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PROTECTIVE EFFECT OF QUERCETIN ON METHOTREXATE INDUCED TOXICITY IN WISTAR RATS

Krunal Solanki [*1], Jignesh Patel [1], Dhruv Desai [1] and Manisha Chaudhari [2]

Department of Veterinary Pathology [1], College of Veterinary Science, Navsari, Kamdhenu University, Vejalpore - 396450, Gujarat, India.
School of Sciences [2], P. P. Savani University, Surat - 394125, Gujarat, India.

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Correspondence to Author:
Dr. Krunal Solanki

MVSc. Scholar,
Department of Veterinary Pathology,
College of Veterinary Science,
Navsari, Kamdhenu University,
Gujarat, India

E-mail: krunalsolanki12345@gmail.com

ABSTRACT: Methotrexate (Mtx) is used to treat a number of conditions, including certain types of malignancy. Therefore, the purpose of this study was to investigate the potential protective impact of flavonoid quercetin on toxicity caused by methotrexate using hematological, biochemical, histopathological and oxidative stress approaches. There were four different groups, each group consisting of ten rats. The group 1 was a control, and animals were treated with 0.5 ml of carboxymethyl cellulose. Group 2 and 3 were treated with methotrexate (0.250 mg/kg); quercetin (50 mg/kg) and group 4 were treated with methotrexate (0.250 mg/kg) + quercetin (50 mg/kg). All drugs were given orally via oral gavage once daily for 28 days. At end of the trial, blood samples were collected from all of the animals for hematological, biochemical and oxidative stress parameters estimation. Following the animal's sacrifice, organs were collected for histopathological examination. The levels of TP, ALB, renal indicators like BUN and creatinine and liver enzymes like AST, ALT, and ALP were markedly elevated by methotrexate at dose rate of 0.25 mg/kg. This result was in accordance with Serum levels of Superoxide dismutase, which considerably reduced and lipid peroxidation levels significantly increased in rats treated with methotrexate. Methotrexate remarkably reduced the level of Hb, TEC, PCV and TLC which was corrected by quercetin. Animals treated with methotrexate showed significant histological changes in their liver, kidneys, lungs, spleen, stomach, intestine and testes which quercetin prevented. These results suggest that quercetin has a significant protective effect against methotrexate-induced toxicity in rats.

INTRODUCTION: Methotrexate formerly known as aminopterin is a specific antagonist of folic acid which was first developed in 1940s [1-3]. Methotrexate (MTX) is used to treat psoriasis [4], psoriatic arthritis [5], rheumatoid arthritis in elderly and younger patients [6], acute lymphoblastic leukemia [7] and ectopic pregnancy [8].

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It is also used in inflammatory bowel diseases such as Crohn's disease, ulcerative colitis [9] and chronic inflammatory demyelinating Polyradicul-oneuropathy [10]. Methotrexate is a chemotherapeutic agent which is widely utilized in cancer therapies [11].

It is one of the most frequently used antimetabolic compounds for pediatric malignancies and has proven efficacy in additional cancer types such as osteogenicsarcoma and choriocarcinoma [12, 13]. Methotrexate is also used alone or in combination with other antineoplastic agents in the treatment of breast cancer, epidermoid cancer of the neck and

head as well as lung cancer [14]. Methotrexate side effects can include renal toxicity, liver toxicity, lung fibrosis, myelosuppression, gut lining impairment [15] central nervous system and gonadal toxicity. Lethargy, vomiting sensation, vomiting, cephalalgia, gastroenteritis, minor alopecia, and fever are some of the adverse toxic consequences [16].

Flavonoids are bioactive antioxidants that are ubiquitous in plant materials and do not include any nutrients. Flavonoids alter primary cell activities such as apoptosis, development and segregation due to their profound free radical scavenging function and ability to increase cyclic-GMP-dependent relaxation [17] this includes having therapeutic benefits on heart diseases, cancer, and neurological conditions.

Quercetin has been the subject of numerous scientific studies and is recognized as one of the most extensively researched bioflavonoids. Quercetin, classified as a flavonoid found in high levels in fruits, herbal medications, and vegetables such as potatoes, broccoli, apples, onion, tea, red wine and soybeans. It exhibits wide-ranging biopharmaceutical characteristics [18].

Quercetin demonstrates greater efficacy as an antioxidant than vitamin E, β-carotene, and vitamin C on a molecular basis [19]. It also has cytoprotective effects, inhibiting endothelial apoptosis induced by hepatoprotective effects, oxidants, and neutralizing reactive oxygen species [20, 18]. Chelation of transition metal ions, including iron, prevents the iron-catalyzed fenton reaction [21].

According to epidemiological research, eating foods abundant in flavonoids may minimize the probability of harm caused by hyper-cholesterolemia, heart failure, atherosclerosis, and coronary heart disease [22, 23]. It is sold as a nutrient adjunct having antioxidant, anti-inflammatory, antihistamine, antiviral, and immunomodulatory activities [24]. It also has antifungal, vasorelaxant, and antitumor effects on hippocampus neurons [25]. In ischemia reperfusion injury quercetin scavenges superoxide [26]. In ischemia-reperfusion damage, quercetin scavenges superoxide [26]. However, the effect of quercetin as a dietary supplement remains unclear by toxicity induced by methotrexate. As a result, the current study was planned to investigate the efficacy of quercetin in reducing methotrexate-induced toxicity in Wistar rats.

