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Regular paper

Dietary quercetin abrogates hepatorenal oxidative damage associated with dichloromethane exposure in rats

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Exposure to dichloromethane (DCM), a commonly used chlorinated solvent in industrial settings and for the production of many household products, reportedly elicits detrimental effects in animals and humans. The present study investigated the protective role of dietary quercetin on DCM-induced hepatorenal damage in rats. Experimental rats were orally administered with DCM (150 mg/kg) and 30 min later with quercetin at 10, 20 and 40 mg/kg or none for 7 consecutive days. The results indicated that DCM-mediated significant ($p<0.05$) increases in serum alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase and alkaline phosphatase activities as well as urea and creatinine levels were dose-dependently normalized to the control values in rats co-treated with quercetin. Further, quercetin co-treatment ameliorated DCM-mediated decrease in the hepatic and renal activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase as well as glutathione level in the treated rats. Moreover, quercetin co-treatment markedly reduced lipid peroxidation level and protected against histological changes in liver and kidney of the treated rats. Taken together, quercetin abrogated hepatorenal oxidative damage in DCM-treated rats *via* improvement of antioxidant status and suppression of oxidative damage.

Key words: dichloromethane, quercetin, hepatorenal, oxidative stress, rats

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Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; DCM, dichloromethane; DTNB, 5′, 5′-dithiobis-2-nitrobenzoic acid; GGT, gamma glutamyl transferase; GPx, glutathione peroxidase; GSH, glutathione; GST, glutathione-S-transferase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid; Qt, quercetin

INTRODUCTION

Dichloromethane (DCM; Fig. 1a) also known as methylene chloride is a colorless, volatile liquid with a sweet and pleasant fragrance. It is widely used as a solvent in pharmaceutical, textile and electronics manufacturing industries (Jianming *et al.*, 2014). Moreover, DCM is a major constituent of many household products specifically lubricants, varnish removers and valve cleaners (ATSDR 2000, HPD 2009). However, owing to incorrect handling, storage and disposal practices, DCM is normally released into the environment including surface water, groundwater, drinking water supply wells globally including the United States (Shestakova & Sillanpää, 2013, Hermon *et al.*, 2018).

Acute and chronic exposure to DCM reportedly poses great threat to humans and ecosystems due to its toxicity, persistence, and bioaccumulation in the food chain (Tobajas *et al.*, 2016). DCM, due to its lipophilicity is easily absorbed and metabolized in humans and animals resulting in multiple organ damage (Schlosser *et al.*, 2015). Further, DCM has been shown to undergo both microsomal oxidation and cytosolic glutathione-conjugation pathways in the liver (Evans & Caldwell, 2010; Schlosser *et al.*, 2015). Epidemiological findings indicated that exposure to DCM is common in workplace leading to toxicity (Bonfiglioli *et al.*, 2014). Previous experimental studies demonstrated that DCM is a lung and liver carcinogen in rodents (Condie *et al.*, 1983; Their *et al.*, 1998; Schlosser *et al.*, 2015). However, the precise biochemical mechanisms involved in DCM-mediated hepatic and renal toxicity is not fully understood.

Quercetin, also known as 3,5,7,3',4'-pentahydroxyflavone (Fig. 1b), is a naturally occurring polyphenolic flavonoid commonly found in fruits and vegetables, such as onions, cabbages, berries, wines and apples (Boots *et al.*, 2008, D'Andrea 2015). Quercetin daily dietary intake was estimated to be between 4 to 68 mg/day (Chen *et al.*, 2010). However, its quantity can rise to 200-500 mg/day in people that eat large amount of flavonoid-rich fruits and vegetables (Costa *et al.*, 2013). In fact, quercetin has an important nutraceutical and pharmaceutical applications (Nair *et al.*, 2010; Pandey *et al.*, 2018; Rauf *et al.*, 2018). It is commercially available as a dietary supplement with a recommended dosage of 1 g/day (Harwood *et al.*, 2007). Experimental findings demonstrated that quercetin elicits several health beneficial effects which is related to its ability to scavenge reactive oxygen radicals, inhibition of xanthine oxidase and oxidative stress *in vitro* and *in vivo* (Zizkova *et al.*, 2017; Ebegboni *et al.*, 2019; Houghton *et al.*, 2018). Quercetin reportedly elicits several health beneficial effects on human health specifically, anti-inflammatory, anti-cancer, anti-viral and neuroprotective effects (Adedara *et al.*, 2017; Sharma *et*

