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Full Length Research Paper

Involvement of CYP450 system in hepatoprotective activity of Malaysian Agricultural Research and Development Institute (MARDI)-produced virgin coconut oils

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The present study aims to determine the role of cytochrome P450 (CYP450) enzyme system in hepatoprotective activity of virgin coconut oils produced by Malaysian Agricultural Research and Development Institute (MARDI). Paracetamol (PCM)-induced hepatotoxic rat was used as a model. Liver injury induced by 3 g/kg PCM increased the liver weight and liver enzymes (e.g. alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphate (ALP)) and decreased cell viability indicating liver damage. Histological observation also confirms liver damage indicated by the presence of inflammations and necrosis. Pre-treatment with VCOA or VCOB reversed the significantly ($P < 0.05$) reversed PCM toxic effect. Groups pre-treated with virgin coconut oil (VCOs) followed by inhibitor or inducer of CYP450 demonstrated significant ($P < 0.0.5$) increase in liver weight, liver enzymes levels and decrease in cell viability, which are, however, significantly ($P < 0.05$) less remarkable as compared to group treated with PCM alone. In conclusion, VCO possessed hepatoprotective effect, which is believed to be mediated via a non-CYP450 system and might be associated partly with the antioxidant potential of the oil. Further studies are warranted to determine the actual mechanisms of hepatoprotection involved.

Key words: Malaysian Agricultural Research and Development Institute (MARDI), virgin coconut oil, liver toxicity, paracetamol, cytochrome P450, hepatoprotection.

INTRODUCTION

Liver being the main organ for metabolism is continuously being exposed to various xenobiotics, environmental pollutants and chemotherapeutic agents (Krishna et al., 2007; Ibrahim et al., 2008). Hepatic injury which occurs due to failure or ineffective protective mechanism involved in the natural detoxification process in the liver, if prolonged can lead to liver diseases. Some commercially available drugs that affect liver function

directly or indirectly are at times inadequate to help regeneration of hepatic cells and fail to protect liver from damage (Chattopadhyay, 2003). This problem is further exacerbated by the adverse effects associated with the consumption of these drugs aims for the treatment of liver disorders (Ozbek et al., 2004).

Cytochrome P450 (CYP450) enzymes are the major catalysts and enzymes most commonly involved in the metabolism of drugs, with the other major contributors being uridine dinucleotide phosphate (UDP) glucuronosyl transferases and esterases. CYP450, together with the conjugation enzymes, can influence bioavailability and toxicity, which are two of the most common barriers in drug development today. Of the CYP450s, ~90% of the metabolism can be accounted for by 5 human P450s (e.g. 1A2, 2C9, 2C19, 2D6 and 3A4). The drug metabolism processes, generally, is known to indicate all transformations of a drug by an enzyme(s), wherein the majority of these changes deactivate the drug or other xenobiotic, attenuating its biological activity and perhaps accelerating its clearance from the body. However, CYP450s and other enzymes can also bioactivate chemicals, converting them to reactive products that modify cellular constituents and produce damage (Guengerich, 2005, 2006; Guengerich et al., 2005). In contrast to the P450s involved in sterol metabolism for normal physiological functions, the levels of the xenobiotic-metabolizing CYP450s vary widely, and individuals can be completely devoid of some of these because of genetics. In an individual deficient of the particular CYP450 (poor metabolizer), limited metabolism of the particular drug will result in the toxic accumulation of the drug or its metabolites.

Plant-based hepatoprotective agents have been employed in the traditional medicine of the developing countries (e.g. India, China and Malaysia) since ancient time and are widely sold in the open market (Dash et al., 2007; Sadasivan et al., 2006; Sirajudeen et al., 2006). They have provided an alternative to overcome the inadequate number of reliable modern hepatoprotective drugs (Pramyothin et al., 2007). Some plant-based drugs are widely consumed for the treatment of liver diseases because of their effectiveness, fewer side effects and relatively low cost (Valiathan, 1998). One of the plant-based natural products that have been studied in our laboratory for its potential hepatoprotective effect is virgin coconut oil (VCO). VCO or '*minyak kelapa dara*' as it is known to the Malaysian is produced through a low heat process from freshly harvested, organically grown coconut. The oil has gain a lot of attention lately due to the reports of many pharmacological effects (e.g. antioxidant, antimicrobial and antiviral and antihypercholesterol and antithrombotic) reported (Nevin and Rajamohan, 2006, 2008, 2004). We have recently reported the hepatoprotective, antihypercholesterolemia, antinociceptive and anti-inflammatory activities of the Malaysian Agricultural Research and Development Institute (MARDI)-produced VCOs (Zakaria et al., 2011a, b, 2010). In addition, we have also reported the antiulcer activity of MARDI-produced VCOs (Malarvilli et al., 2009). The hepatoprotective activity of dried (VCOA) and fermented (VCOB) processed VCOs was assessed using paracetamol-induced hepatotoxic model. The association between antioxidant and hepatoprotective mechanisms has been established and proven for VCOs (Chattopadhyay, 2003; Pramyothin et al., 2007; Zakaria et al., 2011b). The objective of the present study was to determine the involvement of CYP450 system in the hepatoprotective activity of VCOA and VCOB using paracetamol-induced hepatotoxic model.

