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Effects of Catechin, Quercetin and Taxifolin on Redox Parameters and Metabolites Linked with Renal Health in Rotenone-toxified Rats

Crown O. O., Ogundele O. O., Akinmoladun A. C.*, Famusiwa C. D., Josiah S. S., Olaleye M. T. and Akindahunsi A. A.

Biochemical Pharmacology and Toxicology Laboratories, Department of Biochemistry, School of Sciences, The Federal University of Technology, Akure, Nigeria

Summary: Nephrotoxicity, with the attendant risk of progression to kidney failure, is a growing problem in many parts of the world. Current orthodox treatment options for nephrotoxicity and kidney failure are limited and there is need for alternative or complementary approaches. This study aimed at evaluating the effect of three structurally related flavonoids, catechin, quercetin and taxifolin on renal redox and metabolite biochemical disturbances in rotenone intoxicated animals. Male Wistar rats were administered 1.5 mg/kg rotenone (s.c.) for ten days followed by post-treatment with catechin (5, 10 or 20 mg/kg), quercetin (5, 10, or 20 mg/kg) and taxifolin (0.25, 0.5 or 1.0 mg/kg) (s.c.), for 3 days. Renal redox indices and levels of renal-related metabolites (creatinine, urea and uric acid) were assessed after sacrifice of animals. Catechin, quercetin and taxifolin significantly attenuated rotenone-induced effects on oxidative stress markers and metabolites linked to renal health. Quercetin was clearly more effective than catechin. The activity demonstrated by taxifolin, despite being administered at the lowest doses, was compelling. The results highlight the potential of these phytochemicals in the management of renal dysfunction. The findings additionally suggest a correlation between the structure of the flavonoids and their activity but also indicate that additional structural considerations beyond conventionally acknowledged ones may be involved.

Keywords*:* Flavonoids, nephrotoxicity, oxidative stress, structure-activity relationship

**Address for correspondence*: acakinmoladun@futa.edu.ng; akinmoladun_afolabi@yahoo.co.uk; +234 803 444 5893

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INTRODUCTION

The kidney performs several important functions including maintenance of homeostasis and regulation of the extracellular environment which involve detoxification and excretion of toxic metabolites and drugs (Kim and Moon, 2012). It is therefore a major target organ for exogenous toxicants. In nephrotoxicity, excretion is impaired owing to toxic effects of chemicals or drugs and this causes damage to the kidney (Kataria *et al*., 2015). The homeostatic function of the organ is impaired and serum levels of important metabolites and electrolytes are disturbed as the kidney becomes unable to rid the body of excess urine and wastes efficiently (Weber *et al*., 2017; Cao *et al*., 2018).

Rotenone is used as a broad-spectrum insecticide and pesticide. It occurs naturally in the seeds and stems of several plants (Gupta, 2012; Pamies *et al.*, 2018). Studies on the effect of rotenone toxicity in the pathogenesis of Parkinson's disease abound but information on its renotoxicity is scanty. Rotenone causes toxicity through inhibition of complex I of the respiratory chain and oxidative stress (Dorman, 2015; Neely *et al*., 2017)

The neurotoxic and nephrotoxic effects of exposure to chemicals in the environment, remains a topic of substantial current concern and interest. The National Institute for Occupational Safety and Health (NIOSH) reports that exposure to neurotoxic chemicals is one of the ten leading causes of work-related disease and injury and that over 25% of the chemicals for which the American Conference of Governmental Industrial Hygienists (ACGIH) has established threshold limit values (TLV) have demonstrated neurotoxicity and nephrotoxicity (Anetor *et al*., 2008; Arnold *et al*., 2016). To compound the problem, available therapies for the treatment and/or management of neurotoxicity and nephrotoxicity are merely symptomatic without addressing the root cause. In addition, they always cause further severe complications. There is therefore a need for viable alternatives. Medicinal plants and phytochemicals appear to be the most promising candidate over the years (Adil *et al*., 2016; Feriani *et al*., 2017).

Flavonoids are water-soluble, polyphenolic compounds found ubiquitously in plants, and are best

known for their multiple biological effects including antioxidant, anti-inflammatory, cardioprotective, anticancer, renoprotective, hepatoprotective as well as neuroprotective properties (Akinmoladun *et al.*, 2015; Kay *et al*., 2015). The biological efficacy of flavonoids has been linked to their structural properties and is related to the number of hydroxyl groups and additional groups on their flavane nucleus (Chen *et al*., 2018; Noshita *et al*., 2018). The structure-activity relationship analysis of phytochemicals can assist in optimizing their therapeutic potential and design of novel molecules with highly improved bioactivity. The aim of this study was therefore to evaluate the effect of the structurally-related flavonoids, catechin, quercetin and taxifolin (Figure 1) on renal redox and metabolite imbalances in rotenone-toxified rats with a view to delineating order of activity and any structure-activity relationships.

