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Synergistic Action of Hesperidin and Quercetin Modulate the Efficacy of CCl4 -Induced Nephrotoxicity in Rat Model

Grace Ime Ekpo[1], Sampson Ekam Victor[1], Ofem Effiom Eteng[2*], Richard Ebena[2], Ndodo Ofonime[1], Ezekiel. U. Umoh[1], Onofiok Luke Uduak[3], Saviour Ufot[4], Ubana Eyong[2]

[1]Department of Medical Biochemistry, University of Uyo Akwa Ibom State Nigeria.
[2]Department of Biochemistry, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
[3]Department of Biochemistry, University of Calabar, Calabar, Nigeria.
[4]Department of Chemical Engineering, University of Surrey, Guildford, United Kingdom.

Abstract

Chemical toxicants are hazardous compounds that can cause oxidative processes that lead to detrimental metabolites for human health when inhaled, consumed, or come into touch with the surface of the skin. This study aimed to assess how the two antioxidants' combined effects altered the hepato-renal damage in rats exposed to CCL4. Quercetin and hesperidin's effects on carbon tetrachloride (CCl4)-induced nephrotoxicity were examined using a rat model. After being exposed to CCL4 (0.5 mg/kg), rats were gavaged with quercetin and hesperidin (2.4, 2.4, and 1.4 mg/kg each) for twenty-one days. To assess several biochemical indicators, samples were collected. Measurements were made of nitric oxide (NO), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA). Renal function was assessed using the concentrations of the electrolytes Na, K, Ca, P, and Cl. The levels of GPx (glutathione peroxidase), GSH (glutathione), and CAT (catalase) were measured for antioxidants, and histopathology was also investigated. After being exposed to carbon tetrachloride (CCl4), the concentrations of the electrolytes Na, K, Ca, P, and Cl as well as the histopathological findings increased significantly ($P < 0.05$), the kidney's levels of H_2O_2, MDA, SOD, CAT, GPX, and GSH. H_2O_2, NO, and MDA levels were significantly decreased after receiving quercetin and hesperidin therapy ($P < 0.05$), while the concentrations of GPX, SOD, GSH, and CAT were considerably ($P < 0.05$) altered in the rats. Hesperidin and quercetin therapy prevented carbon tetrachloride (CCl4)-induced nephrotoxicity. A combination of quercetin and hesperidin work as antioxidants and free radical scavengers together, they may be able to reduce nephrotoxicity occasioned by carbon tetrachloride (CCl4).

Keywords: Nephrotoxicity, Antioxidant, Carbon tetrachloride, Oxidative stress

Corresponding author: Ofem Effiom Eteng
E-mail ✉ ofemeffiom@gmail.com

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Introduction

ROS (Reactive oxygen species) are developed metabolically when medications, hazardous chemicals, or environmental changes are present. This can lead to negative consequences and injuries [1]. ROS antioxidant and production defense are out of balance during oxidative stress, which leads to excessive cell damage. The pathophysiological consequences of ROS are influenced by various factors such as type, concentration, and specific manufacturing sites. High concentrations of ROS damage cells and create new, more reactive radicals when they react with molecules such as DNA, proteins, cell membranes, and other substances [2]. A colorless, heavy,

transparent, and non-flammable commercial liquid known as carbon tetrachloride (CCL4) is frequently employed to cause free radical poisoning in a variety of experimental animal tissues, including the liver, kidneys, lung, blood, heart, brain, and testis [3]. Exposure to CCL4 starts a multifaceted process that contributes to the development of brain injury by increasing oxidative stress and producing free radicals that metabolize CCL4 and increase resistance to toxicity [4]. According to Parasuraman *et al.* the ensuing oxidative stress causes DNA fragmentation, a substantial alteration in cellular metabolism, and the death of the cells through lipid peroxidation [5]. CCl4 (Reactive metabolic trichloromethyl radicals), OOCCl3 (peroxy trichloromethyl radicals), and CCL4 metabolites all cause acute and deleterious tissue damage. Originating from the Latin term "Quercetum," which means "Oak Forest," quercetin (3,3',4',5,7-pentahydroxyflavone) is a member of the flavonol class that the human body is unable to make [6]. It has a yellow hue, dissolves poorly in hot water, dissolves well in lipids and alcohol, and is insoluble in cold water. One of the most popular bioflavonoids for treating inflammatory and metabolic diseases has been reported to be quercetin.

One of the most prevalent dietary flavonoids, it can be found in a wide variety of foods and drinks, including citrus fruits, buckwheat, green leafy vegetables, flowers, nuts, trees, olive oil, broccoli, onions, apples, dark cherries, red wine, and berries like cranberries and blueberries.

Hesperidin (C28H34O15) is a flavanone glycoside that is abundant in citrus fruits, including grapefruits, lemons, and Citrus sinensis (sweet oranges). *Citrus unshiu*, *Ponderosa lemons*, immature sour oranges, and *C. mitis* have also been shown to contain this chemical [7]. It may also be isolated from other plant genera, such as the *Zanthoxylum* species (*Z. avicennae* and *Z. cuspidatum*) [8], Therefore, this study aimed to assess how the two antioxidants' combined effects altered the hepato-renal damage in rats exposed to CCL4.

