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CURATIVE POTENTIAL OF *Zingiber zerumbet* ON PARACETAMOL-INDUCED HEPATOTOXICITY

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ABSTRACT

Zingiber zerumbet is a type of wild ginger known worldwide for its medicinal values. Its constituents found in the rhizome suggest potent antioxidant and anti-inflammatory activities. As such, this study investigated the possible curative effects of *Zingiber zerumbet* rhizome ethanolic extract (ZZ) on Paracetamol (PCM)-induced hepatotoxicity. The plant was obtained from Sendayan, Negeri Sembilan and had been identified by the Biodiversity Unit of Universiti Putra Malaysia (UPM), Malaysia. Its rhizomes were processed and extracted with 80% ethanol. The chemical profile of the extract was determined using Gas Chromatography-Mass Spectrometry (GC-MS) analysis, while hepatotoxic rats were treated orally with varied extract doses (250, 350, and 450 mg/kg bwt) for a week, and N-acetyl-L-cysteine (NAC) was used as reference drug. At the end of the treatment, sera samples were collected for liver function tests (LFTs). The chemical profile of the extract determined via GC-MS analysis revealed three major compounds that contributed to the bioactivity of the plant. Zerumbone had the highest component concentration (95%) in the extract for GC-MS study. Significant improvements in LFT parameters (alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), and total protein (TP) levels) were also observed in groups that received 350 and 450 mg/kg bwt extract, wherein the 350 mg/kg bwt dose appeared to be the most effective dose that reduced liver enzyme markers in PCM-induced hepatotoxic rats. Overall, *Z. zerumbet* exhibited the most optimum hepatocurative potential in enhancing liver functions amidst PCM-induced hepatotoxic rats at 350 mg/kg bwt dose.

Key words: *Zingiber zerumbet*, paracetamol (PCM), hepatotoxicity, liver function test (LFT), zerumbone

INTRODUCTION

The genus *Zingiber* is distributed mainly in Asia and is native to India and Penisular Malaysia. *Zingiber zerumbet* is a type of wild ginger that belongs to the family Zingiberaceae and commonly known as 'Lempoyang' by the locals. This tuber root plant is confined to the tropics of Asia, Malaysia, and the Pacific Islands. Its rhizomes are widely used as food flavouring and appetiser in various Malay cuisines, while its extracts have been used in Malay traditional medicines to treat various types of ailments, namely inflammatory and pain-mediated diseases, worm infestation, and diarrhoea. Apart from possessing anti-nociceptive, anti-ulcer, anti-cancer, anti-microbial, anti-hyperglycaemic, anti-allergic and anti-platelet activities (Yob *et al*., 2011), *Z. zerumbet* is also known for its high antioxidant properties (Ruslay *et al*., 2007). The plant has been reported to possess the richest source of kaempferol, which is responsible for exhibiting strong antioxidant activities (Lako *et al*., 2007; Booh *et al*., 2015).

A prior study on ginger crude extract demonstrated insignificant changes in liver enzymes after 4-week treatment, which suggested nil hepatotoxic effect (Chaung *et al*., 2008). An acute and 28-day subchronic oral toxicity tests for the plant also reported no toxic signs or death, including insignificant variance in haematological and biochemical analyses on male and female rodents (Chang *et al*., 2012).

This study screened the bioactive compounds of *Z. zerumbet* rhizome ethanolic extract (ZZ) and determined its hepatocurative potential through its ability to enhance liver function test (LFT) in paracetamol (PCM)-induced hepatotoxicity. The screening of bioactive compounds led to the

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identification of potential plant metabolites involved in biological activities, whereas its ability to enhance LFT in hepatotoxic rats determined its hepatocurative capacity.

MATERIALS AND METHODS

Chemicals

Absolute ethanol (HmbG, UK), distilled water, N-acetyl-L-cysteine (NAC) (Sigma-Aldrich, USA), PCM (Panadol® GSK), and sodium chloride (NaCl) (HmbG, UK) were used in this study. All the chemicals and reagents used were of analytical grade.

Plant materials

Whole plants were collected from Jabatan Pertanian Sendayan, Negeri Sembilan in February 2017 during rainy and humid weather. The whole plant was identified by Firdaus Ismail, a resident botanist at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. A voucher specimen (SK 3145/17) was deposited at the herbarium of the Laboratory of Natural Products, IBS, UPM, Malaysia.

Selection of mature rhizomes

Mature rhizomes were selected based on the number of axillary branches of the plant, as well as the colour intensity and odour of the rhizomes. Mature rhizomes have more than 6 axillary branches (Shah & Raju, 1976), while the rhizome has intense yellow shade and stronger odour (Iijima & Joh, 2014). For analyses purpose, the rhizomes were separated from the whole plant.

