

Methyl jasmonate and diphenyl diselenide attenuate diethylnitrosamine and carbon tetrachloride-induced nephrotoxicity in rats via modulation of inflammation and oxidative stress processes

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Date Received: 07-06-2023

Date Accepted: 28-10-2024

DOI: <https://doi.org/10.48198/NJPAS/23.B05>

ABSTRACT

The pathogenesis of toxicity-mediated renal injury has been linked to reactive oxygen species. Therefore, intervention with antioxidant agents as part of therapeutic strategies is necessary. This study investigated the modulatory effects of Diphenyldiselenide (PhSe)₂ and Methyl Jasmonate (MJ) against Diethylnitrosamine (DEN) and Carbon tetrachloride (CCL₄)-induced nephrotoxicity in male *Wistar* rats. Forty-eight (48) rats were assigned into six groups of eight animals each. Group 1 served as control, Group 2 received DEN (100mg/kg) + CCL₄ (0.5mg/kg), Group 3 received [DEN+CCL₄] and treated with MJ (50 mg/kg), Groups 4 and 5 received [DEN+CCL₄] and treated with (PhSe)₂ (5 mg/kg and 10 mg/kg), and Group 6 received [DEN+CCL₄] and treated with quercetin (50mg/kg). Administration of DEN and CCL₄ increased serum urea, creatinine and kidney injury molecule-1 (KIM-1) by 97%, 62% and 73%. Furthermore, activities of glutathione-S-transferase, superoxide dismutase and catalase significantly ($P < 0.05$) decreased in [DEN+CCL₄] rats. The BCL-2 associated X, caspases -3 and -9 increased in rats administered-[DEN+CCL₄]. In addition, TNF- α increased by 117%, myeloperoxidase and nitric oxide level increased by 385% and 65%, respectively in [DEN+CCL₄]-administered rats. Histology showed mild vascular congestion and infiltration of inflammatory cells in interstitial spaces in [DEN+CCL₄] rats. Interestingly, treatment with methyl jasmonate and diphenyl diselenide attenuated kidney function markers, reduced tissue inflammation and apoptosis status of [DEN+CCL₄]-rats. Methyl jasmonate and diphenyldiselenide abate nephrotoxicity caused by combined administration of DEN- and CCL₄. via induction of antioxidative and anti-inflammatory activities.

Keywords: Methyljasmonate, Diphenyldiselenide, Diethylnitrosamine, carbon tetrachloride

Introduction

Environmental exposure to hazardous chemicals and its effects on the renal system have recently garnered attention due to an increase in

chemically-induced nephrotoxicity and the potential for exposed individuals to experience kidney injury (Kuzma *et al.*, 2021). Research has shown that kidney function is severely

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compromised by oxidative stress, which is caused by a number of factors, such as life style, occupational exposure, environmental toxicants, etc., causing the reactive oxygen species (ROS) to upset the balance between antioxidants and ROS (Krata *et al.*, 2018). The Diethylnitrosamine (DEN) and Carbon tetrachloride (CCl₄) are environmental and occupationally encountered chemicals that have the potential to damage the kidneys (Habashy *et al.*, 2021). It has been reported that the distribution of DEN and CCl₄ in renal tissue is much higher in kidney than in the liver (Hassan *et al.*, 2015). A key explanation for the toxic effects of CCl₄ in the renal tissues is the activation of the cytochrome P450 system to generate trichloromethyl and chloride radicals, highly reactive free radicals which binds to electrons in the tissues, resulting in protein peroxidation and DNA damage (Alkreathy *et al.*, 2014).

Oxidative stress is a feature of several kidney pathologies and complications are linked to oxidative stress mediators, and inflammation (Ganie *et al.*, 2011). The main mechanisms of DEN and CCl₄-induced nephrotoxicity have been identified to include oxidative stress, inflammation, and apoptosis (Adeyanju *et al.*, 2018). Due to their reactivity causing overproduction of reactive oxygen species, DEN and CCl₄ may target and change a variety of components, including proteins, lipids, and DNA (Hawkins and Davies, 2019).

Additionally, it has been documented that an increase in ROS is linked to the activation of a number of crucial signaling pathways that limit caspases activities, prevent their execution, and subsequently activate pathways that leads to nephrotoxicity (Barnett and Cummings, 2019).

Antioxidants and anti-inflammatory agents commonly found in natural products have been suggested to mitigate chemically-induced renal toxicity (Reyes-Gordillo *et al.*, 2018). Due to their capacity to combat oxidative stress and inflammation, natural products may be efficient

chemopreventive agent to attenuate nephrotoxicity (Fang *et al.*, 2021). Diphenyl diselenide (PhSe)₂ is an organo-selenium, powerful antioxidant, antiviral, and immuno-modulatory agent (Domracheva *et al.*, 2017). Experimental investigations have shown that (PhSe)₂ reduces the levels of pro-inflammatory cytokines and ROS in a variety of pathologies (Fulco *et al.*, 2020; Marques *et al.*, 2021). In the contrary, at a higher dosage of (PhSe)₂, it has been reported that this agent potentiates HgCl₂ nephrotoxicity in animals (Brandao *et al.*, 2011). Therefore, there is the paucity of information on the effect of (PhSe)₂ on other model of nephrotoxicity in animals. The jasmonate family includes methyl jasmonate (MJ), which is present in jasmine plants (Farooqi *et al.*, 2012). Studies on human cancer cell lines have shown the anti-cancer capacities of MJ and its mechanism of action (Bo *et al.*, 2022). In addition, other biological activities of MJ are known (Umukoro and Olugbemide, 2011; Yousefi *et al.*, 2020). This study therefore, was designed to investigate the effects of (PhSe)₂ and MJ on DEN and CCl₄-induced nephro-toxicity in rats.