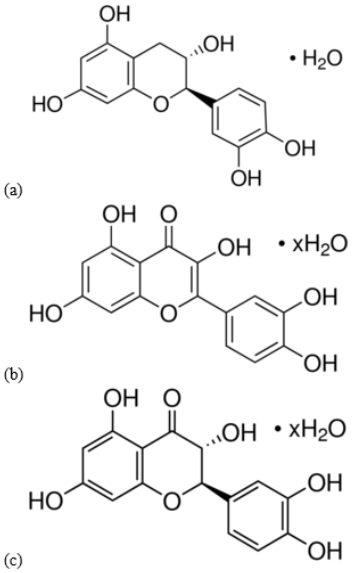

Figure 1: Chemical structures of (a) catechin, (b) quercetin and (c) taxifolin. (Akinmoladun et al., 2018).

MATERIALS AND METHODS

Chemicals and Reagents

Rotenone, (±)-catechin hydrate (trans-3,3′,4′,5,7-pentahydroxyflavane hydrate) ($C_{15}H_{14}O_6 \cdot xH_2O$), quercetin hydrate (3,3′,4′,5,6-pentahydroxyflavone hydrate) ($C_{15}H_{10}O_7 \cdot xH_2O$), (±)-taxifolin hydrate (3,3′,4′,5,7-pentahydroxyflavanone hydrate or dihydroquercetin hydrate) ($C_{15}H_{12}O_7 \cdot xH_2O$), 2,4-dinitrophenyl hydrazine (DNPH), xanthine, NAD^+, epinephrine, 2,4,5-tripyridyl-s- triazine (TPTZ), 2,4-dinitrophenyl hydrazine (DNPH), reduced nicotinamide-dinucleotide (NADH), 1-chloro-2, 4-dinitrobenzene (*CDNB*) and tetramethylbenzidine (TMB), were obtained from Sigma-Aldrich (St-Louis, MO, USA). Other chemicals and reagents used for this research were of analytical grade and obtained from standard sources.

Animal treatment and experimental groups

Male Wistar rats weighing 200±30 g housed at the Animal House of the Department of Biochemistry, The Federal University of Technology, Akure, Nigeria, were used for the study. They were fed standard rat chow and water *ad libitum*. The animals were divided into eleven groups with twelve animals per group. Animals were handled and used in accordance with the NIH Guide for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011).

Rotenone, catechin, quercetin and taxifolin were dissolved in corn oil (vehicle) and administered subcutaneously to animals. Animals in group I (negative control) were administered vehicle (corn oil) only for 13 days. Group II (ROT) animals were administered 1.5 mg/kg rotenone (Thiffault *et al*., 2000) for 10 days followed by 3 days of administration of vehicle, and served as the positive control group. Animals in groups III ($ROT+CAT_5$), IV ($ROT+CAT_{10}$) and V ($ROT+CAT_{20}$) were administered 1.5 mg/kg rotenone for 10 days followed by 5, 10 and 20 mg/kg catechin (Vazquez Prieto *et al*., 2015; Tu *et al*., 2018), respectively, for 3 days. Animals in groups VI ($ROT+QUE_5$), VII ($ROT+QUE_{10}$) and VIII ($ROT+QUE_{20}$) were administered 1.5 mg/kg rotenone for 10 days followed by 5, 10 and 20 mg/kg quercetin (Nabavi *et al*., 2012; Vazquez Prieto *et al*., 2015), respectively, for 3 days while animals in groups IX ($ROT+TAX_{0.25}$), X ($ROT+TAX_{0.5}$) and XI ($ROT+TAX_{1.0}$) were administered 1.5 mg/kg rotenone for 10 days followed by 0.25, 0.5 and 1.0 mg/kg taxifolin (Arutyunyan *et al*., 2013; Wang *et al*., 2006), respectively, for 3 days. After the last treatment, animals were euthanized, the kidneys removed and processed for biochemical estimations. Smaller doses were used for taxifolin based on previous works and this appeared justifiable because of subsequent unpublished observations during investigations in our laboratory.