Materials and Methods

Reagents and chemicals

The utilized CCl4 was supplied by Pfizer International (NY, USA). The kit of Griess reagent was provided by Cayman Chemical, located in Ann Arbor, Michigan, USA. Potassium dihydrogen phosphate (KH2PO4), potassium persulfate (K2S2O8), potassium chloride (KCl), dipotassium hydrogen phosphate (K2HPO4), sodium nitroprusside (C5FeN6Na2O), hydrogen peroxide (H2O2), the potassium persulfate (K2S2O8), Ellman's reagent was supplied by Aldrich Sigma Chemical Company (St. Louis, MO, USA). Instead of using Lab Kit Biochemical Kits (Barcelona), biochemical test kits from Randox Laboratories Limited (Crumlin, UK) were utilized for the quantification of Na, Ca, K, and Pand Cl. All extra chemicals and reagents, unless otherwise noted, were analytical grade and acquired from British Drug Houses in Poole, UK.

Animal model

Experimental animals of albino male gender In the Biochemistry's Animal House Department at the Calabar University in Calabar, Nigeria, Wistar rats were grown artificially. 150–200 g was the weight range of the rats. Rats were housed using a plastic hanging cage with standard cycles of 12-hour light/12-hour dark, and a regulated temperature of 25 °C. Fresh water and standard pellet meal were provided to the rats without restriction. Two weeks were given to the animals to acclimate before the studies began. The NAS (Science National Academy) "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2010) provided guidelines that all of the animals were treated with care. The institution has authorized the researcher's experiment under number 17/042144175.

Experimental protocol

30 rats were divided into five (n = 6) groups at random according to the treatments they received. Before testing, hesperidin and quercetin were added to the vehicle, which was sweetened condensed milk diluted 1:6 in H2O. For fourteen days in a row, the animals were given oral aliquots of quercetin and hesperidin at different concentrations (2.4, 2.4, and 1.4 mg/kg bw) between 8.00 and 9.00 h every day. It has been demonstrated that these different doses of quercetin and hesperidin shield rats against oxidative stress, inflammation, and liver damage. The vehicle was given to the rats that were CCL4 alone and the normal control, as indicated in **Table 1**. All groups (II-6), except the normal control group I, which received freshly sterile injection water, were subsequently given an intraperitoneal (i.p.) CCL4 injection (0.5 mg/kg) formulated in sterile injection water.

Table 1. Treatment protocols

Groups (n = 6)	Treatment
A	Control (administered vehicle)
B	CCL4 (0.5 mg/kg b.wt., respectively)
C	Hesperidin (2.4 mg/kg b.wt.)
D	Quercetin (2.4 mg/kg b.wt.)
E	Hesperidin (1.2 mg/kg b.wt.)

Serum preparation

Precisely 24 hours after the injection of CCL4, the animals were slaughtered under light ether anesthesia, meaning that the CCL4 would have fully metabolized by then. After

piercing the heart, blood samples were taken using basic centrifuge tubes. Serum was generated by centrifugation at 3000 g for ten minutes using an MSE tabletop centrifuge. The clear supernatant was used to estimate serum enzymes.

Tissue preparation

Rats were used and their kidneys were quickly extracted. A portion that was weighed after being dried with filter paper, was rinsed with ice-cold 1.15 percent KCl. Ten percent homogenates were prepared by dicing the kidney into small pieces using scissors in nine ice-cold potassium phosphate buffer volumes (pH 7.4, 0.1 M). Following that, the liver and kidney were homogenized using a Teflon pestle homogenizer (Thomas Scientific, Swedesboro, NJ, U.S.). A homogenate aliquot was centrifuged for 15 minutes at 12,000 g at 4 °C in a TGW16 Micro Centrifuge (Tingtai, China). This process produced the PMF (Post Mitochondrial Fractions). We kept the supernatant at 20 °C until we needed it for biochemical testing.

The levels of calcium, sodium, potassium, phosphorus, and chloride were measured and analyzed using Bethlot Searcy's method to assess nephrotoxicity. The ions sodium (Na+) and calcium (Ca+) levels were measured by the guidelines provided on the kits of diagnostic that were acquired from Randox Laboratories (UK) established using a direct spectrophotometric technique.

Hepatic lipid peroxidation concentration determination

Malondialdehyde (MDA), a byproduct of oxidative damage and cell membrane lipid peroxides, was used in spectrophotometric methods to evaluate kidney lipid peroxidation at 532 nm. MDA also triggered the production of reactive compounds that are sensitive to thiobarbituric acid (TBARS) (MDA TBA-MDA + TBA adduct). Using the MDA molar extinction coefficient of 1.56 105 M 1 cm 1, TBARS content was calculated and expressed as nmol MDA formed/mg protein.

Determination of hepatic nitric oxide radical (NO)

In biological tissues, NO synthase catalyzes the five-electron oxidative conversion of arginine to citrulline, which results in NO. NO is converted into nitrite and nitrate by oxygen. Nitrite levels in cells are a good indicator of nitric oxide synthesis and, in turn, of inducible nitric oxide synthase activity. According to Green *et al.* nitrite reacts with a dye like Griess reagent (0.2% N-(1-naphthyl) ethylenediamine dihydrochloride) to produce color and a purple azoic molecule [NO NO2, + sulphanilamide diazonium salt, + N-(1-naphthyl) ethylenediamine Azo dye] that raises the NO concentration in the hepatic PMF [9].

