

Original Research Paper

Antioxidant effect of methanol extract of plant ebolo *Crassocephalum crepidioides* during paracetamol-induced toxicity in Wistar rats

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Abstract

The effect of methanol extracts *Crassocephalum crepidioides* against Paracetamol (PCM)-induced oxidative stress was investigated. Wistar rats were divided into five groups of six rats. Group 1: control, 10% DMSO, group 2: 250 mg/kg/bwt PCM, group 3: 300 mg/kg/bwt methanol extract of *C. crepidioides* leaves (MECL), group 4: 250 mg/kg/bwt PCM + 300 mg/kg/bwt MECL and group 5: 250 mg/kg/bwt PCM + 50 mg acetylcysteine (NAC) for two weeks. Groups 4 and 5 were pre-administered with 300 mg/kg/bwt MECL and 50mg NAC, respectively, for one week before the co-administration with PCM. Kidney damage was measured by evaluating serum urea and creatinine, while antioxidant status was assessed by evaluating serum glutathione (GSH) level, glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase activities. Oxidative stress was determined from malondialdehyde (MDA) levels. PCM had no significant effect on serum urea and creatinine levels but significantly decreased glutathione levels, SOD and catalase activities, while the activity of glutathione-S-transferase and level of malondialdehyde (MDA) was increased significantly, indicating the presence of oxidative stress. Co-administration with MECL or NAC reversed these effects. In conclusion, *C. crepidioides* protects against PCM-induced oxidative stress in the Kidneys of Wistar rats.

Keywords

Antioxidants, *C. crepidioides*, rat kidney, malondialdehyde, paracetamol, toxicity, detoxification, drug

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Antioksidativni učinek metanolnega ekstrakta rastline *Crassocephalum crepidioides* med strupenostjo paracetamola na podgane Wistar

Izvilleček

Raziskali smo učinek metanolnih izvlečkov rastline *Crassocephalum crepidioides* na oksidativni stres, ki ga povzroča paracetamol (PCM). Podgane Wistar so bile razdeljene v pet skupin po šest podgan: skupina 1: kontrola, 10 % DMSO, skupina 2: 250 mg PCM /kg telesne teže, skupina 3: 300 mg/kg telesne teže metanolnega izvlečka listov *C. crepidioides* (MECL), skupina 4: 250 mg PCM /kg telesne teže + 300 mg MECL/kg telesne teže in skupina 5: 250 mg PCM/kg telesne teže + 50 mg acetilcisteina (NAC) dva tedna. Skupini 4 in 5 sta pred sočasnim dajanjem PCM en teden prejemale 300 mg MECL/kg telesne teže oziroma 50 mg NAC. Poškodbe ledvic smo merili z oceno koncentracije sečnine in kreatinina v serumu, antioksidativni status pa smo ocenili z oceno ravni glutationa (GSH), aktivnosti encima glutation-S-transferaze (GST), superoksid dismutaze (SOD) in katalaze v serumu. Oksidativni stres je bil določen na podlagi ravni malondialdehida (MDA). PCM ni pomembno vplival na raven sečnine in kreatinina v serumu, vendar je pomembno zmanjšal raven GSH, aktivnosti SOD in katalaze, medtem ko sta se aktivnost GST in raven MDA pomembno povečali, kar kaže na prisotnost oksidativnega stresa. Sočasna uporaba MECL ali NAC je zmanjšala te učinke. Zaključimo lahko, da metabolni ekstrakti rastline *C. crepidioides* zmanjšajo oksidativni stres, ki ga v podganah povzroči PCM.