Materials and Methods

Chemicals

Diethylnitrosamine (DEN), carbon tetrachloride (CCl₄), diphenyl diselenide (PhSe)₂ and methyl jasmonate (MJ) were procured from British Drug House (BDH), Poole, UK. All other chemicals and reagents used in this study are of pure quality.

Animals and Treatment

Male *Wistar* rats weighing between 80-85g were acquired from animal breeding unit at the Faculty of Basic Medical Sciences, University of Ibadan. Animals were held in properly aerated rat cages and maintained at ambient temperature (28^oC) under a regulated 12:12 light/dark phases. The animals were nourished with standard laboratory feed and water *ad libitum*. Experimental protocol, rats handling and treatment were approved on the 19th of November 2015 by the University of

Ibadan Animals' Ethics Committee (UI-ACUREC/App/2015/061). All methods were carried out in accordance with relevant guidelines and regulations.

Experimental design

A total of forty-eight rats were allowed to acclimatize for two weeks and were assigned into six groups of eight rats each. Group 1 served as control and received corn oil only, Group 2 received DEN (100 mg/kg) + CCL₄ (0.5 mg/kg), Group 3 received DEN + CCL₄ and treated with methyl jasmonate (50 mg/kg), Group 4 and 5 received DEN + CCL₄ treated with diphenyl diselenide at 5 mg/kg and 10 mg/kg, respectively, and Group 6 received DEN + CCL₄ treated with Quercetin (50 mg/kg). The DEN and CCL₄ were administered intraperitoneally once per week for a period of three weeks after which methyl jasmonate, diphenyl diselenide and quercetin were administered via oral gavage thrice weekly for another three consecutive weeks.

Preparation of tissues and serum

Food was withheld from the animals overnight after twenty-four hours following the final dose of methyl jasmonate, diphenyl diselenide, and quercetin. Animals were sacrificed through cervical dislocation. Blood was collected by ocular puncture using capillary tubes into plain sample bottles, and centrifuged at a speed of 3,000 g for 15 minutes to obtain serum. The kidney tissues were meticulously harvested, rinsed in 1.15% potassium chloride (KCL) buffer, blotted using filter paper, and the kidney's weight was taken and noted. For histological evaluation, one of the kidney pair was fixed in Boiun's solution, and the other pair was homogenized in phosphate buffer (0.1 M, pH 7.4). The resultant homogenate was subjected to cold centrifugation at 10,000 g for 20 minutes to obtain post-mitochondrial fraction (PMF), which was used to assess various biochemical parameters.

Biochemical assays

Renal function markers

Serum urea was determined according to the method of Kumar *et al.* (2018) in which ammonia was measured photometrically by Barthelot's reaction. Also, serum creatinine was determined according to the method of Wolfgangs (Wolfgang *et al.*, 2004).

Protein determination

Levels of renal and serum protein were estimated using bovine serum albumin (BSA) as a reference in a method described by Kresge (2005).

Assay of Superoxide Dismutase activity

The activity of renal superoxide dismutase activity was evaluated following the autoxidation of adrenaline by superoxide radical produced by xanthine oxidase in a method described by McCord and Fridovich, modified by Weydert *et al.* (2010). The reaction mixture contained an aliquot of 0.2 mL of the diluted sample and 2.5 mL of 0.05 M carbonate buffer (pH 10.2). The reaction began by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture which was quickly mixed by inversion. The optical density was estimated at 480 nm every 30 seconds for 150 seconds.

Assessment of Catalase activity

Renal catalase activity was estimated according to the method described by Weydert *et al.* (2010). The reaction contained 20 μ L of phosphate buffer (50 mM and pH 7.0), and 10 μ L of 19 mM H₂O₂ with 50 μ L of the sample added. The mixture was left to run for a period of 3 mins. The reaction process was stopped when 20 μ L of dichromate in acetic acid solution was added, after which it was subjected to heating in a boiling water bath for 10mins. The resulting solution was left to cool and the optical density taken at 570nm.

Estimation of Glutathione-S- Transferase activity

Renal glutathione-S-transferase activity was

determined according to the method of Habig using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate, modified by Moatamedi *et al.* (2014). The reaction mixture contained 30 μ L reduced glutathione, 150 μ L of CDNB and 2.82 mL of 0.1 M phosphate buffer. The optical density was read at 340 nm against blank at 60 seconds interval for 3 mins. Activity of GST was determined using 9.61 $\text{mmol}^{-1} \text{cm}^{-1}$ extinction coefficient.

Reduced glutathione (GSH)

The level of renal GSH was estimated by the method of Boutolleau *et al.* (1997). Succinctly, 2 mL of 4% sulphosalicylic acid solution was added to the diluted test sample (1mL) to deproteinize it and then centrifuged for 15mins at 3000g. Thereafter, 0.5 mL of the supernatant was added to 4.5mL of Ellman's reagent, and readings were taken within 5 mins at 412 nm using spectrophotometer.

Determination of Total thiol

The Boutolleau *et al.* (1997) method was used to determine total sulphhydryl level in the renal PMF samples. An aliquot of the sample was added to Ellman's [5',5'-dithiobis-(2-nitrobenzoic acid)] reagent and readings were taken at optical density of 412nm after 30 minutes of incubation.

Estimation of Nitric Oxide Level

The levels of nitrite in the samples were evaluated following the Griess reaction as described by Palmer (Palmer *et al.*, 1988). Briefly, 0.5 mL of sample was incubated with 0.5 mL of Griess's reagent [0.2% Naphthyl-ethylenediamine dihydrochloride and 2% sulfanilamide in 5% phosphoric acid] at room temperature for 20mins. The optical density was measured at 550 nm spectrophotometrically.