Biochemical Estimations

The kidneys of the sacrificed rats were excised, washed in ice cold 1.15% (v/v) potassium chloride solution, blotted with filter paper and weighed. They were then homogenized in phosphate buffered saline PBS (pH 7.4) (1:10 w/v) using a Teflon homogenizer. The resulting homogenate was centrifuged at 10,000 x g at 4ºC for 30 min to obtain the supernatant which was used for biochemical analyses. The amount of protein in samples was estimated according to Lowry *et al*. (1951). Extent of lipid peroxidation was evaluated by measuring the formation of thiobarbituric acid reactive substances (TBARS) (Varshney and Kale, 1990). Protein carbonyl (PC) content in the kidney was determined according to the method of Levine *et al.* (1990). The method of Beutler *et al.* (1963) was followed in estimating the level of reduced glutathione

(GSH). Glutathione transferase (GST) activity was evaluated as previously described (Habig *et al.*, 1974). The ferric reducing antioxidant power (FRAP) assay was performed according to Benzie and Strain (1996). The activity of superoxide dismutase (SOD) was determined as previously described (Kakkar *et al*., 1984). Xanthine oxidase activity was measured using a previously described spectrophotometric method (Prajda and Weber, 1975). Myeloperoxidase (MPO) activity was evaluated as previously reported (Eiserich *et al*., 1998). Lactate dehydrogenase (LDH) activity was assayed as previously described (McKee *et al*., 1972). Creatinine level was estimated using an assay kit obtained from Agappe Diagnostics (Switzerland) based on Bowers and Wong (1980). Urea and uric acid levels were estimated using assay kits obtained from Randox Diagnostics (Switzerland) based on Jung *et al*. (1975) and Krieg *et al*. (1986), respectively.

Statistical Analysis

Results were analyzed using appropriate analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. In all the tests, $p<0.05$ was taken as criterion for statistical significance. The statistical software used to analyze the data was GraphPad Prism 6.01 (GraphPad Software Inc., CA, USA).

RESULTS

While it appears difficult to directly compare the effects of all three flavonoids because of the different doses of taxifolin employed for the study, results obtained still present valuable insights into their relative efficacy. A direct comparison of catechin and quercetin is straightforward and should be seen as the main focus while taxifolin could be considered a reference compound.

Redox homeostasis was altered by rotenone intoxication as reflected in changes to enzymic and non-enzymic antioxidants and other oxidative stress indicators. There was significant increase in lipid peroxidation (Figure 2I) and protein carbonyl level (Figure 2II) coupled with reduction in GSH level (Figure 2III) and ferric reducing antioxidant power (Figure 2IV), in rotenone-administered, positive

