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TOXICOLOGICAL ASSESSMENT OF REFINED PALM-PRESSED MESOCARP FIBRE OIL

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ABSTRACT

Refined palm-pressed mesocarp fibre oil (PMFO) is a rich source of phytonutrients, which merits further exploration as a functional food component. Current study aimed to evaluate the cytotoxicity and genotoxicity of refined PMFO using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and alkaline comet assay, respectively. Acute oral toxicity assessment was performed on two groups of rats using a stepwise procedure based on OECD 423. Animals were observed for clinical effects at 30 min, hourly for 4 and 6 hr post dosing and once daily up to day 14. There were no inhibitory concentration (IC_{50}) values observed for refined PMFO treated V79-4 Chinese hamster lung cells as compared to positive control hydrogen peroxide (H_2O_2), where a concentration response effect was seen following treatment with H_2O_2 with an IC_{50} value of 1.568 mM. Refined PMFO has no capability to induce direct deoxyribonucleic acid (DNA) strand breakage in V79-4 cells. In addition, refined PMFO showed no-observed-adverse-effect level (NOAEL) up to 2000 mg/kg body weight of the animal. Taken together, our study suggests that refined PMFO has great potential to be used in functional applications such as in food, dietary supplements and pharmaceutical products.

Keywords: acute oral toxicity, cytotoxicity, DNA damage, genotoxicity, palm-pressed mesocarp fibre oil.

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INTRODUCTION

Palm-pressed mesocarp fibre oil (PMFO) is a residual oil extracted from palm-pressed mesocarp fibre, a by-product obtained from palm oil milling process. Crude palm oil (CPO) is typically recovered from the mesocarp of palm fruits by dry method using hydraulic press or a screw press (Obibuzor *et al.*, 2012). Following this process, 5%-6% of oil still remains as a ratio to the dry matter of mesocarp fibre (Obibuzor *et al.*, 2012). This residual oil can be recovered by several methods *e.g.*, solvent extraction (Neoh *et al.*, 2011), supercritical carbon dioxide extraction (Lau *et al.*, 2006; Putra *et al.*, 2019), enzymatic reaction (Noorshamsiana *et al.*, 2017), soxhlet method (Neoh *et al.*, 2011; Putra *et al.*, 2019), reflux method (Neoh *et al.*, 2011) and residual oil recovery system (Subramaniam *et al.*, 2013). The valuable phytonutrients in the residual oil are urged to be recovered before subjecting the mesocarp fibre as energy source for palm oil mills (Choo *et al.*, 1996).

The PMFO is richer in phytonutrients as compared to CPO (Teh and Lau, 2021). Refined PMFO was extracted from pressed mesocarp using

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n-hexane while CPO was obtained by mechanical pressing of mesocarp. A significant quantity of carotenes (1716-2083 ppm), tocols (900-1200 ppm) (Abd Majid *et al.*, 2012), sterols (4509-8490 ppm) (Choo *et al.*, 1996) and squalene (1117-9690 ppm) (Lau *et al.*, 2008) remain in the residual oil of PMFO as compared to CPO. Inferior to PMFO, CPO contains 599-619 ppm of carotenes, 600-800 ppm of tocols (Abd Majid *et al.*, 2012), 250-620 ppm sterols (Choo *et al.*, 1996) and squalene (250-540 ppm) (Loganathan *et al.*, 2010). The tocols composition also differs between PMFO (54% of α-tocopherol, 19% of α-tocotrienol, 17% of γ-tocotrienol, and 10% of δ-tocotrienol) and CPO (21% of α-tocopherol, 25% of β-tocotrienol, 37% of γ-tocotrienol, and 17% of δ-tocotrienol) (Abd Majid *et al.*, 2012). Ninety percent of carotenoids found in CPO are in the form of α- and β-carotenes (35% and 56%, respectively) (Choo *et al.*, 1996). In the case of PMFO, α- and β-carotenes only constitute to 50% (19% and 31%, respectively). Interestingly, higher amounts of lycopene (14.10%), phytoene (11.90%), δ-carotene (7.60%), δ-carotene (6.90%), neurosporene (3.38%), and γ-carotene (2.70%) are found in PMFO as compared to CPO (Choo *et al.*, 1996). In terms of sterols, both PMFO and CPO have similar compositional profile of β-sitosterol (56%-59%), campesterol (19%-22%) and stigmasterol (18%-20%) (Choo *et al.*, 1996).

