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Nephroprotective effects of *Zingiber zerumbet* Smith ethyl acetate extract against paracetamol-induced nephrotoxicity and oxidative stress in rats

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Abstract: Paracetamol (PCM) overdose can cause nephrotoxicity with oxidative stress as one of the possible mechanisms mediating the event. In this study, the effects of ethyl acetate extract of *Zingiber zerumbet* rhizome [200 mg per kg of body weight (mg/kg) and 400 mg/kg] on PCM-induced nephrotoxicity were examined. Rats were divided into five groups containing 10 rats each. The control group received distilled water while other groups were treated with extract alone (400 mg/kg), PCM alone (750 mg/kg), 750 mg/kg PCM+200 mg/kg extract (PCM+200-extract), and 750 mg/kg PCM+400 mg/kg extract (PCM+400-extract), respectively, for seven consecutive days. The *Z. zerumbet* extract was given intraperitoneally concurrent with oral administration of PCM. Treatment with *Z. zerumbet* extract at doses of 200 and 400 mg/kg prevented the PCM-induced nephrotoxicity and oxidative impairments of the kidney, as evidenced by a significantly reduced (P<0.05) level of plasma creatinine, plasma and renal malondialdehyde (MDA), plasma protein carbonyl, and renal advanced oxidation protein product (AOPP). Furthermore, both doses were also able to induce a significant increment (P<0.05) of plasma and renal levels of glutathione (GSH) and plasma superoxide dismutase (SOD) activity. The nephroprotective effects of *Z. zerumbet* extract were confirmed by a reduced intensity of renal cellular damage, as evidenced by histological findings. Moreover, *Z. zerumbet* extract administered at 400 mg/kg was found to show greater protective effects than that at 200 mg/kg. In conclusion, ethyl acetate extract of *Z. zerumbet* rhizome has a protective role against PCM-induced nephrotoxicity and the process is probably mediated through its antioxidant properties.

Key words: *Zingiber zerumbet*, Antioxidant, Oxidative stress, Nephrotoxicity, Paracetamol
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1 Introduction

Paracetamol (PCM) or acetaminophen was first discovered in 1889 and is a widely used nonprescriptive analgesic and antipyretic agent (Brown, 1968). PCM toxicity is one of the major causes of poisoning worldwide (Gunnell *et al*., 2000), and its overdose is commonly associated with hepatic (Nelson, 1995) and renal damages (Placke *et al*., 1987). PCM toxicity is mediated by the activity of its reactive metabolite known as *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which is detoxified by intracellular glutathione (GSH) (Borne, 1995). Therefore, an overdose of PCM will saturate the conjugation pathways of GSH and cause depletion of cellular GSH. This subsequently led to a

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reduced capacity of GSH to detoxify NAPQI. Increased level of NAPQI mediates oxidative damage, and thus enhances cellular injuries and organ dysfunction, including renal damage (Hart *et al*., 1994).

Although the occurrence of nephrotoxicity is less common than that of hepatotoxicity as reported by Blakely and McDonald (1995), a number of studies have documented that PCM-induced renal tubular damage and acute renal failure can occur even in the absence of liver damage (Carpenter and Mudge, 1981), and can be the primary manifestation of PCM toxicity (Boutis and Shannon, 2001). As PCM overdose is shown to mediate severe renal damage, which can be life-threatening, antidotes or treatments that could offer maximum protection against PCM-induced renal injury are therefore required. To date, *N*-acetyl-cysteine (NAC) has been used clinically for the treatment of PCM toxicity (Dilger and Baker, 2007).

Current evidence suggests that PCM-induced hepatorenal injury is mediated by oxidative damage (Das *et al*., 2010; Kheradpezhouh *et al*., 2010). Therefore, the alternative treatments for PCM toxicity could be achieved using a natural compound with antioxidant activity. *Zingiber zerumbet* (L.) Smith, or locally known as lempoyang or wild ginger, belonging to the Zingiberaceae family (Saadiah and Halijah, 1995) has been shown to possess a number of biological activities, including anti-cancer (Huang *et al*., 2005; Rashid *et al*., 2005; Sharifah Sakinah *et al*., 2007; Abdul *et al*., 2008), anti-inflammatory (Jaganath and Ng, 2000; Somchit and Shukriyah, 2003), antimicrobial (Abdul *et al*., 2008; Kaderi *et al*., 2010), and antioxidant properties (Ruslay *et al*., 2007). *Z. zerumbet* has been shown to contain flavonoid compounds that exhibit the antioxidant properties (Pietta, 2000), and the ethyl acetate extract of the plants has been shown to exhibit strong antioxidant activities (Ruslay *et al*., 2007). In a recent study, zerumbone, which is the active compound of the *Z. zerumbet* rhizome, has been shown to protect against cisplatin-induced renal dysfunction by preventing lipid peroxidation and preserving antioxidant (Ibrahim *et al*., 2010).

In this study, we determined the potential protective effects and antioxidant activities of ethyl acetate extract of the *Z. zerumbet* rhizome against PCM-induced nephrotoxicity. The effects were determined by measuring the levels of plasma creatinine (indicator of renal function), endogenous antioxidants [GSH and superoxide dismutase (SOD)], and oxidative stress markers [malondialdehyde (MDA), advanced oxidation protein product (AOPP), and protein carbonylation], and by histological change analysis.

2 Materials and methods

2.1 Plant materials

Fresh rhizomes (7 kg) of *Z. zerumbet* were collected from Temerloh, Pahang, Malaysia and authenticated by a plant taxonomist at Department of Botany, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM), and were deposited as a voucher specimen at the herbarium of UKM, Bangi, Selangor, Malaysia. The specimen was cleaned and chopped into small pieces and then air-dried at room temperature for three days.