MATERIALS:

Drugs and Chemicals: Methotrexate and quercetin (Sigma-Aldrich) were used in experiment. Vehicle used for dose formulation was Carboxyl Methyl Cellulose (CMC).

Animals: A total of 40 Wistar male rats weighing 350-400 g were obtained from Jai Research Foundation located in Vapi, Gujarat. The Institutional Animal Ethical Committee (IAEC) approved the experiment on rats (No. 080-VCN-VPP-2019). The rats were housed in a room where environmental conditions were regulated, maintaining a temperature of 22 ± 3º C, humidity levels between 30% to 70%, and a balanced 12-hour cycle of light and darkness. The rats were given ten days to acclimatize in this environment prior the experiment commenced. The animals were placed in cages made of polypropylene with solid bottoms and accommodating five male rats per cage with autoclaved rice husk as bedding materials. The experimental rats were fed on standard rodent pellets and given *ad libitum* clean, filtered drinking water in polypropylene bottles.

Experimental Design: Ten male rats were randomly assigned to four separate groups for the experimental procedure as follows.

Group I: Normal control (Carboxy methyl cellulose, 0.5 ml).

Group II: Methotrexate 0.250 mg/kg.

Group III: Methotrexate 0.250 mg/kg + Quercetin 50 mg/kg.

Group IV: Quercetin 50 mg/kg.

The control group, served as group I, received a dosage of 0.5 ml of carboxymethyl cellulose (CMC). Rats in group II were administered methotrexate at a dose of 0.250 mg/kg of body weight. Group III rats received a combination of methotrexate (0.250 mg/kg body weight) and quercetin (50 mg/kg body weight). Rats in group IV were administered quercetin solely, with a dosage of 50 mg/kg of body weight. All animals

were orally administered for a duration of four weeks. The rats were monitored each day for any unusual physical or behavioral alterations and mortality throughout the experimental period of 28 days. Weighing rats was carried out on day one and at the end of every week. Rats were anesthetized with isoflurane to collect blood from the retro-orbital venous plexus. Blood samples were obtained using rat capillaries on the 0th, 15th, and 28th days of the experiment. These samples were collected in vials containing K2 EDTA for hematological analysis and in plain vials for serum separation. The serum was separated by allowing the blood to clot at the environmental temperature, subsequently centrifugation at 1000 rpm for 10 minutes at 4°C. This separated serum was then used for analyzing oxidative stress and biochemical parameters.

At the conclusion of the experiment, the rats were terminated by an overdose of ether inhalation. A thorough necropsy assessment was performed for each rat from different groups. Organs such as the liver, kidneys, heart, spleen, lungs, intestines, stomach, and testes were gathered and stored in 10% neutral buffered formalin for subsequent histopathological analysis.

Methods:

Body Weight Measurement: All the rats were individually weighed at the initiation of dosing (1st day) and thereafter at 15th and 28th day of the study. The body weight was expressed as mean fourteen days weight and percent body weight change. The final body weight of the rats was measured on the day of necropsy.

Hematological Parameters: Hematological parameters were analyzed by hematology analyzer (Model: Exigo, automatic hematology analyzer). An array of parameters was examined, encompassing Total Leukocyte Count (TLC/WBC count), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), and Differential Leukocyte Count.

Biochemical Parameters: Levels of ALP, ALT, AST, Albumin, Creatinine, BUN, Total Protein, and Albumin were assessed utilizing a semi-automatic biochemical analyzer (Micro lab Instrument, Model: ARX-3) in conjunction with diagnostic kits, adhering to the guidelines outlined in the kit literature (Brand: Randox, Manufacturer: Randox Laboratories Ltd).

Assessment of Oxidative Stress: For assessing the adverse effects of oxidative stress, the membrane peroxidative damage in erythrocytes i.e. lipid peroxidation and its counteracting enzyme activities i.e. superoxide dismutase was determined. The red blood cells (RBCs) and serum, separated through centrifugation, were utilized to evaluate oxidative stress-related parameters, specifically lipid peroxidation (LPO) and superoxide dismutase (SOD). These assessments were conducted using the methodology outlined by Rehman (1984) [27] for LPO and Competitive-ELISA (C-ELISA) with kits from Elabsciences, USA, for SOD.

Histopathological Examination: The heart, kidneys, liver, spleen, lungs, intestines, stomach, and testes were excised and fixed in 10% neutral buffered formalin. Subsequently, they were embedded in paraffin, sliced into thin layers measuring 5-6 micrometers, and then dyed using hematoxylin and eosin. Observation of the slides was performed under a light microscope, and any pathological findings or lesions were recorded.

Statistical Analysis: All collected data underwent statistical analysis utilizing SPSS 16.0 statistical software (SPSS, Inc., 2007). Intergroup variances were assessed using one-way analysis of variance (ANOVA) followed by Duncan's test. Statistical significance was established with a significance threshold of $P < 0.05$.Mean values were presented alongside the standard error (SE) and the total number of observations (N).