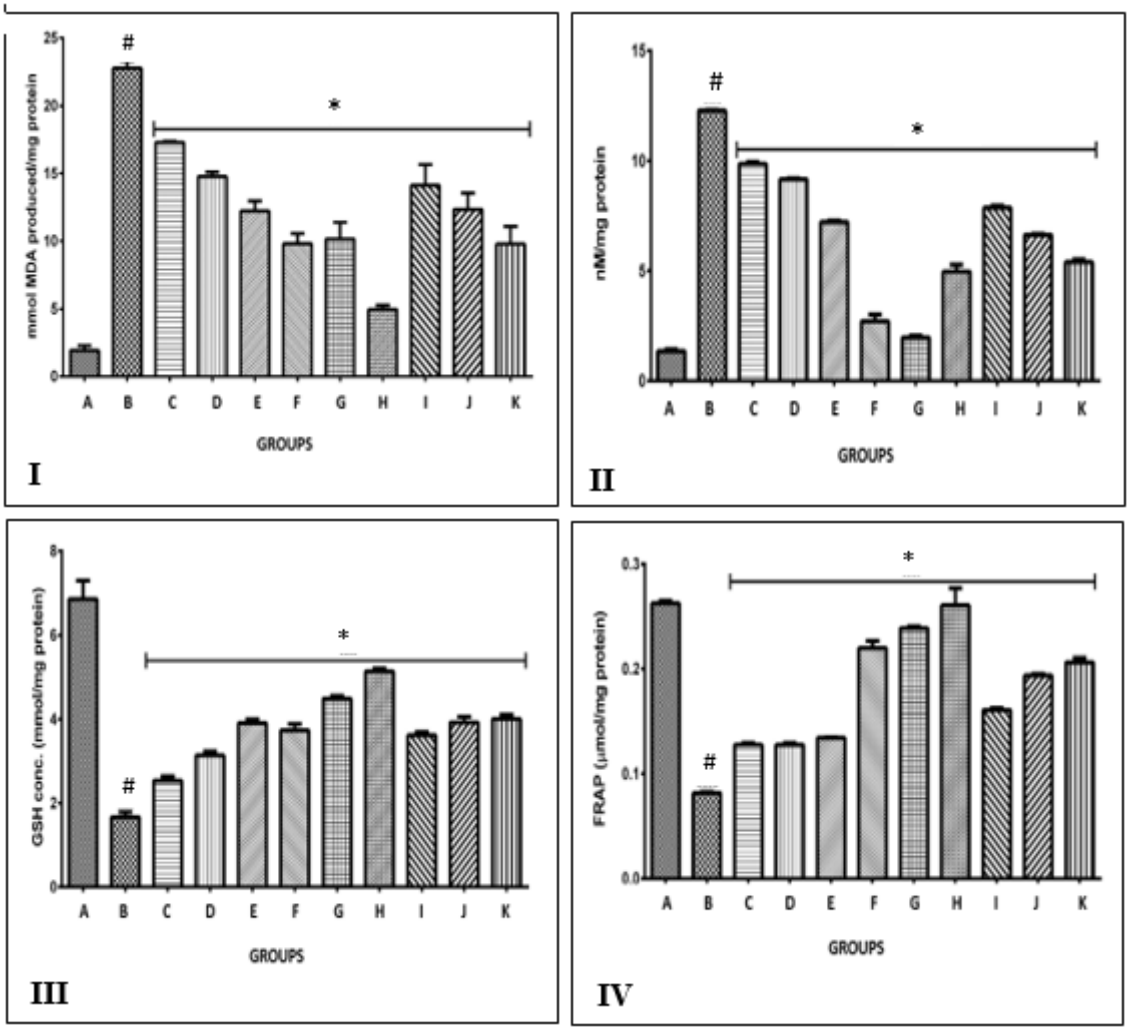

Figure 2: Effect of catechin, quercetin and taxifolin post treatment on (I) lipid peroxidation, (II) protein carbonyl content, (III) Reduced glutathione level and (IV) ferric reducing antioxidant power in kidneys of rotenone-intoxicated rats. Results are expressed as mean ± SD (n=12). $^{\#}p<0.0001$ vs control; $^{*}p<0.0001$ vs rotenone. . A= Control, B= ROT, C= ROT+CAT_5, D= ROT+CAT_{10} E= ROT+CAT_{20} F=ROT+QUE_5, G= ROT+QUE_{10}, H= ROT+$QUE_{20,}$ I= ROT+$TAX_{0.25}$, J= ROT+$TAX_{0.5}$, K= ROT+$TAX_{1.0}$. ROT: Rotenone; CAT: Catechin; QUE: Quercetin; TAX: Taxifolin

