

Protective Role of Quercetin Against Rotenone- Induced Hepato and Nephrotoxicity in Swiss Albino Mice

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Abstract

Rotenone is well known environmental neurotoxin used to induce Parkinson's disease (PD) model. Numerous studies are investigated its toxicity on the brain but few studies are available that examined its toxicity on the liver and kidney. Therefore, the main aim of the present work was to explore the toxicity of rotenone on the liver and kidney and its protection through quercetin. Administration of rotenone orally at the dose of (5mg/kg b.w daily for 60 days) caused a significant increase in the levels of liver function and renal function biomarkers as compared to controls. A significant increase in the level of lipid peroxidation, nitric oxide, and decrease in the levels of reduced glutathione, reduction in the activities of catalase and superoxide dismutase were observed in the liver and kidney as compared to control. The histopathological and SEM study in rotenone-treated mice showed alteration and signs of inflammation in the liver and kidney. While co-treatment of quercetin orally (30 mg/kg b.w for 60 days) together with rotenone, reversed the above parameters. In conclusion, rotenone significantly damages the liver and kidney, and the administration of quercetin along with rotenone shown a protective role. This study provides a new insight into where rotenone-induced liver and kidney dysfunction could be successfully protected by quercetin.

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Introduction

Most of the human health problems and environmental pollutions are caused by the use of pesticides to improve crop production and pest control in agriculture [1]. Despite their fame and prevalent use, pesticides have to indicate serious warnings towards the health risks of farmers who mix and use pesticides or work in treated areas [2]. Several pesticides such as paraquat, maneb, dieldrin, and rotenone are a possible hazard to humans due to their documented neurotoxicity in animals. [3]. The study has been also reported that depression and neurotoxicity were caused in agriculture workers exposed to pesticides [4].

Rotenone is an organic pesticide extracted from *Leguminosae* plants. Now rotenone known as an environmental toxin causes neurological disorders like PD [5]. Mechanism of action involves rotenone inhibits the mitochondrial complex I, as a result, reactive oxygen species (ROS) are produced which leads to oxidative stress, reducing ATP production [6], lowering the membrane potential of mitochondria [7], and finally causes cellular death [8]. Oxidative stress produced by rotenone has been strongly implicated in the pathophysiology of PD [9]. ROS generation is a natural physiological process in different organs. But, excessive generation of ROS can be damaging to the hepatic system [10] and nephritic system [11]. The usual function the the brain is intimately associated with the normal function of the liver and kidney. Dysfunction of the liver and kidney plays a critical role in the development of neuro disorders and cognitive impairment [12,13]. Liver function abnormality leads to deficient detoxification thus permitting neurotoxins like manganese, ammonia, and other chemicals to pass in the cerebral circulation. Chronic kidney disease (CKD) is related to a high prevalence of cerebrovascular disorders like stroke, intracerebral microbleeds, and cognitive impairment. This condition has been found not only in patients with end-stage renal disease but also in patients with slight or reasonable CKD [14]. Recently a finding suggests that liver dysfunction from mild steatohepatitis to cirrhosis and hepatic encephalopathy leads to, microglial activation and loss of Purkinje neurons in the cerebellum of patients with Steatohepatitis [15]. Cognitive alterations and dementia

were found in patients with chronic kidney disease [16]. Increased oxidative stress in the liver of mice was found when treated with rotenone [17]. Rotenone administration caused oxidative damage in renal tissue, causing overproduction of malonaldehyde and ROS, reduction in glutathione, blocking SOD, and glutathione peroxidase activity in rats.

Flavonoids as strong free radical scavengers have fascinated the remarkable interest of researchers [18, 19] as possible therapeutics, against diseases induced by free radical [20]. Quercetin (3,5,7,3',4 pentahydroxyflavone), plant-derived flavonoids found in vegetables and fruits. They have been documented to possess several pharmacological activities involving anti-inflammatory, antiallergic, and antioxidant properties [19, 21]. Quercetin can directly scavenge the superoxide anion and blocks numerous superoxide-producing enzymes like xanthine oxidase [22-24] or the neutrophil membrane NADPH oxidase complex [25]. Among the other antioxidant like Vitamin E and Vitamin C, quercetin is stronger than these [26-28]. Therefore, the main aim of the present study is to investigate the potential protective effects of quercetin toward histopathological and biochemical changes induced by rotenone in the liver and kidney. This study also helps in prevent subsequent neuro disorders due to compromised function of the liver and kidney. Our finding contributing to a novel approach in the treatment of neurological disorders caused by liver and kidney dysfunction through quercetin.

Materials and Methods

Chemicals

Hydrochloric acid (HCl), ethanol, sodium hydroxide (NaOH), Trichloroacetic acid (TCA), 5,5'-dithiobis 2-nitrobenzoic acid (DTNB), di-nitrophenyl-hydrazine (DNPH), Bovine serum albumin, Folin- ciaculate, paraffin wax, and quercetin were purchased from HiMedia Laboratories (Mumbai, India). Potassium dihydrogen orthophosphate (KH₂PO₄), sodium chloride, and ethylene diamine tetraacetate (EDTA) were purchased from LOBA, Chemie, India. Rotenone was purchased from MP Biomedicals, France.

Experimental Animals and Treatment

Adult *Swiss albino* mice, 3-4 months old weighing (25-30 g) were purchased from the College of Veterinary Science and Animal Husbandry Mhow, India, and acclimatized for 2 weeks before starting the experiment. Mice were housed in polypropylene cages under standard hygiene conditions, at temperature between 24-28°C with a 12-hour light/dark cycle, and were given standard food and water *ad libitum*.

The mice were randomly divided into three groups having 6 mice in each group. In group I, mice were treated with vehicle and served as the control, in group II, mice were treated with rotenone (5 mg/kg dissolved in sunflower oil) for 60 days, group III were co-treated with quercetin (30 mg/kg dissolved in sunflower oil) and rotenone for 60 days.

For the biochemical study, after the last day of treatment, four mice from all the experimental groups were anesthetized under ether anesthesia and blood was collected by cardiac puncture. Blood was used for serum preparation and the liver and kidney were excised rapidly and washed with a chilled saline solution. Tissue was weighed and homogenized in homogenate buffer phosphate buffer 0.1 M PH 7.4 (10% w/v) using Teflon homogenizer (Remi Stirrer, 125S/U, Bombay India). Homogenate was centrifuged (1000 rpm for 10 min at 4 °C) to remove the debris and the supernatant was taken out. The supernatant was again centrifuged (12,500 rpm for 15 min at 4 °C), and the pellet was discarded. The clear supernatant fluid was taken for analysis of Lipid peroxidation (LPO), Nitric Oxide (NO), Superoxide dismutase (SOD), Catalase, and Glutathione (GSH) assays. All procedures were done in accordance with Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and the study was approved by the Institutional Animal Ethical Committee (IAEC No. 379/GO/ReBi/S/01/CPCSEA), Dr. Harisingh Gour University, Sagar (MP), India.

Markers of Liver and kidney Functions

Blood was collected by cardiac puncture into, serum gel separator tubes (Thyocare) and centrifuged at 3000 rpm for 10 min and serum was used for the assessment of liver function markers and kidney function markers. Activities of serum alkaline

phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the level of urea and creatinine were measured by the Thyocare laboratory Sagar (M.P).