RESULTS:

Hematological Parameters: Significant decrease in the haematological values like Hb, TEC and PCV on 28th days of experiment was recorded in the methotrexate treated group of rats (Gr.2) as compared to different treatment groups of rats (Gr.1, Gr.3, Gr.4). However, the total leukocyte count (TLC) values showed a decline on the 15th and 28th days of the experiment in comparison to the control group of rats (Group 1).

However, no significant differences were observed in hematological values such as MCV, MCH, MCHC, and DLC (neutrophils, eosinophils, basophils, lymphocytes, and monocytes).

Biochemical Parameters: The serum of rats treated with methotrexate (Gr.2) exhibited notable elevations in the levels of AKP, ALT, AST, BUN, total protein, creatinine and albumin, indicating significant increases at 15th and 28th day of experiment in contrast to the control group of rats (Gr.1) while in combination of methotrexate and quercetin treated group of rats (Gr.4) the enzyme activity was not significantly increased compared to control group of rats (Gr.1). Quercetin, when given alone did not significantly affected the enzyme activity in serum of rats.

Oxidative Stress: Methotrexate treatment resulted in a marked elevation of serum lipid peroxidation (LPO) levels at the end of the experiment in rats of Group 2 compared to those in the control Group 1. While in combination of methotrexate and quercetin treated group of rats (Gr.4) lipid peroxidation (LPO) level did not significantly increased compared to rats of Gr.1.

TABLE 1: VALUES OF BIOCHEMICAL ENZYME ACTIVITIES IN DIFFERENT TREATMENT GROUPS AT DIFFERENT TIME INTERVALS

Parameters Studied	Groups N=10	0 Day	15th Day	28th Day
AST (U/L)	Gr.1	112.87^{a} ± 5.06	107.86^{a} ± 4.27	112.42^{a} ± 6.21
	Gr.2	122.29^{a} ±6.38	181.11^{c} ±7.88	188.18^{c} ± 7.37
	Gr.3	113.16^{a} ±3.80	100.9^{a} ± 3.12	119.91^{a} ± 6.71
	Gr.4	109.69^{a} ±3.38	150.91^{b} ± 1.62	140.43^{b} ± 7.03
ALT (U/L)	Gr.1	21.57^{a} ±0.59	21.32^{a} ± 0.47	21.74^{a} ± 0.60
	Gr.2	20.39^{a} ±0.87	26.47^{b} ± 1.37	28.10^{b} ± 1.35
	Gr.3	21.03^{a} ±0.53	20.97^{a} ± 0.50	22.67^{a} ± 1.06
	Gr.4	21.39^{a} ±0.91	21.51^{a} ± 0.50	22.27^{a} ± 1.90
AKP (U/L)	Gr.1	168.89^{a} ±13.24	184.29$^{a \pm}$ 10.35	192.35^{a} ±11.25
	Gr.2	192.35^{a} ±11.25	219.75^{b} ± 6.82	230.97^{b} ± 7.34
	Gr.3	171.54^{a} ±11.91	162.49^{a} ± 7.66	183.22^{a} ±5.14
	Gr.4	184.26^{a} ±10.35	206.63^{b} ± 5.04	184.48^{a} ±12.59
Creatinine (mg/dL)	Gr.1	0.64^{a} ±0.01	0.68^{a} ± 0.03	0.67^{a} ± 0.04
	Gr.2	0.61^{a} ±0.02	0.89^{b} ± 0.09	0.91^{b} ± 0.06
	Gr.3	0.65^{a} ±0.02	0.59^{a} ± 0.02	0.66^{a} ± 0.02
	Gr.4	0.60^{a} ±0.02	0.70^{a} ± 0.03	0.72^{a} ± 0.04
BUN (mg/dL)	Gr.1	11.09^{a} ±0.41	9.89^{a} ± 0.28	10.77^{a} ± 0.69
	Gr.2	10.43^{a} ±0.47	12.32^{b} ± 0.61	13.09^{b} ± 0.72
	Gr.3	9.99^{a} ±0.29	10.11^{a} ± 0.19	11.22^{a} ± 0.41
	Gr.4	10.01^{a} ±0.18	10.52^{a} ± 0.32	10.72^{a} ±0.30
TP (mg/dL)	Gr.1	6.27^{a} ±0.10	5.92^{a} ± 0.14	6.09^{a} ± 0.15
	Gr.2	6.06^{a} ±0.10	7.11^{b} ± 0.36	7.20^{b} ± 0.41
	Gr.3	5.90^{a} ±0.12	6.06^{a} ± 0.17	6.34^{a} ± 0.10
	Gr.4	6.02^{a} ±0.15	6.33^{a} ± 0.21	6.57^{ab} ± 0.29
ALB (mg/dL)	Gr.1	3.62^{a} ±0.12	3.27^{a} ± 0.05	3.54^{a} ± 0.11
	Gr.2	3.54^{a} ±0.11	3.96^{b} ± 0.13	4.02^{b} ± 0.15
	Gr.3	3.33^{a} ±0.08	3.62^{ab} ± 0.10	3.52^{a} ± 0.06
	Gr.4	3.60^{a} ±0.08	3.73^{b} ± 1.77	3.69^{ab} ± 0.15