Ključne besede

antioksidanti, *C. crepidioides*, ledvice podgan, malondialdehid, paracetamol, strupenost, detoksifikacija, zdravlilo

Introduction

Nephrotoxicity presents as a defect in kidney-specific detoxification and excretory function. It may be due to the effects of endogenous or exogenous toxins on the kidney (Kim and Moon, 2012). The toxicity of drugs in the kidney is of great concern because patients remain exposed to various drugs that may result in acute and chronic kidney injury (Perazella, 2018). Paracetamol, also called acetaminophen (APAP), is safe when taken at therapeutic doses. However, its overdose can result in renal and hepatic damage (Ishitsuka et al., 2020). Besides the liver, the kidney is the next target organ of PCM toxicity because it receives ample blood supply. It also concentrates, secretes, and metabolically activates certain drugs (Pathan et al., 2013). High doses of PCM reduces the vitality of tubular epithelial cell resulting in kidney damage. ROS are generated by chemicals such as PCM; therefore, oxidative stress is responsible for PCM toxicity (Ucar et al., 2013).

The primary toxicity of PCM lies in its metabolism (Mazaleuskaya et al. 2015). About 90% of PCM is conjugated to glucuronic acid and sulphate, rendering it

inactive and easily excreted in the urine. About 2% is excreted unchanged, while about 5-9% is metabolized to reactive N-acetyl-*p*-benzoquinone imine (NAPQI) by cytochrome P450 enzymes. This metabolite is conjugated with glutathione, producing a non-toxic derivative that is excreted in the urine. PCM overdose has long been treated with acetylcysteine (NAC); however, adverse effects such as anaphylactoid reactions, which may include bronchospasm and hypotension, have been shown to occur in some cases during NAC administration (Bateman and Dear, 2019). In PCM overdose, the metabolic pathways of the liver become saturated; thus, large amounts of unmetabolized PCM get to the kidneys. This is activated to NAPQI by renal P450, resulting in nephrotoxicity (Mazaleuskaya et al. 2015). The detoxification of NAPQI by GSH results in its depletion in the kidneys and liver, thereby leading to ROS production and, subsequently, oxidative stress. This eventually results in lipid peroxidation and DNA fragmentation (El-Shafey et al. 2015). In the kidney medulla, another enzyme, prostaglandin endoperoxide synthetase (PGES), activates PCM to NAPQI (Mazer and Perrone, 2008). Excessive NAPQI binding to mitochondrial and proximal tubule

protein causes mitochondrial dysfunction and cell death (Mazaleuskaya et al. 2015).

Phytochemicals such as flavonoids and tannins with antioxidant properties have been proven to protect the kidneys against the toxic effects of acetaminophen overdose (Tienda-Vázquez et al. 2019). *Crassocephalum crepidioides* (Benth.) S. Moore (known as Ebolo among the indigenous Yoruba-speaking people of southwest Nigeria) is widely grown in tropical Africa, where its leaves and stems are used as herbs and consumed as a vegetable (Omoregie et al. 2015). Arawande et al. (2013) reported that it contains various phytochemicals such as phenol, oxalate, saponin and tannin.

PCM is widely used as a prescription, and it is readily accessible over the counter (Ishitsuka et al., 2020). This, together with the narrow therapeutic index of PCM, makes an overdose fairly common. *C. crepidioides* possesses antioxidant and anti-inflammatory properties; hence it may serve as a suitable solution to PCM-induced nephrotoxicity (Bello et al. 2019), especially as prophylaxis in those who regularly consume it and eventually indulge in the chronic use of PCM or administer it as an overdose due to ailment such as toothache. Hence, the aims of this study are to confirm the nephrotoxic effect of PCM and to provide a remedy to PCM-induced nephrotoxicity through the regular use and consumption of *C. crepidioides* leaves in diets.

Material and Methods

Plant collection and extract preparation

Crassocephalum Crepidioides (Ebolo) leaves were bought from a market in Ilara Mokin, Akure, Ondo State, Nigeria. The plants were authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria. The leaves were rinsed with water, sliced into smaller parts, air-dried and kept away from direct sunlight. A sample of *C. crepidioides* leaves weighing 333g was blended and soaked in 3330 ml of 70% methanol (Sigma-Aldrich) for 72 hours, after which it was filtered through Whatman No. 1 filter paper with 11µm pore size. The extract obtained was dried using a rotatory evaporator and refrigerated until required.