2.2 Plant extraction

The air-dried rhizomes of *Z. zerumbet* were sequentially soaked at room temperature in *n*-hexane, ethyl acetate, and then methanol for 72 h. The resultant extracts were filtered and evaporated to dryness in vacuo to yield crude extracts of hexane, ethyl acetate, and methanol. All the crude extracts were stored at 4 °C until tested for bioassay. Prior to use, the *Z. zerumbet* ethyl acetate extract was dissolved in dimethyl sulfoxide (DMSO) and diluted in phosphate buffer saline (PBS; pH 7.4).

2.3 Experimental protocol

All procedures involving the use of laboratory animals were reviewed and approved by the Animal Ethics Committee of UKM. Fifty male Sprague-Dawley rats (230–250 g) were obtained from the UKM Animal Resource Unit. The animals were housed in a controlled environment with room temperature and a 12-h light-dark cycle. Animals were fed mouse pellet and fresh water ad libitum for a week prior to experiments. Rats were randomly divided into five groups containing 10 animals each and all treatments were given daily for seven days. PCM was administered orally, while *Z. zerumbet* extracts at 200 and 400 mg per kg of body weight (mg/kg) were

delivered intraperitoneally concurrent with PCM administration. The selected doses of *Z. zerumbet* extract were based on Hemabarathy *et al*. (2009) who showed the hepatoprotective effects of aqueous extracts of *Alpina Galanga* (Zingiberaceae family) on the PCM-induced hepatotoxicity. Rats in Group I served as the control group and were administered distilled water only. Groups II received 400 mg/kg *Z. zerumbet* extract alone, while Group III received 750 mg/kg PCM alone. In Group IV, rats were treated with 750 mg/kg PCM and 200 mg/kg *Z. zerumbet* extract. Meanwhile, rats in Group V were treated with 750 mg/kg PCM and 400 mg/kg *Z. zerumbet* extract. On Day 8, all animals were weighed and anaesthetized with diethyl ether.

2.4 Sample preparation

Blood was collected via cardiac puncture using a 10 ml syringe and 25G needle after 24 h of last treatment. Blood was collected in EDTA tubes and plasma was obtained after centrifugation at 3 000 r/min for 10 min at 4 °C. Plasma sample was stored at −20 °C until further analysis. The kidney was obtained after the rat was sacrificed, washed in ice-cold 11.5 g/L KCl to remove blood, and quickly blotted and weighed on digital scale (B303 Mettler-Toledo, Switzerland). For histological studies, the kidney was preserved in 10% formalin while for biochemical analysis; the kidney was minced into small pieces and homogenate with 11.5 g/L KCl in 4 ml/g ratio using homogenizers (Ultra Turrax T25).

2.5 Biochemical analysis

Creatinine level was determined according to Jaffe's reaction, based on creatinine reaction with picric acid in alkaline solution to form a red solution measured spectrophotometrically at 520 nm (Slot, 1965). The level of creatinine was extrapolated from the standard graph and the values were then stated as milligram per deciliter (mg/dl).

SOD activity was assayed according to the method as described by Beyer and Fridovich (1987) based on the reaction of SOD with nitrotetrazolium blue chloride (NBT·2HCl), Triton-X 100 and riboflavin and the absorbance was measured with a spectrophotometer at 560 nm. One unit (1 U) of SOD was defined as the amount of SOD required for 50% inhibition of NBT·2HCl reduction.

GSH was quantified using 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) according to the protocols as described by Ellman (1959). The reaction between GSH and DTNB formed a yellow-colored complex that was measured spectrophotometrically at 420 nm.

MDA was determined based on the production of thiobarbituric acid reactive substances (TBARS), the absorbance of which was measured spectrophotometrically at 532 nm (Esterbauer and Cheeseman, 1990). The concentration of TBARS was determined from the extrapolated standard curve of serially diluted 1,1,3,3-tetraethoxypropane (TEP). The final concentration of MDA was then expressed in nmol/g protein.

Protein carbonyls were estimated according to the procedure as described by Levine *et al*. (1990) based on the reaction of carbonyl compounds with 2,4-dinitrophenyl hydrazine (2,4-DNPH) for 1 h and precipitations with 20% trichloroacetic acid (TCA, 0.2 kg/L). The released carbonyl compounds were measured by spectrophotometer at 380 nm. The amounts of carbonyls are calculated based on the total amount of protein contents in each sample.

AOPP of renal homogenate was measured according to Witko-Sarsat *et al*. (1996). Renal homogenate was centrifuged at 2 500 r/min for 10 min at 40 °C. The supernatant was then added to a reaction mixture containing 50% acetic acid and 1.16 mol/L potassium iodide (KI) in PBS solution. The absorbance was recorded at 340 nm and the concentration of AOPP was determined from the extrapolated standard curve of serially diluted AOPP standard solution using 500 μmol/L chloramines stock.

2.6 Histological analysis

Examination of renal histology was performed according to routine histology techniques. Briefly, after the animal was sacrificed, the kidney was harvested, rinsed in normal saline, and sectioned into small pieces. The sectioned tissue was then fixed in 10% formalin, dehydrated in stepwise with increasing concentration of ethanol solution (50% to 100%), and embedded in paraffin. Using a microtome, tissue sections of 4-μm thickness were produced, fixed overnight on the slide, subsequently stained with hematoxylin and eosin (H&E), and were then observed under a light microscope (Olympus BX41, Japan).