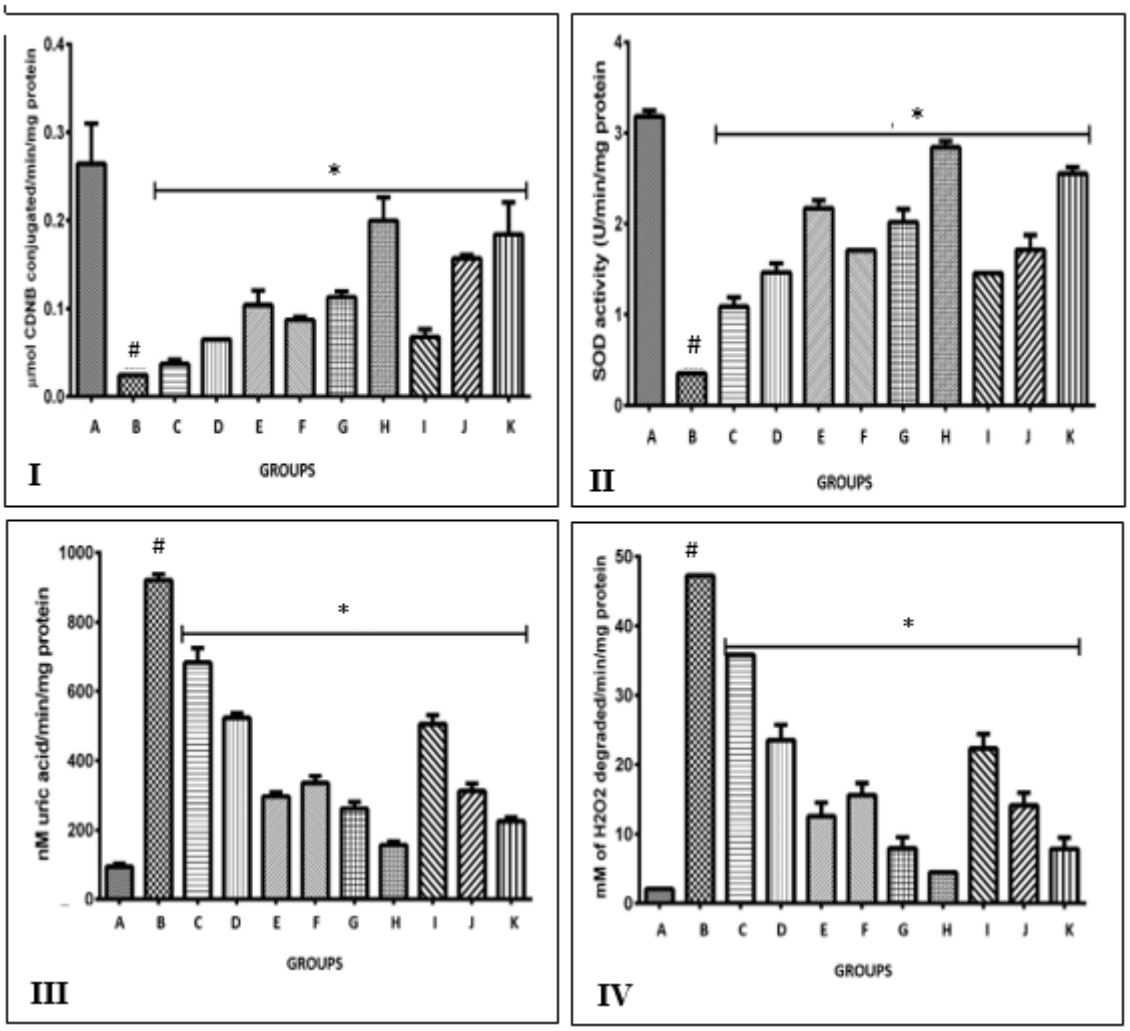

Figure 3: Effect of catechin, quercetin and taxifolin post treatment on renal glutathione transferase (I), superoxide dismutase (II), xanthine oxidase (III) and Myeloperoxidase (IV) activities in rotenone-intoxicated rats. Results are expressed as mean ± SD (n=12). #p<0.0001 vs control; *p<0.0001 vs rotenone. A= Control, B= ROT, C= ROT+CAT_5, D= ROT+CAT_{10} E= ROT+CAT_{20} F=ROT+QUE_5, G= ROT+QUE_{10}, H= ROT+QUE_{20}, I= ROT+$TAX_{0.25}$, J= ROT+$TAX_{0.5}$, K= ROT+$TAX_{1.0}$ ROT: Rotenone; CAT: Catechin; QUE: Quercetin; TAX: Taxifolin.

control group compared with the vehicle treated group (negative control). Rotenone-induced alterations to these parameters were significantly attenuated in the flavonoid treated groups in a dose dependent manner. Quercetin showed superior activity to catechin in tests evaluating extent of lipid peroxidation, protein carbonyl level, GSH level, and FRAP. At 20 mg/kg, quercetin showed the best protection in tests evaluating extent lipid peroxidation, GSH level, and FRAP. Quercetin at 10 mg/kg showed best activity in the test to determine protein carbonyl level with a tendency towards prooxidative effect at 20 mg/kg. Quercetin at 5 mg/kg showed better activity than 20 mg/kg catechin except in the test for GSH level where they appeared equipotent.Activities of the enzymic antioxidants, GST and SOD, were decreased by rotenone administration but restored by post-treatment with catechin, quercetin and taxifolin, dose-dependently (Figures 3I and 3II). In both assays, quercetin (20 mg/kg) displayed best activity and catechin (5 mg/kg), the least. The activity shown by quercetin (10 mg/kg) appear comparable with that shown by catechin (20 mg/kg). On the other hand, the activities of the prooxidant and pro-inflammatory enzymes, XO and MPO, were increased in rotenone-intoxicated control animals. This increase was corrected in animals post-treated with catechin, quercetin and taxifolin in a dose-dependent manner with quercetin demonstrating a clear superior activity to catechin (Figures 3III and 3IV). It could be observed that catechin, quercetin and taxifolin selectively regulated renal antioxidant and prooxidant factors to confer protection against rotenone induced redox imbalance. Antioxidant factors (GSH, GST and SOD) were augmented while prooxidant factors (lipid peroxides, XO and MPO) were suppressed by the flavonoids.