Experimental animals and treatments

Experimental animals

The Wistar rats used for these experiments were obtained from the Central Animal House, Department of Physiology, College of Health Sciences, Federal University of Technology, Akure. The animals were housed in the animal housed under standard laboratory conditions and had access to water and standard laboratory rat chow *ad libitum*. They were acclimatized for two weeks and, thereafter, treated as described below. The conduction of this study was in accordance with the International Ethical Norms on Animal Care and Used as contained in NIH publication/80-23, revised in 2010.

Animal treatments

The experiment consisted of 30 male rats aged 18 weeks with a weight of between 280-300 g each. They were divided into five groups (1, 2, 3, 4 and 5) of six rats per group. Group 1 served as control, and they were administered 10% DMSO. Dried extract, PCM and NAC were dissolved in 10% DMSO. Group 2 animals were administered methanol extract of *C. crepidioides* leaves (MECL) (300 mg/kg bwt/day). Group 3 was administered 250 mg/ kg bwt/day of PCM. These treatments lasted for two weeks. Group 4 was pretreated with MECL for one week and thereafter co-administered with 300 mg/kg bwt/day of MECL and 250 mg/ kg bwt/day of PCM for two weeks, while group 5 was pretreated with NAC for one week and thereafter was co-administered with 50 mg/kg bwt/day of N-acetylcysteine (NAC) and 250 mg/ kg bwt/day of PCM for two weeks. Pre-administration of MECL is a model that assumes that the regular consumption of *C. crepidioides* in diet before exposure to PCM toxicity may confer protection against subsequent PCM toxicity. The experiment aims toward the protective effect of regular intake of *C. crepidioides* on PCM-induced nephrotoxicity. NAC was used in order to compare its effect on PCM toxicity with that of MECL since NAC has been used for a long time as an antidote to PCM toxicity due to its ability to supply cysteine for the replenishment of GSH that is used for NAPQI detoxification. It also protects against oxidative stress through its electron-donating properties (Waring, 2012).

All administration was carried out by oral gavage. The animals maintained their diets for this period. The rats were fasted for 12 hours, and treatments were withdrawn from them 24 hours prior to sacrifice.

Table 1. Experimental Grouping. PCM (paracetamol); MECL (methanol extract of *C. crepidioides* leaves), NAC (N-acetylcysteine)Tabela 1. Eksperimentalne skupine: PCM (paracetamol); MECL (metanolni ekstrakt listov *C. crepidioides*), NAC (N-acetilcistein)

Group	Treatment
Group 1	10% DMSO (Control) for two weeks
Group 2	300 mg MECL /kg bwt/day for two weeks
Group 3	250 mg PCM / kg bwt/day for 2 weeks
Group 4	Pre-administration with 300 mg MECL /Kg bwt/day for one week before co-administration with 300 mg MECL /kg bwt/day and 250 mg PCM/ kg bwt/day for 2 weeks
Group 5	Pre-administration with 50 mg NAC /Kg bwt/day for one week before co-administration with 50 mg NAC /kg bwt/day and 250 mg PCM / kg bwt/day for 2 weeks

Collection of Blood Samples and Preparation of Rat Post Mitochondria Fractions of Kidney

Animals were killed by decapitation, and blood was taken into plain tubes by cardiac puncture. Blood samples were centrifuged for 10 minutes at 3000g to obtain the serum. After sacrifice, the kidney was immediately removed and rinsed in a cold solution of 1.15% KCl (Sigma-Aldrich). The post-mitochondria fraction was obtained by homogenizing 1.4 g of tissue in 4.2 ml of sodium phosphate buffer pH 7.4. The homogenate was centrifuged in a cold centrifuge for 10 minutes at 10,000 x g. The filtrate was used for enzyme assay.

Biochemical Analysis (Evaluation of Organ Damage and Antioxidant Status)

Kidney damage was determined by the evaluation of serum urea and creatinine using the Randox kit, oxidative stress was evaluated by the determination of the malondialdehyde level and antioxidant status was evaluated by determination of SOD activity, GST activity, catalase activity and GSH content.

