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In vivo Toxicity Assessment of Refined Red Palm-pressed Mesocarp Olein in Sprague-Dawley Rats

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Abstract: Refined red palm-pressed mesocarp olein (PPMO) is recovered from palm-pressed mesocarp fiber, which is a by-product from palm oil mill. Its utilization in food industry is extremely limited even though it contains various phytonutrients. Thus, this study aimed to evaluate its toxicity effects by using the male Sprague-Dawley rat model. The rats were administered with a single dose of 2 g/kg PPMO in an acute toxicity study while administered with 2, 1, or 0.5 g/kg PPMO daily for 28 days in a sub-chronic toxicity study. The mortality, oral LD_{50} value, clinical observation, body and organ weight, hematological and biochemical analyses, pathological and histopathological examinations were assessed. The overall outcomes indicated that PPMO is non-toxic up to 2 g/kg and considered safe to be used in food application, especially as functional food ingredient and supplement attributed to its phytonutrients. Besides, this study provides an insight in alternative utilization of the wastes from palm oil mill.

Key words: biochemical analysis, hematological analysis, histopathological examination, lethal dose, palm oil mill waste

1 Introduction

Oil palm is found abundantly in Indonesia and Malaysia where both countries supply approximately 85% of palm oil in the world[1]. The crude palm oil can be extracted from mesocarp fruit of oil palm and the palm-pressed mesocarp is a by-product of crude palm oil extraction process[2,3]. The crude palm-pressed mesocarp oil is obtained from screw-pressing process in the mill and typically comprised of 5-7% residual oil in palm-pressed mesocarp[2,3]. This residual oil could be refined to eliminate the undesirable components and achieve better quality mesocarp oil, subsequently fractionated to obtain refined red palm-pressed mesocarp olein (PPMO)[4]. Figure 1 illustrated the production flow of PPMO in an oil palm mill.

The chemical composition analyses show that PPMO contains minimal undesirable components[4-6]. On the contrary, it has been found to contains various types of phytonutrients, including vitamin E, carotenoids, tocopherol, tocotrienols, squalene, phytosterol and coenzyme Q10[4-6]. However, its applications are limited to non-food industry such as cosmeceutical products, supplementary ingredients for animal feed formulation and alternative carrier oil in personal care products[5,7]. The commercial oils such as perilla (*Perilla frutescens*) seed oil[8], fermented virgin coconut (*Cocos nucifera*) oil[9], grugru palm (*Acrocomia aculeata*) pulp oil[10], urucuri palm (*Attalea phalerata*) fruit oil[11] and pequi (*Caryocar brasiliense*) fruit oil[12] were assessed for their food safety properties in terms of toxicities by using animal models. There is no previous report on the safety usage of PPMO in terms of food application, thus this study aimed to carry out toxicological assessment of PPMO through animal model.

The assessment and evaluation of toxic characteristics of an oral substance is usually measured by sub-chronic toxicity after obtaining the preliminary toxicity data from acute toxicity study in an animal model[13-15]. Acute toxicity describes the adverse effects of a substance arising from single doses within a brief period, typically less than 24 hours. The adverse effects are likely to occur within 14 days of administration of the substance[16]. On the other hand, sub-chronic toxicity aims to investigate the adverse effects of possible health hazards that are likely to result from repetitive exposure of orally administrated substance over a limited period of time[14]. Previous studies on the acute and sub-chronic toxicity of edible oils such as perilla seed oil and fermented virgin coconut oil were assessed through body weight, clinical observation, mortality, lethal dose, hematological parameters, biochemical parameters,

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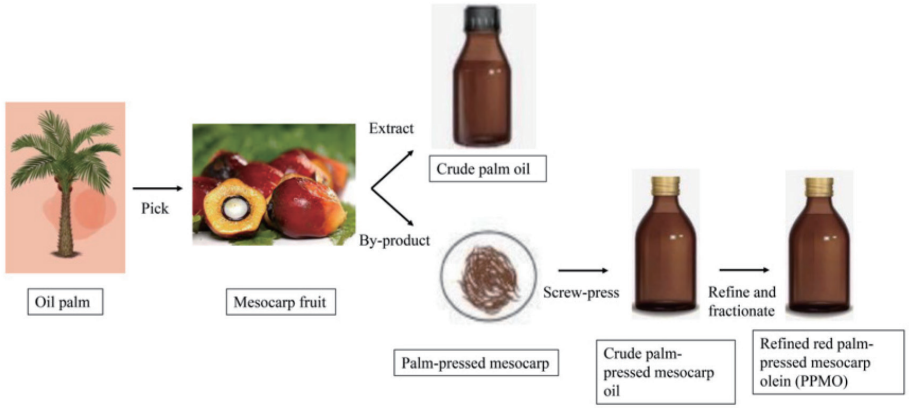

Fig. 1 The production process of refined red palm-pressed mesocarp olein (PPMO).

pathological indicators, organ weight and histopathological indicators of the animals[8, 9]. This study reported the thresholds of PPMO in terms of animal exposure and safety by conducting the acute and sub-chronic toxicity studies of PPMO in Sprague-Dawley rats. The outcomes of this study unveiled the potential applications of PPMO in the food industry.

