ASIAN J NAT PROD BIOCHEM
Volume 22, Number 1, June 2024
Pages: 19-26

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f220103

Hesperidin and quercetin modulate carbon tetrachloride (CCl_4) induced hepatotoxicity in male rats

GRACE IME EKPO[1], OFEM EFFIOM ETENG[2,♥], VICTOR SAMPSON EKAM[1], MARYJANE UKAMAKA ONYEMAIZU[3], NDODO OFONIME[1], OBINAJU EFFIONG BLESSING[1], ONOFIOK LUKE UDUAK[4], ANITIE EYO ROBERT[5], SAVIOUR UFOT[2], UBANA EYONG[2]

[1]Department of Medical Biochemistry, University of Uyo. P.M.B 1017, Uyo, Akwa Ibom State, Nigeria
[2]Department of Biochemistry, Federal University of Agriculture. P.M.B 2240, Abeokuta, Ogun State, Nigeria
Tel.: 234-803-337439, ♥email: ofemeffiom@gmail.com
[3]Department of Biochemistry, University of Calabar. PMB 1115 Etagbor, Cross River State, Calabar, Nigeria
[4]Department of Chemical Engineering, University of Surrey. Guildford, United Kingdom
[5]Depatrment of Medical Biochemistry, Alex Ekwueme Federal University. Ndufu-Alike Ikwo, Abakaliki, Ebonyi State, Nigeria

Manuscript received: 12 December 2023. Revision accepted: 19 March 2024.

Abstract. *Ekpo GI, Eteng OE, Ekam VS, Onyemaizu MU, Ofonime N, Blessing OE, Uduak OL, Robert AE, Ufot S, Eyong U. 2024. Hesperidin and quercetin modulate carbon tetrachloride (CCl_4) induced hepatotoxicity in male rats. Asian J Nat Prod Biochem 22: 19-26.* Quercetin and hesperidin are bioactive chemicals that have shown considerable promise in both conventional and herbal medicine due to their significant impact on treating various human diseases. Oxidative stress is linked to the etiology of many liver disorders. Quercetin-containing foods and fruits include kales, onions, berries, apples, cherries, and other citrus fruits like grapefruit, oranges, lemons, and mandarins. The purpose of this study is to examine the potential therapeutic benefits of quercetin and hesperidin on oxidative stress-induced hepatotoxicity using male rats treated with carbon tetrachloride (CCl_4). A total of (30) male rats weighing 160-180 g were divided into 5 groups (n = 6). For twenty-one days, rats were gavaged with quercetin and hesperidin (2.4 and 2.4 mg/kg each) after being exposed to CCl_4 (0.5 mg/kg). Samples were taken to evaluate several biochemical markers. Nitric oxide (NO), Hydrogen peroxide (H_2O_2), and Malonialdehyde (MDA) levels were assayed for oxidative damage. The enzymes alanine transferase (ALT) and alkaline phosphatase (ALP) activity were estimated to represent liver function. Glutathione peroxidase (GPx), Catalase (CAT), and Glutathione (GSH) levels were evaluated for antioxidants, and histopathology was also assessed. CCl_4 increased significantly ($P < 0.05$) the concentration of MDA, NO, H_2O_2, SOD, CAT, GPX, GSH, and also, the activities of ALT, ALP, and AST in serum. Following treatment with quercetin and hesperidin, significantly ($P < 0.05$) reduced MDA, NO, H_20_2, ALT, AST, and ALP activities and modified SOD, CAT, GPX, GSH, in rats substantially ($P < 0.05$). Hepatotoxicity caused by carbon tetrachloride (CCl_4) was protected by quercetin and hesperidin treatment. This study concludes that by recovering from ROS-mediated oxidative stress, the injection of quercetin and hesperidin helps to offset the damage that CCl_4-induced hepato-renal ailments generate. Hence, quercetin and hesperidin supplements may be recommended as an adjunctive natural therapy because they can scavenge free radicals and prevent hepato-renal damage.

Keywords: Antioxidant, carbon tetrachloride (CCl_4), hepatotoxicity, oxidative damage

INTRODUCTION

The condition known as hepato-renal syndrome (or toxicity) is linked to the development of kidney failure as well as severe liver damage when exposed to carbon tetrachloride (CCl_4) by multiple routes such as ingestion, inhalation, and cutaneous absorption (Khan et al. 2012). This disorder is frequently observed in people who have severe liver failure. Symptoms include weight gain, jaundice, confusion, delirium, nausea, vomiting, dementia, reduced urine output, dark urine, swollen abdomen, and decreased antioxidant-mediated body defense (Tabeshpour et al. 2020). The severity of this condition is thought to be caused by decreased urine production, which leads to the buildup of nitrogenous waste materials. These materials then help to produce reactive oxygen species, which interact with macromolecules to cause organ damage (Low et al. 2015). Enhancing the body's antioxidant capacity, which comes from natural sources like fruits and vegetables, has also been demonstrated to help lessen the effects of hepatorenal syndrome (Douglas et al. 2020). Although it is well known that nutrition can prevent a wide range of diseases, including infectious ones, it seems that modern medicine, especially when treating infectious diseases, has overlooked or undervalued this aspect of health care (Aune et al. 2018)