Determination of Malondialdehyde level

The breakdown product of LPO is a thiobarbituric acid reactive substance (TBARS). The level of LPO was assessed by the reaction of malondialdehyde with TBARS, by the method of Buege and Aust (1978). 3ml containing 1g of the test sample and 9 ml of stock reagent were mixed in a cooked test tube and heated for 15 minutes in a boiling water bath. After cooling at room temperature, the

precipitate was removed by centrifugation at 1000 x g for 10 minutes, and the absorbance of the supernatant was measured using a spectrophotometer at 532nm against a blank containing all the reagents except the test sample.

The MDA concentration in moles /g tissue (converted to µg/g tissue) was calculated from the absorbance using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ according to the method of Adam-Vizi and Seregi (1982).

Determination of SOD activity

SOD activity was determined by the method of Misra and Fridovich (1972). This measures the ability of SOD to inhibit the auto-oxidation of adrenaline to adrenochrome by superoxide anion.

To 0.2 ml of the tissue, homogenates were added to 2.5 ml of 0.05 carbonate buffer (pH 10.2). The reaction was started by the addition of 0.3 ml of freshly prepared 0.3 mM epinephrine to the mixture, which was quickly mixed by inversion. The blank was prepared by replacing the tissue with 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds. The absorbance of the sample was measured at 450 nm using a spectrophotometer. The % inhibition was determined from the equation below.

$$\% \text{ inhibition} = \frac{\text{increase in absorbance of substrate}}{\text{increase in absorbance of the blank}} \times 100$$

One unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline during 1 minute

Determination of GST activity

The activity of GST was estimated by the method of Habig et al. (1974). The method involves the conjugation of the substrate (1-chloro-2,4-dinitrobenzene) with reduced glutathione by GST. Absorption read at 340 nm is a measure of GST activity. 30 µl of reduced glutathione (0.1 M) was added to 150 µl of CDNB (20 mM), and 2.79 ml of 0.1 M phosphate buffer (pH 6.5), 30 µl of serum was then added. The blank was prepared as above with 2.82 ml of 0.1 M phosphate buffer without the serum. The absorbance was read against the blank at 340 nm every 60 seconds for 3 minutes. The temperature was maintained at approximately 31°C. GST activity was calculated in µ mole/min/mg protein using the extinction coefficient of CDNB (9.6 mm⁻¹ cm⁻¹).

Determination of GSH level

GSH content was determined by the method of Beutler et al. (1963). The method involves the reaction of 5,5 – dithiobis – (2-nitrobenzoic acid) (Ellman's reagent) with sulfhydryl compounds such as GSH to produce a stable yellow colour. An aliquot of homogenate was deproteinized by the addition of an equal volume of 4 % sulphosalicylic acid. This was centrifuged at 4,000 rpm for 10 minutes. 0.5 ml of the supernatant obtained was added to 4.5 ml of Ellman's reagent, and the absorbance read at 412 nm. The blank was prepared by the addition of 0.5 ml of 4 % sulphosalicylic acid to 4.5 ml of Ellman's reagent. The concentration of reduced glutathione (GSH) is proportional to the absorbance at 412 nm. Concentrations of GSH were determined from the standard curve (plot of absorbance against standard concentrations of GSH).

Determination of Catalase activity

Catalase activity was determined according to the method of Sinha (1971). This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H₂O₂, with the formation of perchromic acid as an unstable intermediate. The chromate acetate then produced is measured colourimetrically at 570-610nm. The reaction mixture contained 4 ml of H₂O₂ solution (800 µmoles), 5 ml of phosphate buffer, and pH 7.0 in a 10 ml flat bottom. The reaction was started with the addition of 1 ml of diluted sample. The reaction was stopped at different periods of time at an interval of

60 seconds by the addition of 2ml of dichromate acetic acid mixture to a portion (1ml) of the reacting mixture and thereafter heated for 10 minutes. The remaining H₂O₂ in the portion was determined by measuring chromic acetate colourimetrically and extrapolating the value on the standard curve. To each of the varying concentrations of standard H₂O₂ was added 2ml of dichromate/acetate. The mixtures were heated to produce a stable green due to the formation of chromic acetate. After cooling at room temperature, the volume of the reaction mixture was made up to 3ml and the optical density was measured with a spectrophotometer at 570nm. The concentrations of varying concentrations of standard H₂O₂ were plotted against absorbance.