2 Materials and Methods

2.1 Preparation of refined red palm-pressed mesocarp olein (PPMO)

The crude palm-pressed mesocarp oil was obtained from a local palm oil mill in July 2020. The oil was then refined and fractionated into PPMO according to methods reported by Nur Sulihatimarsyila (2019) using a combination of processes including degumming, bleaching, and deacidification[5]. PPMO, a reddish yellow oil with high oxidation stability, low levels of free fatty acids and food contaminants[4], was obtained and stored at a temperature of −4℃.

2.2 Animal study

The animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of University Putra Malaysia (UPM) with a reference number of UPM/IACUC/AUP-R009/2020. The study was carried out in accordance with National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). Male Sprague-Dawley rats were purchased from UPM. A total of ten 5-week-old rats with the weight range of 180 - 220 g were used in the acute toxicity study while twenty 5-week-old rats with the weight range of 160 - 275 g were used in the sub-chronic toxicity study. All the rats were acclimatized to laboratory conditions for 7 days before the experiments. The rats had free access to normal diet (Altromin 1324; Altromin, Germany) and ultra-sterile water *ad libitum*. The standard form of the normal diet was pellet squared (10 mm) and its composition was 11.3% moisture, 5.9% crude ash, 6.1% crude fibre, 4.1% crude fat, 19.2% crude protein and 53.4% nitrogen-free extractives. All the rats were kept in an environmentally controlled breeding room with 22 ± 4℃ temperature, 60 ± 20% relative humidity and 12 h light/ 12 h dark cycle throughout the study in the Comparative Medicine and Technology Unit (COMeT) of UPM.

2.3 Acute toxicity study

The toxicological properties of PPMO were investigated through a 14-day, single-dose, oral acute toxicity study, in compliance with the No. 423 OECD (Organization for Economic Cooperation and Development) Guidelines[15]. Ten Sprague-Dawley rats were randomly divided into 2 groups, which are treatment group and control group with n = 5 rats per group. The oral gavage was given once to the rats in the treatment group with a dosage of 2 g/kg (body weight) of PPMO on the first day of acute toxicity study while the control group rats were given 2 g/kg (body weight) of water. The rats were monitored closely after the gavage administration (30 min, 4 h and 14 days) for clinical signs of toxicity and mortality. The body weights of the rats were recorded daily by using an electronic balance (FX-1200, A & D, Japan). At the end of 14 days, LD_{50} value was obtained according to the mortality. The rats were then euthanized through an intraperitoneal injection of an overdose of ketamine (Narketan™ 10, Germany) (0.1 g/kg) and xylazine (Ilium-Xylazil-100, Troy Laboratories PTY LTD, Australia) (0.01 g/kg). After that, the necropsy was conducted to classify the gross pathological alterations of main organs, including eyes, stomach, intestine, cecum, rectum, reproductive, urinary, heart, kidney, lung, liver, and spleen.

2.4 Sub-chronic toxicity study

2.4.1 Administration procedure

The sub-chronic toxicity study of PPMO was assessed according to No. 407 OECD Guidelines[14]. Twenty male Sprague-Dawley rats were randomly divided into three treatment groups and a control group with five rats in each

group. The rats in the treatment groups were orally administrated with PPMO by gavage daily for 28 days at the doses of 2, 1, 0.5 g/kg/day (body weight) while the rats in the control group were administered with the water in the same volume as PPMO. At the end of the 28-day experiment, 6 mL of blood was drawn from all the rats by cardiac puncture after anesthesia by using ketamine (0.1 g/kg) and xylazine (0.01 g/kg). The blood samples were transferred into vacuum blood collection tubes containing ethylenediaminetetraacetic acid (EDTA) and non-EDTA for hematological and biochemical analyses, respectively. All the rats were then euthanized by intravenous injection of an overdose of ketamine (0.1 g/kg) and xylazine (0.01 g/kg). The carcasses were used for subsequent pathological and histopathological examinations.

2.4.2 Body weight

During the 28 days of treatment, the body weight of each rat was measured once a week. The body weight gain rate was calculated by using the formulae below.

(Final body weight - Initial body weight)/ Initial body weight × 100%

2.4.3 Mortality and clinical observations

The mortality and clinical signs of toxicity of the rats were observed twice a day, morning and afternoon. The observation indicators included body surface characteristic changes in the skin, fur, eyes and mucous membranes, behavioral changes in posture, mobility, responsiveness, appetite, breathing and resting, as well as possible toxic manifestations such as tremor, convulsions, salivation, and diarrhea.