Extract preparation

The rhizomes were thoroughly cleaned to remove soil residues and other contaminants. Next, the rhizomes were cut into small pieces and shade-dried for a week at room temperature. Upon complete dry, the rhizomes were pulverised with an electrical blender (National, Malaysia) to produce coarse powder. About 2 kg of the coarse powder was wrapped in a thin cloth and extracted using 4 L of 80% ethanol for seven days at room temperature. The mixture was occasionally shaken. Next, the extract was filtered through filter paper (pore size: 11 µm) and evaporated to dryness using a rotary evaporator (BUCHI, Switzerland) to eliminate any remaining alcohol. The crude extract generated from this process was freeze-dried (CHRIST, Germany) to remove water from the extract in order to yield approximately 233.2 g of powdered residue. The powdered residue was kept at -4°C refrigerator (Panasonic, Malaysia) until further use and suspended in distilled water prior to usage (Hong *et al*., 2016).

Gas Chromatography and Mass Spectrometry (GS-MS)

The powdered ZZ extract was diluted in 80% ethanol at a ratio of 1:100 and was filtered (pore size 0.2 µm). The GC-MS data were recorded with Agilent Technologies 6890N Network GC System prepared at Universiti Teknologi MARA (UiTM), Shah Alam, Selangor, Malaysia. The gas chromatograph was fitted with an Alltech ECONO-CAP™-EC™-5 (30 mm × 0.25 mm i.d. × 0.25 µm) capillary columns with 5 mm guard column. The oven temperature was set to 40°C for 2 mins, then to 100°C at 8°C per min, next to 280°C at 3°C per min, 300°C at 10°C per min, and held for 3.5 min; whereas the injector, the transfer line, and the ion source temperatures were fixed at 220°C, 250°C, and 200°C, respectively; including an electron voltage at 70eV. The run-time was 69.50 minutes and the pressure values at the front and back inlet were 7.07 psi and 16.11 psi, respectively. Purified helium was used as the carrier gas at a flow rate of 1.2 mL per min. The injection volume was 0.2 µL, whereas the split ratio was 50:1 (Jiang *et al*., 2006). The sample was injected thrice. The contained compounds were identified by comparing to retention times and mass spectra data derived from the database of National Institute of Standard and Technology (NIST).

Experimental animals

Male Sprague Dawley rats (approximately five weeks old; 150-200 g) were procured from a registered local supplier. The rats were housed in polycarbonate cages (15×25 cm) with stainless steel covers (two rats in a cage with wood shavings as bedding), at approximately 26–28°C under dark (12-hours) and light (12-hours) cycles with access to standard rodent pellets and water *ad libitum*. The rats were also provided with enrichment and acclimatised to their environment. The study was carried out in the Postgraduate Physiology Laboratory, UiTM, Shah Alam, Selangor, Malaysia, wherein the protocol and procedures for animal handling were approved by the UiTM CARE (Committee for Animal Research and Ethics) (Ref Number: UiTM CARE 203/2017).

Experimental design

Twenty-four rats were randomly divided into 6 groups (n=4). All rats, excluding Group 1 (normal control), were induced to hepatotoxicity via oral administration of 750 mg/kg bwt PCM for seven days prior to treatments with NAC and ZZ. The rats were then treated daily with extract and reference drugs via oral gavage for seven days. The selected treatment protocol was adopted and slightly modified based on a prior study (Hamid *et al*., 2011).

Group 1 was treated with 1% body weight of saline for seven days and served as normal control.

Meanwhile, Group 2 was treated with 1% body weight of saline and served as a negative control. Next, Group 3 was given 50 mg/kg bwt of NAC and served as positive control (Mahmood *et al*., 2014), whereas groups 4 until 6 were administered with treatments of *Z. zerumbet* extract (ZZ) at doses of 250 mg/kg bwt (ZZ 250), 350 mg/kg bwt (ZZ 350), and 450 mg/kg bwt (ZZ 450), respectively for seven days.

At the end of the treatment period, the rats were sacrificed and their blood samples were collected. The blood samples were stored in plain tubes and were allowed to clot for 30 mins. After that, the blood samples were centrifuged (CHRIST, Germany) at 5000 rpm for 10 mins to collect sera. The sera samples were analysed for biochemical analysis of serum aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total protein (TP) levels.