The monomolecular velocity constant K for the decomposition of H₂O₂ by catalase was determined by using the equation for a first-order reaction.

$$K = \frac{1}{t} \log \frac{S_0}{S}$$

Where S₀ = initial concentration of H₂O₂ and S = concentration of H₂O₂ at 1min intervals. The values of K were plotted against time in minutes, and the velocity constant of catalase K₍₀₎ at 0 minutes was determined by extrapolation.

The catalase content of enzyme preparation was expressed in terms of catalase feiahigkeit or "Kat f" (which is equivalent to micromole of H₂O₂ consumed per min mg protein) according to Von Euler and Josephson (1927):

$$\text{Kat f} = \frac{K_0}{\text{Mg protein/ml}}$$

Statistical Analysis

The results were expressed as Mean ± SEM. The sample sizes were small; thus, a normality test was carried out on the data using a statistical technique that was done by the application of Sapiro-Wilk test using IBM SPSS statistic version 26. P < 0.05 was considered significant. The p values obtained were greater than 0.05, indicating that the data do not show evidence of non-normality, hence the use of a parametric test for comparison of means. Analysis of data was done by one-way analysis of variance (ANOVA) and LSD post hoc test by Fischer. Differences among groups were considered to be significant at a P-value of < 0.05. All analyses were carried out using GraphPad Prism version 8.0 (GraphPad® Inc, CA, USA).

Results

As shown in Table 2, the serum urea and creatinine concentrations for the group administered 250 mg/ kg but/ day PCM were not significantly different from the control group and the other test groups. It also shows that the Kidney MDA level was significantly higher ($p < 0.05$) in the group administered 250 mg PCM with respect to the control group, while in the MECL and NAC co-administered group, the MDA level was not significantly ($p < 0.05$) different from the control group.

Table 3 shows that kidney GST activity was significantly higher ($p < 0.05$) in the group administered 250 mg/ kg bwt/day PCM compared to the control group and was significantly ameliorated with the co-administration of MECL and NAC.

Table 3 also indicated that the activity of SOD was significantly ($p < 0.05$) lowered in the 250 mg/ kg bwt/

day PCM group in relation to the control group. However, co-administration of PCM with MECL and with NAC significantly increased ($p < 0.05$) the SOD activity when compared with the 250 mg/ kg bwt/day PCM group. The trend observed for catalase activity was similar to that of SOD activity, although catalase activity was not significantly ($p < 0.05$) different from the control group in MECL co-administered groups.

The GSH level shown in Table 3 indicates a significant ($p < 0.05$) reduction of GSH level in the 250 mg/ kg bwt/ day PCM administered group in relation to the control but co-administration of PCM with the extract significantly ($p < 0.05$) increase the GSH levels in relation to the PCM group. Co-administration of NAC also significantly ($p < 0.05$) increased the GSH levels in relation to the PCM group, and no significant increase was observed between the NAC co-administered group and the control.