LDH activity (Figure 4I) was significantly increased due to rotenone administration but this was ameliorated by post-treatment with catechin, quercetin

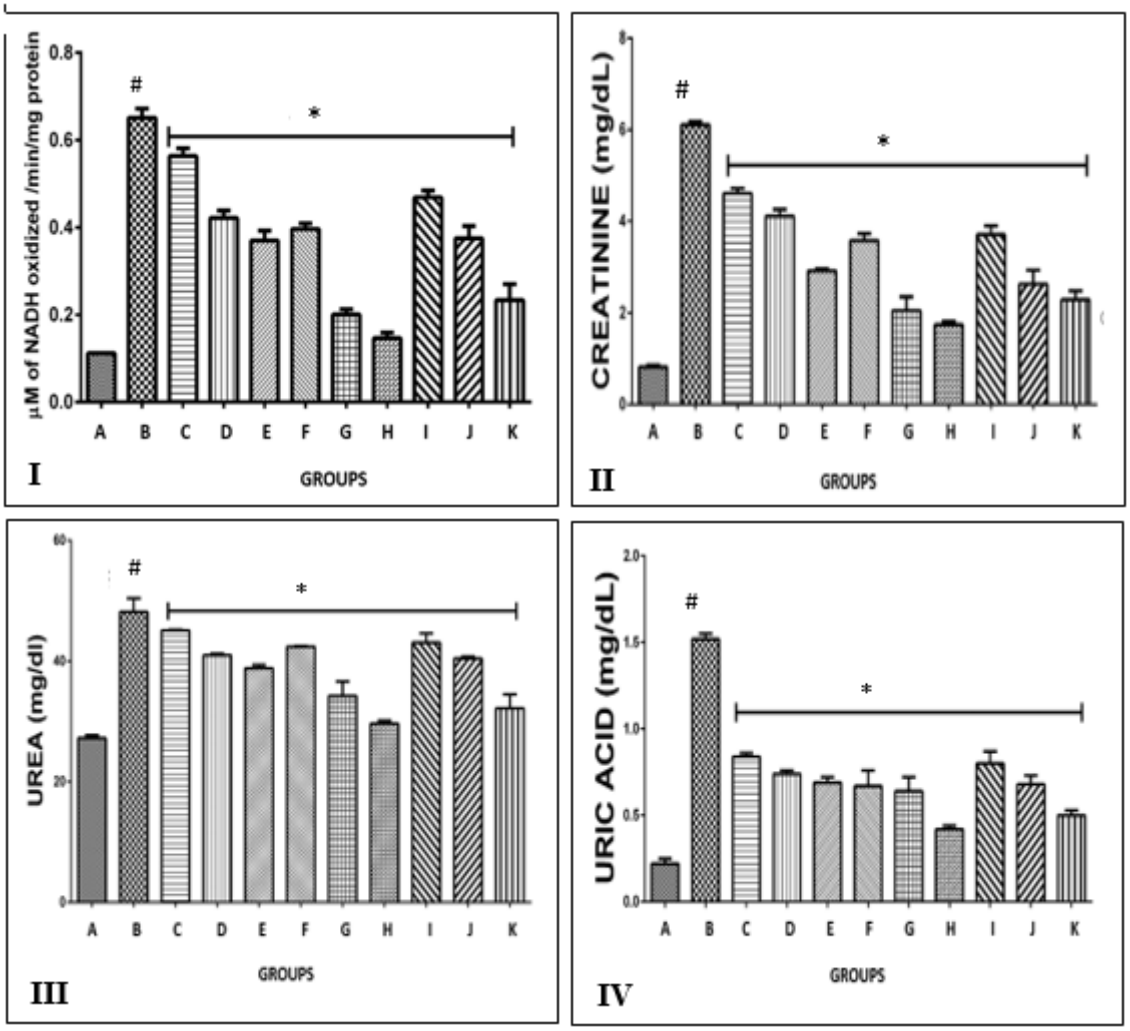

Figure 4: Effect of catechin, quercetin and taxifolin post treatment on lactate dehydrogenase activity in kidney (I), serum level of creatinine (II), serum Urea level (III) and Serum level of uric acid (IV) of rats subjected to rotenone intoxication. Results are expressed as mean ± SD (n=12). #p<0.0001 vs control; *p<0.0001 vs rotenone. A= Control, B= ROT, C= ROT+CAT_5, D= ROT+CAT_{10} E= ROT+CAT_{20} F=ROT+QUE_5, G= ROT+QUE_{10}, H= ROT+QUE_{20}, I= ROT+$TAX_{0.25}$, J= ROT+$TAX_{0.5}$, K= ROT+$TAX_{1.0}$ ROT: Rotenone; CAT: Catechin; QUE: Quercetin; TAX: Taxifolin.