Myeloperoxidase Activity

Myeloperoxidase (MPO) is a marker of polymorphonuclear leukocyte accumulation. Its activity was evaluated by the modified method of Trush (Trush *et al.*, 1994). Briefly, the reaction

mixture contained 200 μ L of *O*-dianisidine, 50 μ L of diluted H_2O_2 and 7 μ L of the sample. The optical density of the solution was measured every 30 seconds for 3 mins at 470 nm using spectrophotometer.

Determination of TNF- α , Interleukin-1 β (IL-1 β) and kidney injury molecule-1 (KIM-1)

The analytical kits used in this study followed the Sandwich-ELISA principle (Andreucci *et al.*, 2017). Briefly, standards or samples were added to the micro-ELISA plate wells (pre-coated with an antibody specific to rat's kim-1, TNF- α or IL-1 β), which then reacted with the specific antibody. A biotinylated detection antibody specific for rat's kim-1, TNF- α or IL-1 β and Avidin-Horseradish Peroxidase (HRP) conjugate was added successively to each micro-plate well and incubated. Free components were then washed away and the substrate was added to each well. Only those wells that contained rat's kim-1, TNF- α or IL-1 β -biotinylated detection antibody and Avidin-HRP conjugate appeared in colour. The enzyme-substrate reaction was terminated by the addition of stop solution and the colour changed to pink or yellow. The optical density (OD) was measured at a wavelength of 450 nm. The OD is proportional to the concentration of rat's kim-1, TNF- α or IL-1 β in the samples, and was obtained by extrapolation from standard curves.

Immunohistochemical analysis

The kits for immunohistochemical assay for expressions of Caspase-3, Caspase-9, p53, Bax, Bcl-2 and COX-2 were obtained from ElabScience China. The modified method of Chakravarthi *et al.*, (2010) was used for the assay and the principle is based on the binding of a primary antibody (dilution of 1:100 or as given by the manufacturer) to a specific antigen. The antibody-antigen complex formed was incubated with a secondary, enzyme-conjugated antibody. In the presence of substrate and chromogen, the enzyme acted on the substrate to generate coloured deposits at the sites of antibody-antigen binding which was observed

under a binocular microscope. Cells with specific distinct colour in the cytoplasm, cell membrane or nuclei, depending on the antigenic sites were considered positive, and compared with external controls. Antigen retrieval was performed by heating in a citric acid buffer (pH 6.0) at 100 °C for 15 mins.

Histopathology

Kidneys already fixed in Boiun's solution was dehydrated in 95% ethanol and cleared in xylene before embedded in paraffin. Micro sections (3-4 µm) were prepared and stained with haematoxylin and eosin (H&E) dye, and were examined under a light microscope (Olympus, USA) by a Histopathologist blinded to the study design.

Statistical analysis

Data were presented as Mean ± Standard Deviation and analysed by one-way analysis of variance followed by the post-hoc Duncan's multiple range test for analysis of biochemical data using SPSS (22.0) and GraphPad Prism 5.0. The differences were taken to be significant at p -value < 0.05.

Result

Effect of DEN and CCL₄ on body weight, organ's weight and serum biochemical indices

In table 1, the alteration of DEN+CCL₄- induction and treatment of MJ and (PhSe)₂ on animal body and kidney weight were illustrated. A significant decrease in body weight was observed in the DEN and CCL₄-induced animals (group 2) relative to control (group 1). There was considerable increase in the body weight of animals administered MJ and (PhSe)₂ (Table 1). On the contrary, the kidney weight of DEN and CCL₄-induced animals demonstrated a drastic elevation when compared to control (group 1). However, MJ and (PhSe)₂ treatment in DEN and CCL₄-induced animals significantly caused kidney weight reduction (Table 1). The DEN and CCL₄ increased serum

urea, creatinine and kidney injury molecule-1 (KIM-1) levels by 97%, 62% and 73% respectively, relative to controls (Figures 1 and 2). However, post-treatment with MJ and (PhSe)₂ at low and high doses markedly decreased KIM-1, urea and creatinine levels across the treated groups when compared to the [DEN+CCL₄] group.

Effect of MJ and (PhSe)₂ on inflammatory markers in [DEN+CCL₄]-administered rats

The level of tumor necrosis factor- alpha (TNF- α) and interleukin-1β (IL-1β) increased significantly (p <0.05) by 117% and 81% in [DEN+ CCL₄] rats relative to control, while post-treatment with MJ, (PhSe)₂ and Que decreased the levels of TNF- α and IL-1β by 43%, 60%, 51% and 43%, 50%, 45% respectively, when compared to the [DEN+ CCL₄] group (Figure 4 and 5). The DEN and CCL₄ significantly (p <0.05) elevated the activity of myeloperoxidase (MPO) and level of nitric oxide (NO) when compared to controls. Precisely, MPO and NO increased by 381% and 65%, respectively. Post-treatment with MJ and (PhSe)₂ (at both doses) and Quercetin drastically decreased (p <0.05) MPO and NO when compared to the [DEN+ CCL₄] rats (Figure 6 and 7).

