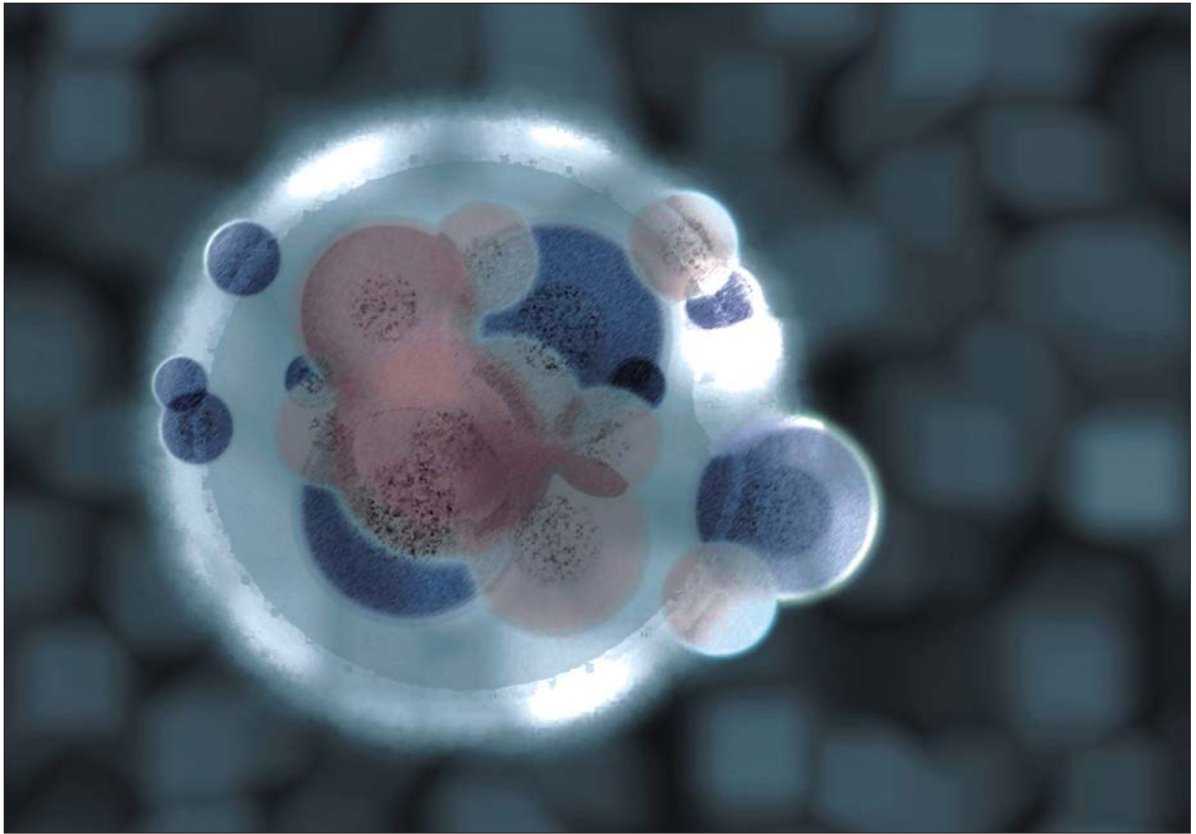

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The Effect of Pretreatment of Zerumbone on Fatty Liver Following Ethanol Induced Hepatotoxicity

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Abstract: This study was designed to determine whether zerumbone, an essential bioactive compound isolated from *Zingiber zerumbet* Smith protects against early ethanol induced liver injury in rats. Male Sprague-Dawley rats were administered with 0.05% (v/v) to 0.5% (v/v) of zerumbone for 14 days. Following the final dosage of zerumbone, the animals were administered with 50% (v/v) ethanol for 14 days. We have observed that pre-treatment of zerumbone had suppressed fatty liver formation following ethanol 50% (v/v) administration. Meanwhile, rats that were treated with ethanol only, found to show significant level of focal vacuolated fatty liver with focal necrosis in the mid zonal region. Therefore, fatty liver development was found to be extensively reduced in animals that were pretreated with zerumbone.