Statistical analysis

All data were expressed as the mean ± standard error of the mean (mean ± SEM) and the gathered data were analysed using One-Way ANOVA. The variables were the positive control drug; NAC and the various concentrations of ZZ treatments were applied to varied rat groups. The data were analysed by using SPSS (version 24) system.

RESULTS AND DISCUSSION

Phytochemical screening via GC-MS

GC-MS revealed three major peaks in its chromatogram. Other compounds present in minute quantities were excluded due to insufficient mass spectrum quality and relative concentration data that disabled them from being adequately identified (DeHaven, 2010). The varied mass-to-charge ratios from each peak in the chromatogram were matched to the compounds humulene, humulene epoxide II, and zerumbone. Grassmann (2005) asserted that zerumbone, humulene, and humulene epoxide II are all classed as sesquiterpenes, which refers to a group of 15 carbon compounds derived by the assembly of three isoprenoid units. Humulene and humulene epoxide II were derived from farsenyl diphosphate (FPP) from the essential oils of *Humulus lupulus*, whereby both compounds possess anti-inflammatory properties (Fernandes *et al*., 2007; Chaves *et al*., 2008). Zerumbone has been extensively studied on cancer cell lines (Sidahmed *et al*., 2015) and displayed cytotoxic activity on cancer cells without affecting the normal cell line (Abdelwahab *et al*., 2011; 2012). Sidahmed *et al.* (2015) reported that zerumbone pre-treatment attenuated TPA-induced (tumour promoting factor) ROS production in mice epidermis.

Unlike flavonoids, an essential oil with sesquiterpenes as its predominant compound exerted weak antioxidant activity (Zakia-Bey *et al*., 2016). This is because; sesquiterpenes have lower direct antioxidant activity due to its electrophilic nature. On the other hand, humulene and humulene epoxide II are non-electrophilic, thus causing them to possess higher direct antioxidant activity, but less significant impact, when compared to zerumbone. This is because zerumbone has the highest component concentration in ZZ ethanolic extract, thus generating more influence as an active compound among all the three screened compounds. Unlike humulene and humulene epoxide II, zerumbone has electrophilic analogue that bears α,β-unsaturated carbonyl moiety (Shin *et al*., 2011). Thus, the compound exhibited a lower direct antioxidant activity. Prior studies reported that α,β-unsaturated carbonyl moiety possesses the ability to decrease oxidative stress by activating drug-metabolising enzymes via cytoprotective genes. The codes of genes for NADP(H) quinone oxidoreductase I and proteins appear to increase cellular antioxidant activity in mitochondria and glutathione synthesis. It also activates phase II drug-metabolising enzymes (Choi & Alam, 1996; Shin *et al*., 2011; Tang *et al*., 2014; Kundu *et al*., 2016).

Table 1 presents the quantitative screening of ZZ that displayed retention time, molecular weight, molecular formula, and composition percentage of the ZZ. The composition percentage was considered as a measure of component concentration. In this

Table 1. Constituents of rhizome ethanolic extract from *Zingiber zerumbet*

No.	RT	Area %	Name	Quality	Molecular formula	Molecular weight (g/mol)
1	27.05	2.94	Humulene	58	$C_{15}H_{24}$	204.35
2	27.41	1.68	Humulene epoxide II (1R,3E,7E,11R)-1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	91	$C_{15}H_{24}O$	220.35
3	31.92	95.37	Zerumbone 2,6,10-Cycloundecatrien-1-one,2,6,9,9-tetramethyl-,(E,E,E)-	81	$C_{15}H_{22}O$	218.17

screening, zerumbone recorded the highest component concentration in ZZ (95.37%), followed by humulene (2.94%), and humulene epoxide II (1.68%). The outcomes are in agreement with those reported by Srivastava *et al*. (2000), Yu *et al*. (2008), and Sulaiman *et al*. (2010) on the essential oils of *Z. zerumbet* cultivated from India, Japan, and Indonesia. Interestingly, the similarity between studies that describe the highest content of zerumbone usually applied rhizome as the main source of their studies. This is supported by Bhuiyan *et al*. (2009), who claimed that the highest value of zerumbone is indeed found in the rhizome. Chien *et al*. (2008) asserted that the content of zerumbone was found richer in mature rhizomes, which refers to the plant material used in this study.

Table 2 shows the comparison of phytochemical compounds found in *Z. zerumbet* by other authors based on geographical areas. This concurs with past studies conducted by Nigam and Levi (1963), and Damodaran and Dev (1968), where the same three compounds were discovered in the essential oils of *Z. zerumbet* cultivated in India and Bangladesh. Similarly, Yu *et al*. (2008) and Batubara *et al*. (2013) investigated the phytochemicals in the essential oils of *Z. zerumbet* harvested in Japan and Indonesia. Contrary to the study performed by Lako *et al*. (2007), high contents of kaempferol were found in ethyl acetate fractions of *Z. zerumbet* obtained from Fiji, although the study did not detect the presence of this flavonoid in the ZZ cultivated in Malaysia.

