Role Of Oxidative Stress In The Protective Effects Of Zingiber Zerumbet Smith Ethyl- Acetate Extract Against…

Article · January 2011

9 authors, including:

Asmah Hamid
National University of Malaysia
24 PUBLICATIONS **76** CITATIONS

SEE PROFILE

Siti Balkis Budin
National University of Malaysia
64 PUBLICATIONS **478** CITATIONS

SEE PROFILE

Khairana Husain
National University of Malaysia
19 PUBLICATIONS **128** CITATIONS

SEE PROFILE

Zariyantey Abd Hamid
National University of Malaysia
24 PUBLICATIONS **210** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Project   Sexuality and Disability View project

Project   SEANUTS Malaysia View project

Role Of Oxidative Stress In The Protective Effects Of Zingiber *Zerumbet* Smith Ethyl-Acetate Extract Against Paracetamol-Induced Hepatotoxicity In Sprague-Dawley Rats

[1]Asmah Hamid, [1]Siti Balkis Budin, [1]Rafidah Aini Pakri Mohamed, [1]Noraida Abd Manaf, [3]Nor Yuliani Yuhana, [2]Khairana Husain, [1]Zariyantey Abd Hamid, [1]Jamaludin Mohamed.

[1]Department of Biomedical Science, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.
[2]Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.
[3]Lincoln Collage, Kuala Lumpur, Malaysia.

Abstract: *Zingiber zerumbet* is known to possess antioxidant, anti-inflammatory and anticancer activities. The aim of this study was to investigate the hepatoprotective effects of ethyl acetate extract of *Z. zerumbet* rhizome against paracetamol-induced hepatotoxicity. Fifty male Sprague-Dawley rats were divided into five groups consisted of control, 750 mg/kg paracetamol, 400 mg/kg *Z. zerumbet* extract, paracetamol plus 200 mg/kg *Z. zerumbet* extract and paracetamol plus 400 mg/kg *Z. zerumbet* extract. Paracetamol were administered orally for seven days concurrent with *Z. zerumbet* extract intraperitoneally. Administration of ethyl acetate *Z. zerumbet* extract at doses of 200mg/kg and 400mg/kg protected paracetamol-induced hepatotoxicity as evidenced by significantly reduced ($p<0.05$) activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Treatment with *Z. zerumbet* at similar doses were also able to significantly increased ($p<0.05$) the superoxide dismutase (SOD) activities and reduced glutathione (GSH) levels in liver homogenate. The increment of malondialdehyde (MDA) and protein carbonyl in liver homogenate were significantly inhibited ($p<0.05$) by administration of ethyl acetate extract of *Z. zerumbet*. Administration of *Z. zerumbet* at the dose of 400 mg/kg were found showed better hepatoprotective effects as compared to 200 mg/kg. In conclusion ethyl acetate extract of *Z. zerumbet* have the potential in preventing paracetamol-induced hepatotoxicity and this hepatoprotective effects was probably mediated through the reduction in oxidative stress.

Key words: Hepatotoxicity, **a**ntioxidants, oxidative stress, paracetamol, *Zingiber zerumbet*.

INTRODUCTION

Paracetamol (PCM) is an excellent antipyretic and analgesic drug and is proven safe when taken in therapeutic doses, however overdoses can causes hepatotoxicity (Larson, 2007). Hepatic injury in paracetamol toxicity is induced by reactive metabolite, N-acetyl-p-benzo-quinoneimine (NAPQI) (Nelson, 1990). The increment of NAPQI decreased intracellular reduced glutathione (GSH) which enable NAPQI to bind with cellular protein and leads to hepatocellular injury (Jollow *et al.,* 1973). Aminotransferase enzymes (alanine aminotransferase: ALT, aspartate aminotransferase: AST) and alkaline phosphatase (ALP) which significantly elevated in hepatotoxicity condition are considered as a good marker of hepatocellular injury (Yanpallewar *et al*., 2003).

Depletion of GSH in paracetamol induced-hepatotoxicity is also a consequence of high reactive oxygen species formation (Mourelle *et al*., 1991). Generation of reactive oxygen species (ROS) and depletion of GSH in the early stage of hepatic metabolism of paracetamol has been proposed as the possible mechanism mediating hepatocellular injury process (Manov *et al*., 2002).

