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Hepatoprotective Action of Zerumbone Against Paracetamol Induced Hepatotoxicity

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This study is conducted to investigate the possible effect of zerumbone towards hepatoprotective activity against paracetamol intoxication. Male Sprague-Dawley rats were randomly divided into six groups consisted of 3-5 animals. Group I was administered with 0.2% zerumbone for 14 days prior to 3 g kg⁻¹ paracetamol administration. Group II was given paracetamol only and group III was given 200 mg kg⁻¹ of silymarin and paracetamol. Group IV was administered with zerumbone only and finally group V was treated with corn oil and 40% sucrose buffer as vehicle treated group. Animals were sacrificed at 4 and 24 h post treatment following diethyl ether. There was no significant changes in liver enzyme activities as well as histological observations at 4 h after paracetamol administration. Meanwhile, 24 h after paracetamol administration, the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were found to be reduced in rats that were pretreated with zerumbone compared to group that was treated with paracetamol only. Correspondingly, there was no hepatocellular necrosis observed in rats that were pretreated with zerumbone. The results obtained may have suggested that zerumbone exert hepatoprotective activities against paracetamol induced hepatotoxicity.

Key words: *Zingiber zerumbet*, hepatotoxicity, paracetamol, acetaminophen

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INTRODUCTION

Overdosage of paracetamol (PCM) leads to the saturation of conjugation pathway leading to glutathione depletion and increase in the formation of toxic reactive metabolites (Liu and Klaassen, 1996; Arana *et al.*, 2001; Takahashi *et al.*, 2008; Schnackenberg *et al.*, 2008). High level of reactive metabolites increase the level of hepatotoxicity with increase level of protein adducts formation (Pumford *et al.*, 1997), mitochondrial dysfunction and oxidative stress (Masubuchi *et al.*, 2005).

Zerumbone (ZER) is a major bioactive sesquiterpene from *Zingiber zerumbet* Smith. The compound has been reported to have anti-inflammatory (Szabolcs *et al.*, 2007; Murakami and Ohigashi, 2006) and antiproliferative potential (Sadhu *et al.*, 2007; Xian *et al.*, 2007; Huang *et al.*, 2005). The compound has been implicated as one of the most promising chemopreventive agent against colon and skin cancer (Murakami *et al.*, 2004).

Recent study has suggested that the compound is also cytotoxic to pancreatic cancer cells (Hosoya *et al.*, 2008). Zerumbone has also been indicated as a potential phytochemical for regulating atherosclerosis (Eguchi *et al.*, 2007) by attenuating the expression of scavenger receptors (SRS) and lectin-like oxidized low-density lipoproteins (ox-LDL). The compound has also been reported to have antioxidant activities by attenuating reactive oxygen and nitrogen species generation (Murakami *et al.*, 2003; Murakami and Ohigashi, 2006; Kim *et al.*, 2008).

Consequently, this study was to evaluate the possible role of zerumbone, given as a supplement diet in protecting the liver following a single overdosage of PCM.

MATERIALS AND METHODS

Zerumbone was isolated and confirmed as previously described by Fakurazi *et al.* (2008a). The compound was kept at -20°C until further use.

Animals: Adult male Sprague-Dawley rats, 200-230 g purchased from Faculty of Veterinary Medicine, Universiti Putra Malaysia and acclimatised at the animal house of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. All animals were kept for one week under the same laboratory conditions, with food and water *ad libitum*. All experimental procedures were carried out with the approval of Animal Care and Use Committee (ACUC) regulation.

Hepatoprotective study: The study is conducted according to the method described by Fakurazi *et al.* (2008b). Study consisted of two (2) phases of treatment in which the animals were pretreated with 0.2% (v/v) zerumbone for 14 days and challenged with 3 g kg⁻¹ of paracetamol (PCM) 24 h after the last zerumbone treatment. Animals were sacrificed at 4 and 24 h after PCM administration.

