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PII: S0928-4680(18)30063-4
DOI: https://doi.org/10.1016/j.pathophys.2018.07.002
Reference: PATPHY 951

To appear in: *Pathophysiology*

Received date: 26-4-2018
Revised date: 3-7-2018
Accepted date: 7-7-2018

Please cite this article as: Akinmoladun AC, Oladejo CO, Josiah SS, Famusiwa CD, Ojo OB, Olaleye MT, Catechin, quercetin and taxifolin improve redox and biochemical imbalances in rotenone-induced hepatocellular dysfunction: Relevance for therapy in pesticide-induced liver toxicity?, *Pathophysiology* (2018), https://doi.org/10.1016/j.pathophys.2018.07.002

Catechin, quercetin and taxifolin improve redox and biochemical imbalances in rotenone-induced hepatocellular dysfunction: Relevance for therapy in pesticide-induced liver toxicity?

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Abstract

Hepatotoxicity occurs as a result of adverse effects of some xenobiotics on the liver, which is often the target tissue of toxicity for environmental chemicals. Rotenone, used as a natural pesticide, is an environmental poison reported to cause organ toxicity. This study investigated the protective effect of three flavonoids, catechin, quercetin and taxifolin (2,3-Dihydroquercetin) in rotenone-induced hepatotoxicity. Male Wistar rats were administered rotenone for 10 days followed by post treatment with catechin (5, 10 and 20 mg/kg), quercetin

(5, 10 and 20 mg/kg) or taxifolin (0.25, 0.5 and 1 mg/kg), respectively, for 3 days. Bioindices of oxidative stress and hepatocellular injury were measured in serum and tissue homogenate of animals. Rotenone intoxication produced liver damage in rats as reflected in alterations to activities/levels of enzymic and non-enzymic oxidative stress markers and enzymes linked with inflammation, as well as the transaminases, gamma glutamyl transpeptidase, bilirubin, and lactate dehydrogenase. Catechin, quercetin and taxifolin post treatment significantly attenuated these ($p<0.0001$) rotenone-induced imbalances. Comparatively, quercetin displayed the best apparent ameliorative activity. It clearly showed superior activity to catechin. However, taxifolin appeared to show comparable activity to quercetin and better activity than catechin in some of the assays despite being administered at considerably lower doses. The results provide insight on the relative efficacy and structure-activity relationships of the selected flavonoids in ameliorating liver damage and also indicate that additional structural and metabolic factors may be involved in the structure-activity relationships of flavonoids.

Keywords: Flavonoids, Hepatotoxicity, Rotenone, Structure-activity relationship

1. Introduction

The liver plays an important role in energy metabolism, and in the biotransformation of xenobiotics, which makes it vulnerable to toxicity or injury [1,2]. Liver damage caused by certain xenobiotics is a recognized toxicological problem referred to as hepatotoxicity. Several molecular mechanisms involving mitochondrial impairment, oxidative stress and apoptotic or necrotic cell death are primarily involved in drug-induced hepatocyte injury and the way in which intracellular organelles are affected defines the pattern of liver disease [3,4]. The mitochondrion is a frequent target of hepatotoxic xenobiotics with immediate effects on cell integrity and function [5,6].

There is a growing concern over the deleterious effect of environmental and agricultural toxicants on human health, for example organ toxicity resulting from exposure to pollutants. Pesticides could present danger to non-target animal species, including humans and the liver, being the primary xenobiotic metabolizing organ is more susceptible to injury [7–10]. Rotenone is an odourless, colourless, crystalline broad-spectrum insecticide, piscicide, and pesticide of botanical origin which causes toxicity through inhibition of complex I of the respiratory chain and oxidative stress [11–14]. Many studies have examined the effect of 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 liver [15–19].

There is an increasing focus on the bioactivity of flavonoids and their prophylactic and therapeutic effects in human pathologies including hepatic dysfunction. Also, the number of known individual flavonoids has continued to increase. In addition, biological activities of these flavonoids have been shown to correlate with their structural features such as degree of hydroxylation, other substitutions and conjugations [20–26]. However, there is paucity of studies on such structure-function relationships. In particular, the structure-activity relationships of the structurally-related flavonoids, catechin, quercetin and taxifolin have not been investigated. Hence, the present research, evaluated the effect of catechin, quercetin and taxifolin on rotenone-induced hepatotoxicity in rats.

2. Materials and methods

2.1 Chemicals

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) ($C_{15}H_{12}O_7 \cdot xH_2O$), reduced glutathione (GSH), glutamic acid, 5′,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), sodium pyruvate, adenosine triphosphate (ATP),

benzylamine hydrochloride (BAHC), reduced nicotinamide-dinucleotide (NADH), β-nicotinamide adenine dinucleotide phosphate reduced (NADPH), 2,4-dinitrophenyl hydrazine (DNPH), epinephrine, acetylcholine iodide, 2,4,5-tripyridyl-s-triazine (TPTZ), tetramethylbenzidine (TMB), xanthine, sodium azide and α-ketoglutarate were obtained from Sigma-Aldrich (St-Louis, MO, USA). All other chemicals and reagents used for this research were of analytical grade and obtained from standard suppliers.

2.2 Animal grouping and treatment

Adult male Wistar rats weighing 200±30 g bred and housed at the primate colony of the Animal House of the Department of Biochemistry, The Federal University of Technology Akure, Nigeria, were used. They were fed with 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, 2011.

Rotenone, catechin, quercetin and taxifolin were dissolved in corn oil and administered to animals subcutaneously (s.c). Doses of toxicant and flavonoids were based on literature and previous works in our laboratory The animals were divided into negative control group (administered vehicle only), positive control group (intoxicated with rotenone), and post-treated groups administered catechin (5, 10 and 20 mg/kg) [27,28], quercetin (5, 10 and 20 mg/kg) [28,29] or taxifolin (0.25, 0.5 and 1 mg/kg) [30,31] after rotenone intoxication. Administration of rotenone (1.5 mg/kg body weight) [32] was carried out for 10 days followed by post treatment with the varying doses of flavonoids for 3 days. Twenty four hours after the last administration, animals were euthanized, blood was collected for serum preparation and livers were excised and processed for biochemical estimations. The amount of protein in a given sample was estimated using the method described by Lowry [33].

2.3 Evaluation of markers of hepatocellular injury

Blood was collected by cardiac puncture into EDTA tubes, properly mixed and centrifuged at 3000 g for 15 min. The clear supernatant was collected and used for estimation of liver function markers. Direct bilirubin level and activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were estimated using assay kits obtained from Agappe Diagnostics (Switzerland GmbH) following the instructions of the manufacturer.

2.4 Evaluation of markers of oxidative stress, inflammation and tissue damage

Livers were excised, washed in ice cold 1.15% 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 4°C for 30 min to obtain the supernatant which was used for biochemical analyses.