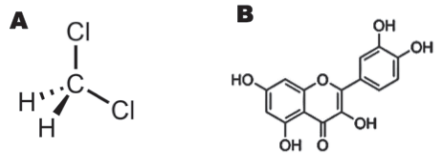

Figure 1. Chemical structures of the tested compounds: (A) Dichloromethane (DCM) and (B) Quercetin.

al., 2018; Babaei *et al*., 2018). Hitherto, there is no study in literature on the possible influence of quercetin on hepatic and renal toxicity induced by DCM. Thus, the present study investigated, for the first time, the role of quercetin on hepatorenal toxicity following exposure to DCM in male Wistar rats. To achieve this, we evaluated some hepatic and renal toxicity indices, antioxidant enzyme activities, lipid peroxidation along with histological alterations in the liver and kidney of experimental rats.

MATERIALS AND METHODS

Chemicals and reagents. Quercetin, dichloromethane (DCM), glutathione (GSH), thiobarbituric acid (TBA), epinephrine, 1-chloro-2,4-dinitrobenzene (CDNB), 5',5'-dithiobis-2-nitrobenzoic acid (DTNB) and hydrogen peroxide (H_2O_2) were procured from Sigma-Aldrich Company, St. Louis, Missouri, United States. All other chemicals used in this study were of analytical grade and were purchased from the British Drug Houses (Poole, Dorset, UK). The kits used for the assessment of liver and kidney tests were purchased from Randox Laboratories Limited (UK).

Animal model and care. Forty male Wistar rats (7 weeks old, 147±4 g) used for this study were purchased from the breeding colony of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The rats were kept in plastic cages located in a well-ventilated vivarium with natural photoperiod of 12-hr light: 12-hr dark cycle. The rats had free access to rat chow and water and were adequately cared for according to the conditions specified in the 'Guide for the Care and Use of Laboratory Animals' by the National Institute of Health. All experimental protocols were performed after approval by the University of Ibadan Ethical Committee. The rats were acclimatized for a week before commencement of the experiment.

Experimental design. The rats were randomly allotted to six groups of eight rats each and were treated for 7 consecutive days. Rats of group one were orally administered corn oil alone at 2 ml/kg. Group two rats were orally administered quercetin alone at 40 mg/kg whereas group three rats were orally administered DCM alone at 150 mg/kg. Groups four, five and six rats were orally co-administered DCM (150 mg/kg) and quercetin at 10, 20 and 40 mg/kg, respectively. Quercetin and DCM were separately dissolved in corn oil. Administration of DCM was done 30 min before quercetin treatment in the groups that received both compounds. The time interval between the treatments was to circumvent direct interaction between the compounds. The doses of DCM (150 mg/kg) and quercetin (10, 20 and 40 mg/kg) used in the present study were chosen from pilot study and previously published data (Adedara *et al*., 2017). Twenty-four hours following the final treatment, the animals were weighed and the samples of blood were taken from retro-orbital venous plexus into plain tubes. Thereafter, the rats were sacrificed using the cervical dislocation technique. Serum samples were subsequently prepared by centrifuging the clotted blood at 3000×*g* for 10 minutes. The serum samples were kept frozen at –20°C until assessment of liver and kidney function biomarkers. Moreover, liver and kidney samples were immediately excised, weighed and processed for biochemical and histological analyses.

Analysis of liver and kidney function indices. Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were analyzed to assess the liver functional status whereas serum urea and creatinine levels were analyzed to assess to kidney functional status using available commercial kits.