Both the PMFO and CPO contains predominantly palmitic acid (PMFO: 30.9%-32.6%, CPO: 39.5%-39.8%) and oleic acid (PPFO: 24.5%-25.1%, CPO: 35.6%-38.7%). PMFO is unique as it is rich in medium chain triglyceride namely lauric acid (20.0%-23.6%) which is trace in CPO (0.2%-0.3%) (Abd Majid *et al.*, 2012). The presence of lauric acid in PMFO is due to broken kernels trapped in mesocarp fibre (Abd Majid *et al.*, 2012; Sulihatimarsyila *et al.*, 2019).

To date, there is no scientific evidence on the safety assessment of PMFO nor refined PMFO. There is a recent publication on the blending of PMFO with CPO on oil quality and nutritional profile (Hasliyati *et al.*, 2021), yet toxicological aspects have not been investigated. Although chemically refined PMFO is proven to be safe for food application (Sulihatimarsyila *et al.*, 2019), however, there is no biological evidence to support this claim. Hence, there exists a necessity to study the toxicity aspects of refined PMFO to further exploit their functional applications in food, dietary supplements and pharmaceutical. Thus, the present study was designed to examine the safety assessment of refined PMFO based on its toxicological potential using both cell culture and animal acute study models. The terms of refined PMFO or PMFO are used throughout the manuscript to indicate the difference between refined PMFO and PMFO.

MATERIALS AND METHODS

Materials

The refined PMFO used in the current study was refined according to the published method as described by Sulihatimarsyila *et al.* (2019; 2020). The optimum conditions for the refining process comprised of water degumming using 5.0 v/v% of water at 90°C, followed by acid degumming using 1.0 weight % of phosphoric acid at 90°C, bleaching using 0.1 weight % of natural bleaching earth at 105°C and deacidification at 110°C at 0.1 mtorr (Lau *et al.*, 2006). The refined PMFO was supplied by Kim Loong Palm Oil Mills Sdn. Bhd., Johor, Malaysia.

Cell Line, Standards, Chemicals and Reagents

V79-4 Chinese hamster lung cells (*Cricetulus griseus* fibroblast, CCL-93TM) were purchased from the American Type Culture Collection (ATCC) (Montgomery, USA). Myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1); monopalmitin, monostearin, and monoolein; 1,2- and 1,3-dipalmitin, 1,2- and 1,3-distearin, and 1,2- and 1,3-diolein; tripalmitin and squalene; β-sitosterol, campesterol, stigmasterol, and cholesterol; low melting points agarose (LMA) and normal melting point agarose (NMA); acetone, uranyl acetate, hydrogen peroxide (H_2O_2), ethidium bromide solution and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent were obtained from Sigma Aldrich (Montgomery, USA). Dubelcco Modified Eagle Media (DMEM), fetal bovine serum (FBS), penicillin–streptomycin, glutaraldehyde, and phosphate buffer saline were purchased from Gibco- Thermo Fisher Scientific, Inc. (England, United Kingdom). The silylating reagent N,O-bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (BSTFA) was purchased from Fluka Chemicals (Buchs, Switzerland). All solvents were purchased from Merck and were of chromatographic or analytical grade. A TLC plate coated with silica gel 60 F254 (20 × 20 cm; Merck 1.05715.0001) was obtained from Merck (Darmstadt, Germany).

Cell Culture

The Chinese hamster lung V79-4 fibroblast cells were cultured in DMEM supplemented with 10% FBS and maintained at 37°C in a 5% carbon dioxide (CO_2) incubator. After reaching confluency, cells were detached via trypsinisation (0.025% trypsin).

