ORIGINAL ARTICLE

Evaluation of Acute Toxicity Induced by Supercritical Carbon Dioxide Extract of *Canarium odontophyllum* (CO) Miq. Pulp Oil in SPF Sprague Dawley Rats

Nurdiyana Abdul Manap[1], Azrina Azlan[1], Hazilawati Hamzah[2], Sharida Fakurazi[3], Noor Atiqah Aizan Abdul Kadir[1]

[1] Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2] Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3] Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

ABSTRACT

Introduction: Different solvents extraction was used to extract the good fatty acid composition of Dabai fruits. Nevertheless, solvents extraction may exhibit harmful effects. The present study was aimed to evaluate the safety of using supercritical carbon dioxide extraction (SCO2) of dabai pulp oil by acute toxicity study in Specific Pathogen Free (SPF) Sprague-Dawley (SD) rats. **Methods:** The CO pulp oil extract was prepared by SCO2 extraction of the freeze-dried pulp and was administered orally to SPF SD rats (consisted of 5 rats/sex/group) at upper limit dose 5000 mg/kg body weight (BW) for 14 days. The study includes the control and treatment groups, each consisting of 5 male and female rats. The rats were fed and allowed to drink sterilized water ad libitum. Fatty acid composition (FAC) of the extract was determined using GC-FID. Electrolytes and biochemical parameters in blood, as well as relative organs weight were measured. **Results:** The extract at a single dose of 5000 mg/kg did not cause any acute toxicity effects or mortality to the treatment of rats during observation periods in 14 days. FAC of the SCO2 extracted oil exhibited high content of palmitic and linoleic acids. The relative organs weights (ROW) and histopathology of rats were within normal range. **Conclusion:** Thus, the LD50 was estimated to be more than 5000 mg/kg of CO pulp oil extract and can be considered for further investigation for its therapeutic efficacy in a larger animal model.

Keywords: *Canarium odontophyllum*, Oil extract, Supercritical extraction, Acute toxicity

Corresponding Author:
Azrina Azlan, PhD
Email: azrinaaz@upm.edu.my
Tel: +603-89472466

INTRODUCTION

Canarium odontophyllum (CO) Miq., also known as dabai fruit is a fruit-bearing tree of the genus Canarium in the family Burseraceae. This fruit pulp contains 6.5-33.1 g of fat per 100g of pulp (1, 2) which produce the unique taste sensation of this infamous fruit is a prized seasonal delicacy in Sarawak, Malaysia and love by the local. The fruit's pulp is popular mainly just eaten by soaking in warm water and seasoned with salt or soy sauce (3). Other than the basic style of dabai consumption, in Sarawak some food products have been developed, prepared and commercialise from dabai fruits such as frozen dabai fruits and pulp, dabai juice, dabai mayonnaise, dabai fruits preserved in sugar as halwa, dabai crackers, preserved dabai pulp, dabai sauce, and flavoring ingredient such as dabai paste (4, 5). The taste and aroma intensity of fat in CO pulp oil has led to the popularity of this fruit (6). The fruit's extract has been proven scientifically for their available natural antioxidant with good fatty acid composition (7). Recent studies showed that CO pulp oil extract has been found to have the potential as future nutritive oils (8, 9) and has health promoting properties of essential fatty acids (10).

Extraction of plant materials can be done by various extraction procedures (11). Previous studies on CO pulp oil extract using a different type of solvents (3, 12) (petroleum ether and hexane) have recently been performed to determine antioxidants and fatty acid composition. Nevertheless, supercritical carbon dioxide (SCO_2) extraction has not yet been done on CO pulp to produce oil. This is the first study conducted on CO pulp oil extracted using SCO_2, where the extraction process does not involve any use of the toxic solvent. Furthermore, toxicity evaluation of CO pulp oil becomes more important as they are consumed widely

as a favourite food among Sarawakian, Sabahan and Bruneian (5). Besides, identification of potential toxicity effect of the extracted oil is essential as baseline information for further development of CO pulp oil for prevention of lifestyle diseases in human. Therefore, this study aims to determine the safety and acute toxicity induced by supercritical carbon dioxide extracted CO pulp oil using SPF Sprague Dawley rats.