2.7 Statistical analysis

Statistical analysis was done using SPSS version 18.0. Data were analyzed using Shapiro-Wilk normality test and one way analysis of variance (ANOVA) was used for comparison between groups followed by post-hoc Tukey test. Data were expressed as mean±standard deviation (SD) and $P<0.05$ showed statistically significance.

3 Results

3.1 Effects of *Z. zerumbet* extract on body weight, kidney weight, and food and water intake

In all experimental groups, no mortality was observed during the period of the study. Reduced water intake and food consumption, as well as reduced body weight, were observed in PCM group as compared to control (Fig. 1a). In contrast, groups receiving PCM concurrent with administration of either 200 or 400 mg/kg of *Z. zerumbet* extract weighed significantly higher ($P<0.05$) than PCM group. Meanwhile, no significant alteration of body weight was recorded from rats treated with 400 mg/kg of extract only. Furthermore, the kidney weight was significantly lower ($P<0.05$) in all treated groups as compared to the control, and the reduction was markedly noticeable in the PCM group (Fig. 1b).

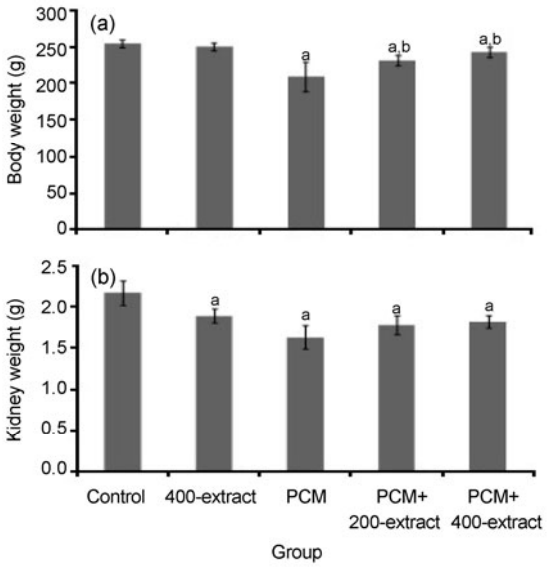

Fig. 1 Effects of ethyl acetate extract of *Z. zerumbet* on body weight (a) and kidney weight (b) of rats after seven days of treatments

Each column represents mean±SD (n=10). [a] $P<0.05$ versus control group; [b] $P<0.05$ versus PCM group. 400-extract, PCM, PCM+200-extract, and PCM+400-extract groups were treated with 400 mg/kg extract alone, 750 mg/kg PCM alone, 750 mg/kg PCM+200 mg/kg extract, and 750 mg/kg PCM+400 mg/kg extract, respectively

3.2 Effect of *Z. zerumbet* extract on creatinine level

A significantly increased ($P<0.05$) level of plasma creatinine was observed in plasma samples of the PCM group (Fig. 2). However, supplementation with *Z. zerumbet* extract at 200 and 400 mg/kg significantly prevented ($P<0.05$) further elevations of creatinine, with obvious effects observed in the PCM+400 mg/kg extract group. There were no nephrotoxic effects observed in rats administered 400 mg/kg of the *Z. zerumbet* extract alone, as evidenced by no significant difference in plasma creatinine levels as compared to the control group.

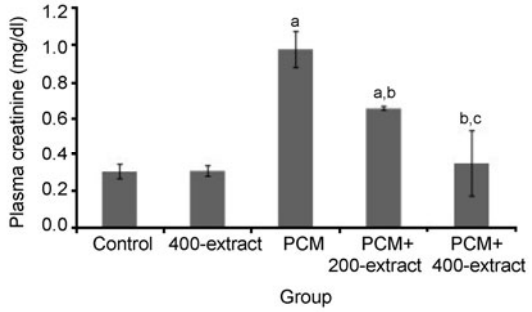

Fig. 2 Effects of ethyl acetate extract of *Z. zerumbet* on plasma creatinine in rats after seven days of treatments

Each column represents mean±SD (n=10). [a] $P<0.05$ versus control group; [b] $P<0.05$ versus PCM group; [c] $P<0.05$ versus PCM+200-extract group

3.3 Effect of *Z. zerumbet* extract on antioxidant status

Administration of PCM for seven consecutive days caused a significant reduction ($P<0.05$) of GSH level in both plasma and renal homogenate (Figs. 3a and 3b). In contrast, administrations with either 200 or 400 mg/kg of *Z. zerumbet* extract significantly prevented ($P<0.05$) PCM-induced GSH reductions, with greater effects demonstrated in the PCM+ 400 mg/kg extract group, which was able to maintain the level of GSH despite the presence of PCM. Furthermore, supplementation of 400 mg/kg of *Z. zerumbet* extract alone showed no significant difference in plasma and renal levels of GSH as compared to the control group.

