IJPSR (2021), Volume 12, Issue 6 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 22 June 2020; received in revised form, 24 November 2020; accepted, 04 May 2021; published 01 June 2021

# THE POTENTIAL PROTECTIVE EFFECT OF STRAWBERRY EXTRACT AGAINST INDOMETHACIN-INDUCED LIVER TOXICITY AND GASTRIC ULCERATION IN RATS: BIOCHEMICAL, HISTOPATHOLOGICAL AND GENETIC STUDIES

Soha A. Hassan [1], Sahar M. Abo Elwafa [2], Hatim A. El-Baz [3], Mohammed A. Hussein [* 4] and Naglaa A. Gobba [5]

Basic Science Department [1], Faculty of Dentistry, October 6 University, Sixth of October City, Egypt.
Forensic Medicine and Clinical Toxicology Department [2], Faculty of Medicine, Benha University, Benha City, Egypt.
Biochemistry Department [3], Genetic Engineering and Biotechnology Division, National Research Center, Cairo, Egypt.
Biochemistry Department [4], Faculty of Applied Medical Sciences, October 6 University, Sixth of October City, Egypt.
Pharmacology Department [5], Faculty of Pharmacy, MUST University, Sixth of October City, Egypt.

**Keywords:**

Straw berry, Hepatoprotective, Gastroprotective, Indomethacin, Antioxidant enzymes, Inflammatory mediators, Ultrastructural changes.

**Correspondence to Author:**
**Mohammed Abdalla Hussein**

Prof. of Biochemistry and Vice Dean for Post-graduate Studies and Scientific Research, Faculty of Applied Medical Sciences, October 6 University, Egypt.

**E-mail:** prof.husseinma@o6u.edu.eg

**ABSTRACT:** The objective of this study was to determine the hepatoprotective effect of strawberry extract (150 mg/kg.b.w) in rats against Indomethacin sodium-induced gastric ulceration. Oral indomethacin administration (5 mg/kg.b.w.) resulted in a substantial rise in total plasma cholesterol, triglycerides (HDL), factor-alfa (TNF-α), nitric oxide (NO), liver and stomach necrosis, tumor necrosis factor-alfa (TNF-α), nitric oxide (NO), thiobarbaturic acid reactive substances (TBARS), 8-hydroxy 2 deoxyguanosine (8-OHdG) and NF kappaB. Also, treatment of rats with Indomethacin led to a significant decrease in liver and stomach high mobility group box 1 (HMGB1) gene expression as well as a decrease in the levels of GSH, superoxide dismutase (SOD), catalase (CAT). The obtained results supported by histopathological data obtained by light and electron microscope which revealed that strawberry extract (150 mg/kg.b.w.) prevent liver tissue damage and stomach ulceration through increasing of GSH, SOD and CAT activities and decrease significantly TBARs, TNF-α, NO, 8-OHdG, NF kappaB levels, and HMGB1 gene expression. Conclusion: These results suggest that strawberry extract contains bioactive phenolics, minerals, and vitamins, which may be effective in enhances the protection of liver toxicity and treatment of ulceration by its radical-scavenging effect, antioxidant and anti-inflammatory activity.

**INTRODUCTION:** NSAIDs are one of the most common groups of drugs that induce oxidative stress and produce various tissue damage such as gastrointestinal damage, nephrotoxicity, and hepatotoxicity [1-3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(6).3120-33

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(6).3120-33

Gastric mucosa injury was devolved by misused of NSAIDs due to the inhibition of cyclooxygenase enzyme (COX) and suppression of prostaglandin-mediated effects on mucosal protection [4].

Indomethacin is one of the NSAIDs that was synthesized specifically to treat the inflammatory responses [5] *via* Inhibition of COX and blocks production of prostanoid metabolites [5, 6]. The major cellular sources of prostaglandins in the liver are endothelial, and Kupffer cells hepatocytes rapidly metabolize COX products [7]. Indomethacin leads to mitochondrial oxidative stress associated with the

generation of intra-mitochondrial reactive oxygen species (ROS), which induces an imbalance of oxidants and antioxidants status in living system [6].

Gastrointestinal toxicity was produced by Indomethacin via increasing gastric acid secretion and interfered with mucosal cell regeneration. Also, it inhibits PGE2 and gastric nitric oxide synthesis and produces free radicals, led to induce of gastric apoptosis [8].

High mobility group box 1 (HMGB1) is a DNA-binding nuclear protein that can be released into the extracellular milieu during states of cellular stress or damage and subsequently activate the immune system and promote inflammation [9, 10].

HMGB1 represents a major challenge to improve the treatment of acute/chronic inflammation as well as infection and ischemia-reperfusion-induced injury. Many studies prove that the developing of HMGB1 inhibitors like natural products [11-13].

Strawberry is a crop cultivated worldwide; its cultivars are specifically adapted to the different areas [14]. It is the best source of minerals such as manganese, potassium**,** magnesium, copper, iron and phosphorus [15], ascorbic acid [16], thiamine, riboflavin, niacin, vitamin B6, vitamin K, vitamin A and vitamin E [17], folate [18], flavonols, catechins, hydroxycinnamic acids, ellagitannins, and ellagic acid have also been associated with the beneficial effect of strawberries on human health [19]. The most significant contributors of the antioxidant capacity of strawberries are considered to be ascorbic acid, ellagitannins, and anthocyanins [20].

*In-vivo* tests have been conducted with extract berry fruits to determine, for example, its hepatoprotective [23], hypolipidemic, hypoglycemic, and antioxidant activity [24]. But there are no reports about the hepato-and gastroprotective activity of the strawberry extract.

As an extension of our studies on the biological importance of neutral products [25-27], we wish to evaluate the therapeutic potential of the strawberry extract on indomethacin-induced ulcerative gastritis in a rat model. Therefore, in the present study, we sought to understand whether the regulation of HMGB1 through strawberry is a good strategy to reduce gastric inflammation.

## MATERIALS AND METHODS:

### Dose of Strawberry Extract:

1. Strawberry extract was purchased from Virgin Extracts (TM), China. Strawberry was given to rats with 150mg/ kg.b.w. daily for 7 days by oral gastric gavage tube.

2. Indomethacin (100%) was obtained from Merck Ltd., Germany. All reagents used were of analytical grade.

**Experimental Set-Up:** This experiment was carried out to examine the protective potential of the strawberry extract against Indomethacin-induced liver toxicity and gastric inflammation in a rat model. Groups of animals, each consisting of 8 rats, were treated daily for 7 days as follows:

**Group I:** Normal; was given saline orally for 7 days.

**Group II:** Positive control; was treated with Indomethacin (5 mg/kg b.w.) suspended in saline orally in a single daily dose for 7 days [21]**.**

**Group III:** was treated with strawberry extract (150 mg/kg.b.w, orally) dissolved in saline for 7 days [22].

**Group IV:** was treated with strawberry extract (150 mg/kg.b.w, orally) + indomethacin (5 mg/kg b.w.) suspended in saline for 7 days.

**Blood Samples:** Blood samples were collected at the end of experimental period in dry, clean, and screw capped tubes, also, plasma was separated by centrifugation at 2500 r.p.m for 15 min. Serum was separated by automatic pipette and received in dry sterile samples tube and kept in a deep freeze t -20°C until used for subsequent biochemical analysis (triglyceride [28], total cholesterol [29] and HDL-C [30]).

**Tissue Specimens (Liver and Stomach):** At the end of the experiment, rats of each group were sacrificed by cervical decapitation. The abdomen and chest were opened and the liver and stomach specimens were quickly removed and opened gently using a scrapper, cleaned by rinsing with ice-cold isotonic saline to remove any blood cells, clots, then blotted between 2 filter papers and divided into two parts one part for biochemical estimation of liver and stomach GSH [31], superoxide

dismutase (SOD) [32], Catalase (CAT) [33], TBARS [34], TNF-α [35], Nitric oxide [36], 8-hydroxy 2 deoxy-guanosine (8-OHdG) [37] and NF kappaB [38].

**Real-Time PCR:** Total RNA was isolated from rats liver and stomach tissues using the RNeasy kit (Invitrogen, California, USA), and 1 mg of total RNA was reverse transcribed by a High-Capacity cDNA Reverse Transcription Kit (Agilent Technologies, CA, USA) and the integrity of RNA was analyzed by gel imaging system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The RT-PCR amplifications were done with an ABI PRISM 7300 Sequence Detection System using the SYBR Green kit (Applied Biosystems). Specific primers were identified using the Primer Express v.3.0 (Applied Biosystems) provided with the ABI Prism7300 sequence detector.

The following primers were used:

**HMGB1** forward (fwd) primer: 5'-GCCTCGCG GAGGAAAATC -3'; **HMGB1** reverse (rvs) primer: 5'-AAGTTTGCACAAAGAATGCATAT GA-3'

**GAPGH** (fwd) primer 5'-AACTTTGGCATT GTGGAAGG-3'; **GAPGH** (rvs) primer 5'- CACA TTGGGGGTAGGAA AC -3'.

HMGB1 gene expression level of mRNA was assessed using the standard curve method and GAPDH was used for normalization.

**Histopathological Assessment:** Liver and stomach were extracted. Specimen from liver and glandular stomach were preserved in formalin 10% then routinely processed to be embedded in paraffin. 5-mm thick paraffin sections were cut and stained with hematoxylin-eosin (HE) for light microscopic analysis according to the method of Bancroft and Steven [39]. Histopathological scoring in liver for degree of (congestion, ductular proliferation, portal tract fibrosis, portal inflammation, lobular inflammation, degeneration, apoptosis) was scored as: 0 = absent; 1 = slight; 2 = moderate; and 3 = severe [40]. Histopathological scoring in glandular stomach for degree of (congestion, ulceration, inflammation, submucosal edema) was scored as: 0 = absent; 1 = slight; 2 = moderate; and 3 = severe. Histopathological evaluation was performed in six sections per slide from all animals in each group.

**Ultrastructure Analysis:** Tissue spacemen from liver and stomach were processed as described in Oliveira *et al*. [41] Tissues were diced and fixed in 2.5% glutaraldehyde for 4 h and subsequently washed in phosphate buffer (pH 7.4), post-fixed in 1% osmium tetroxide in phosphate buffer (pH 7.4) and dehydrated using alcohol series. Then, the tissues were washed with propylene oxide and embedded in epoxyresin embedding media Epon resin (Epon 812; Fluka Chemie, Switzerland). The samples transferred to polyethylene capsules and placed in oven at 60°C for 24 h to insure polymerization. Ultrathin-sections (60-70 nm) were cut using ultramicrotome and investigated using transmission electron microscope JEOL-JEM 2100 TEM operated at 80 KV.

