.41, 21.9±2.04 and 29.2±2.87 respectively. Value of F which indicates difference between groups was 2082.22 and was statistically significant whereas $P > 0.001$. The Mean ± SD values of ALT in the control group, second group, third group and fourth group were 34.15±2.85, 85.45±3.13, 26.6±2.54 and 34.05±3.06 respectively. Value of F which indicates difference between groups, was 1742.94 and was statistically significant, whereas $p > 0.001$. The Mean ± SD values of ALK.Ph in the control group, second group, third group and fourth group were 74.9±3.19, 211.4±3.66, 58.15±4.10 and 99.45±5.67 respectively. Value of F which indicates difference between groups, was 5245.89 and was statistically significant, whereas $p > 0.001$. The Mean ± SD values of albumin in the control group, second group, third group and fourth group were 4.53±0.27, 6.53±0.25, 4.37±0.36 and 5.22±0.16 respectively. Value of F which indicates difference between groups, was 254.79 and was statistically significant, whereas $p > 0.001$.

Hepatic histopathological findings by light microscope

Examination of hepatic tissues in the rats of the first group (control), showed normal hepatic architecture (Figure 1A and B) with normal distribution of collagen and small amount of wavy fibrils (Figure 2A) and normal positive reaction of PAS based on mucopolysaccharide granules in the cytoplasm of hepatocytes (Figure 3A). But hepatic tissues in rats of the second group which received indomethacin alone, showed loss of hepatic architecture, increase the number of coarse, pink vacuoles in the cytoplasm, inflammatory cellular infiltration with small contracted and fragmented pycknotic nuclei around the central vein, necrosis of hepatocytes, degenerated kupffer cells and fibrosis of

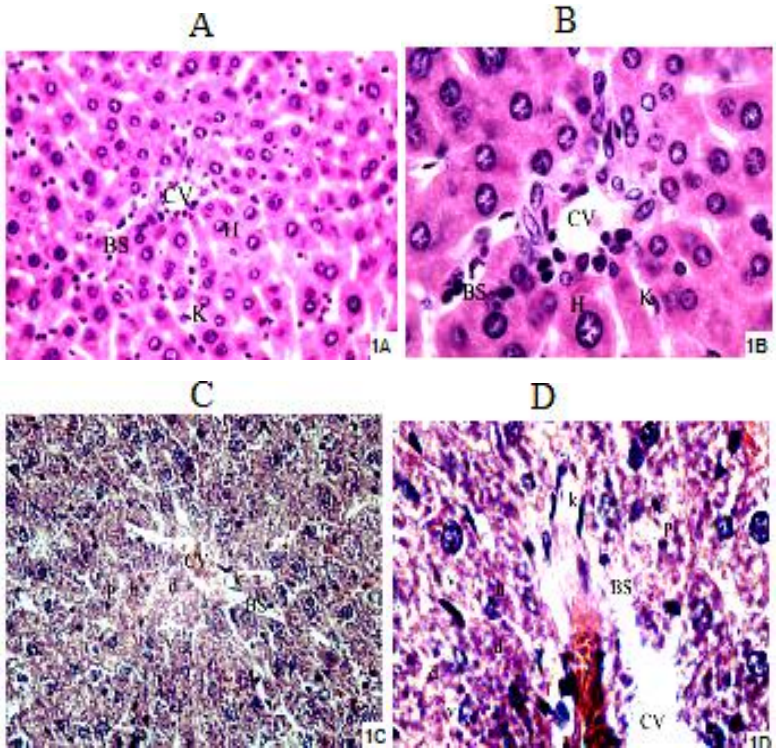


Figure 1. (1A) A photomicrograph of transverse section in the control rat liver showing normal hexagonal hepatic lobules with portal triads at the vertices and a central vein (CV) in the middle. Hepatocytes (H) are arranged into hepatic cords and separated by adjacent blood sinusoids (BS) which contain Kupffer cells (k) (H&E ×400). **(1B)** A photomicrograph of transverse section in the control rat liver showing normal hexagonal hepatic lobules with portal triads at the vertices and a central vein (CV) in the middle. Hepatocytes (H) are arranged into hepatic cords and separated by adjacent blood sinusoids (BS) which contain Kupffer cells (k) (H&E ×1000). **(1C)** A photomicrograph of transverse section in the second group rat liver showing widening of central vein (CV), foamy hepatocyte cytoplasm filled with vacuoles (v), necrosis of some hepatocytes (h) and contracted, pycknotic nuclei with condensed chromatin (P). Widening of blood sinusoids (BS) with degenerated area (d) (H&E ×400). **(1D)** A photomicrograph of transverse section in the second group rat liver showing widening of central vein (CV), foamy hepatocyte cytoplasm filled with vacuoles (v), necrosis of some hepatocytes (h) and contracted pycknotic nuclei with condensed chromatin (P). Widening of blood sinusoids (BS) with degenerated area (d) (H&E ×1000).

central vein (Figure 1C and D) with thick bundles of wavy collagen fibers which consists of sporadic or fused fibrils (Figure 2B) and marked low positive reaction of PAS in comparison with control group (Figure 3B). Transverse section of hepatic tissues in the rats of the third group which received gum arabic only showed normal hepatic architecture (Figure 1E and F) with normal distribution of collagen and small amount of wavy fibrils (Figure 2C) and normal positive reaction of PAS (Figure 3C). Hepatic tissues in the rats of the fourth group which received indomethacin and gum arabic showed marked reduction of hepatic degeneration with hypertrophy of some hepatocytes and mild kupffer cells hypertrophy (Figure 1G and H) with normal distribution of collagen fibers (Figure 2D) and positive reaction of PAS (Figure 3D).

Renal biochemical findings

Table 2 represents mean ± SD values of renal function tests in the rats. Mean ± SD values of urea in control group, second group, third group and fourth group were 27.7±4.39, 63.8±2.54, 23.2±2.01 and 25.65±5.44 respectively. Value of F which indicates the difference between groups was 496.77 and was statistically significant, whereas $p > 0.001$. Mean ± SD values of creatinine in control group, second group, third group and fourth group were 0.88±0.25, 1.72±0.11, 0.76±0.36, and 0.71±0.10 respectively. Value of F which indicates the