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MATERIALS AND METHODS

Samples of VCOs

Dried and fermented processed VCOs, labelled as VCOA and VCOB, respectively, were provided by Dr. Kamariah Long from the Malaysian Agriculture Research and Development Institute (MARDI), Serdang, Selangor and were store at room temperature before use.

Preparation of VCOA

Preparation of VCOA was performed according to the methods described by Seow and Gwee (1997) with several modifications. Briefly, coconut milk emulsion was centrifuged before chilling and thawing to allow better packing of the coconut oil globules. The temperature used were 10 and -4°C for chilling and freezing process, respectively while the thawing process was carried out in a water bath at 40°C, until the coconut cream reached room temperature (25°C).

Preparation of VCOB

Preparation of VCOB was performed according to the methods described by Che Man et al. (1997) with several modifications. Pure culture of *Lactobacillus plantarum* 1041 IAM was used to extract coconut oil. Grated coconut meat and water at 30°C was mixed in ratio of 1:1 and allowed to settle for 2 to 6 h. Coconut milk emulsion was then separated by adjusting pH of the coconut milk emulsion between pH 3 and 5.6.

Chemicals

Paracetamol (PCM; Sigma Chemicals, USA) was diluted in glycerol to the concentration of 3 g/kg. Proadifen (SKF 525A; Sigma Aldrich, USA) and phenobarbital (PBT; Sigma Aldrich, USA) was prepared

at the dosage of 25 and 50 mg/kg, respectively by dissolving each of them in distilled water.

Experimental animal

Male Sprague-Dawley rats weighing between 180 to 220 g were used in the present study. The animals were obtained from the Veterinary Animal Unit, Universiti Putra Malaysia (UPM) and housed at the Animal House, Faculty of Medicine and Health Science, UPM. The animals were kept in polypropylene cages with wood shaving, fed with standard pellet and water *ad libitum* and maintained in a 12 h light/dark cycle at 27 ± 2°C. At all times the rats were cared for in accordance with principles and guidelines for the care of laboratory animals and ethical guidelines for investigations of experimental pain in conscious animals as adopted from Zimmermann (1983).

Role of CYP450 on hepatoprotective activity of VCOA and VCOB in PCM-induced rats

Animals were weighed and randomly divided into eight groups of six rats each. Four groups were separately used to study the effect of inhibitor or inducer of CYP450 on VCOs hepatoprotective activity. Each groups was pre-treated orally (p.o.) for seven days according to the procedures described earlier. Normal saline (NS), SKF 525A at the dosage of 25 mg/kg or PBT at the dosage of 50 mg/kg was injected intraperitoneally (i.p.) for 3 consecutive days starting on the 5th day after the administration of NS, 10 ml/kg VCO A and VCO B until 7th day (Somchit et al., 2006; Marchand et al., 1970). On the 8th day, the animal from group 1 and group 2 were treated with vehicle of 10 ml/kg (glycerol) and animal from group 3 and 4 received 3 g/kg PCM. Percentage changes in body weight were measured 48 h after the administration of vehicle or toxin using the following formula:

Change in body weight (%) = 100 × [($Weight_n$ – $Weight_{initial}$)/ $Weight_{initial}$] (Özbek et al., 2004).

Blood sample (3.0 ml) was collected by cardiac puncture using sterile disposable syringe for biochemical studies. The animals were sacrificed and liver weight/100 g body weight were measured and fixed in 10% formalin for histopathology studies.

Biochemical studies

Blood (3 ml) was collected into plain tube and were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and utilized for the estimation of various biochemical parameters, namely, alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphate (ALP)A (Somchit et al., 2005).

Histopathology studies

Small pieces of liver tissues in each group were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H&E). These sections were examined under light microscope for histologically change and percentage of cell viability using the following formula:

Cell viability (%) = 100 × [(viable cell/(viable + death cell)] under a light microscope.

Statistical analysis

The result are expressed as mean ± S.E.M. One-way analysis of variance (ANOVA) and Turkey Post Hoc test analysis was used to determine statistical significance difference of samples mean and P value < 0.05 is considered significant.

RESULTS

Effect of inhibitor and inducer of CYP450 metabolizing system on body weight of hepatotoxic rats pretreated with VCOA or VCOB

Effects of SKF 525A and PBT on the percentage change in body weight of rats pretreated with VCOA or VCOB followed by treatment with PCM are as shown in Table 1. In the non-induced groups receiving NS, SKF 525A or PBT alone as in the (NS + NS + vehicle)-treated, (NS + SKF 525A + vehicle)-treated and (NS + PBT + vehicle)-treated groups, no significant change in percentage of body weight were observed. In the PCM-induced groups receiving NS, SKF 525A or PBT as in the (NS + NS + PCM)-treated and (NS + PBT + PCM)-treated, but not (NS + SKF 525A + PCM)-treated groups, only the first two groups demonstrated significant ($P < 0.05$) increase in the percentage change of body weight.

In the non-induced groups pretreated with 10 ml/kg VCOA (10 VCOA) followed by SKF 525A or PBT as in (10 VCOA + NS + vehicle)-treated, (10 VCOA + SKF 525A + vehicle)-treated and (10 VCOA + PBT + vehicle)-treated groups, no significant change in percentage of body weight were observed. However, in the PCM-induced groups pretreated with 10 VCOA followed by SKF 525A or PBT, only (10 VCOA + NS + PCM)-treated group exhibited significant ($P < 0.05$) reduction in the percentage change of body weight when compared against the (NS + NS + PCM)-treated group, while the (10 VCOA + SKF 525A + PCM)-treated and (10 VCOA + PBT + PCM)-treated groups exerted significant ($P < 0.05$) increase in the percentage change of body weight when compared with the respective (NS + SKF 525A + PCM)-treated and (NS + PBT + PCM)-treated groups (Table 2).