Estimation of hydrogen peroxide (H_2O_2) based on Montgomery and Dymock

Megaplex Red's oxidation by HRP is the basis for the hydrogen peroxide quantification assay. In summary, the colorless megaplex red probe is oxidized in a 1:1 stoichiometry to a colored product (resorufin) in the presence of HRP and H2O2. A spectrophotometer set at 570 nm or a fluorometer that can excite at 530–560 nm and emit light at about 590 nm can be used to measure resorubidin [10].

Determination of antioxidants

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

Glutathione peroxidase (GPx) activity determination

By employing GSH as a co-factor to determine the remaining content of GSH during the hydrogen peroxide breakdown, the GPx activity was detected spectrophotometrically at 420 nm using the methodology of Marklund and Marklund (H2O2 + 2GSH →2H2O + GSSG). 9.6 0.017 mM− 1 cm− 1 GSH molar extinction coefficient (Σ) was used to represent the GPx-specific activity as nmol of residual GSH/min/mg protein or Units/mg protein [11].

Reduced glutathione (GSH) concentration determination

By monitoring the chromophoric product 2-nitro-5-thiobenzoate (TNB) rate synthesis following the Ellman's reagent DTNB [5,5-dithiobis (2-nitrobenzoic acid)] reduction, the GSH concentration was measured spectrophotometrically at 412 nm. The method of Hadwan and Abed indicates that the yellow-colored complex formed intensity is directly proportional to the -SH group amount [12]. By the reduced glutathione's free sulphydryl group (2GSH + DTNB TNB + GSSG). The values of GSH were expressed as GSH/mg protein using a 9.6 0.017 mM 1 cm 1 GSH molar extinction coefficient.

Superoxide dismutase (SOD) activity determination

By assessing how pyrogallol, an SRIM (Superoxide-Reacting Indicator Molecule) that rivals SOD for the reaction with superoxide in alkaline media, is suppressed from autoxidation, SOD activity was evaluated spectrophotometrically at 420 nm. Using the procedure outlined by El-Boshy *et al.* (pyrogallol/SOD + O2 • + 2H+ H2O2 + O2). 8.0 105 M 1 cm 1 pyrogallol molar extinction coefficient was used to calculate the pyrogallol auto-inhibition/min/mg protein or units/mg protein used to express the specific activity of SOD [13].

Determination of catalase (CAT) activity

The method of [14], involved measuring the hydrogen peroxide (2H2O2 2H2O + O2) oxidation rate at 374 nm to spectrophotometrically quantify catalase activity. Using the H2O2 molar extinction value, the catalase-specific activity was represented as mmol H2O2 degraded/min/mg protein or Units/mg protein of 43.6 M 1 cm 1.

Histopathological investigation

Following the animals' sacrifice, the kidney tissues were detached and secured in a 10% neutral formaldehyde solution. The pancreatic tissues were fixed for 72 hours, then dehydrated in a graded alcohol series, embedded in paraffin, and sectioned by the use of a microtome (Leica RM2125RT, Germany) into 5 mm thicknesses. The parts underwent histopathologic examination. The sections were stained using Crossman-modified Mallory's triple staining for that analysis, and a light microscope with a camera attachment (Nikon Eclipse i50, Tokyo, Japan) was used to take a picture.

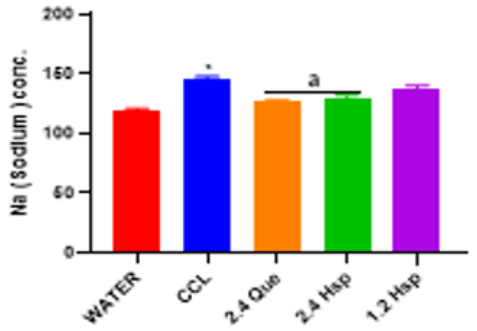
Statistical analysis

Data were expressed in each group using the mean ± standard deviation (SD) of five replicates. A variance Analysis (ANOVA) was used to determine the homogeneity of the groups. Duncan's Multiple Range Test was used to distinguish groups with heterogeneity. The statistical significance threshold was considered less than 0.05. The SPSS version 20 was used to perform all of the statistics. Plotting of graphs was done with GraphPad Prism 8 (GraphPad Software Inc., San Diego, USA).

Results and Discussion

Renal damage indicators are rejuvenated by hesperidin and quercetin pretreatment.

Effects of hesperidin and quercetin **(Figure 1)** on renal markers unique to the kidney in rats given CCL4. The administration of CCL4 causes a considerably increased ($p < 0.05$) concentration of Na, K, Ca, P, and Cl when compared to the standard control. Following treatment with quercetin and hesperidin, the concentration of the indicators dramatically decreased in comparison to the control. Thus, when administered at doses of 2.4, 2.4, and 1.4 mg/kg to the CCL4-intoxicated group, the two phytochemicals significantly ($p < 0.05$) decreased the altered concentration of Na, K, Ca, P, and Cl, which were released into the blood as a CCL4-mediated renal injury result.