Effect of MJ and (PhSe)₂ on antioxidant status of DEN and CCL₄- induced nephro-toxicity

The [DEN+CCL₄] significantly (P <0.05) decreased renal superoxide dismutase and catalase activities relative to controls, while post-treatment with MJ and (PhSe)₂ 1 increased these antioxidant enzymes by 27% and 28%; 22% and 20%, respectively (Figure 8 and 9). In addition, DEN and CCL₄ administration significantly (p < 0.05) decreased the activity of glutathione-S-transferase (GST) by 1.5 folds relative to the control group. Post-treatment with MJ, (PhSe)₂ (at both doses) and Que drastically elevated GST activities by 2.1, 2.0, 1.6 and 2.2 folds respectively (Figure 10). The DEN and CCL₄ decreased the total sulphhydryl (TSH) level by 15%, while treatment with MJ, (PhSe)₂ and Que elevated TSH levels by 16%, 26% and 20% respectively (Figure 11).

Effect of MJ and (PhSe)₂ on cyto-architecture and apoptotic proteins in [DEN+CCL₄] administered rats

Histology showed mild vascular congestion and infiltration of inflammatory cells in interstitial spaces in [DEN+CCL₄] rats (Figure 7). Also, the immuno-histochemistry revealed a strong expression of Caspase-3, Caspase-9, p53 and Bax

activities in the DEN and CCL₄ administered rats (Figures 8, 9, 10 and 11). Post-treatment with MJ, (PhSe)₂ and Que restored the cyto-architecture and decreased expression of caspase-3, caspase-9, p53 and Bax across the treated groups. In the same manner, DEN and CCL₄-administration elevated COX-2 while post-treatment with MJ, (PhSe)₂ and Que down-regulated this protein in the treated groups (Figure 12).

Table 1: Effects of methyl jasmonate and diphenyl Diselenide on body weight and organo-somatic weight of the kidney in male *Wistar* rats.

Treatments	Weight (g)			Kidney wgt (g)	Organo-somatic Weight (as %)
	Initial	final	Weight gain		
CONTROL	97.38±14.42	141.23±8.32	43.85±12.78	1.08±0.06	0.76±0.06
DEN+CCL ₄	101.13±10.43	128.20±20.32	27.07±12.88*	1.69±0.10	1.32±0.13*
DEN+CCL ₄ +MJ	95.50±6.74	146.50±38.33	51.00±36.03**	1.14±0.20	0.78±0.26**
DEN+CCL ₄ +(PhSe) ₂ 1	100.88±16.16	147.00±10.86	46.12±21.79**	1.12±0.15	0.76±0.13**
DEN+CCL ₄ +(PhSe) ₂ 2	103.63±9.50	135.80±19.58	32.17±20.08**	1.07±0.07	0.81±0.16**
DEN+CCL ₄ +Que	100.5±12.57	121.25±7.85	20.75±15.57**	0.94±0.08	0.77±0.07**

DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, DS1- Diphenyl diselenide (5mg/kg), DS2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8). *Significantly different from control (p<0.05), ** significantly different from DEN+CCL₄(p<0.05)

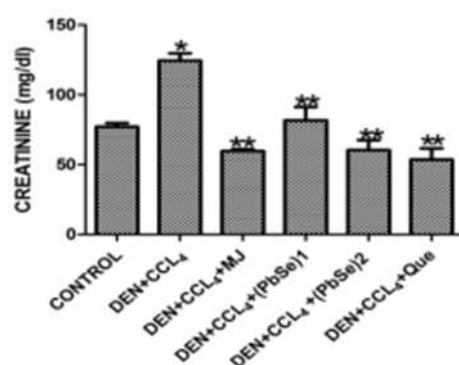


Fig. 1: Effect of methyl jasmonate and diphenyl diselenide in *Wistar* rats treated with DEN and CCL₄ on serum creatinine level. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values

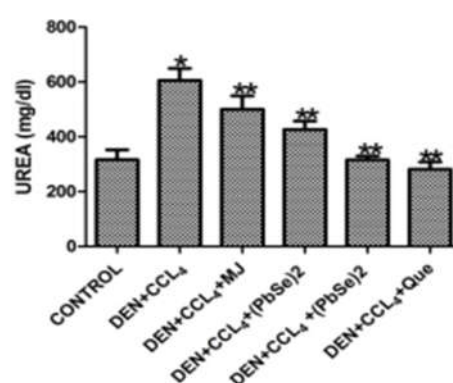


Fig. 2 : Effect of methyl jasmonate and diphenyl diselenide in *Wistar* rats treated with DEN and CCL₄ on serum urea level. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8), *significantly different from control (p<0.05), ** significantly different from DEN+CCL₄(p<0.05)

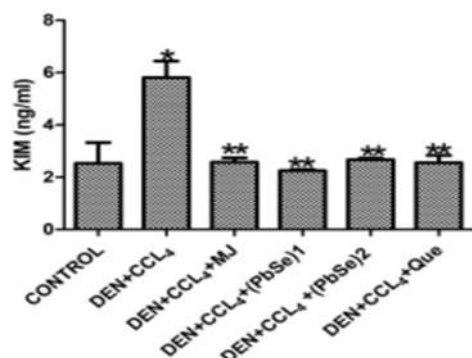


Fig 3: Effect of methyl jasmonate and diphenyl diselenide in *Wistar* rats treated with DEN and CCL₄ on KIM levels. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8), *significantly different from control (p<0.05), ** significantly different from DEN+CCL₄(p<0.05).