TABLE 2: VALUES OF ANTIOXIDANT ENZYME ACTIVITIES IN DIFFERENT TREATMENT GROUPS AT DIFFERENT TIME INTERVALS

Parameter studied	Days	Gr.1 N=10	Gr.2 N=10	Gr.3 N=10	Gr.4 N=10
		Gr.1 N=10	Gr.2 N=10	Gr.3 N=10	Gr.4 N=10
LPO(ng/ml)	28th day	1.59^{a} ± 0.47	1.93^{b} ± 0.13	1.59^{a} ± 0.18	1.65^{a} ± 0.95
SOD(ng/ml)	28th day	3.64^{a} ±0.09	2.85^{b} ± 0.20	3.77^{a} ±0.22	3.42^{a} ± 0.20

Superoxide dismutase (SOD) levels was significantly reduced in the sera of methotrexate treated rats (Gr.2) at the end of an experiment compared with those in control (Gr.1) and other groups. While in combination of methotrexate and quercetin treated group of rats (Gr.4) superoxide dismutase (SOD) level did not significantly decreased compared to rats of Gr.1. Only quercetin treated group (Gr.3) did not show any significant variation in SOD level.

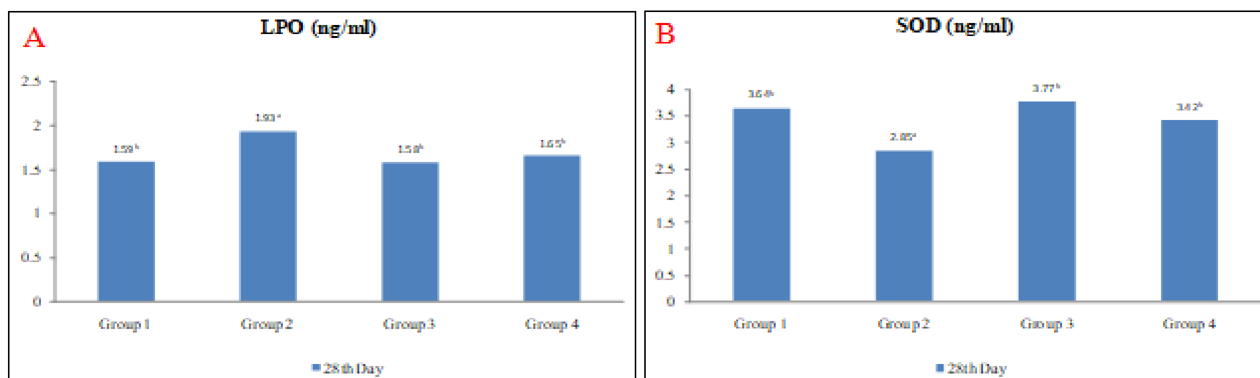

FIG. 1: THE IMPACT OF QUERCETIN ON ALTERATIONS INDUCED BY METHOTREXATE IN LPO (A) AND SOD (B) LEVELS. THE MEAN VALUES WITH STANDARD ERROR (SE) ARE PRESENTED FOR TEN RATS IN EACH GROUP. DIFFERENCES BETWEEN BARS LACKING A SHARED SUPERSCRIPT LETTER (A, B) INDICATE SIGNIFICANT DISTINCTIONS AT P<0.05 DMRT.

Pathological Changes: Lungs of methotrexate treated group of rats (Gr. 2) were hemorrhagic. No any other significant gross pathological lesions were observed in various organs (liver, kidneys, heart, spleen, stomach, intestine and testes) in all groups (Gr.1 to Gr.4) of rats.