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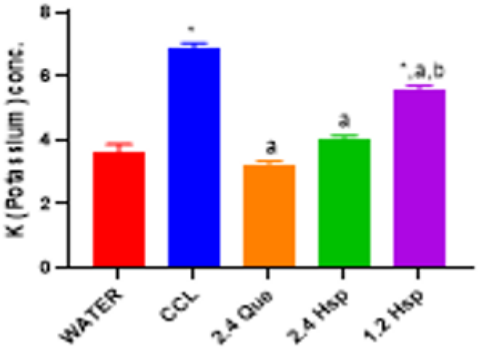

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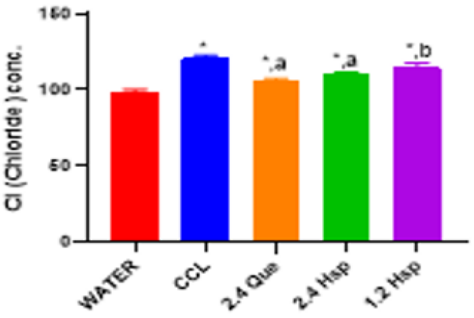

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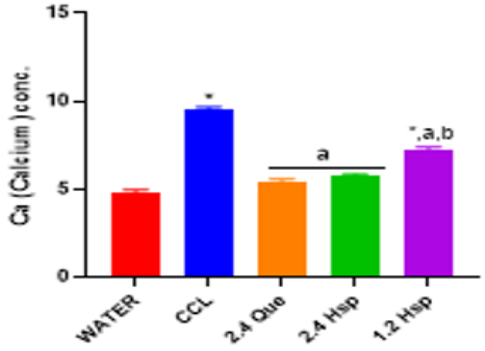

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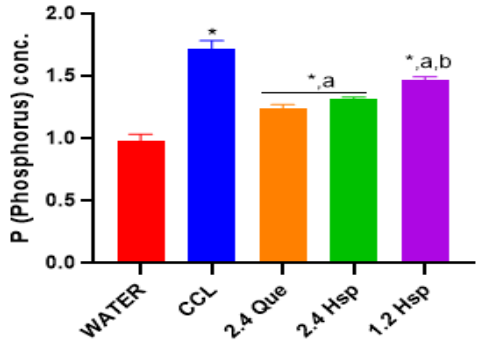

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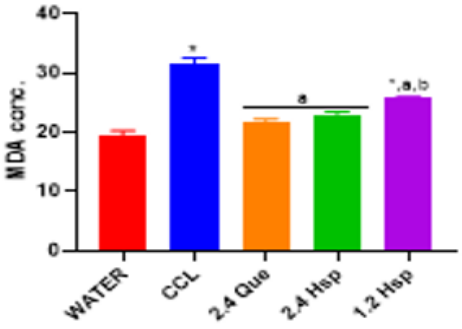
Figure 1. Effect of quercetin and hesperidin pretreatment on CCL4 mediated decrease in kidney function test (a) Na, (b) K, (c) Cl, (d) Ca, and (e) P. Bar represents mean ± SEM (n=5). Bar with different letters is significantly different at P < 0.05

Quercetin and hesperidin pretreatment attenuate the effect of lipid peroxidation induced by CCL4

Figure 2 reveals the effects of quercetin and hesperidin on oxidative damage markers in the kidneys of rats exposed to CCL4. The kidneys of the CCL4-exposed group had considerably greater ($p < 0.05$) amounts of MDA, NO, and H202 than the control group. Pre-treatment with hesperidin and quercetin (2.4, 2.4, and 1.4 mg/kg) led to a significant decrease in all the markers compared to the normal control.



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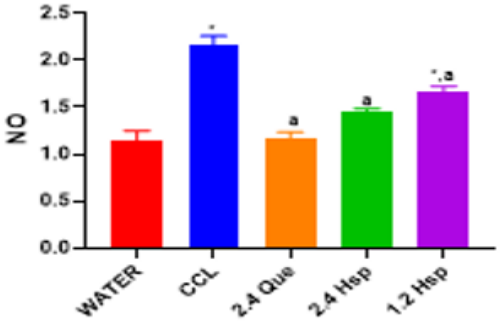

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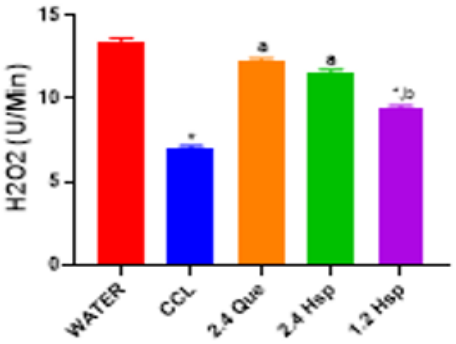

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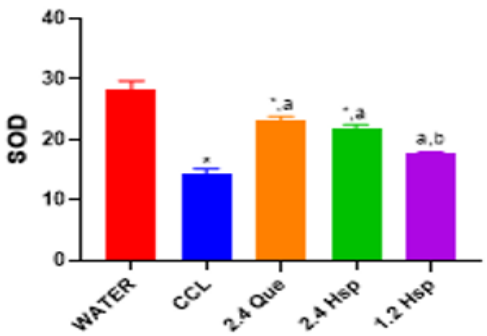
Figure 2. Quercetin and hesperidin pretreatment effects on CCL4 mediated increase in oxidative stress markers in rats on kidney parameters. (a) MDA level, (b) Nitric oxide, and (c) Hydrogen peroxide. Bars represent mean ±SEM (=5). Bars with different letters are significantly different at P < 0.05.