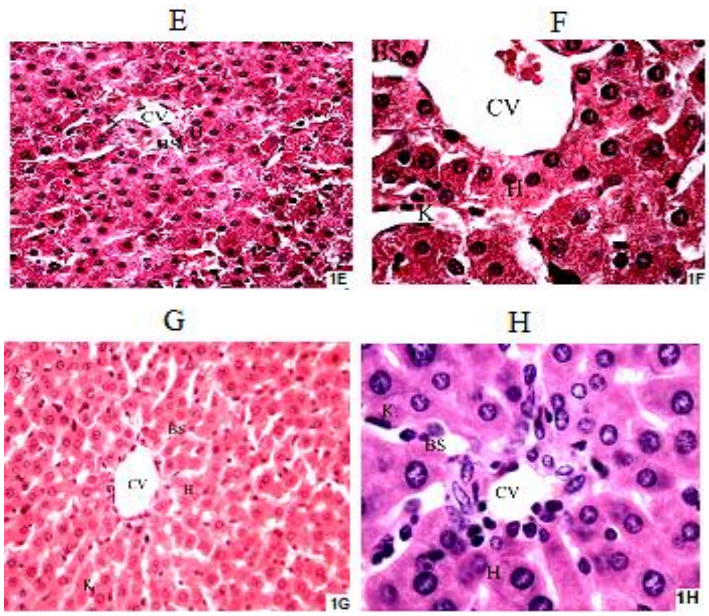


Figure 1. (1E) A photomicrograph of transverse section in the third group rat liver showing normal hexagonal hepatic lobules with portal triads at the vertices, central vein (CV) in the middle. Hepatocytes (H) are arranged into hepatic cords and separated by adjacent blood sinusoids (BS) which contain Kupffer cells (k) (H&E ×400). **(1F)** A photomicrograph of transverse section in the third group rat liver showing normal hexagonal hepatic lobules with portal triads at the vertices and central vein (CV) in the middle. Hepatocytes (H) are arranged into hepatic cords and separated by adjacent blood sinusoids (BS) which contain Kupffer cells (k) (H&E ×1000). **(1G)** A photomicrograph of transverse section in the fourth group rat liver showing less nearly normal central vein (CV) and hepatocytes (H) with normal nuclei and Kupffer cell (K) (H&E × 400). **(1H)** A photomicrograph of transverse section in the fourth group rat liver showing hepatocytes with less vacuolated cytoplasm and separated by blood sinusoids (BS) which contain Kupffer cells (k) (H&E ×1000).

difference between groups was 81.89 and was statistically significant, whereas $p > 0.001$.

Renal histopathological findings by light microscope

Examination of renal tissues in the rats of the first group (control) showed normal renal structure (Figure 4A and B) with normal distribution of collagen (Figure 5A) and normal positive reaction of PAS (Figure 6A). But renal tissues in rats of the second group which received indomethacin alone, showed enlargement of vascular glomeruli, widening the glomerular capsular space, atrophied of some vascular glomeruli and degeneration of epithelial lining of most renal tubules (Figure 4C and D) with marked fibrosis of vascular glomeruli, degenerated epithelial lining Bowman's capsule with oedema and fibrosis of tubular epithelium cells (Figure 5B), and marked reduction of the positive reaction of PAS in comparison with control group (Figure 6B). Transverse section of renal tissues in the rats of the third group which received gum arabic only showed normal renal structure (Figure 4E and F) with normal distribution of collagen (Figure 5C) and normal positive reaction of PAS (Figure 6C). Renal tissues in the rats of the fourth group which received indomethacin and gum arabic showed reduction in the vasculature of the renal glomeruli, appearance of glomerular capsular space, decrease the oedema of both the proximal and distal convoluted tubular epithelium, lack of the fibrosis in the Bowman's capsule (Figure 4G and H) with normal distribution of collagen deposition and connective tissues fibers (Figure 5D) and positive reaction of PAS (Figure 6D).

Retinal histopathological findings by light microscope

Examination of retinal tissues in the rats of the first group (control), showed normal differentiation of retinal cell layers including inner limiting membrane, nerve fiber layer, thick inner plexiform layer, inner nuclear layer, thin

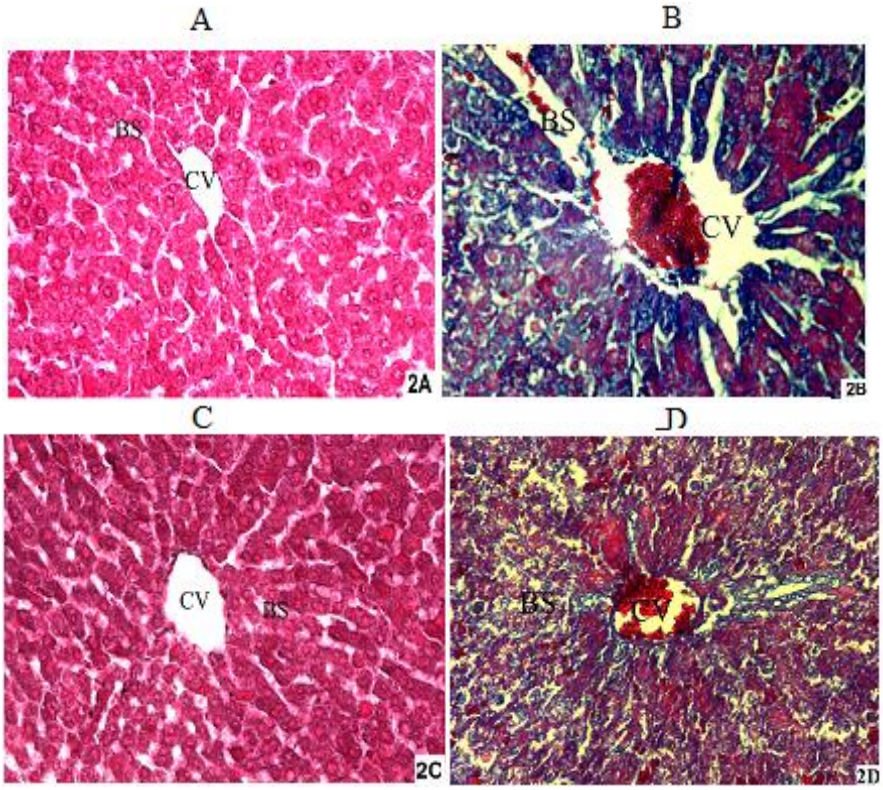


Figure 2. (2A) A photomicrograph of transverse section in the control rat liver showing normal hepatic structures, central vein (CV) and blood sinusoids (BS) (Mallory ×400). **(2B)** A photomicrograph of transverse section in the second group rat liver showing dense bundles of single or fused wavy collagen fibrils around the central vein (CV) and in-between blood sinusoids (BS) (Mallory ×400). **(2C)** A photomicrograph of transverse section in the third group rat liver showing normal hepatic structures, central vein (CV) and blood sinusoids (BS) (Mallory ×400). **(2D)** A photomicrograph of transverse section in the fourth group rat liver showing nearly normal distribution of collagen, with normal central vein (CV) and blood sinusoids (BS) (Mallory ×400).

outer plexiform layer, outer nuclear layer, photoreceptor cell layer, gangilionic cell layer and pigmented epithelium (Figure 7A and B). But retinal tissues in rats of the second group which received indomethacin alone, showed dramatic degenerative lesions of nuclear cell layers including pyknosis, vacuolar degeneration and karyolysis in different areas with ill-differentiated nuclear layer, abundant distribution of vacuoles in the photoreceptor layer, thin outer plexiform cell, a thin layer of few numbers of ganglion cells at the periphery of the inner plexiform cell layer in comparison with control group, massive degenerative lesions in the vacuolated nerve fiber layers (Figure 7C and D). Retinal tissues in the rats of the third group which received gum arabic only showed normal differentiation of retinal cell layers including inner limiting membrane, nerve fiber layer, gangilionic cell layer, thick inner plexiform layer, inner nuclear layer, thin outer plexiform layer, outer nuclear layer, photoreceptor cell layer and pigmented epithelium (Figure 7E and F). Retinal tissues in the rats of the fourth group which received indomethacin and gum arabic showed nearly normal differentiation of retinal cell layers including inner limiting membrane, nerve fiber layer, gangilionic cell layer, thick inner plexiform layer, inner nuclear layer, thin outer plexiform layer, outer nuclear layer, photoreceptor cell layer and pigmented epithelium (Figure 7G and H).