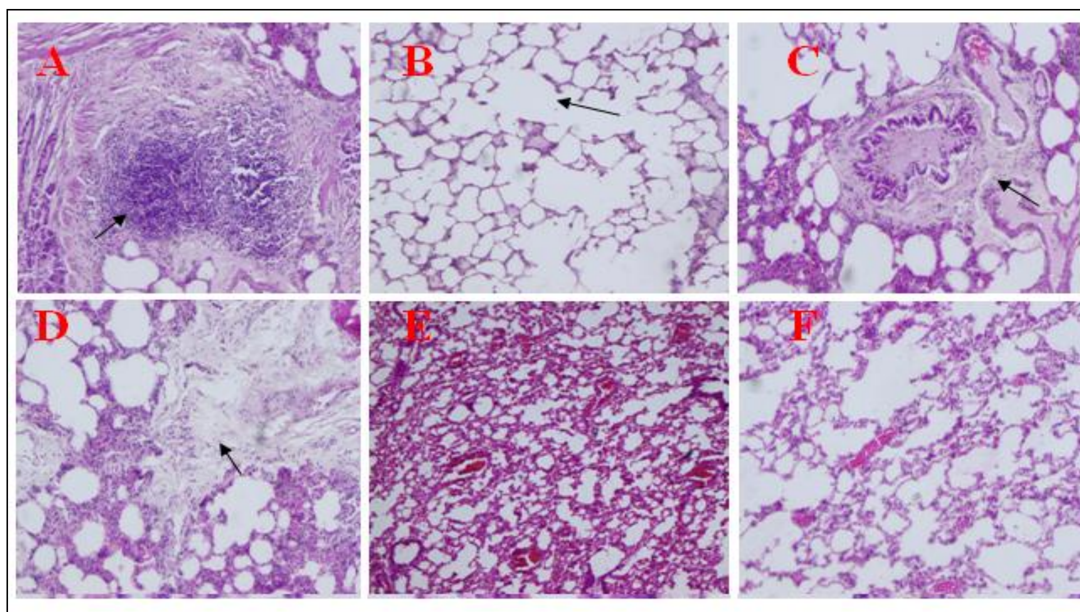

FIG. 2: REPRESENTATIVE PHOTOMICROGRAPH OF LUNGS SECTIONS STAINED WITH H&E. GROUP II SHOWING (A) MONONUCLEAR CELLS INFILTRATION (B) PULMONARY EMPHYSEMA (C) BRONCHUS FILLED WITH PROTEINACEOUS FLUID (D) PROLIFERATION OF SMOOTH MUSCLE IN LUNG (E) SEVERE HAEMORRHAGES IN LUNGS. GROUP III SHOWING (F) MILD HAEMORRHAGES

Treatment with methotrexate caused karyomegaly of hepatocytes, mono nuclear cells infiltration in liver, vacuolar degenerative changes in hepatocytes and hemorrhages, cholangiofibrosis and hypertrophy of hepatocytes **Fig. 3 A, B, C, D, E**. In kidney methotrexate caused glomerular atrophy, desquamation of tubular epithelium, tubular atrophy, fatty changes and severe tubular hemorrhages, initiation of fibrosis, tubular hyperplasia, tubular dilatation in medulla and cortex region **Fig. 4 A, B, C, D, E**. Histopathological lesions in lungs treated with methotrexate were severe hemorrhages, emphysema, peri-bronchial mononuclear cell infiltration, thickening of inter alveolar septa, mononuclear cell infiltration **Fig. 1 A, B, C, D, E**. Microscopically the spleen of the methotrexate treated rats revealed rarefaction of white pulp, degenerative lymphocyte and rarefaction of white pulp **Fig. 5 A, B**. Histopathological findings observed in stomach of Gr.2 rats were necrosis, vacuolation in gastric gland and fibrosis around gastric gland **Fig. 6 A, B**. Microscopically the intestine of the methotrexate treated rats of Gr.2

revealed marked necrosis of intestinal villi, destruction of intestinal villi and mild hemorrhages and vacuolation in the epithelial cells of intestinal villi **Fig. 7 A, B**. Microscopically the testes of the methotrexate treated rats of Gr.2 revealed atrophy of seminiferous tubule, marked necrotic and degenerative changes, presence of proteinaceous fluid and necrosis of seminiferous tubules, mild hemorrhages, sloughing of germ cell of seminiferous tubule **Fig. 8 A, B**.

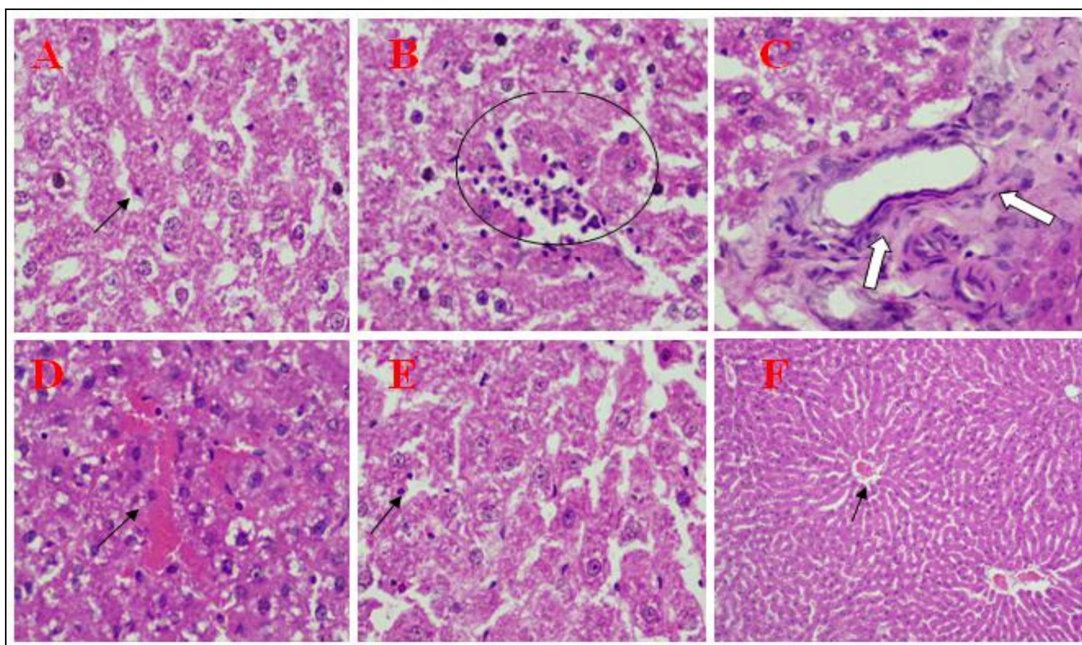

FIG. 3: REPRESENTATIVE PHOTOMICROGRAPH OF LIVER SECTIONS STAINED WITH H&E. GROUP II SHOWING (A) KARYOMEGALY OF HEPATOCYTES (B) MONONUCLEAR CELLS INFILTRATION (C) CHOLANGIOFIBROSIS (D) VACUOLAR DEGENERATIVE CHANGES IN HEPATOCYTES AND HEMORRHAGES (E) APOPTOSIS. GROUP III SHOWING (F) MILD DEGENERATIVE CHANGES AND CENTRAL VEIN CONGESTION