**Statistical Analysis:** The obtained data were statistically analyzed and using the statistical package for social science (SPSS, 13.0 software (2012) [42] for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making multiple comparison among the groups for testing the inter-grouping homogeneity.

**RESULTS:** After treatment with Indomethacin, the level of plasma cholesterol (TC) and triglycerides (TG) were significantly ($P<0.05$) increased as well as significantly decreased of HDL-C as compared to the control **Table 1**. Rat receiving strawberry extract used in this study (150 mg/kg.b.w.) showed a significant decrease the plasma cholesterol (TC) and triglycerides (TG) as well as a significant increase of HDL-C compared to the group that received Indomethacin ($p< 0.05$).

**Table 2** showed the oral administration of Indomethacin at 5 mg/kg.b.w. resulted in a significant increase in liver and stomach tumor necroses factor -α (TNF-α), nitric oxide (NO), and thiobarbituric acid reactive substances (TBARS) compared to the normal control group ($p<0.05$). Rat receiving an extract of strawberry (150 mg/kg.b.w.) showed a significant decrease in TNF-α, NO and TBARS compared to the group that received Indomethacin ($p< 0.05$).

**Table 3** showed the oral administration of Indomethacin at 5 mg/kg.b.w. resulted in a

significant decrease in liver and stomach reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) compared to the normal control group ($p< 0.05$). Rat receiving an extract of strawberry (150 mg/kg.b.w.) showed a significant increase in liver and stomach reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) compared to the group that received Indomethacin ($p<0.05$).

**TABLE 1: PLASMA LEVEL OF TOTAL CHOLESTEROL (TC), TRIGLYCERIDES (TG) AND HDL-C IN NORMAL AND EXPERIMENTAL GROUPS OF RATS**

| No. | Groups | TC (mg/dl) | TG (mg/dl) | HDL-C (mg/dl) |
|---|---|---|---|---|
| (I) | Normal 3ml distilled water | 234.5 ± 10.4 | 195.05 ± 11.80 | 30.76 ± 1.89 |
| (II) | Positive control Indomethacin (IND) (5 mg/kg.b.w) | 370.32 ± 9.06* | 265.75 ±15.33* | 19.54 ±2.76* |
| (III) | Strawberry extract (150 mg/kg.b.w.) | 225.49 ± 15.48* | 210.49 ± 8.70* | 28.60 ± 4.10* |
| (IV) | Strawberry extract (150 mg/kg.b.w.) + IND (5 mg/kg.b.w) | 287.50 ± 16.4* | 216.42 ± 13.65* | 25.66 ± 2.50* |

Indomethacin (IND) was given orally as a daily dose of 5 mg/kg.b.w. It was given to all groups except I. Strawberry extract was orally given daily for 7 days and the last dose was given 1 h before indomethacin administration. Blood samples were collected 24 h after indomethacin administration. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at *p< 0.05*

**TABLE 2: LEVEL OF LIVER AND STOMACH TUMOR NECROSES FACTOR -α (TNF-α), NITRIC OXIDE (NO) AND THIOBARBATURIC ACID REACTIVE SUBSTANCES (TBARS) IN NORMAL AND EXPERIMENTAL GROUPS OF RATS**

| No. | Groups | TNF-α (Pg/ g tissue) | | NO (Umol/ g tissue) | | TBARS nmol/ g tissue | |
|---|---|---|---|---|---|---|---|
| | | Liver | Stomach | Liver | Stomach | Liver | Stomach |
| (I) | Normal 3ml distilled water | 10.43 ± 0.25 | 19.80 ± 1.64 | 11.66 ± 1.68 | 15.46 ± 0.97 | 4.25 ± 0.75 | 8.09 ± 0.54 |
| (II) | Positive control Indomethacin (IND) (5 mg/kg.b.w) | 29.70 ±1.32* | 54.37 ±3.28* | 25.87 ±3.10* | 37.60 ±2.18* | 11.63 ±1.03* | 25.44 ±1.80* |
| (III) | Strawberry extract (150 mg/kg.b.w.) | 12.64 ± 1.08* | 25.40 ± 3.21* | 12.75 ± 1.89* | 17.50 ± 2.06* | 5.44 ± 0.55* | 11.65 ± 1.25* |
| (IV) | Strawberry extract (150 mg/kg.b.w.) + IND (5 mg/kg.b.w) | 15.43 ± 2.10* | 29.65 ± 3.40* | 14.26 ± 1.68* | 21.70 ± 1.82* | 7.20 ± 0.81* | 15.85 ± 2.06* |

Indomethacin (IND) was given orally as a daily dose of 5 mg/kg.b.w. It was given to all groups except the normal one. Strawberry extract was orally given daily for 3 weeks and the last dose of each was given 1 h before indomethacin administration. Organs were collected 24 h after indomethacin administration. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at *p< 0.01*

**TABLE 3: LEVEL OF LIVER AND STOMACH REDUCED GLUTATHIONE (GSH) AND ACTIVITIES OF SUPEROXIDE DISMUTASE (SOD) AND CATALASE (CAT) IN NORMAL AND EXPERIMENTAL GROUPS OF RAT**

| No. | Groups | GSH (mg/ g tissue) | | SOD (U/gm tissue) | | CAT (U/gm tissue) | |
|---|---|---|---|---|---|---|---|
| | | Liver | Stomach | Liver | Stomach | Liver | Stomach |
| (I) | Normal 3ml distilled water | 10.76 ± 1.26 | 3.65 ± 0.78 | 210.68 ± 8.97 | 79.54 ± 5.48 | 110.65 ± 8.60 | 45.32 ± 2.76 |
| (II) | Positive control Indomethacin (IND) (5 mg/kg.b.w) | 4.37 ± 0.65* | 1.27 ±0.18* | 176.09 ±10.50* | 36.98 ±3.11* | 45.48 ±3.75* | 15.40 ±1.27* |
| (III) | Strawberry extract (150 mg/kg.b.w.) | 8.65 ± 0.95* | 3.16 ± 0.42* | 205.08 ± 14.73* | 70.05 ± 3.99* | 105.32 ± 5.96* | 39.80 ± 2.25* |
| (IV) | Strawberry extract (150 mg/kg.b.w.) + IND (5 mg/kg.b.w) | 7.95 ± 0.43* | 2.98 ± 0.40* | 198.70 ± 17.75* | 62.38 ± 4.76* | 94.00 ± 7.33* | 35.09 ± 3.18* |

Indomethacin (IND) was given orally as a daily dose of 5 mg/kg.b.w. It was given to all groups except the normal one. Strawberry extract was orally given daily for 7 days and the last dose of each was given 1 h before indomethacin administration. Organs were collected 24 h after indomethacin administration. Values are given as mean ± SD for groups of eight animals each. * Significantly different from the normal group at *p< 0.05*

**Table 4** showed that oral administration of Indomethacin (5 mg/kg.b.w.) resulted in a significant increase in liver and stomach of 8-hydroxy 2 deoxyguanosine (8-OHdG) and NF kappa B when compared to the normal control group ($p< 0.05$). Supplementation of strawberry extract at 150 mg/k.g.b.w. resulted in a significant decrease in liver and stomach of 8-hydroxy 2 deoxyguanosine (8-OHdG) and NF kappaB when compared to the group that received Indomethacin ($p< 0.05$).

**TABLE 4: LEVEL OF LIVER AND STOMACH 8-HYDROXY 2 DEOXYGUANOSINE (8-OHdG) AND NF KappaB IN NORMAL AND EXPERIMENTAL GROUPS OF RATS**

| No. | Groups | 8-OHdG (ng/ g tissue) | | NF kappaB (ng/gm tissue) | |
|---|---|---|---|---|---|
| | | Liver | Stomach | Liver | Stomach |
| (I) | Normal 3ml distilled water | 1.6 ± 0.15 | 2.1 ± 0.3 | 0.62 ± 0.7 | 0.35 ± 0.28 |
| (II) | Positive control Indomethacin (IND) (5 mg/kg.b.w) | 4.5 ± 0.31* | 6.1 ± 0.33* | 6.4 ± 1.0* | 5.8 ± 0.21* |
| (III) | Strawberry extract (150 mg/kg.b.w.) | 3.6 ± 0.23* | 5.45 ± 0.2* | 5.1 ± 0.53* | 4.9 ± 0.39* |
| (IV) | Strawberry extract (150 mg/kg.b.w.) + IND (5 mg/kg.b.w) | 4.1 ± 0.11* | 5.1 ± 0.60* | 5.9 ± 0.5* | 5.1 ± 0.37* |

Indomethacin (IND) was given orally as a daily dose of 5 mg/kg.b.w. It was given to all groups except the normal one. Strawberry extract was orally given daily for 7 days and the last dose of each was given 1 h before indomethacin administration. Organs were collected 24 h after indomethacin administration. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p < 0.05$

Liver and stomach high mobility group box-1 (HMGB1) in the indomethacin group were higher than those in the control group (P<0.01). Treatment with strawberry extract significantly decreased the expression levels of HMGB1 (P<0.05) as compared to indomethacin group (**Charts 1-3**).