Over the years, research has evaluated the role and possible mechanisms by which most chemical agents induce tissue damage, especially in the liver and kidney, which are directly responsible for the metabolism and excretion of chemicals following exposure (El-Boshy et al. 2017). Among the compounds mentioned is carbon tetrachloride (CCl_4), one of the principal xenobiotics that have been demonstrated to produce both acute and chronic tissue damage with a well-established hepatotoxicity (Xu et al. 2010). Douglas et al. (2020) state that quercetin is a polyphenolic flavonoid component widely found in red wine, tea, onions, berries, apples, broccoli, cherries, and

red grapes. According to Douglas et al. (2020), quercetin's high solubility, bioavailability, and capacity to form complexes or combine to generate certain unique preparations utilized for human health care contribute to its medicinal value. According to Tabeshpour et al. (2020), hesperidin (HSP), a pharmacologically active subclass of flavonoids (flavonoid aglycone), is widely distributed in citrus species, including blood orange, orange, lemon, and lime. According to Tabeshpour et al. (2020), it is a disaccharide derivative that has hesperidin substituted by a 6-O-(alpha-L-rhamnopyranosyl)-beta-D-glucopyraosyl moiety at position 7 by a glycosidic linkage with a diphenol structure with the molecular formula C28H34O15. According to the paper by Zanwar et al. (2018), it has been shown that flavanone exhibits various of pharmacological activities such as anti-inflammatory antioxidant, analgesic, anticarcinogenic, antiviral, and anti-coagulant. It is utilized in herbal formulations. Recent studies on the protective effects of hesperidin have shown that, as a major dietary polyphenol, its ability to guard against organ damage is well known (de Aja et al. 2020). It is used as a supplemental agent in complementary therapy protocols with success because of its biological and pharmacological properties, which are effective in lowering lipid levels, scavenging free radicals, and acting as powerful antioxidant, anti-inflammatory, anti-carcinogenic, and anti-hypertensive agents (Sedky et al. 2017). Hesperidin's antioxidant properties protect the testicles from cadmium poisoning and regulate the hepatic synthesis of cholesterol by blocking the action of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, as per a study by Saleh et al. (2022). The vital roles that bioactive chemicals from plants play in combating various human diseases have led to their widespread usage in conventional and botanical medicine. Calas, onions, berries, apples, red grapes for quercetin, and a variety of citrus fruits, including oranges, lemons, mandarins, and grapefruit for hesperidin, are excellent sources of them (Low et al. 2015). They have higher antioxidant activity due to their high solubility and bioavailability. Examples of this activity include their effect on glutathione (GSH), enzymatic activity, signal transduction pathways, and the elimination of Reactive Oxygen Species (ROS) brought on by chemical and environmental toxicological factors, such as the CCl_4 used in this study. Therefore, the study aimed to clarify how hesperidin and quercetin can work in concert to lessen the harmful effects of CCl_4.

MATERIALS AND METHODS

Chemicals and reagents

Pfizer International (NY, USA) provided the CCl_4 that was used. Randox Laboratories Limited (Admore, Crumlin, Co-Antrim, UK) produced the alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (-GT), and total protein kits. Cayman Chemical (Ann Arbour, Michigan, USA) supplied the Griess reagent kit, Potassium persulfate ($K_2S_2O_8$), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium chloride (KCl), sodium nitroprusside ($C_5FeN_6Na_2O$), hydrogen peroxide (H_2O_2), butylated hydroxytoluene, 2-deoxy-D-ribose, 2-thiobarbituric acid (TBA), and Aldrich Sigma Chemical Company (St. Louis, MO, USA) provided Ellman's reagent. Randox Laboratories Limited (Crumlin, UK) biochemical test kits were used instead of Lab Kit Biochemical Kits (Barcelona) to estimate urea, uric acid, and creatinine. All other chemicals and reagents, except those noted otherwise, were of the analytical grade and were bought from British Drug Houses in Poole, UK.

Animal model

Male albino test subjects In the Animal House of the Department of Biochemistry at the University of Calabar in Calabar, Nigeria, Wistar rats were produced artificially. The weight of the rats varied between 150 and 200 grams. They were housed in a plastic hanging cage with standard 12-hour light/12-hour dark cycles in a rat housing that was appropriately ventilated and maintained at 25°C. Fresh water and standard pellet meal were provided to the rats without restriction. The animals were allowed to acclimate for two weeks before the start of the tests. The National Academy of Science's (NAS) "Guide for the Care and Use of Laboratory Animals" by National Research Council (2010), provided guidelines that all of the animals were treated with care. The institution has accepted the researcher's experiment under number 17/042144175.

Experimental protocol

Therefore, according to their treatments, 30 rats were randomly divided into five (n = 6) groups. Before testing, hesperidine and quercetin were added to the vehicle, sweetened condensed milk diluted 1:6 in water. For 14 days consecutively, the animals consecutively were given oral aliquots containing doses of hesperidin and quercetin (2.4, 2.4, and 1.4 mg/kg bw) every day between 8.00 and 9.00 AM. These different doses of quercetin and hesperidin have been demonstrated to shield rats against oxidative stress, inflammation, and liver damage (Seema et al. 2021). The vehicle was given to the rats that were CCl_4 alone and the normal control, as indicated in Table 1. Except for normal control group I, which just received sterile injection water, all of the groups (II - 6) were subsequently given an intraperitoneal (i.p.) injection of CCl_4 (0.5 mg/kg) formulated in sterile injection water.

Preparation of serum

At the end of the experiment, precisely 24 hours after the CCl_4 administration, the animals were slaughtered under light ether anesthesia, meaning the CCl_4 would have fully metabolized by then. After piercing the heart, blood samples were taken using basic centrifuge tubes. Serum was created by centrifuging it at 3000 g for 10 minutes using an MSE bench centrifuge. The clear supernatant was used to estimate serum enzymes.