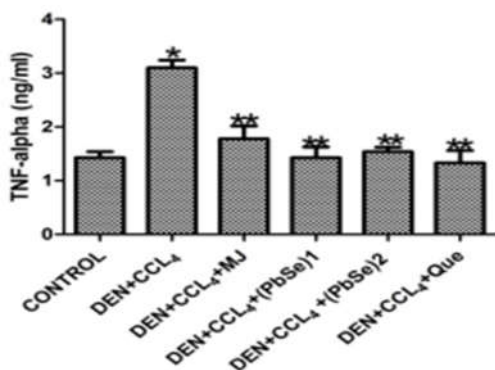


Fig. 4: Effect of methyl jasmonate and diphenyl diselenide in *Wistar* rats treated with DEN and CCL₄ on serum TNF-α level. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8). *Significantly different from control (p<0.05), ** Significantly different from DEN+CCL₄(p<0.05)

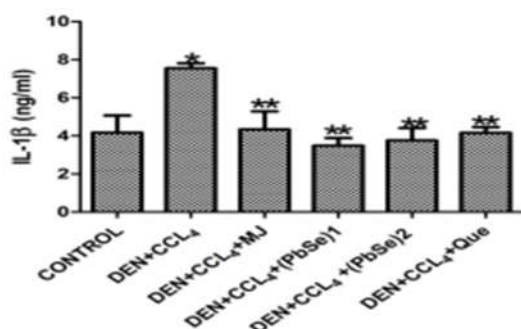


Fig. 5: Effect of methyl jasmonate and diphenyl diselenide in *Wistar* rats treated with DEN and CCL₄ on serum IL-1β level. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8). *Significantly different from control (p<0.05), ** Significantly different from DEN+CCL₄(p<0.05)

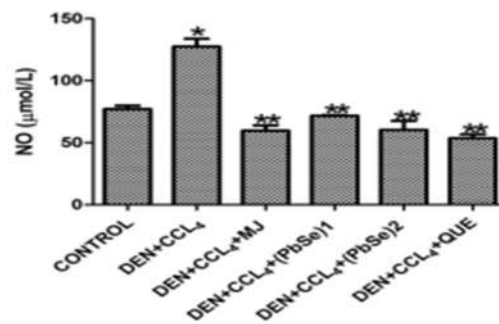


Fig. 6: Effect of methyl jasmonate and diphenyl diselenide in *Wistar* rats treated with DEN and CCL₄ on nephronal levels of nitric oxide (NO). DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8). *Significantly different from control (p<0.05), ** Significantly different from DEN+CCL₄(p<0.05)

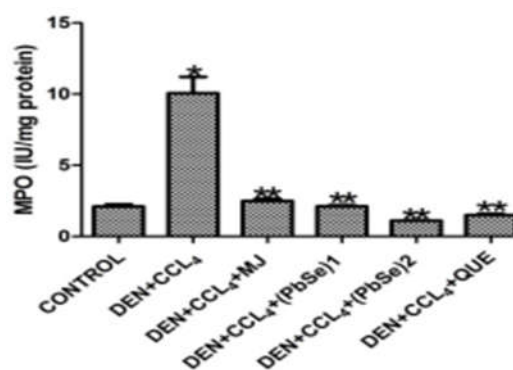


Fig. 7: Effect of methyl jasmonate and diphenyl diselenide in *Wistar* rats treated with DEN and CCL₄ on nephronal myeloperoxidase activity. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8). *Significantly different from control (p<0.05), ** Significantly different from DEN+CCL₄(p<0.05)

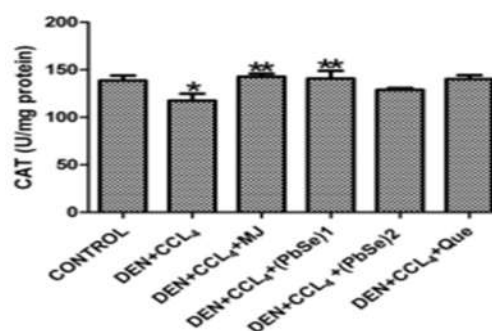


Fig 8: Effect of methyl jasmonate and diphenyl diselenide in *Wistar* rats treated with DEN and CCL₄ on catalase (CAT) activity. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8). *Significantly different from control (p<0.05), ** Significantly different from DEN+CCL₄(p<0.05).

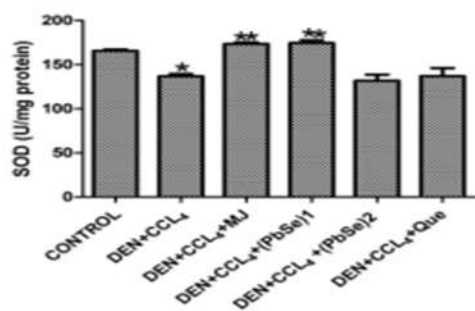


Fig. 9: Effect of methyl jasmonate and diphenyl diselenide in Wistar rats treated with DEN and CCL₄ on superoxide dismutase (SOD) activity. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8). *Significantly different from control (p<0.05), ** significantly different from DEN+CCL₄ (p<0.05).

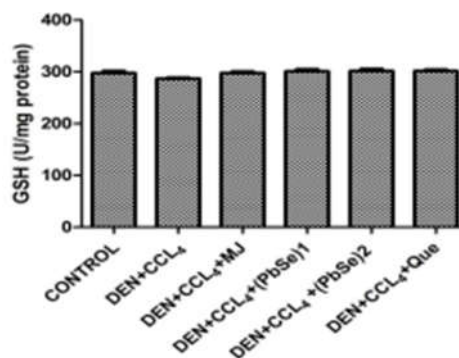


Fig. 12: Effect of methyl jasmonate and diphenyl diselenide in Wistar rats treated with DEN and CCL₄ on reduced glutathione (GSH) level. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg).