Biochemical Analysis

Determination of LPO: The level of Lipid peroxidation (LPO) measured by the amounts of MDA present in the homogenate of tissue by the use of the thiobarbituric acid (TBA) colour reaction at 95°C temperature for 40 minutes to produce a TBA-reactive substance and then resultant pink-colored pigment trimethylene complex product was measured maximum absorbance at 532 nm using spectrophotometer by the method of [29]. The results were expressed as nmol MDA/mg protein.

Determination of GSH: The content of Reduced glutathione (GSH) was estimated by the procedure of [30]. DTNB (5,5'-dithiobis (2-nitrobenzoic acid) or Ellman's reagent is reduced by the free sulfhydryl group on GSH molecule to yield generate 5-to-2-nitrobenzoic acid which has a yellow color and can be determined by reading absorbance at 412 nm and expressed as umol/mg protein.

Determination of NO: Nitric Oxide (NO) is produced from arginine by nitric oxide synthase (NOS). Nitrate is converted to nitrite by nitrate reductase. The level of nitric oxide was determined by measuring the accumulation of nitrite using Griess reagent. Nitrite ions react with Griess reagent to form a pink diazo dye by diazonium coupling reaction which is measured at 540 nm as described earlier by [31] and expressed as μMol/mg tissue.

Determination of SOD: Superoxide dismutase (SOD) activity was determined spectrophotometrically at 560 nm using the modified method of Kakkar [32]. Briefly, the assay mixture containing sodium pyrophosphate buffer (0.052M, pH 8.3), nitroblue tetrazolium (300 uM), phenazine methosulfate (186 uM), NADH (780 uM), and appropriately diluted enzyme in the total volume of 3mL was incubated at 37°C for 90 s. The reaction was stopped by the addition of glacial acetic acid. The reaction mixture was mixed vigorously by adding n-butanol and was allowed to stand for 10 min before the collection of the butanol

layer. The intensity of the chromogen in butanol was measured at 560 nm. The SOD activity was calculated in the inhibition rate %.

Determination of Catalase Activity: Catalase was measured by the method as described by [33], spectrophotometrically in a post-mitochondrial fraction using H₂O₂ as a substrate. The activity of the catalase was expressed in mmole/min/mg protein.

Protein Estimation: Protein concentration was estimated by [34] using BSA as a standard.

Histopathological Study

After the last day of treatment, two mice from each treatment group were perfused transcardially with

ice-cold 0.1 M phosphate-buffered saline (PBS) followed by cold paraformaldehyde (4%, w/v) in 0.1 M PBS. After that, the liver and kidney were isolated and processed for paraffin-embedded sectioning. Six-micron sections were cut with a microtome and mounted on clean gelatin-coated slides, and stained by hematoxylin and eosin, and observed under a light microscope.

Scanning Electron Microscopy (SEM)

SEM study of liver and kidney sample was performed on paraffin sections organized for histology. 6 um thick sections of liver and kidney were dried at 37°C for overnight and deparaffinized in xylene for 20 min. After that, sections were rinsed with trichloro-trifluoro-ethane (C₂Cl₃F₃) and the solvent was

Table 1. Effect of rotenone and co-treatment of rotenone and quercetin in the activities of serum hepatic markers in mice. *** (p < 0.001), Significantly differs from control group, ###(p < 0.001), #(p < 0.05) Significantly differs from rotenone treated group.

Parameters	Control	Rotenone	Rotenone + Quercetin
Alkaline phosphatase (U/ml)	62.32±6.16	113.17±3.32***	85.4±2.32###
Aspartate aminotransferase (U/ml)	30.43±3.14	99.83±4.61***	76.60 ±3.98#
Alanine aminotransferase (U/ml)	27.73± 2.25	84.06±2.69***	71.07± 2.30#

Table 2. Effect of rotenone and co-treatment of rotenone and quercetin in the activities of serum kidney function markers in mice. *** (p < 0.001), *(p < 0.05) Significantly differs from control group, ###(p < 0.001), #(p < 0.05) Significantly differs from rotenone treated group.

Parameters	Control	Rotenone	Rotenone + Quercetin
Urea mg/dl	18.94±2.33	31.07±2.13*	25.04±1.54#
Creatinine mg/ dl	0.16±0.02	0.43±0.02***	0.26±0.01###

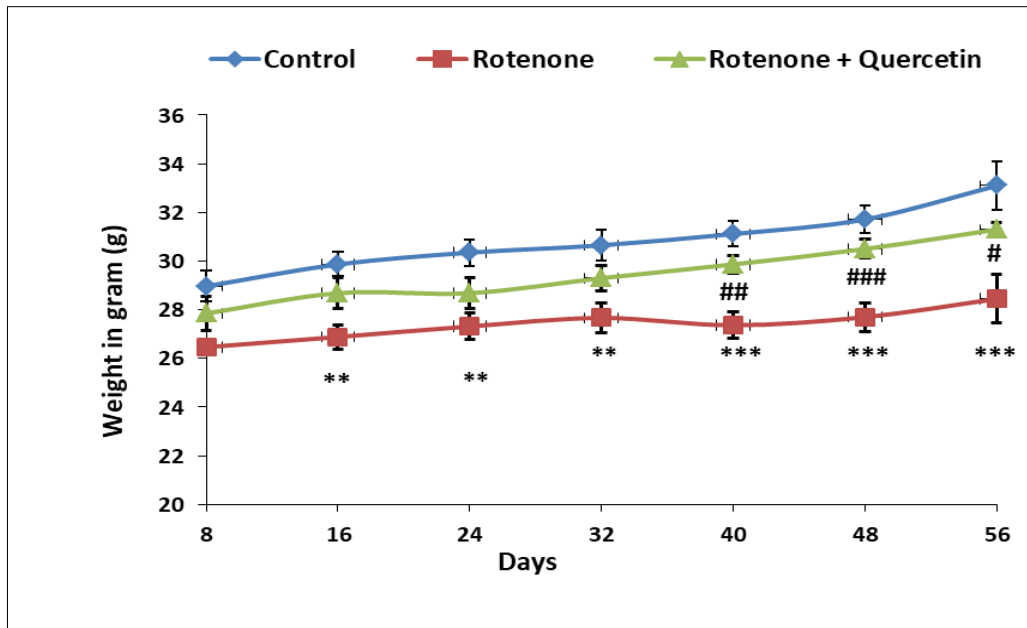


Figure 1. Effect of rotenone and co-treatment of rotenone and quercetin on body weight of mice. The results were expressed as mean±SE (n=06). ***p<0.001), ** (p<0.01) Significantly differs from control group, ###(p<0.001), ##(p < 0.01), #(p < 0.05) Significantly differs from rotenone treated group.