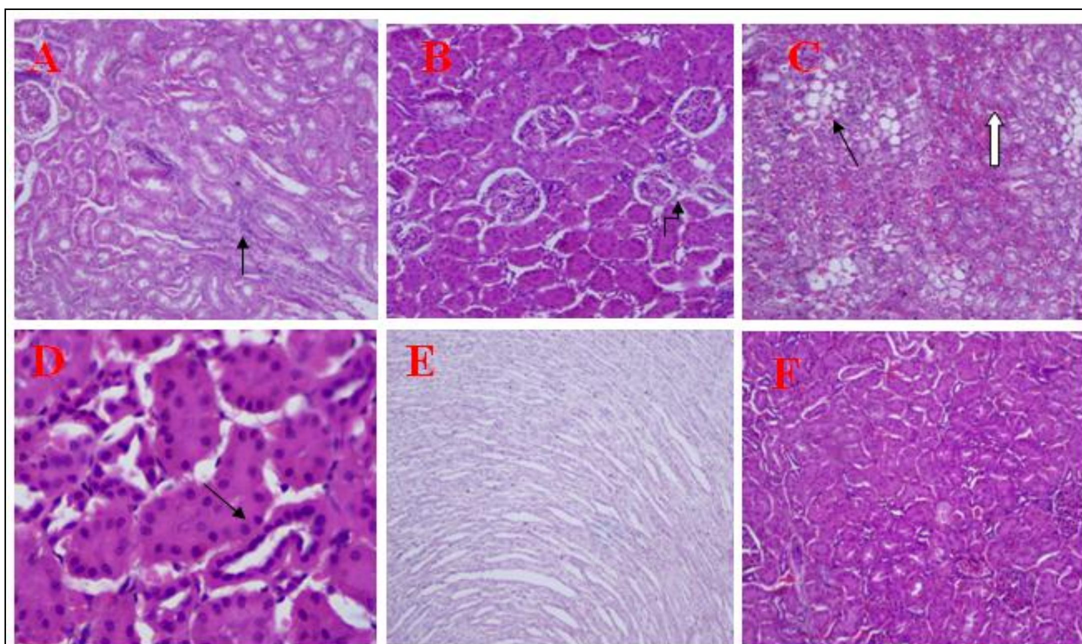

FIG. 4: REPRESENTATIVE PHOTOMICROGRAPH OF KIDNEY SECTIONS STAINED WITH H&E. GROUP II SHOWING (A) DESQUAMATION OF TUBULAR EPITHELIUM, DEGENERATION AND INFILTRATION OF MONONUCLEAR CELLS INFILTRATION (B) GLOMERULAR ATROPHY (C) FATTY CHANGES AND MODERATE TUBULAR HEMORRHAGES (D) TUBULAR ATROPHY (E) TUBULAR DILATATION IN CORTEX REGION OF KIDNEY. GROUP III SHOWING (F) MILD TUBULAR AND GLOMERULAR HEMORRHAGES

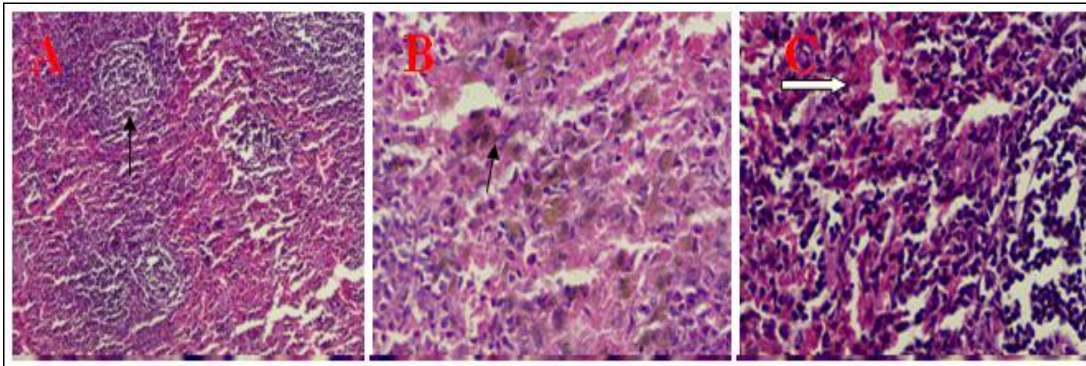

FIG. 5: REPRESENTATIVE PHOTOMICROGRAPH OF SPLEEN SECTIONS STAINED WITH H&E. GROUP II SHOWING (A) RAREFACTION OF WHITE PULP (B) HEMOSIDERIN PIGMENT DEPOSITION. GROUP III SHOWING (C) MILD HEMORRHAGES