Extent of lipid peroxidation was evaluated by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Varshney and Kale [34]. Protein carbonyl (PC) content in the liver was determined according to the method of Levine *et al*. [35]. The method of Beutler *et al.* [36] was followed in estimating the level of reduced glutathione (GSH). Glutathione transferase (GST) was measured by the method of Habig *et al.* [37]. Ferric Reducing Antioxidant Power (FRAP) was determined according to the method of Benzie and Strain [38]. The activity of superoxide dismutase (SOD) in the homogenates was determined by the method of Kakkar *et al.* [39]. Xanthine oxidase activity was measured using the spectrophotometric method of Pradja and Weber [40]. Myeloperoxidase (MPO) activity was evaluated according to the method of Eiserich *et al*. [41] while lactate Dehydrogenase (LDH) activity was assayed as previously described [42].

2.5 Statistical Analysis

Results were analysed using appropriate analysis of variance (ANOVA) followed by Tukey 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).

3. Results

Table 1 shows serum liver function markers of rats subjected to rotenone toxicity but post-treated with quercetin, catechin and taxifolin. There was significant increase in the level of direct bilirubin, activities of gamma glutamyl transferase, alkaline phosphate, alanine aminotransferase, and aspartate aminotransferase as well as total protein concentration in the rotenone intoxicated group compared to negative control group ($p<0.0001$). This increase was significantly ameliorated in the rotenone intoxicated rats post treated with catechin, quercetin and taxifolin.

The levels/activities of hepatic oxidative stress indices of rats subjected to rotenone toxicity and post-treated with catechin, quercetin and taxifolin is shown in Figure 1. There was significant increase in lipid peroxidation (Figure 1A), protein carbonyl content (Figure 1B) and xanthine oxidase activity (Figure 1C) in the rotenone intoxicated group compared to the negative control group ($p<0.0001$) but this was significantly ameliorated in rotenone intoxicated rats post-treated with catechin, quercetin and taxifolin ($p<0.0001$) compared to the positive control group. There was significant decrease in glutathione level (Figure 1D), glutathione transferase activity (Figure 1E), ferric reducing antioxidant power (Figure 1F), superoxide dismutase activity (Figure 1G) and myeloperoxidase activity (Figure 1H) in the rotenone intoxicated group compared to the positive control group ($p<0.0001$) which was significantly corrected in rats post-treated with catechin, quercetin and taxifolin ($p<0.0001$). However, the glutathione level and ferric reducing antioxidant power in the rotenone intoxicated rats post treated with 5 mg/kg catechin was not significantly different when compared to the rotenone intoxicated group.

Figure 2 shows the extent of tissue damage in liver of rats subjected to rotenone toxicity. There was significant increase in lactate dehydrogenase activity (Figure 2) in the rotenone induced group compared to the control group ($p<0.0001$) but this was attenuated by post-treatment with catechin, quercetin and taxifolin.

4. Discussion

The hepatotoxic effect of rotenone and its amelioration by post-treatment with catechin, quercetin and taxifolin, were demonstrated in the present study. Rotenone intoxication caused assault on hepatic antioxidant defense system leading to oxidative stress. Rotenone has been established as a mitochondrial toxin which interferes with the electron transport chain and promotes redox imbalance, inflammation and apoptosis [43–47]. Rotenone toxicity promotes production of excessive reactive oxygen species (ROS) and oxidative stress ensues due to failure of hepatic antioxidant mechanisms to safely neutralize them. In the current study, evidence of hepatic oxidative damage by ROS in rotenone-intoxicated liver is observed in increased level of malondialdehyde (MDA), a specific marker of lipid peroxidation largely produced as a result of peroxidation of polyunsaturated fatty acids [48]. Related to the peroxidation of membrane lipids is the increased activity of LDH which was observed after rotenone intoxication. Elevated levels of LDH are detected in the blood following release from damaged cells in many areas of the body, including the liver and this is suggestive of hepatocellular necrosis [49].

The decreased activity of glutathione transferase in rotenone intoxicated animals also points to hepatocellular injury. Induction of the synthesis of this enzyme is a protective mechanism that occurs in response to xenobiotic exposure [49]. Also, elevated protein carbonyls in positive control animals is a further reflection of hepatic oxidative damage by rotenone since protein carbonyls represent an irreversible form of protein modification due to

oxidative processes. A positive association between protein carbonylation and apoptosis has been demonstrated [50,51].

MPO is another oxidant parameter elevated in oxidative damage situations. It is found in neutrophils and macrophages and catalyses the production of toxic hypochlorous acid involved in hydroxyl radical formation [52]. Like MPO, xanthine oxidase is prooxidative and generates reactive oxygen species such as superoxide radicals and hydrogen peroxide when it catalyzes the oxidation of hypoxanthine to xanthine and subsequently to uric acid [53,54]. MPO and XO have also been implicated in several pathologies in connection with inflammation [55–58]. Therefore, increased MPO and XO activities in this study indicate possible involvement of inflammation in addition to oxidative stress in rotenone hepatotoxicity.

Compromised antioxidant defense which gives rise to oxidative stress and cause damage of biological macromolecules and disruption of normal metabolism and physiology [59] is further reflected in decreased activity of SOD, decreased GSH level and reduced FRAP score observed in rotenone intoxicated animals. These confirm the severity of oxidative stress imposed by rotenone administration on the liver and is in consonance with reports of previous workers [43,44].

Marked increase in serum bilirubin level and activities of alanine amino transferase, aspartate aminotransferase, alkaline phosphatase, and gamma glutamyl transferase which is a consistent mark of liver injury, further indicated the deleterious effect of rotenone on the livers of rats. Alkaline phosphatase (AP), and gamma glutamyltranspeptidase (GGT) when elevated in serum appear to reflect cholestatic injury [60–62] while increased level of total bilirubin is suggestive of jaundice and can be due to metabolic problems in the liver associated with reduced hepatocyte uptake, impaired bilirubin conjugation, or reduced bilirubin secretion [61,63]. Specifically, aminotransferases are markers of hepatocellular injury [63]. ALT is a widely used clinical biomarker of liver injury [64,65]. AST, on the other hand, is a

mitochondrial and cytoplasmic enzyme which is a less specific marker of liver injury because it is predominantly found in other tissues and organs in addition to the liver making it an indicator of a diseased state or injury to multiple tissues [65]. AST is used in combination with other enzymes, for example, ALT to monitor the cause of various liver disorders. The ratio of ALT to AST can be used to differentiate liver damage from other organ damage [65].

Rotenone-imposed hepatocellular dysfunction observed in this study was remarkably corrected by catechin, quercetin and taxifolin. The spectrum of evaluated biochemical indices sufficiently confirmed the hepatotoxicity of rotenone and highlighted the hepatoprotective property of catechin, quercetin and taxifolin. The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability [66,67]. In particular, 5,7-dihydroxyl substitution of the A ring, and a C2-C3 double bond, C4 keto group and C3 hydroxyl substitution of the C ring have been reported to be important with respect to the bioactivities of flavonoids. In addition, a catechol (o-dihydroxy) or pyrogallol (trihydroxy) arrangement on ring B is regarded as additionally essential [68–72].