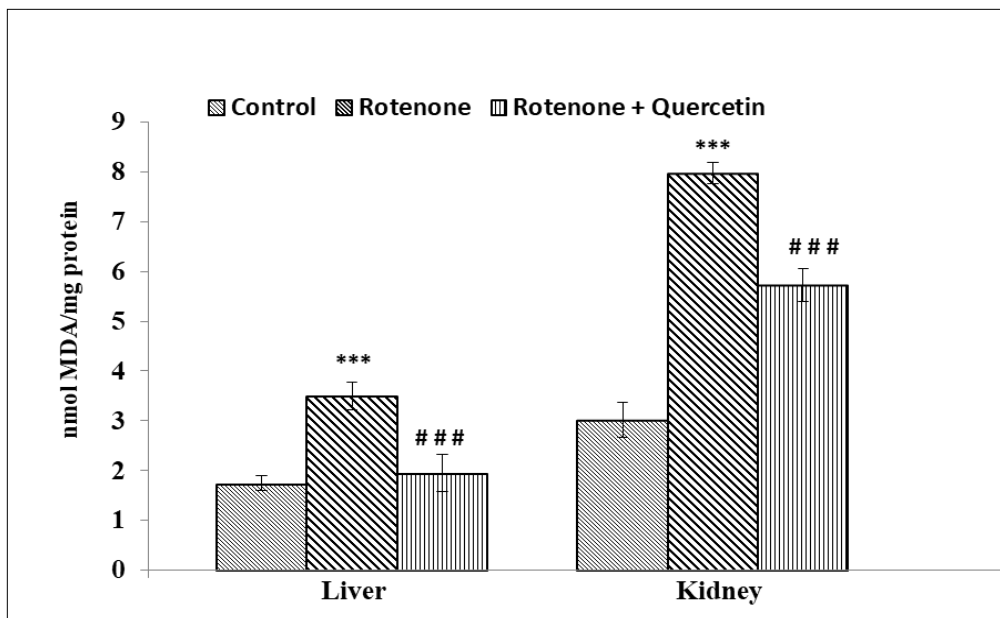


Figure 2. Effect of rotenone and co-treatment of rotenone and quercetin on lipid peroxidation in liver and kidney of mice. The results were expressed as mean±SE (n=04). ***p<0.001) Significantly differs from control group, ###(p<0.001) Significantly differs from rotenone treated group.

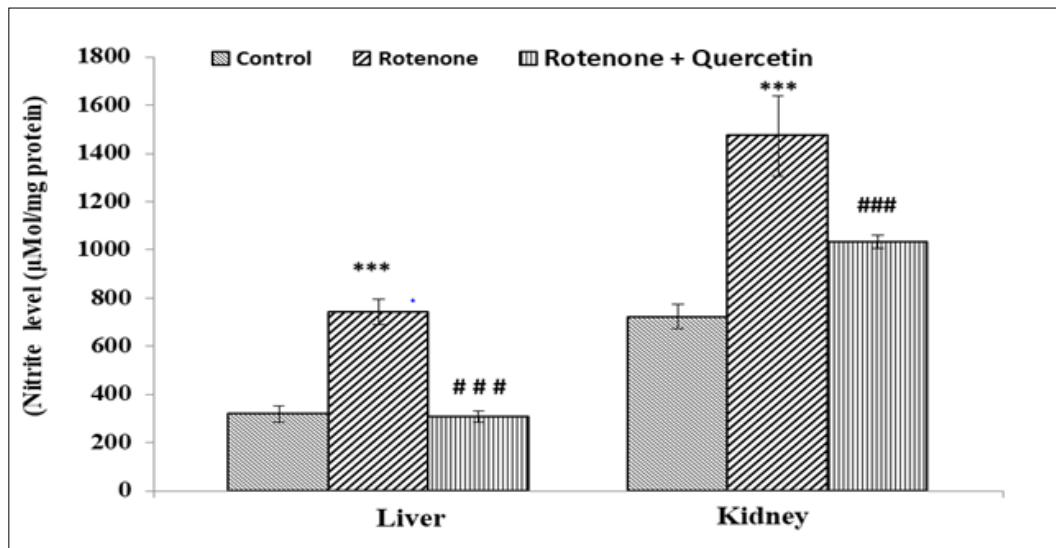


Figure 3. Effect of rotenone and co-treatment of rotenone and quercetin on nitric oxide levels in liver and kidney of mice. The results were expressed as mean±SE (n=04). ***p<0.001) Significantly differs from control group, ###(p<0.05) Significantly differs from rotenone treated group.

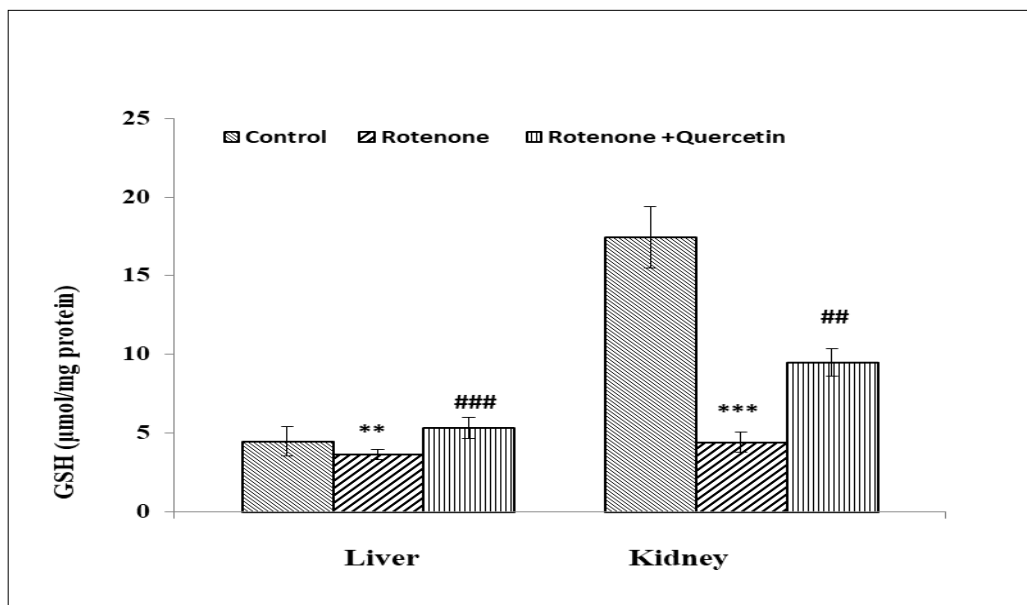


Figure 4. Effect of rotenone and co-treatment of rotenone and quercetin on reduced glutathione in liver and kidney of mice. The results were expressed as mean±SE (n=04). *(p<0.01) Significantly differs from control group, (##p<0.01, ###p<0.001) Significantly differs from rotenone treated group.

seared by evaporation. Then, the backscattered electrons (BSE) were used for noticing the various constituents in the liver and kidney from the surface of seared sections. The surface conductive coating of gold (Au) was done for further visualization. Images were taken at different magnifications i.e. low and high [35] through scanning electron microscopy, FEI Nova NanoSEM 450 made in Netherland.

Statistical Analysis: The statistical significance was determined by one-way analysis of variance (ANOVA) by the use of Sigmaplot version 12.0. Values were expressed as mean \pm standard error of the mean (SEM). The level of significance was set at $p < 0.05$.

Results

Effect on Body Weight

The results of body weight of mice from rotenone treated mice showed that a significant decreased at all different days from day 16th to 32th ($p < 0.01$) and 40th to 56th ($p < 0.001$) as compared to control Figure. 1. Co- administration of quercetin leads to the increased body weight at 40th ($p < 0.01$), 48th ($p < 0.001$) and at 56th ($p < 0.05$) as compared to rotenone alone group Figure. 1.

Effect on the Liver Function Markers

The results of the present study showed that administration of rotenone caused significant increases in levels of liver-function biomarkers, i.e., AST (3.28 folds, $P < 0.001$), ALT (3.03 folds, $p < 0.001$), and ALP (1.81 folds, $p < 0.001$), compared to the control (Table -1). While co-treatment with quercetin along with rotenone decreased the liver-function biomarkers, i.e., AST (1.30 folds, $p < 0.05$), ALT (1.18 folds, $p < 0.05$), and ALP (1.32 folds, $p < 0.01$), compared to the rotenone alone.