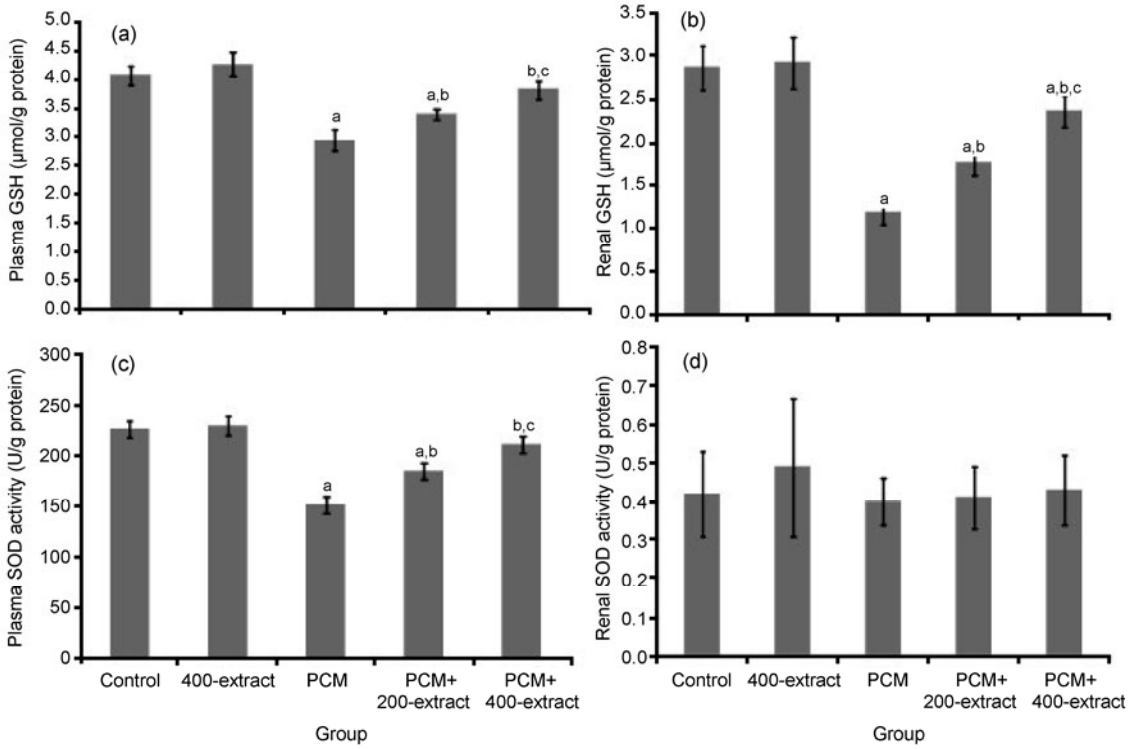

Fig. 3 Effects of ethyl acetate extract of *Z. zerumbet* on the levels of plasma GSH (a), renal GSH (b), plasma SOD activity (c), and renal SOD activity (d) in rats after seven days of treatments

Each column represents mean±SD (n=10). [a] P<0.05 versus control group; [b] P<0.05 versus PCM group; [c] P<0.05 versus PCM+200-extract group

In addition to the GSH level, the activity of SOD enzymes in plasma and renal tissue was also determined. The group treated with PCM alone showed significantly lower (P<0.05) plasma SOD activity as compared to the control (Fig. 3c). Administration of extracts at both doses significantly increased (P<0.05) plasma SOD activity than the PCM group. In addition, supplementation with 400 mg/kg extract together with PCM further increased plasma SOD activity than the PCM+200 mg/kg extract groups, and its plasma SOD activity was almost similar as that in the control group. In contrast to the activity of plasma SOD, the renal homogenate SOD activity did not show any significant difference among all experimental groups (Fig. 3d).

3.4 Effect of *Z. zerumbet* extract on oxidative stress

Administration of PCM for seven consecutive days induced oxidative stress with a significant increase (P<0.05) in the levels of renal homogenate and plasma MDA, plasma protein carbonyl, and renal AOPP (Fig. 4). Concurrent administration of PCM and *Z. zerumbet* extract at 200 and 400 mg/kg, however, reduced the oxidative stress, as evidenced by significantly reduced (P<0.05) level of renal homogenate and plasma MDA, plasma protein carbonyl, and renal AOPP than those in the PCM group. Interestingly, administration of both doses brought the MDA level of renal homogenate nearly to the value of the control group. In addition, no significant induction of oxidative stress was observed in rats administered with 400 mg/kg of the extract alone, as evidenced by no significant difference in the value of renal homogenate and plasma MDA, plasma protein carbonyl, and renal AOPP than in control.

3.5 Effect of *Z. zerumbet* extract on histological features of the kidney

The histological features of the kidneys from various treatments groups are as presented in Fig. 5. Renal sections from the control and 400 mg/kg *Z. zerumbet* group showed no histological changes, as evidenced by the normal appearance of glomeruli and tubules (Figs. 5a and 5b). In contrast, kidney sections