Table 1. Treatment protocols

Groups (n = 6)	Treatment
I	Control (administered vehicle)
II	CCl_4 (0.5 mg/kg b.wt., respectively)
III	Hesperidin (2.4 mg/kg b.wt.)
IV	Quercetin (2.4 mg/kg b.wt.)
V	Hesperidin (1.2 mg/kg b.wt.)

Preparation of tissue

The livers and kidneys of the sacrificed rats were quickly removed. A portion was dried using filter paper, weighed, and rinsed with ice-cold 1.15 percent KCl. To create 10% homogenates, the liver and kidney were chopped with scissors in 9 vol of ice-cold potassium phosphate buffer (0.1 M, pH 7.4). Next, a Teflon pestle homogenizer was used to homogenize the kidney and liver. (Thomas Scientific, Swedesboro, NJ, US). An aliquot of the homogenate was centrifuged at 12,000 g at 4°C for 15 minutes in a TGW16 Micro Centrifuge (Tingtai, China). This process produced the Post-Mitochondrial Fractions (PMF). We kept the supernatant at 20°C until we needed it for biochemical testing.

Biochemical assays

The manufacturer's instructions for the kit are available here. Total bilirubin, total protein, urea, uric acid, and creatinine were measured using spectrophotometry at 546 nm, while the interaction between cupric ions and protein peptide bonds in an alkaline medium to form a colored complex was observed.

Determination of liver integrity biomarkers

Measurement of ALT (alanine transaminase) activity in the blood and liver. ALT catalyzes the transamination of L-alanine to pyruvate, which subsequently combines with 2,4-dinitrophenylhydrazine (DNPH) to produce a brown-colored complex, pyruvate2,4-dinitrophenylhydrazone (PDNPHO) in alkaline medium (L-alanine + α-oxoglutarate → L-glutamate + pyruvate; + DNPH → PDNPHO), the intensity of the brown colored complex, PDNPHO at 340 nm is proportional to the activity of ALT, as described by Reitman and Frankel (1957). Units/mg protein was used to express the ALT-specific activity. The generation of reactive species – H_2O_2 and nitric oxide (NO) – was estimated according to the methods of Junglee et al. (2014). Oxidative degradation of lipids and proteins was assessed as malondialdehyde (MDA), and MDA was determined according to the method of Buege and Aust (1978) as thiobarbituric acid reactive substances (TBARS).

Determination of serum and hepatic aspartate transaminase (AST) activity.

A combination of malate and NAD+ is produced by the combination of AST-catalyzed transamination of aspartate to oxaloacetate and L-glutamate + oxaloacetate; + NADH malate + NAD+, according to Reitman and Frankel (1957). The AST-specific activity was expressed in units/mg of protein.

Determination of serum and hepatic alkaline phosphatase (ALP) activity

ALP catalyzes the hydrolysis of p-nitrophenyl phosphate to pnitrophenol, a yellow chromogen, at a wavelength of 405 nm, according to Akamo et al. (2015) technique. The activity of ALP, quantified in units per milligram of protein, is directly correlated with the intensity of yellow chromogen.

Determination of antioxidants

The liver homogenate's 12,000 g post-mitochondrial fraction was tested for antioxidant content.

Determination of reduced glutathione (GSH) concentration

The GSH concentration was determined spectrophotometrically at 412 nm by tracking the rate of production of the chromophoric product 2-nitro-5-thiobenzoate (TNB) after Ellman's reagent DTNB [5,5-dithiobis(2-nitrobenzoic acid)] was reduced. The method of Jollow et al. (1974) indicates that the intensity of the yellow-colored complex formed is directly proportional to the amount of -SH groups. by the reduced glutathione's free sulphydryl group (2GSH + DTNB TNB + GSSG). The GSH data were expressed as GSH/mg protein using a GSH molar extinction value () of 9.6 0.017 mM 1 cm 1.

Determination of superoxide dismutase (SOD) activity

SOD activity was measured spectrophotometrically at 420 nm by measuring the suppression of autoxidation of pyrogallol, a superoxide-reacting indicator molecule (SRIM) that rivals SOD for its interaction with superoxide in an alkaline media. Applying the method (pyrogallol/SOD + O2 • + 2H+ H2O2 + O2) described by Marklund and Marklund (1974). The units/mg protein or pyrogallol auto-inhibition/min/mg protein was calculated using the pyrogallol molar extinction coefficient () of 8.0 105 M 1 cm 1 to express the specific activity of SOD.

Determination of catalase (CAT) activity

The method of Hadwan and Abed (2016) involved measuring the rate of hydrogen peroxide ($2H_2O_2.2H_2O$ + O_2) oxidation at 374 nm was used to quantify catalase activity spectrophotometrically. The specific activity of catalase was expressed as Units/mg protein or mmol H_2O_2 degraded/min/mg protein using the H2O2 molar extinction value () of 43.6 M 1 cm 1.

Determination of glutathione peroxidase (GPx) activity

Using GSH as a co-factor (H_2O_2 + 2GSH →$2H_2O$ + GSSG), the remaining GSH content during the breakdown of hydrogen peroxide was measured Spectrophotometrically at 420 nm to quantify the GPx activity, following the methodology of Mohandas et al. (1984). Using a GSH molar extinction coefficient (Σ) of 9.6 0.017 mM− 1 cm− 1, GPx-specific activity was reported as Units/mg protein or nmol of residual GSH/min/mg protein.