Hematological findings

Table 3 represents mean ± SD values of complete blood picture in the rats. Mean ± SD values of hemoglobin in the control group which received distilled water, second group which received indomethacin, third group which received gum arabic, fourth group which received indomethacin and gum arabic were 16.09±0.12, 8.34±0.29, 16.15±0.20 and 13.09±0.31 respectively. Value of F which indicates the difference between groups, was 4477.49 and was statistically significant, whereas $p > 0.001$. The Mean ± SD values of white blood

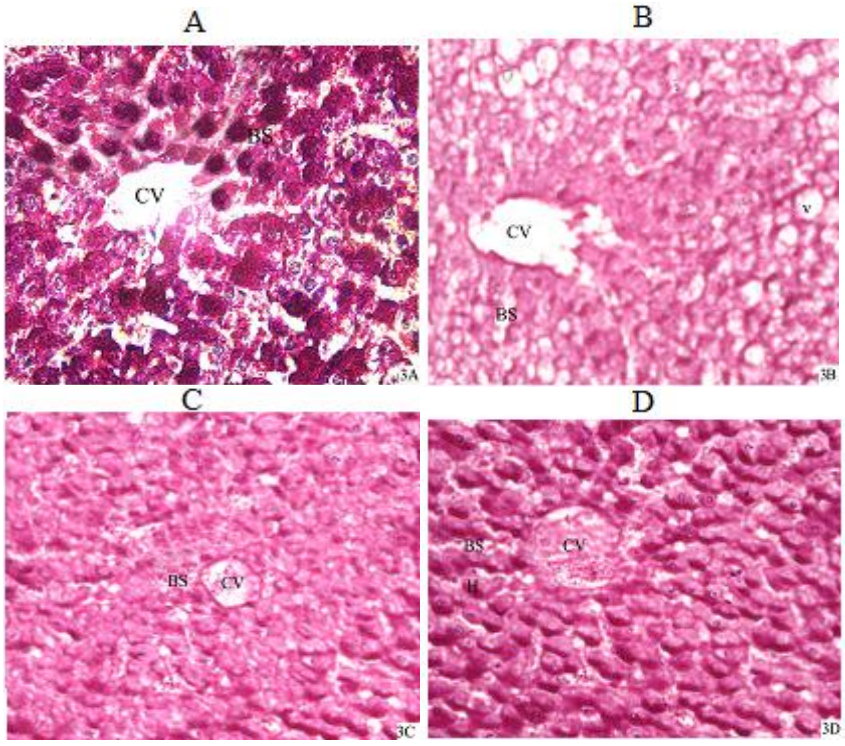


Figure 3. (3A) A photomicrograph of transverse section in the control rat liver showing normal positive reaction of PAS, central vein (CV) and blood sinusoids (BS) (Periodic acid-Schiff's ×400). **(3B)** A photomicrograph of transverse section in the second group rat liver showing marked decrease in PAS reaction with increase in the number of vacuoles (v), widening of central vein (CV) and blood sinusoids (BS) (Periodic acid-Schiff's ×400). **(3C)** A photomicrograph of transverse section in the third group rat liver showing normal positive reaction of PAS, central vein (CV) and blood sinusoids (BS) (Periodic acid-Schiff's ×400). **(3D)** A photomicrograph of transverse section in the fourth group rat liver showing nearly normal positive reaction of PAS, normal central vein (CV) and blood sinusoids (BS) (Periodic acid-Schiff's ×400).

Table 2. Effect of indomethacin used alone or with gum arabic on Mean ± SD of rats renal function tests.

Group / Parameter	First M±S.D	Second M±S.D	Third M±S.D	Fourth M±S.D	F
Urea	27.7±4.39	63.8±2.54*	23.2±2.01	25.65±5.44**	496.77
Creatinine	0.88±0.25	1.72±0.11*	0.76±0.36	0.71±0.10**	81.89

Number per group = 20; SD = standard deviation. First group (control) received distilled water; Second group received 20 mg/kg/day of indomethacin; Third group received 10 gm/kg/day of Gum arabic; Fourth group received 20 mg/kg/day of indomethacin with 10 gm/kg/day of Gum arabic. * = $p < 0.001$ (significant difference in comparison with the control group); ** = $p < 0.001$ (significant difference in comparison with the second group).

cells count in the control group, second group, third group and fourth group were 5.70±0.18, 7.70±0.18, 5.72±0.5417 and 6.27±0.12 respectively. Value of F which indicates difference between groups was 603.565 and was statistically significant, whereas $p > 0.001$. The Mean ± SD values of red blood cells count in control group, second group, third group and fourth group were 5.89±0.57, 3.99±0.20, 5.80±0.99 and 6.12±0.30 respectively. Value of F which indicates difference between groups, was 511.65 and statistical significant whereas $p > 0.001$. The Mean ±SD values of haematocrit in control group, second group, third group and fourth