The animals were randomly divided into groups of 3-5 animals. Group I was treated with zerumbone and PCM. Group II consisted of animals treated with PCM only. Whereas, in group III the animals were administered with 200 mg kg⁻¹ silymarin and PCM in a similar manner. In group IV and group V, the animals were treated with zerumbone only and dose vehicle, respectively. All treatments were conducted via oral intubations. Following diethyl ether, animals were sacrificed and blood was obtained via cardiac puncture. Livers were then removed and fixed for histological processing.

Determination of liver function tests: Liver enzymes such as ALT, AST and ALP were analyzed using specific kits purchased from Roche Diagnostics using a double beam spectrophotometer.

Histopathological analysis: Livers were removed and fixed in 10% (v/v) formal saline and processed according to standard H and E staining procedure. The liver sections were then analyzed for the evidence of possible improvement of histological change in hepatic lesions induced by PCM, when pretreated with zerumbone.

Statistical analysis: Statistical analysis of ALT, AST and ALP was carried out using Statistical Package for the Social Sciences (SPSS) to construct a one-way Analysis of Variance (ANOVA) table to ease interpretation of the results. Tukey post hoc test was later carried out. Significant difference is noted when p<0.05.

RESULTS AND DISCUSSION

For this study the dosage of 0.2% zerumbone given prophylactically was chosen due to the fact that this was the efficient dosage that reduced liver injury in rats when challenged with ethanol (Fakurazi *et al.*, 2008a).

There was no difference of ALT, AST and ALP level between the group treated with paracetamol only and those that were pretreated with zerumbone at 4 h. There was also no significant difference in all other groups. At 24 h, there was a pattern of reduction in the level of liver enzyme in rats pretreated with zerumbone even though statistically it was not significant. Those rats, which were

Table 1: The level of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) at 4 and 24 h

Groups	Mean ALT levels (IU L ⁻¹)		Mean AST levels (IU L ⁻¹)		Mean ALP levels (IU L ⁻¹)	
	4 h	24 h	4 h	24 h	4 h	24 h
Zerumbone+PCM	65.44±3.02	78.70±8.93	270.00±20.57	248.00±20.57	248.60±14.39	214.73±7.570
Corn oil+PCM	58.48±0.98	131.90±21.37	271.25±33.34	268.33±21.67	188.23±11.34	352.73±112.67
Zerumbone	48.70±4.74	60.67±5.21	199.33±40.18	219.33±23.24	178.53±14.37	150.93±15.23
Silymarin+PCM	68.65±20.85	68.47±2.74	305.50±19.50	256.67±21.11	160.60±11.90	174.33±10.60
Corn oil+40% sucrose	62.13±7.76	62.80±7.79	225.67±14.25	211.33±11.67	244.37±55.84	244.13±43.49

At 24 h, there was a pattern of reduction in the level of liver enzyme in rats pretreated with zerumbone although statistically it was not significant. Pretreatment with zerumbone has reduced the level of ALT, AST and ALP compared to those treated with PCM alone