2.4.4. Hematological analysis

The hematological analysis was conducted at the end of sub-chronic toxicity study by using the blood samples collected from the rats. The platelet (PLT), white blood cell (WBC), red blood cell (RBC), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (HGB) and mean corpuscular volume (MCV) levels in blood were evaluated using a hematology analyzer (ADVIA-2120i, Siemens AG, Germany). The packed cell volume (PCV) and plasma protein levels in blood were measured by micro-hematocrit reader (L-550, Phillips-Drucker, United States) and refractometer (10440, American Optical, United States), respectively. The WBC differential count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) of blood was counted by using Neubauer counting chamber under a microscope (Eclipse Ci-E; Nikon, Japan).

2.4.5 Biochemical analysis

Biochemical analysis was performed at the end of subchronic toxicity study by using the serum of blood samples collected from the rats. The alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (ALB), glucose, urea and creatinine levels in serum were assessed using the automated clinical analyzer (Biolis-24i, Premium, Japan). The level of electrolytes, including potassium (K), sodium (Na) and chlorine (Cl) levels in serum were measured by an automated clinical chemistry analyzer (Dimension Xpand Plus, Siemens, United States).

2.4.6 Pathological examination

A complete and thorough gross necropsy was performed on all the rats at the end of sub-chronic toxicity study, which included close inspection of the body's organs, including eyes, stomach, intestine, cecum, rectum, reproductive, urinary, heart, lung, liver, and spleen.

2.4.7 Relative organ weight

The organs, including liver, kidney, spleen, lung and heart of all rats were excised and weighed immediately after dissection and pathological examination to avoid drying. Relative organ weight was determined as below[20].

(Internal organ weight / Final body weight) × 100

2.4.8 Histopathological examination

For the histological processing, the liver and spleen of rats were collected and fixed in 10% neutral-buffered formalin. The fixed tissues were embedded in paraffin blocks by using a paraffin-embedding machine (EG1150C, Leica, Germany). The tissue paraffin blocks were made into sections range of 4 – 8 microns by using a microtome (RM2045, Leica, Germany). The tissue sections were fixed manually on glass microscope slides in a tissue floatation bath (TFB-L-220, General Data Company, United States). Finally, the tissue slides were dehydrated and stained by hematoxylin (H9627, Sigma-Aldrich, United States) and eosin (E4009, Sigma-Aldrich, United States) in an automatic vacuum tissue processor (TP1020, Leica, Germany). Under an optical biological microscope (Eclipse 80i, Nikon, Japan) coupled with a digital camera (DS-Fi1C, Nikon, Japan), the staining tissue sections were examined for histopathological changes. The histopathological analysis involved observing tissue integrity and signs of toxic injury, including degeneration, necrosis, apoptosis, and leukocyte infiltration.

2.5 Statistical analysis

The results were reported as mean value ± standard deviation (SD). The differences between the control group and treatment groups were analyzed using one-way analysis of variance (ANOVA) by SPSS (IBM, 22.0). The p-value of <0.05 was considered statistically significant.

3 Results

3.1 Acute toxicity study of the rats treated with PPMO

3.1.1 Normal body weight gain of the rats

All the rats revealed a reasonable increase in body weight without any significant difference ($p>0.05$) between the control and PPMO treatment group (2 g/kg) throughout 14 days of study (Fig. 2).

3.1.2 No acute mortality of rats

No death phenomenon was observed for the rats in both control and treatment groups throughout the acute toxicity study.

3.1.3 No clinical abnormality of the rats

The rats in both control and treatment groups did not show any irregular behavioral changes associated with toxic signs throughout the 14 days of study.

3.1.4 No pathological changes in the rats

None of the rats revealed gross changes in any body's external surface, orifice, cranial, thoracic, abdominal cavity, eyes, stomach, intestine, cecum, rectum, reproductive, urinary, heart, lung, liver, and spleen in both treatment and control groups.

3.2 Sub-chronic toxicity study of the rats treated with PPMO

3.2.1 Normal body weight gain of the rats

All the rats in control and PPMO treatment (2, 1 and 0.5 g/kg) groups exhibited reasonable body weight increments throughout 28-day of study, as shown in Fig. 3. The rats in control and PPMO treatment groups have shown significant increase in body weighs only from week 0 to week 1. In addition, the body weight of rats fed with 2 and 0.5 g/kg PPMO had increased significantly from week 1 to week 3, continuously. The results showed that the rats are gaining reasonable weighs throughout the study by weeks. Nevertheless, Fig. 4 showed that no significant difference was observed for the body weight gain rate between the control and PPMO treatment groups throughout the study.