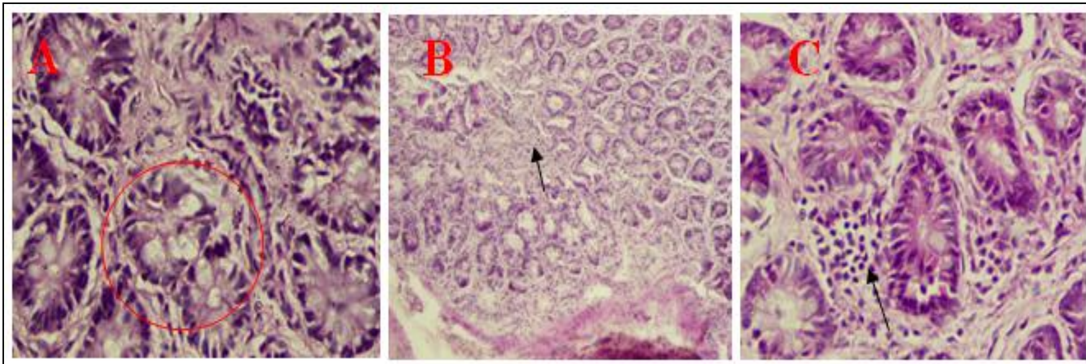

FIG. 6: REPRESENTATIVE PHOTOMICROGRAPH OF STOMACH SECTIONS STAINED WITH H&E. GROUP II SHOWING (A) VACUOLATION IN GASTRIC GLAND (B) FIBROSIS AROUND GASTRIC GLAND. GROUP III SHOWING (C) MILD INFILTRATION OF MONONUCLEAR INFLAMMATORY CELLS

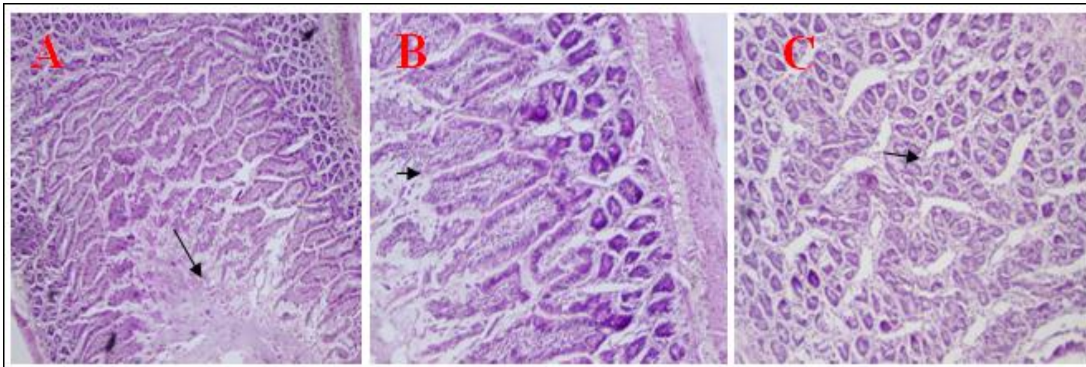

FIG. 7: REPRESENTATIVE PHOTOMICROGRAPH OF INTESTINE SECTIONS STAINED WITH H&E. GROUP II SHOWING (A) MARKED NECROSIS OF INTESTINAL VILLI (B) NECROSIS AND VACUOLATION IN EPITHELIAL CELLS OF INTESTINAL VILLI. GROUP III SHOWING (C) MILD DEGENERATIVE CHANGES

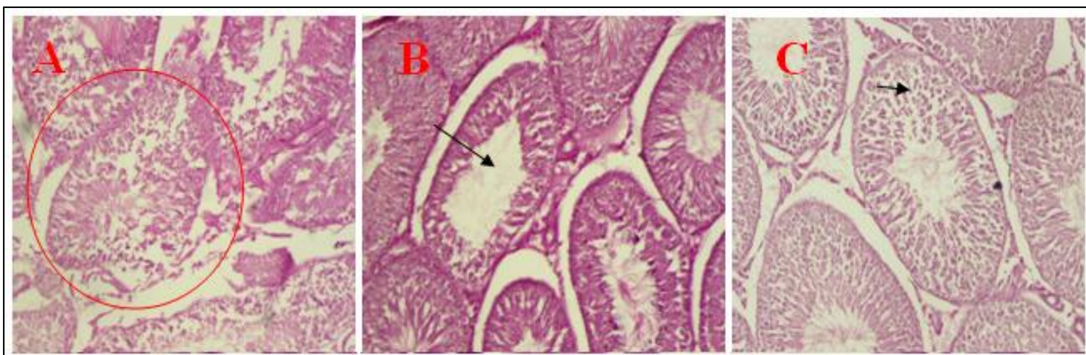

FIG. 8: REPRESENTATIVE PHOTOMICROGRAPH OF TESTES SECTIONS STAINED WITH H&E. GROUP II SHOWING (A) MARKED NECROTIC AND DEGENERATIVE CHANGES (B) SLOUGHING OF GERM CELLS OF SEMINIFEROUS TUBULE. GROUP III SHOWING (C) MILD DEGENERATIVE CHANGES