Histopathological examinations

Dr. Kris Uko, a consultant pathologist at the University of Calabar Teaching Hospital in Nigeria, conducted the

histological analysis of the liver and kidney samples. Small pieces of liver tissue were gathered in 10% neutral buffered formalin for the proper fixing. These tissues were processed and embedded in paraffin wax. Sections of 5 mm thickness that had been cut, mounted, and stained were stained with hematoxylin and eosin. After that, the slices were examined under a light microscope.

Statistical analysis

Data were expressed as the mean ± Standard Deviation (SD) of five replicates in each group. Analysis of Variance (ANOVA) was conducted to test for the level of homogeneity among the groups. Where heterogeneity occurred, the groups were separated using the Duncan Multiple Range Test (DMRT). A p-value of less than 0.05 was considered statistically significant. All the statistics were carried out by SPSS (Statistical Package for Social Sciences) software for Windows version 20 (SPSS Inc., Chicago, Illinois, USA). Graphs were plotted using Graph Pad Prism 8 Software (Graph Pad Software Inc., San Diego, USA).

RESULTS AND DISCUSSION

Quercetin and hesperidin pre-treatment preserved hepatic integrity

It was found that quercetin and hesperidin may reduce CCl_4-mediated liver injury by assessing the activities of ALT, AST, and ALP in the serum (Figure 2). Therapy with CCl_4 markedly ($p < 0.05$) raised the activities of serum ALT (Figure 2), AST (Figure 2.B), and ALT. The findings of comparing each serum's ALP, AST, and ALT levels to the corresponding negative control (normal) group are displayed in Figures 2.C, 2.D, and 2.E, respectively.

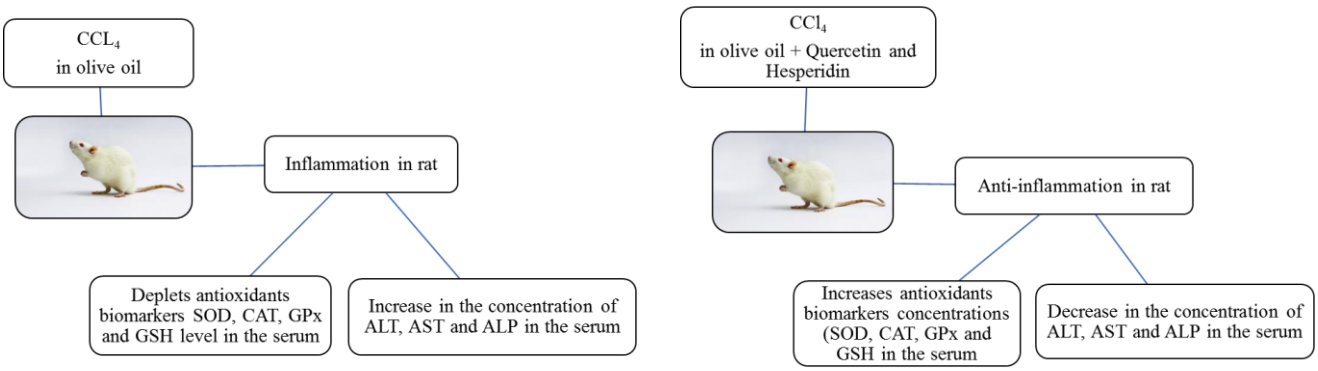
In contrast to the corresponding negative control (normal) group, the function and activity of the hepatic liver enzymes were considerably ($p < 0.05$) decreased following the administration of CCl_4. When quercetin and hesperidin were administered to the CCl_4-intoxicated group at doses of 2.4, 2.4, and 1.4 mg/kg, they significantly ($p < 0.05$) reduced the altered activities of ALT, AST, and ALP that were released into serum as a result of CCl_4-mediated liver injury. By increasing their hepatic activities compared to the CCl_4 alone treated groups, the various doses of quercetin and hesperidin also significantly ($p < 0.05$) increased the hepatic functional activities of ALT, AST, and ALP. In the two compartments, quercetin and hesperidin exhibited a concentration-dependent protective effect against hepatotoxicity generated by CCl_4.

Quercetin and hesperidin pre-treatment prevents the alterations of the oxidative stress markers induced by CCl_4 in rat organs

The effects of a single dose of 0.5 mg/kg CCl_4 were measured on the levels of reduced glutathione and the liver's activities of glutathione-S-transferase, superoxide dismutase, catalase, and glutathione reductase after pre-treatment with 2.4, 2.4, and 1.4 mg/kg quercetin and hesperidin for 21 days in a row (Figure 1). After CCl_4 injection, intracellular GSH levels were considerably ($p < 0.05$) lower than in the healthy control group (Figure 3.A). Conversely, CCl_4-induced decreases in the activities of glutathione reductase (Figure 3), glutathione peroxidase (Figure 3), and catalase (Figure 3) were seen in comparison to the healthy control group. Glutathione-S transferase and superoxide dismutase activities were significantly ($p < 0.05$) increased by CCl_4-intoxication compared to the healthy control group. The oral administration of the rats with quercetin and hesperidin to varying degrees significantly ($p < 0.05$) reverses the CCl_4-mediated changes in the oxidative stress markers.

Histopathological changes in the liver

Even though there were no reported deaths in any of the groups during this experiment, some of the liver tissues from group A (0.4% DMSO) showed a conserved architecture with a noticeable modification observed in the liver and kidney tissues of the CCl_4 exposed groups (Figure 4). After pre-treatment with Q and H, the liver and kidney tissues are clearly repaired, suggesting that the histopathological study indicates a little increase in nucleated cells.