Recently the use of herbal natural product gains active research interest among researcher to study the effects and implication of various natural products in human life. To date, a number of herbs have been proven to have high medicinal value that beneficial for improvement of health status (Fakurazi *et al*., 2008). These include *Kigelia africana, Hibiscus sabdariffa, Alchornea cordifolia* and *Moringa oleifera* which shown to have high antioxidant properties and hepatoprotective effects (Fakurazi *et al*., 2008, Olaleye & Rocha 2008). Previously, *Curcuma longa* has been reported to show hepatoprotective effects against paracetamol-induced toxicity by reducing the formation of pro-inflammatory cytokines and upregulation of its antioxidant activities (Donatus *et al*., 1990). The role of antioxidants derivatives from natural products in mediating hepatoprotective effects has been shown in *M. oleifera* which hepatoprotective activities were achieved by restoring the level of GSH during paracetamol toxicity (Fakurazi *et al*., 2008).

Zingiberaceae family which is widely distributed in Southeast Asia region contains important natural resources for various products development and well known for its medicinal properties (Tushar *et al*., 2010).

Corresponding Author: Siti Balkis Budin. MD, PhD. Department of Biomedical Science, Faculty of Health Sciences Universiti Kebangsaan Malaysia, Kuala Lumpur Malaysia. Fax 03 26929032.
E-mail: balkis6466@yahoo.com.my

Zingiber zerumbet Smith which belongs to the Zingiberaceae family is a herb commonly found in Malaysia and known locally as *lempoyang* (Ruslay *et al*., 2007). Traditionally, the fresh rhizome of *Z. zerumbet* is used as an appetizer and to treat swelling sores, stomach ache and worm infestation. Furthermore, the use of *Z. zerumbet* as an anti-inflammatory (Sulaiman *et al*., 2010) and anticancer agent (Syarifah Sakinah *et al*., 2007) has been also documented. Recently, ethyl acetate extract *of Z. zerumbet* has been found to contain high antioxidant properties (Ruslay *et al*., 2007). However no study has been done on its hepatoprotective effect. Considering the above facts, we embarked on the present study to determine the protective effects of ethyl acetate extract of *Z. zerumbet* rhizome against paracetamol-induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant Materials:
The rhizomes 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 and was deposited as a voucher specimen at the herbarium of UKM, Bangi, Selangor. The specimen was cleaned and chopped into small pieces and then air-dried in room temperature for 3 days.

Extraction:
The air-dried rhizomes of *Z. zerumbet* (7 kg) was sequentially soaked at room temperature in *n*-hexane, ethyl acetate and followed by methanol for 72 hours. The resultant extracts were filtered and evaporated to dryness *in vacuo* to yield crude extracts of hexane, ethyl acetate, and methanol. The ethyl acetate crude extract (3.2 g) obtained were stored at 4ºC until tested for bioassay. Prior to use, the *Z. zerumbet* ethyl acetate extract was dissolved in DMSO and diluted in Phosphate Buffer Solution (PBS) pH 7.4.

Animal Preparation And Treatment Regime:
Male Sprague-Dawley rats (230 to 250g) were obtained from Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia (UKM). The animals were allowed to adapt to laboratory condition for 7 days prior to the experiments, kept in polypropylene cages with wood shavings at an ambient room temperature with 12 h light/dark cycle and access to food and water *ad libitum* without restriction. Animals handling procedure were in accordance with the rules and regulations by the UKM Animal Ethics Committee.

The animals were randomly divided into five groups consisting of 10 rats each. The first group was control group (Control) and only received distilled water, the second group was given 400 mg/ kg *Z. zerumbet* extract (Control 400) and the 3rd group was received oral PCM (20% suspension in distilled water) at the dose of dose of 750 mg/kg for seven consecutive days (PCM group). The 4th and 5th groups were paracetamol plus 200 mg/ kg of *Z. zerumbet* extract (PCM + 200) and paracetamol plus 400 mg/kg of *Z. zerumbet* extract (PCM + 400) respectively in which the extract were given intraperitoneally and concurrently with PCM administration for seven consecutive days. 24 hours after the last treatment body weight were measured and the animals were sacrificed to obtain blood and liver. Livers were quickly excised, washed in ice-cold saline, dried and weighed.