Preparation of Compound and Evaluation of Physical Characteristics

Refined PMFO was first dissolved in dimethyl

sulphoxide (DMSO) at a concentration of 50%. The solution was further diluted in DMEM to 1%(v/v) of concentration as working solution. Total carotene content in the oil sample was analysed using a UV-Vis Spectrometer, model U-2001 (Hitachi Instruments Inc., Tokyo, Japan) according to MPOB Test Method p2.6:2004 (Ainie *et al.*, 2005). Free fatty acid (FFA), monoacylglycerols (MAG), diacylglycerols (DAG), triacylglycerols (TAG), sterols and squalene were determined by using a Hewlett-Packard Series II gas chromatography, model 5890 (Hewlett-Packard, Avondale, USA), as described previously (Nang Lau *et al.*, 2005). Vitamin E content was determined by high-performance liquid chromatography with fluorescence detector (Agilent Technologies, Palo Alto, USA) as described previously (Sulihatimarsyila *et al.*, 2019).

MTT Viability Assay

The cytotoxicity of refined PMFO was determined using MTT assay to assess the amount of formazan crystals formed via a reduction process in the mitochondria (Mosmann, 1983). The cells were seeded in 96-well microplate at 5x 10^4 cells/mL (based on established and published data in our laboratory) (Awang *et al.*, 2015; Siew *et al.*, 2020) and were incubated at 37°C in 5% CO_2 for 24 hr. Media was replaced with treatment medium containing highest concentration of refined PMFO (1%v/v) on the following day and the cells were incubated further for 24 hr. Then 30 µL of sterile MTT solution (5 mg/mL) was added into each well and the plate was incubated for 4 hr. Media and MTT solution were removed after 4 hr and 200 µL of DMSO was added into each well to dissolve the formazan crystals. The plate was shaken for 10 min and the optical density reading of each well was obtained using enzyme-linked immunoassay (ELISA) plate reader at 570 nm wavelength. Graph of viability was plotted against concentration.

Alkaline Comet Assay

The genotoxicity of refined PMFO was assessed using the alkaline comet assay by detecting the primary DNA damage (Tice *et al.*, 2000). The V79-4 cells were seeded in 6-well plate were treated with IC10 [0.162% (v/v)] and IC25 [0.450% (v/v)] following 24 hr of incubation. Cells were also treated with H_2O_2 (positive control, 1.0 mM) for 30 min. Then, the cells were detached, trypsinised and collected for centrifugation at 2500 rpm for 5 min. The supernatant was removed, and the pellet was washed with Ca^{2+}-/Mg^{2+}-free PBS prior to re-centrifugation. Pellets left at the bottom were mixed thoroughly with 80 mL of 0.6% LMA (w/v) and the mixture was pipetted onto hardened 0.6% NMA (w/v) on the slide. Cover slips were placed to spread the mixture and the slides were left on ice for LMA to solidify. Following the removal of the cover slips, the embedded cells were lysed in lysis buffer containing 2.5 M sodium chloride (NaCl), 100 mM Na_2EDTA, 10 mM Tris, and 1% Triton X-100 for 1 hr at 4°C. As for DNA-unwinding procedure, slides were soaked in electrophoresis buffer solution for 20 min at 4°C prior to electrophoresis at 300 mA, 25 V, for 20 min. Subsequently, the slides were rinsed with neutralising buffer for 5 min and stained with 50 µL ethidium bromide solution. Slides were left overnight at 4°C before analysis with fluorescence microscope (Nikon E600, USA) equipped with 515 barrier filter and 560 emission filter. Tail moment (TM) of 50 cells per slide were scored and analysed using COMET assay III (Perceptive Instruments, United Kingdom).