The same patterns of activities were observed for groups pretreated with VCOB (Table 1).

Effect of inhibitor and inducer of CYP450 metabolizing system on liver weight of hepatotoxic rats pretreated with VCOA or VCOB

Effects of SKF 525A and PBT on the liver weight of rats

Table 1. Effects of CYP450 inducer and inhibitor on the percentages change in body weight after treatment with VCOA and VCOB.

Group	Change in body weight (%)
NS + NS + Vehicle	3.47 ± 0.73[c]
NS + NS + PCM	12.01 ± 0.61[a]
NS + SKF 525A + Vehicle	4.11 ± 0.72[c]
NS + SKF 525A + PCM	4.47 ± 0.31[c]
NS + PBT + Vehicle	3.86 ± 0.47[c]
NS + PBT + PCM	25.74 ± 1.57[b]
10 ml/kg VCOA + NS + PCM	3.51 ± 0.82[c]
10 ml/kg VCOA + SKF 525A + Vehicle	6.50 ± 0.99[c]
10 ml/kg VCOA + SKF 525A + PCM	16.83 ± 4.99[a]
10 ml/kg VCOA + PBT + Vehicle	5.67 ± 0.58[c]
10 ml/kg VCOA + PBT + PCM	13.54 ± 1.30[a]
10 ml/kg VCOB + NS + PCM	5.63 ± 0.61[c]
10 ml/kg VCOB + SKF 525A + Vehicle	8.33 ± 0.49[c]
10 ml/kg VCOB + SKF 525A + PCM	18.00 ± 6.97[a]
10 ml/kg VCOB + PBT + Vehicle	6.36 ± 0.54[c]
10 ml/kg VCOB + PBT + PCM	16.16 ± 3.21[a]

Values are mean ± S.E.M. Mean with different letters differ significantly ($P < 0.05$).

Table 2. Effects of CYP450 inducer and inhibitor on the liver weight after pretreatment with VCOA and VCOB.

Group	Liver weight (g/100 g body weight)
NS + NS + Vehicle	3.57 ± 0.48[c]
NS + NS + PCM	5.02 ± 0.32[a]
NS + SKF 525A + Vehicle	3.42 ± 0.34[c]
NS + SKF 525A + PCM	3.92 ± 0.31[c]
NS + PBT + Vehicle	3.44 ± 0.81[c]
NS + PBT + PCM	4.65 ± 0.72[a]
10 ml/kg VCOA + NS + PCM	3.52 ± 0.37[c]
10 ml/kg VCOA + SKF 525A + Vehicle	3.48 ± 0.31[c]
10 ml/kg VCOA + SKF 525A + PCM	4.90 ± 0.63[a]
10 ml/kg VCOA + PBT + Vehicle	4.93 ± 0.16[a]
10 ml/kg VCOA + PBT + PCM	6.82 ± 0.28[b]
10 ml/kg VCOB + PCM	3.48 ± 0.51[c]
10 ml/kg VCOB + SKF 525A + Vehicle	3.81 ± 0.12[c]
10 ml/kg VCOB + SKF 525A + PCM	4.68 ± 0.39[b]
10 ml/kg VCOB + PBT + Vehicle	4.45 ± 0.11[b]
10 ml/kg VCOB + PBT + PCM	6.62 ± 0.18[b]

Values are mean ± S.E.M. Mean with different letters differ significantly ($P < 0.05$).

pretreated with VCOA or VCOB followed by treatment with PCM are as shown in Table 2. In the non-induced groups receiving NS, SKF 525A or PBT alone as in the (NS + NS + vehicle)-treated, (NS + SKF 525A + vehicle)-

Table 3. Effects of CYP450 inducer and inhibitor on the percentage of viable cells in PCM-treated rats.

Group	Percentage of viable cell (%)
NS + NS + Vehicle	93.41 ± 0.87[a]
NS + NS + PCM	11.21 ± 0.44[b]
NS + SKF 525A + Vehicle	92.17 ± 0.59[a]
NS + SKF 525A + PCM	73.11 ± 0.65[c]
NS + PBT + Vehicle	91.09 ± 0.73[a]
NS + PBT + PCM	7.13 ± 0.62[b]
10 ml/kg VCOA + NS + PCM	90.43 ± 0.72[a]
10 ml/kg VCOA + SKF 525A + Vehicle	89.44 ± 0.69[a]
10 ml/kg VCOA + SKF 525A + PCM	19.02 ± 1.06[d]
10 ml/kg VCOA + PBT + Vehicle	91.11 ± 0.96[a]
10 ml/kg VCOA + PBT + PCM	3.29 ± 0.73[b]
10 ml/kg VCOB + NS + PCM	91.07 ± 0.74[a]
10 ml/kg VCOB + SKF 525A + Vehicle	87.55 ± 0.84[a]
10 ml/kg VCOB + SKF 525A + PCM	20.31 ± 1.10[d]
10 ml/kg VCOB + PBT + Vehicle	94.08 ± 1.22$_a$
10 ml/kg VCOB + PBT + PCM	3.22 ± 0.53[b]

Value are mean ± S.E.M. Mean with different letters differ significantly ($P < 0.05$).

treated and (NS + PBT + vehicle)-treated groups, no significant change in liver weight were observed. However, in the PCM-induced groups receiving NS, SKF 525A or PBT alone, only the (NS + NS + PCM)-treated and (NS + PBT + PCM)-treated, but not (NS + SKF 525A + PCM)-treated rats demonstrated significant ($P < 0.05$) increase in liver weight.