Comparatively, quercetin showed the best apparent antioxidant and hepatoprotective properties. Its activity was clearly superior to that of catechin. This is attributable to the presence of the 4-keto group and the C2-C3 double bond which are absent in catechin. The higher protective activity of quercetin compared to catechin is in consonance with results of previous investigations. For example, quercetin displayed better vascular relaxation effect than catechin in porcine coronary artery [73].

From the results obtained, taxifolin demonstrated remarkable hepatoprotection and can arguably be stated to possess the best activity. Although its doses were considerably lower than those of catechin and quercetin, it showed comparable activity to quercetin and superior

activity to catechin in many of the tests. The major structural difference between taxifolin and quercetin is the absence in taxifolin, of the C2-C3 double bond. Some previous reports have ascribed greater activity to quercetin due to its possession of this C2-C3 double bond which is lacking in taxifolin [74,75]. Though the conventionally ascribed structural criteria for effective radical scavenging activity clearly explain the superior bioactive profile of quercetin over catechin in this study, they fail to account for the performance of quercetin in comparison to taxifolin [74,76].

It has been observed that a minor variation in structure can elicit marked difference in activity between flavonoids [77,78]. In addition, instances where flavonoids with fewer structural attributes linked to greater bioactivity demonstrated superior bioactivity to quercetin have been reported. For example, kaempferol which lacks B ring orthohydroxyl group showed better antibacterial and anti-inflammatory activity than quercetin [79,80]. It has been demonstrated that quercetin and taxifolin can show opposite biological activities [77]. In addition, SARs of flavonoids can be influenced by other factors such as the types of assay involved and the nature of vehicles employed.

Results from this study indicate that the SAR of flavonoids could be complex and additional factors beyond the typical criteria for activity could be involved. These factors may influence charge delocalization and energy required for the dissociation of the B ring hydroxyl groups or induce other changes that modulate reactivity, bioavailability and bioefficacy of flavonoids. On the whole, this study provides insight on the relative efficacy and structure-activity relationships of the selected flavonoids in ameliorating rotenone-induced liver damage.

5. Conclusion

The number of individual flavonoids being discovered has continued to increase and there is growing interest to develop them as nutraceutics, functional foods, adjuvants and drugs. It is therefore important to profile these polyphenols for relative effectiveness in various

pathologies so as to optimize the benefits they confer. This study has shown that the studied flavonoids are effective in meliorating hepatic dysfunction, specifically pesticide-induced liver toxicity, with taxifolin and quecetin showing more potency than catechin. The results also indicate that additional structural and metabolic factors not yet clearly defined may be involved in the structure-activity relationships of flavonoids.