Acute Oral Toxicity Study

Acute oral toxicity study was appropriate for the purpose to determine the adverse toxic effect and responses of a test material following oral administration of the test material in rats within a short time period. Hence, the study was performed on two groups of rats using a stepwise procedure. In the first step, three female Sprague-Dawley rats (Test Group) were administered orally with 2000 mg/kg body weight of test substance in 10 mL/kg vehicle (pre-filtered water). Three additional female rats (Control Group) used as control were administered with pre-filtered water (10 mL/kg). Animals were observed for clinical effects at 30 min, hourly up to 4 hr, at 6 hr post dosing and once daily up to day 14. Their body weights were measured on day 1, 7 and at termination (day 14). Following euthanasia, gross necropsy was performed on both the Test Group and Control Group animals. Following the no-observable-adverse-effects-level (NOAEL) findings from the previously tested group (Test Group), three female rats (Continuing Test Group) were administered orally with 2000 mg/kg body weight of test substance in 10 mL/kg vehicle pre-filtered water. Animals were observed for clinical effects at 30 min, hourly up to 4 hr, and at 6 hr post dosing and once daily up to day 2. Their body weights were measured on day 1 and at termination (day 2). On day 2 the animals were sacrificed for necropsy. This study was performed in compliance with the appropriate provision of the OECD (2002), Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4. The procedures of use and care of animals were adhered to approval from Universiti Kebangsaan Malaysia's Animal Ethics Committee (BIOSERASI/UKM/2018/FAEZA/28-JUNE/931-JUNE-2018-JUNE-2019).

Statistical Analysis

All the data were presented as the mean ± standard error of mean (SEM) in at least three independent experiments. Statistical analyses using analysis of variance (ANOVA) was conducted to assess the significance between means followed by a Dunnet's t-test. A *p*-value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Physical Characteristics of Refined PMFO

The physical characteristics of refined PMFO is presented in *Table 1*. The oil was degummed, bleached and deacidified. The phytonutrient contents of refined PMFO are as follows: carotenes (1255 ppm), tocopherols and tocotrienols (1290 ppm), sterols (591 ppm) and squalene (886 ppm). The oil contains 7.79 g/100 g DAG and 92.2 g/100 g TAG. The fatty acid composition is similar to what have been reported for palm olein, which is predominantly high in palmitic acid (C16:0, 36.9 g/100 g) and oleic acid (43.5 g/100 g), with 10.6 g/100 g linoleic acid (C18:2) (Teh and Lau, 2021). The present data is in line with what has been recently reported, where refined PMFO retains substantial amount of total carotene, vitamin E and sterols compared to PMFO (Teh and Lau, 2021). Notably, the concentration of phytonutrients are still substantially higher than that of CPO and commercially available red palm oil as reviewed in our published data (carotenoids, 600-750 ppm and tocols, 717-863 ppm) (Loganathan *et al.*, 2017). This could be explained by the extraction methods, where refined PMFO used in the present study was extracted from pressed mesocarp using n-hexane while CPO was obtained by mechanical pressing of mesocarp. The high amount of carotenes and vitamin E in refined PMFO was due to its high content in the bi-membrane layer of the cell in the membrane and was co-extracted with the residual oil using n-hexane (Lau *et al.*, 2006). The condition subjected to the extraction of PMFO, in particular the deodorisation temperature (which is below 200°C, as compared to conventional CPO deodourisation temperature at 265°C), does not significantly degrade the phytonutrients. The extraction method however, did not modify the fatty acid composition of PMFO as compared to CPO.