In the non-induced groups pretreated with 10 VCOA followed by NS, SKF 525A or PBT, only the (10 VCOA + NS + vehicle)-treated and (10 VCOA + SKF 525A + vehicle)-treated rats showed no significant change in liver weight, while (10 VCOA + PBT + vehicle)-treated group showed significant ($P < 0.05$) increase in liver weight when compared against the (NS + NS + vehicle)-treated and (NS + PBT + vehicle)-treated rats. In the PCM-induced groups pretreated with 10 VCOA followed by NS, SKF 525A or PBT, the (10 VCOA + NS + PCM)-treated group exhibited significant ($P < 0.05$) decrease in liver weight when compared with (NS + NS + PCM)-treated group. The (10 VCOA + SKF 525A + PCM)-treated and (10 VCOA + PBT + PCM)-treated groups demonstrated significant ($P < 0.05$) increase in liver weight when compared with the (10 VCOA + NS + PCM)-treated group. The latter ((10 VCOA + PBT + PCM)-treated group) liver weight was also significantly ($P < 0.05$) increased when compared with the (NS + PBT + PCM)-treated group (Table 2). The same patterns of activities were observed for groups pretreated with VCOB (Table 2).

Effect of inhibitor and inducer of CYP450 metabolizing system on cells viability of hepatotoxic rats pretreated with VCOA or VCOB

Effects of SKF 525A and PBT on the percentage of liver cell viability of rats pretreated with VCOA or VCOB followed by treatment with PCM are as shown in Table 3. Overall, in the PCM-induced groups pretreated with 10 VCOA or 10 VCOB followed by NS, SKF 525A or PBT, only the (NS + NS + PCM)-treated (9%), (NS + PBT + PCM)-treated (7%), (10 VCOA + SKF 525A + PCM)-treated (19%), (10 VCOA + PBT + PCM)-treated (3%), (10 VCOB + SKF 525A + PCM)-treated (20%) and (10 VCOB + PBT + PCM)-treated (3%) groups exhibited significant ($P < 0.05$) decrease in the percentage of cell viability when compared with the non-induced rats (NS + NS + vehicle) (90%). The other PCM-induced groups demonstrated percentage of cell viability that range between 73 to 95%.

Effect of inhibitor and inducer of CYP450 metabolizing system on serum enzymes levels of hepatotoxic rats treated with VCOA or VCOB

Table 4 shows the effects of SKF 525A and PBT on the level of serum enzymes of non-hepatoxic and hepatotoxic rats following pretreatment with VCOA or VCOB. Overall, the PCM-induced groups pretreated with NS, SKF 525A

Table 4. Effects of CYP450 inducer and inhibitor on the serum level of hepatic enzymes after treatment with VCOA and VCOB.

Group	Serum enzyme (U/l)		
	ALT	AST	ALP
NS + NS + Vehicle	2.13 ± 0.58[a]	2.88 ± 0.31[a]	177.31 ± 1.54[a]
NS + NS + PCM	20.62 ± 1.46[b]	21.77 ± 1.79[b]	302.41 ± 3.81[b]
NS + SKF 525A + Vehicle	2.83 ± 0.43[a]	2.54 ± 0.47[a]	175.17 ± 5.81[a]
NS + SKF 525A + PCM	6.03 ± 0.34[c]	14.10 ± 1.07[c]	223.47 ± 12.11[c]
NS + PBT + Vehicle	3.17 ± 0.52[c]	2.94 ± 0.73[a]	172.38 ± 8.71[a]
NS + PBT + PCM	32.71 ± 1.35[d]	39.41 ± 3.86[d]	421.71 ± 10.61[d]
10 ml/kg VCOA + NS + PCM	8.32 ± 0.57[c]	7.14 ± 0.73[e]	213.23 ± 4.61[c]
10 ml/kg VCOA + SKF 525A + Vehicle	0.72± 0.07[a]	1.57 ± 0.14[a]	231.58 ± 21.56[c]
10 ml/kg VCOA + SKF 525A + PCM	1.37 ± 0.11[a]	3.39 ± 0.32[a]	389.85 ± 22.58[d]
10 ml/kg VCOA + PBT + Vehicle	0.48 ± 0.10[a]	1.53 ± 0.10[a]	179.12 ± 17.44[a]
10 ml/kg VCOA + PBT + PCM	6.40 ± 1.73[b]	7.75 ± 0.71[e]	277. 00 ± 7.03[c]
10 ml/kg VCOB + NS + PCM	8.08 ± 0.49[a]	8.37 ± 0.21[e]	203.42 ± 6.11[a]
10 ml/kg VCOB + SKF 525A + Vehicle	0.78 ± 0.05[c]	1.82 ± 0.13[a]	244.51 ± 18.84[c]
10 ml/kg VCOB + SKF 525A + PCM	1.50 ± 0.13[a]	2.80 ± 0.19[a]	401.41 ± 38.03[d]
10 ml/kg VCOB + PBT + Vehicle	0.53 ± 0.07[c]	2.03 ± 0.22[a]	205.33 ± 1.31[c]
10 ml/kg VCOB + PBT + PCM	14.53 ± 1.18[b]	18.85 ± 2.36[c]	306.00 ± 19.94[b]