Quercetin and hesperidin pre-treatment prevents the oxidative stress markers alterations induced by CCL4 in rat organs

Following a 21-day pretreatment with glutathione-S-transferase, superoxide dismutase, glutathione reductase, and catalase, the effects were assessed on the reduced glutathione levels and the activity of these enzymes in the kidney 2.4, 2.4, and 1.4 mg/kg quercetin and hesperidin **(Figure 3)**. A single dose of 0.5 mg/kg CCL4 was used. Intracellular GSH levels were considerably ($p<0.05$) lower following CCL4 injection in comparison to the healthy control group **(Figure 3a)**. Conversely, CCL4-induced reduced the functions of glutathione reductase **(Figure 3)**, glutathione peroxidase **(Figure 3)**, and catalase **(Figure 3)** were seen in comparison to the healthy control group. Remarkably, glutathione-S transferase and superoxide dismutase activities were significantly increased upon CCL4-intoxication compared to the normal control group ($p < 0.05$). The rat's Oral administration with quercetin and hesperidin to varying degrees considerably ($p < 0.05$) reverses the CCL4-mediated changes in the oxidative stress markers.



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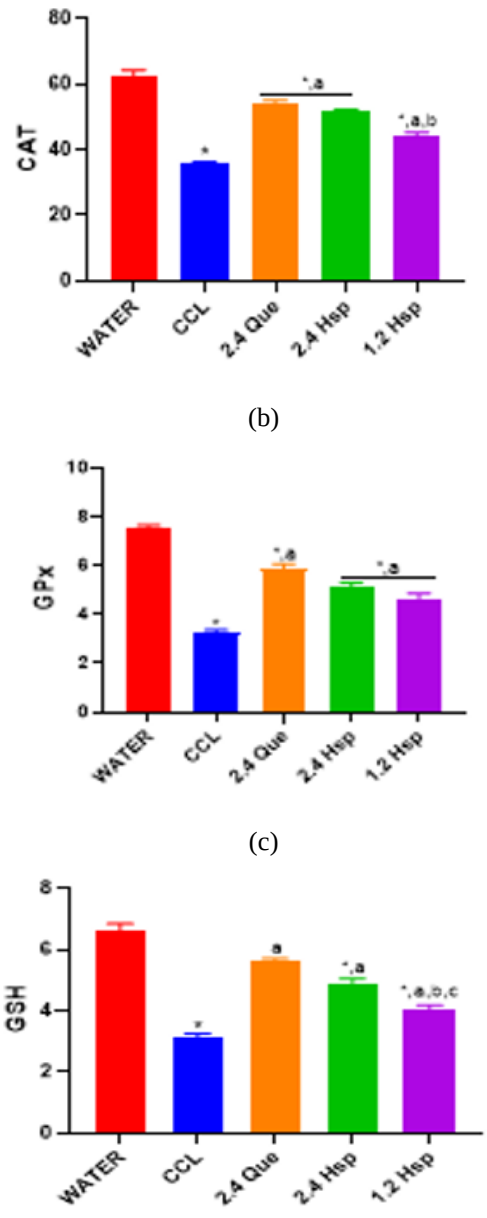

(b)