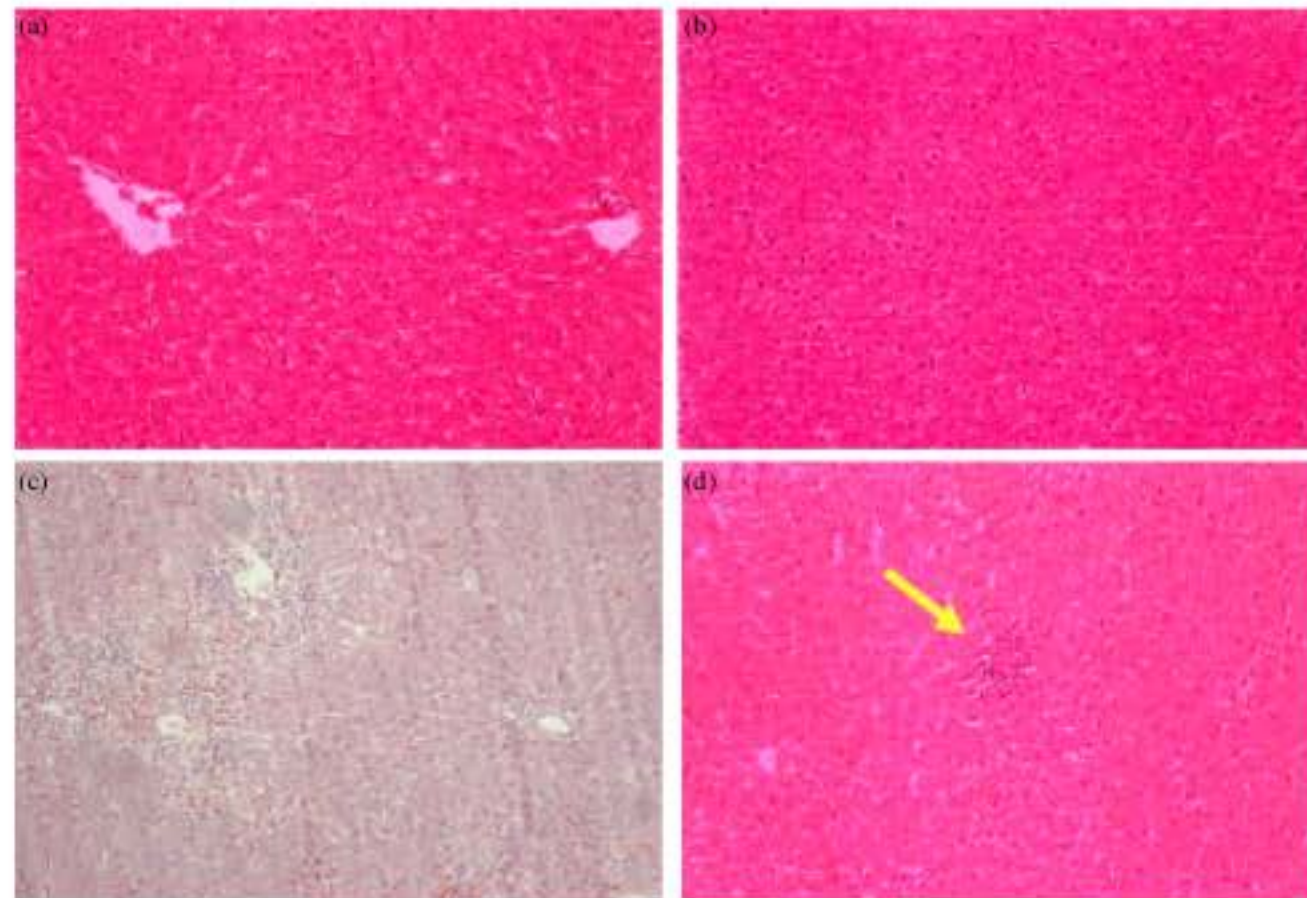


Fig. 1: Histological analysis of the liver section; (a) obtained from rats treated with vehicle (control), (b) of rats treated with zerumbone only. The liver histology was preserved in these groups, (c) liver section from rat treated with 3 g kg⁻¹ APAP sacrificed 24 h post-treatment. The section revealed submassive inflammation with cells undergoing necrosis around perivenular area and (d) liver sections obtained from rats pretreated with zerumbone and challenged with PCM. There was a massive reduction which was only accompanied by a mild focal inflammation (arrow). Magnification: 100x

pretreated with zerumbone prior to PCM administration showed a lower level of liver enzymes activities compared to those rats that were treated with PCM only (Table 1).

The histopathological analysis has showed consistent findings with the liver enzymes. The liver sections obtained from rats from all groups were similar to that of the control at 4 h. However, at 24 h, the liver sections obtained from the rats treated with PCM only showed significant hepatocellular damage with 3 g kg⁻¹ PCM. Changes were clearly seen around the PV area. Intriguingly, the liver sections obtained from those rats that were pretreated with zerumbone showed no prominent necrosis, but with a mild level of inflammation. This has suggested the possible hepatoprotective action of zerumbone against paracetamol induced liver toxicity (Fig. 1a-d).

CONCLUSION

The results that were shown in this study has potentially suggested that zerumbone, to a certain measure was found to augment hepatocellular changes following high dose of hepatotoxin such as PCM.

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