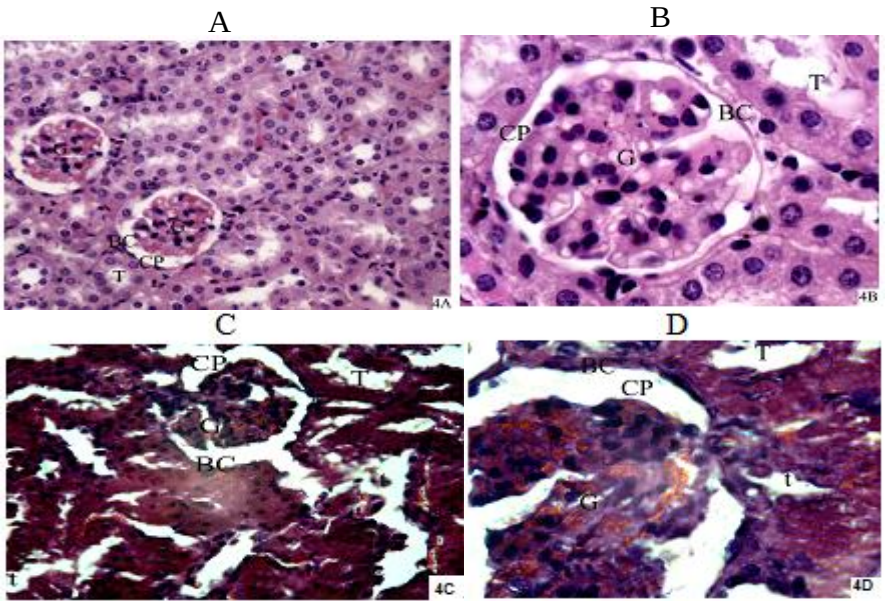


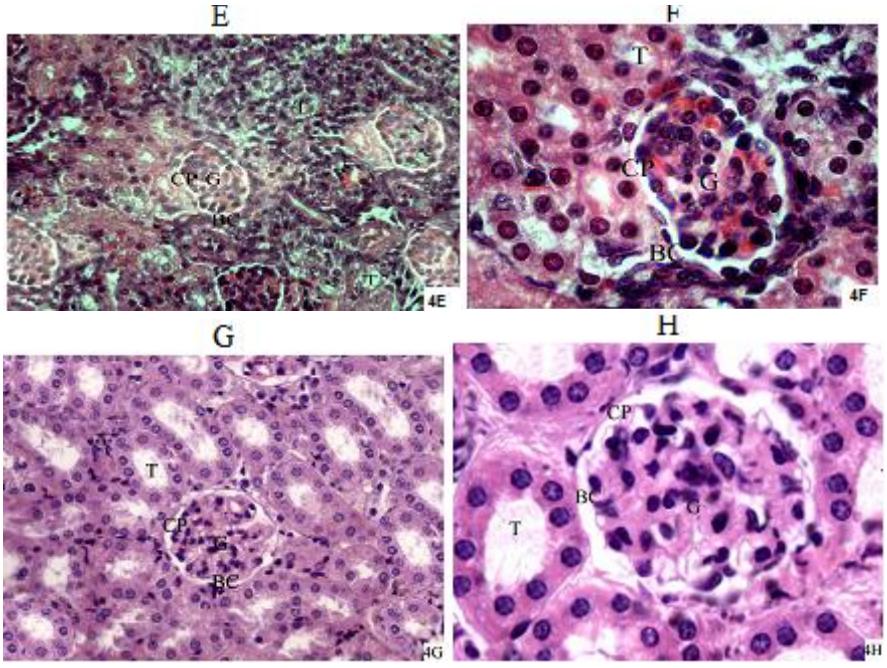
Figure 4. (4A) A photomicrograph of transverse section in the control rat kidney shows normal glomeruli (G), normal glomerular capsular space (CP), with flat epithelium lining the Bowman's capsule (BC) and normal cells in the lining epithelium of the tubules (T) (H&E ×400). **(4B)** A photomicrograph of transverse section in the control rat kidney showing normal glomeruli (G), normal glomerular capsular space (CP), with flat epithelium lining the Bowman's capsule (BC) and normal cells in the lining epithelium of the tubules (T) (H&E ×1000). **(4C)** A photomicrograph of transverse section in the second group rat kidney showing enlarged vascular glomeruli (G), decrease of glomular capsular space (CP), with flat epithelium lining of Bowman's capsule (BC), degeneration of some epithelial lining of tubule (T) and complete destruction of epithelial cells of other tubules (t) (H&E ×400). **(4D)** A photomicrograph of transverse section in the second group rat kidney showing enlarged vascular glomeruli (G), decrease of glomular capsular space (CP), with flat epithelium lining of Bowman's capsule (BC), degeneration of some epithelial lining of tubule (T) and complete destruction of epithelial cells of other tubules (t) (H&E ×1000).

group were 49.2±0.68; 44.22±1.46; 48.86±0.70 and 48.84±0.64 respectively. Value of F which indicates to difference between groups, was 124.406 and was statistically significant, whereas p > 0.001. The Mean ± SD values of platelets count in control group, second group, third group and fourth group were 463.29±1.86, 439.29±8.30, 462.69±1.54 and 463.59±1.45 respectively. Value of F which indicates difference between groups was 148.599 and statistically significant, whereas p > 0.001.

Table 4 represents mean ± SD values of coagulation profile in the rats. Mean ± SD values of prothrombin time in control group which received distilled water, second group which received indomethacin, third group which received gum arabic, fourth group which received indomethacin and gum arabic were 12.21±0.29, 21.59±0.88, 12.21±0.16 and 12.25±0.05 respectively. Value of F which indicates difference between groups, was 1999.118 and statistically significant whereas p > 0.001. The Mean ± SD values of partial thromboplastin time in the control group, second group, third group and fourth group were 22.17±0.27, 18.56±0.25, 22.92±0.53 and 22.63±0.44 respectively. Value of F which indicates difference between groups, was 524.47 and statistically significant whereas p > 0.001. The Mean ± SD values of INR in control group, second group, third group and fourth group were 1.72±0.05, 2.62±0.12, 1.75±0.11 and 1.84±0.87 respectively. Value of F which indicates difference between groups, was 359.631 and statistically significant whereas p > 0.001. The Mean ± SD values of Fibrinogen in control group, second group, third group and fourth group were 259.0±9.93, 639.03±10.91, 150.53±23.66 and 234.43±14.64 respectively. Value of F which indicates difference between groups was 3801.169 and statistically significant whereas p > 0.001. The Mean ± SD values of Prothrombin concentration in control group, second group, third group and fourth group were 82.4±1.27, 42.2±0.83, 86.90±5.23 and 92.35±2.75 respectively. Value of F which indicates difference between groups was 1122.647 and statistically significant whereas p > 0.001.

Antioxidants defense system findings

Table 5 represents mean ± SD values of oxidants and antioxidants parameters in the rats. Mean ± SD values of



(4E) A photomicrograph of transverse section in the third group rat kidney showing normal glomeruli (G) and glomerular capsular space (GS), with flat epithelium lining the Bowman's capsule (BC) and normal cells in the lining epithelium of the tubules (T) (H&E ×400). **(4F)** A photomicrograph of transverse section in the third group rat kidney showing normal glomeruli (G) and glomerular capsular space (GS), with flat epithelium lining the Bowman's capsule (BC) and normal cells in the lining epithelium of the tubules (T) (H&E ×1000). **(4G)** A photomicrograph of transverse section in the fourth group rat kidney showing decrease in the vasculature of the renal glomeruli (G), appearance of the glomerular capsular space (CP), decrease in the oedema of proximal (X) and distal (D) convoluted tubular epithelium, lack of fibroses in the Bowman's capsule (BC) (H & E ×400). **(4H)** A photomicrograph of transverse section in the fourth group rat kidney showing decrease in the vasculature of the renal glomeruli (G), appearance of the glomerular capsular space (CP), decrease in the oedema of proximal (X) and distal (D) convoluted tubular epithelium, lack of fibroses in the Bowman's capsule (BC) (H & E ×1000).

hepatic tissues catalase in control group which received distilled water, second group which received indomethacin, third group which received gum arabic, and fourth group which received indomethacin and gum arabic were 66.83±1.65, 43.71±1.45, 67.27±1.82 and 65.52±3.04 respectively. Value of F which indicates difference between groups was 600.131 and was statistically significant, whereas p > 0.001. Mean ± SD values of renal tissues catalase in the control group, second group, third group, and fourth group were 42.78±1.35, 21.81±0.72, 37.27±1.82 and 38.77±1.39 respectively. Value of F which indicates difference between groups, was 884.689 and statistically significant, whereas p > 0.001. The Mean ± SD values of hepatic tissues peroxidase in the control group, second group, third group and fourth group were 25.51±1.22, 18.34±1.03, 25.89±1.22 and 64.89±3.38 respectively. Value of F which indicates difference between groups was 2291.138 and was statistically significant, whereas p > 0.001. The Mean ± SD values of renal tissues peroxidase in the control group, second group, third group and fourth group were 15.76±1.32, 7.74±0.74, 15.39±1.26 and 14.64±0.95 respectively. Value of F which indicates difference between groups, was 238.956 and statistically significant, whereas p > 0.001. The Mean ± SD values of hepatic tissues GSH in the control group, second group, third group and fourth group were 151.37±7.08, 116.02±3.24, 149.52±4.24 and 148.02±5.27 respectively. Value of F which indicates difference between groups, was 213.314 and was statistically significant, whereas p > 0.001. The Mean ± SD values of renal tissues GSH in the control group, second group, third group and fourth group were 85.92±6.72, 48.77±6.25, 89.02±2.85 and 86.49±5.53 respectively. Value of F which indicates difference between groups, was 240.423 and was statistically significant, whereas p > 0.001. Mean ± SD values of hepatic tissues MDA in the control group, second group, third group and fourth group were 237.8±5.97, 285.3±2.65, 208.80±5.06 and 215.75±5.32 respectively. Value of F which indicates difference between groups was 985.693 and was statistically significant, whereas p > 0.001. Mean ± SD