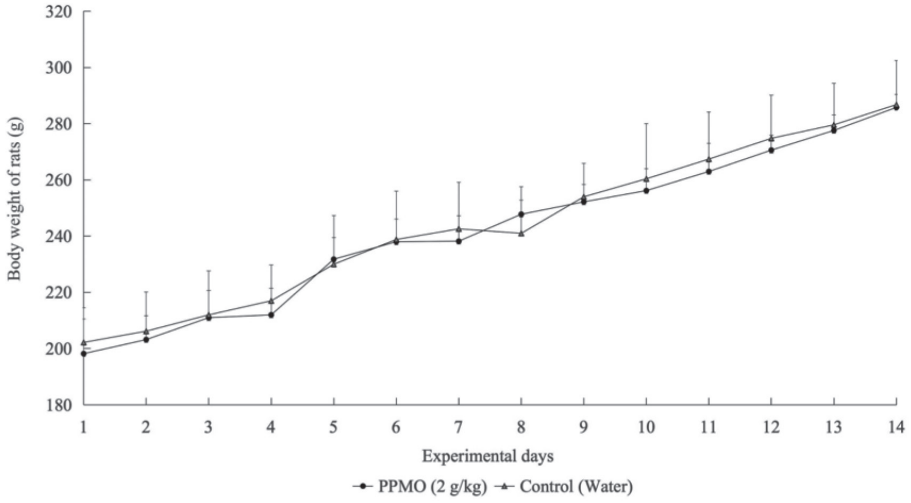

Fig. 2 The body weight of rats with refined red palm-pressed mesocarp olein (PPMO) treatment (2 g/kg) and control during the 14 days of acute toxicity study. For groups of 5 rats, data are means ± SD.

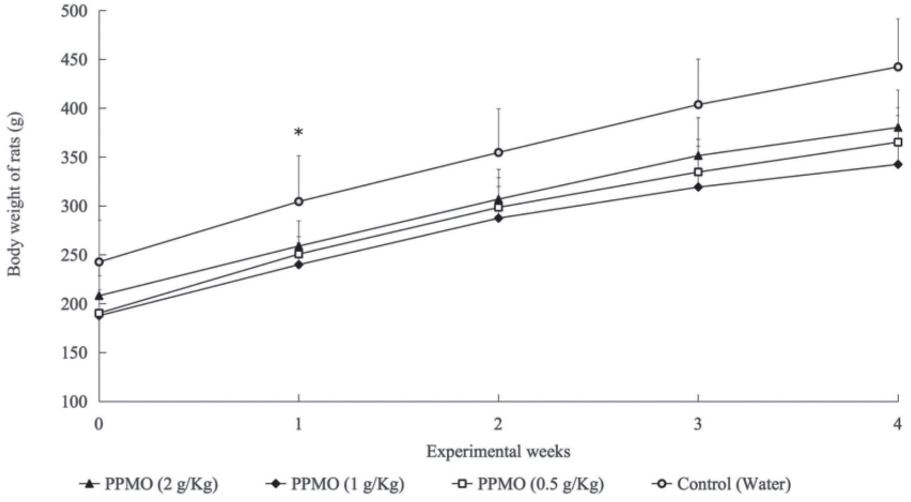

Fig. 3 Body weight of rats during the 28 days of sub-chronic toxicity study for refined red palm-pressed mesocarp olein (PPMO). For groups of 5 rats, data are means ± SD. * denote a significant difference from immediate past week within each individual group ($p < 0.05$).

3.2.2 No sub-chronic mortality and clinical abnormality of the rats

There was no mortality of rats observed in all the PPMO treatment groups and control group. Similarly, there was no abnormalities and no noticeable differences in behavior being recorded.

3.2.3 Normal range of hematological parameters in the rats

For the sub-chronic toxicity study, there was no significant difference found between PPMO treatment groups and control group in terms of the levels of plasma protein, RBC, MCHC, HGB, band neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils and basophils. The values obtained for these hematological parameters from the rats in PPMO treatment groups also fall within the reference ranges, as seen in the Table 1[9, 17–20]. Although the levels of PLT, PCV, MCV and WBC in all the PPMO treatment groups were significantly different ($p < 0.05$) from that of the control group, these levels are still within the reference ranges[9, 17].

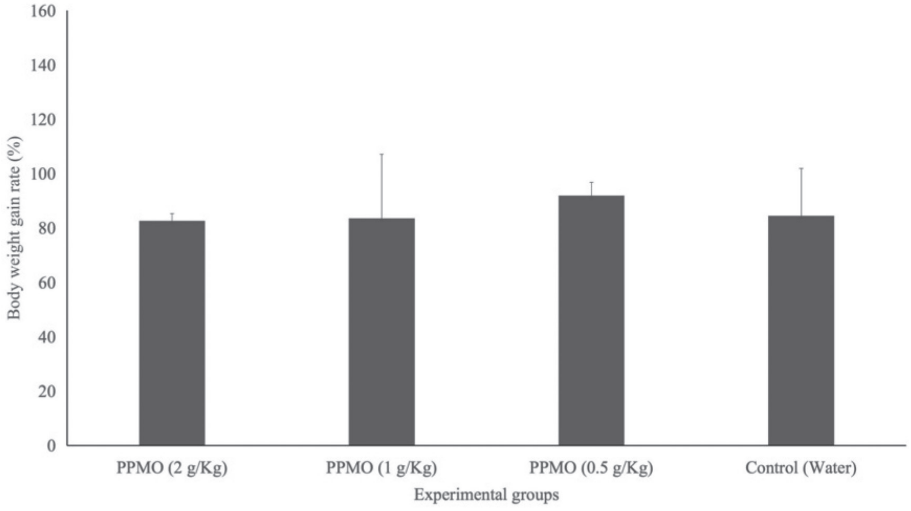

Fig. 4 Body weight gain rate of rats during the 28 days of sub-chronic toxicity study for refined red palm-pressed mesocarp olein (PPMO). For groups of 5 rats, data are means ± SD. No significant differences ($p < 0.05$) were observed between control and PPMO treatment groups.