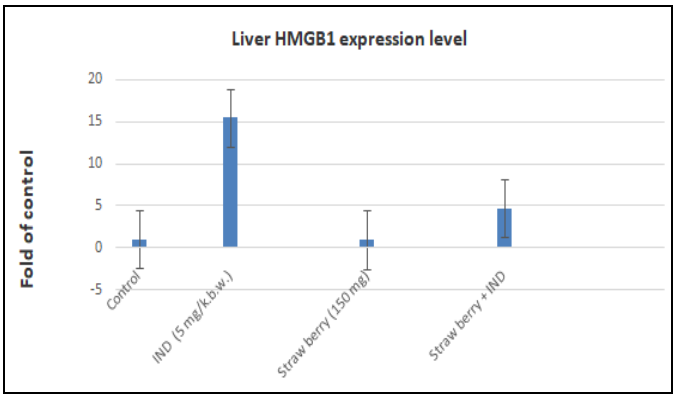


**CHART 1: EFFECT OF STRAW BERRY ON LIVER HIGH MOBILITY GROUP BOX-1 (HMGB1) IN INDOMETHACIN INDUCED STOMACH ULCERATION IN RATS. REPRESENTATIVE BAR DIAGRAM OF THREE INDEPENDENT EXPERIMENTS ARE PRESENTED**

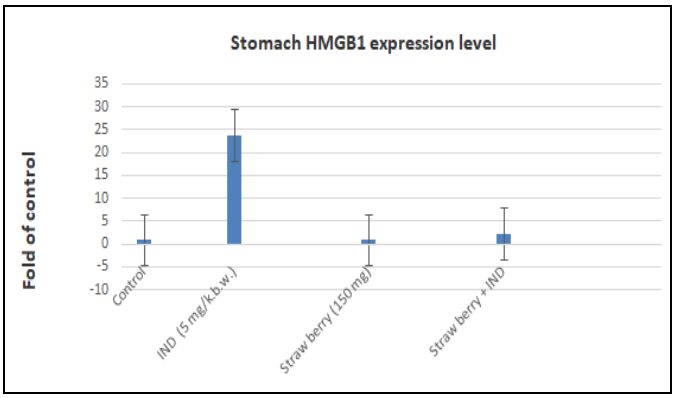


**CHART 2: EFFECT OF STRAW BERRY ON STOMACH HIGH MOBILITY GROUP BOX-1 (HMGB1) IN INDOMETHACIN INDUCED STOMACH ULCERATION IN RATS. REPRESENTATIVE BAR DIAGRAM OF THREE INDEPENDENT EXPERIMENTS ARE PRESENTED**

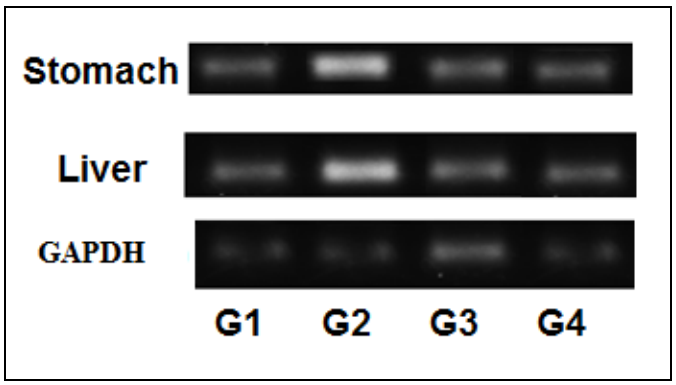


**CHART 3: AN AGAROSE GEL ELECTROPHORESIS SHOWS PCR PRODUCTS OF LIVER AND STOMACH HIGH MOBILITY GROUP BOX-1 (HMGB1) AND GAPDH IN DIFFERENT TREATED GROUPS**

**Histopathological Findings: Fig. 1:** Microscopic pictures of H&E stained liver sections showing normally organized hepatic cords, central veins (CV) and portal areas (PA) (A) in the control negative group. In contrast, marked histo-pathological changes including congestion of portal vein (red arrow), bile duct dilation (black arrow), portal expansion with fibrous tissue (yellow arrow) (B), congested blood vessels (red arrows), degenerated hepatocytes (yellow arrows) (C), congested blood vessels (red arrows), portal lymphocytic inflammation (black arrows) (D) are seen in indomethacin group II. Meanwhile, mild to very mild portal lymphocytic inflammation (black arrows) are seen in group III (E) and group IV (F), respectively. X: 100 bar 100.

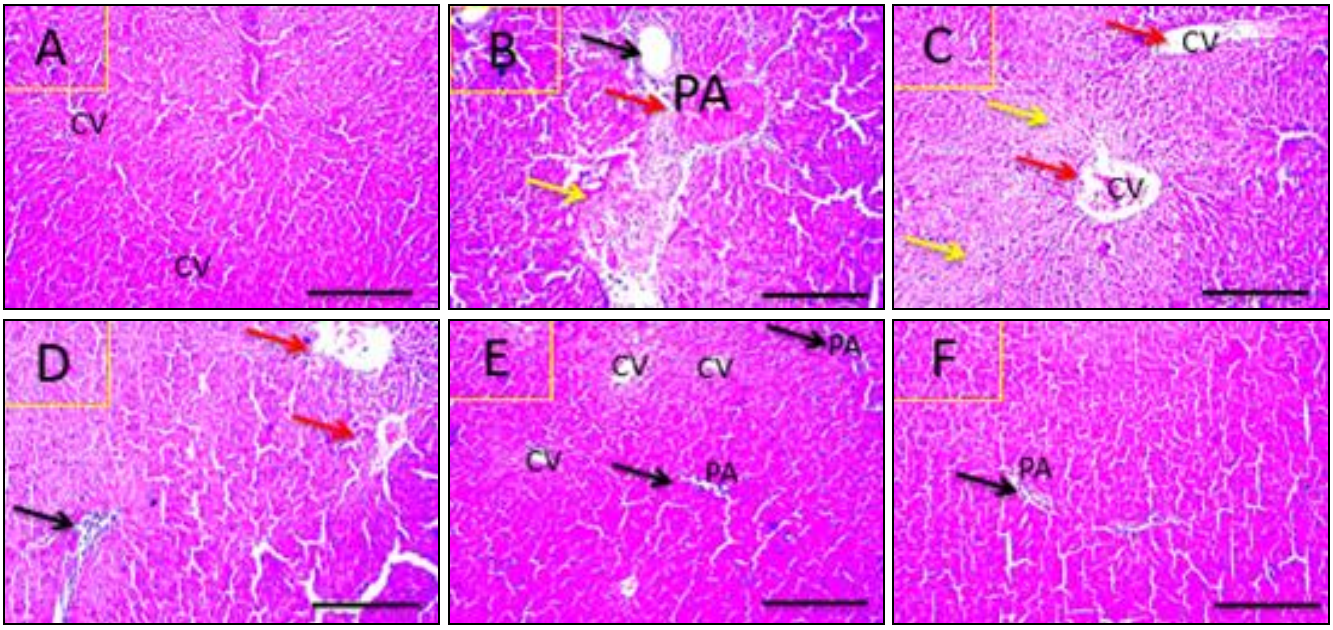


**FIG. 1: MICROSCOPIC PICTURES OF RAT LIVER SECTIONS STAINED WITH H&E IN DIFFERENT STUDIED GROUPS**

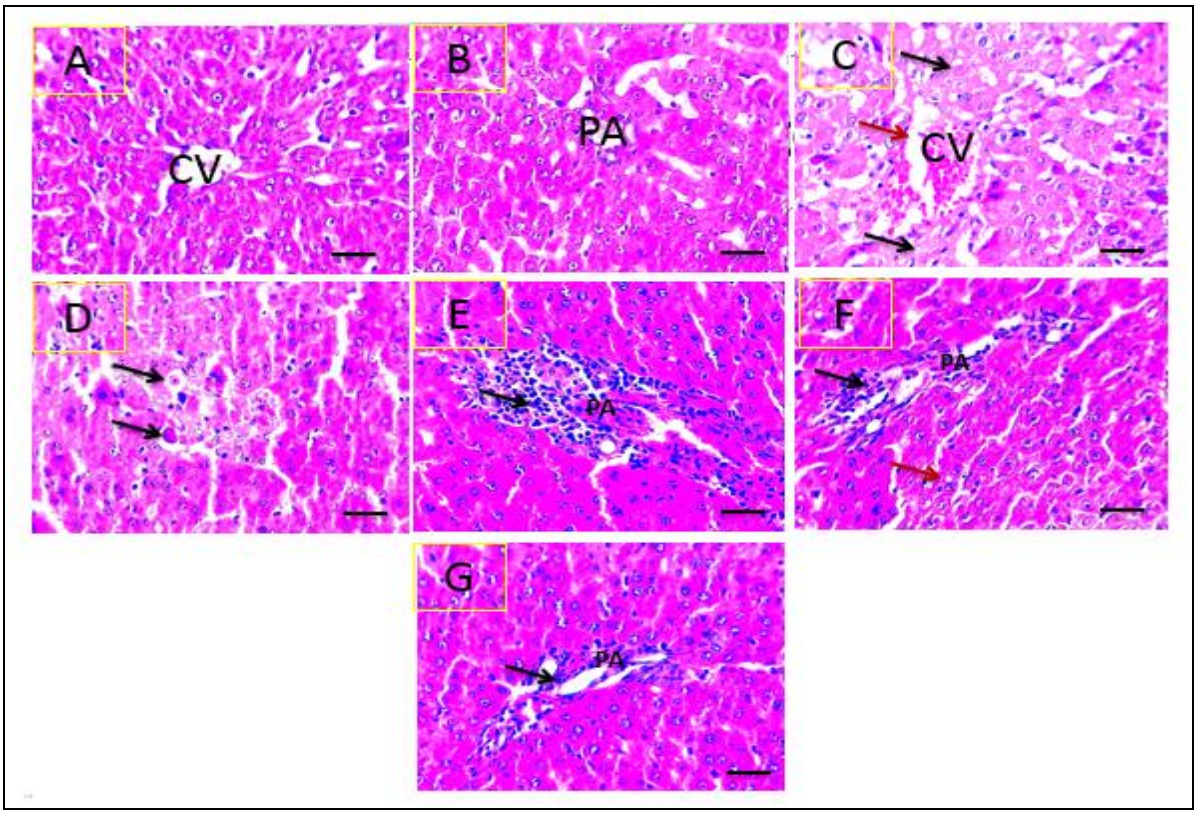


**FIG. 2: MICROSCOPIC PICTURES OF RAT LIVER SECTIONS STAINED WITH H&E IN DIFFERENT STUDIED GROUPS**

**Fig. 3:** Microscopic pictures of H&E stained sections from glandular stomach showing normal mucosa (M), submucosa (SM), and muscular coat (MC) in the control negative group. In contrast, marked histopathological changes including mucosal ulceration (thick black arrow), congestion (red arrow), submucosal edema (asterisk) with few leukocytic cells infiltration (thin black arrows) (B), nuclear pyknosis of glandular cells (yellow arrow) (C&D), focal lymphocytic aggregation in the mucosa (black arrow) (C&E) in indomethacin group II. Meanwhile, cystic gland (yellow arrow), mild congestion (red arrows), and submucosal edema (asterisk) are seen in group III (F). The glandular stomach retained its normal histological picture in group IV (G). X: 100 bar 100 except (D&E) X: 400 bar 50.