Biochemical Assays:
Blood was centrifuged for 15 min at 1000 *g* and the obtained serum was stored at −40 ◦C until further used. Liver homogenate was prepared by homogenizing the tissue in cold KCl (1.15%, pH 7.4, 1:4 w/v) using homogenizer (Ultra Turrax T25). Serum aminotransferases (ALT and AST) were determined using the method by Reitman & Frankel (1957), and serum ALP activities were measured by the method of Boomers & McComb (1975). Serum and liver protein were determined following Bradford assay (Bradford, 1976).

Oxidative Stress Evaluation:
In vivo lipid peroxidation was determined by measuring malondialdehyde (MDA) spectrophotometrically according to the procedure of Stock *et al*., (1976) using various concentration of 1.1.3.3.-tetraethoxypropane (TEP) as a standard curve. Measurement of protein carbonyl was done according to the method of Levine *et al*., (1994) based on their reaction with 2,4- dinitro-phenylhydrazine (DNPH) to form hydrazones which was measured spectrophotometrically at 370 nm. Superoxide dismustase (SOD) activity was assayed according to the method of Beyer and Fridovich (1987) and one unit of SOD is considered as amount of enzymes caused 50 % inhibition of nitro blue tetrazoluem (NBT) reduction. Reduced glutathione (GSH) was determined by the method of Ellman (1959) using 5,5,-dithiobis-2-nitrobenzoic (DTNB) as substrate whereby the homogenate and plasma were precipitated with perchloric acid (5%), centrifuged at 1000 g and the GSH in the supernatant were determined colourimetrically at 412 nm.

Statistical Analysis:

The data was expressed as mean ± standard error of mean (S.E.M.) Statistical analysis was performed using statistical Package for the Social Sciences (SPSS) version 18. The significant differences between groups were determined using one-way analysis of variance (ANOVA) followed by Post-hoc Tukey test with p<0.05 considered as statistically significance different.

Results and Discussion

Throughout the study there was no mortality noted in all rats in the experimental groups. PCM group showed a significant decreased in body weight as compared to the control (p<0.05) and Z. *zerumbet* supplementation caused significantly higher body weight as compared to the PCM group (p<0.05) (Table I). No significant difference in total weight of the liver between all groups was observed. However, PCM groups exhibit significantly higher (p<0.05) in relative weight of liver as compared to control group. Treatment with 200 mg/kg and 400 mg/kg Z. *zerumbet* extract, both groups showed a significant reduction (p<0.05) in relative weight of the liver as compared to PCM group. Furthermore, the administration of 400 mg/kg Z. *zerumbet* extract alone, was able to maintain the relative weight of liver to the value of control group (p>0.05).

Liver enzymes namely ALP, ALT, AST were determined to indicate hepatic function. Administration of PCM alone at the dose of 750 mg/kg for seven consecutive days caused a significant increased the levels of serum ALT, AST and ALP in PCM group as compared to the control group (p<0.05) (Table II). Administration of Z. *zerumbet* ethyl acetate extract at 200 mg/kg and 400 mg/kg however prevented the liver injury as indicated by significant reduction (p<0.05) in the levels of liver enzymes activities. In addition, administration of extract at higher dose (PCM + Z. *zerumbet* 400 mg/kg) showed greater hepatoprotective effects as demonstrated with significantly reduced (p<0.05) activities of ALT, AST and ALP as compared to PCM + Z. *zerumbet* 200 mg/kg group. PCM administration significantly lowered the levels of serum and liver protein and significant increased (p<0.05) of protein levels was also observed in PCM groups treated with both doses of the extract.

The results of oxidative stress markers of liver homogenate showed in Table III. The liver MDA and protein carbonyl was found to be significantly higher (p<0.05) in PCM group as compared to the control group. Although the administration of Z. *zerumbet* extracts at both doses were able to significantly inhibit (p<0.05) the elevation of MDA and protein carbonyl during PCM toxicity, the levels were still significantly higher (p<0.05) as compared to the control group. In addition, treatment with higher dose of Z. *zerumbet* extract was significantly more effective in reducing (p<0.05) the production of liver homogenate MDA and protein carbonyl as compared to PCM + 200 mg/kg group-administered rats.