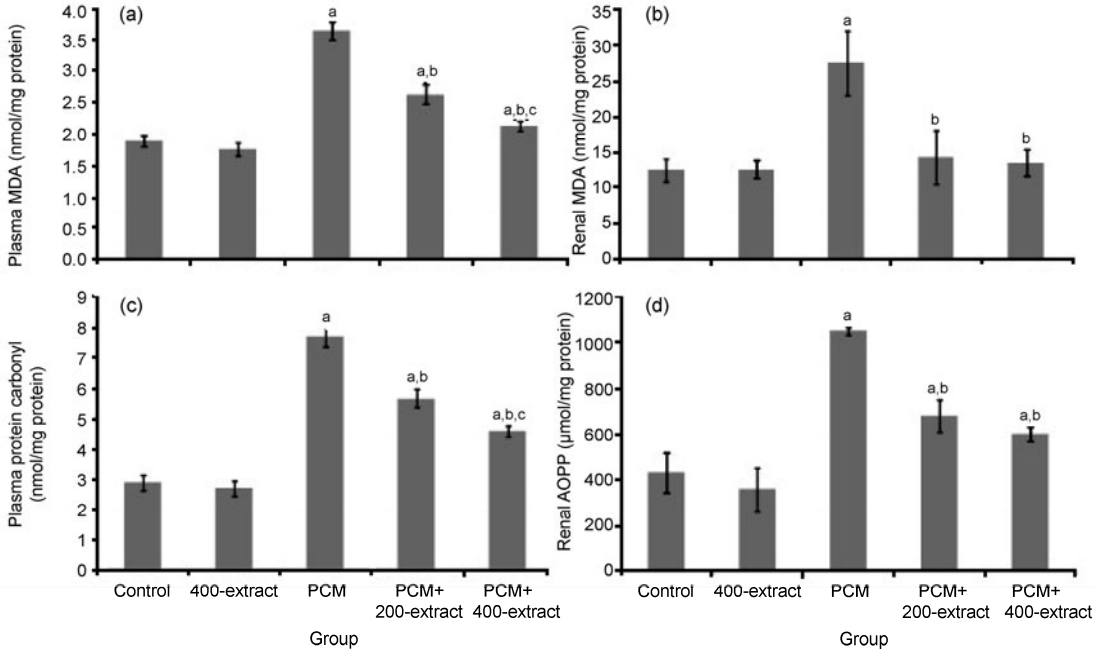

Fig. 4 Effects of ethyl acetate extract of *Z. zerumbet* on the levels of plasma MDA (a), renal MDA (b), plasma protein carbonyl (c), and renal AOPP (d) in rats after seven days of treatments

Each column represents mean±SD (n=10). [a] P<0.05 versus control group; [b] P<0.05 versus PCM group, [c] P<0.05 versus PCM+200-extract group

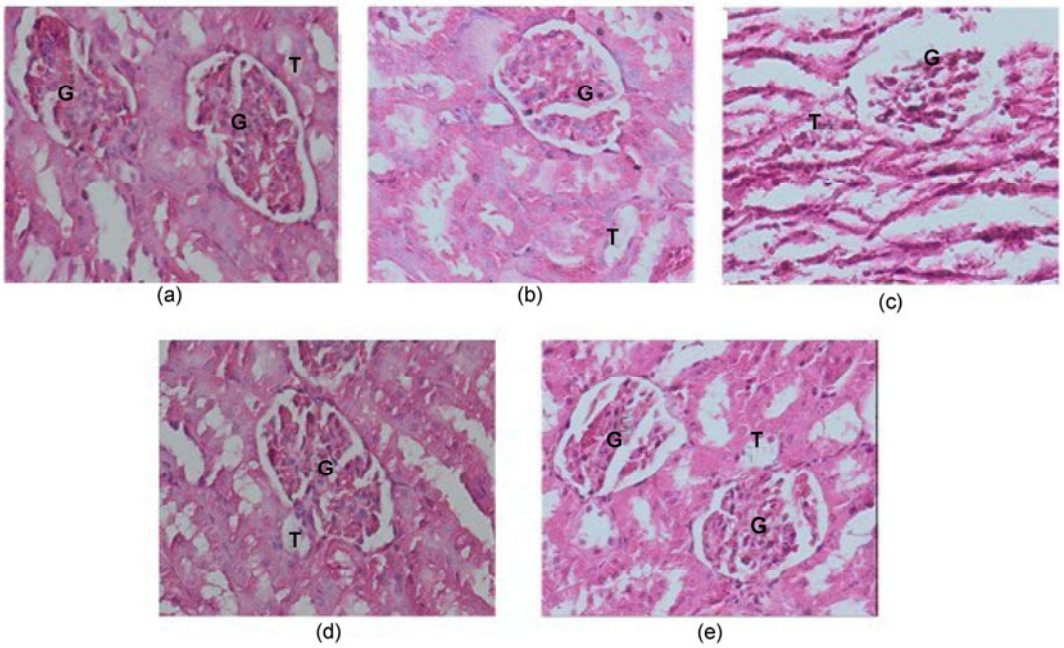

Fig. 5 Kidney sections of each group (hematoxylin and eosin stained)

(a) Control group showing normal appearance of glomeruli (G) and tubules (T); (b) 400 mg/kg extract group exhibiting normal features of glomeruli (G) and tubules (T); (c) PCM group showing severe glomerular (G) and tubular damages (T); (d) PCM+200 mg/kg extract group showing moderate glomerular (G) and tubular destruction (T); (e) PCM+400 mg/kg extract group showing almost normal appearance of glomeruli (G) and tubules (T)

from the PCM group exhibited altered architecture with extensive destruction of glomeruli and tubular structures, as demonstrated by marked necrotic areas (Fig. 5c). On the other hand, administration of *Z. zerumbet* extract at 200 (Fig. 5d) or 400 mg/kg (Fig. 5e) concurrent with PCM administration, showed protective effects on the kidney, as evidenced by the less severe tubular and glomerular damages, with better protection being observed in the PCM+400 mg/kg extract group. These histological observations were in line with biochemical findings and all these results support each other.

4 Discussion

PCM-induced nephrotoxicity has been previously documented by a number of studies (Mitchell *et al.*, 1977; McMurtry *et al.*, 1978; Newton *et al.*, 1983). In this study, we demonstrated that the administration of 750 mg/kg PCM for seven consecutive days was able to induce nephrotoxicity in rats, as demonstrated by the significantly increased level of plasma creatinine. The nephrotoxic effect of PCM was further confirmed by the severely destructed renal tissue, as shown in the histological analysis. This finding is in accordance with that of Abdel-Zaher *et al*. (2007) who showed that the administration of PCM at 750 mg/kg induced nephrotoxicity in rats. Administration of ethyl acetate extract of *Z. zerumbet* concurrent with PCM exposure prevented PCM-induced nephrotoxicity. Furthermore, the protective effect was found to be more remarkable in the PCM+400 mg/kg extract group than in the PCM+200 mg/kg extract group.