Table 2. Effects of MECL on serum urea, creatinine and MDA concentration in PCM-induced nephrotoxicity. PCM (paracetamol); MECL (methanol extract of *C. crepidioides* leaves), NAC (N-acetylcysteine)

Tabela 2. Učinki MECL na serumsko urejo, kreatinin in koncentracijo MDA pri z PCM-inducirani nefrotoksičnosti. PCM (paracetamol); MECL (metanolni ekstrakt listov *C. crepidioides*), NAC (N-acetilcistein)

Group	Urea concentration (mmol/l)	Creatinine concentration (μ mol/l)	MDA concentration μ g/g tissue
G1 (Control)	6.805 $\pm$ 0.205	96.192 $\pm$ 3.372	298.898 $\pm$ 6.8845
G2 (MECL)	3.824 $\pm$ 0.199	80.326 $\pm$ 2.425	180.492 $\pm$ 12.6135 [#]
G3 (PCM 250)	6.910 $\pm$ 0.136	98.475 $\pm$ 3.068	391.583 $\pm$ 18.350*
G4 (PCM + MECL)	5.225 $\pm$ 0.127	90.543 $\pm$ 4.032	277.611 $\pm$ 13.381 [#]
G5 (PCM + NAC)	4.342 $\pm$ 0.188	87.425 $\pm$ 4.25	310.957 $\pm$ 10.725 [#]

* $p < 0.05$ shows that the group is significantly different from the control group

[#] $p < 0.05$ shows that the group is significantly different from the PCM group

Table 3. Effects of MECL on kidney GSH levels, GST, SOD and catalase activities in PCM-induced nephrotoxicity. PCM (paracetamol); MECL (methanol extract of *C. crepidioides* leaves), NAC (N-acetylcysteine)

Tabela 3. Učinki MECL na koncentracijo GSH ter GST, SOD in katalazno aktivnost v ledvicah pri PCM-inducirani nefrotoksičnosti. PCM (paracetamol); MECL (metanolni ekstrakt listov *C. crepidioides*), NAC (N-acetilcistein)

Groups	GST activity (μ mole/min/mg protein)	SOD activity (μ mole/min/mg protein)	Catalase activity (Kattf)	GSH levels (μ g/ml)
G1 (Control)	0.125 $\pm$ 0.0031	7.532 $\pm$ 0.270	0.0721 $\pm$ 0.0021	0.009 $\pm$ 0.0008
G2 (MECL)	0.092 $\pm$ 0.0043 [#]	8.186 $\pm$ 0.270 [#]	0.0952 $\pm$ 0.0023 [#]	0.014 $\pm$ 0.0009 [#]
G3 (PCM 250)	0.170 $\pm$ 0.0013*	2.058 $\pm$ 0.122*	0.0246 $\pm$ 0.0012*	0.003 $\pm$ 0.0008*
G4 (PCM + MECL)	0.118 $\pm$ 0.0056 [#]	3.412 $\pm$ 0.083 [#]	0.0634 $\pm$ 0.0082 [#]	0.006 $\pm$ 0.0004 [#]
G5 (PCM + NAC)	0.109 $\pm$ 0.024 [#]	4.453 $\pm$ 0.064 [#]	0.0534 $\pm$ 0.0043 [#]	0.008 $\pm$ 0.0005 [#]

* $p < 0.05$ shows that the group is different significantly from the control group

[#] $p < 0.05$ shows that the group is different significantly from the PCM group

Discussion

GSTs are toxicologically important enzymes because they catalyze the conjugation of N-acetyl-p-benzoquinone imine (NAPQI) with glutathione resulting in the detoxification of NAPQI (Arakawa et al. 2013). The significant increase in the activity of GST in the 250mg/kg bwt administered PCM group suggests that an overdose of PCM may result in the overactivation of GST. Hernandez et al. (2018) have also shown that exposure to foreign compounds can increase the activity of GST in adaptation to chemical stress. Bello et al. (2019) reported in one of their *in vitro* studies that MECL possesses antioxidant and anti-inflammatory activity. Thus, the decreased activity of the GST observed in MECL co-administered group may be due to the high level of antioxidants in MECL, resulting in reduced chemical stress and, thus, reduced oxidative stress on the cells of the kidney.