Effect on the kidney function markers Levels of biomarkers related to kidney functions, i.e., increased significantly urea (1.64 folds, $p < 0.05$) (Table. 1), and creatinine (3.25 folds, $p < 0.001$) (Table.2) then these of the control. Levels of biomarkers of kidney functions, i.e., decreased significantly when co-treated with quercetin urea (1.24 folds, $P < 0.05$) (Table - 2), and creatinine (1.66 folds, $p < 0.001$) (Table. 2), compared to the rotenone alone group.

Effect on LPO: The results of LPO revealed that

the LPO level was significantly increased in the liver (2.0 folds, $p < 0.001$) and kidney (1.47 folds, $p < 0.001$) in rotenone-treated mice as compared to control Figure.2. Co-treatment with quercetin along with rotenone significantly decreases the LPO level in the liver (1.84 folds, $p < 0.001$) and kidney (1.79 folds, $p < 0.001$) as compared to those treated with rotenone alone.

Effect on NO Levels: NO level was increased significantly (2.34 folds, $p < 0.001$) in the liver and in the kidney (1.79 folds, $p < 0.001$) in the rotenone treated group as compared to control Figure 3. Co-treatment with quercetin along with rotenone decreased the level of NO in the liver (2.41 folds, $p < 0.001$) and in the kidney (1.95 folds, $p < 0.001$) as compared to those treated with rotenone alone.

Effect on GSH: GSH content was significantly decreased (1.22 folds, $p < 0.01$), (3.15 folds, $p < 0.001$) respectively in the liver, kidney of rotenone treated group as compared to control Figure 4. Co-treatment with quercetin along with rotenone significantly increased the level of GSH (1.45 folds, $p < 0.001$), (1.67 folds, $p < 0.01$) respectively as compared to those treated with rotenone alone.

Effect on SOD Activity: The activity of SOD was significantly decreased (1.34 folds $p < 0.001$), (1.24 folds, $p < 0.001$) respectively in the liver, kidney of rotenone treated group as compared to control Figure5. Co-treatment with quercetin along with rotenone significantly increased the activity of SOD (1.15 folds, $p < 0.05$), (1.12 folds, $p < 0.001$) in the liver and kidney respectively as compared to those treated with rotenone alone.

Effect on Catalase Activity: The activity of catalase was significantly decreased (2.02 folds, $p < 0.001$), (2.07 folds, $p < 0.001$) respectively in the liver, kidney of rotenone treated group as compared to control Figure. 6. Co-treatment with quercetin along with rotenone significantly increased the activity of catalase (1.67 folds, $p < 0.001$), (1.60 folds, $p < 0.001$), in liver and kidney respectively as compared to those treated with rotenone alone.

Effect on Histological changes in Liver and Kidney

The histological investigation of liver sections from the control mice Figure -7 (A) showing no

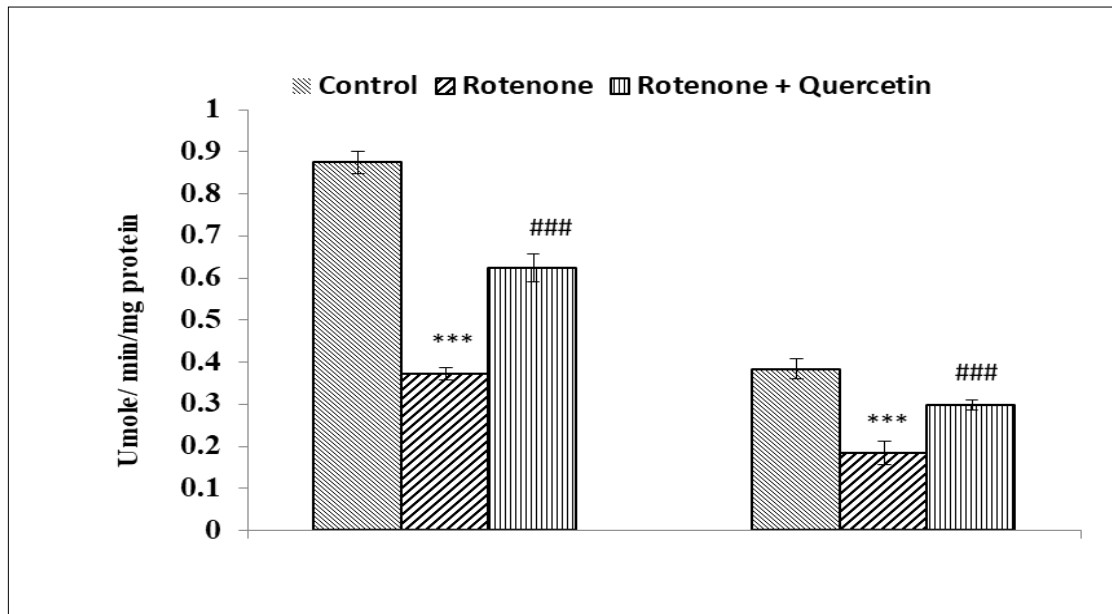


Figure 5. Effect of rotenone and co-treatment of rotenone and quercetin on catalase in liver and kidney of mice. The results were expressed as mean±SE (n=04). ***(p<0.001), Significantly differs from control group, ###(p<0.001) Significantly differs from rotenone treated group.

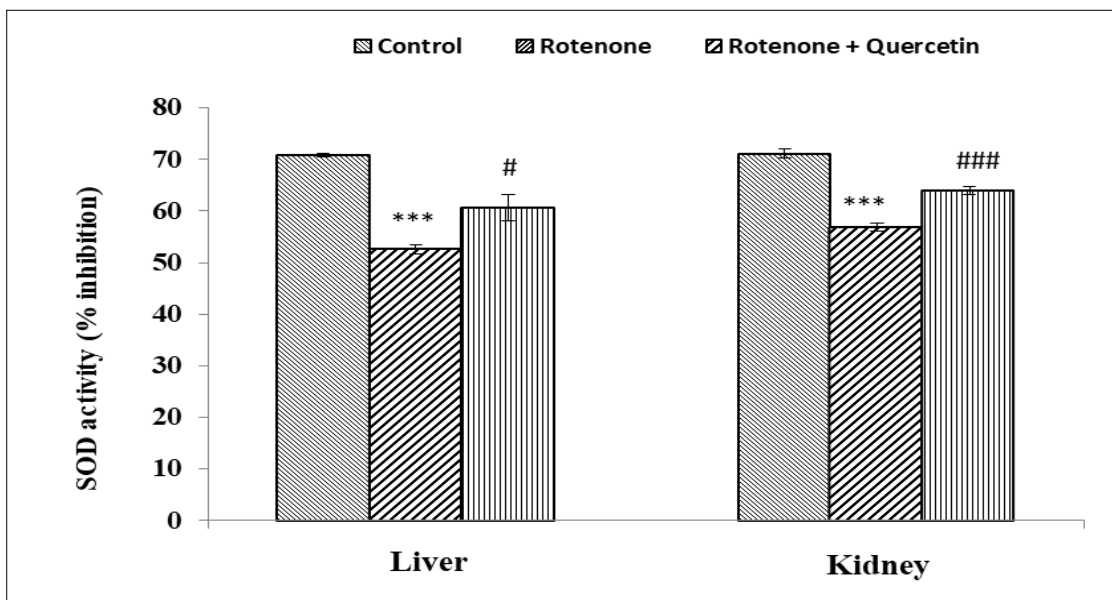


Figure 6. Effect of rotenone and co-treatment of rotenone and quercetin on superoxide dismutase in liver and kidney of mice. The results were expressed as mean±SE (n=04). *** (p < 0.001) Significantly differs from control group, # (p<0.05) Significantly differs from rotenone treated group.