MATERIALS AND METHODS

Plant material

Fresh dabai fruits were homogenously harvested from the Sarikei district by the officer from Agriculture Research Centre (ARC) in Semonggok, Sarawak, Malaysia. Fresh fruits (226 kg) were collected in March 2017 and packed in the sealed airtight containers filled with ice and flown to West Malaysia and transported to Faculty of Medicine and Health Sciences, UPM around the same time of accumulation. The authentication of dabai fruits was administered and recorded the herbarium voucher specimens (S 64872) by research officer of ARC, Semonggok, Sarawak, Malaysia.

Sample preparation

The fully ripe dabai fruits were chosen and cleaned thoroughly. The dabai pulp is hard when ripe, to loosen and easily remove the pulp from the seed, the dabai fruits soaked in warm water at 36°C temperature for 15 minutes. The collected edible portion (a mixture of flesh and skin) of 137.62 kg dabai fruits was cryodesiccated using an industrial scale freeze dryer (The Virtis Company Inc., Gardiner, NY, USA). The mixture of dried dabai flesh and skin (62.46 kg) were stored at -20 °C. Then, the freeze-dried dabai pulp was ground into small particles to increase surface area and extracted using supercritical technology from Supercritical Fluid Centre (SFC), UPM. SCO_2 liquid was used to extract out CO pulp oil from dabai pulp at 40°C temperature and 40 mPa pressures (13). The extracted oil then was purged with liquid nitrogen to remove and reduce oxygen levels in containers and extend shelf life. The dabai oil was stored in an airtight container to maintain the availability of crude compound in the oil.

Fatty acid profiling of SCO_2CO oil

Preparation of fatty acid methyl esters of fatty acid followed IUPAC 2.301 similar to Malaysian Palm Oil Board (MPOB) p3.4 (Acidic Methanolysis Method)

Heavy metal analysis of CO pulp oil extract

The CO pulp oil extract was sent to Food Quality and Safety Research Development, Universiti Kebangsaan Malaysia (UNIPEQ, UKM) for heavy metals (Arsenic, cadmium, lead and mercury) analytical laboratory services. The heavy metals were determined after a microwave digestion method in a closed vessel, in atmospheric aerosols followed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Animals

Ten male and female Specific Pathogen Free (SPF) Sprague-Dawley rats with 135-157 gram in weight at the age of 4-5 weeks old were supplied from the Nomura Siam International Co., Ltd. Thailand and housed at the laboratory animal facility Comparative Medicine and Technology Unit (COMeT), Bioscience Institute, UPM. The animals were divided into two groups (n=5), which are the negative control and treatment group. Two to 3 animals were randomly housed per cages. The rats were fed (standard pelleted diet from Specialty Feeds, Australia) and allowed to drink sterilized water ad libitum. Animals were maintained in an individual ventilated cage (IVC) and controlled environmental condition (well-ventilated cages; a controlled temperature between 21 to 23oC; relative humidity in a range of 50 to 60%; 12-h light/dark photoperiod). Prior to commencing the study, the rats were acclimatized for two weeks. The ethical protocol clearance was sought from the Institutional Animal Care and Use Committee (IACUC), Faculty of Medicine and Health Sciences, UPM (UPM/IACUC/AUP-R051/2015).

Acute oral toxicity study

The acute oral toxicity test for CO pulp oil extract was carried out based on the guideline of Organisation Economic Co-operation and Development Guidelines No. 420 for "Acute Oral Toxicity – Fixed Dose Procedure" following the limit test procedure. The acute oral study comprised of two groups, one control and one treatment group that consisted of 5 female and male rats in each group. Rats (n = 5 females and males/group) were subjected to oral gavage normal saline and one time dose of 5000 mg/kg BW of CO pulp oil extract to the control and treatment group respectively. Body weight of the rats was measured and recorded daily from the beginning until the end of the experiment. The rats were fasted at the end of day 14 overnight and were sacrificed by exsanguination under ketamine (50 mg/kg) and xylazine (10 mg/kg) anaesthesia. Blood samples were collected via intracardiac puncture by using 23 gauge needle and 3 mL syringe through the diaphragm. The blood was withdrawn slowly to prevent the heart from collapsing.