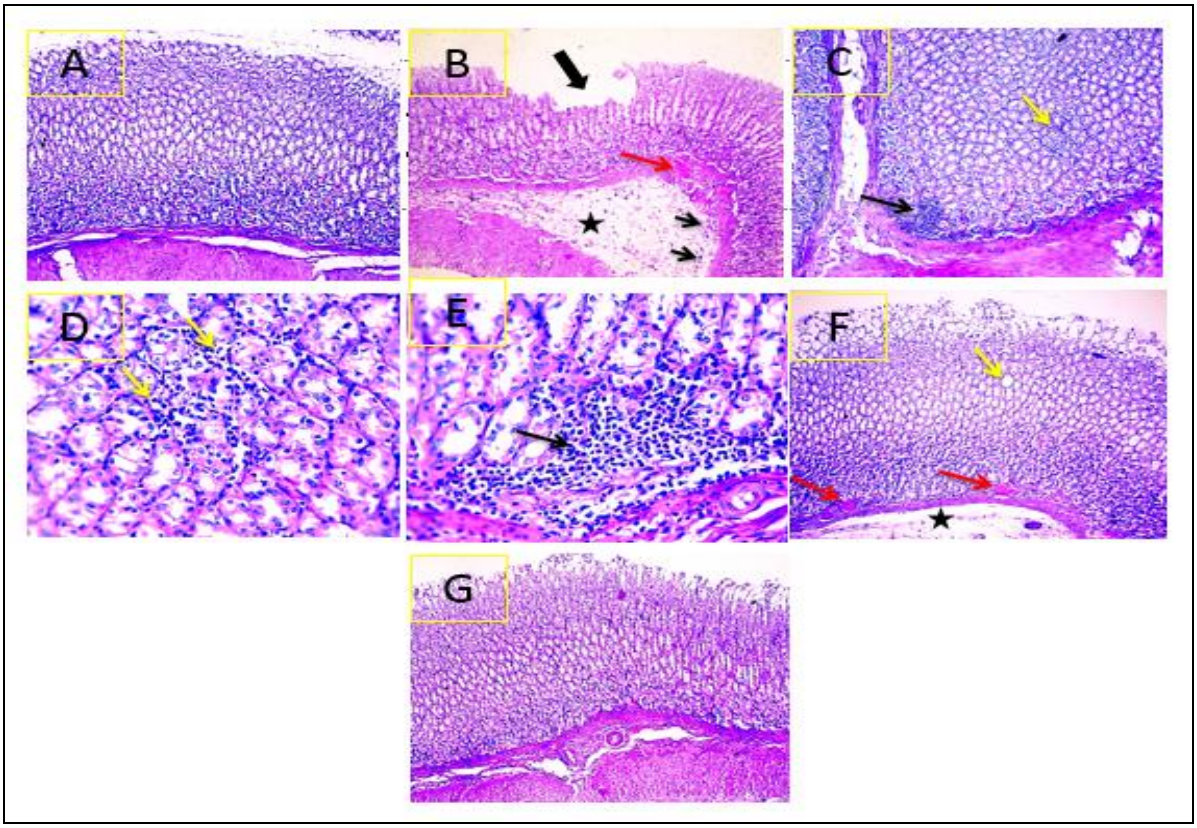


**FIG. 3: MICROSCOPIC PICTURES OF H&E STAINED SECTIONS FROM GLANDULAR STOMACH SHOWING DIFFERENT STUDIED GROUPS**

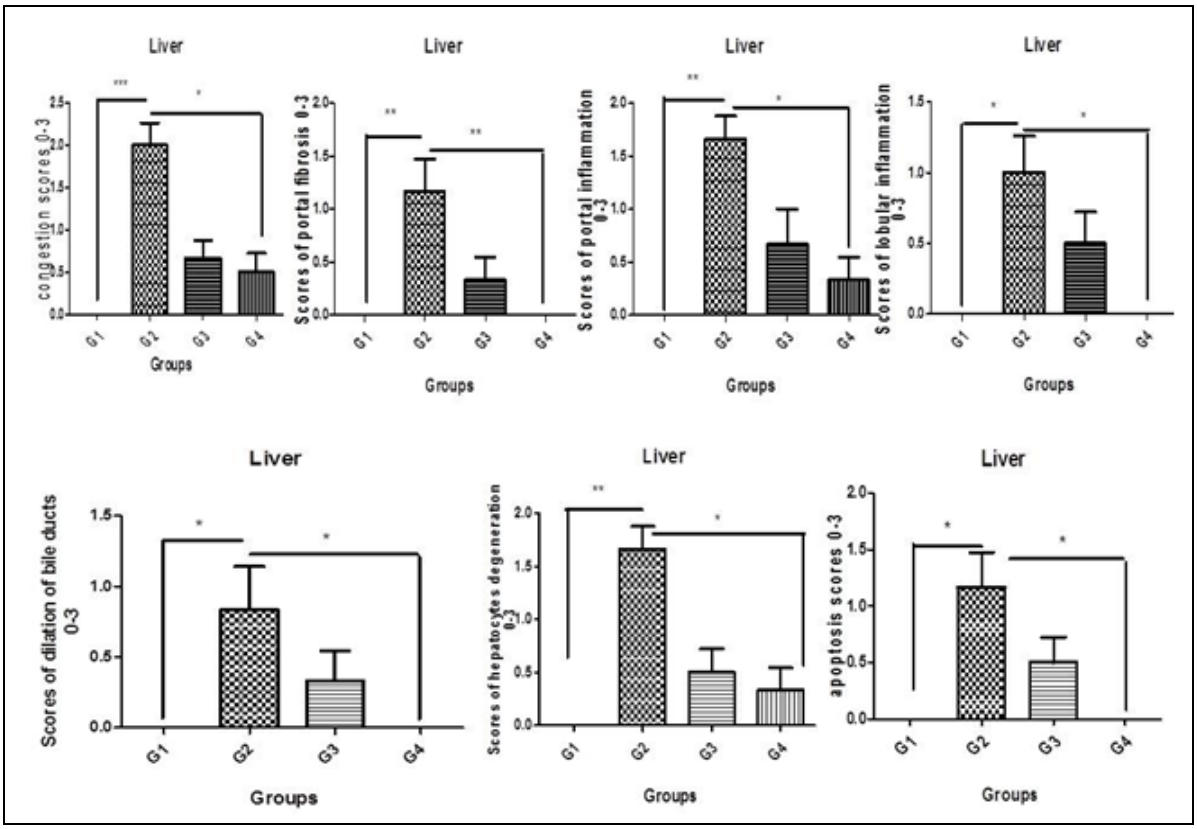


**FIG. 4: STATISTICAL ANALYSIS OF HISTOPATHOLOGICAL SCORES IN THE EXAMINED H&E STAINED LIVER SECTIONS FROM FOUR EXPERIMENTAL GROUPS USING KRUSKALIS-WALLIS TEST FOLLOWED BY DUNN'S METHOD SHOWS REDUCTION OF ALL LESION SCORES IN GROUPS III & IV AND SIGNIFICANTLY IN GROUP IV TREATED WITH STRAWBERRY.** Small stars indicate significantly different means (when $p<0.05$).

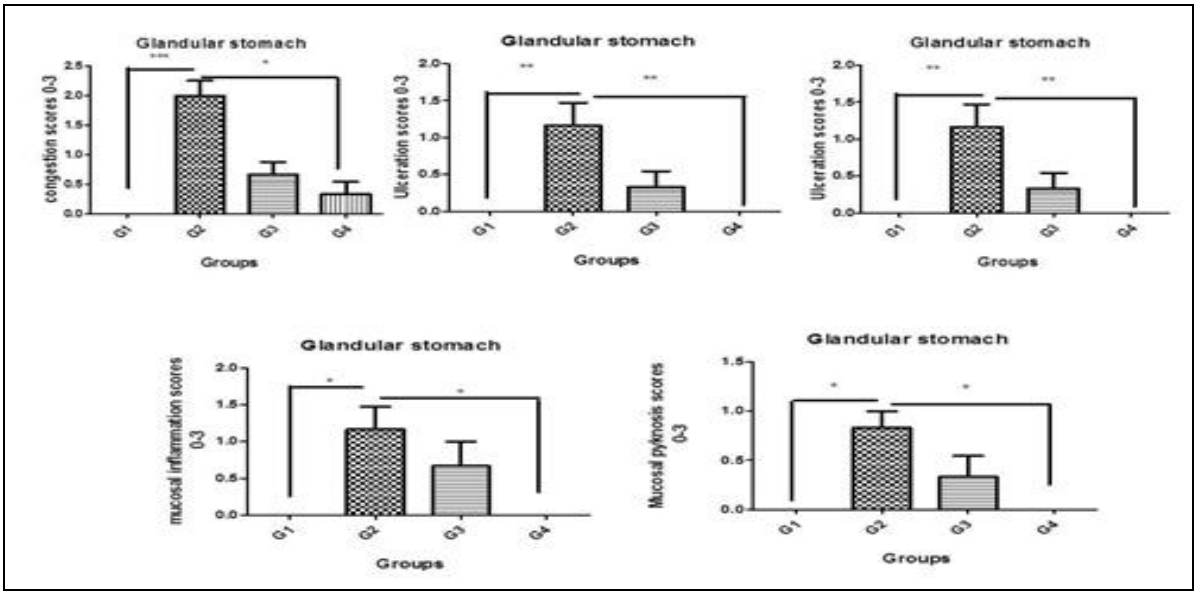


**FIG. 5: STATISTICAL ANALYSIS OF HISTOPATHOLOGICAL SCORES IN THE EXAMINED H&E STAINED GLANDULAR GASTRIC SECTIONS FROM FOUR EXPERIMENTAL GROUPS USING KRUSKALIS-WALLIS TEST FOLLOWED BY DUNN'S METHOD SHOWS REDUCTION OF ALL LESIONAL SCORES IN GROUPS III & IV AND SIGNIFICANTLY IN GROUP IV TREATED WITH STRAWBERRY.** Small stars indicate significantly different means (when $p<0.05$).