Kidney GSH level was depleted in the 250mg/kgbwt administered PCM group. This finding is consistent with those of Das et al. (2010), Demirbag et al. (2010) and Ahmad et al. (2012), in which a decrease in renal GSH concentration was observed in PCM-induced nephrotoxicity. GSH helps in the detoxification of PCM thus, prevent PCM-induced nephrotoxicity (Orji et al., 2020). Therefore, in GSH depletion, which may occur during chronic administration or when high doses are administered, detoxification of NAPQI is reduced, and excess NAPQI that is retained in the kidney may result in renal injury. The increase in GSH level in kidneys by MECL administration may therefore prevent PCM-induced nephrotoxicity. A similar study also showed that *C. crepidioides* increased the GSH level during rifampicin toxicity (Omoregie et al. 2015).

SOD is a key cellular antioxidant responsible for eliminating superoxide radicals (Younus, 2018). This study showed that 250 mg/kg/bwt PCM decreased renal SOD activity, ameliorated by MECL treatment. Demirbag et al. (2010) and Orji et al. (2020) also reported a decrease in rats' renal SOD levels after acute PCM overdose, while *C. crepidioides* were shown to strongly scavenge superoxide anion ($O_2^{\cdot-}$) and hydroxyl radicals ($HO^{\cdot}$) (Bello et al. 2019). Catalase prevents oxidative stress and hydroxyl radicals ($HO^{\cdot}$) formation by dismutating hydrogen peroxide (H_2O_2) to give oxygen and water (Nandi et al. 2019). Administration of PCM reduced catalase activity which was attenuated by MECL. Orji et al. (2020) also observed that paracetamol treatment significantly reduced the activity

of catalase, while Oboh et al. (2021) showed that *C. crepidioides* increased catalase activity in the *Drosophila melanogaster* model of Alzheimer's disease. The reduced activity of SOD and catalase in 250 mg/kg/bwt PCM administered group is an indication of the reduced ability of the animals in this group to scavenge the reactive oxygen species, $O_2^{\cdot-}$, H_2O_2 and the $HO^{\cdot}$ which may eventually result in oxidative stress (Lenzen et al. 2022) in these animals. The increase in the activities of these antioxidant enzymes by administration of *C. crepidioides* may, however, scavenge the reactive oxygen species and prevent oxidative stress.

The results from this study suggest that PCM elevated renal MDA levels. This may be caused by the decrease in GSH level, catalase and SOD activities. Tripathi et al. (2019) and Canayakin et al. (2016) also suggested lipid peroxidation as a mechanism for PCM-induced oxidative stress and tissue damage. Decreased MDA levels observed in the MECL-treated group indicated that MECL may reduce oxidative stress in the kidneys of these animals. Although lipid peroxidation may be involved in the pathogenesis of PCM-induced nephrotoxicity and injury, Demirbag et al. (2010), no significant difference was observed in creatinine and urea levels in all groups. This indicates that although the increase in the level of lipid peroxidation by PCM might result in oxidative stress in the kidney, this might not be enough to cause serious damage to the kidney since serum urea and creatinine are nephrotoxicity markers (Salazar, 2014).

Similarly, co-administration of NAC with PCM attenuates the toxicity of PCM by significantly increasing SOD, Catalase activity and GSH levels. In addition, a significant decrease in GST activity and MDA levels in the kidney occurred in this group. MDA level and GST activity were also significantly decreased in *C. crepidioides*-only administered group relative to the control and relatively to PCM-only administered group, while GSH level, catalase and SOD activities were significantly increased in this group relative to the control and relatively to PCM-only, administered group.

In conclusion, the use of PCM is still unrestricted, as it can be obtained without a doctor's prescription. This study showed that PCM overdose caused oxidative stress in the rat kidney by increasing the MDA level and decreasing the activities of SOD, catalase and GSH level. This may eventually lead to nephrotoxicity and renal damage. Thus the non-restriction of PCM, which makes

it easy for an overdose to be taken, poses a health risk. Hence, the need to protect against PCM-induced nephrotoxicity is in high demand. In this study, MECL increased the activities of SOD, catalase and GSH levels; thus, it is promising in protecting against oxidative stress and nephrotoxicity that may be induced by PCM overdose in the kidney.

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