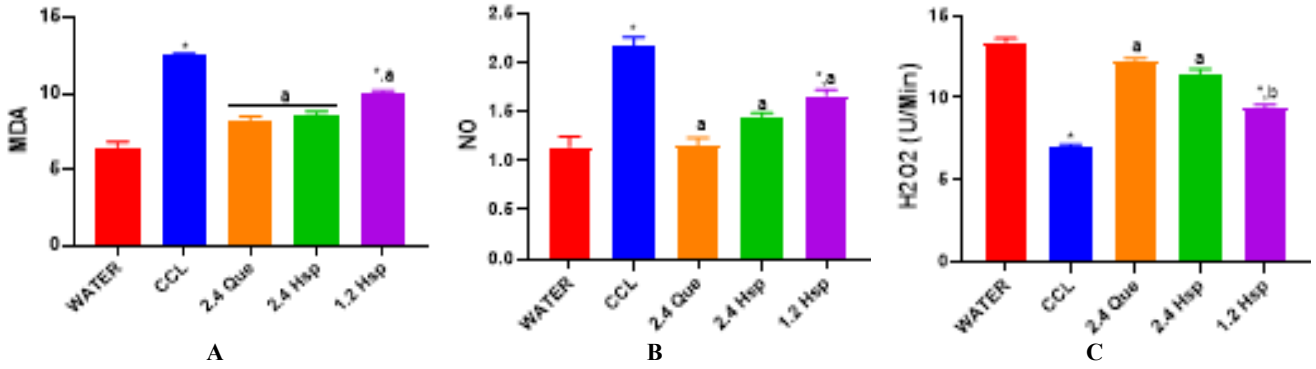


Figure 1. Effects of quercetin and hesperidin pretreatment on CCl_4 mediated increased in oxidative stress markers in rats on kidney parameters. A. MDA level, B. Nitric oxide, C. Hydrogen peroxide. Bars represent mean ± SEM (=5). Bars with different letters are significantly different at P <0.05

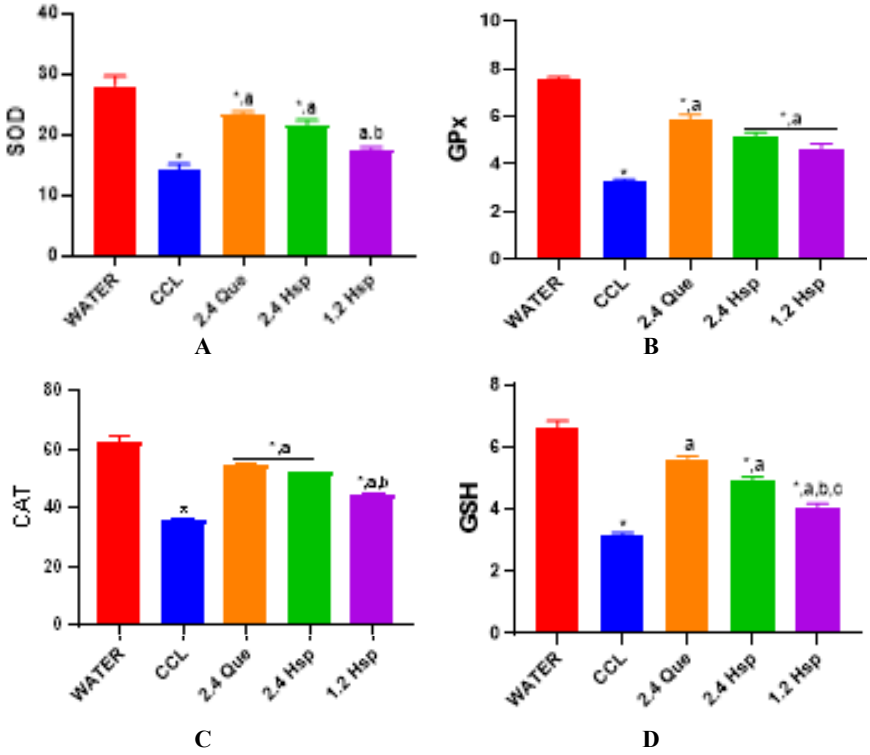


Figure 2. Effects of quercetin and hesperidin pretreatment on CCl_4 mediated increase in antioxidant markers in rats on kidney parameters. A. SOD, B. CAT, C. GPx, D. GSH. Bars represent mean ±SEM (=5). Bars with different letters are significantly different at P<0.05

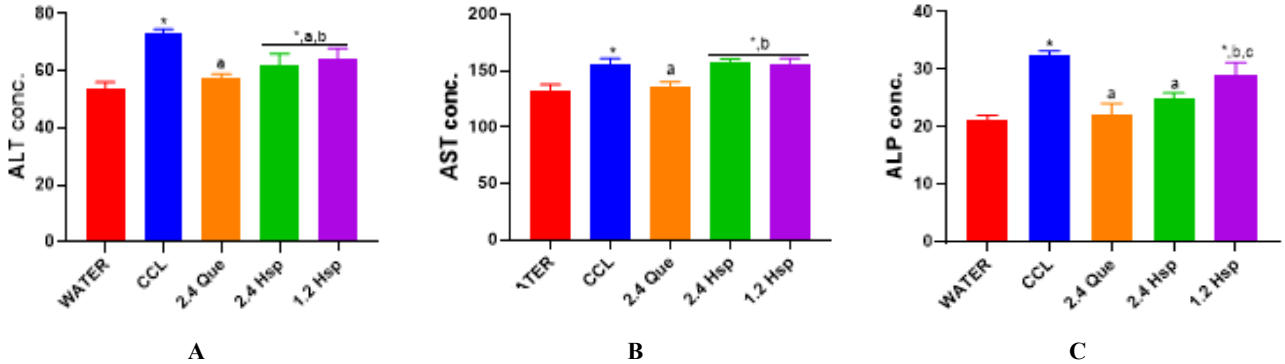


Figure 3. Effects of quercetin and hesperidin pretreatment on CCl_4 mediated increase in liver enzymes markers in rats on kidney parameters. A. AST, B. ALT, C. ALP. Bars represent mean ±SEM (=5). Bars with different letters are significantly different at P<0.05