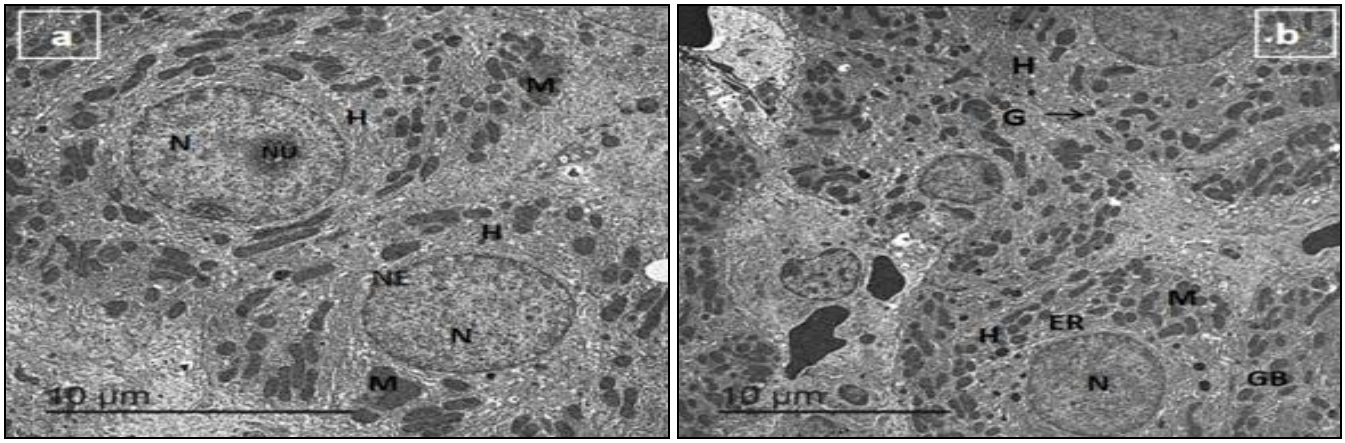


**FIG. 6 (A & B): ELECTRON MICROGRAPHS FROM RAT LIVER IN CONTROL GROUP SHOW NORMAL APPEARANCE OF HEPATOCYTES (H) WITH PROMINENT NUCLEUS (N) AND INTACT NUCLEAR ENVELOP (NE). NOTE: NORMAL FEATURES MITOCHONDRIA (M), GOLGI BODIES (G) AND CISTERNAE OF ENDOPLASMIC RETICULUM**

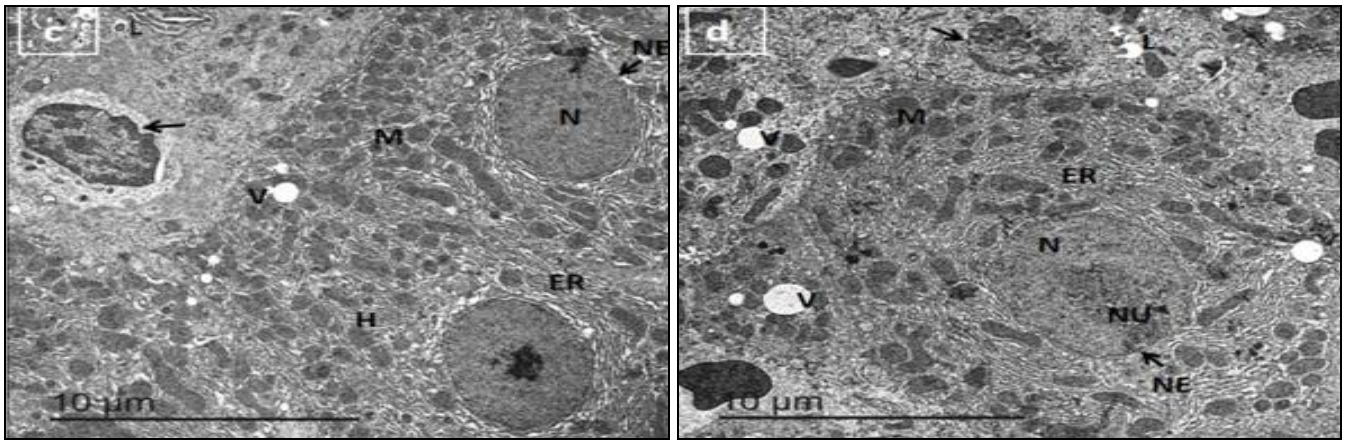


**FIG. 7 (C & D): ELECTRON MICROGRAPHS OF RAT LIVER IN GROUP II TREATED WITH INDOMETHACIN SHOW HEPATOCYTES WITH IRREGULAR NUCLEAR ENVELOPE GIVES THE NUCLEUS (N) ABNORMAL APPEARANCE (H). SOME CELLS SHOW DAMAGED NUCLEI WITH CONDENSED CHROMATIN MATERIAL (ARROWS). SWOLLEN MITOCHONDRIA (M) AND DILATED ENDOPLASMIC RETICULUM (ER) ARE NOTICED. MANY LYSOSOMES (L) AND VACUOLES (V) APPEAR IN THE CYTOPLASM OF HEPATOCYTES**

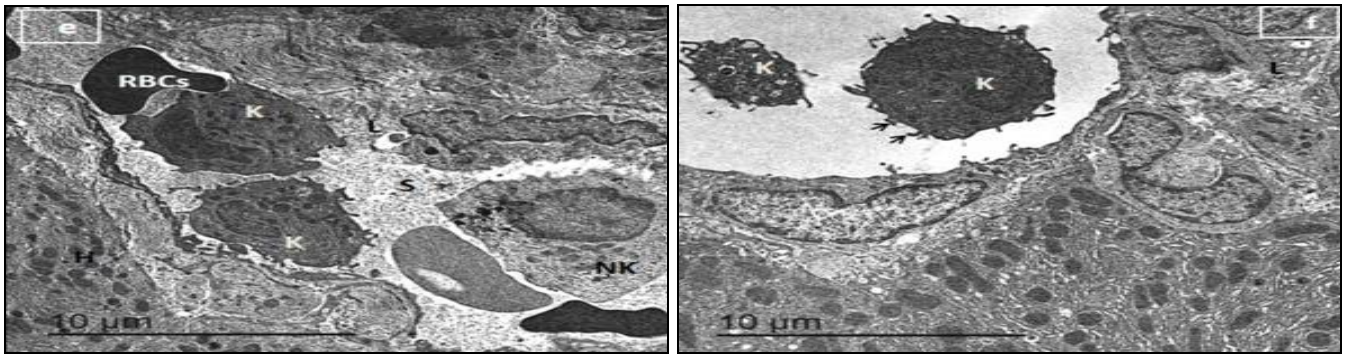


**FIG. 8 (E & F): ELECTRON MICROGRAPH IN GROUP II SHOW SINUSOIDAL SPACE CONTAINS RBCS AND MANY ACTIVE KUPFFER CELLS (K) WITH CYTOPLASMIC PROCESS (ARROWS), IRREGULAR SHAPE NUCLEUS AND MULTIPLE PHAGOCYTOSED MATERIALS. NATURAL KILLER CELLS (NK) AND LYSOSOMES (L) ARE ALSO OBSERVED**

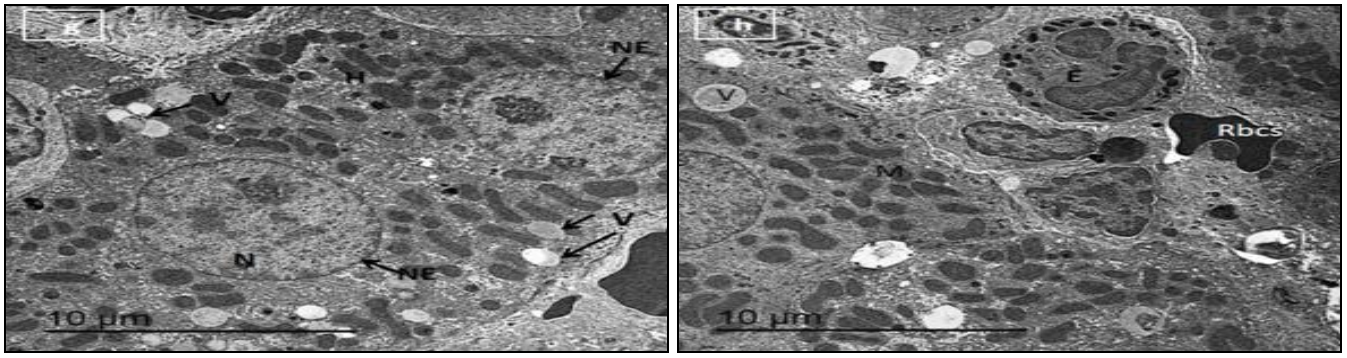


**FIG. 9 (G & H): ELECTRON MICROGRAPH OF RAT LIVER IN GROUP III. FIGURE A SHOW HEPATOCYTES (H) WITH NORMAL FEATURES OF MITOCHONDRIA (M). NUCLEUS EXHIBIT NORMAL STRUCTURE AND REGULAR NUCLEAR ENVELOPE (NE). CYTOPLASMIC VACUOLIZATION (V) ARE NOTICED. EOSINOPHILS (E) AND RBCS SPREADS IN SINUSOIDAL SPACE AS SEEN IN (B)**