Meanwhile the GSH levels in the hepatic tissue homogenate of PCM group was significantly (p<0.05) decreased as compared to the levels measured in the control group and treatment with Z. *zerumbet* extract at both doses prevent the PCM-induced GSH reduction. In addition, group treated with Z. *zerumbet* at the dose of 400 mg/kg showed significantly higher GSH levels than in 200 mg/kg group. Results showed that SOD activities were not altered in PCM treated group and administration of Z. *zerumbet* also did not give any significant changes compared to control group.

According to the biochemical analyses, interestingly we found that rats treated with 400 mg/kg of Z. *zerumbet* alone did not show any significant changes in all parameters when compared to the control group and these indicate that Z. *zerumbet* ethyl acetate extract at the dose of 400 mg/kg did not cause any toxic effects to the experimental animals.

Discussion:

PCM induced-hepatocellular damage is mediated through its reactive metabolite, namely NAPQI which cause acute centrilobular hepatic necrosis that lead to loss of cell function and structural integrity of hepatocellular membrane (Lee *et al*., 1996). Leakage of cellular enzymes into the plasma is a hallmark of hepatic injury or damage and measurement of serum liver enzyme such as ALT, AST and ALP are commonly used as marker enzymes in assessing PCM induced hepatotoxicity (Asha *et al*., 2004; Yen *et al*., 2007). In this study, administration of PCM at a dose of 750 mg/kg for seven consecutive days was able to induce hepatic damage as indicated with the substantial increased in serum liver enzymes and this finding is in accordance with other reported studies (Abdel-Zaher *et al*., 2007; Gardher *et al*., 2002). In addition, marked reduction of serum and protein levels were observed in PCM-treated rat. These events could be mediated through the covalent binding of NAPQI to proteins which has been reported as the causative factors for oxidative damage, proteins denaturation and changes in protein structures that contribute to the reduction of serum and liver protein levels (Hazai *et al*., 2002).

Various medicinal plants have been evaluated for their potential effects as hepatoprotective agent (Ahmed & Khater 2001; Kumar *et al*., 2004). To our knowledge, no studies have been done to investigate the protective role of ethyl acetate extract of Z. *zerumbet* rhizome in PCM-induced hepatocellular damage. Results from the present study demonstrated that concurrent administration of Z. *zerumbet* at 200 mg/kg and 400 mg/kg with

750mg/kg PCM for seven consecutive days was able to suppress PCM induced hepatocellular injury as evidenced by the significant reduced in the levels of serum hepatic enzymes and elevation in protein levels. This indicates that *Z. zerumbet* was able to maintain the structural integrity and functional capacity of the hepatocyte thus preventing enzymes leakage into the plasma and suggest the potential used of *Z. zerumbet* rhizome as hepatoprotective agents during PCM toxicity. Furthermore, administration of *Z. zerumbet* at 400 mg/kg alone showed no significant alteration of the levels of theses liver enzymes which were almost similar as in control group.

Our results are in agreement with previous study which showed that plant extract have the potential in preventing the hepatocellular damage thus inhibit the leakage of hepatic enzymes into the circulation (Ahmed and Khater 2001). In addition, similar effect was also observed when *Moringa oleifera* Lam that was given prior to PCM administration was able to restore plasma liver enzymes to almost normal levels and the hepatoprotective effects were confirmed by reduced histopathological changes with less hepatic cell damage were observed (Fakurazi *et al*., 2008).

GSH is one of the most important non-enzymatic biological antioxidant that present in the liver which responsible for the removal of free radical species such as hydrogen peroxide and superoxide radicals (Prakash *et al*., 2001). GSH is also the main water-soluble cellular antioxidant that serves as the first line of defence mechanism in combating against free radicals species and plays as an important role in the detoxification activities in liver (Kaplowitz, 2000). GSH is essential to maintain the redox environment by detoxifying electrophiles and scavenging free radicals (Ibrahim *et al*., 2010). In PCM toxicity, NAPQI will bind to GSH and being converted to the oxidized form (GSSH) and thus exacerbated the oxidative membrane damage. Furthermore, the generation of reactive nitrogen and oxygen species appears as an early event, which exacerbates intracellular GSH depletion (Manov *et al*., 2002).