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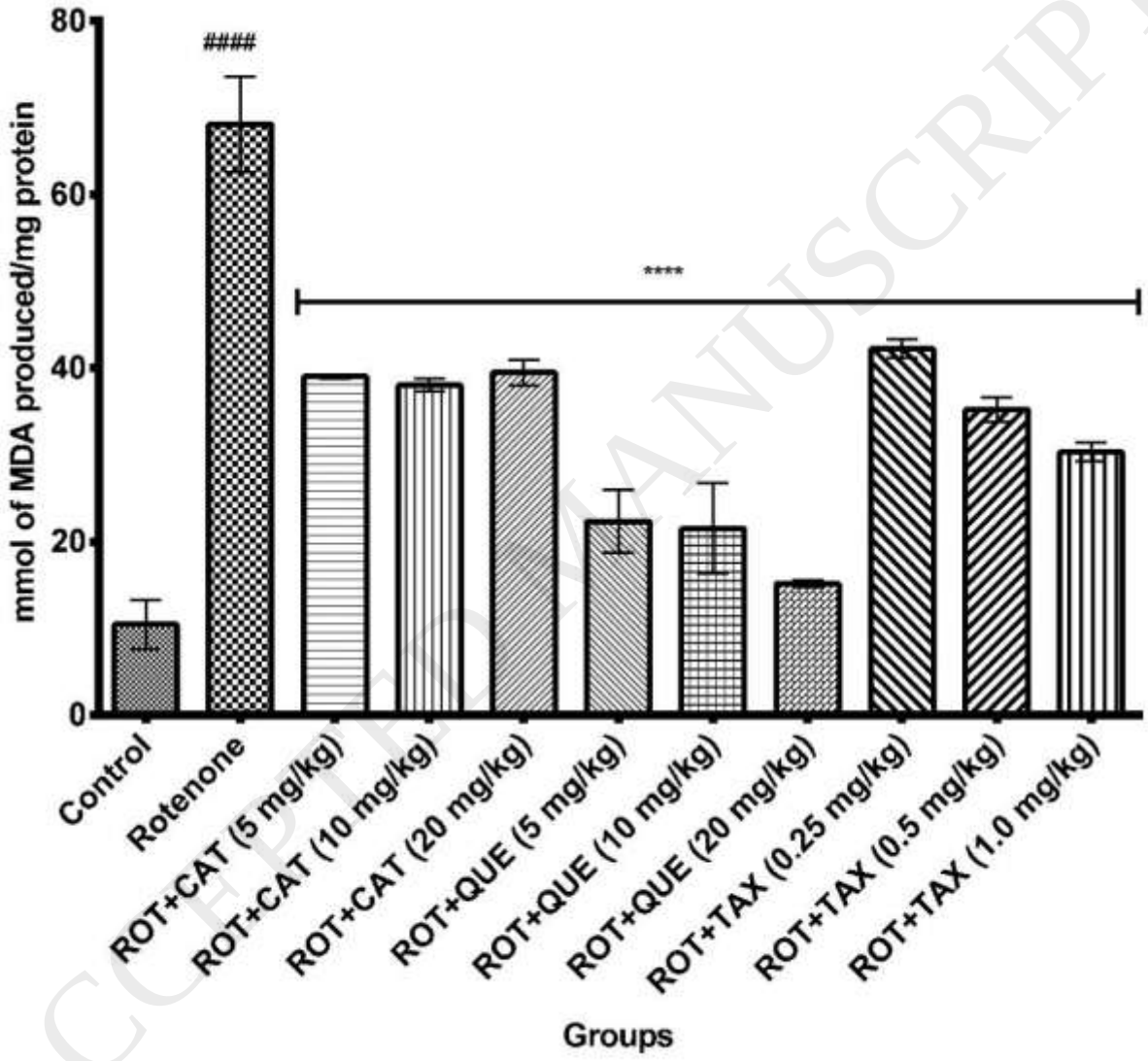
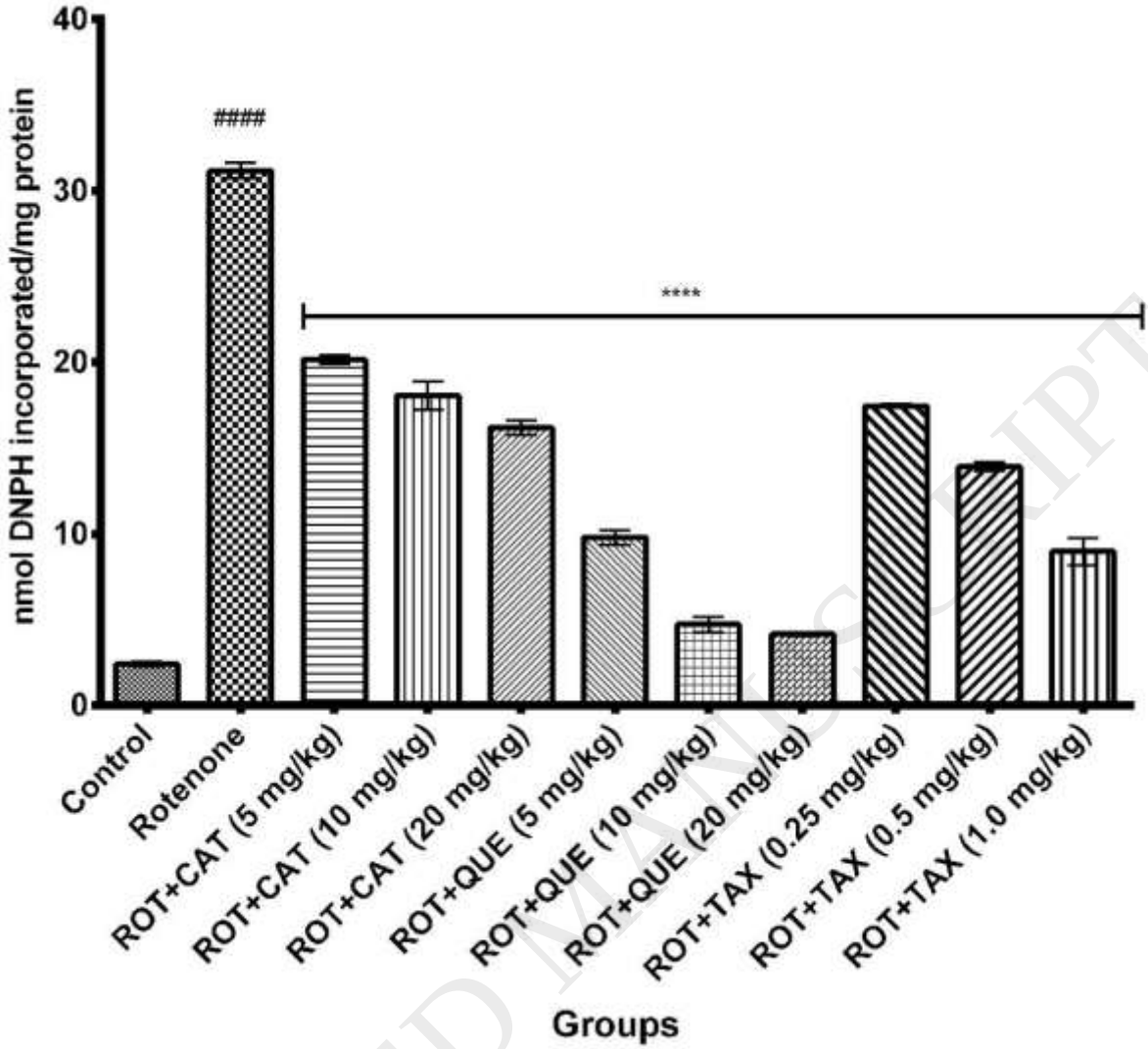