Values are mean ± S.E.M. Mean with different letters differ significantly ($P < 0.05$).

or PBT alone as in the (NS + NS + PCM)-treated, (NS + SKF 525A + PCM) and (NS + PBT + PCM)-treated groups demonstrated significant ($P < 0.05$) increase in the serum ALT, AST and ALP levels. However, pre-treatment of the PCM-induced rats with 10 VCOA followed by NS, SKF 525A or PBT as in the (10 VCOA + NS + PCM)-treated, (10 VCOA + SKF 525A + PCM)-treated and (10 VCOA + PBT + PCM)-treated groups demonstrated significant ($P < 0.05$) decrease in the levels of serum ALT and AST when compared with the (NS + NS + PCM)-treated, (NS + SKF 525A + PCM)-treated and (NS + PBT + PCM)-treated groups, respectively. However, the level of serum ALP of the (10 VCOA + SKF 525A + PCM)-treated group was found to increase significantly ($P < 0.05$) when compared with the (NS + SKF 525A + PCM)-treated group.

Histopathological study of the liver of PCM-induced rats pretreated with VCOA or VCOB after prechallenged with inhibitor and inducer of CYP450 metabolizing system

The histopathological findings on the liver tissues of normal and PCM-induced hepatotoxic rats pretreated with vehicle, VCOA or VCOB followed by SKF 525A or phenobarbital are as shown in Figures 1 and 2. The (NS + NS + vehicle)-treated group represents normal group with normal liver histology, while the (NS + NS + PCM)-treated group represents the hepatotoxic group wherein the liver histology demonstrated necrosis and inflammation. The (10 VCOA + NS + vehicle)-treated, (10 VCOB + NS + vehicle)-treated, (10 VCOA + SKF 525A + vehicle)-treated, (10 VCOA + PBT + vehicle)-treated, (10 VCOB + SKF 525A + vehicle)-treated or (10 VCOB + PBT +v ehicle)-treated groups were found to show normal liver histology (data not shown). On the other hand, the liver histology of (10 VCOA + SKF 525A + PCM)-treated, (10 VCOA + PBT + PCM)-treated, (10 VCOB + SKF 525A + PCM)-treated and (10 VCOB + PBT + PCM)-treated groups demonstrated the presence of necrosis and inflammation in contrast to the (10 VCOA + NS + PCM) and (10 VCOB + NS + PCM)-treated groups, respectively (Figures 3 and 4). In contrast to these groups, the (10 VCOA + NS + PCM) and (10 VCOB + NS + PCM)-treated groups exhibited normal liver histology (Figure 2).

DISCUSSION

PCM, in therapeutic doses, is normally and predominantly eliminated in mammalian species via four parallel competing mechanisms. The two major mechanisms are

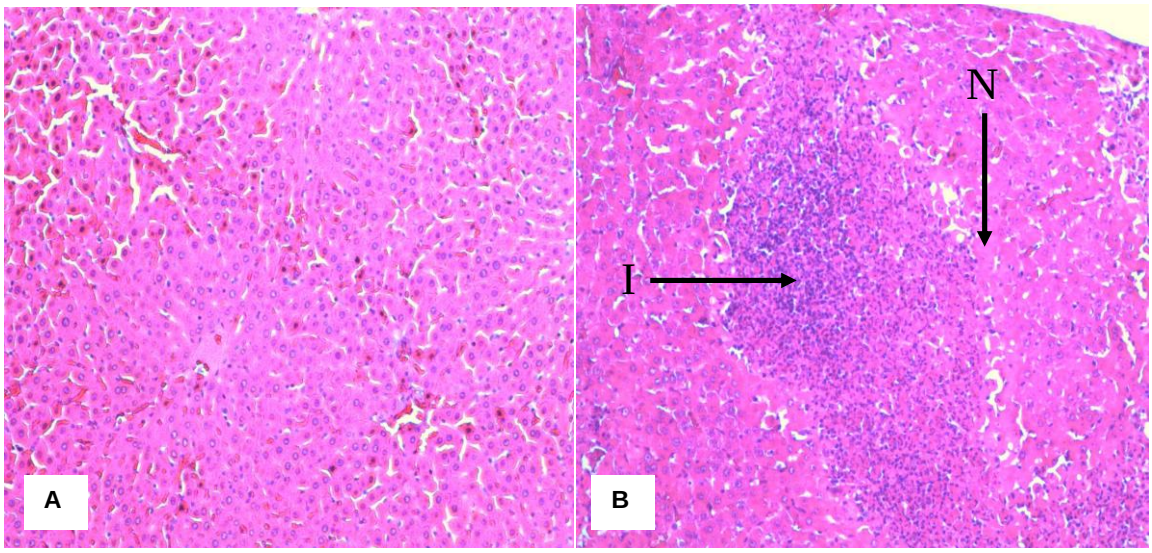

Figure 1. (A) Section of the liver tissue of control group (NS + NS + vehicle) showing normal histology (40x); (B) Section of the liver tissue of rats treated with (NS + NS + 3 g/kg PCM) (p.o.) showing tissue necrosis (N) and inflammation (I) (40x). (Liver sections of groups treated with (10 ml/kg VCOA + NS + vehicle) or (10 ml/kg VCOB + NS + vehicle), which showed normal histology were not shown).