Table 1: Effects of Z. zerumbet ethyl acetate extract on mean body weigh total and relative weight of liver in rats given 750 mg/kg/day PCM for 7 consecutive days.

Group	Body weight (g)	Total weight of liver (g)	Relative weight of liver
Control	255.33 ± 5.47	10.53 ± 0.26	0.043 ± 0.001
Z. zerumbet (400 mg/kg)	251.17 ± 4.92	10.28 ± 0.41	0.039 ± 0.002
PCM	210.33± 20.22*	10.71 ± 0.17	0.051 ± 0.002*
PCM + *Z. zerumbet* (200 mg/kg)	232.17 ± 7.57*#	10.48 ± 0.13	0.045 ± 0.002#
PCM + *Z. zerumbet* (400 mg/kg)	244.17± 7.34#	10.50 ± 0.16	0.043 ± 0.002#Φ

Values are expressed as mean ± SEM, n=10 animals for each group. * significantly different compared to the control group, #significantly different compared to PCM groups, Φ significantly different compared to PCM + 200 mg/kg *Z. zerumbet* (all significant value is at p<0.05).

Table 2: Effect of *Zingiber zerumbet* ethyl acetate extract on liver enzymes and total protein levels in rats given 750 mg/kg/day PCM for 7 consecutive days.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total serum protein (mg/ml)	Total liver protein mg/ml
Control	24.02 ± 8.79	20.70 ± 4.23	61.03 ± 4.96	109.38 ± 18.85	122.38 ± 20.76
Z. zerumbet (400 mg/kg)	20.37 ±1.83	22.93 ±2.07	68.72 ±3.09	107.38 ±17.65	121.88 ± 24.34
PCM	64.17 ± 4.60*	110.20 ±12.14*	127.88 ± 8.59*	54.88 ± 10.95*	55.21 ± 6.60*
PCM + *Z.zerumbet* (200 mg/kg)	52.07±3.92*#	98.40 ± 13.32*#	119.60±6.46*	109.54 ± 13.74#	96.69±15.90#
PCM + *Z. zerumbet* (400 mg/kg)	24.93 ± 2.40Φ	81.23 ± 13.64*#Φ	79.10 ± 7.12*#Φ	113.54 ±14.69#	119.88 ±23.08#

Values are expressed as mean ± SEM. n=10 animals for each group. * significantly different compared to the control group, # significantly different compared to PCM groups, Φ significantly different compared to PCM + 200 mg/kg *Z. zerumbet* (all significant value is at p<0.05).

Table 3: Effect of *Zingiber zerumbet* ethyl acetate extract on oxidative stress status of liver homogenate in rats given 750 mg/kg/day PCM for 7 consecutive days.

Groups	Protein carbonyl (mmol/g protein)	MDA (nmol/g protein)	SOD (u.e/min/g protein)	GSH (mmol/g tissue)
Control	0.14 ± 0.02	8.27 ± 0.73	14.1 ± 1.29	4.28 ± 0.10
Z. zerumbet) (400mg/kg)	0.10 ± 0.01	8.04 ± 0.53	13.6 ± 1.49	3.65 ± 0.38
PCM	0.41 ± 0.04*	25.58 ± 1.10*	13.33 ± 1.61	2.72 ± 0.14*
PCM + *Z. zerumbet* (200mg/kg)	0.22 ± 0.01*#	19.02 ± 0.83*#	13.57 ± 0.81	5.22 ± 0.38#
PCM + *Z. zerumbet* (400mg/kg)	0.20 ± 0.02*#	14.59 ± 0.84*#Φ	14.67 ± 0.69	6.24 ± 0.11*#

Values are expressed as Mean ± SEM. n=10 animals for each group * significantly different compared to the control group, # significantly different compared to PCM groups Φ significantly different compared to PCM + 200 mg/kg Z. zerumbet (all significant value is at p<0.05).