The variation in phytochemical compounds of *Z. zerumbet* discovered in past studies was due to the different geographical areas, where the wild ginger was sourced. Aside from the varying cultivars, distinct environmental conditions in different geographical locations also could affect the outcome of the chemical contents garnered from the materials (Norhamidar *et al*., 2018). For instance, the presence of oxygenated sesquiterpene (humulene epoxide II) is an indicator of high humidity in the environment where the samples were collected (de Silva *et al*., 2015). Other environmental conditions include temperature and soil pH (Liu *et al*., 2015). Apart from that, the different solvents used also may cause variation in the extracted bioactive compounds. This is because; different solvents affected the yield and the type of active compounds garnered as the extraction of antioxidants is highly affected by the polarity of the solvent used (Boeing *et al*., 2014; Sharif & Taha Bennett, 2016).

In vivo hepatocurative study

Effect of ZZ on liver function test (LFT)

In this study, liver enzyme markers ALP, ALT, AST, and TP were measured using LFT. Administration of ZZ at varied doses was able to reduce the liver enzymes in PCM-induced hepatotoxic rats. PCM-induction caused an elevation in the ALP level, which indicated hepatitis and cirrhosis of the liver (Ellis, 2017). After being treated with the extract, the levels of ALP in ZZ 350 (125.75 ± 20.35 U/L) and ZZ 450 (127.25 ± 12.53 U/L) groups were reduced and comparable to the positive control group (ALP = 97.63 ± 8.36) ($p>0.05$) which is shown in Figure 1. Increment in ALT levels due to PCM-induction was also successfully reduced by the extracts in ZZ 350 (59.75 ± 3.09 U/L) and ZZ 450 (59.29 ± 5.78 U/L) groups, which were also comparable to the positive control group (ALT = 44.25 ± 8.26 U/L). The decrease in ALT levels in

Table 2. Variation in phytochemical compounds found in *Zingiber zerumbet* by other authors

Study	Rhizome source	Climate	Extraction solvent	Compounds extracted
Nigam and Levi, 1963	India	Tropical	Water	Borneol, α-pinene, camphor, linalool, zerumbone, limonene, α-humulene and β-caryophyllene.
Damodaran and Dev, 1968	Bangladesh	Subtropical monsoon	Water	Humulene epoxide I, II and III, humulenol I and II and caryophyllene oxide.
Lako *et al*., 2007	Fiji	Tropical	Ethyl acetate	Kaempferol
Yu *et al*., 2008	Japan	Temperate	Water	Zerumbone, α-humulene, humulene epoxide I and II, β-Caryophyllene, caryophyllene oxide and β-eudesmol
Sulaiman *et al*., 2010	Indonesia	Tropical	Water	Borneol, α-pinene, camphor, linalool, camphene, eucalyptol, β-terpinene and β-phellandrene.
Batubara *et al*., 2013	Indonesia	Tropical	Water	Zerumbone, sabinene and β-myrcene.
This study	Malaysia	Equatorial	Ethanol	Humule, humulene epoxide II and zerumbone