Table 1 Hematological parameters of rats in sub-chronic toxicity study.

Parameters	Unit	Experimental groups (PPMO)				Reference range	Reference
		2 g/kg	1 g/kg	0.5 g/kg	Control (water)		
Plasma proteins	g/L	64.80 ± 3.63	64.00 ± 3.74	62.40 ± 2.19	65.20 ± 1.10	60.00 - 80.00	18
PLT	$x10^9$/L	783.00 ± 95.08	786.40 ± 66.81	721.20 ± 39.86*	877.00 ± 91.34	574.00 - 1253.00	9
RBC	$x10^{12}$/L	8.20 ± 0.17	8.27 ± 0.33	8.45 ± 0.35	8.29 ± 0.34	7.20 - 9.20	9
MCHC	g/dL	32.26 ± 0.92	32.14 ± 0.28	31.84 ± 0.18	32.29 ± 0.19	25.41 - 80.55	17
HGB	g/L	153.40 ± 4.72	149.80 ± 3.70	152.80 ± 6.98	146.60 ± 5.59	137.00 - 172.00	9
PCV	L/L	0.48 ± 0.03	0.47 ± 0.01	0.48 ± 0.02*	0.45 ± 0.02	0.40 - 0.50	9
MCV	fL	57.99 ± 1.98*	56.42 ± 1.60	56.81 ± 1.58	54.79 ± 1.52	29.41 - 123.07	17
WBC	10^9/L	9.05 ± 1.18*	10.28 ± 1.46*	10.30 ± 1.30*	15.02 ± 1.38	1.98 - 11.06	9
Band neutrophils	%	1 ± 0	1 ± 0	1 ± 0	1 ± 0	0 - 5	20
Segmented neutrophils	%	15.20 ± 1.10	13.60 ± 1.67	14.00 ± 2.38	15.60 ± 0.89	6.14 - 22.95	19
Lymphocytes	%	77.80 ± 1.30	79.20 ± 2.39	79.40 ± 3.91	75.80 ± 1.64	69.68 - 86.89	19
Monocytes	%	5.00 ± 0.71	5.20 ± 0.84	4.80 ± 0.84	6.80 ± 0.84	3.77 - 10.82	19
Eosinophils	%	1.00 ± 0.71	1.00 ± 0.00	0.80 ± 0.84	0.80 ± 1.30	0.54 - 3.39	19
Basophils	%	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 - 1	17

PPMO: Refined red palm-pressed mesocarp olein; PLT: Platelet; RBC: Red blood cell; MCHC: Mean corpuscular hemoglobin concentration; HGB: Hemoglobin; PCV: Packed cell volume; MCV: Mean corpuscular volume; WBC: White blood cell. For groups of 5 rats, data are means ± SD. * denotes significant difference between control and PPMO treatment groups ($p < 0.05$).

3.2.4 Normal range of biochemical parameters in the rats

There was no significant difference found for the serum Na, K, Cl, urea, ALT, ALB and glucose levels between the PPMO treatment and control groups for the sub-chronic toxicity study (Table 2). Even though significant difference was found in between the control group and PPMO treatment groups for the serum creatinine, AST and TP levels, the detected levels are within the reference ranges[9, 17, 19].

3.2.5 No pathological changes in the rats

There was no major variations or toxic changes observed on the external surface, orifice, cranial, thoracic, abdominal cavities, eyes, stomach, intestine, cecum, rectum, reproductive, urinary, heart, kidney, lung, liver, and spleen of all the rats during the pathological examination.

3.2.6 Normal relative organ weight of the rats

The results of relative organ weight is similar to that of pathological examination, no significant difference was found between the PPMO treatment groups and control group in the relative organ weight, including heart, lung, liver, spleen, and kidney (Table 3).

3.2.7 No histopathological abnormality in the rats

Histopathological examination of the livers revealed that the hepatic lobules showed irregular polygons and the hepatic plates were clearly visible in both the PPMO treatment and control groups. The bile ducts, hepatic arterioles and portal venules were visible in the portal area of liver without obvious fibrous tissue hyperplasia and inflammatory cell infiltration in all the rats, as shown in Fig. 5. Besides that, histopathological examination of the spleens collected from all the rats showed that the capsule was intact, marginal sinus was orderly, splenic trabecular structure was intact, and the ratio of red and white pulp was normal (Fig. 5).