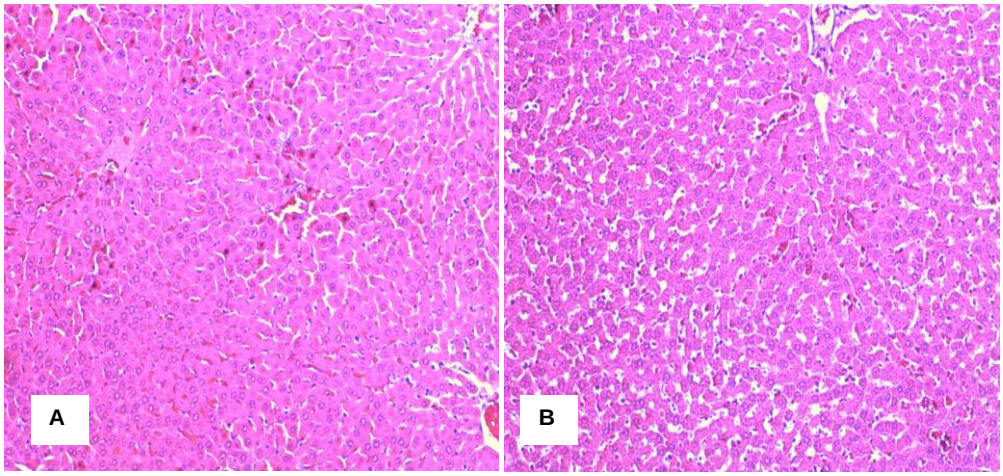

Figure 2. (A) Section of the liver tissue of group pre-treated with (10 ml/kg VCOA + NS + PCM) showing normal histology (40x); (B) Section of the liver tissue of group pre-treated with (10 ml/kg VCOB + NS + PCM) showing normal histology (40×). (Liver sections of groups treated with (10 ml/kg VCOA + SKF 525A + vehicle), (10 ml/kg VCOA + PBT + vehicle), (10 ml/kg VCOB + SKF 525A + vehicle) or (10 ml/kg VCOB + PBT + vehicle), which showed normal histology were not shown).

capacity-limited processes that lead to the formation of PCM sulfate and PCM glucuronide. The two minor pathways are first-order processes for the renal excretion of unchanged drug and the metabolism by cytochrome P450-mediated oxidation to a reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI) (Hazai et al., 2002). NAPQI is a toxic metabolite that is rapidly reduced by glutathione (GSH) and excreted in the urine as cysteine and mercapturic acid conjugates of PCM. When taken in overdose, PCM tend to induce serious hepatic

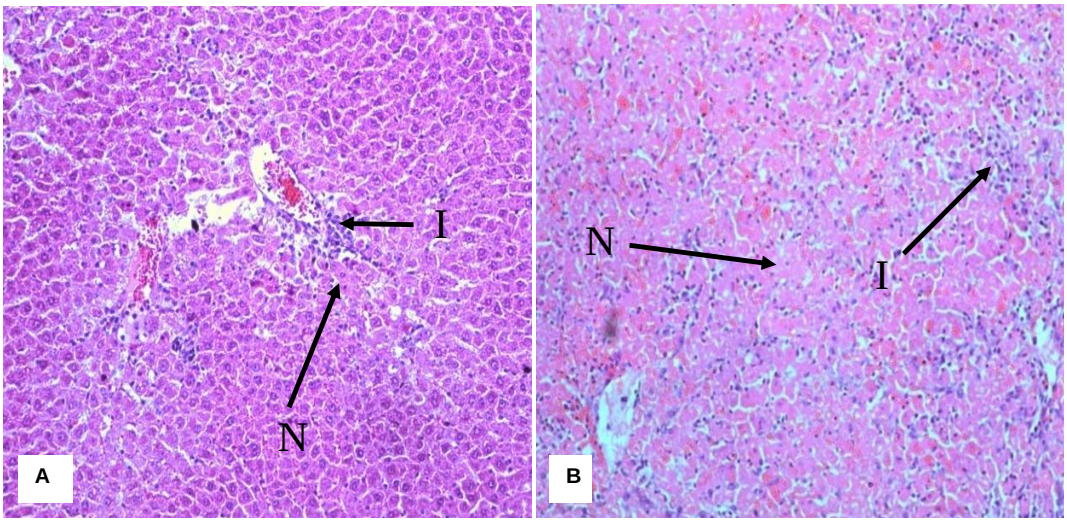

Figure 3. (A) Section of the liver tissue of group pre-treated with (10 ml/kg VCOA + SKF 525A + PCM) showing inflammation and necrosis (40x); (B) Section of the liver tissue of group pre-treated with (10 ml/kg VCOA + PBT + PCM) showing inflammation and necrosis (40×).