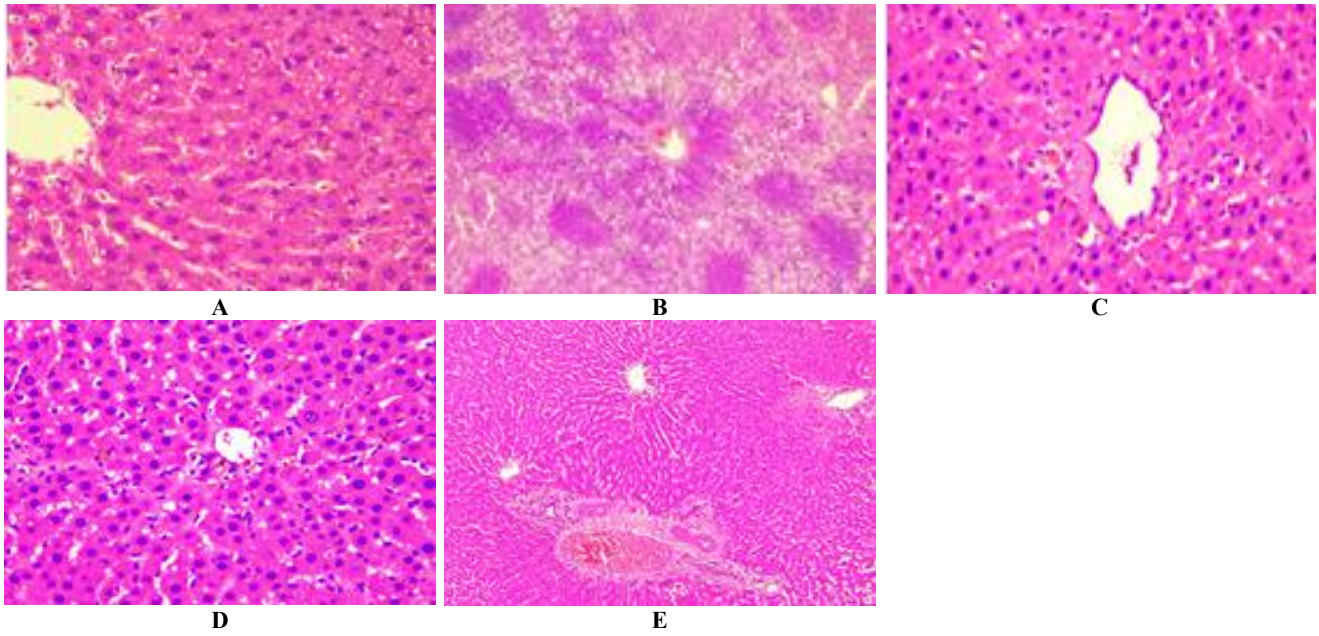


Figure 4. Liver histology of control and CCL_4-exposed rats treated with quercetin and hesperidin. Group A: 0.4% DMSO, Group B: CCL_4; 0.5 mg/kg, Group C: Quercetin (2.4 mg/kg), Group D: Hesperidin (2.4 mg/kg), Group E: Hesperidin 1.4 mg/kg). Photomicrograph of the liver section showing arrays of hepatocytes (black arrows) severe diffused fatty degeneration (#) with both a mixed microvesicular (mi), macrovesicular (ma) steatosis and hepatocellular ballooning degeneration (asterisk). Also seen was a congested blood vessel (V) necrotic liver cell (Red arrow). Haematoxylin and Eosin (H&E) Stain. X100 and x400 magnification on the CCL4 mediated group upon treatment with quercetin and hesperidin changes showed hepatocytes (black arrows), Central Vein (CV) and average-sized sinusoidal spaces (Thin Blue arrow). No pathological lesion was seen. H&E stain. x400 magnification

Discussion

Reactive Oxygen Species (ROS) produced by a biological system are not balanced with the system's capacity to detoxify the reactive intermediates or repair the damage that results in the impacted tissues quickly (El-Boshy et al. 2017). This imbalance is known as oxidative stress. According to a study by Medline (2022), this led to the accumulation of oxidative stress indicators and a notable decrease in antioxidant indicators.