4 Discussion

According to Dietary Guidelines for Americans (2015-2020), the recommended intake of edible oil of humans is 25 g/day[21]. Thus, a corresponding dose conversion from humans to rats was done to determine the dosage of PPMO

Table 2 Biochemical parameters of rats in sub-chronic toxicity study.

Parameters	Unit	Experimental groups (PPMO)				Reference range	Reference
		2 g/kg	1 g/kg	0.5 g/kg	Control (water)		
Na	mmol/L	143.20 ± 1.64	141.60 ± 1.14	142.40 ± 0.89	141.40 ± 2.30	135.00 - 145.00	49
K	mmol/L	5.14 ± 0.27	5.40 ± 0.39	4.88 ± 0.20	5.00 ± 0.46	3.80 - 6.11	9
Cl	mmol/L	100.20 ± 1.92	101.20 ± 1.3	99.20 ± 1.3	99.00 ± 2.45	98.00 - 106.00	9
Urea	mmol/L	6.86 ± 0.61	6.78 ± 0.36	6.46 ± 0.66	6.32 ± 0.36	4.32 - 8.97	19
Creatinine	μmol/L	38.70 ± 0.91*	40.14 ± 2.05	39.64 ± 3.03*	42.74 ± 2.19	32.36 - 47.90	19
ALT	U/L	50.70 ± 9.59	51.62 ± 3.45	50.54 ± 6.03	49.28 ± 10.09	24.00 - 172.00	9
AST	U/L	174.74 ± 34.76*	170.76 ± 46.25*	147.58 ± 14.10	121.46 ± 6.58	0.20 - 838.30	17
ALB	g/L	31.60 ± 0.64	31.42 ± 0.58	31.82 ± 0.50	31.12 ± 0.96	26.85 - 34.55	19
TP	g/L	72.68 ± 1.62*	72.70 ± 2.58*	71.89 ± 3.36*	77.82 ± 1.32	56.00 - 76.00	9
Glucose	mmol/L	10.48 ± 3.97	11.68 ± 1.86	11.86 ± 2.16	11.90 ± 1.86	3.89 - 11.56	9

PPMO: Refined red palm-pressed mesocarp olein; K: Potassium; Na: Sodium; Cl: Chlorine; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALB: Albumin; TP: Total protein. For groups of 5 rats, data are means ± SD. * denotes significant difference between control and PPMO treatment groups ($p < 0.05$).

Table 3 Relative organ weight of the rats in sub-chronic toxicity study.

Relative organ weight (g)	Experimental groups (PPMO)			
	2 g/kg	1 g/kg	0.5 g/kg	Control (water)
Heart	0.39 ± 0.05	0.40 ± 0.05	0.39 ± 0.03	0.39 ± 0.04
Lung	0.48 ± 0.02	0.50 ± 0.05	0.53 ± 0.09	0.51 ± 0.01
Liver	3.83 ± 0.55	3.79 ± 0.31	3.92 ± 0.28	4.04 ± 0.22
Spleen	0.26 ± 0.03	0.28 ± 0.03	0.26 ± 0.03	0.24 ± 0.02
Kidney	0.81 ± 0.15	0.80 ± 0.02	0.79 ± 0.09	0.78 ± 0.03

PPMO: Refined red palm-pressed mesocarp. For groups of 5 rats, data are means ± SD. No significant differences ($p < 0.05$) were observed between control and PPMO treatment groups.

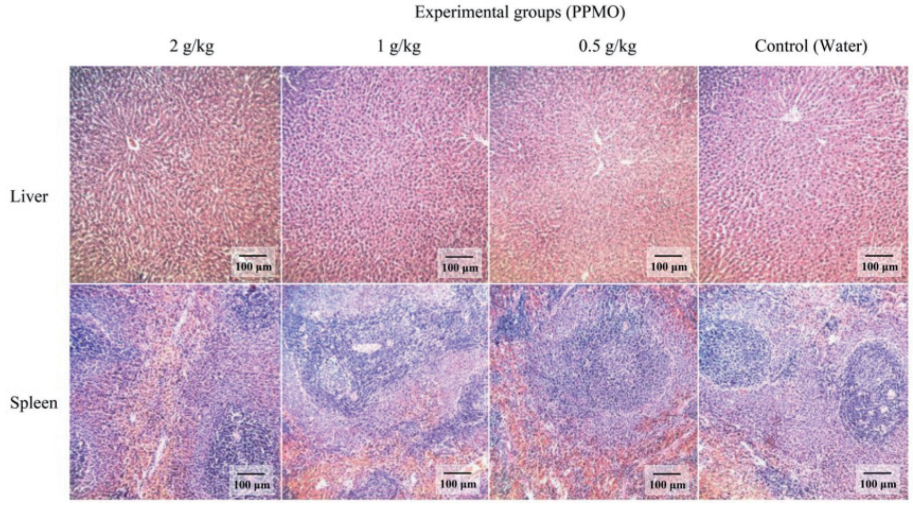

Fig. 5 Histopathological examination diagram of liver and spleen collected from the rats after completion of sub-chronic toxicity study at 100 × magnification. PPMO: Refined red palm-pressed mesocarp olein.