From this study, the decreased level of GSH has been observed in paracetamol-treated group, hence were in favour of the possibility that the overproduction of NAPQI in PCM hepatotoxicity, and excess generation of free radical species lead to oxidative stress condition with subsequent depletion in GSH levels. However, *Z. zerumbet* administration appeared to cause less oxidative stress condition as evidenced in this study showing that ethyl acetate extract of *Z. zerumbet* did increase the levels of GSH. According to Chan *et al.,* (2001), Nrf2 (nuclear factor erythroid 2-related factor 2) played an essential role for detoxification of acetaminophen. In addition a study conducted by Nakamura *et al.,* (2004) revealed that Zerumbone (ZER), a sesquiterpene compound from *Z. zerumbet* could boost Nrf2 function which plays an essential role in the defense against oxidant and electrophilic chemical insults. Nrf2 will mediate glutathione (GSH) synthesis pathway as it binds to antioxidant response element (ARE) of the phase II enzymes genes. In agreement with these findings, a study conducted by Ibrahim *et al.,* (2010) revealed that rats treated with *Z.zerumbet* had elevated level of renal GSH in cisplatin induced nephrotoxicity in rats models.

Previous study reported that the ROS mediated hepatotoxicity can be effectively prevented by administration of compound possessing antioxidants (Fakurazi *et al.,* 2008; Olaleye & Rocha 2008; Hemabarathy *et al.,* 2002). Family Zingiberaceae was known to possess highly antioxidant activity and highly medicinal value (Tushar *et al.,* 2010). In support with our finding, previously study also reported that the extract of *Alpania galangal* , a member of zingiberaceae family also possess hepatoprotective effects by reducing the oxidative stress in paracetamol induced toxicity rats (Hemabarathy *et al.,* 2009). *Z. zerumbet* treatments were able to ameliorate the PCM induced hepatocellular damage and therefore the hepatoprotective effects were most probably due antioxidant activities of its compound. Previous finding reported that ethyl acetate extract of *Z. zerumbet* rhizome rich with flavonoid glycosides, the derivatives of kaempferol, and possess antioxidant property (Ruslay *et al.,* 2007). Ruslay *et al.,* (2007) had measured the total antioxidant activity from rhizome of *Z.zerumbet* using ferric thiocyanate (FTC) assay and thiobarbituric acid (TBA) assay which revealed that the antioxidant activity of ethyl acetate extract from rhizome of *Z.zerumbet* was the most significant.

Excess production of free radicals attack the biological molecules such as DNA, proteins and lipid and in which lipid and protein oxidation has been suggested as a mechanism that lead to hepatocellular injury (Genet *et al.,* 2000). In addition, the depletion in intracellular GSH in PCM toxicity condition, causes high levels of reactive electrophilic metabolites bind massively to the biological molecules (Kaplowitz, 2000). PCM toxicity led covalent binding of NAPQI with sulphydryl group of proteins results in the protein oxidation and cell necrosis (Lin *et al.,* 1997). MDA is one of the main products of lipid peroxidation that is widely used as a biomarker of oxidative stress in determination of the total lipid peroxidation in cell and tissues (Yagi, 1998). In agreement with the previous study (Gamal *et al.,* 2003), the present study shows that the depletion of hepatic GSH leads to oxidation damage of lipid, and protein, and also failure of the antioxidant defense system as evidence by increased in MDA and protein carbonyl levels in the liver and plasma together with reduced SOD activities in PCM treated rats and these finding suggested that tissue injury had occurred.

The ethyl acetate extract of *Z. zerumbet* rhizome used in the present study able to minimize the oxidative damage in plasma and liver of *Z. zerumbet*-treated PCM group. These results suggested the hepatoprotective effect of *Z. zerumbet* against paracetamol-induced hepatoxicity which most probably mediated through its antioxidant properties. The ability of *Z. zerumbet* to reduce protein and lipid oxidation and to increase the antioxidant defense mechanism are believed as the possible mechanism that preserve the cell function through inhibition of oxidative cellular damage through oxidative stress. Furthermore the probable mechanism by which *Z. zerumbet* exerts its protective effect against paracetamol -induced hepatocellular injury is also could be mediated by its ability to accelerate detoxification and excretion of hepatotoxic reactive metabolite products.

The *Z. zerumbet* used in this study shows the hepatoprotective effects in dose dependent manner as evident with better hepatoprotective effects was demonstrated at higher dosage (400mg/kg) of *Z. zerumbet* than in rats received lower dosage (200mg/kg). In addition, administration of *Z. zerumbet* ethyl acetate extract at 400mg/kg that was given alone in the absence of paracetamol showed no hepatotoxicity effects as indicated by the levels of hepatic enzymes and oxidative stress markers that were almost similar to the control group. This finding is believed to be mediated through its higher antioxidant properties that prevent PCM-induced liver damage.