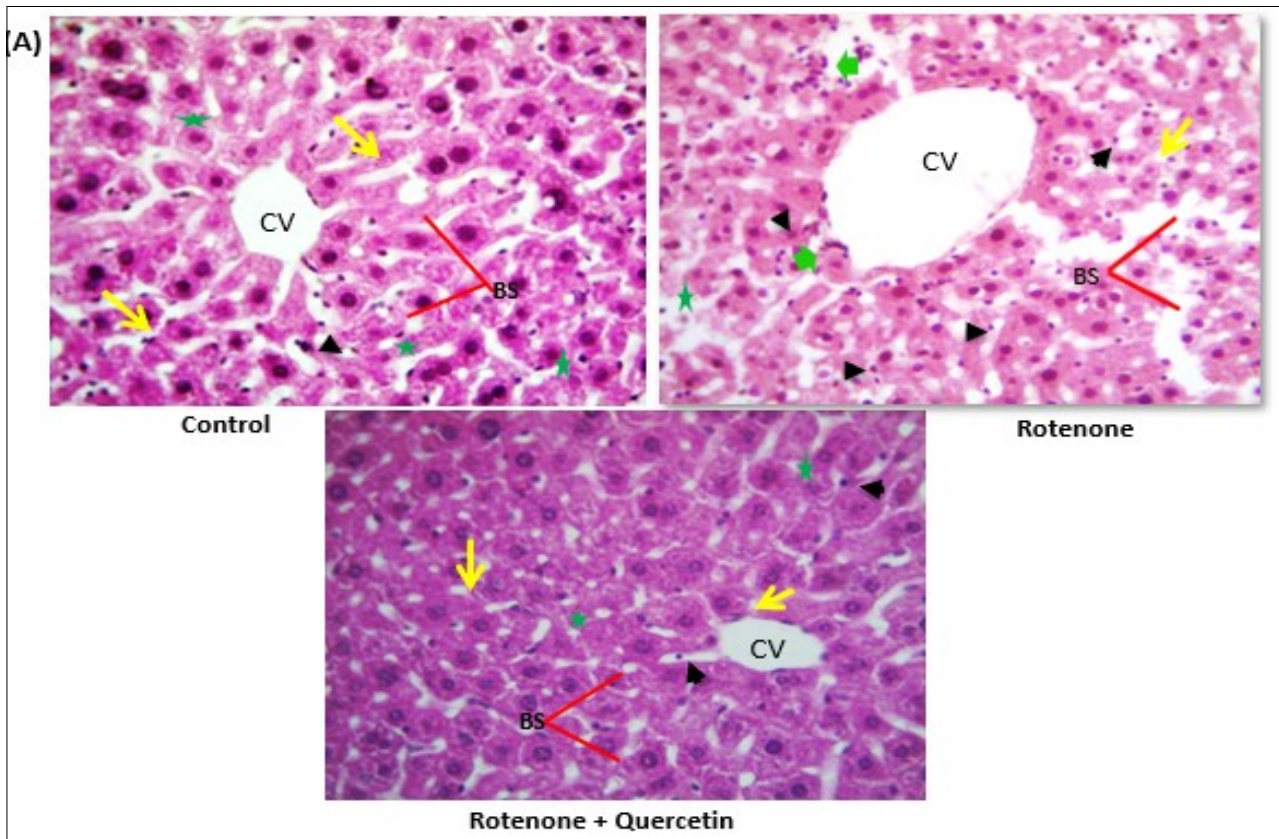


Figure 7 (A). Effect of rotenone and co-treatment of quercetin and rotenone on histology of liver of mice. Control liver showing normal appearance classic hepatic strands (yellow arrows) with hepatocytes (green asterisk) of control mice separated by blood sinusoids (Bs) (red line); Central vein (CV). Rotenone treated group, showing degranulation of hepatocytes cytoplasm, loss of hepatic strands (yellow arrows); abnormality in sinusoids (red line); shrinkage of hepatocytes (green asterisks), Leucocytes infiltration (green arrow head) and Kupper cells activation (black arrow head). Rotenone + quercetin treated group showing classical hepatic strands (yellow arrows); normal structure of blood sinusoids (Bs); central vein (Cv); normal appearance of hepatocytes (green asterisks) and least number of Kupper cells activation (black arrow head). Tissues sections (about 5 μ m) were prepared, stained with haematoxylin and eosin (H&E). Magnification, 40 \times

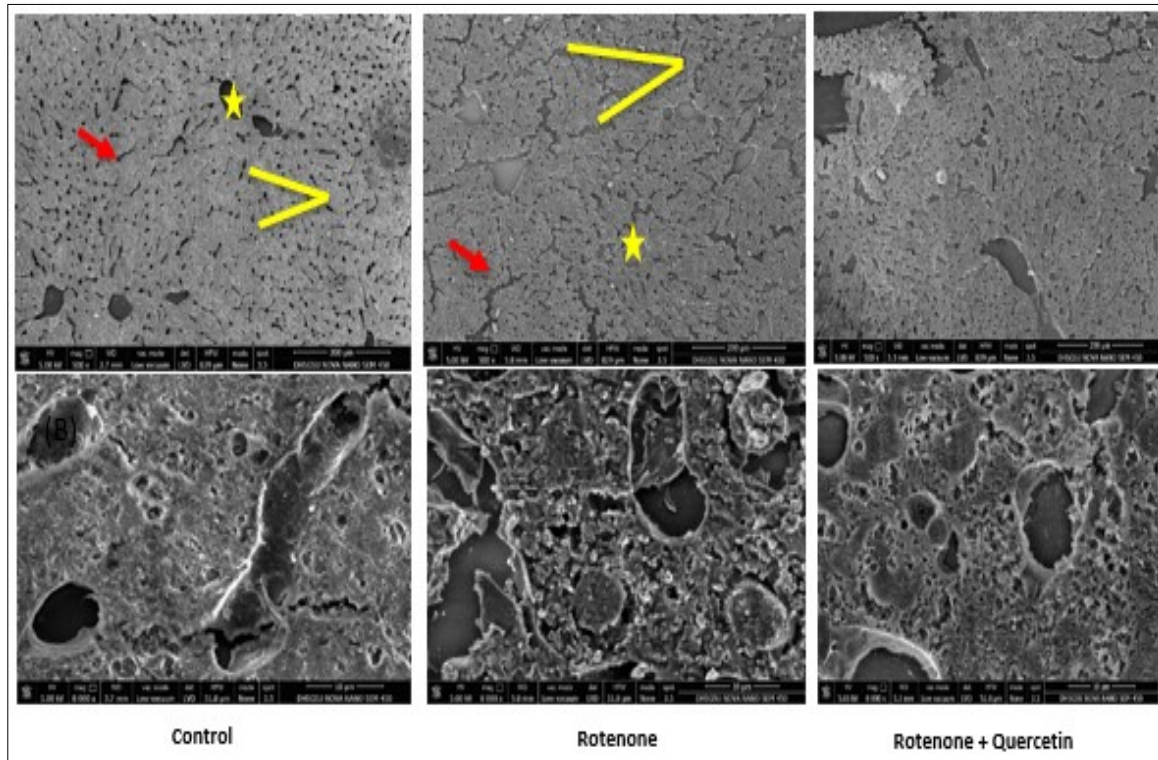


Figure 7 (B). Scanning electron micrographs of liver of rotenone and co- administration of quercetin mice . Control group showing normal appearance of hepatocytes strands (red arrow), central vein (yellow star); surrounding the normal sinusoids (yellow line), Rotenone administered group, shows loss of hepatic strands (red arrows); abnormality in sinusoids (Yellow line). Rotenone + quercetin treated group showing classical hepatic strands ,normal structure of blood sinusoids , central vein and normal appearance of hepatocytes .Scale Bar 200 µm and 10 µm.