in rats[22]. A dose of 2 g/kg of body weight was used in acute toxicity study and it falls within the recommended dose in No. 425 OECD Guidelines[15]. The toxicity indicators, including body weight, mortality, clinical observation and pathological examination are commonly used in the *in vivo* acute toxicity studies[9, 23, 24]. On the other hand, the sub-chronic toxicity was conducted and the final doses used in the Sprague-Dawley rats were 2, 1 and 0.5 g/kg, based on the maximal dose of PPMO (2 g/kg) obtained in the acute toxicity study, in addition to No. 407 OECD Guidelines[14]. The common indexes used in the evaluation of sub-chronic toxicity study are body weight, relative organ weight, mortality, clinical observation, hematological analysis, biochemical analysis, pathological examination and histopathological examination[25].

Early symptoms and markers of toxicity and allergic effects are often associated with the changes in body weight where a loss in weight indicates side effects or adverse reactions of a substance[26]. A weight loss of more than 10% postulates the possibility of severe toxicity of the substance[27]. The increased body weight of the rats in both PPMO treatment and control groups throughout the experimental period of acute and sub-chronic toxicity studies showed that the likelihood of the rats to the exposure of potentially toxic substances is low[24]. No significant change in the body weight gain rate of rats was observed in the treatment and control groups, implying that an oral administration of PPMO up to 2 g/kg had no toxicity effect on the growth and development of rats. The outcomes were in line with the literature data which focused on the quality assessment of PPMO where none of the contaminants included gum, waxes, trace metals and free fatty acids (FFA) were detected[4]. The sustaining body weight gain of rats is likely to be attributed to the removal of contaminants during the PPMO refine process, resulting in a good source of oil for food applications and dietary nutritional supplements.

The LD_{50} value is a single lethal dose that kills half of the number of animals and has become a widely recognized basis for the comparison and classification of toxic samples, including new drugs, food additives, cosmetic ingredients, household products, industrial chemicals and pesticides[15, 28]. It allows a substance to be ranked and classified according to the Globally Harmonised System[15, 28]. In both acute and sub-chronic toxicity studies, no rat was found to be dead until the termination, illustrating that the LD_{50} value of PPMO is higher than the dose of 2 g/kg in Sprague-Dawley rats. The LD_{50} values reported for the plant fruit oils, such as grugru palm pulp oil[10], urucuri palm fruit oil[11] and pequi fruit oil[12] were also greater than 2 g/kg. Furthermore, based on the requirements of the Guideline on Acute Oral Toxicity Testing, an LD_{50} value of 2 g/kg is graded as a classification No. 5, which is the lowest toxicity level[15]. Thus, PPMO can be considered as a safe oral substance since its oral LD_{50} is greater than 2 g/kg. Moreover, any altered clinical observation is considered as an early sign of toxicity and adverse reactions of the toxic substances[26]. The clinical observation results revealed that the rats in the PPMO treatment groups did not produce any alteration in the appearance and behavioral patterns of the rats when compared to the control group, in both acute and sub-chronic toxicity studies. The outcomes of the toxicity study on PPMO are comparable to the commercial cooking oils such as perilla seed oil and fermented virgin coconut oil, which did not show clinical abnormality on the appearance and behavior of rats[8, 9].

The hematopoietic system is highly sensitive to poisons and thus having a high predictive value for toxicity evaluation on the substances administered into human and animal[29, 30]. Irregular hematological parameters are known

as substance-induced tissue injury and widely used as the diagnostic predictor in the hospital[19, 21]. Plasma protein presents in the blood plasma to maintain the colloidal osmotic pressure[31]. The detected plasma protein levels of the rats indicated that none of the rat administered with PPMO showed the plasma osmotic pressure alteration in sub-chronic toxicity study. PLT is a component of blood that reacts to bleeding from blood vessel injury by clumping, thereby initiating a blood clot[32]. The detected PLT levels suggested that the rat administrated with PPMO did not alter the blood coagulation function in sub-chronic toxicity study. RBC and its related indicators (MCHC, HGB, PCV and MCV) can diagnose anemia and other related conditions of hematopoietic function[33]. The levels of RBC, MCHC, HGB, PCV and MCV indicated that PPMO does not affect the development of blood cells in the rats up to the dose of 2 g/kg. On the other hand, WBC is comprised of band neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils and basophils[34]. An increase in WBC count may be associated with allergic reactions, infections and cancer[35]. The levels of WBC, band neutrophils, segmented neutrophils, lymphocytes, monocytes, eosinophils and basophils in the hematology analysis showed that PPMO did not cause allergic reactions or infection to the rats in sub-chronic toxicity study. These results were similar to those commercial cooking oils such as perilla seed oil and fermented virgin coconut oil in terms of non-toxicity effects, suggesting that PPMO is safe to be used as an edible oil[8, 9].