Assessment of hepatic and renal antioxidant status. Liver and kidney samples were homogenized in four volumes of 50 mM Tris/HCl buffer (pH 7.4). The homogenates were subsequently centrifuged at 12000×*g* for 15 min at 4°C and the supernatants were used for biochemical evaluations. Hepatic and renal protein concentration was assayed using bovine serum albumin as standard according to established method (Bradford 1976).

Assay of superoxide dismutase activity. Superoxide dismutase (SOD) activity was assayed by evaluating the inhibition of autooxidation of epinephrine according to Misra and Fridovich (Misra & Fridovich, 1972). Succinctly, the reaction mixture consisted of 20 μL of the tissue sample, 2.5 mL of 0.05 M carbonate buffer (pH 10.2) and 300 μL of 0.3 mM epinephrine. The increase in absorbance at 480 nm was monitored for 150 s at interval of 30 s using a UV-visible spectrophotometer (Shimadzu, Japan).

Assay of catalase activity. Catalase (CAT) activity was assayed by monitoring the rate of disappearance of H_2O_2 according to Aebi (Aebi, 1984). Succinctly, the reaction medium contained 1.8 mL of 50 mM phosphate buffer (pH 7.0), 180 μL of 300 mM H_2O_2, and 20 μL of the tissue sample. The reaction was analyzed at 240 nm for 2 min at intervals of 10 s using a UV-visible spectrophotometer (Shimadzu, Japan).

Assay of glutathione level. Glutathione (GSH) level was assayed according to Ellman (Ellman, 1959). Tersely, an aliquot of tissue homogenate was mixed (1:1) with 10% TCA to precipitate the protein. The resulting supernatant was then centrifuged at 5000×*g* for 5 min and the free thiol groups were assessed in the supernatant. The reaction mixture containing 50 μL of the tissue sample, 450 μL phosphate buffer and 1.5 mL of 0.1 mM DTNB was incubated for 10 min at room temperature. The absorbance of 200 μL of the mixture was then measured at 412 nm using a SpectraMax plate reader (Molecular Devices, CA, USA).

Assay of glutathione peroxidase activity. Glutathione peroxidase (GPx) activity was assayed according to Rotruck and coworkers (Rotruck *et al*., 1973). Tersely, the assay mixture comprising of 50 μL phosphate buffer, 20 μL of 4.0 mM GSH, 10 μL of 2.5 mM H_2O_2, 10 μL of 10.0 mM sodium azide and 5 μL of the tissue sample was prepared and the total volume was made up to 200 μL with distilled water. Subsequently, the assay mixture was incubated at 37°C for 3 min, the reaction was terminated by adding 50 μL of TCA (10%) followed by centrifugation at 5000×*g* for 5 min. The residual GSH level was then assayed spectrophotometrically at 412 nm using 50 μL of the supernatant, 200 μL of disodium hydrogen phosphate (0.3 M) solution and 50 μL of 0.1 mM DTNB.

Assay of glutathione-S-transferase activity. Glutathione-S-transferase (GST) activity was assayed using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate according to Habig and others (Habig *et al*., 1974). Succinctly, the reaction mixture consisted of 270 μL of a cocktail (20 mL of 0.25 M phosphate buffer, pH 7.0, 500 μL of 0.1 M GSH and 10.5 mL of distilled water), 20 μL of tissue sample and 10 μL of 25 mM CDNB. The reaction was monitored at 340 nm for 5 min at intervals of 30 s using a SpectraMax plate reader (Molecular Devices, CA, USA).

Assay of lipid peroxidation. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) during an acid-heating reaction according to standard procedure (Farombi *et al*., 2000). Briefly, reaction mixture consisted of 800 µL of phosphate buffer, 200 µL of tissue sample, 500 µL of 10% TCA and 500 µL of 0.75% TBA in 0.1 M HCl. The mixture was subsequently heated at 90°C for 20 min, cooled to room temperature and centrifuged at 5000×*g* for 10 min. The absorbance of the resulting supernatant was read at 532 nm and the MDA level was calculated using the extinction coefficient (Σ) of 1.56×10^5 L/mol/cm.