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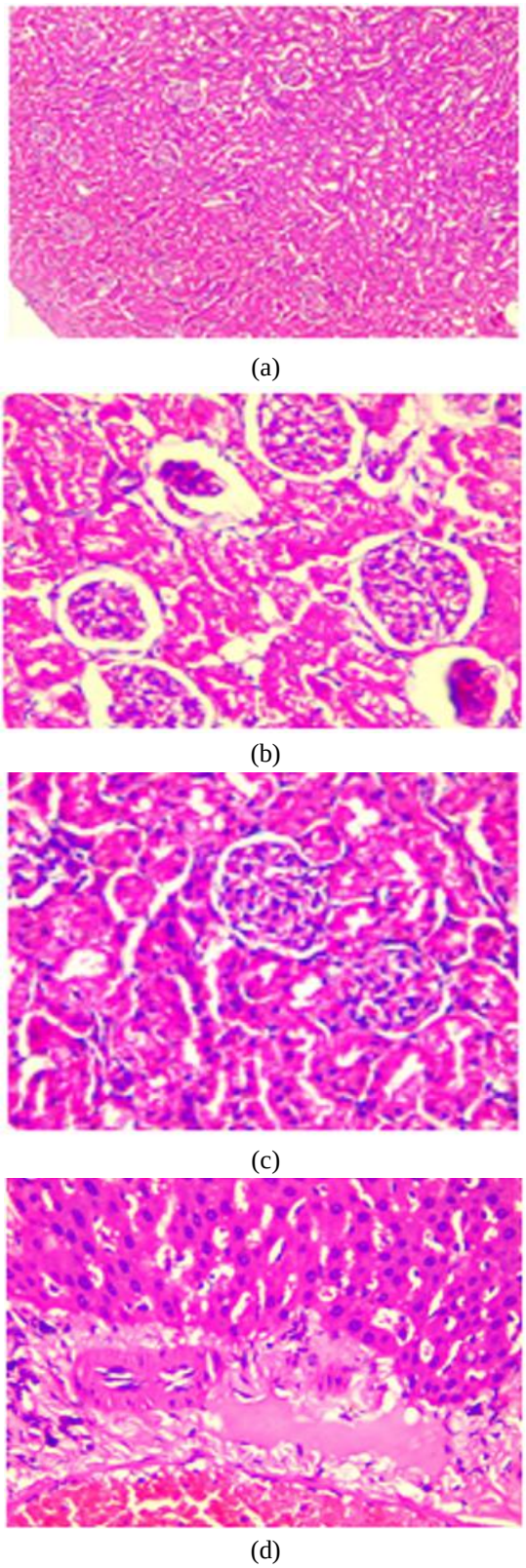
Figure 3. Effect of quercetin and hesperidin pretreatment on CCL4 mediated decrease in antioxidants in rats in the kidney. (a) The activities of superoxide dismutase, (b) the activities of catalase, (c) glutathione peroxidase, and (d) glutathione transferase. Bars represent mean±SEM (n = 5). Bars with different letters are significantly different at $P < 0.05$.

The outcomes of the histopathological investigation of the kidney are shown in ***Figure 4****.*

Even though there were no reported deaths in any of the groups during this experiment, a portion of the kidney tissues from group A (0.4% DMSO) presented a conserved architecture with a noticeable modification being observed in the renal tissues of the CCL4 exposed groups. After receiving quercetin and hesperidin pretreatment, the kidney's renal tissues are repaired, suggesting that the histopathological study indicates a slight increase in nucleated cells.



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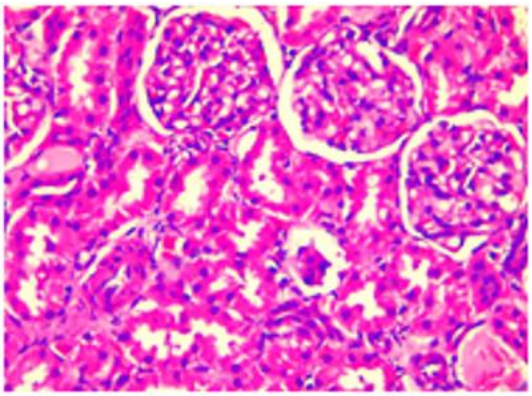

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Histopathological changes

Figure 4. Control kidney histology and CCL4-exposed rats treated with quercetin and hesperidin. Group A: 0.4% DMSO; GROUP B: CCL4 0.5 mg/kg; GROUP C: 2.4 mg/kg Q +H GROUP D: 2.4 mg/kg Q +H Group E: 1.2 mg/kg hesperidin changes in renal cortex tissue of experimental and control groups. E and H, ×400. Control group: renal tissue revealed no crystallization sign. DEP+BPS group: tubular crystal deposits (arrows) with secondary tubular dilatation (arrowhead), leukocyte reaction, and epithelial damage were found. In hesperidin-treated and quercetin rats, renal tissue revealed a small crystal formation.

All histological architectural evidence showed normal kidney tissue with normal glomeruli (GM) and renal tubule (RT). No pathological changes were seen. Haematoxylin and Eosin (H & E) stain. x400 magnification.

Numerous environmental elements that exist in gaseous, liquid, semi-solid, and solid phases that are easily absorbed by organisms through eating, skin contact, diffusion, and inhalation have been shown to change an organism's normal physiological condition. These compounds are known as xenobiotics, and when they enter an organism, the unintended chemical response triggers the system to change from its normal condition [15]. CCl4 (Carbon tetrachloride) is a well-known chemical that is harmful to the liver and kidneys. Its biotransformation into two free radicals by the cytochrome P450 system is the root cause of its hepatotoxicity. The first metabolite, a trichloromethyl free radical, interacts with O2 to produce a trichloromethylperoxy free radical, a second metabolite, or it can take out hydrogen atoms to produce chloroform. It joins proteins and lipids to generate covalent adducts. Lipid peroxidation of membranes results from this chain of events, which damages the liver and kidneys.