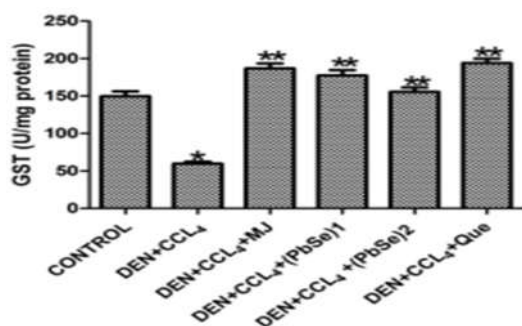


Fig. 10: Effect of methyl jasmonate and diphenyl diselenide in Wistar rats treated with DEN and CCL₄ on glutathione-S-transferase (GST) activity. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8). *Significantly different from control (p<0.05), ** significantly different from DEN+CCL₄ (p<0.05).

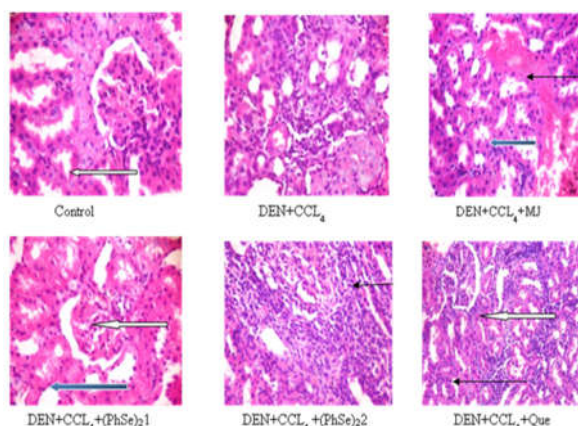


Fig. 13: Photomicrograph showing the effect of methyl jasmonate and diphenyl diselenide on nephron integrity of DEN and CCL₄-administered Wistar rats

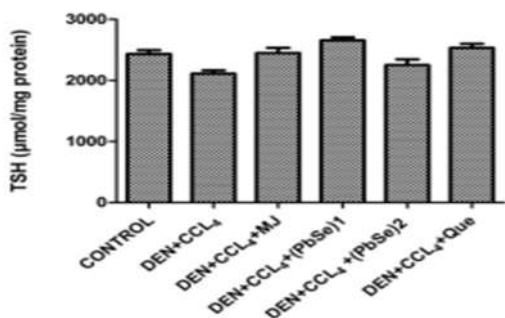


Fig. 11: Effect of methyl jasmonate and diphenyl diselenide in Wistar rats treated with DEN and CCL₄ on total thiol (TSH) level. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg).

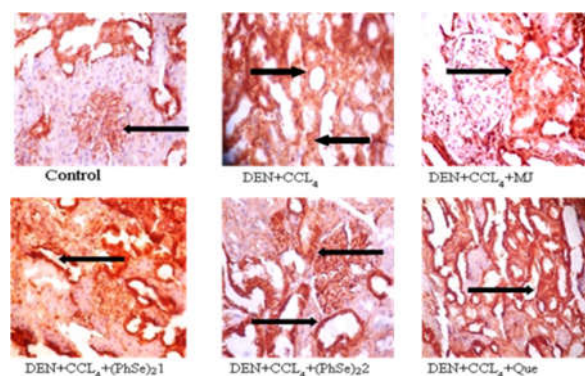


Fig. 14: Immunohistochemical staining of Caspase-3 activity in the kidney tissue of DEN and CCL₄-administered rats treated with methyl jasmonate and diphenyl diselenide. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). The black arrow showing expression of caspase-3.

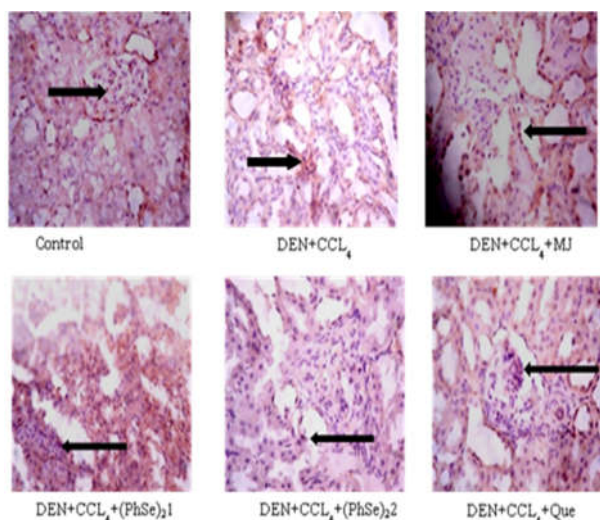


Fig. 15: Immunohistochemical staining of caspase-9 activity in the kidney tissue of DEN and CCL₄-administered rats treated with methyl jasmonate and diphenyl diselenide. DEN- Diethyl nitrosamine, CCL₄- Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₁- Diphenyl diselenide (5mg/kg), (PhSe)₂- Diphenyl diselenide (10mg/kg). The black arrow showing expression of caspase-9.

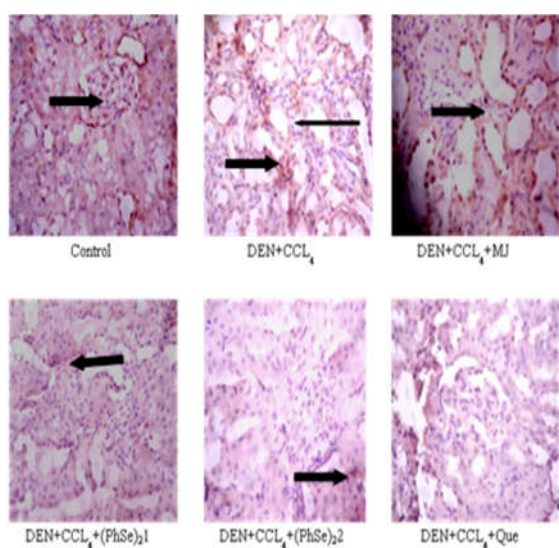


Fig. 16: Immunohistochemical staining of p53 activity in the kidney tissue of DEN and CCL₄-administered rats treated with methyl jasmonate and diphenyl diselenide. DEN- Diethyl nitrosamine, CCL₄- Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₁- Diphenyl diselenide (5mg/kg), (PhSe)₂- Diphenyl diselenide (10mg/kg). The black arrow showing expression of p53