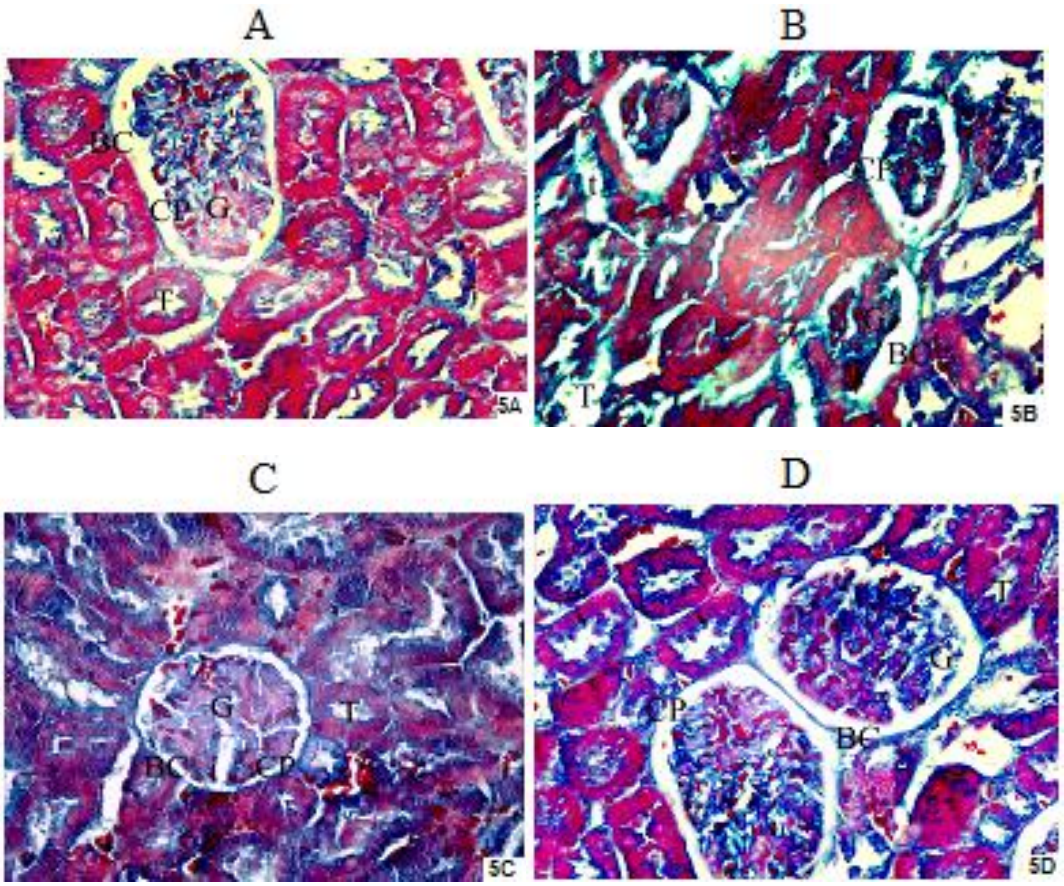


Figure 5. (5A) A photomicrograph of transverse section in the control rat kidney showing normal glomeruli (G), flat epithelium lining glomerular capsule (BC) with distinct capsular space (CP) and normal tubules (T) (Mallory ×400). **(5B)** A photomicrograph of transverse section in the second group rat kidney showing fibrosis and enlarged vascular glomeruli (G), widening of glomerular capsular space (CP), with degenerated epithelial lining Bowman's capsule (BC), oedema and fibrosis of tubular epithelium cells (T) and complete degenerated tubules (t) (Mallory ×400). **(5C)** A photomicrograph of transverse section in the third group rat kidney showing normal glomeruli (G) and flat epithelium lining glomerular capsule (BC) with distinct capsular space (CP) and normal tubules (T) (Mallory ×400). **(5D)** A photomicrograph of transverse section in the fourth group rat kidney showing nearly normal distribution of collagen, normal vascular glomeruli (G) and glomerular capsular space (CP), with normal lining Bowman's capsule (BC), and tubular epithelium cells (T) (Mallory ×400).

values of renal tissues MDA in control group, second group, third group and fourth group were 137.05±6.22, 188.6±5.37, 109.55±5.16 and 115.2±6.26 respectively. Value of F which indicates difference between groups was 776.573 and was statistically significant, whereas p > 0.001. Mean ± SD values of hepatic tissues nitric oxide in the control group, second group, third group and fourth group were 89.16±1.67, 97.92±0.86, 83.47±2.04 and 82.87±1.47 respectively. Value of F which indicates difference between groups was 393.573 and was statistically significant, whereas p > 0.001. Mean ± SD values of renal tissues nitric oxide in the control group, second group, third group and fourth group were 47.28±3.55, 57.32±1.39, 42.97±1.61 and 42.42±5.07 respectively. Value of F which indicates difference between groups was 88.604 and was statistically significant, whereas p > 0.001. Mean ± SD values of hepatic tissues superoxide dismutase in the control group, second group, third group and fourth group were 21.55±0.78, 12.06±1.08, 21.86±0.91 and 21.81±0.84 respectively.

Value of F which indicates difference between groups was 561.239 and was statistically significant whereas p > 0.001. Mean ± SD values of renal tissues superoxide dismutase in the control group, second group, third group and fourth group were 15.09±1.12, 10.21±1.03, 15.71±0.91 and 14.76±0.71 respectively.

Value of F which indicates difference between groups was 155.247 and was statistically significant, whereas p > 0.001.