and taxifolin. The performance of catechin and quercetin revealed the same trend of superior activity of quercetin with 5 mg/kg quercetin showing comparable activity to 20 mg/kg catechin. Renal functional imbalance was obvious in the positive control group administered rotenone without flavonoid post-treatment. Serum concentrations of creatinine, urea and uric acid which are principal metabolites that give insight on renal health, were significantly increased in rotenone-administered positive control group when compared with that of the negative control group. However, the rotenone-induced increase in the level of these metabolites was significantly corrected by post-treatment with catechin, quercetin and taxifolin in a dose-dependent fashion (Figures 4II, 4III and 4IV). Again, in all three tests, quercetin, especially at 20 mg/kg demonstrated the best activity compared to catechin.

Taxifolin, at the doses employed showed remarkable activity in this investigation. For example, in the lipid peroxidation test, the activity of 1 mg/kg taxifolin surpassed that of catechin even at the highest dose and was comparable to that of 5 and 10 mg/kg quercetin. In the test for protein carbonyl level, 1 mg/kg taxifolin showed superior activity to catechin at 20 mg/kg and in the FRAP test, 0.25 mg/kg taxifolin showed better activity than 20 mg/kg catechin. Also, taxifolin at 0.5 mg/kg proved superior to 10 mg/kg quercetin in the glutathione transferase assay while in the assay for myeloperoxidase activity, and determination of creatinine and urea levels, 1 mg/kg taxifolin appear equipotent with 10 mg/kg quercetin and superior in the uric acid test.

DISCUSSION

Rotenone, a potent mitochondrial toxin blocks mitochondrial complex I (NADH: ubiquinone oxidoreductase) activity causing accumulation of a large number of free radicals and ROS which leads to oxidative damage (Bonet-Ponce *et al*., 2016). Renal

tissues are vulnerable to injury by rotenone, probably because of their high metabolic state, active enzymes, and massive oxygen demand (Meng *et al*., 2016; Jiang *et al*., 2017). Flavonoids are best known for their antioxidant property and can prevent oxidant-induced injury in various ways (Taşlı *et al*., 2018).

The effects of rotenone on MDA level, protein carbonyl content, GSH level, FRAP score, and activities of GST, SOD, XO, MPO and LDH observed in this study, are consistent with the pathological features of nephrotoxicity (Feriani *et al*., 2017; Jiang *et al*., 2017; Taşlı *et al*., 2018). In rotenone renotoxicity, free radical overload resulting from mitochondrial dysfunction causes oxidative stress seen in a drastic reduction in the level of the non-enzymic antioxidant, GSH as well as decreased activities of the enzymic antioxidants, SOD and GST (Magaji *et al*., 2012). This is also accompanied by an increase in protein carbonyl content, a consequence of protein denaturation by the free radicals. Attenuating effect of catechin, quercetin and taxifolin on rotenone-induced redox disturbances indicates their potential to mitigate mitochondrial dysfunction and ensuing redox disturbances.

FRAP is an index of the antioxidant capacity of various biological samples (Wootton-Beard *et al*., 2011) while XO and MPO are prooxidant enzymes. Xanthine oxidase generates reactive oxygen species such as superoxide radical and hydrogen peroxide when it catalyzes the oxidation of hypoxanthine to xanthine. Therefore, increase in the activity of xanthine oxidase indicates further accumulation of free radicals (Romagnoli *et al*., 2010). MPO generates reactive oxygen species and produces hypochlorous acid from hydrogen peroxide and chloride anion during neutrophil respiratory burst (Degrossoli *et al*., 2018). MPO also oxidizes tyrosine to tyrosyl radical using hydrogen peroxide as an oxidizing agent (Dai *et al*., 2018; Degrossoli *et al*., 2018). Both hypochlorous acid and tyrosyl radical are cytotoxic and pathogenicidal but hypochlorous acid may also cause oxidative damage in host tissue if over-produced (Tian *et al*., 2017; Dai *et al*., 2018). The modulation of FRAP and the activities of XO and MPO by the flavonoids under consideration, further confirmed their redox-stabilizing property. In addition to being pro-oxidant enzymes, XO and MPO have been implicated in inflammatory processes (Zhao *et al*., 2017; Aldemir *et al*., 2018). Therefore, the effect of catechin, quercetin and taxifolin on the activities of XO and MPO may also reflect their anti-inflammatory potential (Magaji *et al*., 2012; Topal *et al*., 2016; Kalai-Selvi and Nagarajan, 2018).