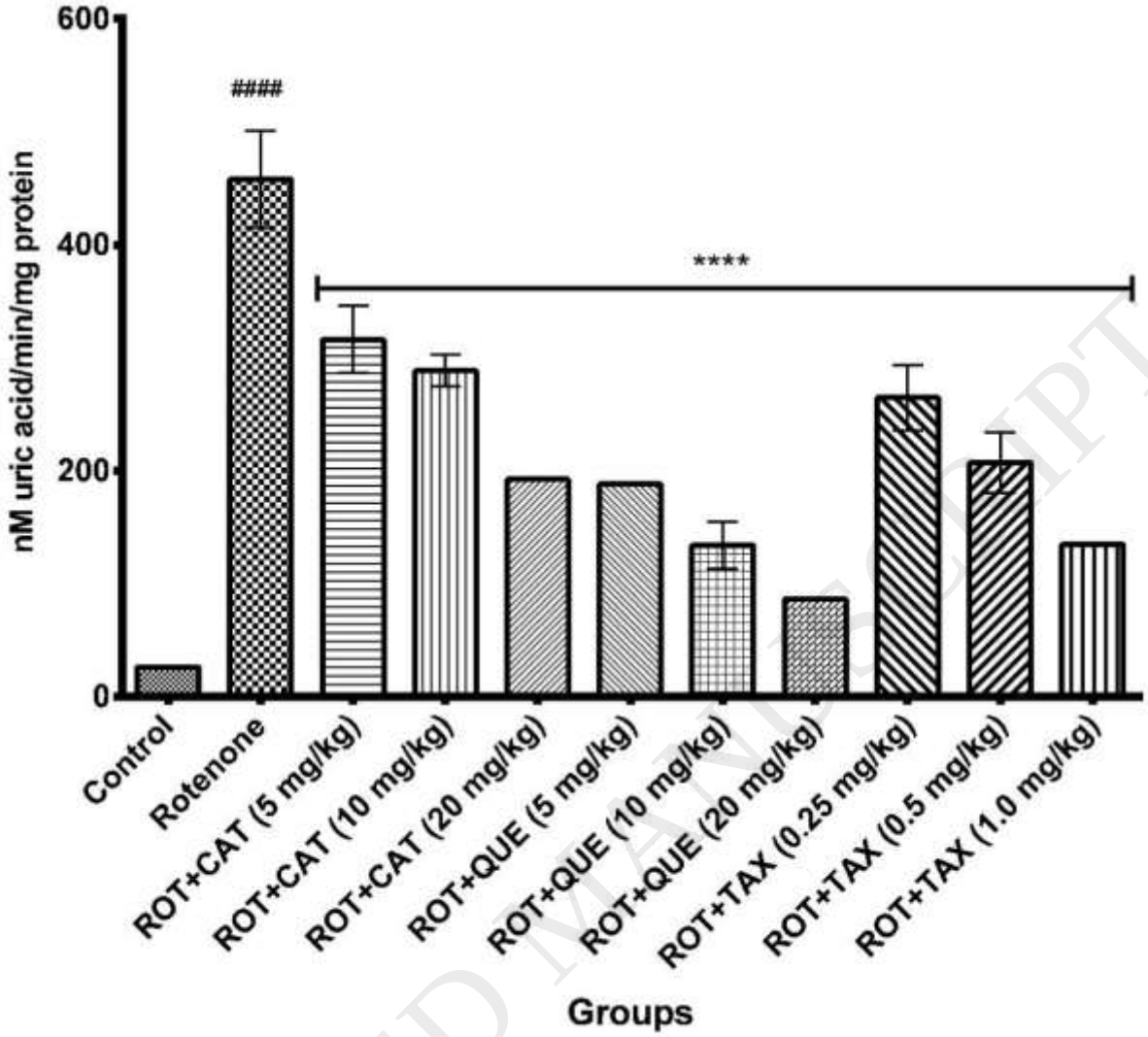
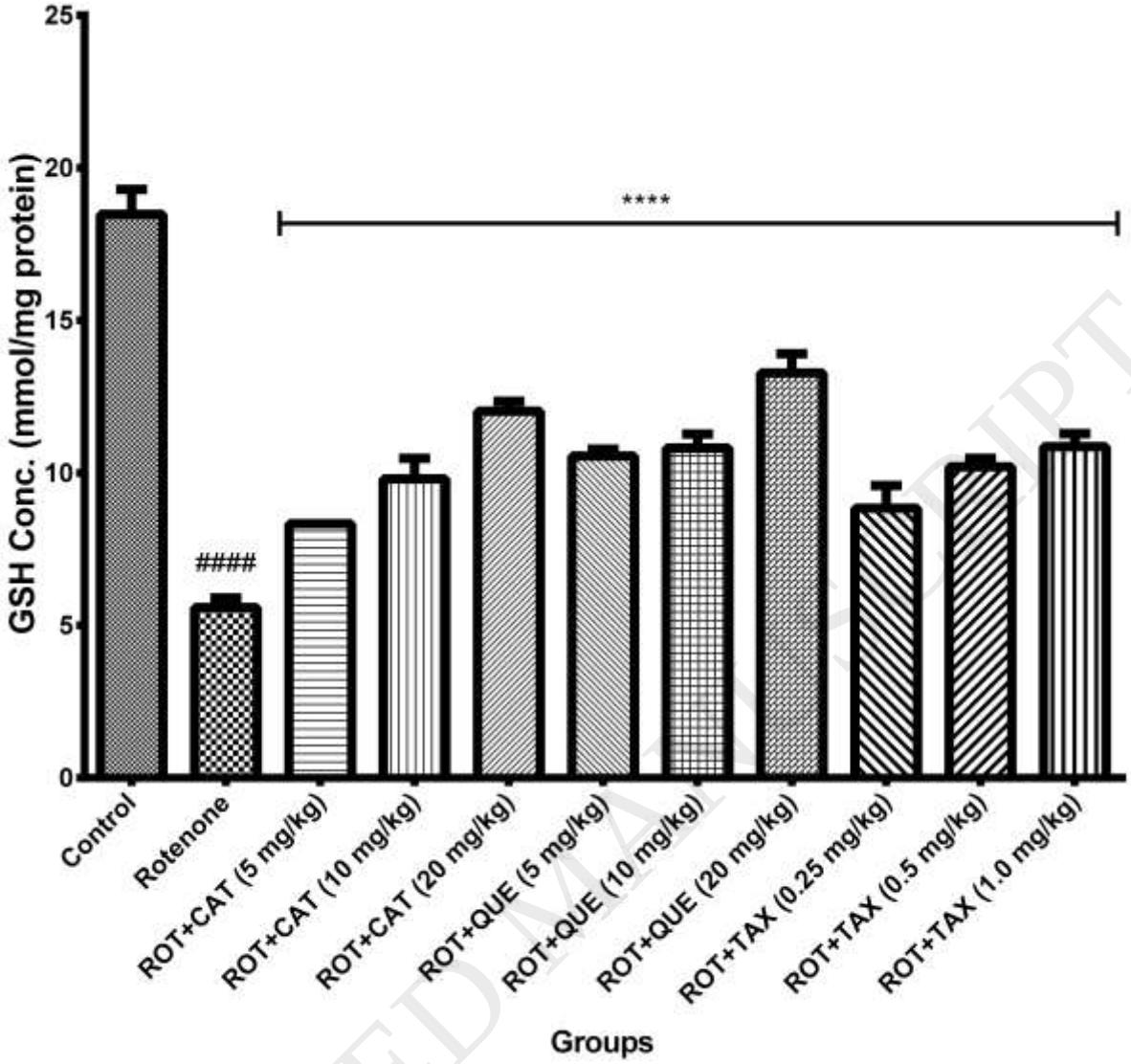
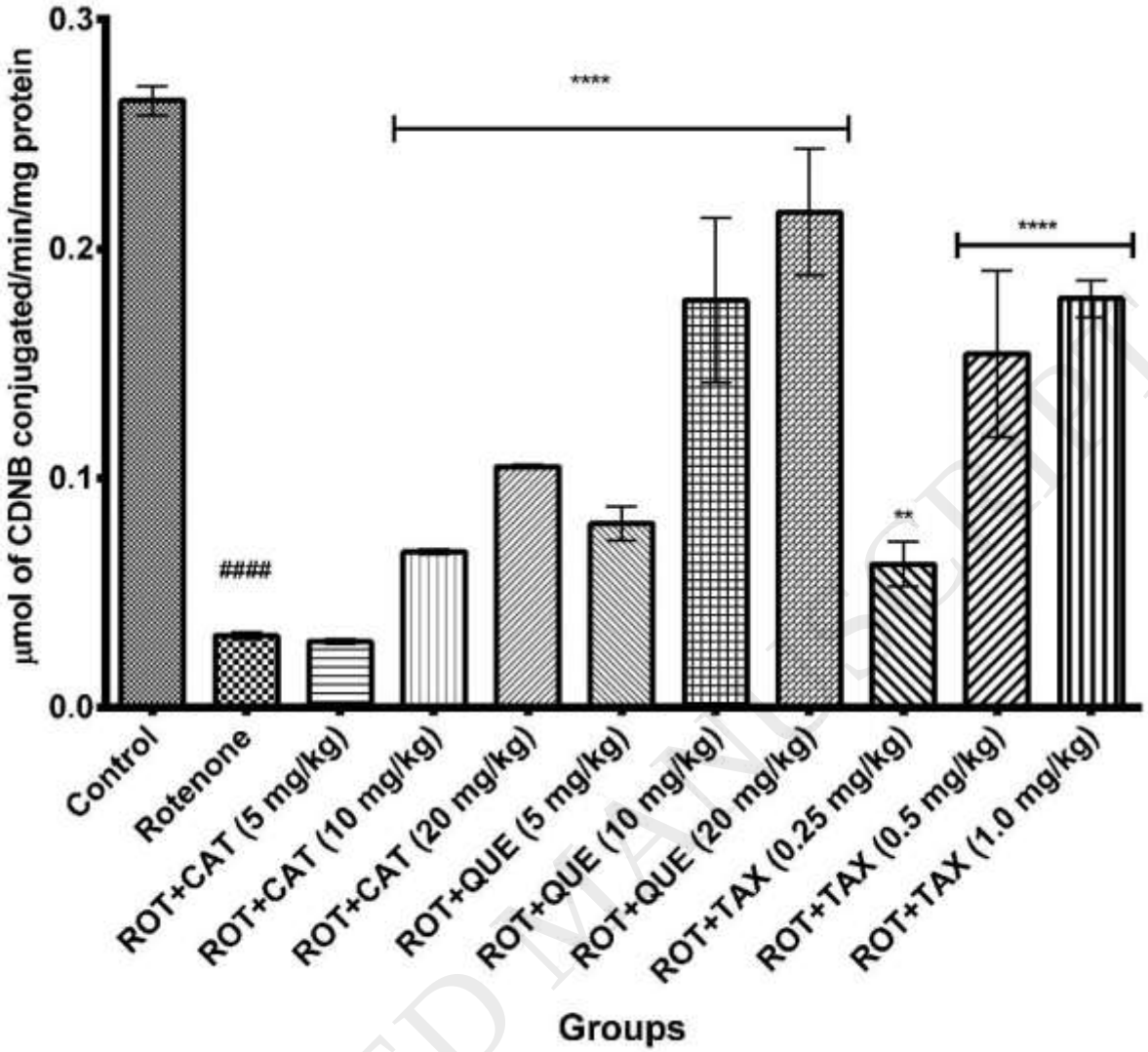
Figure 1