Histological examination. Representative samples of the liver and kidney were processed through routine stages of fixation, dehydration, clearing, infiltration and embedding before sectioning to 5 µm thickness using a Rotary Microtome (Leica RM2125 RTS, Germany). The slides were stained with haematoxylin and eosin according to standard protocol (Bancroft & Gamble, 2008). All the slides were coded before examination under a light microscope (Leica DM 500, Germany) by pathologists.

Statistical analyses. Data were presented as mean ± standard deviation. Statistical analyses were executed using one-way analysis of variance test followed by Bonferroni's post-test using GRAPHPAD PRISM 5 software (Version 4; GraphPad Software, La Jolla, California, USA). Statistical significance was set at $p<0.05$.

RESULTS

Effect of quercetin on biomarkers of hepatic and renal damage in DCM-treated rats

There were no treatment-related effect on the body weight gain and the relative weight of the liver and kidney in all the treatment groups. Renal toxicity was verified by assessing the serum levels of urea and creatinine whereas hepatotoxicity was verified by assessing the serum activities of AST, ALT, GGT and ALP. In comparison with the control rats, DCM-treatment resulted in a significant ($p<0.05$) increase in all the indices of hepatic and renal damage in the serum (Fig. 2). However, administration of quercetin to the DCM-treated rats caused a marked reduction to near normal in the serum activities of these biomarkers. Specifically, DCM administration increased the serum activities of AST, ALT, GGT and ALP by 193%, 167%, 185% and 212%, respectively, when compared with the control rats. On the other hand, co-administration of Qt_{10}, Qt_{20} and Qt_{40} to the DCM-treated rats caused a dose-dependent restoration in ALT activity by 51%, 69% and 75% and AST activity by 36%, 60% and 73% respectively, in the treated animals. Similarly, GGT activity was restored by 41%, 60% and 74% whereas ALP activity was restored by 46%, 58% and 79%, respectively, following co-administration of Qt_{10}, Qt_{20} and Qt_{40} to DCM-treated rats. Moreover, DCM administration alone increased serum urea and creatinine levels by 294% and 108%, respectively, when compared with the control rats. However, post-administration of Qt_{10}, Qt_{20} and Qt_{40} to the DCM-treated animals decreased the urea level by 48%, 64%, and 85%, respectively, whereas creatinine level was reduced by 50%, 70 % and 80%, respectively.

Effect of quercetin on hepatic and renal antioxidant status in DCM-treated rats

Figure 3 shows the influence of quercetin administration on antioxidant enzymes, GSH and lipid peroxidation in the kidney and liver of rats. Acute exposure to DCM alone resulted in a significant ($p<0.05$) decrease in the SOD, CAT, GPx and GST activities and GSH level in the kidney and liver of the treated rats. Con-