Biochemistry analysis

The blood was collected via intracardiac puncture and kept in the plain tube (Serum blood collection tube, BD Vacutainer). The blood samples were allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 4°C for 15 min and the following parameters were measured: sodium, potassium, chloride, urea, creatinine, total protein, total bilirubin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride, and cholesterol. The test was sent to and carried out at Veterinary Haematology and Clinical Biochemistry laboratory of Veterinary Laboratory

Services Unit (VLSU) Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM).

Relative organ weight (ROW)

The rats were dissected after exsanguination and their vital organs such as brain, heart, stomach, spleen, liver, and kidneys were excised, observed for any signs of abnormality or lesions, and clean using normal saline (0.9% NaCl) immediately and weighed (absolute weight). The following equation was used to calculate the relative organ weight:

$$\frac{\text{Absolute organ weight (g)}}{\text{Final body weight of rat on sacrifice day (g)}} \times 100 \quad (14)$$

The organs were then preserved in prepared 10% formalin for histopathological examination.

Histopathology

Representative tissue of liver organ was taken and processed to make histology slide using standard procedure (dehydrated in alcohol 24 hours, embedded in paraffin wax, and cut into 4 µm thick sections) and was stained with Hematoxylin and Eosin. The slides were examined using a light microscope (Olympus BX 51, Tokyo, Japan).

Statistical analysis

Statistical significance was analyzed using independent sample t-tests between control and treatment group with the probability of $p < 0.05$ as appropriate and expressed as mean ± standard deviation (SD). Data management and analysis were performed using SPSS software version 22.

RESULTS

Acute toxicity testing

Physical observation of both control and treated groups throughout the study appeared healthy. Physical abnormalities of hair coat, eye colour, salivation, touch response, tail pinch, and grip strength were not detected in gross observation. No mortality was observed in any of the groups. The effects of CO pulp oil extract on the body weight changes are shown in Table I. The weight gain throughout the study was gradually increased in both groups. The treatment group BW were not significant difference as compared to the control group. The CO pulp oil extract did not affect body weight changes in the treatment group.

Table I: Weekly mean body weight (g) of rats receiving single dose of CO pulp oil extract for 14 days.

Organs	Control	CO pulp oil extracts 5000mg/kg BW
Male		
Acclimatisation week 1	157.6±7.5	153.8±11.4
Acclimatisation week 2	207.4±8.0	204.4±18.5
Treated week 1	252.0±15.6	260.8±11.5
Treated week 2	303.8±22.1	315.2±19.8
Female		
Acclimatisation week 1	142.6±14.1	135.0±9.8
Acclimatisation week 2	174.8±15.7	170.4±13.6
Treated week 1	207.2±17.4	188.0±27.4
Treated week 2	223.8±15.7	213.2±19.0

Values are expressed as mean ± SD (n = 5/sex/group). No significant difference compared to control (t-test).

Fatty acid compositions

The fatty acid composition (%) of the SCO_2 CO oil is summarized in Table II. Among the saturated,

Table II: Fatty acid composition (%) of CO pulp oil extract.