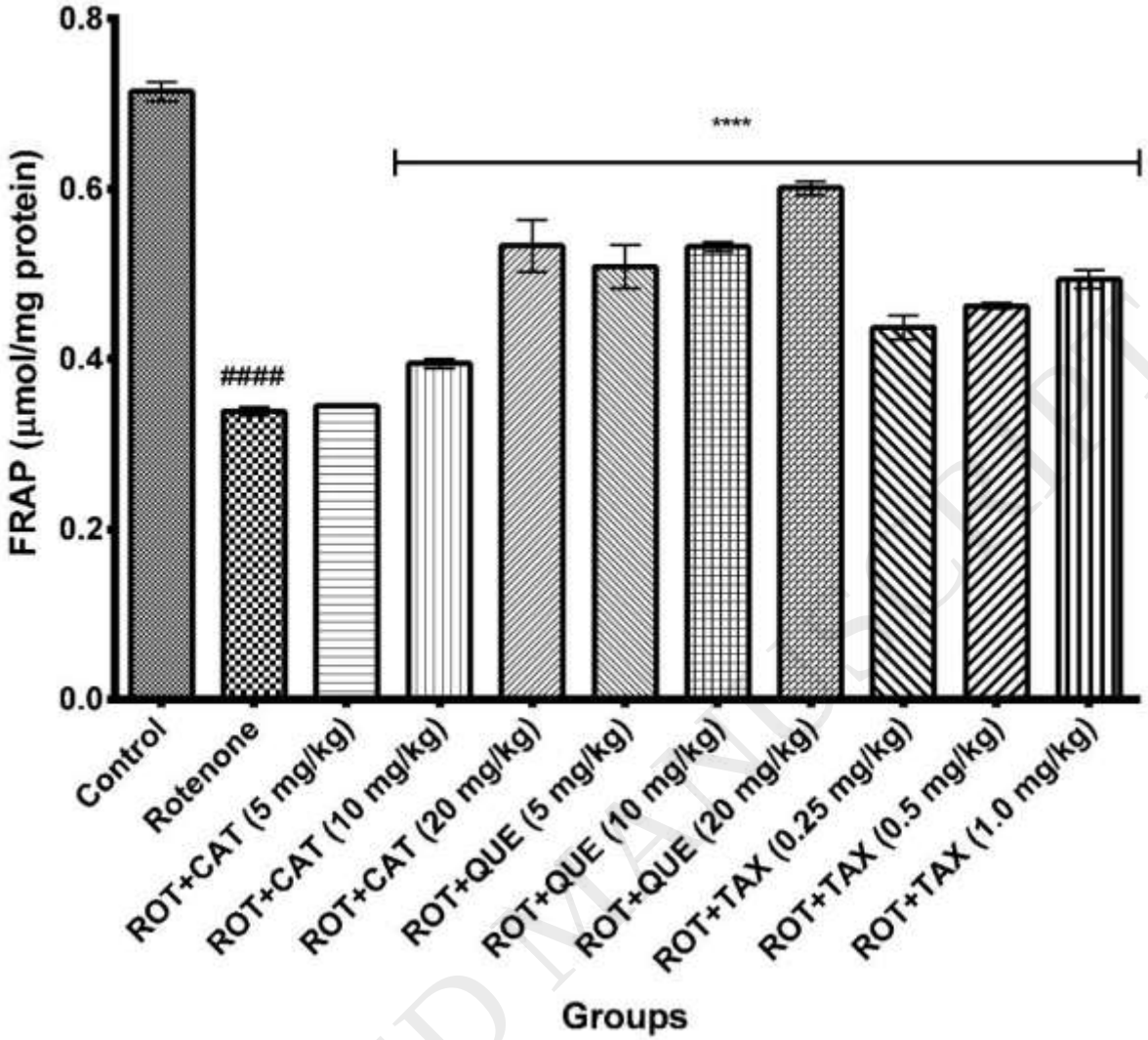


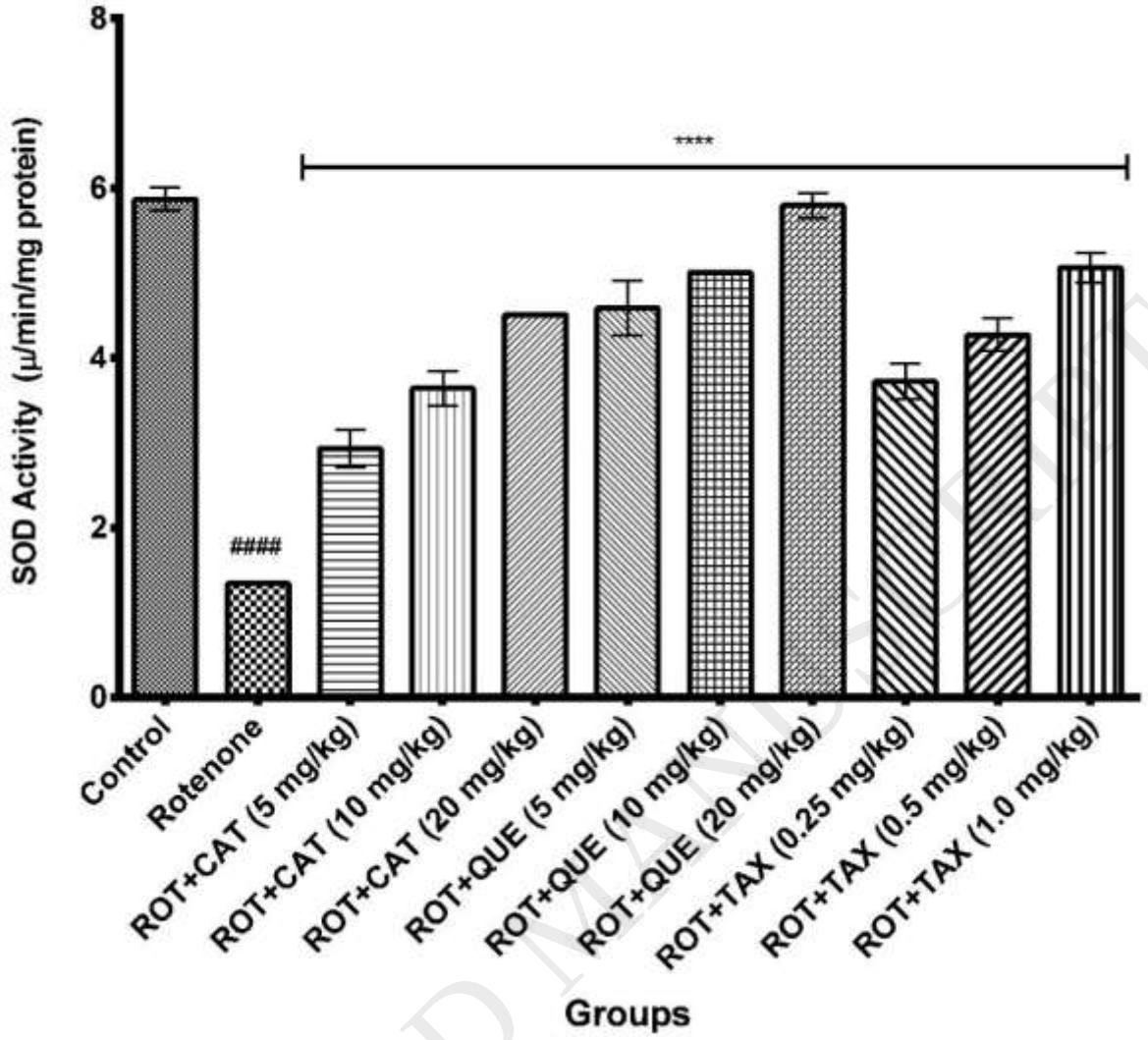












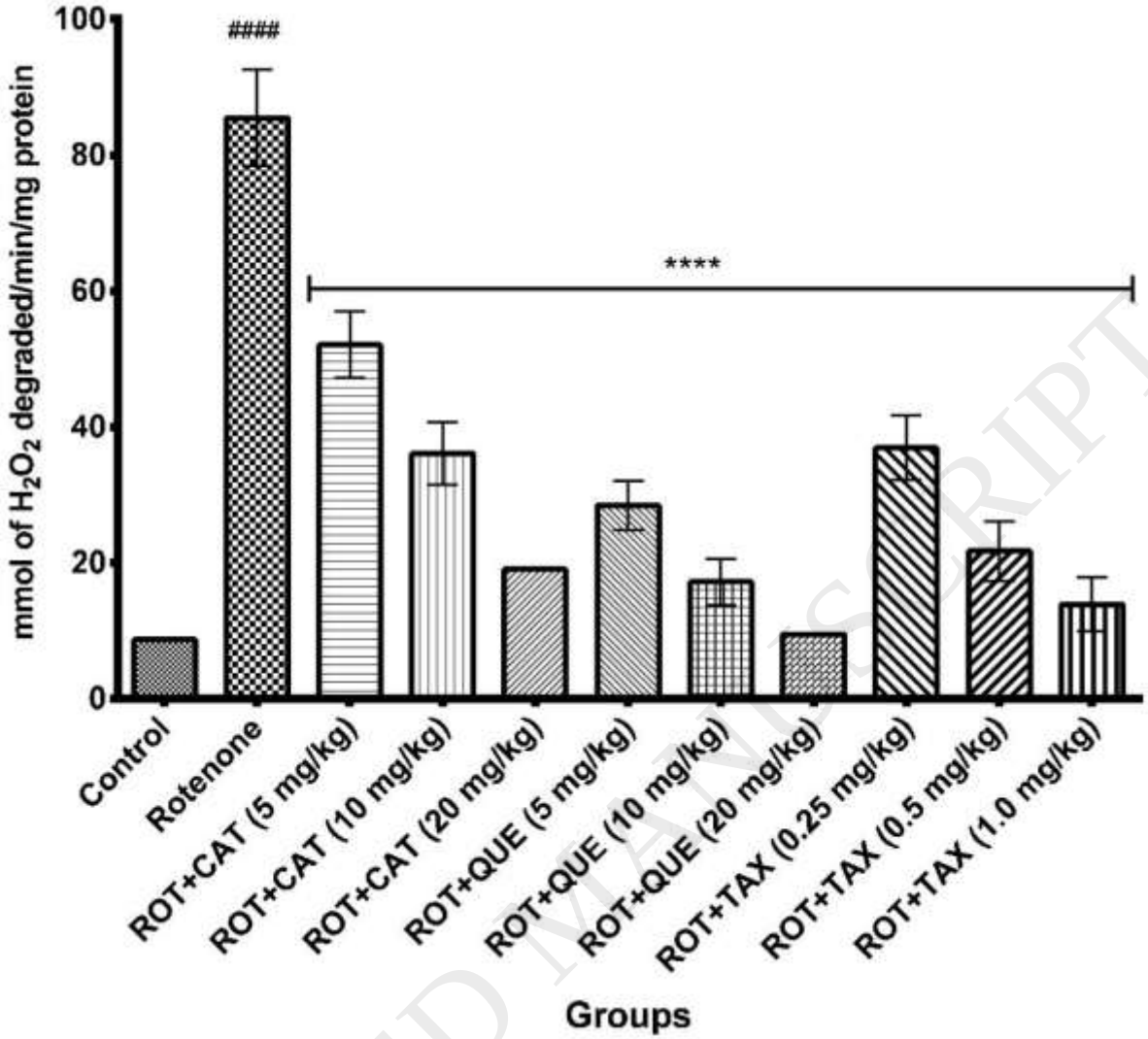


Figure 2

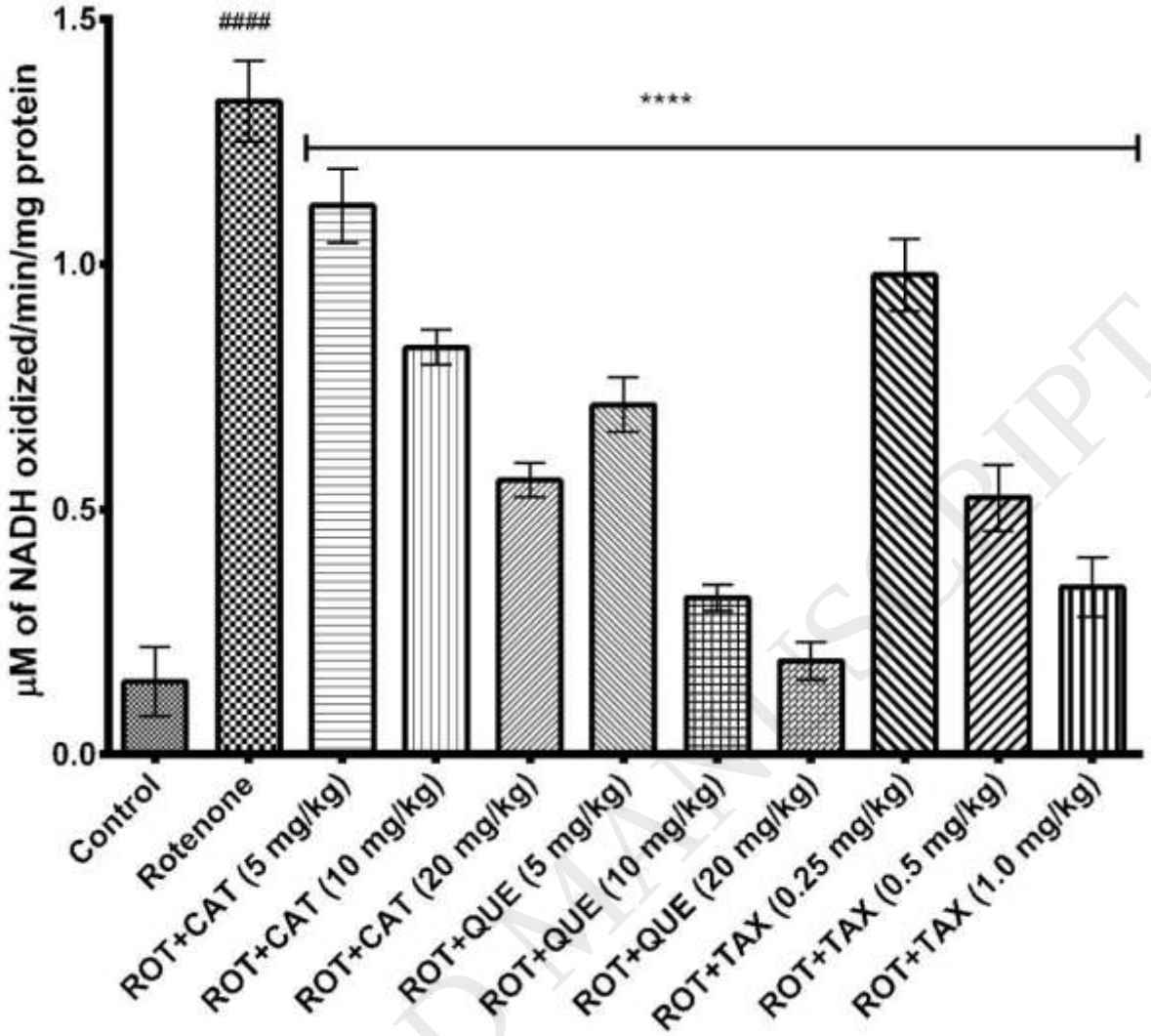
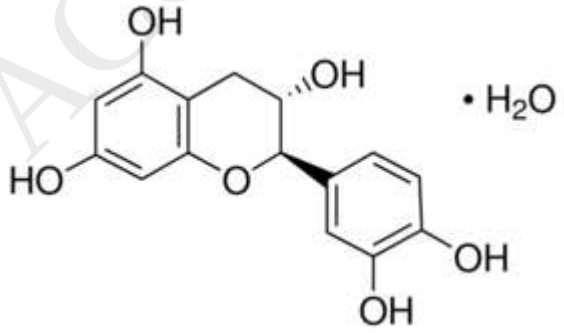
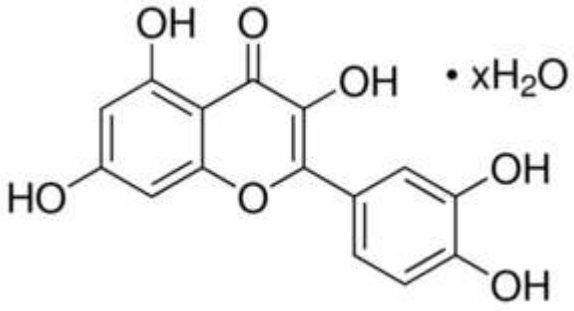
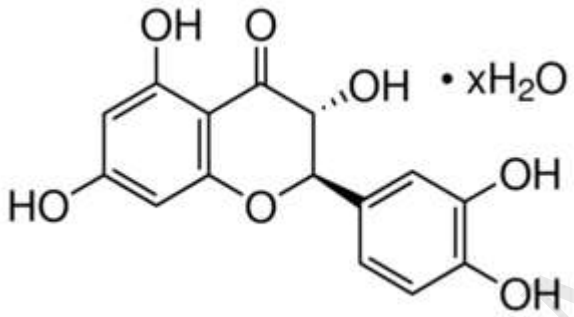


Figure 3







GROUP	DBIL	GGT	ALP	ALT	AST	TP
CONTROL	1.34±0.16	8.69±0.27	8.71±0.65	5.53±0.41	52.64±3.70	2.72±0.07
ROTENONE	3.42±0.09$^{\#\#\#\#}$	56.17±1.91$^{\#\#\#\#}$	64.63±1.94$^{\#\#\#\#}$	16.29±0.82$^{\#\#\#\#}$	424.04±1.65$^{\#\#\#\#}$	7.99±0.03$^{\#\#\#\#}$
ROT + CAT (5 mg/kg)	2.86±0.23****	46.90±0.27****	56.83±1.30****	15.12±0.82****	277.16±5.35****	7.18±0.15****