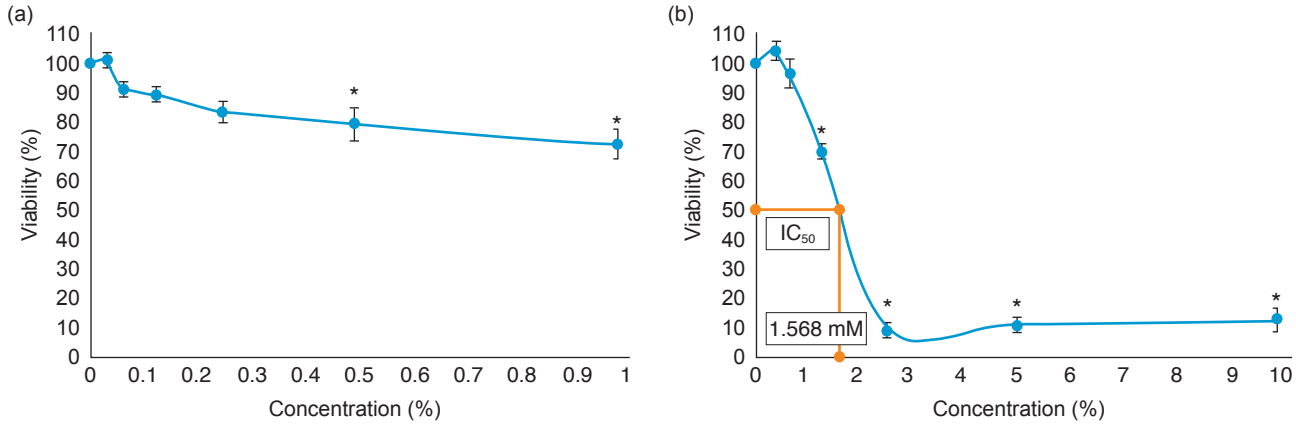
TABLE 1. PHYSICAL CHARACTERISTICS OF REFINED PALM-PRESSED MESOCARP FIBRE OIL[1]

Parameter	Weight
Free fatty acids (g/100 g)	0.2
Fatty acid composition (g/100 g)	
C10:0	0.1
C12:0	2.6
C14:0	1.5
C16:0	36.9
C16:1	0.5
C18:0	3.6
C18:1	43.5
C18:2	10.6
C18:3	0.6
C20:0	0.1
Acylglycerol composition (g/100 g)	
Monoacylglycerol	0.05
Diacylglycerol	7.79
Triacylglycerols	92.17
Phytonutrients (mg/kg)	
Carotenoids	1 255
Tocols	1 290
Sterols	591
Squalene	886

Note: [1]Results are presented in means ±SE of at least three separate experiments.

MTT Viability Assay

The cytotoxic effects of refined PMFO against V79-4 fibroblast cells as assessed by MTT assay following 24 hr treatment are shown in *Figure 1*. V79-4 cells showed decrease in viability in a concentration-dependent manner following 24 hr treatment with refined PMFO. The results show that at the highest concentration of refined PMFO (1%v/v), (72.65 ± 4.1) % of viable cells were observed (*Figure 1*). A concentration response effect was seen following treatment with H_2O_2 with an inhibitory concentration (IC_{50}) value of 1.568 mM. No IC_{50} values were observed for refined PMFO treated V79-4 cells as compared to positive control (H_2O_2) (*Figure 1*). H_2O_2 was used to validate the experimental procedure. Based on the results obtained, refined PMFO has no cytotoxic effect using a widely acceptable method (MTT assay) in V79-4 fibroblast cells. Our group has previously studied the cytotoxicity of PMFO against three human cancer cell lines, namely Hep-G2, LS174T and SK-MEL-28, and no cytotoxic effect has been reported (Teh *et al.*, 2021). The results were supported by a study conducted on cytotoxic effect oil palm kernel protein hydrolysates (OPKHs) against Hep-G2 cells where no cytotoxicity was observed. The growth simulation and prolongation of cells could occur due to the presence of oleic acids in OPKHs that also present in oil palm-based oil extracts (Chang *et al.* 2014). Other than that, this could also be possibly explained by the synergistic and antagonistic effects of the components in PMFO, which warrants further investigation.



Note: [1] The results are expressed as mean ± SE of at least three independent experiment.
* Significant difference (p<0.05) as compared to negative control.

Figure 1. Cytotoxicity of (a) refined palm-pressed mesocarp fibre oil (PMFO), and (b) H_2O_2 against V79-4 cells with 24 hr of incubation.