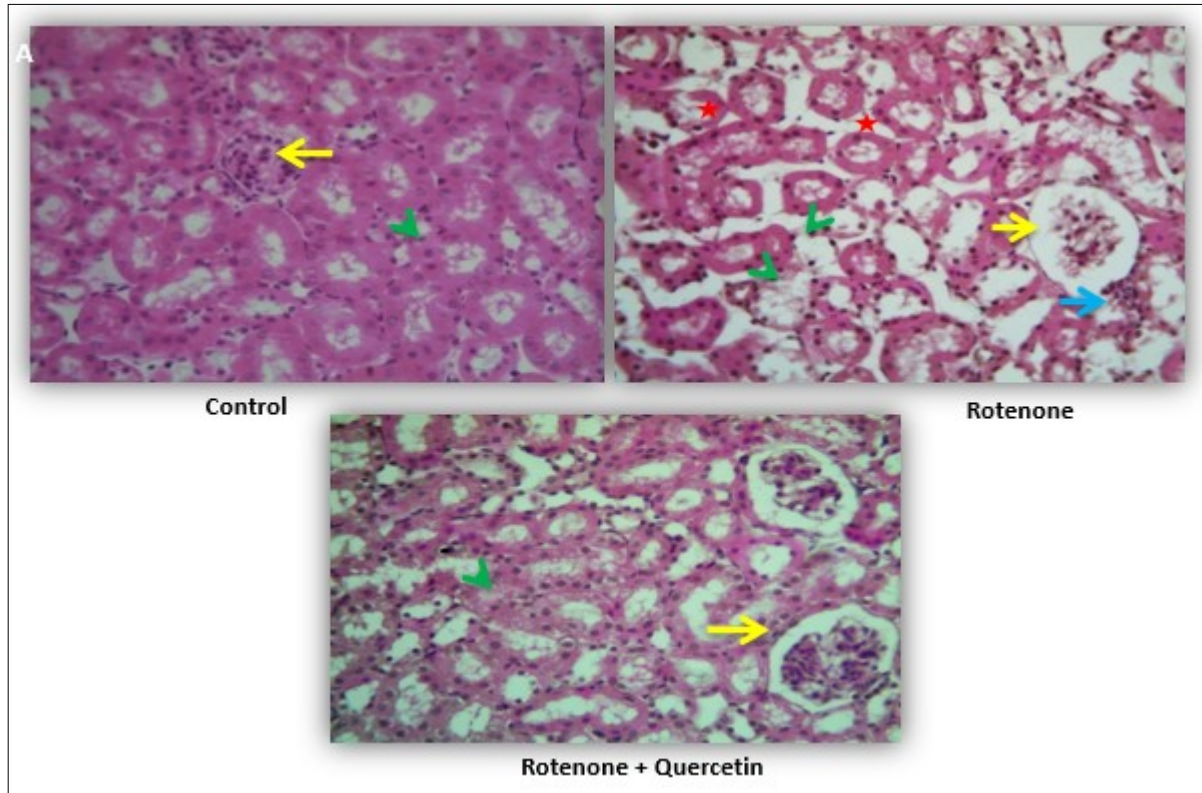


Figure 8. Effect of quercetin and co-treatment of quercetin and rotenone on histological alteration of mice kidney following exposure to rotenone.(A) Control group showing normal architecture of renal corpuscles with their glomeruli (Yellow arrows) and renal tubules (green arrow head). Photomicrograph of kidney treated with rotenone showing alteration in renal corpuscles (yellow arrows); degeneration of renal tubules (green arrow head) and necrosis and wide spacing of tubules with atrophy of their lining epithelium (red star), focal interstitial chronic inflammation infiltration of lymphocytes and mononuclear cell interstitium (blue arrow). Photomicrograph of kidney treated with rotenone + quercetin showing normal appearance of renal corpuscles and renal tubules. Kidney tissue sections were stained with hematoxylin and eosin method (40x).

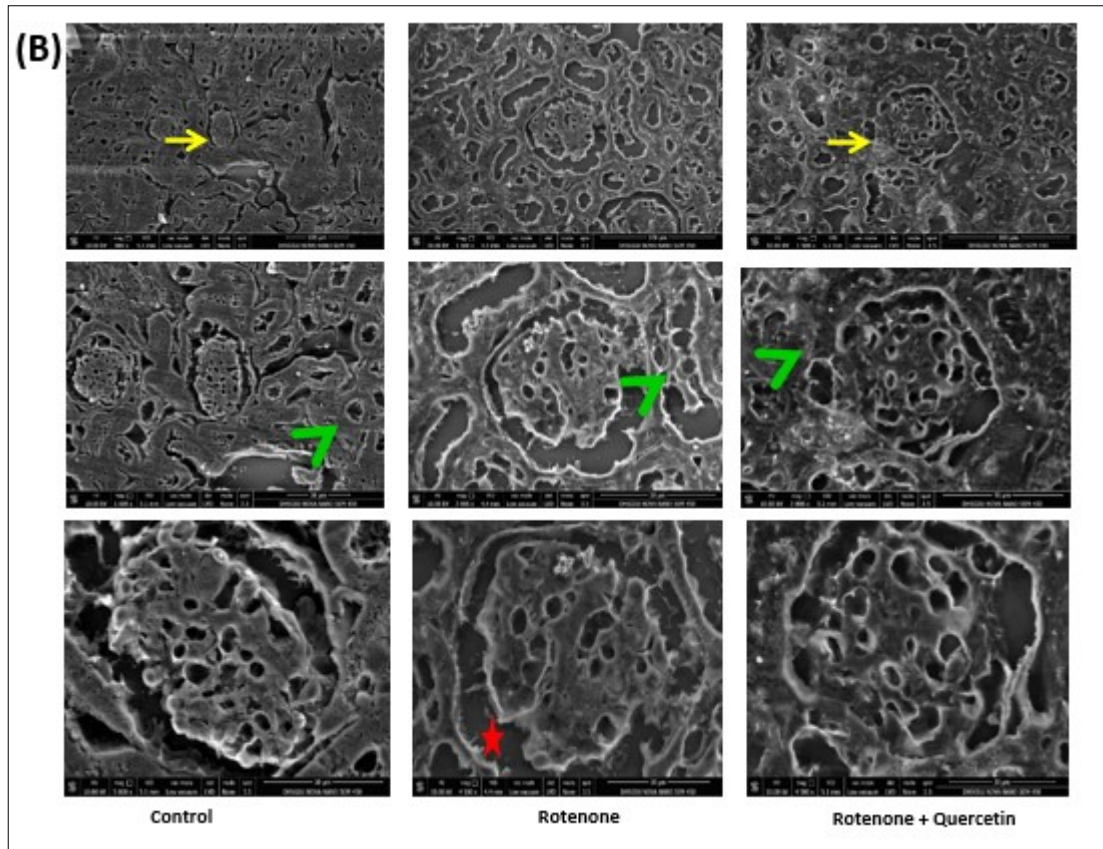


Figure 8 (B). Scanning electron micrographs of kidney of rotenone and co- administration of quercetin mice . Control group showing normal architecture of renal corpuscles with their glomeruli (Yellow arrows) and renal tubules (green arrow head). Photomicrograph of kidney treated with rotenone showing alteration in renal corpuscles (yellow arrows); degeneration of renal tubules (green arrow head), wide spacing of tubules with atrophy of their lining epithelium (red star).Photomicrograph of kidney treated with rotenone + quercetin showing normal appearance of renal corpuscles and renal tubules.