ROT + CAT (10 mg/kg)	2.70±0.06****	39.57±1.36****	34.38±1.94****	9.88±0.00****	168.39±4.52****	6.17±0.12****
ROT + CAT (20 mg/kg)	2.42±0.07****	33.20±2.73****	28.42±1.30****	8.14±0.00****	140.18±9.05****	6.09±0.03****
ROT + QUE (5 mg/kg)	2.22±0.11****	40.53±1.09****	38.96±0.65****	12.22±0.82****	121.28±5.35****	4.42±0.03****
ROT + QUE (10 mg/kg)	1.72±0.15****	28.18±3.82****	15.13±1.94****	6.69±0.41****	108.19±10.69****	4.10±0.01****
ROT + QUE (20 mg/kg)	1.53±0.28****	13.51±0.55****	10.08±1.30****	5.24±0.00****	74.74±9.46****	3.19±0.02****
ROT + TAX (0.25 mg/kg)	2.26±0.03****	42.65±0.27****	46.75±1.30****	14.54±0.82****	223.07±7.81****	6.62±0.13****
ROT + TAX (0.5 mg/kg)	1.84±0.02****	32.23±2.46****	26.58±2.59****	7.27±0.41****	146.29±6.99****	5.46±0.12****
ROT + TAX (1.0 mg/kg)	1.73±0.05****	21.81±0.27****	18.79±0.65****	6.11±0.41****	120.70±5.35****	5.32±0.09****

Table 1: Effects of catechin, quercetin and taxifolin post-treatment on some liver function indicators in serum of rats subjected to rotenone toxicity

Results are expressed as mean ± SD (n=12). ####p<0.0001vs control; ****p<0.0001vs rotenone. ROT: Rotenone; CAT: Catechin; QUE: Quercetin; TAX: Taxifolin DBIL: direct bilirubin, GGT: gamma glutamyl transferase, ALP: alkaline phosphate, ALT: alanine aminotransferase, AST: aspartate aminotransferase TP: total protein