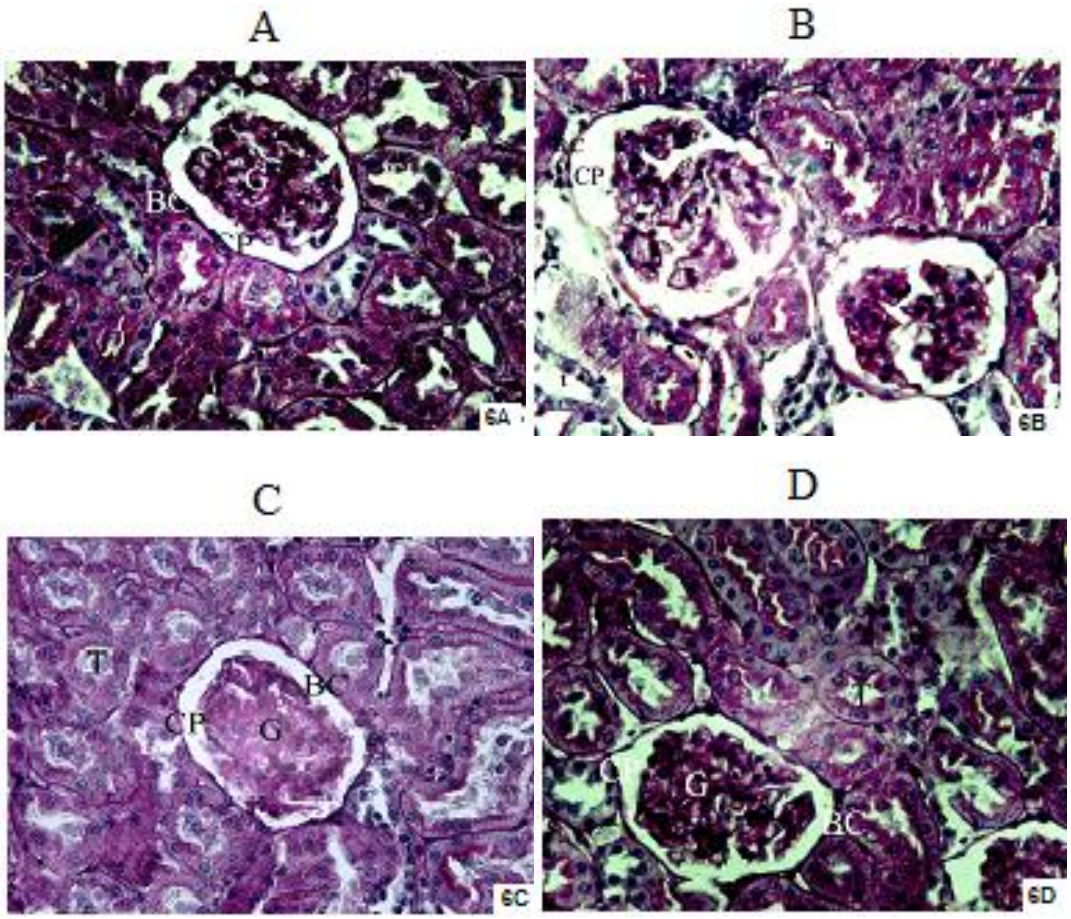


Figure 6. (6A) A photomicrograph of transverse section in the control rat kidney showing normal positive reaction of PAS, normal vascular glomeruli (G), glomerular capsular space (CP), with normal lining Bowman's capsule (BC), and tubular epithelium cells (T) (Periodic acid-Schiff's ×400). **(6B)** A photomicrograph of transverse section in the second group rat kidney showing marked decrease in PAS reaction with degenerated vascular glomeruli (G), widening glomerular capsular space (CP), irregular lining Bowman's capsule (BC) and tubular epithelium cells (T) (Periodic acid-Schiff's ×400). **(6C)** A photomicrograph of transverse section in the third group rat kidney showing normal positive reaction of PAS, normal vascular glomeruli (G), glomerular capsular space (CP), normal lining Bowman's capsule (BC) and tubular epithelium cells (T) (Periodic acid-Schiff's ×400). **(6D)** A photomicrograph of transverse section in the fourth group rat kidney showing nearly normal positive reaction of PAS with normal vascular glomeruli (G), glomerular capsular space (CP), nearly normal lining Bowman's capsule (BC) and tubular epithelium cells (T) (Periodic acid-Schiff's ×400).

DISCUSSION

Indomethacin is considered potent and wider used drug as non-steroidal anti-inflammatory. It is used as the alternative solution in patients who are resistant to other drugs. But manifestations of its toxicity limit its use and then there is an inevitable desire for searching about ameliorated agents for its toxicity when there is necessity for its use. Thus, the present study investigates systemic toxicity of indomethacin and how we can ameliorate this toxicity by using the gum arabic.

The present study indicated that there is a statistical significant increase of serum urea and creatinine in the second group which received indomethacin alone in comparison to the control group. This is consistent with the study of Vijayalakshmi et al. (2011). Renal histopathological findings support significant increase of renal biomarkers levels. It showed fibrosis of glomeruli with degeneration of renal tubules epithelial lining and Bowman's capsule. This study showed that statistical significant increase of renal oxidants parameters such as MDA and nitric oxide decreased renal antioxidants parameters such as catalase, peroxidase, GSH and superoxide dismutase. This is consistent with the study of Hemieda et al. (2004) and Polat et al. (2010) who confirmed that indomethacin toxicity leads to renal

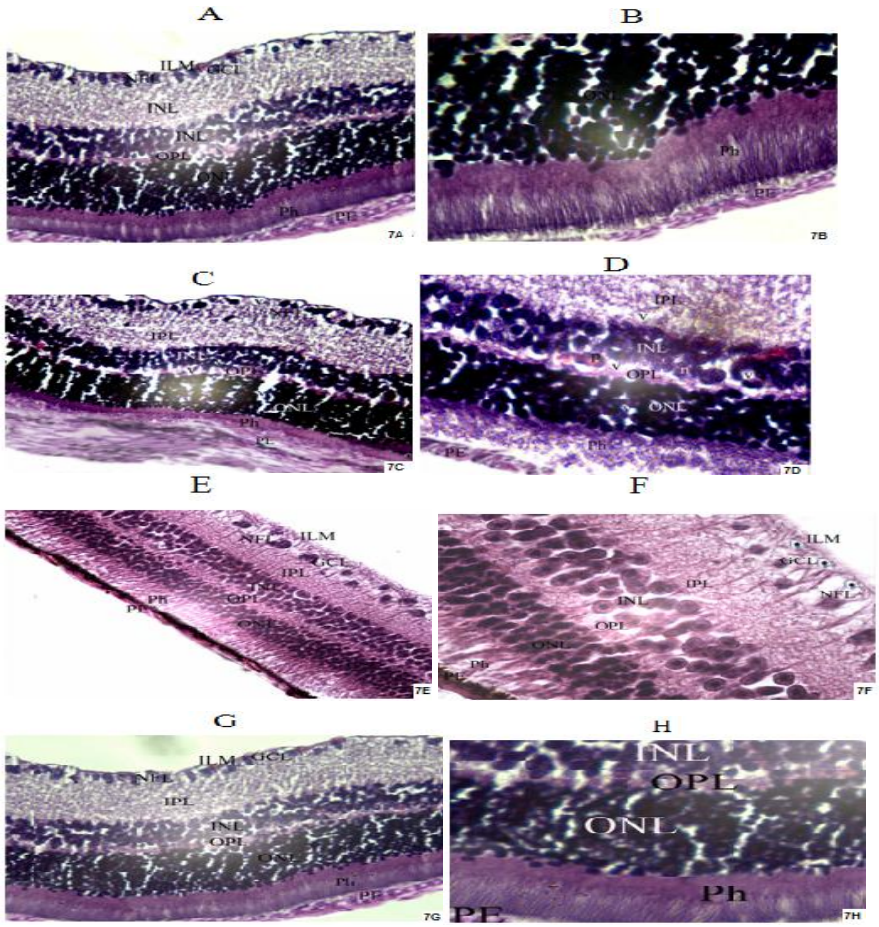


Figure 7. (7A) A photomicrograph of section in the control rat retina showing normal differentiation of retinal cell layers including inner limiting membrane (ILM), nerve fiber layer (NFL), gangilionic cell layer (GCL), thick inner plexiform layer (IPL), inner nuclear layer (INL), thin outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor cell layer (Ph) and pigmented epithelium (PE) (H&E ×400). **(7B)** A photomicrograph of section in the control rat retina showing normal differentiation of retinal cell layers, outer nuclear layer (ONL), photoreceptor cell layer (Ph) and pigmented epithelium (PE) (H&E ×1000). **(7C)** A photomicrograph of section in the second group rat retina showing vacuolated nerve fiber layer (NFL), massive reduction of both inner (INL) and outer (ONL) nuclear cell layers, massive atrophy of inner plexiform layer (IPL), ill differentiated nuclear layer (NL) associated with massive decrease of nuclear cells (H&E ×400). **(7D)** A photomicrograph of section in the second group rat retina showing vacuolated nerve fiber layer (NFL), massive reduction of both inner (INL) and outer (ONL) nuclear cell layers, massive atrophy of inner plexiform layer (IPL), ill differentiated nuclear layer (NL) associated with massive decrease of nuclear cells (H&E ×1000). **(7E)** A photomicrograph of section in the third group rat retina showing normal differentiation of retinal cell layers including inner limiting membrane (ILM), nerve fiber layer (NFL), gangilionic cell layer (GCL), thick inner plexiform layer (IPL), inner nuclear layer (INL), thin outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor cell layer (Ph) and pigmented epithelium (PE) (H&E ×400). **(7F)** A photomicrograph of section in the third group rat retina showing normal differentiation of retinal cell layers, outer nuclear layer (ONL), photoreceptor cell layer (Ph) and pigmented epithelium (PE) (H&E ×1000). **(7G)** A photomicrograph of section in the fourth group rat retina showing nearly normal differentiation of retinal cell layers including inner limiting membrane (ILM), nerve fiber layer (NFL), gangilionic cell layer (GCL), thick inner plexiform layer (IPL), inner nuclear layer (INL), thin outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor cell layer (Ph) and pigmented epithelium (PE) (H&E ×400). **(7H)** A photomicrograph of section in the fourth group rat retina showing nearly normal differentiation of retinal cell layers, inner nuclear layer (INL), thin outer plexiform layer (OPL), outer nuclear layer (ONL), photoreceptor cell layer (Ph) and pigmented epithelium (PE) (H&E ×1000).