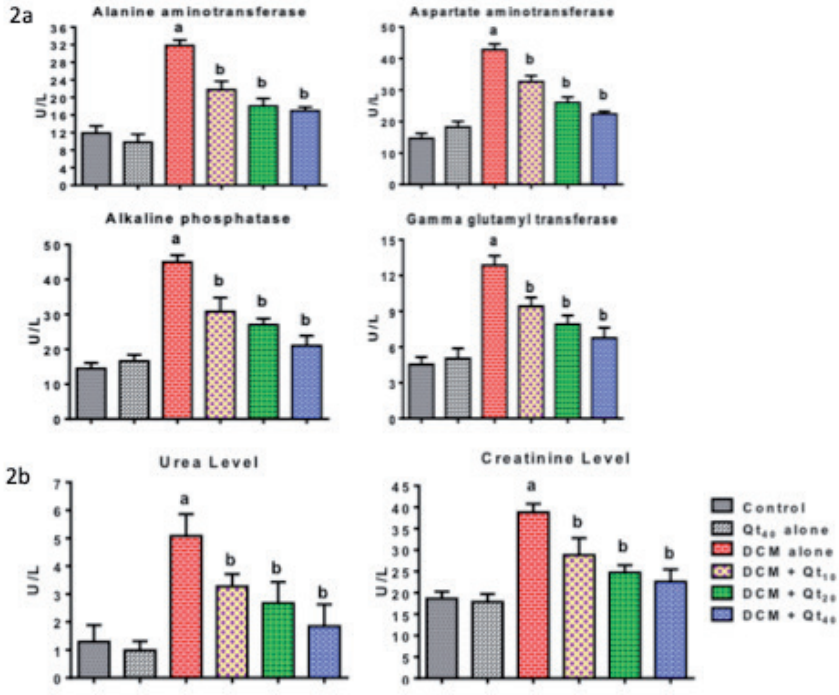

Figure 2. Effects of quercetin on biomarkers of (a) hepatic and (b) renal damage in DCM-treated rats.
DCM, 150 mg/kg dichloromethane; Qt_1, Qt_2 and Qt_3 denote 10, 20 and 40 mg/kg of quercetin, respectively. Each bar is mean ± S.D. of ten rats per group [a]Values differ significantly from control ($p<0.05$). [b]Values differ significantly from DCM alone group ($p<0.05$).

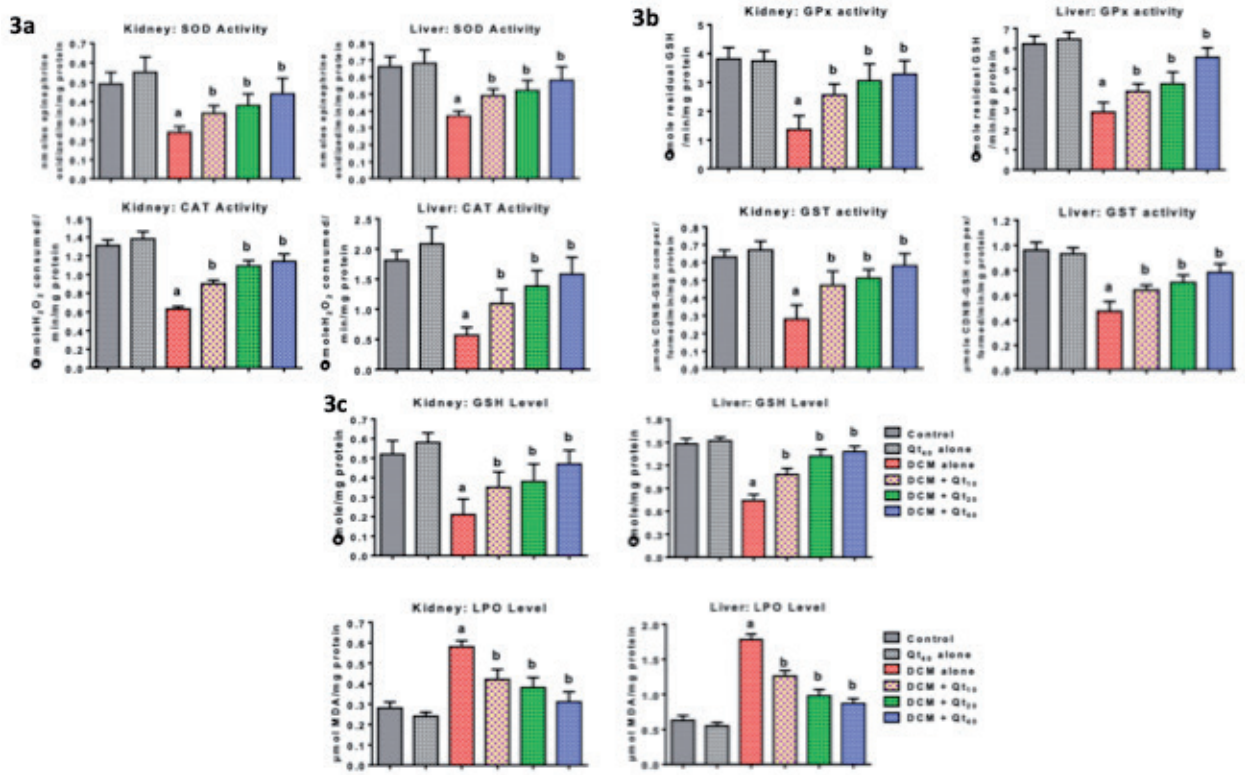

Figure 3. Effects of quercetin on (a) SOD, CAT, (b) GPx, GST, (c) GSH and LPO levels in liver and kidney of DCM-treated rats.
DCM, 150 mg/kg dichloromethane; Qt_1, Qt_2 and Qt_3 denote 10, 20 and 40 mg/kg of quercetin, respectively. Each bar is mean ± S.D. of ten rats per group [a]Values differ significantly from control ($p<0.05$). [b]Values differ significantly from DCM alone group ($p<0.05$).