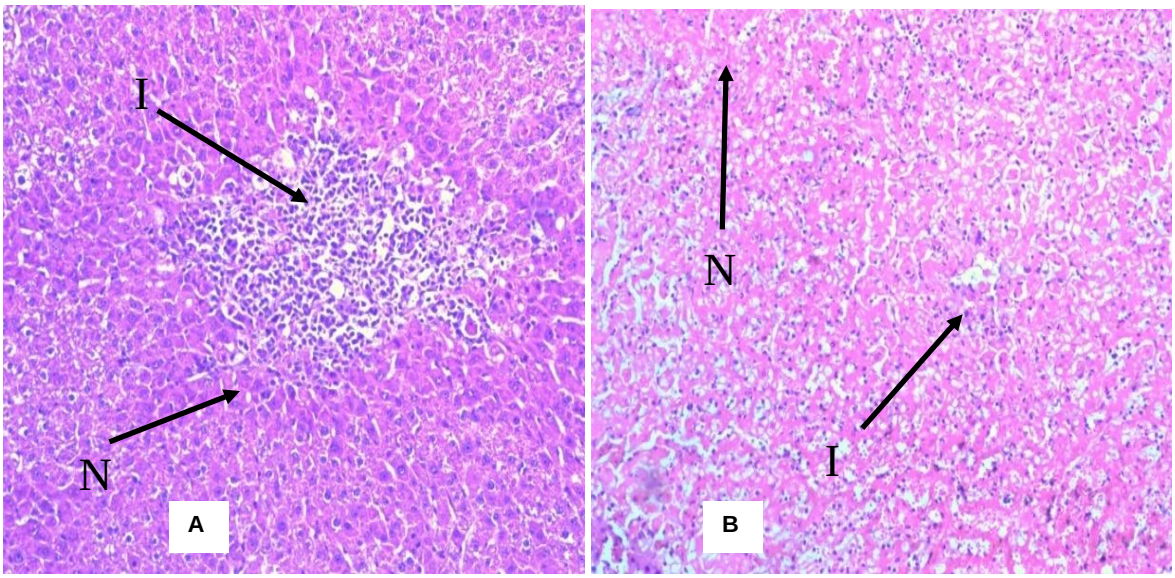

Figure 4. (A) Section of the liver tissue of group pre-treated with (10 ml/kg VCOB + SKF 525A + PCM) showing inflammation and necrosis (40×); (B) Section of the liver tissue of group pre-treated with (10 ml/kg VCOB + PBT + PCM) showing inflammation and necrosis (40×).

injury. The PCM-induced hepatotoxicity has been related to cytochrome P450 mixed function oxidases-mediated formation of NAPQI and depletion of hepatic glutathione (Kumar et al., 2004). During the overdose of PCM, the conjugation pathways become saturated leading to the formation of greater amounts of NAPQI. Furthermore, when hepatic GSH is depleted, free NAPQI binds covalently to macromolecules (proteins and DNA) and cellular proteins to produce protein adducts (Somchit et al., 2005), and also, by oxidizing lipids and altering homeostasis of calcium after depletion of glutathione causing acute hepatocellular necrosis (Jafri et al., 1999). According to Mehendale (2000), PCM-induced liver injury could be accounted for by at least three types of cell death

namely (1) mechanism-based cell death; (2) cell death triggered by lytic enzymes (death proteins) released by dead or dying cell; and (3) apoptosis.

Two indicators of the presence of hepatotoxicity include increase in liver weight and elevation of serum liver enzymes (Rasheed et al., 1994; Kaplowitz, 2001). The latter is due to leakage of cellular enzymes, particularly ALP, AST and ALT, which are found in the cytosol into blood plasma as a result of damage to the plasma membrane of liver cell (Kumar et al., 2004). According to Kumar et al. (2004) measurement of those enzymes' level in the serum is a useful quantitative marker to describe the extent and type of hepatocellular damage. In the present study, PCM, at the toxic dose increased the liver weight and elevated the serum levels of the respective hepatic enzymes (Jafri et al., 1999). In addition to these, PCM also increased the rats' body weight, reduced the number of viable cells and these findings are further supported by histological studies that showed marked sign of inflammation and gross necrosis of the centrilobular hepatocytes characterised by nuclear pyknosis, karyolysis and eosinophilic infiltration in liver pre-treated with PCM alone. Pre-treatment with either VCOA or VCOB, on the other hand, reduced the percentage of body weight, liver weight and serum levels of ALT, AST and ALP, while increasing the hepatocytes viability in PCM-induced hepatotoxic rats indicating the oils potential as hepatoprotective agents against PCM-induced liver toxicity. Histological study of liver tissue of PCM-induced rats pre-treated either with VCOA or VCOB demonstrated normal liver architectures, which confirmed the hepatoprotective potential of the oil. SKF 525A, an inhibitor of CYP450 activity, when given alone did not change the parameters measured but managed to reduce PCM hepatotoxic effects as seen with reduced percentage change of body weight and serum enzymes level, insignificant change in liver weight and increased cell viability. However, the VCOA and VCOB hepatoprotective activity against PCM-induced liver damage were significantly reduced by SKF 525A as seen with increase in percentage change of body weight and liver weight, serum enzymes level and reduced cell viability. SKF 525A only increased the ALP level, but reduced the ALT and AST levels. The ability of SKF 525A to significantly reduce the hepatoprotective effect of VCOA or VCOB was confirmed by histological studies that show the presence of inflammation and necrosis in the liver tissue. On the other hand, PBT, an inducer of CYP450 activity, given alone did not change the parameters measured but enhanced the PCM-induced hepatotoxicity as demonstrated by the increase in percentage change of body weight and serum enzymes level. However, PBT did not change the liver weight and cell viability of hepatotoxic rats. When given with either VCOA or VCOB, PBT also reduced the oils hepato-hepatoprotective effects as indicated by the increase in percentage change of body weight and liver weight and reduced cell viability. However, PBT only increased the ALP level, but not the ALT and AST levels, in VCOA-treated hepatotoxic rats, while in VCOB-treated hepatotoxic rats, PBT increased the levels of ALT, AST and ALP. The ability of PBT to reverse hepatoprotective effect of both oils was confirmed by histological studies that show the presence of inflammation and necrosis in the liver tissue. AST and ALT are enzymes produced in the liver to assist the production of proteins and the metabolism of amino acids. When the liver is damaged or inflamed, these enzymes leak into the bloodstream. On the other hand, ALP is known as cholestatic enzyme, and elevated levels of this enzyme usually indicate a problem with the bile ducts that transport bile from the liver to the gall bladder and the intestines. The level also tends to be higher in diseases associated with injury to the bile-secreting part of the liver's activity. High levels of ALP indicates signs of diverse conditions, such as bile duct blockage, gallstones, alcoholic liver disease, nonalcoholic fatty liver disease, primary sclerosing cholangitis, liver tumors, drug-induced liver disease and primary biliary cirrhosis (Amerman, 2011). Thus, it is suggested that the increased ALP level seen after pre-treatment with SKF 525A or PBT could be related to the drug-induced liver disease, which leads to blocking of the bile flow in smaller bile channels within the liver (Sinomedresearch, 2011).