	FAME	% in fat
	Saturated fatty acids	
C4	Butryic	0.00 ± 0.00
C6	Caproic	0.00 ± 0.00
C8	Caprylic	0.05 ± 0.00
C10	Capric	0.01 ± 0.00
C11	Undecanoic	0.01 ± 0.01
C12	Lauric	0.74 ± 0.06
C13	Tridecanoic	0.00 ± 0.00
C14	Myristic	0.28 ± 0.00
C15	Pentadecanoic	0.03 ± 0.00
C16	Palmitic	41.56 ± 0.10
C17	Heptadecanoic	0.11 ± 0.00
C18	Stearic	4.31 ± 0.01
C20	Arachidic	0.10 ± 0.00
C21	Henicosanoic	0.02 ± 0.01
C22	Behenic	0.23 ± 0.06
C23	Tricosanoic	0.11 ± 0.00
C24	Lignoceric	0.10 ± 0.01
Total SFA		47.65 ± 0.11
	Monounsaturated fatty acids	
C14:1	Myristoleic	0.03 ± 0.02
C15:1	Cis-10-pentadecenoic	0.04 ± 0.01
C16:1	Palmitoleic	0.63 ± 0.02
C17:1	Cis-10-heptadecanoic	0.03 ± 0.00
C18:1n9t	Elaidic 9trans)	0.00 ± 0.00
C18:1n9c	Oleic	39.37 ± 1.01
C20:1n9	Cis-11-eicosenoic	0.07 ± 0.01
C22:1n9	Erucic	0.03 ± 0.03
C24:1	Nervonic	0.20 ± 0.19
Total MUFA		40.38 ± 0.79
	Polyunsaturated fatty acids	
C18:2n6t	Linolelaidic (trans)	0.00 ± 0.00
c18:2n6c	Linoleic (cis)	12.54 ± 1.03
C18:3n6	α-linolenic	0.12 ± 0.02
C18:3n3	A-linolenic	0.44 ± 0.06
C20:2	Cis-11,14-eicosadienoic	0.00 ± 0.00
C20:3n6	Cis-8,11,14-eicosatrienoic	0.00 ± 0.00
C20:3n3	Cis-11,14,17-eicosatrienoic	0.00 ± 0.00
C20:4n6	Arachidonic	0.01 ± 0.01
C20:5n3	Cis-5,8,11,14,17-eicosapentaenoic	0.00 ± 0.00
C22:2	Cis-13,16-docosadienoic	0.00 ± 0.00
C22:6n3	Cis-4,7,10,13,16,19-docosahexaenoic	0.00 ± 0.00
Total PUFA		13.11 ± 1.10

Values represent the Mean ± SD (n = 3)

monounsaturated and polyunsaturated fatty acids; palmitic (41.56±0.10) and linoleic (12.54±1.03) acids are the most abundant fatty acids in the extract.

Heavy metal analysis

The heavy metals availability of the CO pulp oil extract is as summarized in Table III. Lead (Pb) level in the oil is highest among the metals followed by arsenic (As) and cadmium (Cd). However, mercury (Hg) was not detected in the oil extract.

Table III: Heavy metal analysis of CO pulp oil extract.

Heavy metal	mg/kg
Arsenic	0.007±0.003
Cadmium	0.003±0.001
Lead	0.018±0.015
Mercury	ND

Values are the mean of triplicate analysis. (ND = not detected)

Biochemistry analysis

The effects of acute administration of CO pulp oil extract on serum biochemistry parameters are presented in Table IV. The results showed that ingestion of the CO pulp oil extract did not show any significant difference from the control group on serum electrolytes: sodium, potassium, and chloride. Moreover, CO pulp oil extract had no effects on the kidney (urea and creatinine) and liver function parameters (ALP, AST, and ALT). The lipid profiles (LDL, HDL, triglyceride and cholesterol) were not statistically significant difference. No significant changes in total protein and total bilirubin were noted.

Table IV: Effect of CO pulp oil extract on biochemical parameters in acute oral toxicity study.

	unit	Control	CO oil extracts 5000mg/kg BW
Male			
Sodium	mmol/L	146.0±2.0	146.4±1.3
Potassium	mmol/L	5.2±0.7	5.0±0.4
Chloride	mmol/L	103.6±2.1	102.8±1.5
Urea	mmol/L	7.2±0.5	7.0±0.7
Creatinine	µmol/L	41.4±5.0	41.8±1.6
Total protein	g/L	64.0±3.7	63.8±1.5
Total bilirubin	µmol/L	1.5±0.3	1.6±0.3
ALP	U/L	191.8±12.9	229.8±61.0
AST	U/L	119.6±47.2	100.0±12.9
ALT	U/L	60.2±22.6	43.0±4.9
LDL	mmol/L	0.3±0.0	0.3±0.1
HDL	mmol/L	2.1±0.3	2.2±0.1
Triglyceride	mmol/L	1.3±0.3	1.0±0.3
Cholesterol	mmol/L	2.4±0.4	2.5±0.9
Female			
Sodium	mmol/L	145.0±3.5	148.0±4.4
Potassium	mmol/L	4.5±0.4	4.2±0.1
Chloride	mmol/L	103.2±2.0	106.6±3.6
Urea	mmol/L	6.9±1.1	6.6±1.1
Creatinine	µmol/L	37.4±2.1	38.6±4.5
Total protein	g/L	62.9±5.9	62.9±1.8
Total bilirubin	µmol/L	1.5±0.6	1.2±0.2
ALP	U/L	129.0±30.7	119.2±14.4
AST	U/L	79.0±34.1	109.2±31.7
ALT	U/L	38.0±7.9	42.2±5.6
LDL	mmol/L	0.3±0.5	0.3±5.9
HDL	mmol/L	2.6±0.3	2.3±0.4
Triglyceride	mmol/L	1.1±0.4	1.1±0.5
Cholesterol	mmol/L	2.9±0.4	2.5±0.3