histopathological alteration indicating normal architecture of hepatocytes cells (green asterisks), intact hepatic strands (yellow arrows), Blood sinusoid (Bs, red line), and central vein (cv). Rotenone treated group, showing degranulation of hepatocytes cytoplasm, loss of hepatic strands (yellow arrows), abnormality in sinusoids (red line), shrinkage of hepatocytes (green asterisks), leucocytes infiltration (green arrow head), and Kupper cells activation (black arrowhead). When co-treated with quercetin along with rotenone showing classical hepatic strands, the normal structure of blood sinusoids (Bs), central vein (cv), and the normal appearance of hepatocytes (green asterisks) and least no of Kupper cells activation. Inflammatory cellular infiltration was found more around the central vein in the rotenone treated group. The results of present studies suggested that quercetin protects against liver injury due to rotenone-induced inflammation and histological alteration.

The histopathological investigation of the kidney of control mice showed normal architecture of renal corpuscles with their glomeruli and renal tubules. Rotenone treated mice showing alteration in renal corpuscles, diffuse hydropic degeneration of renal tubular epithelium, multifocal chronic interstitial nephritis, considered by lymphocyte infiltration, mononuclear cells in interstitium and necrosis Figure. 8 (A). While mice treated with quercetin along with rotenone showing a normal appearance of renal corpuscles and renal tubules.

Scanning Electron Microscopy of Liver and kidney

Scanning electron microscopy (SEM) has enhanced our understanding of the complex morphology of the liver and kidney. Investigation of the liver tissue at low magnification resulted in the inclusive remark of the histological construction of the functional liver unit. In SEM analysis of control mice, the hepatocytes strands are seen in the normal manner. As shown by the photomicrograph, a row of hepatocytes lies between two sinusoids, (Figure. 7 (B)). Rotenone administrated mice revealed broken hepatocytes strand and irregular shape of central vein and blood sinusoids as compared to control group. Co-administration of quercetin effectively restores the morphology of liver hepatocytes cells with the intact structure of

hepatocytes strands and blood sinusoids (Figure.7 (B)).

Scanning Electron Microscopy of Kidney

Scanning electron microscopy (SEM) has suggested about complex morphology of kidneys of mice. SEM investigation of control mice shown the typical structure of renal corpuscle and renal tubules (Figure. 8(B)). Rotenone administrated mice displayed irregularities in renal corpuscle and renal tubules with widespread spacing in epithelium cells compared with control mice. Co-administration of quercetin fruitfully avert the morphology of the kidney (Figure.8 (B)).

Discussion

The present work investigated the ameliorative role of quercetin against rotenone-induced alteration in the liver and kidney of mice at various biochemical parameters and histological levels. Treatment of rotenone for 60 days leads to the increased LPO, NO also known as the inflammatory marker, and a noticeable reduction in the content of GSH, activities of catalase, SOD, and histological changes in liver and kidney were observed. The liver and kidney showed signs of increased oxidative stress. Taken together, Co-administration of quercetin decreased oxidative stress and ameliorate rotenone toxicity.

Evidence from the earlier study suggested that exposure to pesticides altered the antioxidant defense system in the brain, heart, and kidney of rats by producing oxidative stress, leading to severe damage [36-38]. Rotenone a well-known environmental toxin induces oxidative stress by inhibiting complex I of the electron transport chain. Recently, in our lab, we reported that exposure to rotenone was confirmed to caused oxidative stress in both *in vitro* as well as *in vivo* mice models [17]. Various studies have observed that quercetin, a natural antioxidant has strong free radical scavenging activity and sthe could protect against oxidative damage [39].

Measurement of body weight is the key indicator of development and physical growth of the organisms. In PD gastrointestinal dysfunction is the main characteristics during rotenone administration with other symptoms such as weight loss, constipation and early satiety [40] Treatment of rotenone could be linked with late in gastric emptying which is the cause of

reduced body weight of animals[41]. Rotenone administrated rats have been shown reduced body weight continuously during administration of rotenone and significant decreased in body weight were found at the end of 4th week[42]. Supporting these finding in our present work , we observed a significant reduction in body weight in rotenone administrated mice. These outcome significantly reversed by co-treatment with quercetin along with rotenone. Our study consistence with other published data in which quercetin has been found to Prevents body weight loss against toxicity of superparamagnetic iron oxide nanoparticles in rats [43]

The liver is the primary target of ingested oxidants and also the main tissue involves in the protection against oxidative stress. Liver dysfunction is associated with increased levels of serum enzymes, which are the suggestive sign of cellular leakage and loss of functional integrity of cellular membrane in the liver [44]. High levels of AST and ALT are well parameters to identify liver damage [45]. Serum GGT and ALP levels are also related to the status and function of hepatic cells. In the present study elevated levels of hepatic markers like ALP, ALT, and AST in the serum proposed that an extensive liver injury was caused by rotenone. Indicating rotenone causes structural and functional damage to the cell membrane and enhanced membrane permeability leading to the leakage of hepatic enzymes into the blood. It is well established that rotenone administration significantly increased the serum hepatic marker enzyme [46]. Co-administration of quercetin decreased the above liver function biomarkers in blood serum. Our result has been supported by another study, quercetin reduced the liver function biomarkers from acute liver injury in rats induced by composite factors [47]. Administration of quercetin effectively decreased the activities of these enzymes in the present study. This can be attributed to the antioxidant property of quercetin and membrane-stabilizing property.

The kidney is the vital organ of the body it plays a major role in homeostasis and regulates the extracellular environment that helps in the excretion and detoxification of drugs and metabolites, which are harmful to the body[48]. For this reason, it is important for exogenous toxicants. By the negative impact of

chemicals or drugs, excretions get altered which leads to nephrotoxicity [49]. When the function of the kidney is altered and the homeostatic function disturbed it is unable to get rid of body excess waste[50, 51]. In a study, it has been reported that treatment of rotenone caused the increased serum urea and creatinine and MDA level, protein carbonyl content, and reduction in GSH level, and activities of, GST, SOD, MPO, and LDH have been observed [52]. Rotenone toxicity also caused an enhanced level of serum urea and creatinine, which are central metabolites associated with renal health [53]. At the time of skeletal muscle metabolism creatinine, is formed by the spontaneous and irreversible reaction. Increased creatinine level in kidneys connected with renal damage [54]. One of the major incidents in nephrotoxicity is an increase in the level of serum urea due to alteration in kidney function [55].