Serum biochemical analysis is the most widely used diagnostic index in clinical practice[25]. The electrolytes, Na, K and Cl are essential for maintaining the homeostasis of normal cells and organs[36]. The detected levels of Na, K and Cl indicated that PPMO did not alter the homeostasis of rats. Besides that, kidney is an important detoxifying organ and is very sensitive to toxic ingredients[37]. It can convert and produce huge quantity of toxins in the renal tubules because of the large amount of blood flowing to the kidneys[37]. The elevation of urea and creatinine indicates significant impairment of functional kidney[30]. The detected urea and creatinine levels indicated that PPMO did not alter the urea synthesis and kidney function. Another organ that has been used for the toxicity assessment is liver. In general, the liver injury and possible toxicity of the liver, particularly the damage of parenchymal liver cells, are measured using transaminases such as AST and ALT[38]. ALT is closely associated with liver function where an increase of ALT level in serum is considered as the first sign of liver damage[26, 39]. The detected serum ALT level suggested that PPMO did not alter the liver function. Furthermore, liver damage results in major elevations in the blood of AST[39]. The detected AST level revealed that PPMO did not cause any liver damage in sub-chronic toxicity study. Any deterioration in liver function may result in hepatocyte injury since liver is a major site of protein synthesis[40]. A decrease in serum ALB concentration is likely to be resulted from impaired hepatocellular activity or hepatocellular infection[40, 41]. The detected serum ALB level indicated that PPMO did not cause any impaired hepatocellular function of the rats in sub-chronic toxicity study. The liver is also important for glucose metabolism by converting glucose into liver glycogen for storage and regulation of the release of free glucose into the blood[30]. The detected serum glucose level showed that PPMO did not deteriorate the liver function of the rats. These results indicated that PPMO is safe to be used orally since the data obtained are similar to perilla seed oil and fermented virgin coconut oil, which are widely used as cooking oil where both of these oils and PPMO did not show toxicity effects in terms of homeostasis, kidney function and liver function[8, 9].

The ingestion of toxic substances usually damages organs[42]. Heart, liver, kidneys, spleen and lungs are the organs that will exhibit metabolic responses if toxic agents were administered into the body[43]. During pathological examination in the acute and sub-chronic toxicity studies, PPMO did not affect the appearance, morphological and size of the organ, revealing that no toxic metabolic responses were generated by PPMO. Organ weight is an extremely sensitive toxicity factor[24], thus significant variations in organ weight between the animals in the control and treatment groups indicate an exposure to toxic substance, although no morphological changes is observed in these organs[44]. Therefore, the relative organ weight to measure the organ-to-body weight ratio is used to diagnose any potential organ damage due to toxic substances[23, 45]. In the sub-chronic toxicity study, the PPMO treatment groups did not induce a change in relative organ weight of rats when compared to the control group, indicating that PPMO has no potential damage on the organ of rats.

Histopathological examination could further identify the variations in the composition of internal organ cells which are not reflected by the changes in hematological and biochemical analysis[46]. Liver damage can lead to several toxicological reactions, such as vomiting, diarrhea, weight loss and death[47]. Histopathological examinations of the liver indicated the PPMO with the dose up to 2 g/kg did not results in any liver damage in the sub-chronic toxicity study. Besides that, spleen is a biological target of direct and indirect toxicity and its damage is involved in many systemic diseases, particularly immune system[48]. The histological examination of the spleen could reveal the toxic effects of substances on the structure of visceral cells[48]. Histological examinations of the spleen indicated no toxicity effect was observed for the rats administered with PPMO in the sub-chronic toxicity study.

5 Conclusions

The present study revealed that no mortality of rats was seen in both acute and sub-chronic toxicity studies up to a dose of 2 g/kg PPMO. The LD_{50} value of PPMO was found to be greater than 2 g/kg which is classified as the lowest toxicity level, equivalent to classification No. 5 in the No. 425 OECD Guidelines. No obvious abnormality was observed in terms of appearance and behavior of all the rats, as well as no significant difference in body weight gain in both acute and sub-chronic toxicity studies. Hematological and biochemical analyses showed that an orally administered PPMO at the doses of 2, 1, 0.5 g/kg did not result in apparent effect on the hematopoietic system, electrolyte balance, kidney and liver of rats. Pathological and histopathology examinations revealed that the architecture of the internal organs did not change substantially in PPMO after 28 days of sub-chronic toxicity study. Thus, PPMO is considered as a safe edible oil with non-toxicity and could be used as food ingredient and supplement. The outcomes of this study provide a critical insight on the safety dose of PPMO for the food applications, as well as utilization of oil palm by-products for natural resources conservation. Further studies on the clinical toxicology of PPMO and its nutrition and therapeutic effectiveness are highly recommended.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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