Values are expressed as mean ± SD (n = 5/sex/group). No significant difference compared to control (t-test).

Organ weight changes

As shown in Table V, organ weight changes were calculated. Administration of CO pulp oil extract to both groups did not generate any statistically significant differences between control and treatment groups in either male or female rats.

Table V: Relative organ weights of rats receiving single dose of CO pulp oil extract for 14 days.

Organs	Control	CO pulp oil extracts 5000mg/kg BW
Male		
Brain	0.6±0.0	0.6±0.0
Heart	0.3±0.1	0.3±0.0
Liver	3.4±0.4	3.3±0.2
Spleen	0.3±0.1	0.2±0.0
Kidney left	0.3±0.1	0.3±0.0
Kidney right	0.3±0.1	0.3±0.0
stomach	0.6±0.1	0.5±0.1
Female		
Brain	0.8±0.2	0.9±0.1
Heart	0.4±0.1	0.4±0.0
Liver	3.2±0.2	3.3±0.3
Spleen	0.3±0.0	0.3±0.0
Kidney left	0.3±0.0	0.4±0.0
Kidney right	0.3±0.0	0.4±0.0
stomach	0.6±0.1	0.6±0.1

Values are expressed as mean ± SD (n = 5/sex/group). No significant difference compared to control (t-test).

Histopathology

Histological examination under light microscopy of the control and CO pulp oil extract group revealed no significant changes as well as showed a normal structure in all the organs examines including liver as compared to the control group. Some parts of the picture from the histopathology are shown in Figure 1.

DISCUSSION

In Malaysia, especially for the Sarawak and Sabah communities, indigenous dabai fruits are prevalent and love by the communities. Scientific evidence on CO pulp oil extraction has been widely reported, as solvent extraction has high extractability, especially using solvents such as ethyl acetate (15), petroleum ether (16), n-butanol (15), methanol (10) and ethanol (3). However, it has some limitations especially the existence of residual solvent in the final oil and harmful volatiles