To date, a number of studies have suggested the involvement of oxidative stress as the mediator of PCM-induced nephrotoxicity (Das *et al*., 2010; Kheradpezhouh *et al*., 2010; Yousef *et al*., 2010). Hart *et al.* (1994) pointed out that PCM toxicity was shown to enhance NAPQI production. The accumulated NAPQI induces greater formation of free radicals, which are responsible in mediating cellular damages and renal toxicity.

Elevated levels of MDA in tissue have been regarded as an indicator for cellular damage due to excess lipid peroxidation processes that occur during malfunction of the antioxidant defense system (Kaplowitz, 2000). In our study, administration of 750 mg/kg PCM caused significant elevations of plasma and renal MDA. In contrast, treatment with ethyl acetate extract of *Z. zerumbet* abrogated this elevation with greater protection being shown with the 400 mg/kg extract. The ability of *Z. zerumbet* to protect lipid peroxidation is in agreement with Ruslay *et al.* (2007), who demonstrated that ethyl acetate fractions of *Z. zerumbet* possess remarkable lipid peroxidation inhibition and radical scavenging activities. Pietta (2000) also showed that the ethyl acetate fraction of *Z. zerumbet* contains flavonoid glycosides known as afzelin and diacetylafzelin, and derivatives of kaempferol, a 3-flavonol that was known to possess antioxidant property. In addition, Ibrahim *et al.* (2010) reported that pretreatment with zerumbone, a natural compound isolated from fresh rhizomes of *Z. zerumbet* protected against cisplatin-induced nephrotoxicity by attenuating the production of MDA and simultaneously promoting GSH production. Therefore, we believe that the effects manifested by the crude ethyl acetate extract of *Z. zerumbet* to overcome PCM-induced nephrotoxicity in our study were mediated by the presence of these bioactive compounds.

Excess reactive oxygen species (ROS) can promote protein oxidation, forming protein carbonyls (Chevion *et al*., 2000; Margetis *et al*., 2009) and AOPP, which have been described as reliable markers to estimate the degree of oxidant-mediated protein damage (Alderman *et al*., 2002). The significant elevation of plasma protein carbonyls and renal homogenate AOPP levels in the PCM group indicates the presence of oxidative stress and upregulation of NAPQI production, which stimulates protein oxidations. Concurrent delivery of *Z. zerumbet* extract at both doses, however, reduced significantly the production of plasma protein carbonyls and renal homogenate AOPP, and this demonstrates the capacity of the *Z. zerumbet* extract to overcome ROS production, presumably through its antioxidants property.

GSH is a well-known non-enzymatic antioxidant that is responsible as the frontier defense mechanism during oxidative stress and the depletion of intracellular GSH in a prolonged oxidative stress (Kaplowitz, 2000). As demonstrated in our findings, a significant decline in both plasma and renal homogenate GSH levels in PCM-treated rats is in line with the previous finding that oral administration of 750 mg/kg PCM to

rats for seven days reduced renal cellular contents of GSH (Abdel-Zaher *et al.*, 2007). The administration of *Z. zerumbet* ethyl acetate extract at 200 and 400 mg/kg preserved GSH levels and the effects were remarkably effective especially in rats received 400 mg/kg *Z. zerumbet* extract. Ibrahim *et al.* (2010) reported that zerumbone compound in *Z. zerumbet* extract indirectly induces the biosynthesis of GSH and provides a protective intracellular mechanism, presumably as a free radical scavenger for toxic agents. Hence, we can speculate that the *Z. zerumbet* extract in our study may induce the synthesis of endogenous GSH, which protects against PCM-induced oxidative damage and greater effects at 400 mg/kg treated rats are presumably conferred through higher amounts of zerumbone present in the extracts.

Administration of the *Z. zerumbet* extract at 200 and 400 mg/kg was again able to maintain the SOD activity during PCM overdose. Improvement in SOD activities does provide evidence to an overall improvement in endogenous antioxidant defense system (Hemabarathy *et al.*, 2009). This relates to the fact that *Z. zerumbet* has been shown to have high antioxidant activities through induction of the endogenous antioxidants that reduce free radical activity (Nakamura *et al.*, 2004). In contrast, SOD activities measured from renal homogenate were not significantly different among all groups. This finding is supported by Hemabarathy *et al*. (2009) who demonstrated that no significant alteration of liver SOD levels was observed during PCM-induced hepatotoxicity. This finding suggests that GSH is responsible as the foremost antioxidant defense mechanism against PCM-induced oxidative damage, and the significant alteration of GSH levels will appear before the depletion of SOD activities.

As demonstrated in this study, rats exposed to PCM in the absence of the *Z. zerumbet* extract showed severe cellular damage. In contrast, concurrent treatment with the *Z. zerumbet* extract managed to protect against severe cellular lesions, with only mild or moderate degeneration of glomerular and tubular kidney cells observed. In agreement with biochemical parameters, the best protection was manifested in rats treated with 400 mg/kg of the extract. Finally, it is also demonstrated that administration of ethyl acetate extract of *Z. zerumbet* at 400 mg/kg in rats possessed no nephrotoxicity effects, as evidenced by biochemical and histological analyses. However, despite no nephrotoxic evidence, rats administered with 400 mg/kg extract of *Z. zerumbet* alone showed significantly reduced kidney weight as compared to control rats. Therefore, at this stage, we cannot ascertain the definitive reason for the above condition; however, we suggest that the *Z. zerumbet* extract, when administered at 400 mg/kg, may affect the kidney without remarkable alteration of renal histology. Thus, the administration of the *Z. zerumbet* extract at 400 mg/kg or higher in rats may possess a risk of toxicity, and further study is required to elucidate this issue.