The current study's findings demonstrated that the injection of CCl_4 resulted in an imbalance in the level of oxidative stress indicators tested, as seen by differences between the CCl_4 and the water groups. According to El-Boshy et al. (2017), peroxides and free radicals, which harm all parts of the cell, including proteins, lipids, and DNA, are produced when there is a disruption in the normal redox state of cells. According to the results of this investigation, CCl_4 administration was able to cause damage to cellular components, increase the generation of reactive oxygen species, and entirely reduce their clearance from the system. These reported damages may help to explain them. However, other results showed that serum CCl_4 poisoning led to a considerable decrease in antioxidant levels, including SOD, CAT, GPx, and GSH. On the other hand, CCl_4 injection and liver and kidney homogenates cause an increment in AST, ALT, ALP, SOD, and CAT activities. Therefore, a high level of ALT may indicate liver damage, which could be brought on by toxic chemicals or certain drugs that restrict blood supply to the liver. While ALP levels are reported to be a sign of liver disease or specific bone disorders, AST is directly linked to heart issues that are associated with damage to the kidneys or liver (Medline 2022). The current study's results corroborate the earlier report since exposure to CCl_4, which has a well-documented history of hepatorenal toxicity, led to a rise in the levels of these enzymes. All live cells include the enzyme superoxide dismutase (SOD), which aids in the breakdown of potentially hazardous oxygen molecules within the cells and guards against cellular damage. Because it plays a part in detoxifying harmful produced O_2 in the system, its concentration has been found to increase in excessive accumulation of reactive oxygen species. According to (Biju et al. 2014), one of the host defense mechanisms facilitating a quick rise in oxygen uptake may be the protective and adaptive mechanisms against oxidative stress that are developing in the tissue, which could explain the increase in SOD. This report is consistent with the findings in liver and kidney homogenates from the current study, which indicated that the system produced more SOD and its activity to protect itself and promote O_2 clearance from the system as a result of the damage caused in the tissues by CCl_4; this resulted in low substrate in the serum and reduced SOD activity. Similar decreases in serum levels of catalase (CAT) were also observed; they have been linked in earlier studies to CAT gene mutations, and the decreases encourage the accumulation of hazardous levels in specific cells Medline (2022). CAT is responsible for maintaining an optimal amount of the molecule in the cell, which is linked to crucial cellular signaling pathways, by using hydrogen

peroxide, a non-radical ROS, as its substrate and neutralizing it by breakdown (El-Boshy et al. 2017). However, the rise in CAT activity observed in the tissues in this study may result from the body's attempt to correct the disruption in the antioxidant levels and reduce the negative effects of reactive species and their byproducts. CAT is essential for reducing oxidative stress to a great degree since they break down cellular hydrogen peroxide to create oxygen and water. The present study's observation of elevated CAT activity indicates the deleterious consequences of administering CCl_4.

The present study indicate that serum levels of glutathione peroxidase (GPx), a family of peroxide-active enzymes whose primary biological function is to protect the body from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and free hydrogen peroxide to water, were decreased. This study suggests that CCl4 toxicity caused a reduction in GPx concentration, which may indicate disease condition, but higher concentrations of GPx were recorded in the tissues. El-Boshy et al. (2017) also reported a similar reduction and stated that the measured reduction may contribute to vitiligo and most other disease conditions, including diabetes. As stated in the study by Dibal et al. (2018), reducing oxidative stress, which can exacerbate symptoms of various chronic illnesses, including autoimmune disease, is one of the potential health benefits of boosting GPx concentration. The rise in this study could be attributed to a systematic process used by the system to counteract the tissue-damaging effects of CCl_4. Glutathione (GSH), which is known as the body's master antioxidant because of its function in cell protection, is another significant antioxidant that is of interest in the current investigation. The report claims that it immediately quenches radical centers on DNA and other biomolecules, as well as other free radicals with oxygen centers and reactive hydroxyl centers. This enzyme's decrease has been linked to long-term exposure to pollutants, alcohol, chemicals, cadmium, and other medical conditions. This could account for the serum result that showed a decrease, indicating that the administration of CCl4 induced a medical state, including liver illness. Glutathione plays a role in detoxifying endogenous and xenobiotic substances and the excretion of oxidative molecules. This could explain the rise in tissue homogenate seen in the current study. Nonetheless, dietary antioxidants support the antioxidant activity of cells (El-Boshy et al. 2017). By scavenging active oxygen and free radicals and neutralizing lipid peroxides, antioxidants and anti-inflammatory drugs play a crucial role in the fight against CCl_4 intoxication. This raises antioxidant levels, restores the structural integrity of injured organs, and improves ameliorative effects. This was observed in both the liver and kidney but not in the serum for SOD, CAT, GPx, and GSH; it was also shown in a dose-dependent manner in the hesperidin groups. The groups treated with 2.4 mg/kg quercetin showed substantial results. According to a study by Dibal et al. (2018), elevated levels of AST, ALP, and ALT in the liver indicate a dysfunctional liver condition with an unbalanced level of oxidative stress markers. nevertheless, most research indicates that this outcome could result from a disordered lipid profile in the liver. Furthermore, the results should support the findings of Dibal et al. (2018), which showed that quercetin has the potential to significantly reduce ALT, AST, and ALP activity in hepatorenal injury; similarly, Apaydin et al. (2018) reported hesperidin's ability to ameliorate chemically induced hepatorenal toxicity. Treatment with plant extracts should also be able to restore these effects, particularly in the groups treated with 2.4 mg/kg quercetin.

In conclusion, this current study concludes that by recovering from ROS-mediated oxidative stress, the injection of quercetin and hesperidin helps to offset the damage that CCl_4-induced hepato-renal ailments generate. The quercetin and hesperidin supplements may be recommended as an adjunctive natural therapy because they can scavenge free radicals and prevent hepato-renal damage.

ACKNOWLEDGEMENTS

We gratefully recognise the contributions made by the study team and the entire laboratory staff of the Department of Biochemistry, University of Calabar, Nigeria.

REFERENCES

Akamo JA, Ugbaja RN, Amah GH, Fabuluje I, Edaferiemu JO, Lepzem NG, Oyekale KO. 2015. The relationship between indices of hepatocellular injury and anthropometric measurements in some Babcock University Students, Ilisan, Ogun State, Nigeria. Braz J Biol Sci 2 (3): 23-37.