Table 3. Effect of indomethacin used alone or with gum arabic on Mean ± SD of rats complete blood picture.

Parameter \ Group	First M±S.D	Second M±S.D	Third M±S.D	Fourth M±S.D	F
HB	16.09±0.12	8.34±0.29*	16.15±0.20	13.09±0.31**	4477.49
WBCs	5.70±0.18	7.70±0.18*	5.72±0.5417	6.27±0.12	603.565
RBCs	5.89±0.57	3.99±0.20*	5.80±0.99	6.12±0.30**	511.65
HCT	49.2±0.68	44.22±1.46*	48.86±0.70	48.84±0.64**	124.406
PLAT	463.29±1.86	439.29±8.30*	462.69±1.54	463.59±1.45**	148.599

Number per group = 20; SD = standard deviation; HB = Hemoglobin; WBCs = White blood cells; RBCs = Red blood cells; HCT = Haematocrit; PLAT = Platelet. First group (control) received distilled water; Second group received 20 mg/kg/day of indomethacin; Third group received 10 gm/kg/day of gum arabic; Fourth group received 20 mg/kg/day of indomethacin with 10 gm/kg/day of gum arabic. * = $p < 0.001$ (significant difference in comparison with the control group); ** = $p < 0.001$ (significant difference in comparison with the second group).

Table 4. Effect of indomethacin used alone or with gum arabic on Mean ± SD of rats' coagulation profile.

Parameter \ Group	First M±S.D	Second M±S.D	Third M±S.D	Fourth M±S.D	F
PT	12.21±0.29	21.59±0.88*	12.21±0.16	12.25±0.05**	1999.118
PTT	22.17±0.27	18.56±0.25*	22.92±0.53	22.63±0.44**	524.47
INR	1.72±0.05	2.62±0.12*	1.75±0.11	1.84±0.87**	359.631
Fibrinogen	259.0±9.93	639.03±10.91*	150.53±23.66	234.43±14.64**	3801.169
Prothrombin concentration	82.4±1.27	42.2±0.83*	86.90±5.23	92.35±2.75**	1122.647

Number per group = 20; SD = standard deviation; PT = Prothrombin time; PTT = Partial prothrombin time; INR = International normalised ratio. First group (control) received distilled water; Second group received 20 mg/kg/day of indomethacin; Third group received 10 gm/kg/day of gum arabic; Fourth group received 20 mg/kg/day of indomethacin with 10 gm/kg/day of gum arabic. * = $p < 0.001$ (significant difference in comparison with the control group); ** = $p < 0.001$ (significant difference in comparison with the second group).

structural and functional abnormalities based on release of the reactive oxygen species (ROS) and then occurrence of renal oxidative damage.

Our results showed statistical significant increase of liver function tests (AST, ALT, ALK.Ph and albumin), necrosis of hepatocytes, degenerated kupffer cells and fibrosis of central vein in the second group which received indomethacin alone in comparison to the control group. These results are consistent with those of Abatan et al. (2006) who confirmed hepatotoxic effect of indomethacin. Study of oxidants and antioxidants parameters in hepatic tissues (MDA, nitric oxide, catalase, peroxidase, GSH, and superoxide dismutase) explained that pathogenesis of indomethacin induced liver damage depending on the increase of hepatic lipid peroxidation associated with decrease of GSH in the hepatic tissues (Masubuchi et al., 1998; Klaassen, 2001).

The current study observed that hematological parameters values of complete blood picture (hemoglobin, red blood cells, haematocrit and platelets) in the second group which received indomethacin alone showed statistical significant decrease in comparison to those of the control group. Also, the white blood cells count showed statistical significant decrease in its value. These results are consistent with those of Adedapo and Aiyelotano (2001) who explained that reduction of hemoglobin, blood cell count and haematocrit is due to blood loss especially from small bowel because of the ability of indomethacin to cause cyclooxygenase enzyme inhibition, mitochondrial dysfunction and oxidative stress which leads to hemorrhagic anemia in agreement with Fukumoto et al. (2011). The increase of white blood cells count is caused by relative increase of neutrophils due to the stress following the hemorrhage. These results are in contrast with results of Abatan et al. (2006) except for white blood cells count.

The present study showed statistical significant difference for coagulation profile parameters (prothrombin time, partial thromboplastin time, prothrombin concentration, INR, platelet and fibrinogen) in the second group which received indomethacin alone in comparison to the control group. According to Silva et al. (2012), the statistical significant increase of fibrinogen level is multifactorial due to tissue injury, increase of its synthesis

Table 5. Effect of indomethacin used alone or with gum arabic on Mean ± SD of oxidants and antioxidants parameters in the rats.

Parameter		Group				
	Organ	First M±S.D	Second M±S.D	Third M±S.D	Fourth M±S.D	F
Catalase	Liver	66.83±1.65	43.71±1.45*	67.27±1.82	65.52±3.04**	600.131
	Kidney	42.78±1.35	21.81±0.72*	37.27±1.82	38.77±1.39**	884.689
Peroxidase	Liver	25.51±1.22	18.34±1.03*	25.89±1.22	64.89±3.38**	2291.138
	Kidney	15.76±1.32	7.74±0.74*	15.39±1.26	14.64±0.95**	238.956
GSH	Liver	151.37±7.08	116.02±3.24*	149.52±4.24	148.02±5.27**	213.314
	Kidney	85.92±6.72	48.77±6.25*	89.02±2.85	86.49±5.53**	240.423
MDA	Liver	237.8±5.97	285.3±2.65*	208.80±5.06	215.75±5.32**	985.693
	Kidney	137.05±6.22	188.6±5.37*	109.55±5.16	115.2±6.26**	776.573
Nitric oxide	Liver	89.16±1.67	97.92±0.86*	83.47±2.04	82.87±1.47**	393.573
	Kidney	47.28±3.55	57.32±1.39*	42.97±1.61	42.42±5.07**	88.604
Superoxide Dismutase	Liver	21.55±0.78	12.06±1.08*	21.86±0.91	21.81±0.84**	561.239
	Kidney	15.09±1.12	10.21±1.03*	15.71±0.91	14.76±0.71**	155.247

Number per group = 20; SD = standard deviation; GSH = Glutathione; MDA = Malondialdehyde. First group (control) received distilled water; Second group received 20 mg/kg/day of indomethacin; Third group received 10 gm/kg/day of gum arabic; Fourth group received 20 mg/kg/day of indomethacin with 10 gm/kg/day of gum arabic. * = $p < 0.001$ (significant difference in comparison with the control group); ** = $p < 0.001$ (significant difference in comparison with the second group).

in the liver after the release of interleukins TNF-α, IL-1 and IL-6 or because of fibrinogen degradation products which increase fibrinogen formation. But other results are in contrast with Silva et al., (2012) because there is a statistical significant increase of prothrombin time and INR which are associated with statistical significant decrease of prothrombin concentration, partial thromboplastin time and platelets count. According to the coagulation profile abnormalities of indometacin toxicity, there is a liability for risk of hemorrhage and thromboembolic event (disseminated intravascular coagulation) based on the ability of indomethacin to decrease the production of prostaglandin by cyclooxygenases inhibition (COX-1 and COX-2); and this is consistent with Rocca and Fitzgerald (2002), and Taiwo and Conteh (2008).