5 Conclusions

The administration of *Z. zerumbet* ethyl acetate extract was found to have protective effects and antioxidant activities against PCM-induced nephrotoxicity, as evidenced by the biochemical status and histological findings. The most remarkable effects were observed when the *Z. zerumbet* extract was delivered at 400 mg/kg as compared to a lower dose of 200 mg/kg of the extract. This indicates that the amount of antioxidant compounds present in *Z. zerumbet* contributes significantly to its antioxidant property. Together, the absence of renal damage and supportive evidence of its antioxidant properties may suggest the potential applications of *Z. zerumbet* as an alternative antidote against PCM-induced nephrotoxicity, as well as a novel antioxidant.

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References

Abdel-Zaher, A.O., Abdel-Rahman, M.M., Hafez, M.M., Omran, F.M., 2007. Role of nitric oxide and reduced glutathione in the protective effects of aminoguanidine, gadolinium chloride and oleanolic acid against acetaminophen-induced hepatic and renal damage. *Toxicology*, **234**(1-2):124-134. [doi:10.1016/j.tox.2007.02.014]

Abdul, A.B., Abdelwahab, S.I., Al-Zubairi, A.S., Elhassan, M.M., Murali, S.M., 2008. Anticancer and antimicrobial activities of zerumbone from the rhizomes of *Zingiber zerumbet*. *Int. J. Pharmacol.*, **4**(4):301-304. [doi:10.3923/ijp.2008.301.304]

Alderman, C.J., Shah, S., Foreman, J.C., Chain, B.M., Katz, D.R., 2002. The role of advanced oxidation protein products in regulation of dendritic cell function. *Free Radic. Biol. Med.*, **32**(5):377-385. [doi:10.1016/S0891-5849(01)00735-3]

Beyer, W., Fridovich, I., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal. Biochem.*, **161**(2):559-566. [doi:10.1016/0003-2697(87)90489-1]

Blakely, P., McDonald, B.R., 1995. Acute renal failure due to acetaminophen ingestion: a case report and review of the literature. *J. Am. Soc. Nephrol*., **6**(1):48-53.

Borne, R.F., 1995. Nonsteroidal Anti-Inflammatory Drugs. *In*: Foye, W.O., Lemke, T.L., Williams, D.A. (Eds.), Principles of Medicinal Chemistry. Williams & Wilkins, p.535-580.

Boutis, K., Shannon, M., 2001. Nephrotoxicity after acute severe acetaminophen poisoning in adolescents. *Clin. Toxicol*., **39**(5):441-445. [doi:10.1081/CLT-100105413]

Brown, R.A., 1968. Hepatic and renal damage with paracetamol overdosage. *J. Clin. Pathol.*, **21**(6):793.

Carpenter, H.M., Mudge, G.H., 1981. Acetaminophen nephrotoxicity: studies on renal acetylation and deacetylation. *J. Pharmacol. Exp. Ther*., **218**(1):161-167.

Chevion, M., Berenshtein, E., Stadtman, E.R., 2000. Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic. Res.*, **11**(33): S99-S108.

Das, J., Ghosh, J., Manna, P., Sil, P.C., 2010. Taurine protects acetaminophen-induced oxidative damage in mice kidney through APAP urinary excretion and CYP2E1 inactivation. *Toxicology*, **269**(1):24-34. [doi:10.1016/j.tox.2010.01.003]

Dilger, R.N, Baker, D.H., 2007. Oral *N*-acetyl-L-cysteine is a safe and effective precursor of cysteine. *J. Anim. Sci*., **85**(7):1712-1718. [doi:10.2527/jas.2006-835]

Ellman, G.L., 1959. Tissue sulphydryl groups. *Arch. Biochem.*, **82**(1):70-77. [doi:10.1016/0003-9861(59)90090-6]

Esterbauer, H., Cheeseman, K.H., 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. *Meth. Enzymol*., **186**:407-421. [doi:10.1016/0076-6879(90)86134-H]

Gunnell, D., Murray, V., Hawton, K., 2000. Use of paracetamol (acetaminophen) for suicide and nonfatal poisoning: worldwide patterns of use and misuse. *Suicide Life Threat. Behav.*, **30**(4):313-326.

Hart, S.G., Beierschmitt, W.P., Wyand, D.S., Khairallah, E.A., Cohen, S.D., 1994. Acetaminophen nephrotoxicity in CD-1 mice. I. Evidence of a role for in situ activation in selective covalent binding and toxicity. *Toxicol. Appl. Pharmacol.*, **126**(2):267-275. [doi:10.1006/taap.1994.1116]

Hemabarathy, B., Budin, S.B., Feizal, V., 2009. Paracetamol hepatoxicity in rats treated with crude extract of *Alpinia galanga*. *J. Biol. Sci.*, **9**(1):57-62. [doi:10.3923/jbs.2009.57.62]

Huang, G.C., Chien, T.Y., Chen, L.G., Wang, C.C., 2005. Antitumor effects of zerumbone from *Zingiber zerumbet* in P-388D1 cells in vitro and in vivo. *Planta Med.*, **71**(3): 219-224. [doi:10.1055/s-2005-837820]

Ibrahim, M.Y., Abdul, A.B., Ibrahim, T.A., AbdelWahab, S.I., Elhassan, M.M., Mohan, S., 2010. Attenuation of cisplatin-induced nephrotoxicity in rats using zerumbone. *Afr. J. Biotechnol.*, **9**(28):4434-4441.

Jaganath, I.B., Ng, L.T., 2000. Herbs: The Green Pharmacy of Malaysia. Vinpress Sdn. Bhd, Malaysia, p.95-99.