Rotenone-induced increase in LDH activity was an indication of nephrotoxicity and renal injury (Hsiao *et al*., 2009; Piel *et al*., 2015). Impairment of the electron transport chain as a result of complex I inhibition (Birsoy et al., 2015) drastically reduces ATP synthesis. This leads to increased anaerobic respiration and accumulation of lactic acid because the physiological system switches to energy production through conversion of pyruvate to lactic acid, a reaction catalyzed by LDH (Piel *et al*., 2015). The studied flavonoids remarkably attenuated rotenone-induced alteration to LDH activity suggesting the protection of renal tissue from rotenone toxicity.

Rotenone toxicity elevated serum levels of creatinine, urea, and uric acid which are important metabolites associated with renal health (Sindhu *et al*., 2015; Amin *et al*., 2017). Creatinine, an anhydride of creatine, is formed by spontaneous and irreversible reaction during skeletal muscle metabolism. Serum creatinine is a kidney related variable that indicate renal toxicity or damage (Sindhu *et al*., 2015; Amin *et al*., 2017). Urea is formed by the liver and considered the main end product of protein catabolism in carnivorous and omnivorous species. Serum urea levels can be a reliable indication of renal function as a decrease in the rate of excretion of urea produces an increase in the concentration of serum urea, a key event in nephrotoxicity (Hassan *et al*., 2017). Uric acid is produced by the breakdown of purines and by synthesis from 5-phosphoribosyl pyrophosphate (5-PRPP) and glutamine. Uric acid is passed in the urine in humans but in other mammals, it is further oxidized to allantoin before excretion. Accumulation of uric acid as a result of poor excretion and elimination is an indication of nephrotoxicity (Sindhu *et al*., 2015; Amin *et al*., 2017). The increased serum levels of these kidney function markers may be related to oxidative stress and inflammation from rotenone toxicity (Pedraza-Chaverrí *et al*., 2003; Silan *et al*., 2007; Soliman *et al*., 2007; Sindhu *et al*., 2015) and the ameliorative action of the flavonoids indicates their free-radical scavenging, antioxidant and anti-inflammatory properties (Abdel-Raheem *et al*., 2009).

Many studies have examined rotenone toxicity on the central nervous system, especially in the pathogenesis of Parkinson's disease, but few have investigated the effects of rotenone on the kidney. The ability of rotenone to cause kidney injury was reported by Jiang *et al*. (2017) who carried out histopathological assessment along with other evaluations. Results from their study showed that rotenone caused dilation of the renal corpuscles and tubules, denuded epithelial lining and cytoplasmic blebs as well as congestion in the epithelial cells. The impairment in the renal cells could account for the significant increase in the serum concentrations of creatinine, urea and uric acid observed in our study following rotenone induction.

Quercetin apparently showed the best ameliorative activity in all tests when compared to catechin. Quercetin possesses the classical advantageous structural features adduced for strong bioactivity of flavonoids. These include C2-C3 double bond, 3-OH

group and 4-keto group on the C-ring, all of which are absent in catechin (Rice-Evans *et al*., 1996; Csepregi *et al*., 2016). Results of the present study agree with previous reports on the superior bioactivities of quercetin compared to catechin (Hayek *et al*., 1997; Jaiswal and Rizvi, 2014; Murakami *et al*., 2015). Quercetin has also been rated to be a more bioactive flavonoid than taxifolin although the main structural difference between the two is the absence of the C2-C3 double bond in the latter. Purported reasons for this have been advanced in previous reports (Makena *et al*., 2009; Weidmann, 2012; Osorio *et al*., 2013).

As earlier stated, although a direct comparison of the activity of quercetin and catechin seem implausible in the context of the present study, the overall activity demonstrated by taxifolin is compelling. This appear a bit intriguing since the C2-C3 double bond which is present in quercetin but lacking in taxifolin is a key advantageous structural feature for strong bioactivity in flavonoids (Trouillas *et al*., 2006). The remarkable activity shown by taxifolin at the low doses employed could point to the involvement of yet unclarified factors in the structure-activity relationships of flavonoids and warrants further investigation.

In conclusion, the results of this study demonstrated the positive modulation of redox environment and metabolites associated with renal health by catechin, quercetin and taxifolin in rotenone-toxified rats and pointed to the renoprotective effects of post-treatment with the flavonoids.

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