Key words: *Zingiber zerumbet*, zerumbone, hepatotoxicity, hepatoprotective, ethanol

INTRODUCTION

Largely, studies have progressed to identify substances that may reduce the severity of injury seen in the liver following alcohol consumption (Cahill *et al*., 2002; Castilla *et al*., 2004). To counteract the vulnerability of the liver to oxidative challenges during alcohol consumption, various interventions have been put forward such as to reinforcing the endogenous antioxidant defence system (Koch *et al*., 2000; Ozaras *et al*., 2003).

Zerumbone (ZER) is a bioactive sesquiterpene isolated from tropical rhizomes of *Zingiber zerumbet* Smith (Murakami *et al*., 1999). In some Southeast Asian countries, the rhizomes of the plant are used as a traditional medicine for antiinflammatory and as condiments (Murakami *et al*., 2002). This naturally occurring compound has been implicated as one of the promising chemopreventive agents against colon and skin cancer (Kirana *et al*., 2003). ZER was reported to inhibit the proliferation of human colonic adenocarcinoma cell lines in a dose dependent manner (Huang *et al*., 2005).

Interestingly, ZER was found to induce GSH-related phase II enzymes including glutathione-S-transferase (GST) (Nakamura *et al*., 2004). GSTs are a family of soluble proteins, which conjugate xenobiotics with glutathione. Metabolites formed after glutathionylation are more hydrophilic and thus biologically inactive and readily excreted in bile or urine as conjugates. This enzyme plays a major role in the cellular detoxification of oxidative damaging, genotoxic and carcinogenic chemicals (Nakamura *et al*., 2004). Therefore, this study was intended to evaluate the possible role of zerumbone, when given as a supplement diet, in protecting liver from impairment following exposure to ethanol.

MATERIALS AND METHODS

Isolation of zerumbone: Zerumbone is obtained from a local wet market and authenticated by a local botanist, Institute of Bioscience, Universiti Putra Malaysia. Zerumbone (99% purity) was isolated from the rhizomes *Zingiber zerumbet*. About 1.3 g zerumbone was obtained from 1 kg fresh rhizomes *Z. zerumbet*. The fresh blended

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rhizomes (1 kg) of *Z. zerumbet* were soaked into hexane for 3 days. The hexane was filtered to remove the particles. The soaking was repeated for three times to ensure maximum amount of zerumbone was recovered. Several crystallizations and column chromatography of the hexane fraction were carried out to obtain pure of zerumbone. The chemical identity was confirmed by comparison of its mass spectra and NMR spectral data with those earlier reported by Matthes *et al.*, (1980).

Animals: The study was performed in adult male Sprague-Dawley rats, 200-230 g purchased from Faculty of Veterinary Medicine, Universiti Putra Malaysia and kept 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.

Induction of fatty liver: Initially, rats were grouped and treated with 20, 40 and 50% (v/v) ethanol, given orally once a day for 2, 4, 6 and 14 days. The body weights were monitored and recorded for signs of direct toxicity. For each of the groups, rats were sacrificed according to the duration and frequency of dosing earlier mentioned. Livers were removed and blotted dry. Livers were fixed in 10% formol saline and processed for Haematoxylin and Eosin (H and E) stain section according to standard procedure.

Hepatoprotective study: In another study, rats were randomly divided into groups of treatment with 0.05, 0.1, 0.2 and 0.5% (w/v) of zerumbone in 10% (v/v) Tween 80, via oral intubation for 14 days. Following the final dosage of zerumbone, the animals received 50% of ethanol orally for 14 days. The control animals received an equivalent amount of Tween 80 in a similar manner. Twenty four hours after the final ethanol administration, the animals were euthanised and livers were removed. Livers were fixed in 10% formol saline and processed for Haematoxylin and Eosin (H and E) stain section according to standard procedure.

Histopathological analysis: The liver sections were then analysed by a pathologist for the evidence of fatty liver following ethanol administration compared to control. Possible improvements of histological change in fatty liver and hepatic lesions induced by ethanol, when pretreated with zerumbone were compared with the control groups.