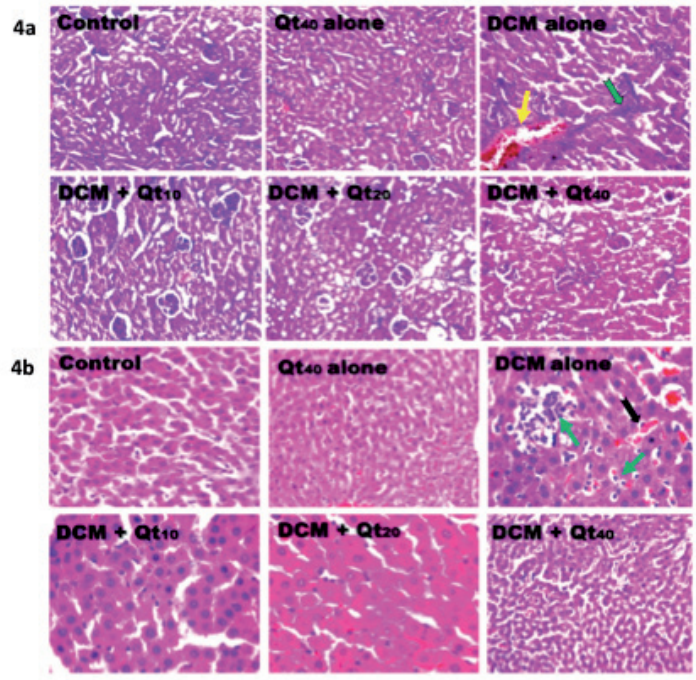

Figure 4. Representative photomicrographs of the kidney and liver from control and experimental rats.
(**a**) Kidney of control and quercetin alone rats showing normal morphology. Kidney of rats treated with DCM alone showing congestion of vessels (yellow arrow) and infiltration of inflammatory cells (green arrow). Kidney of rats co-exposed to DCM and quercetin at 10, 20 and 40 mg/kg appear somewhat similar to control. (**b**) Liver of rats treated with DCM alone showing marked disseminated congestion (black-notched arrow), focal area of lymphoid aggregate and mild infiltration of zone 2 by inflammatory by cells (green arrow). Liver of rats co-exposed to DCM and quercetin at 10, 20 and 40 mg/kg showing appear somewhat similar to control. Original magnification: 200x.

versely, administration of quercetin mitigated against the decrease in the SOD, CAT, GPx and GST activities as well as GSH level, and re-established the hepatic and renal levels to near normal in DCM-treated rats. Furthermore, administration of DCM alone caused a significant increase in the levels of MDA, an established known index of lipid peroxidation, in the kidney and liver of the treated rats. However, administration of quercetin to DCM-treated rats markedly reduced MDA levels in the liver and kidney when compared with DCM alone group. DCM administration alone increased MDA level in the liver and kidney by 183% and 105%, respectively, when compared with the control rats. Administration of Qt_{10}, Qt_{20} and Qt_{40} to the DCM-treated caused renal MDA level to decrease by 53%, 66%, and 90 % whereas hepatic MDA level was reduced by 45%, 70 % and 79%, respectively.