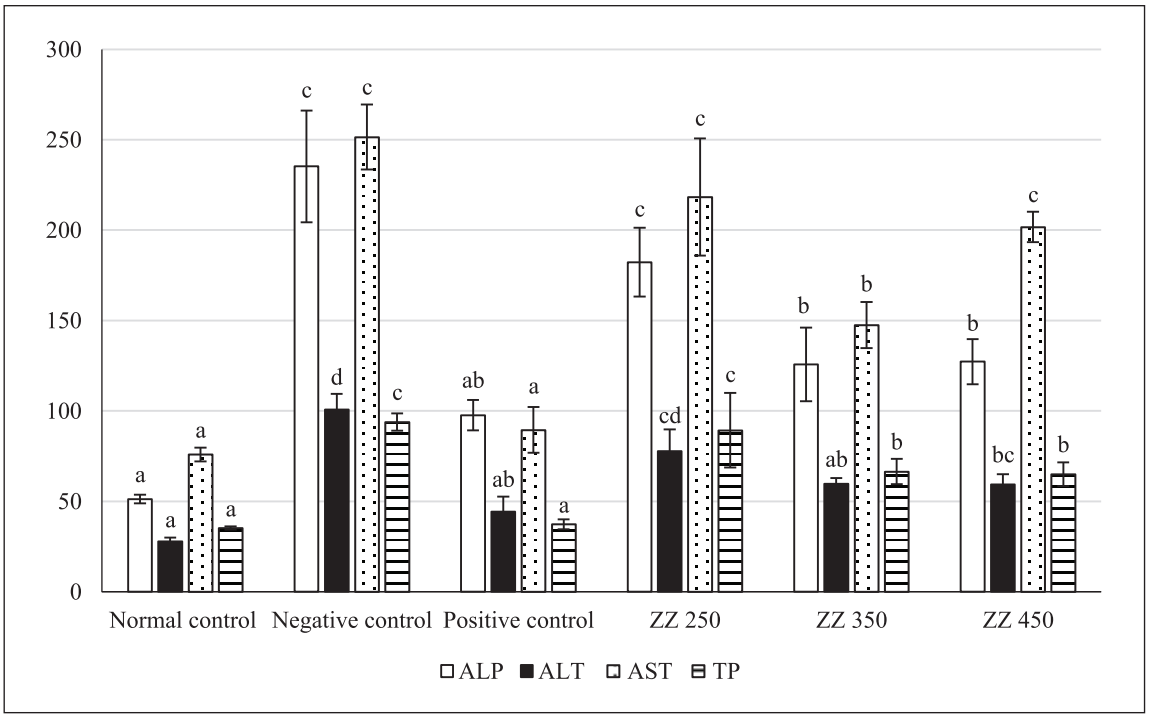

Fig. 1. Effect of various doses of *Z. zerumbet* rhizome ethanolic extract on liver function test (LFT).
Note: Values are expressed as means ± SEM, n=4. Superscripts ^{abcd} denote significant difference at $p<0.05$ between the groups.

this study signified that toxin-induced liver damage was improved in the damaged hepatocytes (Pathak, 2017). The treatment of ZZ in PCM-induced hepatotoxic rats also suggested improvement of liver damage as the AST levels in ZZ 250 (218.25 ± 32.40 U/L) ZZ 350 (147.50 ± 12.78 U/L), and ZZ 450 (201.75 ± 8.43 U/L) groups decreased. Nevertheless, the values did not significantly differ from the negative control ($p<0.05$). Pathak (2017) asserted that although the elevation of AST indicates liver damage, the serum can be found in other organs, such as the kidney and heart. Hence, the insignificant reduction in AST levels could be due to cellular damages that occurred in other organs as well. In general, the TP level rose in blood, as the body responded to inflammation. However, the TP levels in all groups treated with ZZ displayed insignificant variance with the negative control ($p>0.05$).

ZZ treatment had been proven to be effective even at 350 mg/kg bwt in ameliorating hepatocellular injury, as evidenced by the substantial reduction in the levels of hepatic enzymes. Since the ZZ ethanolic extract used in this study contained the highest concentration of zerumbone, it could have played a significant role in the expression of the antioxidant regulatory gene (Shin *et al.,* 2011) and aided with decrease levels of hepatic enzymes. This study and other hepatoprotective studies (Fakurazi *et al.,* 2009; Hamid *et al*., 2018) also highlighted the ability of zerumbone to suppress inflammatory processes due to PCM overdosage, apart from decreasing the hepatocytes tendency to go through necrotic processes.

Hence, it can be concluded that ZZ possesses the ability to maintain cellular integrity and functional capacity of hepatocytes, thus preventing enzyme leakage into the blood. As a conclusion, the optimum concentration of zerumbone in 350 mg/kg bwt dose had boosted the ability of the extract to address the PCM-induced radical accumulation.

CONCLUSION

In conclusion, the screening of bioactive compounds in ZZ via GC-MS identified three potential hepatocurative compounds: humulene, humulene epoxide II, and zerumbone. Zerumbone had the highest component concentration in the ZZ extract and the carbonyl moiety in its structure displayed indirect antioxidant activities. The effects of ZZ on liver functions in PCM-induced hepatotoxic rats exhibited significant improvements in LFT parameters (ALP, ALT, and AST) for 350 and 450 mg/kg bwt ZZ-treated groups. Although the LFT of PCM-induced hepatotoxic rats treated with 450 mg/kg bwt ZZ dose exerted insignificant variance, when compared to 350 mg/kg bwt ZZ dose, a lower dose of ZZ seemed to be effective in exerting its hepatocurative effects. Overall, ZZ exhibited hepatocurative potential by enhancing liver functions in PCM-induced hepatotoxic rats.

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