Apaydin FG, Kaltalioğlu K, Balabanli B, Coskun Cevher Ş. 2018. Morin and hesperidin ameliorate cisplatin-induced hepatotoxicity and nephrotoxicity in rats: A histopathological study. Gazi Univ J Sci 31 (2): 399-406.

Aune D, Keum N, Giovannucci E, Fadnes LT, Boffeth P, Greenword DC. 2018. Dietary intake and bllod c oncentration of antioxidants and the risk of cardiovascular disease, total cancer and all cause mortality: a systematic review and dose-response meta-analysis of prospective studies. Am J Clin Nutr 108 (5): 1069-1091. DOI: 10.1136/bmj.i2716.

Biju SD, Garg S, Mahony S, Wijayathilaka N, Senevirathne G, Meegaskumbura M. 2014 DNA barcoding, phylogeny and systematics of Golden-backed frogs (Hylarana, Ranidae) of the Western Ghats-Sri Lanka biodiversity hotspot, with the description of seven new species. Contrib Zool 83 (4): 269-335. DOI: 10.1163/18759866-08304004.

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Method Enzymol 30: 302-310. DOI: 10.1016/S0076-6879(78)52032-6.

de Aja JS, Dost A F M, Kim C F. 2020. Alveolar progenitor cells and the origin of lung cancer. Key *Symposium.* J Intern Med 289: 629-635, DOI: 10.1111/joim.13201.

Dibal NI, Garba SH, Jacks TW. 2018. Onion peel quercetin attenuates ethanol-induced liver injury inmice by preventing oxidative stress and streatosis. Biomed Res Ther 9 (6): 5102-5112. DOI: 10.15419/bmrat.v9i6.745.

Douglas AS, Pere G, Patrick SK. 2020. Hepatorenal syndrome: Pathophysiology, diagnosis, and management BMJ 370: m2687. DOI: 10.1136/bmj.m2687.

El-Boshy ME, Abdelhamidb F, Richab E, Ashshia A, Gaitha M, Qustya N. 2017. Attenuation of CCl_4 Induced oxidative stress, immunosuppressive, hepatorenaldamage by fucoidan in rats. J Clin Toxicol 30 (1): 13-24. DOI: 10.1177/0394632017694898.

Hadwan MH, Abed HN. 2016. Data supporting the spectrophotometric method for the estimation of catalase activity. Data Brief 6: 194-199. DOI: 10.1016/j. dib.2015.12.012.

Jollow DJ, Mitchell JR, Zampaglione NA, Gillette JR. 1974. Bromobenzeneinduced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11 (3): 151-169. DOI: 10.1159/000136485.

Junglee S, Urban L, Sallanon H, Lopez-Lauri F. 2014. Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. Am J Anal Chem 5 (11): 730. DOI: 10.4236/AJAC.2014.511081.

Khan R, Khan M, Sahreen S. 2012. CCl_4-induced hepatotoxicity: Protective effect of rutin on p53, CYP_2E_1 and the antioxidative status in rat. Complement Altern Med 12: 178-184. DOI: 10.1186/1472-6882-12-178.

Low G, Alexander GJM, Lomas DJ. 2015. Hepatorenal syndrome: Aetiology, DIAGNOSIS, AND TREATMENT. Gastroenterol Res Pract 2015: 207012. DOI: 10.1155/2015/207012.

Marklund S, Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47 (3): 469-474. DOI: 10.1111/j.1432-1033.1974.tb03714.x.

Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. 1984. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: Possible implications in analgesic nephropathy. Biochem Pharmacol 33 (11): 1801-1807. DOI: 10.1016/0006-2952(84)90353-8.

National Research Council. 2010. Guide for the Care and Use of Laboratory Animals. National Academies Press, Nigeria.

Reitman S, Frankel S. 1957. A colorimetric method for the Determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28 (1): 56-63. DOI: 10.1093/ajcp/28.1.56.

Saleh N, Allam T, Korany R, Abdelfattah MS, Omran MA, Eldaim MAA, Hassan AM, El-Borai NB. 2022. Protective and therapeutic efficacy of hesperidin versus cisplatin against ehrlich ascites carcinoma-induced renal damage in mice. Pharmaceuticals 15: 294. DOI: 10.3390/ph15030294.

Sedky A, Mahboub F, Elsawy H, Eid R. 2017. Protective potential of quercetin on Cd-induced hepatorenal damage. Pol J Environ Stud 26 (5): 21972205. DOI: 10.15244/pjoes/68954.

Seema S, Rohilla KK, Kalyani VC, Babbar P. 2021. Prevalence and contributing factors for adolescent obesity in present era: Cross-sectional Study. J Family Med Prim Care 10: 1890-1894. DOI: 10.4103/jfmpc.jfmpc_1524_20.

Tabeshpour J, Hosseinzadeh H, Hashemzaei M, Karimi G. 2020. A review of the hepatoprotective effects of hesperidin, a flavanonglycoside in citrus fruits, against natural and chemical toxicities. DARU J Pharm Sci 28: 305-317. DOI: 10.1007/s40199-020-00344-x.

Xu C, Bailly-Maitre B, Reed JC. 2010. Endoplasmic reticulum stress: Cell life and death decisions. J Clin Invest 15 (10): 26564. DOI: 10.1172/JCI26373.

Zanwar P, Heyn PC, McGrew G, Raji M. 2018. SIG proceedings paper in word Format. In: Proceedings of ACM Human-Habitat for Health conference, Boulder, CO USA, October 2018 (H3'18): 9. DOI: 10.1145/3279963.3279971.