Effects of quercetin on DCM-induced histopathological lesions in liver and kidney of rats

The representative photomicrographs of the kidney and liver of experimental rats are depicted in Fig. 4. The glomeruli and hepatocytes of control rats appeared normal with well-preserved morphology, in contrast to the kidney of rats administered DCM alone that exhibited congestion of vessels (yellow arrow) and infiltration of inflammatory cells (green arrows). The liver of rats administered DCM alone exhibited marked disseminated congestion (black notched arrow), focal area of lymphoid aggregate with mild infiltration of zone 2 by inflammatory cells (green arrow). The lesions identified in kidney and liver of DCM-treated rats was independent of the vehicle corn oil. However, the kidney and liver of rats co-administered with quercetin exhibited normal structure. The renal and hepatic cells of rats co-administered with Qt_{10}, Qt_{20} and Qt_{40} appeared structurally and functionally normal.

DISCUSSION

This study demonstrates the beneficial role of quercetin on DCM-induced hepatorenal toxicity in rat and provides evidence that this effect is due to quercetin's antioxidant mechanism of action. DCM treatment resulted in elevated renal and hepatic MDA level. MDA and 4-hydroxynonenal are biomarkers of lipid peroxidation (Demir *et al*., 2011), attacking distant targets and covalently binding with biomolecules (Levent *et al*., 2006). Reduction in MDA level in rats co-treated with quercetin indicates its ability to protect against DCM mediated lipid peroxidation, due to its antioxidant activity (Xiao *et al*., 2018). DCM treatment also resulted in decreased hepatorenal antioxidant enzymes (SOD, CAT, GPx), essential for superoxide radical biotransformation to H_2O_2, thus preventing its damaging effects (Klaunig *et al*., 2011) on one hand and cytotoxicity by transforming H_2O_2 to water and oxygen (Kohen & Nyska, 2002; Adedara *et al*., 2012) on the other hand. Decrease in antioxidant enzymes results in ROS production. ROS bioaccumulation aggravates oxidative stress, disrupts cellular membrane integrity and induces cytotoxic damage (Ratliff *et al*., 2016; Pizzino *et al*., 2017). DCM treated rats; antioxidant activities were enhanced by quercetin co-treatment. Thus, the restoration of hepatorenal antioxidant capacity in quercetin-treated rats is attributed to its free radical scavenging ability.

Glutathione is well known to interact directly with ROS using its thiol group or act as a co-substrate during biochemical conjugation of xenobiotics by GST to prevent cellular oxidative damage (Singh *et al*., 2002). Reduction in GSH level and GST activity in the target organs of DCM-treated rats suggests utilization of GSH and GST inhibition. Co-treatment with quercetin reversed this trend, further highlighting the benefit of quercetin.

Elevated hepatic transferases in serum manifest hepatocellular injury and dysfunction (Kaplan, 1993; Lin *et al*., 2003; Adedara *et al*., 2010; Owumi *et al*., 2019). Since they are located primarily in hepatocytes, their increased serum activities indicate hepatocyte membranes injury. Similarly, elevation in serum urea and creatinine levels indicates renal dysfunction observed in DCM-treated rats. Increased creatinine level connotes impairment in glomerular filtration rate of kidney, whereas elevation of the urea level signifies diminished re-absorption at the renal epithelium (Adedara *et al*., 2012). Quercetin effectively chemo-protects rats against DCM-mediated oxidative damage, decreases hepatorenal injury biomarkers, possibly *via* preservation of membrane integrity and enhancing endogenous antioxidant capacity.

Histopathological examination of the kidney and liver supports biochemical data on DCM-induced oxidative injury in treated rats. Kidneys of the treated animals showed vascular congestion and infiltration of inflammatory cells, whereas the liver presented marked disseminated congestion and focal area of lymphoid aggregate. Co-treatment with quercetin limited DCM-induced tissues injury.

Overall, biochemical and histological data showed that quercetin conferred dose-dependent protection against DCM-induced hepatorenal injury. Conclusively, DCM mediated induction of oxidative stress is correlated with hepatorenal impairment. Quercetin abrogated this trend by mechanisms associated with inhibition of lipid peroxidation and enhancement of antioxidant defense status. Dietary supplementation with quercetin may hold promise against hepatorenal injury due to the exposure to DCM or related chemical compounds.

Conflict of interest

The authors have no conflicts of interest to declare.

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