Conclusion:

Present results suggested that oral administration of ethyl acetate extract of Z. zerumbet protects rats from PCM-induced hepatotoxicity and the effect is most probably mediated through the reduction of oxidative stress. These observations suggest that *Z. zerumbet* could be applied in clinical application in which cellular damage is a consequence of oxidative stress.

ACKNOWLEDGEMENT

Declaration of Interest:

The authors report no conflicts of interest. All the authors are responsible in writing and contents of this article.

REFERENCES

Abdel-Zaher, A.O., M.M. Abdel-Rahman, M.M. Hafez and F.M. Omran, 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: 124-134.

Ahmed, M.B. and M.R. Khater, 2001. Evaluation of the protective potential of *Ambrosia maritima* extract on acetaminophen-induced liver damage. J. Ethnopharmacol., 75: 169–174.

Asha, V.V., S. Akhila, P.J. Wills and A. Subramoniam, 2004. Further studies on the antihepatotoxic activity of Phyllanthus maderaspatensis Linn. J. Ethnopharmacol., 92: 67–70.

Beyer, W.F. and I. Jr Fridovich, 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem.,161: 59-66.

Bowers, G.N. and R.B. Jr McComb, 1975. Measurement of total alkaline phosphatase activity in human serum. Clin. Chem., 21: 1988-1995.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Chan K., X.D. Han and Y.W. Kan, 2001. An important function of Nrf2 in combating oxidative stress: Detoxification of acetaminophen. Proc. Natl. Acad. Sci. USA., 98: 4611-4616.

Donatus, I.A., Sardjoko and N.P. Vermeulen, 1990. Cytotoxic and cytoprotective activities of curcumin. Effects on paracetamol-induced cytotoxicity, lipid peroxidation and glutathione depletion in rat hepatocytes. Biochem. Pharmacol., 39: 1869-1875.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77.

Fakurazi, S., I. Hairuszah and U. Nanthini, 2008. Moringa oleifera Lam prevents acetaminophen induced liver injury through restoration of glutathione level. Food. Chem. Toxicol., 46: 2611–2615.

Gamal, el-din, A.M, A.M. Mostafa, O.A. Al-Shabanah, A.M. Al-Bekairi and M.N. Nagi, 2003. Protective effect of arabic gum against acetaminophen-induced hepatotoxicity in mice. Pharmalogical. Res., 48: 631–635.

Gardner, C.R., J.D. Laskin, D.M. Dambach, M. Sacco, S.K. Durham, M.K. Bruno, M.K. Gordon, D.R. Gerecke, P. Zhou and D.L..Laskin, 2002. Reduced hepatotoxicity of acetaminophen in mice lacking inducible nitric oxide synthase: potential role of tumor necrosis factor-alpha and interleukin-10. Toxicol Appl Pharmacol., 184: 27–36.

Genet, S., R.K. Kale and N.Z. Baquer, 2000. Effects of free radicals on cytosolic creatine kinase activities and protection by antioxidant enzymes and sulfhydryl compounds. Mol. Cell. Biochem., 210: 23–28.

Hazai, E., L. Vereczkey and K. Monostory, 2002. Reduction of toxic metabolite formation of acetaminophen. Biochem. Biophys. Res .Commun., 291: 1089–1094.

Hemabarathy, B., S.B. Budin and V. Faizel, 2009. Paracetamol hepatotoxicity in rats treated with crude extract of *Alpinia galanga*. J. Biol. Sciences, 9: 57-62.

Ibrahim M.Y., A.B. Abdul, T.A.T. Ibrahim, S.I Abdel Wahab, M.M. Elhassan and S. Mohan, (2010). Attenuation of cisplatin-induced nephrotoxicity in rats using zerumbone. African Journal of Biotechnology , 4434-4441.

Jollow, D.J., J.R. Mitchell, W.Z. Potter, D,C. Davis, J.R. Gillette and B.B. Brodie, 1973. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. J. Pharmacol. Exp. Ther.,187: 195–202.

Kaplowitz, N., 2000. Mechanism of liver cell injury. J. Hepatol., 32: 39–47.