NAPQI has been known to be the cause of liver toxicity associated with overdose of PCM and is produced by the CYP450 system, particularly CYP2E1. Pre-challenging the VCOs-treated hepatotoxic rats with CYP450 inhibitor, SKF 525A, was found to maintain the presence of inflammation and necrosis in the liver when compared with PCM-induced group treated only with VCOs, which showed normal liver architecture. Unexpectedly, SKF 525A that is supposed to inhibit the formation of NAPQI and help the VCOs to maintain the normal liver architecture that failed to do so and this is further supported by increase in liver weight and level of serum enzymes. The mechanism responsible for this activity was not yet understood. However, it is suggested that the VCOs might somehow act as inhibitor towards the action of SKF 525A against CYP2E1, which in turn lead to increase NAPQI production. On the other hand, the ability of PBT to reduce VCOA and VCOB hepatoprotective effect was expected as PBT indirectly involved in the conversion of PCM to NAPQI via stimulation of the CYP450 system.

The inability of either SKF 525A or PBT to fully inhibit the VCOs hepatoprotective activity suggested that several possible mechanisms could partly be involved in the observed hepatoprotective activity of the oils. Mehendale (2000) have claimed that any potential mechanisms must involve activation of peroxisomes

proliferators activated receptor-α (PPAR-α), which is a member of the nuclear superfamily. PPAR-αs are ligand-activated transcription factors activated by xenobiotics and are highly expressed in hepatocytes. In earlier study, Manautou et al. (1996) demonstrated that the nuclear receptor PPAR-α is required for clofibrate's hepatoprotection against PCM-induced liver toxicity. They also showed that the protection against PCM-induced hepatotoxicity was abolished in PPAR-α knockout mice and that regardless of which cellular mechanism is involved in hepatoprotective activity of clofibrate, all depend on PPAR-α receptor activation.

Another possible mechanism that could contribute to the observed hepatoprotective activity of VCOs is the oils antioxidant activity (Zakaria et al., 2010; Nevin and Rajamohan, 2006, 2008; Marina et al., 2009). According to Mehendale (2000), the increase antioxidants and oxyradical quenching enzymes, such as catalase, could protect against oxyradical-mediated cell death. Compounds like PPs characteristically induce antioxidant defenses, such as catalase, which may bolster the ability of hepatocytes to detoxify oxidant-induced injury and thereby prevent cell death. Furthermore, compounds possessing immunomodulatory and anti-inflammatory properties, like VCOA and VCOB, have been suggested to possess antioxidant properties and would be a good hepatoprotective candidate (Malarvilli et al., 2009; Devasagayam and Sainis, 2002). This is supported by claim that the combination of hepatoprotective effect and antioxidant activity synergistically prevents the process of initiation and progress of hepatocellular damage (Gupta et al., 2006). Other possible mechanisms by which those VCOs exert their protective action against PCM-induced hepatocellular metabolic alterations include stimulation of hepatic regeneration via an enhanced synthesis of protein and glycoprotein or accelerated detoxification and excretion (Kumar et al., 2004), stabilization of the hepatocellular membrane and prevention of the process of lipid peroxidation (Mujeeb et al., 2009). However, further studies are warranted before we could conclude on the exact mechanism(s) involved in the hepatoprotective activity of the VCOs.

In conclusion, the hepatoprotective effect of MARDI-produced VCOs does not involved modulation of the CYP450 systems, and, thus, suggested to be partly attributed to its antioxidant activity. This report may serve as a basis for further studies into the exact hepatoprotection mechansim of VCOs.

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