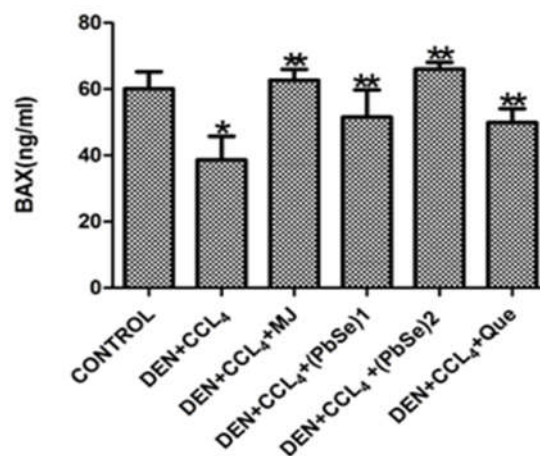


Fig. 17: Effect of methyl jasmonate and diphenyl diselenide in Wistar rats treated with DEN and CCL₄ on serum BAX activity. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₁- Diphenyl diselenide (5mg/kg), (PhSe)₂- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8), *Significantly different from control (p<0.05), ** Significantly different from DEN+CCL₄ (p<0.05).

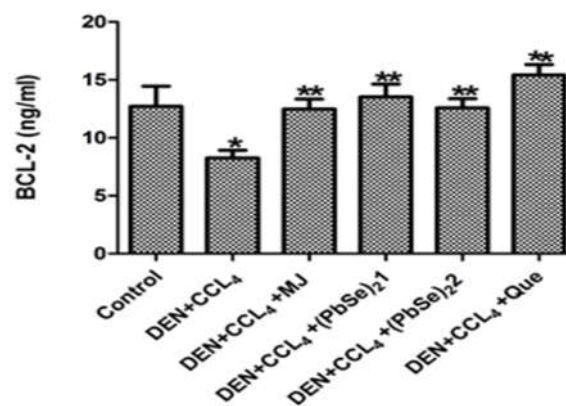


Fig. 18: Effect of methyl jasmonate and diphenyl diselenide in Wistar rats treated with DEN and CCL₄ on serum BCL-2 activity. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₁- Diphenyl diselenide (5mg/kg), (PhSe)₂- Diphenyl diselenide (10mg/kg). Values are expressed as Mean ± SD (n=8), *Significantly different from control (p<0.05), ** Significantly different from DEN+CCL₄ (p<0.05).

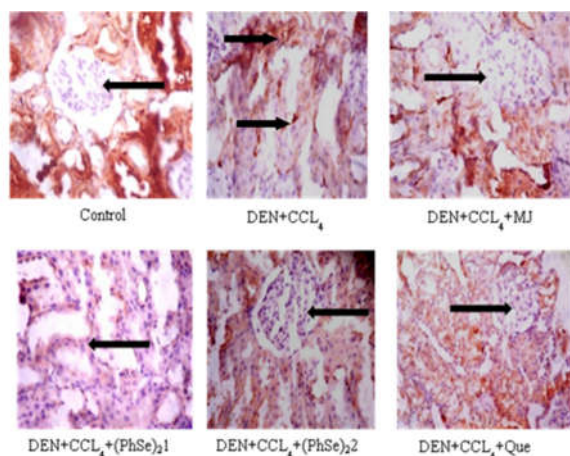


Fig. 19: Immunohistochemical staining of COX-2 activity in the kidney tissue of DEN and CCL₄-administered rats treated with methyl jasmonate and diphenyl diselenide. DEN- Diethyl nitrosamine, CCL₄-Carbon tetrachloride, MJ- Methyl jasmonate, (PhSe)₂1- Diphenyl diselenide (5mg/kg), (PhSe)₂2- Diphenyl diselenide (10mg/kg). The black arrow showing expression of COX-2.

DISCUSSION

Research into molecular mechanism of kidney diseases has increasingly focused on oxidative stress in the recent years (Habashy *et al.*, 2021). According to previous study, DEN- and CCL₄-induced nephrotoxicity have been attributed to oxidative stress (Adeyanju *et al.*, 2018). In experimental animal models, DEN and CCL₄ are frequently employed to cause organ toxicities, such as nephrotoxicity, cardiotoxicity, and hepatotoxicity. A study performed by Ganie *et al.* (2011) documented that CCL₄ exposure led to kidney damage due to reactive oxygen species. In the current study, an investigation was conducted to determine if methyl jasmonate and diphenyldiselenide could attenuate DEN and CCL₄-induced nephrotoxicity in rats. The investigation of the body and kidney weight in animals are the two preliminary indicators in evaluating the kidney's pathological state. Accordingly, drastic reduction in body weight and increased kidney weight was noted in the experimental rats challenged with DEN and CCL₄ which could associate with chronic kidney injury. However, methyl jasmonate, diphenyl diselenide intervention restored the body and kidney weight, indicating improved appetite and food

consumption, hence body weight reduction was prevented.