Electrolytes are necessary for basic bodily functions such as producing and conducting action potentials in the nerves and muscles and preserving electrical neutrality in cells. Significant electrolytes include sodium, potassium, and chloride in addition to bicarbonates, magnesium, calcium, and phosphate. We obtain electrolytes via our diet and beverages. An imbalance in these electrolytes can result in either high or low amounts. Elevations or decreases in electrolyte levels can cause serious health problems and interfere with regular body processes. After being exposed to carbon tetrachloride, the concentration of potassium (K) amplified largely ($P < 0.05$); however, the concentration was comparatively reduced after treatment with quercetin and hesperidin. This result is consistent with that of Toy *et al*. who found that exposure to carbon tetrachloride increased potassium levels [16]. A kidney disease or damage could be the cause of the rise. After being exposed to CCl4, the concentrations of calcium (CA), phosphorus (P), and chloride (Cl) all showed a substantial rise ($P < 0.05$). This increase may have been brought on by kidney disease or dehydration [17]. Reported electrolyte levels that were similar. According to this study, kidney injury occurred because there was an overall increase in renal electrolyte content in the CCl4-exposed group. Additionally, there was a relative increase in response to quercetin and hesperidin therapy, suggesting their reno-protective qualities. The rise in electrolyte concentration following exposure to carbon tetrachloride was documented in a study by Burton *et al.* on the attenuation of Hepatonephrotoxicity and CCl4-induced oxidative Stress by Saudi Sidr honey in rat models [17]. Treatment with Pleurotus ostreatus nearly restored the effect to normal [18]. Found a substantial increase in serum sodium levels after exposure to carbon tetrachloride. The reduction of sodium levels was observed in groups treated with quercetin and hesperidin, respectively, after exposure to streptozotocin (STZ) as reported by Pompella *et al.* in their study "the hesperidin and quercetin effect on NF-κB, oxidative stress, and SIRT1 levels in an STZ-induced experimental diabetes model [19]."

For various reasons, comprehending the idea of a pro-oxidant–antioxidant equilibrium is essential to understanding oxidative stress [20]. Initially, it highlights that the disruption could stem from modifications on either end of the balance, such as reactive oxygen species' excessive production or inadequacies in antioxidant defenses [19, 20]. It also draws attention to the homeostatic ROS concentrations [17]. Despite being initially identified by biologists as potentially hazardous by-products of aerobic metabolism, ROS are now understood to have vital roles as secondary messengers in numerous intracellular signaling pathways [20] both kidneys exhibit an increase in MDA levels. According to Sies *et al*. MDA (Malondialdehyde) is a significant reactive aldehyde that is produced when PUFA (Biological Membrane Polyunsaturated Fatty Acid) peroxides [20]. Lipid peroxidation's intermediate product, MDA, is employed as a marker of tissue damage resulting

from a cascade of events [19]. When it interacts with thiobarbituric acid, reddish compounds are created. The pathophysiology of osmotic fragility, heightened membrane rigidity, decreased erythrocyte survival, and alterations in lipid fluidity have been linked to lipid peroxidation.

Hepatocyte membranes' lipid peroxidation by CCl4 free radical derivatives has been proposed as one of the main mechanisms of CCl4-induced hepatotoxicity [14]. This theory is supported by the current study's finding that Group II rats (those given CCl4 alone) had higher kidney and serum MDA levels. Consequently, the quercetin and hesperidin-treated groups' MDA levels were maintained at almost normal levels. Curiously, the study offers more proof that quercetin and hesperidin may have reno-protective effects.

According to the current study, exposure to CCL4 increased nitric oxide (NO). By activating soluble guanylate cyclase (GC) in the vascular smooth muscle and inhibiting cytochrome c oxidase, nitric oxide (NO) influences two important components of O_2 supply and demand: blood flow and vascular tone. The rise could have resulted from pulmonary system abnormalities such as pneumonitis, bronchitis, bronchiolitis, emphysema, and potentially methemoglobinemia. The administration of hesperidin and quercetin considerably lowered the NO level. The results are consistent with those of Recknagel and Glende and Sies *et al.* [18, 20]. The group treated with CCL4 saw a considerable drop in hydrogen peroxide (H_2O_2) content. Both oxidative biosynthesis reactions and host defense depend on hydrogen peroxide. The body's natural tendency to fight oxidative stress and the oxidative stress caused by CCL4 may be the cause of the rise. This result is consistent with that of Kivrak *et al.* and El-Boshy *et al.* still [6, 13]; the treatment of hesperidin and quercetin comparatively decreased the hydrogen peroxide level.

The body produces antioxidants through a variety of processes to combat oxidative stress; these antioxidants can be obtained externally from diets or naturally occurring in the body [15]. According to Sies *et al.* antioxidants have three main functions: they neutralize excess free radicals, shield cells from their harmful effects, and help prevent disease [20]. The kidney's GST, SOD, CAT, GPx, and GSH levels significantly decreased after exposure to CCL4. These enzymes are part of the Phase II detoxification family and work to shield cellular macromolecules from reactive electrophile attack. The decline may have been brought on by signs of oxidative stress or a compromised immune system. In particular, GSTs catalyse the GSH (Glutathione Conjugation) to an endogenous wide range and foreign electrophilic chemicals.

One of the main non-protein thiols found in living things, glutathione is essential for the coordination of the body's natural defense mechanisms against free radicals. Through its redox and detoxifying activities, it likely plays a role in maintaining the regular structure and cell function [16]. Necrosis begins when reserves of reduced glutathione (GSH) are noticeably depleted. GSH is essential for the detoxification of the CCl4 reactive toxic metabolites [17]. The current study's findings, which show lower GSH levels in Group II rats (given CCl4) compared to normal, are in line with those of previous researchers. According to Pompella *et al.* quercetin and hesperidin treatment comparatively increase the levels of the enzymes, indicating that quercetin and hesperidin reversed the effect of CCL4 [19]. Reduced GSH levels may result from GSH increased utilization by antioxidant enzymes and scavenging H_2O_2.