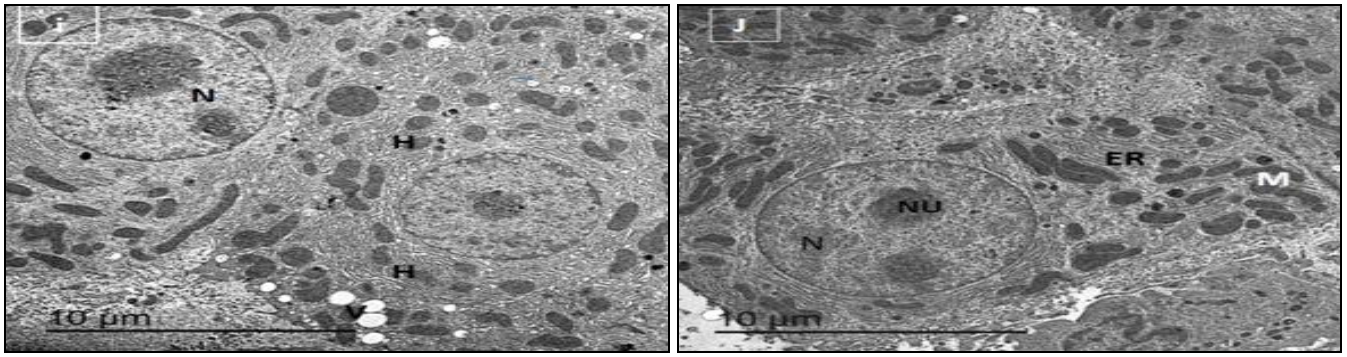


**FIG. 10 (I & J): ELECTRON MICROGRAPHS OF RAT LIVER IN GROUP IV SHOW NORMAL APPEARANCE OF HEPATIC TISSUE**

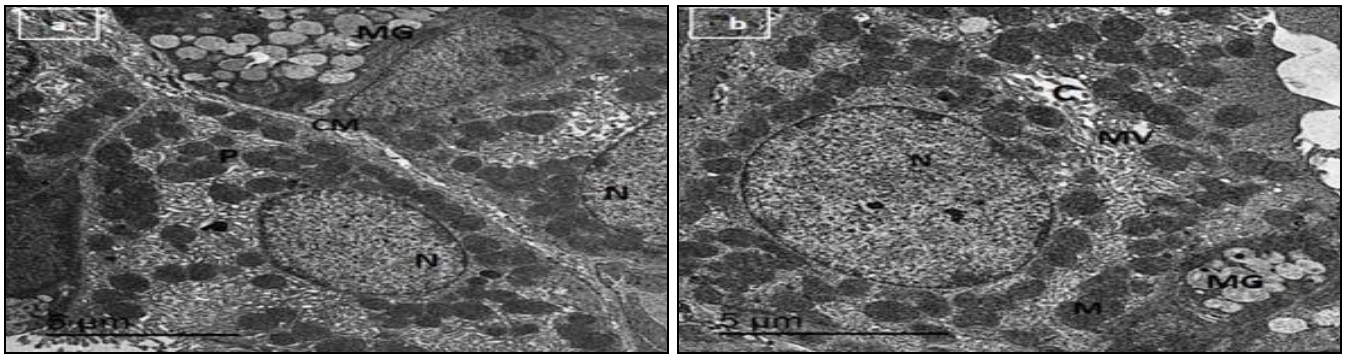


**FIG. 11 (A&B): ELECTRON MICROGRAPH OF CONTROL GROUP (GROUP I) IN RAT STOMACH MUCOSA. THE SECTIONS SHOW NORMAL CELLULAR ARCHITECTURE, WITH INTACT CELL MEMBRANES (CM). PARTIAL CELL (P) EXHIBITS A HEALTHY NUCLEUS (N) WITH REGULAR SHAPE NUCLEAR ENVELOPE (NE), WELL-DEFINED NUCLEOLI (NU) AND CHROMATIN IS FINELY DISTRIBUTED. MITOCHONDRIA (M) APPEARS IN LARGE NUMBERS WITH INTACT CRISTAE. THE INTRACELLULAR CANAL (C) LINED WITH CHARACTERISTIC MICROVILLI (MV) AND MUCIN GRANULES MG NEAR LUMEN**

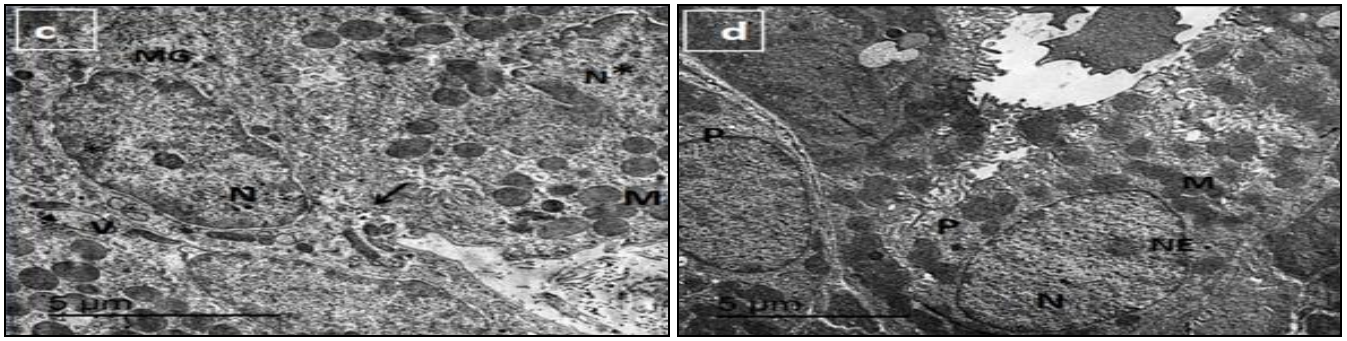


**FIG. 12 (C&D): ELECTRON MICROGRAPHS OF RAT STOMACH FUNDIC MUCOSA TREATED WITH INDOMETHACIN (GROUP II) SHOWS DISRUPTION OF CELL ARCHITECTURE AND DISSOCIATION OF CELL MEMBRANES IN CHIEF CELL, PYKNOTIC NUCLEUS (N) WITH CONDENSED CHROMATIN (*), AND VACUOLATED CYTOPLASM(V) AND DEGENERATED ZYGOMATIC GRANULES (Z) WHILE ADJACENT CELL SHOW TOTALLY DEGENERATED NUCLEUS (N*) AND DISCONTINUOUS CELL MEMBRANE (DM). NOTICE THE ENLARGEMENT OF MITOCHONDRIA AND DISAPPEARANCE OF CRISTAE AND CELL MEMBRANE DEGENERATION (ARROW). D: PARIETAL CELL SHOWS IRREGULAR NUCLEAR ENVELOP, DEGENERATED MITOCHONDRIA**

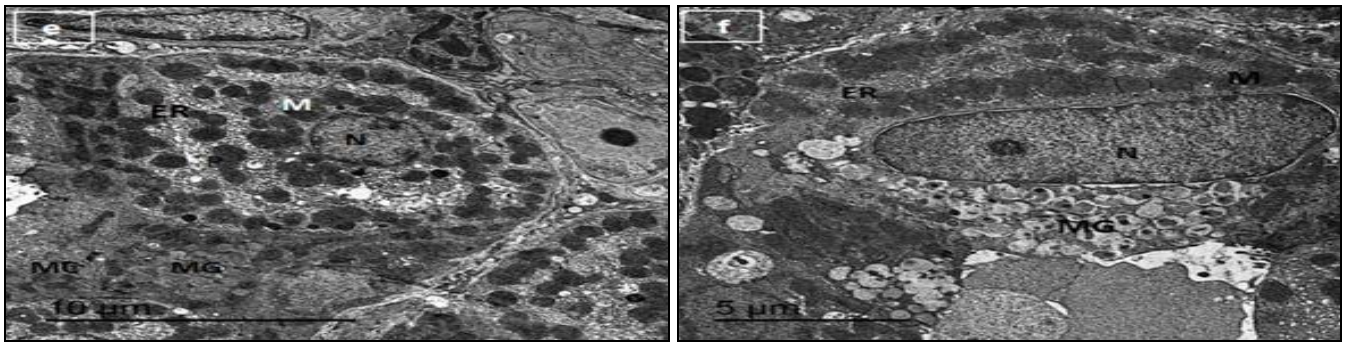


**FIG. 13 (E & F): ELECTRON MICROGRAPHS IN GROUP III RAT STOMACH MUCOSA. E: SHOW INTACT PARIETAL CELL WITH NORMAL NUCLEUS WHILE VACUOLES APPEAR IN CYTOPLASM. NUMEROUS MITOCHONDRIA ARE NOTICED. F: MUCOUS GRANULES (MG) ARE FOUND IN THE ADJACENT MUCOUS CELL (MC)**

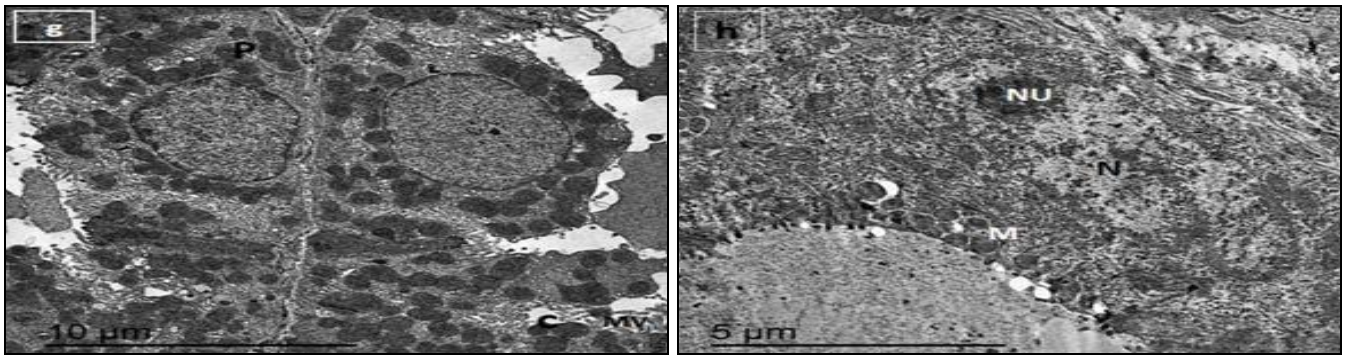


**FIG. 14 (G & H): SHOW TWO ADJACENT PARIETAL CELL AND MUCOUS SECRETING CELL RESPECTIVELY IN GROUP IV. ALL CELLS SHOW NORMAL APPEARANCE**

**DISCUSSION:** The oxidative stress of Indomethacin in the animal has been reported *via* free radicals generation and depletion of antioxidant [1]. Such event consequently results in overwhelmed antioxidant defense system in experimental animal model [1, 43].

An imbalance between free radicals production and antioxidant defense system results in oxidative stress, which further deregulates cellular functions leading to pathological conditions [44, 45]. In the present study, the reduced activity of antioxidant enzymes in indomethacin-treated rats is a clear manifestation of excessive formation of free radicals resulting in hepatic and mucosa epithelia damage.

Clinical observations have shown that long-term treatment with NSAIDs correlates with the onset or aggravation of congestive liver toxicity failure [45].

The first source of protection of the body against free radicals and other oxidants are antioxidants, which help cells to neutralize the excess formation of radical species [46].