Our study showed degeneration of nuclear cell layers and nerve fibers layers in the rat retina in the second group which received indomethacin alone. These results are consistent with those of Steven (2004) who showed that indomethacin metabolizes in ocular tissue and its metabolite attaches the vital ocular macromolecules and then decrease the reduced glutathione concentration leading to retinopathy.

In the present study, all manifestations of systemic toxicity induced in the second group by administration of high dose of indomethacin alone because it mediates cytotoxicity by glutathione depletion and release the reactive oxygen species (ROS) according to Kaplan et al. (2012).

These manifestations are modulated by administration of the gum arabic with indomethacin in the fourth group because there is a statistical significant difference in all parameters (liver and renal function tests, complete blood picture and coagulation profile, oxidants and antioxidants parameters) with improved hepatic, renal and retinal histopathological findings in the fourth group in comparison with the second group which received indomethacin alone. These results are consistent with those of Badreldin et al. (2009) who indicated that gum arabic is a strong antioxidant so it has a protective effect against lipid peroxidation. Our results showed that statistical significant decrease of oxidants parameters such as malondialdehyde and nitric oxide is as a result of administration of gum arabic with indomethacin because it has free radical scavenging properties. Gum arabic leads to statistical significant increase of antioxidants parameters such as catalase, peroxidase, superoxide dismutase and glutathione in consistency with the studies of Al kenanny et al. (2012), and Gado and Aldahmash (2013) who confirmed antioxidants properties of gum arabic.

Conclusion

High dose of indomethacin leads to systemic toxicity which represents hepatic, renal and retinal histopatholgical changes, disturbance of liver and renal function tests associated with abnormalities of complete blood picture and coagulation profile in adult albino rats. Administration of gum arabic as antioxidant agent ameliorates toxicity manifestations induced by indomethacin.

RECOMMENDATIONS

According to the results of this study, use of indomethacin may be under precautions for its dose. If there is a necessity for its high dose use, we should use protective agents such as gum arabic because it modulates its toxicity based on its antioxidant properties.

REFERENCES

Abatan MO, Lateef I , Taiwo VO (2006). Toxic effects of non-steroidal anti-inflammatory agents in rats. Afr. J. Biomed. Res., 9: 219-223.

Abd-Allah AR, Al-Majed AA, Mostafa AM, Al-Shabanah OA, Gamal El-Din A , Nagi MN (2002). Protective effect of Arabic gum against cardiotoxicity induced by doxorubicin in mice: a possible mechanism of protection. J Biochem Mol Toxicol., 16(5):254–259.

Adedapo AA, Aiyelotano O (2001). Effect of chronic administration of indomethacin on haematological parameters in rats. Afr. J. Biomed. Res., 4: 159-160.

Alexander RH, Griffith JM (1992). Clinical/Nutritional Biochemistry. Basic Biochemical Methods. 2nd ed., Wiley-Liss, New York. John Wiley & Sons.

Al-kenanny ER, Al-Hayaly LK, Al-Badrany AG (2012). Protective effect of Arabic gum on liver injury experimentally induced gentamycin in mice. Kufa J. Vet. Med. Sci., 3(1): 174-189.

Badreldin HA, Ziada A, Blunden G (2009). Biological effects of gum Arabic: A review of some recent research. Food Chem. Toxicol., 47: 1-8.

Bain PJ (2003). Liver. In: Latimer KS, Mahaffey EA, Prasse KW (Eds.). Duncan and Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 4th ed. Ames, Iowa State University Press, pp. 193-214.

Bancroft JD, Gamble M (2002). Theory and Practice Histological Techniques, 5th ed., Churchill Livingstone. New York, Edinburgh and London, pp. 126 and 173-175.

Calame W, Weseler AR, Viebke C, Flynn C, Siemensma AD (2008). Gum Arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. Br. J. Nutr., 100(6): 1269–1275.

Davalos A, Gomez-Cordoves C, Bartolome B (2003). Commercial dietary antioxidant supplements assayed for their antioxidant activity by different methodologies. J. Agric. Food Chem., 50: 5909–5914.

Fukumoto K, Naito Y, Takagi T, Yamada S (2011). Role of tumor necrosis factor- ∞ in the pathogenesis of indomethacin-induced small intestinal injury in mice. Int. J. Mole. Med., 27:353-359.

Gado AM , Aldahmash BA (2013). Antioxidant effect of Arabic gum against mercuric chloride-induced nephrotoxicity. Drug Design, Dev. Therapy, 7: 1254-1252.

Goodrum LJ, Patel A, Leykam JF, Kieliszewski MJ (2000). Gum arabic glycoprotein contains glycomodules of both extensin and arabinogalactanglycoproteins. Phytochemistry, 54(1): 99–106.

Hemieda FA, Elmissiry MA, Badawy ME , Goda AA (2004). Partial suppressive effect of melatonin on indomethacin-induced renal injury in rat. Indian J. Exp. Biol., 42, 63-67.

Kaplan KA, Odabasoglu F, Halici Z, Cadirci E, Atalay F , Cakir A (2012). Alpha-lipoic acid protects against indomethacin-induced gastric oxidative toxicity by modulating antioxidant system. J. Food Sci., 77(11): 224-230.

Klaassen CD (2001). Casarett and Doull's Toxicology : The basic science of poison . 6th eds the McGrau-Hill Companies Inc. New York.

Masubuchi Y, Saito H, Horie T (1998). Structural requirements for the hepatotoxicity of non-steroidal anti-inflammatory drugs in isolated rat hepatocytes. J Pharmacol Exp. Therapy, 287: 208.

McKenzie M (2010). Clinical Laboratory Hematology, 2nd ed., Prentice Hall, pp. 411-422.

Musu M, Fincol G, Antoncci R, Polati E, Sannal D, Evangelista M, Ribuffo D, Schweiger V , Fanos V (2011). Acute nephrotoxicity of NSAID from the foetus to the adult, Eur. Rev. Med. Pharmacol. Sci., 15: 1461-1472.

Polat B, Suleyman H, Alp HH (2010). Adaptation of rat gastric tissue against indomethacin toxicity. Chem. Biol. Interact., 7:186(1): 82-89.

Rocca B, Fitzgerald GA (2002). Cyclooxygenases and prostaglandins: shaping up the immune response. Int. Immunopharmacol., 2: 603-630.

Silva MA, Rao VS, Souza CM, Neves JCS, Menezes DB, Santos FA, Andrade GM (2012). Evaluation of thalidomide against indomethacin-induced small intestinal damage and systemic toxicity in rats. Biomed. Res., 23 (1): 125-133.

Steven, MT (2004). Oxidative stress plays an important role in the pathogenesis of drug-induced retinopathy. Exp. Biol. Med., 229: 607.

Taiwo VO, Conteh OL (2008). The rodenticidal effect of indomethacin: pathogenesis and pathology. Vet. Archive., 78(2):167-178.

Vijayalakshmi P, Kanagavalli U, Jayanthi M (2011). Effect of melatonin on indomethacin induced nephrotoxicity in rats. Int. J. Universal. Pharm. Life Sci. 1(2): 174-182.