The kidney section histology of a normal control animal shows typical renal diseases, such as epithelial destruction, degeneration, and modular tubules without any indication of crystallization. Hesperidin and quercetin pretreatment at a dose of 2.4 mg/kg each has demonstrated a possible nephron-protective effect and decreased the kidney's degenerative alterations **(Figure 4)**. The resulting finding is consistent with the research conducted by Sies *et al.* who found that F. assafoetida had a nephron-protective effect on CCl4-induced damage in rats [20].

The findings of histological investigations to extract quercetin and hesperidin also directly demonstrated the extract's reno-protective effects and the renotoxicity effects of CCl4. Lastly, the results of these investigations offered support for the results of the biochemical study.

Conclusion

Taking into account the prevalence of health issues among Nigerians caused by an increasing frequency of chemical exposure. Based on their combined dosage of 2.4 mg/kg, these investigations conclude that quercetin and hesperidin have a weakening effect on nephrotoxic damage, suggesting that they have kidney-protective qualities.

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References

1. Usui T, Foster SS, Petrini JH. Maintenance of the DNA-damage checkpoint requires DNA-damage-induced mediator protein oligomerization. Mol Cell. 2009;33(2):147-59.
2. Petković J, Žegura B, Stevanović M, Drnovšek N, Uskoković D, Novak S, et al. DNA damage and alterations in expression of DNA damage responsive genes induced by TiO2 nanoparticles in human hepatoma HepG2 cells. Nanotoxicology. 2011;5(3):341-53.
3. Alkreathy HM, Khan RA, Khan MR, Sahreen S. CCl 4 induced genotoxicity and DNA oxidative damages in rats: Hepatoprotective effect of Sonchus arvensis. BMC Complement Altern Med. 2014;14:1-7.
4. Hafez MM, Al-Shabanah OA, Al-Harbi NO, Al-Harbi MM, Al-Rejaie SS, Alsurayea SM, et al. Association between paraoxonases gene expression and oxidative stress in hepatotoxicity induced by CCl 4. Oxid Med Cell Longev. 2014;2014:92-112.
5. Parasuraman S, Kumar EP, Kumar A, Emerson SF. Free radical scavenging property and diuretic effect of triglize, a polyherbal formulation in experimental models. J Pharmacol Pharmacother. 2010;1(1):38-41.
6. Kivrak İ, Duru ME, Öztürk M, Mercan N, Harmandar M, Topçu G. Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of Salvia potentillifolia. Food Chem. 2009;116(2):470-9.
7. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: Possible implications in analgesic nephropathy. Biochem Pharmacol. 1984;33(11):1801-7. doi:10.1016/0006-2952(84)90353-8
8. Li X. Solvent effects and improvements in the deoxyribose degradation assay for hydroxyl radical-scavenging. Food Chem. 2013;141(3):2083-8. doi:10.1016/j.foodchem.2013.05.084
9. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem. 1982;126(1):131-8. doi:10.1016/0003-2697(82)90118-x
10. Jollow DJ, Mitchell JR, Zampaglione NA, Gillette JR. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology. 1974;11(3):151-69. doi:10.1159/000136485
11. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74.
12. Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. Data Br. 2016;6:194-9. doi:10.1016/j. dib.2015.12.012
13. El-Boshy M, Abdelhamidb F, Richab E, Ashshia A, Gaitha M, Qustya N. Attenuation of CCl4 Induced oxidative stress, immunosuppressive, HepatorenalDamage by fucoidan in rats. J Clin Toxicol. 2017;7(3):1-6.
14. Jayaraman J, Veerappan M, Namasivayam N. Potential beneficial effect of naringenin on lipid peroxidation and antioxidant status in rats with ethanol-induced hepatotoxicity. J Pharmacy Pharmacol. 2009;61(10):1383-90. doi:10.1211/jpp/61.10.0016
15. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. Nature. 2008;454(7203):455-62.
16. Toy H, Camuzcuoglu H, Camuzcuoglu A, Celik H, Aksoy N. Decreased serum prolidase activity and increased oxidative stress in early pregnancy loss. Gynecol Obstet Invest. 2010;69(2):122-7.
17. Burton GJ, Yung HW, Cindrova-Davies T, Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and early onset preeclampsia. Placenta. 2009;30:43-8.
18. Recknagel RO, Glende EA. Carbon tetrachloride hepatotoxicity: An example of lethal cleavage. CRC Crit Rev Toxicol. 1973;2(3):263-97.
19. Pompella A, Sies H, Wacker R, Brouns F, Grune T, Biesalski HK, et al. The use of total antioxidant capacity as surrogate marker for food quality and its effect on health is to be discouraged. Nutrition. 2014;30(7-8):791-3.
20. Sies H, Berndt C, Jones DP. Oxidative stress. Ann Rev Biochem. 2017;86:715-48.