RESULTS

Isolation of zerumbone: In this experiment, zerumbone was confirmed based on ^{13}C NMR based on ^{13}C NMR spectra, there are 15 carbon signals matched with the No. of carbon in sesquiterpene type compound. The ^{13}C signals at δ203.8 showed the present of ketone group which is corresponding to C-8 in the compound. There are six sp_2 carbon signals at δ135.9, δ124.6, δ148.1, δ137.6, δ126.8 and δ160.3 corresponding to the carbons in the olefin groups. Four carbon signals at the high field region (δ14.9, δ11.4, δ23.9 and δ29.1), showed the present of 4 methyl groups in the compound. The rest of carbon signals are from 3 methylene groups (δ42.1, δ39.1 and δ24.1) and one quaternary carbon signal (δ37.5). These ^{13}C signals showed a very close matching with the published data as stipulated in Table 1 and Fig. 1. Therefore, the compound isolated is unambiguously confirmed as zerumbone which is isolated from *Zingiber zerumbet*.

Induction of fatty liver: Following 20 and 40% ethanol administration for 2, 4 and 6 days, there was no observational change in the hepatocytes morphology (results not shown). Histologically, there was no evidence of fatty liver at these dosages and within the study

Table 1: ^{13}C NMR data of zerumbone (100.5 MHZ, $CDCl_3$ as solvent)

Carbon	Chemical shift of ^{13}C signal from zerumbone (δ) (Matthes *et al.*, 1980)	Chemical shift of ^{13}C signal from isolated zerumbone (δ)
1	42.2	42.1
2	125.0	124.6
3	136.1	135.9
4	39.4	39.1
5	24.3	24.1
6	148.5	148.1
7	137.8	137.6
8	203.8	203.8
9	127.1	126.8
10	160.4	160.3
11	37.8	37.5
12	15.2	14.9
13	11.7	11.4
14	24.1	23.9
15	29.4	29.1