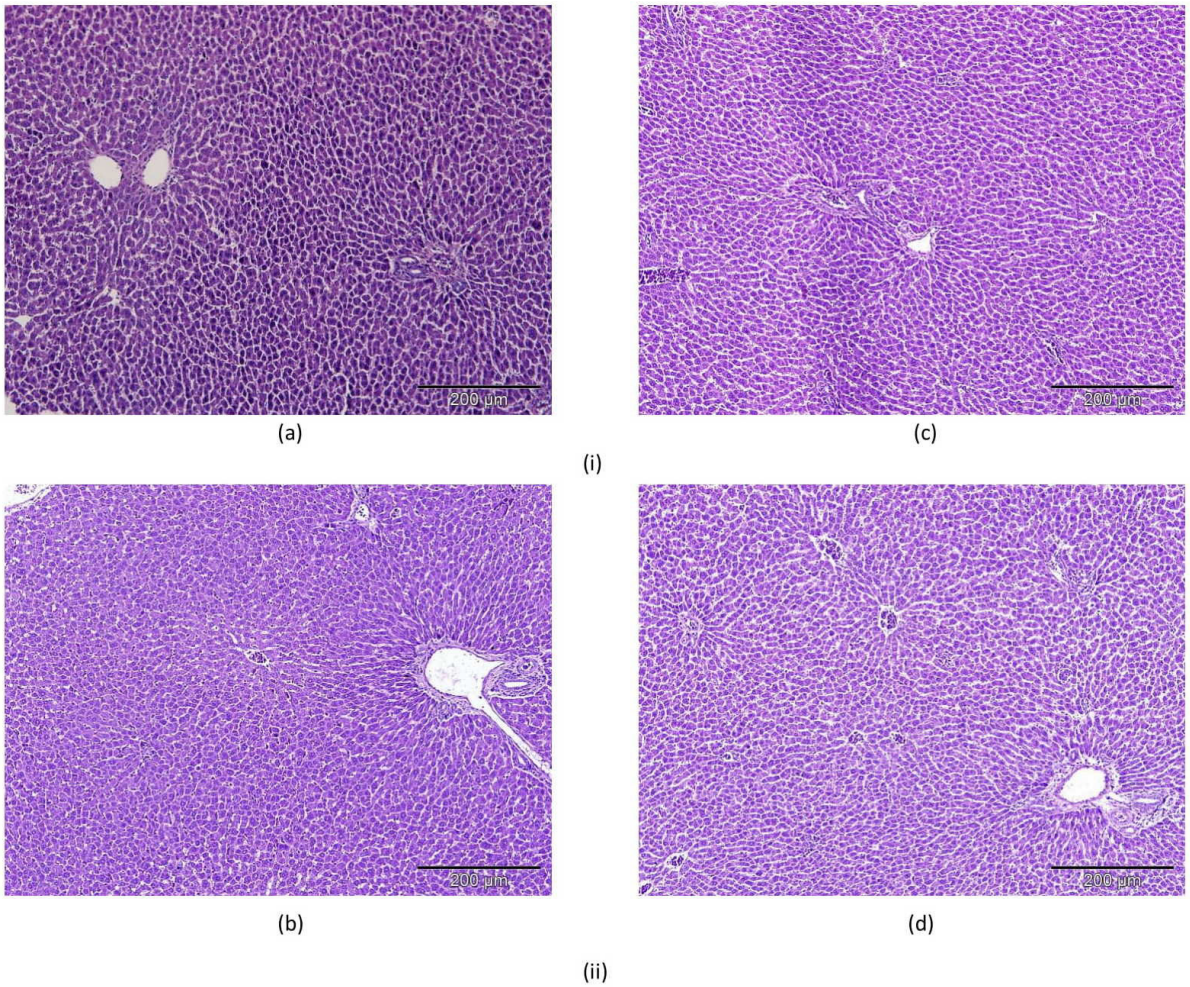

Figure 1: Histological structure of liver from control (i) and CO pulp oil 5000 mg/kg BW extract (ii) group of male and female SPF SD rats in acute oral toxicity study. (a) and (b): male; (c) and (d): female. The structure showed there were normal as compared to the control (H&E staining).

solvent emission that affect health and the environment (17). Furthermore, the safety studies are still lacking and limited data are available and no reports were found on the point of toxicity CO pulp oil extract. Therefore, it is fundamental to evaluate the toxicity effect of CO pulp oil extracted using supercritical carbon dioxide (a green technology) as solvent-free extraction (18) in animals to ensure its safety.

Metals such as arsenic, cadmium, lead and mercury are among the heavy metal found in the most food supply because of their presence in the air, water and soil uptake by the plants and animals. The analysis of metal in the extracted oil was essential to confirm that no outside factor contributes to toxicity signs in the study. Based on the Maximum Permitted Concentration as prescribed in the Malaysian Food Act (1983) and the Food Regulation (1985) (19), the permitted levels of heavy metals are; 0.1 mg/kg for As, 1 mg/kg for Cd, 0.1 mg/kg for Pb and 0.05 µg/kg for Hg. These metals were in a trace amount in the CO pulp oil extract, where values were much lower than the permitted level, indicating the extracted CO oil is considered non-toxic and safe to be consumed. This result corroborates with the findings of previous work in Canarium ovatum, Engl by Arenas and Trinidad (2017) (20).

The significant component of the extracted CO pulp oil was fat with main fatty acids being palmitic (41.56±0.10) and linoleic (12.54±1.03) acids that are slightly higher than solvent extracted oil (10) at 36.05±0.05 and 11.75±0.02 respectively. There were also no unusual fatty acids detected in the extracted oil (21). The richness of MUFAs in the dabai pulp contribute to the unique creamy taste of dabai (5, 6) and led to the development of many commercialise dabai food products. A similar FAC in both extraction methods (SCO2 and solvent) has not been reported previously on acute toxicity test. The acute toxicity data is necessary before conducting a subchronic toxicity study. Then, herein we presented the evaluation of acute toxicity study of CO pulp oil extract for the first time.