The current study revealed that indomethacin (5 mg/kg.b.w.) induced liver toxicity and stomach injury as indicated by elevations in plasma liver and stomach damage markers. Lipid peroxidation may also damage membranes in other cells, altering important elements of control for blood pressure and liver rate. Given that increased cholesterol and triglycerides as well as decreased HDL cholesterol (HDL-C). Both Britton *et al.*, [47] and Brunet *et al.*, [48] found the same results.

In line with other NSAIDs, a study done by Melvin and Edwards (1999) demonstrated that injection of rats with Indomethacin caused hyperlipidemia which was represented by a marked increase in serum lipid profiles including TG and TCh with a concomitant decreased in HDL-C in relation to control ones [49].

The antioxidant effect is a well-established function of strawberry or flavonoids found in it [50]. In addition, strawberry procyanidins have been found to scavenge reactive carbonyls by the formation of adducts [51], and the current study demonstrated elevation of tumor necroses factor -α (TNF-α), nitric oxide (NO), thiobarbituric acid reactive substances (TBARS), 8-hydroxy 2 deoxyguanosine (8-OHdG), NF kappaB and High mobility group box 1 (HMGB1) in Indomethacin treated animals. The anti-inflammatory properties of strawberries are in fact, due to their proanthocyanidin content, which has a protective effect on the liver and mucosal surface lesions induced by indomethacin [52] and elevation of food intake and absorption.

The significant increase in the level of liver and stomach TNF-α, NO, TBARS, 8-OHdG), NF kappaB, and HMGB1 in indomethacin-treated groups indicates ongoing peroxidative stress and compromised antioxidant defense mechanisms [53].

The mechanism of indomethacin-induced mitochondrial injury seems to involve the generation of ROS, causing oxidative stress to hepatocytes as proposed by Sokol *et al.*, [54] and number of structurally related NSAIDs do produce oxidative stress, and the underlying mechanisms have been suggested to be based on peroxidase-catalyzed production of NSAID radicals, which in turn can oxidize GSH and NAD(P)H. NSAID radicals can undergo redox cycling.

In other systems, gastric mucosa lesions are elevated by extracellular TNF-α, and NO [55], in particular, has been shown to cause acute cell death in a variety of *in-vitro* and *in-vivo* conditions [56]. The finding that Diclofenac sodium administration results in a rapid increase in TNF-α and NO plasma levels [57] and that inhibition of TNF-α synthesis and release protects from NSAID-induced gastropathy [58] suggests that this cytokine plays a major role in mediating gastric mucosal injury in NSAID-treated rats. However, because NSAIDs stimulate TNF-α and NO release (present results), their site of action must be located downstream of this cytokine.

Hussein, [59] has reported that the concentration of lipid peroxides increases in the tissues of indomethacin-treated rats. In the present study, TBARS levels in the liver and stomach were significantly lower in thestrawberry–treated groups compared to the indomethacin-treated control group. The present results were confirmed with Boshra and Hussein [60].

The current study showed that Indomethacin induced oxidative stress in liver and stomach tissues of rats as evidenced by a significant reduction in the activities of reduced glutathione (GSH) metabolizing enzymes, SOD as well as CAT in cardiac tissues of indomethacin intoxicated rats versus normal healthy ones.

This effect may consider one of the indomethacin mechanisms induced liver toxicity. GR is the key enzyme in the conversion of oxidized glutathione (GSSG) back to the reduced form (GSH).

GSH, a non-enzymatic antioxidant, has an important role in scavenging the electrophilic moieties produced by toxic chemicals and conjugate them to fewer toxic products. CAT is an antioxidant enzyme widely distributed in all animal tissues. It decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [61]. Thus, the less amount of GSH production due to inhibition of its metabolizing enzymes together with a reduction of CAT activity in cardiac of diclofenac intoxicated rats may reduce the capacity of the tissue to protect itself from the diclofenac induced oxidative tissue damage. Our result is supported by some authors who stated the reduction in the levels of hepatic and renal

enzymatic and non-enzymatic antioxidants of animals injected with indomethacin [62].

The present study described the normal structure of liver and gastric mucosa in rats and the effect of Indomethacin under light and electron microscope. Also, this study investigated the protective role of strawberry extract. The indomethacin-induced cellular and nuclear damage in both liver and gastric tissue as described in our results.

The impact of indomethacin injection on liver tissue includes cytoplasmic degeneration and vacuolation in addition to lymphocytic inflammation. Inflammatory and apoptotic changes were also noticed in different hepatic cells.

The Indomethacin induced ulceration in the gastric mucosa, which becomes eroded, and subsequently, the muscularis mucosae became exposed to gastric secretion. These findings are confirming the previously mentioned results in the current work and in aligned with the results of Barnett *et al.,* [63,] who observed massive necrosis, epithelial sloughing, and superficial ulcers after indomethacin administration. Wallace [64], reported that non-steroidal anti-inflammatory drugs (NSAIDs) induce stomach ulcers and bleeding by impairing the restitution process and inactivating several growth factors that are important in the mucosal defense and repair.

The previous studies suggested that one of the important mechanisms by which indomethacin damage gastric mucosa is the increased production of reactive oxygen species (ROS), which has a critical role in the gastric ulceration process. Lipid peroxidation resulted from indomethacin damages membrane proteins. The damage to membrane proteins decreases the membrane's permeability, the activities of enzymes and receptors, and the activation of inflammatory cells [65, 66].

Administration of both Indomethacin and strawberry extract induces less damage and inflammatory reactions in hepatic and gastric mucosal tissue, as revealed in both light and TEM examination. This finding also confirms the decrease in Level of liver and stomach tumor necroses factor -α (TNF-α), nitric oxide (NO) and thiobarbituric acid reactive substances (TBARS) as well as Level of liver and stomach 8-hydroxy 2 deoxyguanosine (8-OHdG) and NF kappaB in the group of rat received strawberry extract with Indomethacin.

**CONCLUSION:** From the obtained results, it could be concluded that strawberry extract was effective in protection against liver toxicity induced by Indomethacin in rats. Strawberry extract received the highest oxygen radical absorbing capacity (ORAC) value and exhibited superior antioxidant properties, and it was able to ameliorate plasma oxidative stress biomarkers as well as an enzymatic and non-enzymatic antioxidant defense system in liver and stomach tissues.

**ACKNOWLEDGEMENT:** Declared none

**COMPETING INTEREST:** The authors declare no conflict of interest, financial or otherwise.

**FUNDING:** The authors are not currently in receipt of any research funding.

## REFERENCES:

1. Rothwell PM, Cook NR and Gaziano JME: Effects of aspirin on risks of vascular events and cancer according to bodyweight and dose: analysis of individual patient data from randomised trials. Lancet 2018; 392: 387.
2. Tomic Z, Milijasevic B and Sabo A: Diclofenac and ketoprofen liver toxicity in rat. Eur J Drug Metab Pharmacokinet 2008; 33(4): 253-60.
3. Quadri KA and Rufus O: Kolaviron Diminishes Diclofenac-Induced Liver and Kidney Toxicity in Wistar Rats Via Suppressing Inflammatory Events, Upregulating Antioxidant Defenses, and Improving Hematological Indices. Dose Response 2020; 18(1): 1-12.
4. Abdel-Daim MM, Eltaysh R, Hassan A and Mousa SA: Lycopene attenuates tulathromycin and diclofenac sodium-induced cardiotoxicity in mice. IJMS 2018; 19(2): 344.
5. Cheng YT, Lu CC and Yen GC: Phytochemicals enhance antioxidant enzyme expression to protect against NSAID-induced oxidative damage of the gastrointestinal mucosa. Mol Nutr Food Res 2016; 4: 34-41.
6. Soliman NA, Zineldeen DH, Katary MA and Ali DA: N-acetylcysteine a possible protector against indomethacin-induced peptic ulcer: crosstalk between antioxidant, anti-inflammatory, and antiapoptotic mechanisms. Can J Physiol Pharmacol 2016; 14: 1-8.
7. Ibrahim FM, Attia HN, Aly YA, Ahmed KA and Ramadan MF: Biochemical characterization, anti-inflammatory properties and ulcerogenic traits of some cold-pressed oils in experimental animals. Pharm Biol 2017; 55: 740-48.
8. Matsui H, Shimokawa O, Kaneko T, Nagano Y and Rai K: Hepathophysiology of non-steroidal anti-inflammatory drug (NSAID)- induced mucosal injuries in stomach and small intestine. J Clin Biochem Nutr 2011; 48: 107-11.
9. Harris HE, Andersson U and Pisetsky DS: HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. Nat Rev Rheumatol 2012; 8: 195-202.