Kaderi, M.G., Habib, M.R., Nikkon, F., Yeasmin, T., Rashid, M.A., Rahman, M.M., Gibbons, S., 2010. Zederone from the rhizomes of *Zingiber zerumbet* and its antistaphylococcal activity. *Latin Am. Caribbean Bull. Med. Arom. Plants*, **9**(1):63-68 (in Spanish).

Kaplowitz, N., 2000. Mechanism of liver cell injury. *J. Hepatol.*, **32**(S1):39-47. [doi:10.1016/S0168-8278(00)80414-6]

Kheradpezhouh, E., Panjehshahin, M.R., Miri, R., Javidnia, K., Noorafshan, A., Monabati, A., Dehpour, A.R., 2010. Curcumin protects rats against acetaminophen-induced hepatorenal damages and shows synergistic activity with *N*-acetyl cysteine. *Eur. J. Pharmacol*., **628**(1-3):274-281. [doi:10.1016/j.ejphar.2009.11.027]

Levine, R.L., Williams, J., Stadtman, E.R., Shacter, E., 1990. Carbonyl assays for determination of oxidatively modified proteins. *Meth. Enzymol.*, **233**:346-357. [doi:10.1016/S0076-6879(94)33040-9]

Margetis, P.I., Antonelou, M.H., Petropoulos, I.K., Margaritis, L.H., Papassideri, I.S., 2009. Increased protein carbonylation of red blood cell membrane in diabetic retinopathy. *Exp. Mol. Pathol.*, **87**(1):76-82. [doi:10.1016/j.yexmp.2009.04.001]

McMurtry, R.J., Snodgrass, W.R., Mitchell, J.R., 1978. Renal necrosis, glutathione depletion, and covalent binding after acetaminophen. *Toxicol. Appl. Pharmacol.*, **46**(1):87-100. [doi:10.1016/0041-008X(78)90139-4]

Mitchell, J.R., McMurtry, R.J., Statham, C.N., Nelson, S.D., 1977. Molecular basis for several drug-induced nephropathies. *Am. J. Med.*, **62**(4):518-526. [doi:10.1016/0002-9343(77)90407-7]

Nakamura, Y., Yoshida, C., Murakami, A., Ohigashi, H., Osawa, T., Uchida, K., 2004. Zerumbone, a tropical ginger sesquiterpene, activates phase II drug metabolizing enzymes. *FEBS Lett.*, **572**(1-3):245-250. [doi:10.1016/j.febslet.2004.07.042]

Nelson, S.D., 1995. Mechanisms of the formation and disposition of reactive metabolites that can cause acute liver injury. *Drug Metab. Rev.*, **27**(1-2):147-177.

Newton, J.F., Yoshimoto, M., Bernstein, J., Rush, G.F., Hook, J.B., 1983. Acetaminophen nephrotoxicity in the rat. 1. Strain differences in nephrotoxicity and metabolism. *Toxicol. Appl. Pharmacol.*, **69**(2):291-306. [doi:10.1016/

0041-008X(83)90311-3]

Pietta, P.G., 2000. Flavonoids as antioxidants. *J. Nat. Prod.*, **63**(7):1035-1042. [doi:10.1021/np9904509]

Placke, M.E., Wyand, D.S., Cohen, S.D., 1987. Extrahepatic lesions induced by acetaminophen in the mouse. *Toxicol. Pathol.*, **15**(4):381-387. [doi:10.1177/019262338701500401]

Rashid, R.A., Hawariah, A., Pihie, L., 2005. The antiproliferative effects of *Zingiber zerumbet* extracts and fractions on the growth of human breast carcinoma cell lines. *Malays. J. Pharma. Sci.*, **3**(1):45-52.

Ruslay, S., Abas, F., Shaari, K., Zainal, Z., Maulidiani, Sirat, H., Israf, D.A., Lajis, N.H., 2007. Characterization of the components present in the active fractions of health gingers (*Curcuma xanthorrhiza* and *Zingiber zerumbet*) by HPLC-DAD-ESIMS. *Food Chem.*, **104**(3):1183-1191. [doi:10.1016/j.foodchem.2007.01.067]

Saadiah, M.S., Halijah, I., 1995. Zingiberaceae. Proceedings of the National Convention on Herbal Medicine. Forest Research Institute Malaysia, Kuala Lumpur, p.205-207.

Sharifah Sakinah, S.A., Handayani, S.T., Azimahtol, L.P., 2007. Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. *Cancer Cell Inter.*, **7**(1):4. [doi:10.1186/1475-2867-7-4]

Slot, C., 1965. Plasma creatinine determination. A new & specific Jaffe reaction method. *Scand. J. Clin. Lab. Invest.*, **17**(4):381-387. [doi:10.3109/00365516509077065]

Somchit, M.N., Shukriyah, M.H., 2003. Anti-inflammatory property of ethanol and water extracts of *Zingiber zerumbet*. *Ind. J. Pharmacol.*, **35**:181-182.

Witko-Sarsat, V., Friedlander, M., Capeillere-Blandin, C., Nguyen-Khoa, T., Nguyen, A.T., Zingraff, J., Jungers, A.T., Descamps-Latscha, B., 1996. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney Int.*, **49**(5):1304-1313. [doi:10.1038/ki.1996.186]

Yousef, M.I., Omar, S.A., El-Guendi, M.I., Abdelmegid, L.A., 2010. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, liver and kidney functions and haematotoxicity in rat. *Food Chem. Toxicol*., **48**(11):3246-3261. [doi:10.1016/j.fct.2010.08.034]