An acute toxicity study of in vivo CO pulp oil extract is necessary in order to evaluate the approximate safe dosage so that it can be used as a reference in future research. Initial steps for determination of LD50 is usually conducted during the evaluation of the toxic

signs. Data from the acute toxicity study may (i) present fundamental information on the characteristics and regulation of a substance; (ii) help in dose determination in animal studies; and (iii) help determine LD50 values that provide advantages of the potential useful dose of a discovery substances (22, 23). Additionally, if the high dose does not produce mortality, no addition acute testing will be conducted (24). In principle, the limit test method is not intended for determining a precise LD50 value, but it serves as a suggestion for classifying the crude extract based on the expectation at which dose level the animal is expected to survive (23).

In this study, CO pulp oil extract at a dose of 5000 mg/kg had no adverse effect on the treatment group up to 14 days of observation. CO pulp oil extract did not affect the body weight of the treatment group when compared to the control group. Chemical compounds can be as a sensitive indicator of direct toxic effects which altered the weight changes in organs (25). In our study, analysis of relative organ weight resulted in no statistically significant differences between the treated and control groups in both sexes for 14 days.

The biochemical parameters (electrolytes, kidney profile, and liver profile) showed that CO pulp oil extract did not induce toxicity, as we did not observe any significant differences in these serum samples between the treated and control groups in both sexes. The normal results showed that CO pulp oil extract does not cause any damage to the organs. Lipid parameters (LDL, HDL, triglyceride and cholesterol) were not significantly different as compared to the control group. This results may be due to the short period of the study besides the fatty acids in CO oil are common fatty acids as in other oils. The normal results of liver profile strongly suggest that the acute administration of CO pulp oil extract did not develop any morphological changes in the histopathology observations of liver tissue.

CONCLUSION

We may conclude that oral administration of CO pulp oil extract to healthy SPF rats for 14 days did not cause adverse effects in both male and female rats of a maximum dose of 5000 mg/kg. The heavy metals were in trace level and considered safe to consume. The CO pulp oil extract did not induce toxicity on biochemical parameters and relative organs weight at this highest dose. Thus, this extract did not altered any histopathological changes at acute oral toxicity. However, further investigations on subacute and subchronic toxicity should perform for detail safety profile of CO pulp oil extract.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Universiti Putra Malaysia for the financial support given under the UPM Innovation Development Research Grant (Vot No. 9449700).

REFERENCES

1. Chua HP, Nicholas D, Yahya MA. Physical properties and nutritional values of dabai fruit (Canarium odontophyllum) of different genotypes. Journal of Tropical Agriculture and Food Science. 2015;43(1):1-0.
2. Azrina A, Amin I, Muhammad I. Proximate composition and characterization of fat in Canarium odontophyllum. InProceedings of the 10th Asean Food Conference 2007 Aug.
3. Mokiran NN, Ismail A, Azlan A, Hamid M, Hassan FA. Effect of dabai (Canarium odontophyllum) fruit extract on biochemical parameters of induced obese–diabetic rats. journal of functional foods. 2014 May 1;8:139-49.
4. Chua HP, Daniel N. Malaysian Agricultural Research and Development Institute. Product Development from Dabai Fruit [serial online]. MARDI publication 5(2014): 51 - 61. Available from: http://ebuletin.mardi.gov.my/buletin/05/Dabai.pdf
5. Rahman MN, Thajudin NA. Product development of Kembayau (Canarium odontophyllum) exotic fruit sauce. Journal of Tropical Resources and Sustainable Science. 2015;3(1):19-28.
6. Running CA, Craig BA, Mattes RD. Oleogustus: the unique taste of fat. Chemical senses. 2015 Jul 3;40(7):507-16.
7. Chew LY, Khoo HE, Amin I, Azrina A, Lau CY. Analysis of phenolic compounds of dabai (Canarium odontophyllum Miq.) fruits by high-performance liquid chromatography. Food Analytical Methods. 2012 Feb 1;5(1):126-37.
8. Kadir NA, Azlan A. Dietary Fatty Acids and Edible Fruit Oil: Potential Therapy for Heart. Pertanika Journal of Scholarly Research Reviews. 2017;3(2).
9. Shakirin FH, Azlan A, Ismail A, Amom Z, Yuon LC. Antiatherosclerotic effect of Canarium odontophyllum Miq. fruit parts in rabbits fed high cholesterol diet. Evidence-Based Complementary and Alternative Medicine. 2012;2012.
10. Azlan A, Prasad KN, Khoo HE, Abdul-Aziz N, Mohamad A, Ismail A, Amom Z. Comparison of fatty acids, vitamin E and physicochemical properties of Canarium odontophyllum Miq.(dabai), olive and palm oils. Journal of Food Composition and Analysis. 2010 Dec 1;23(8):772-6.
11. Azmir J, Zaidul IS, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MH, Ghafoor K, Norulaini NA, Omar AK. Techniques for extraction of bioactive compounds from plant materials: a review. Journal of Food Engineering. 2013 Aug 1;117(4):426-36.
12. Shakirin FH, Prasad KN, Ismail A, Yuon LC, Azlan A. Antioxidant capacity of underutilized Malaysian Canarium odontophyllum (dabai) Miq. fruit.