10. Hirsiger S, Simmen HP, Werner CM, Wanner GA and Rittirsch D: Danger signals activating the immune response after trauma. Mediators Inflamm 2012; 15: 1-10.
11. Andersson U and Tracey KJ: HMGB1 is a therapeutic target for sterile inflammation and infection. Annu Rev Immunol 2011; 29: 139-62.
12. Hussein MA, Aboelwafa SM and Abosalem HM: The Possible Protective Effect of Spirulina Platensis Extract against Nicotine Induced-Lung Toxicity in Rats 2020; 14(10): 1-8.
13. El Gizawy HA, Hussein MA and Abdel-Sattar E: Biological activities, isolated compounds and HPLC profile of Verbascum nubicum. Pharmaceutical biology 2019; 57: 485-97.
14. Neri D, Baruzzi G, Massetani F and Faedi W: Strawberry production in forced and protected culture in Europe as a response to climate change. Canadian Journal of Plant Sciences 2012; 92: 1021-36.
15. Giampieri F, Tulipani S, Alvarez JM, Quiles JL, Mezzetti B and Battino M: The strawberry: Composition, nutritional quality, and impact on human health. Nutrition 2012; 28: 9-19.
16. Singh R, Gupta RK, Patil RT, Sharma RR, Asrey R, Kumar A and Jangra KK: Sequential foliar application of vermicompost leachates improves marketable fruit yield and quality of strawberry (*Fragaria ananassa* Duch.). Scientia Horticulurae 2010; 124: 34–39.
17. Tulipani S, Alvarez JM, Busco F, Bompadre S, Quiles JL, Mezzetti B and Battino M: Strawberry consumption improves plasma antioxidant status and erythrocyte resistance to oxidative haemolysis in humans. Food Chemistry 2011; 128: 180-86.
18. Tõnutare T, Keert K, Szajdak L and Moor U: Composition of commercially produced organic and conventional strawberries. Nutrition and Food Science 2014; 44: 562-75.
19. Tulipani S, Romandini S, Bompadre S, Capocasa F and Mezzetti B: Variation in strawberry micronutrients, phytochemical and antioxidant profiles: the combined effect of genotype and storage. Acta Horticulturae 2009; 842: 867-71.
20. Aaby K, Ekeberg D and Skrede G: Characterization of phenolic compounds in strawberry (*Fragaria ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. Journal of Agricultural and Food Chemistry 2007; 55: 4395-4406.
21. Liao X, Wang L, Yang C, He J, Wang X and Guo R: Cyclooxygenase mediates cardioprotection of angiotensin-(1–7) against ischemia/reperfusion-induced injury through the inhibition of oxidative stress. Mol Med Rep 2011; 4: 1145-50.
22. Abdel-Maksoud H, Hussein MA and Barakat M: Cranberry extract as a functional food in treatment of myocardial toxicity induced by nicotine in Rats 2015; 5(4): 1174-80.
23. Ali HA, Mahmoud OM, Hussein MA and Mohammed MS: Biochemical effect of cranberry extract on experimentally induced myocardial infarction Benha Veterinary Medical Journal 2017; 32(1): 59-66.
24. Hassan SA, Sabry D, Hussein MA: Protective effect of cranberry extracts against oxidative stress and DNA damage induced by diclofenac sodium in kidney of male albino rate. Chinese Medicine 2017; 8: 113-31.
25. El-gizawy A, Mosaad YO, Gobba NA and Hussein MA: Chemical Composition of the Essential oil of the Leaves of *Pimenta diocia* (L.) Merr. & *Pimenta racemosa* (Mill.) cultivated in Egypt and evaluation of their in-vitro antioxidant and antidiabetic. International journal of phytomedicine 2018; 4: 226-34.
26. Hussein MA, Kasser AK, Mohamed AT, Eraqy TH and Asaad A: Resveratrol Nanoemulsion: A promising protector against ethinylestradiol-induced hepatic cholestasis in female rats. J Biomol Res Ther 2020; 8: 1-7.
27. Hussein MA: Cardioprotective effects of astaxanthin against isoproterenol-induced cardiotoxicity in rats. J Nutr Food Sci 2015; 5: 1-6.
28. Fossati P and Prencipe L: Serum triacylglycerols determined calorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 1: 2077-80.
29. Burnstein M, Selvenick H and Morfin R: Rapid method for isolation of lipoprotein from human serum with polyanions. J Lipid Res 1970; 11: 583-95.
30. Allain C, Poon LS and Chan CS: Enzymatic determination of total serum cholesterol. ClinChem. 1974; 4: 470-75.
31. Chanarin I: Textbook of Laboratory Haematology: An Account of Laboratory techniques, Churchill Livingstone, New York, 1989; 107.
32. Marklund S and Marklund D: Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469.
33. Sinha AK: Colorimetric assay of catalase. J Anal Biochem 1972; 47(2): 389-94.
34. Nichans WH and Samulelson B: Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968: 6: 126-30.
35. Beyaert R and Fiers W: Tumor Necrosis Factor and Lymphotoxin. In Cytokines, A.R.M.-S. a. R. Thorpe, eds. Academic Press, San Diego 1998; 335-60.
36. Miranda KM, Espey MG and Wink DA: A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001; 5: 62-71.
37. Hupa KJ, Stein K and Schneider R: AIM2 inflammasome-derived IL-1β induces postoperative ileus in mice. Sci Rep 2019; 9: 10602-11.
38. Li S, Sui X, Guo L, Liu X, Li H, Gao L and Cai S: Root Extract of Polygonum cuspidatum Siebold & Zucc. Ameliorates DSS-Induced Ulcerative Colitis by Affecting NF-kappaB Signaling Pathway in a Mouse Model via Synergistic Effects of Polydatin, Resveratrol, and Emodin. Front Pharmacol 2018; 9: 347- 55.
39. Bancroft GD and Steven A: Theory and Practice of Histological Technique, 4th ed. Churchill Livingstone, New York 1983; 99-112.
40. Hernández R, Díaz M, López V, López F, Yáñez L, Vidrio S, Aranda A, and Chagoya V: Balance between oxidative damage and proliferative potential in an experimental rat model of CCl4-induced cirrhosis: protective role of adenosine administration. Hepatology 1997; 26: 1100-10.
41. Oliveira LZ, Hossepian VF, Levenhagen MA, Santos RM, Assumpcao TI, Jacomini JO, Andrade AF, Arruda RP and Beletti ME: Transmission electron microscopy for characterization of acrosomal damage after Percoll gradient centrifugation of cryopreserved bovine spermatozoa. J Vet Sci 2011; 12: 267-72.
42. SPSS (2012). (SPSS 15), Inc., Chicago, IL, USA.
43. Sayanti B, Susri RC, Subrata S and Sandip KB: Healing Properties of Some Indian Medicinal Plants against IndomethacinInduced Gastric Ulceration of Rats, Journal of Clinical Biochemistry and Nutrition 2007; 41(2): 106-14.

44. Gege-Adebayo GI, Igbokwe UV, Shafe MO, Akintayo CO and Mbaka DI: Anti-ulcer effect of *Ocimum gratissimum* on Indomethacin induced ulcer and percentage of superoxide dismutase on wistar rats. Journal of Medicine and Medical Science 2013; 4(1): 8-12.
45. Melvin VS and Edwards P: Coregulatory proteins in steroid hormone receptor action: the role of chromatin high mobility group proteins HMG-1 and -2. Steroids 1999; 64: 576-86.
46. Zeiko I, Mariam T and Folz R: Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2) and EC-Sod (SOD3) genes structures, evolution and expression, Free Radical Protol Medicine 2002; 33(3): 337-49.
47. Teixeira V, Valente H and Casal S: Blood antioxidant and oxidative stress biomarkers acute responses to a 1000-m kayak sprint in elite male kayakers. J Sports Med Physical Fitness 2013; 53(1): 71-79.
48. Britton RS, Bacon BR and Recknagel RO: Lipid peroxidation and associated hepatic organelle dysfunction in iron overload. Chem. Phys. Lipids 1987; 45: 207-39.
49. Brunet S, Thibault L, Delvin E, Yotov W, Bendayan M and Levy E: Dietary iron overload and induced lipid peroxidation are associated with impaired plasma lipid transport and hepatic sterol metabolism in rats. Hepatology 1999; 29: 1809-17.
50. Mehta R, Shang A and O'brien PJ: Preventing cell death induced by carbonyl stress, oxidative stress or mitochondrial toxins with vitamin B anti-AGE agents. Mol Nutr Food Res 2008; 52: 379-85.
51. Côté J, Caillet S, Doyon G, Sylvain JF and Lacroix M: Bioactive compounds in cranberries and their biological properties. Crit Rev Food Sci Nutr. 2010; 50: 666-79.
52. Howell AB, Leahy M, Kurosawa E and Guthrie N: *In vivo* evidence that cranberry proanthocyanidins inhibit adherence of p-fimbriated E. coli bacteria to uroepithelial cells. Federation of American Societies for Experimental Biology Journal 2001; 15: A284.
53. Khan IH and Edward N: Pancreatitis associated with diclofenac. Postgrad Med J 1993; 69: 486-87.
54. Sokol RJ, Straka MS, Daha R, Devereaux MW, Yerushalmi B and Gumpricht E: Role of oxidant stress in the permeability transition induced in rat hepatic mitochondria by hydrophobic bile acid. Pediatric Res 2001; 49: 519-31.
55. Eissner G, Flohlhuber F and Grell M: Critical involvement of transmembrane tumor necrosis factor in endothelial programmed cell death mediated by ionizing radiation and bacterial endotoxin. Blood 1995; 86: A4184.
56. Salvesen GS and Dixit VM: Caspases: Intracellular signaling by proteolysis. Cell 1997; 91: 443-6.
57. Appleyard CB, McCafferty DM, Tigle AW, Swain G and Wallace JL: Tumor necrosis factor mediation of NSAID-induced gastric damage: role of leuKocyte adherence. Am J Physiol 1996; 270: G42-8.
58. Fiorucci S, Santucci L and Gerli R: NSAIDs upregulate β2-integrin expression on human neutrophils through a calcium-dependent pathway. Aliment Pharmacol Ther 1997; 11: 671-80.
59. Hussein MA: Purslane Extract Effects on Obesity-Induced Diabetic Rats Fed a High-Fat Diet. Mal J Nut 2010; 3: 419-29.
60. Boshra SA and Hussein MA: Cranberry extract as a supplemented food in treatment of oxidative stress and breast cancer induced by n-methyl-n-nitrosourea in female virgin rats. Int J Phytomed 2016; 8: 217-27.
61. Ding G: Cardioprotection from oxidative stress in the newborn heart by activation of PPARγis mediated by catalase. Free Radic Biol Med 2012; 53: 208-15.
62. Baravalia Y, Vaghasiya Y and Chanda S: Hepatoprotective effect of Woodfordia fruticosa Kurz flowers on diclofenac sodium induced liver toxicity in rats. Asian Pacific JTropical Med 2011; 4: 342-46.
63. Barnett K, Bell CJ, Mc Knight W, Dicay M, Sharkey KA and Wallace JL: Role of cyclooxygenase-2 in modulating gastric acid secretion in the normal and inflamed rat stomach. Am J Physiol Gastrointest Liver Physiol 2000; 279: 1292-97.
64. Wallace JL: How do NSAIDs cause ulcer disease Baillieres best Pract Res Clin Gasteroenterol 2000; 14: 147-59.
65. Nam JH, Kim IK, Lee MR, Hong SJ and Ahn JH: Free radicals enzymatically triggered by Clonorchissinensis excretory-secretory products cause NF-KB-mediated inflammation in human cholangiocarcinoma cells. Int J Parasitol 2012; 42: 103-13.
66. Fiorucci S, Antonelli E and Morelli A: Mechanism of non-steroidal anti-inflammatory drugs-gastropathy. Dig. Dig Liver Dis 2001; 2: 35-43.


**How to cite this article:**
Hassan SA, Elwafa SMA, El-Baz HA, Hussein MA and Gobba NA: The potential protective effect of strawberry extract against indomethacin-induced liver toxicity and gastric ulceration in rats: biochemical, histopathological and genetic studies. Int J Pharm Sci & Res 2021; 12(6): 3120-33. doi: 10.13040/IJPSR.0975-8232.12(6).3120-33.