Kumar, G., G.S. Banu, P.V. Pappa, M. Sundararajan and M.R. Pandian, 2004. Hepatoprotective activity of *Trianthema portulacastrum* L.against paracetamol and thioacetamide intoxication in albino rats. J. Ethnopharmacol., 92: 37–40.

Larson, A.M., 2007. Acetaminophen hepatotoxicity. Clin. Liver Dis., 11: 525–548.

Lee, S.S., J.T. Buters, T. Pineau, P. Fernandez-Salquero and F.J. Gonzalez, 1996. Role of CYP2E1 in hepatotoxiciy of acetaminophen. J. Biol. Chem., 271: 12063-12067.

Levine, R.L., J.A. Williams, E.R. Stadtman and E. Shacter, 1994. Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol., 233: 346-357.

Lin, C.C., D.E. Shieh and M.H. Mah, 1997. Hepaprotective effect of the fractions of Ban-zhi-lian on experimental liver injuries in rats. J. Ethnopharmacol., 56: 193-200.

Manov, I., M. Hirsh and T.C. Ianccu, 2002. Acetaminophen hepatotoxicity and mechanisms of its protection by *N*-acetylcysteine: a study of Hep3B cells. Exp. Toxicol. Pathol., 53: 489–500.

Mourelle, M., D. Beales and A.E. McLean, 1991. Prevention of paracetamol-induced liver injury by fructose. Biochem. Pharmacol., 41: 1831–1837.

Nakamura, Y., C. Yoshida, A. Murakami, H. Ohigashi, T. Osawa and K. Uchida, 2004. Zerumbone, a tropical ginger sesquiterpene, activates phase II drug metabolizing enzymes. FEBS Lett., 572: 245-250.

Nelson, S.D., 1990. Molecular mechanisms of the hepatotoxicity caused by acetaminophen. Seminar Liver Disease, 10: 267-278.

Olaleye, M.T. and B.T. Rocha, 2008. Acetaminophen-induced liver damage in mice: effects of some medicinal plants on the oxidative defense system. Exp. Toxicol. Pathol., 59: 319–327.

Prakash, J., S.K. Gupta, V. Kochupillai, N. Singh, Y.K. Gupta and S. Joshi, 2001. Chemopreventive activity of *Withania somnifera* in experimental induced fibrosarcoma tumors in Swiss Albino mice. Phytother. Res.,15: 240-244.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56–63.

Ruslay, S., F. Abas, K. Shaari, Z. Zainal, Maulidiani, H. Sirat, D.A. Israf and N.H. Lajis, 2007. Characterization of the components present in the active fractions of health gingers (*Curcuma xanthorrhiza* and *Zingiber zerumbet*). Food. Chem,. 104: 1183-1191.

Stock, H., A. Freeman, P. Gorog and B. Kovac, 1976. Simple and specific test for measuring lipid peroxides in plasma. Anal. Biochem., 65: 32-37.

Sulaiman, M.R., E.K. Perimal, M.N. Akhtar, A.S. Mohamad, M.H. Khalid, N.A. Tasrip,F. Mokhtar, Z.A. Zakaria, N.H. Lajis and D.A. Israf, 2010. Anti-inflammatory effect of zerumbone on acute and chronic inflammation models in mice. Fitoterapia, 81: 855-858.

Syarifah Sakinah, S.A., S.T. Handayani and L.P. Azimatol Hawariah, 2007. Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. Cancer Cell Int., 7: 4.

Tushar Basak, S., G.C. Sarma and L. Rangan, 2010. Ethnomedical uses of Zingiberaceous plants of Northeast India. J. Ethnopharmacol., 132: 286-296.

Yagi, K., 1998. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol. Biol., 108: 101-106.

Yanpallewar, S.U., S. Sen, S. Tapas, M. Kumar, S.S. Raju and S.B. Acharya, 2003. Effect of Azadirachta indica on paracetamol-induced hepatic damage in albino rats. Phytomedicine, 10: 391–396.

Yen, F.L., T.H. Wu, L.T. Lin and C.C. Lin, 2007. Hepatoprotective and antioxidant effects of Cuscuta chinensis against acetaminophen-induced hepatotoxicity in rats. J. Ethnopharmacol., 111: 123–128.