Quercetin when given independently was found to be safe, showing no detectable histopathological alterations in the liver. Conversely, rats that received a combination of methotrexate and quercetin displayed mild lung hemorrhages. **Fig. 2F**, mild degenerative changes and central vein congestion in liver **Fig. 3F**, Mild tubular and glomerular hemorrhages in kidney **Fig. 4F**, mild hemorrhages in spleen **Fig. 5C**, Mild infiltration of mononuclear inflammatory cells in stomach **Fig. 6C** and mild degenerative changes in intestine and testes **Fig. 7** & **8 C.**

DISCUSSION: In present study, the decrease in blood parameter values observed in the group of rats treated with methotrexate (Gr.2) could potentially be attributed to bone marrow suppression. In addition, free radical induced red cell damage and profuse bleeding due to intestinal injury by methotrexate could have contributed for lowered hemoglobin and erythrocyte count [28]. In contrast, Patel *et al*. (2014) [29] found a notable reduction in lymphocytes and monocytes, alongside a considerable rise in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in male rats treated with methotrexate (Gr.2).

Elevated levels of hydrogen peroxide, lipid peroxidation and free radicals are accountable for the toxicity associated with methotrexate. By damage of antioxidant mechanisms, hepatotoxicity, cellular impairment and disruption of cell membrane integrity supposed to do deterioration of plasma membrane of hepatic parenchyma leads to liberation of ALT and AST from cell to blood serum. So, it refers to deterioration of liver structure and cell death [30]. AKP enzyme is another marker of liver injury so the enzyme is raised due to bile ducts obstruction so the bile will ultimately flow out of or into the liver causes increased AKP in serum [31]. Above mentioned changes in AST, ALT and AKP caused by methotrexate were in agreement with those observed in methotrexate induced toxicity [29, 17, 29-34]. Renal damage may be developed by imparted levels of nitroxidative stress, oxidative stress parameters and down regulation of TNF- α/ kB/ COX-2 inflammatory pathways in methotrexate treated rats [17]. In this study, methotrexate-treated rats exhibited markedly elevated levels of BUN and creatinine, indicating a substantial reduction in renal filtration rate, which may be attributed to the nephrotoxic effects of methotrexate. This is confirmed by histopathological examination of the kidney tissues revealing mild fatty changes and severe tubular hemorrhages, tubular atrophy and glomerular atrophy. Similar findings of increase creatinine and BUN were also reported by [17, 29, 35]. In this experimental study, the combined administration of methotrexate and quercetin did not result in an elevation in serum creatinine levels in rats, indicating an ameliorative effect. Consistent observations were also noted in prior research studies [18, 36-38].

Quercetin caused a decline in lipid peroxidation within cell membranes, consequently reducing the liberation of AST and ALT enzymes to the bloodstream [39]. The current study revealed reduced levels of hepatic enzymes (AST and ALT) due to quercetin, a finding consistent with results reported by Afifi *et al*. (2018), Gupta *et al*. (2010); Selvakumar *et al*. (2012); De David *et al*. (2011); Uzun and Kalender (2013) and El Nekeety *et al*. (2014) [36, 38-46].

The increased presence of malondialdehyde (MDA) in the serum of rats subjected to methotrexate treatment signifies the activation of lipid peroxidation pathways and the consequent generation of excessive free radicals. MDA levels serve as a reliable marker of tissue injury and the extent of lipid peroxidation. The noticed elevation in lipid peroxidation levels among the methotrexate-treated group of rats (Gr.2) in our study concurs with previous research findings [35]. Quercetin's lipophilic properties enable it to permeate lipid layers, facilitating its interaction with various intracellular pathways. This mechanism involves the inhibition of lipoprotein receptor-1 (LOX-1) activity and the interruption of chain reactions initiated by free radicals, thereby attenuating lipid peroxidation within cell membranes. In the present study, administration of quercetin in Group 4 resulted in the eventual restoration of serum lipid peroxidation (LPO) levels to normal. Similarly, several studies have documented a notable reduction in LPO levels in rats following treatment with quercetin [41, 44, 46-48]. Most important potential side effect of methotrexate is testicular damage which induced by oxidative stress. It can damage the germ cells and

structure of testes which leads to infertility, decreased sperm count, sperm DNA damage, defective spermatogenesis, defective spermatogenesis and damage in seminiferous tubules of testes [49]. These changes were in agreement with the histopathology findings, as methotrexate caused necrosis and sloughing of germ cells which was significantly ameliorated by quercetin.

CONCLUSION: According to our research, quercetin effectively mitigated the damage caused by methotrexate to the liver, kidneys, lungs, spleen, stomach, intestines, and testes by controlling hematological and biochemical parameters (AKP, ALT, AST, Urea, creatinine, TP, albumin and CBC) which were subsequently followed by a decrease in oxidative damage. Our study indicates that quercetin shows significant potential as an ameliorative agent against the adverse effects of methotrexate and potentially other hazardous substances. However, additional research is required to clarify the exact protective mechanisms and long-term health benefits of quercetin.

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CONFLICTS OF INTEREST: The authors have no conflict of interest to declare.

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