Research has been found that treatment of rotenone induces ROS production and structural damage in renal proximal tubule derived from OK cells [11]. Another study also reported that rotenone administration leads to oxidative stress, causing an increased level of malonaldehyde, and ROS, and blocking SOD and glutathione peroxidase (GSH-Px) activity. Along with this due to rotenone treatment reduction in the membrane potential of mitochondrial and increased voltage-dependent anion channel (VDAC), caspase-3, and caspase-9 protein levels in the kidney were found [56]. In the present study increased levels of urea and creatinine were observed in rotenone administrated mice signifying severe renal damage. The enhanced degree of these kidneys functions markers in serum linked to oxidative stress and inflammation from rotenone-induced damaged [53, 57]. The serum biochemistry results obtained from this study also showed an increased level of urea and creatinine suggesting severe renal damage occurred in mice treated with rotenone. Our result supported by another study increased levels of urea and creatine were observed in rotenone-intoxicated rats [58]. Co-administration of quercetin decreased the level of urea and creatine. Consistent with our work quercetin reduces serum creatinine and urea nitrogen levels against the toxicity of a mixture of four

organophosphate pesticides [59].

Excessive generation of ROS encourages oxidative stress following pesticide exposure, leads to lipid peroxidation [60]. In view of the present results, mice administered rotenone displayed a significant enhancement of MDA content in the liver and kidney when compared with the control group. These results are in agreement with the earlier findings that reported enhance levels of MDA in the liver and kidney of mice exposed to rotenone [10]. There was an enhanced level of the LPO, indicating an attack on membrane lipids by intracellular ROS metabolites [61]. Promotion at the LPO level may be one of the primary mechanisms due to pesticide exposure [60]. LPO has participated in numerous unfavorable effects in cells like enhanced membrane rigidity and osmotic fragility [62]. Co-administration of quercetin protects against rotenone-induced LPO. The free radical scavenging effects of quercetin may be linked with the presence of two hydroxyl groups in the β -ring of its molecule [63]. Quercetin possesses antiperoxidative properties due to the presence of polyunsaturated substitution on the β -ring together with 2,3 double bond, a free 3-hydroxyl substitution, and a 4-keto group in its structure [64]. Several studies reported that LPO was blocked by quercetin administration [65]. Hence, in our study co-administration of quercetin effectively scavenges free radicals, blocks LPO, and protects the liver and kidney tissue from the rotenone-induced oxidative damage (Figure. 2).

NO is a signaling molecule, which controls several physiological and pathophysiological processes in the immune system, nervous system, and liver [66, 67]. It was documented that rotenone increases the content of NO [68]. It was also reported that rotenone treatment increases the NO level in the liver and kidney [17, 69]. Consistent with this study in the present study we also found increased NO content in liver and kidney increased level of NO also shown the sign of inflammation in liver and kidney induced by rotenone. Co-administration of quercetin decreased the NO content in the liver and kidney (Figure - 3). In our previous study, we also observed that the level of NO enhanced in the cerebellum of mice when treated with rotenone and reduced significantly when co-treated with

quercetin [70].

GSH is a non-enzymatic member of the antioxidant defense system and serves as a free radical scavenger, but it also participated in liver and kidney protection against various toxicity as a substrate for antioxidant enzymes GSH-Px and GST [71]. Our finding has shown that reduction in GSH level in the liver and kidney of rotenone-treated mice are linked with its primary role in oxidative stress condition. Reduction in GSH may enhance the susceptibility of the liver and kidney to pesticides toxicity [72]. The administration of rotenone reduced GSH content in the liver which is in accordance with other published studies [17]. Co-administration of quercetin restores the GSH content as compared to the rotenone alone group. Quercetin enhances GSH dependent protection and prevents the depletion of thiols during oxidative stress [73]. In this study, co-administration of quercetin exerted an antioxidative effect, reversing the alteration in liver and kidney levels of LPO and GSH these effects might have been contributed to the observed different organ protection. The antioxidant role of quercetin may include the inhibition of free radical, [46] Scavenging of O_2^- , OH^- , peroxy radicals, and peroxy nitrite [74].

SOD is known to be the first line of defense against the harmful effects of free radicals and it scavenges ROS by catalyzing the dismutation of superoxide to H_2O_2 . Studies have shown that rotenone significantly decreased SOD activities in the liver [46]. The inhibition of SOD activity may be the consequence of increased flux of superoxide in cells which may be the reason for the enhanced lipid peroxidative indices in our present work. Catalase serves as a preventive antioxidant and plays a major role in the prevention of the harmful effects of LPO. In the present study, there is a significant decrease in the activities of catalase and SOD in the liver and kidney of rotenone-treated mice were found. While co-treatment of quercetin enhanced the activity of catalase and SOD in the liver and kidney. Our study has an agreement with another study where oxidative stress-induced toxicity, by doxorubicin and cyclophosphamide in rat kidney and liver protected by quercetin [75].

The toxicity of rotenone was also confirmed by histological studies. Evidence from the study suggested

that histological alteration in rat liver was observed in the rotenone exposed group [69]. In this study, histological analysis revealed that rotenone induces striking pathological alteration in the liver, and signs of inflammatory response were observed as indicating by Kupper cell activation and leucocytes infiltration as compared to the control group. Co-treatment with quercetin leads to appear normal architecture of hepatocytes cell and reduction in Kupper cell activation were observed. Quercetin was found to reversed histological alteration induced by a high-fat diet in mice [76]. Exertion of metabolite products occurs through the renal tubular epithelium, which might lead to xenobiotic-induced cell damage and hydropic degeneration of tubular epithelium and also able to induce inflammatory cytokine to excite lymphocytes and mononuclear cells infiltrated lesion. In the kidney, rotenone showed severe damage in renal corpuscles, renal tubules as compared to control while co-treatment with quercetin restore the normal appearance of renal corpuscles and renal tubules in the kidney. Protective effects of quercetin on histological changes in kidneys were observed, toxicity induced by methotrexate in rats [77]. Scanning electron microscopic study further strongly supported our histological result of liver and kidney where we found severe damage and liver and kidney and protective effects of quercetin. These results suggest that rotenone is hepatotoxic and nephrotoxic and co-administration of quercetin successfully protected liver and kidney damage caused by rotenone.

Conclusion

In conclusion, the outcome of the present study showed that rotenone caused alteration in liver and kidney function biomarkers together with this also altered the biochemical parameters and induced inflammatory response as indicated by an increased level of No and presence of inflammatory cells. Medicinal plants with strong pharmacological properties have been found to play a key role in the health care system of huge amounts of the world's populations. Comprehensive research on the chemistry and pharmacology of components of plant origin are essential and this may ultimately lead to the finding of medicine that can be used in dealing with numerous diseases. Biological products with antioxidant,

anti-inflammatory activities allow the prevention of cells and tissues against harmful effects of free radicals by averting or governing the procedure of harm. In our study co-administration of quercetin leads to a reduction in damaged caused by rotenone. The possible mechanism of quercetin as a hepatoprotective and nephroprotective may be due to its anti-oxidant and anti-inflammatory. These results also suggest that toxicity of rotenone not only limited to the brain as reported by other literature but also affect the other organ which further contributes in neurological disorders due to liver and kidney dysfunction as suggested by various published data. Quercetin significantly protected the liver and kidney dysfunction, suggesting that quercetin can protect liver and kidney damage, maybe it further inhibit neurological disorders associated with liver and kidney dysfunction. This study might be helpful to the pharmacologist in the development of the drug in the treatment of PD through targeting the liver and kidney also. Further investigations are required to find out molecular mechanisms of quercetin and their action against rotenone or other pesticides with adverse effects on non-target organisms.

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Declaration of Competing Interests

All authors declare that there is no conflict of interest.

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