Urea and creatinine are both important biochemical markers for assessing kidney function. This present findings demonstrated DEN and CCL₄ induction drastically increased urea and creatinine, which are nitrogenous metabolic waste products eliminated from the blood by the kidney (Etim *et al.*, 2014). Elevation of these markers is a reflection of kidney metabolism instability. According to Makni *et al.* (2012), the increase in these parameters might be caused by structural damage to the renal corpuscle and tubule tissues. Following intervention with methyl jasmonate and diphenyl diselenide, a considerable decrease in creatinine and urea was seen, probably due to the improvement of pathological lesions in which the renal tubules appeared normal and more importantly, methyl jasmonate and diphenyl diselenide (at high dose) elicited similar protective effect as quercetin. These results are consistent with previously documented data by Elsaywy *et al.* (2019) who reported that rutin ameliorates CCL₄-induced hepatorenal toxicity and hypogonadism in male rats. This effect may be attributed to the free radical scavenging capacity of methyl jasmonate as reported from our previous studies (Adaramoye *et al.*, 2017; Akanni *et al.*, 2017).

Free radicals have been found to be produced during metabolism of DEN and CCL₄ in the kidney, liver, brain, and lungs (Hamed *et al.*, 2012). As a result of these free radicals, proteins are denatured, lipids are peroxidized, and cells undergo apoptosis in these tissues (Hismiogullari *et al.*, 2015). Lipid peroxidation, glutathion-s-transferase, catalase, superoxide dismutase and reduced glutathione are vital biomarkers for the assessment of oxidative stress. In animals, reduced glutathione serves as a powerful endogenous antioxidant that inhibits cellular damage by scavenging lipid peroxides and free radicals (Krata *et al.*, 2018). Our findings confirmed that administration of DEN and CCL₄ promotes oxidative stress which was evidenced by pronounced elevation of malondialdehyde and depletion of glutathion-s-transferase, glutathione

peroxidase, catalase and superoxide dismutase activities. The reduction in the antioxidant status of the rats is in consonance with the findings of Suzuki *et al.* (2015). Oral administration of methyl jasmonate and diphenyl diselenide caused a significant decrease in malondialdehyde and a marked increase in enzymatic and non-enzymatic antioxidant defense systems of the rats.

Oxidative stress may also cause inflammation in tissues via several pathways and mechanisms (Tarique *et al.*, 2016). In the normal kidney, KIM-1, a sensitive and specific marker for the presence of tubular damage, is not expressed (Peters *et al.*, 2011). However, after various types of renal injury, its expression is up-regulated and significantly elevated in proximal tubular epithelial cells. The severity, therapeutic response, and prognosis of a number of kidney disorders, have all been shown to be strongly linked with blood and urinary KIM-1 levels (Vaidya *et al.*, 2011). Given that KIM-1 is highly up-regulated protein in the proximal tubules, increase in kidney injury molecule-1 (KIM-1) observed in DEN and CCL₄ administered rats, provides a clear indication that the combination of these chemical is nephrotoxic. This result is in consistent with Lei *et al.* (2017) who reported KIM-1 expression was markedly elevated in both acute and chronic kidney injury which plays a pivotal role in macrophage activation via the MAPK pathway in kidney disease. Myeloperoxidase, a polymorphonuclear lysosomal enzyme predominantly present in the neutrophils and monocytes is involve in the oxidation of a variety of aromatic compounds by utilizing hydrogen peroxide (H₂O₂) produced by the neutrophils to give substrate radicals needed for oxidative stress. Our findings demonstrated increased in myeloperoxidase activity and nitric oxide levels upon administration of DEN and CCL₄. Taken together, our data indicates that, post-treatment with methyl jasmonate and diphenyl diselenide exhibited nephro-protection by lowering the elevated myeloperoxidase activity, nitric oxide and KIM-1 levels.

There is a clear link between oxidative stress and inflammatory cytokines in the current study. Administration of DEN and CCL₄ caused increase in IL-1 β and TNF- α in the blood of the rats. By activating different mechanisms, oxidative stress may enhance inflammation cytokine bursts. We found that methyl jasmonate and diphenyl diselenide significantly reduced IL-1 β and TNF- α , probably through the suppression of oxidative stress. Furthermore, Ranneh *et al.* (2017) reported that the activation of NF- κ B upregulates the gene expression of COX-2 activity in DEN and CCL₄-induced kidney injury. Similarly, in our study, we observed an increase in COX-2 expression in kidney tissues of the DEN and CCL₄-administered rats, whereas post-administration of methyl jasmonate and diphenyldiselenide produced a remarkable reduction COX-2 activities in the renal tissues.

Apoptosis can be mediated by few different signaling pathways that are associated with numerous regulatory molecules. During apoptosis, cells are programmed to die, and under normal condition, the cellular turnover in adult kidney is low, so apoptosis does not occur often. As a result of kidney injury, apoptotic reactions are highly active to remove damaged cells that have become irreparable (Galluzzi *et al.*, 2018). In the current study, we reported elevation in p53, BAX, caspases-3 and -9 levels in renal tissues in the group administered DEN and CCL₄, which indicates cellular damage in the kidney. These findings are in agreement with that of Hwang *et al.* (2013) and Hassan *et al.* (2015), who reported DEN and CCL₄-induced hepatic and renal damage in animals. Oral administration of methyl jasmonate and diphenyl diselenide decreased p53, BAX, caspases-3 and -9 levels, thus conforming their protective capacity in the rats. Histopathological examination of the renal cortex showed a compromised structure and vascular congestion in DEN and CCL₄ administered rats, while post-treatment with methyl jasmonate and diphenyl diselenide attenuated the lesions in these

tissues. In conclusion, methyl jasmonate and diphenyl diselenide improved renal function by suppressing oxidative stress, inflammation, and apoptotic effects of DEN and CCl₄ in renals of the rats.

Page | 4797 Declaration of conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability statement

All data generated or analyzed during this study are included in the manuscript file.

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