Journal of Food Composition and Analysis. 2010 Dec 1;23(8):777-81.
13. Hurtado-Benavides AM, Secorбns FJ, Ibбcez E, Reglero G. Countercurrent packed column supercritical CO2 extraction of olive oil. Mass transfer evaluation. The Journal of supercritical fluids. 2004 Jan 1;28(1):29-35.
14. Halim SZ, Abdullah NR, Afzan A, Rashid BA, Jantan I, Ismail Z. Acute toxicity study of Carica papaya leaf extract in Sprague Dawley rats. Journal of Medicinal Plants Research. 2011 May 18;5(10):1867-72.
15. Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure. OECD Guidelines for the Testing of Chemicals, Section 4. 2002.
16. Prasad KN, Chew LY, Khoo HE, Kong KW, Azlan A, Ismail A. Antioxidant capacities of peel, pulp, and seed fractions of Canarium odontophyllum Miq. fruit. BioMed Research International. 2010 Sep 20;2010.
17. Khoo HE, Azlan A, Ismail A, Abas F. Influence of different extraction media on phenolic contents and antioxidant capacity of defatted dabai (Canarium odontophyllum) fruit. Food Analytical Methods. 2012 Jun 1;5(3):339-50.
18. Kate AE, Singh A, Shahi NC, Prakash O, Singh TP. Novel eco-friendly techniques for extraction of food based lipophilic compounds from biological materials. Natural Products Chemistry & Research. 2016 Jul 7.
19. Legal Research Board, Food Act 1983 and Food Regulations 1985, International Law Book Services, Kuala Lumpur, Malaysia, 2008.
20. Arenas EH, Trinidad TP. Heavy metal and microbiological profiles of defatted pili (Canarium ovatum, Engl.) pulp meal residue and acute oral toxicity of its ethanolic extract in mice. International Food Research Journal. 2017 Aug 1;24(4).
21. Nagao T, Shimada Y. Microbial production of rare unsaturated fatty acids and alcohols. Lipid Technology. 2010 Nov;22(11):250-2.
22. Dhanavathy G, Jayakumar S. Acute and Subchronic Toxicity Studies of Swertiamarin A lead Compound Isolated from Enicostemma Littorale. blume in wistar rats. Biosciences Biotechnology Research Asia. 2017 Mar 25;14(1):381-90.
23. Yuet Ping K, Darah I, Chen Y, Sreeramanan S, Sasidharan S. Acute and subchronic toxicity study of Euphorbia hirta L. methanol extract in rats. BioMed research international. 2013;2013
24. National Research Council (NRC), "Toxicity testing for assessing environmental agents," Interim Report, National Academies Press., Washington, DC, USA, 2006.
25. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, Johnson JK, Schafer K. Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. Toxicologic pathology. 2007 Aug;35(5):742-50.