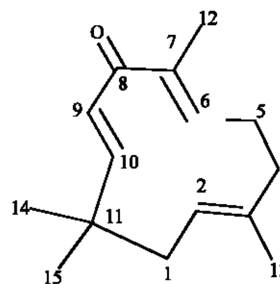

Fig. 1: The chemical structure of zerumbone

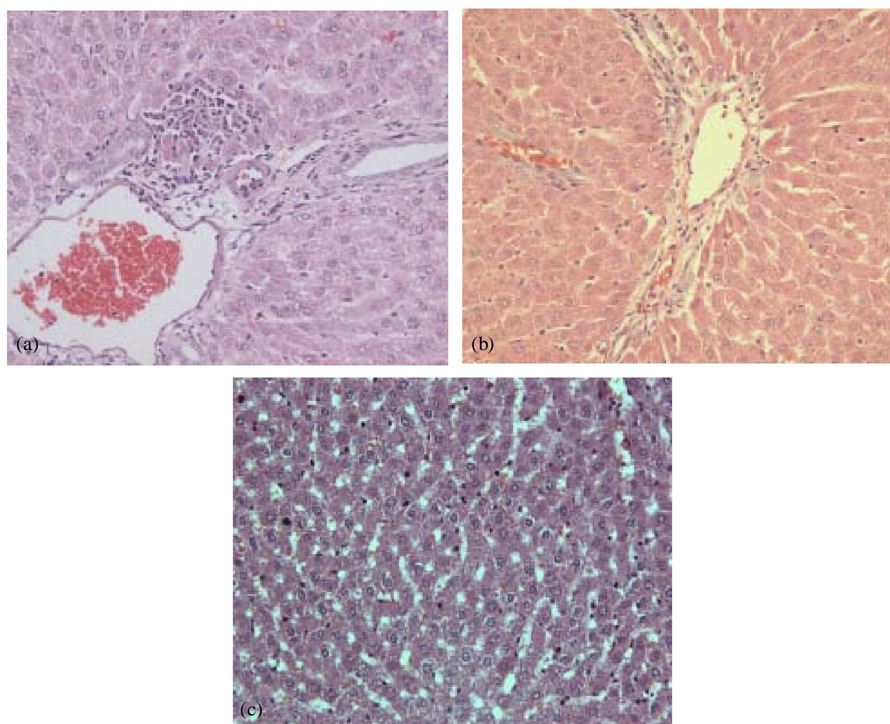

Fig. 2: Hepatoprotective action of zerumbone against ethanol induced hepatotoxicity in rats. Liver sections were stained with H and E stain according to a standard procedure, (a) Liver section obtained from rats treated with 50% (v/v) ethanol only for 14 days. Mild and focal inflammations with lymphocytes accumulation were observed. The cytoplasm of the hepatocytes appeared condensed and pinkish with shrunken nucleus. Focal vacuolated fatty change was evidenced, (b) Liver section obtained from rats that were pre-treated with 0.5% (w/v) zerumbone for 14 days and 50% (v/v) for 14 days ethanol. No fatty change was observed, with a very mild inflammation at the perivenular area and (c) Liver sections obtained from rats pretreated with 0.2% zerumbone for 14 days and then 50% ethanol for another 14 days. Generally, the liver section observed to be normal. Magnification 200x

duration. When the rats were administered with 50% ethanol for 14 days, there was a significant changes in the liver morphology observed under the light microscope (Fig. 2a). Focal injury of the hepatocytes with localization of lymphocytes infiltration and inflammatory cells was evidenced in the periportal region. Development of focal nerosis was observed in the midzonal region. Local vacuolated fatty change was observed in the hepatocytes in the midzonal and centrilobular region. The cytoplasm of the hepatocytes appeared condensed and pinkish with shrunken nucleus, suggesting the possible development of apoptotic cells.

Hepatoprotective study: Consequently, 50% ethanol was used in the subsequent study to investigate the hepatoprotective activity of zerumbone. The evidence of fatty liver was found to be reduced when rats were pretreated with 0.2 and 0.5% (v/v) zerumbone before challenged with ethanol. There was less or no development of fatty liver in the liver sections obtained from these groups of rats (Fig. 2b, c). However, higher dose of 0.5% (v/v) has induced the development of inflammatory cells panlobularly. When rats were treated with lower amount of zerumbone, that was the 0.05 and 0.1% (v/v), the fatty liver was still observed panlobularly, with lymphocytes infiltration and moderate inflammation was also evidenced. A mild and focal inflammation was observed in the liver section obtained from rats treated with Tween 80.

DISCUSSION

Pretreatment of zerumbone before challenging with ethanol has considerably reduced the development of fatty liver and the accumulation of lymphocytes leading to inflammation. This has suggested the possible hepatoprotective effect of zerumbone against ethanol induced fatty liver. Hepatoprotective actions of other plant extracts have also been reported. It has been shown that the plant extracts may have exhibited antioxidant actions in order to exert hepatoprotective activity.

Administration of ethanol to rats has lowered the antioxidant capacity of the rat liver as shown by the decreased in the activity of antioxidant enzymes (Srivastava and Shivanandappa, 2006). The administration of ethanol at 50% (v/v) for 14 days might have led to the depletion of glutathione level, an important factor in contributing to hepatotoxicity (Fernandez-Checa *et al*., 2002). Pretreatment of rats with zerumbone extract might have boosted the level of antioxidant enzymes and increase in the activity of the enzymes in the liver. Possible explanation to this study is supported by a reported study by Nakamura *et al*. (2004) which suggested that zerumbone might be a potential activator of phase II metabolizing enzymes. The plant has also been reported to have induced the expression of proinflammatory cytokine genes (Murakami *et al*., 2004). This biochemical property may have explained the presence of inflammatory cells seen in the liver sections treated with zerumbone. Following observation and analysis, pre-treatment with zerumbone was found to hinder the process of ethanol induced hepatotoxicity. The formation of fatty liver was found to cease in groups of rats that were treated with zerumbone prior to ethanol challenged.

CONCLUSION

Zerumbone to a certain extent has been found to ameliorate the hepatocellular changes following high doses of ethanol suggesting a hepatoprotective action of the substance.

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