Alkaline Comet Assay

The detection of primary DNA damage induced by refined PMFO in V79-4 cells was assessed by Alkaline Comet assay. Negative control cells displayed low levels of background damage. Scoring for the DNA damage was done based on TM values. As shown in *Table 2*, V79-4 negative control cells has TM of (0.432 ± 0.09). As shown in *Table 2*, there was a slight increase with no significant difference in TM at IC_{25}, (0.468 ± 0.09) of refined PMFO treated cells respectively at 24 hr as compared to negative control cells. This data showed that refined PMFO has no capability to induce direct DNA strand breakage in V79-4 cells. A significant (p<0.05) increase in DNA damage shown by TM was observed in H_2O_2 treatment at 1 mM, which served as positive control (*Table 2*). This data indicates that refined PMFO does not induce DNA damage, this may partly be explained by the fact that PMFO is the power house of antioxidants, with a recent published data from our group to support this (Teh *et al.*, 2021). It has been reported that the bouquet of phytonutrients in PMFO has free radical scavenging effects as assessed using α, α-diphenyl-β-picrylhydrazyl (DPPH), H_2O_2 and nitric oxide (NO) radicals scavenging assay when compared with extra virgin coconut oil and red palm oil (Teh *et al.*, 2021). The presence of vitamin E, particularly tocotrienols and tocopherols, are known for their potent antioxidant activity. Moreover, carotenoids and co-enzyme Q10 are proven to scavenge H_2O_2 radicals effectively. A previous study also acknowledged the antioxidant capacity of the extracts of oil palm fruit through H_2O_2 scavenging assay (Balasundram *et al.*, 2005). This finding further confirms that the oil palm-based edible oil, is rich in antioxidants that acts on radicals. Carotenoids and tocotrienols, the two most abundantly available antioxidants in PMFO have been reported to exert protective effects in various diseases using animal and human models as reviewed by our group (Fu *et al.*, 2014; Loganathan *et al.*, 2017).

TABLE 2. LEVEL OF DNA DAMAGE ON V79-4 TREATED CELL LINE WITH R REFINED PALM-PRESSED MESOCARP FIBRE OIL[1]

Item	Level of DNA damage (Arbitrary unit)
	(TM) 24 hr
Negative control	0.432 ± 0.08
Refined PMFO (IC_{10})	0.343 ± 0.04
Refined PMFO (IC_{50})	0.468 ± 0.09
	(TM) 30 min
Positive control (H_2O_2)	*9.762 ± 1.41

Note: [1]The results are the means ±SE of at least three separate experiments (*p<0.05 *vs.* negative control). PMFO - palm-pressed mesocarp fibre oil; TM - tail moments.

Acute Oral Toxicity

There was no difference observed between female Sprague-Dawley rats treated with refined PMFO at 2000 mg/kg body weight and control (with 10 mL/kg body weight of pre-filtered water) on the behavioural observations (over the 14-day interval). General clinical observations (namely skin and fur, eyes, respiratory effect, mucous membrane, motor activity, tremor, convulsion, walking behaviours, and diarrhoea) were normal for both treated and control animals (*Table 3*). No mortality or any toxic symptoms was observed. Furthermore, necropsy results including observations in all eight separate organs (namely brain, kidneys, lungs, liver, stomach, spleen, heart, and pancreas) showed no notable abnormalities. No significant differences in body weight were

observed between rats treated with refined PMFO treatment and control (*Figure 2)*. Percent of increment in rat's body weights for treated and control showed no significant difference. There was no remarkable difference in mean percentage of organ to animal body weight between rats treated with refined PMFO and control. The heart, kidneys, liver, lungs and spleens are the first organs to display metabolic responses to toxic substances. Organ weights are useful indicator of any kind of toxicity, in particular in drug administration. It is important to note that without further morphological changes in acute toxicity, differences in organ weight between treated and control animals may occur (Ara and Usmani, 2015). Taken together the data observed, the administration of refined PMFO does not exert acute oral toxicity impact in rats. This is in line with an extensive review published by our group on the toxicological and nutritional assessment of CPO and red palm oil (Loganathan *et al.* 2017). In addition, a study conducted in weanling Wistar/NIN albino rats using CPO, refined palm oil and peanut oil has shown that the vegetable oils tested using a diet containing 10% dietary fat has no adverse effects on growth rate, feed efficiency, digestibility and fat absorption after a 90-day feeding period (Manorama and Rukmini, 1991).

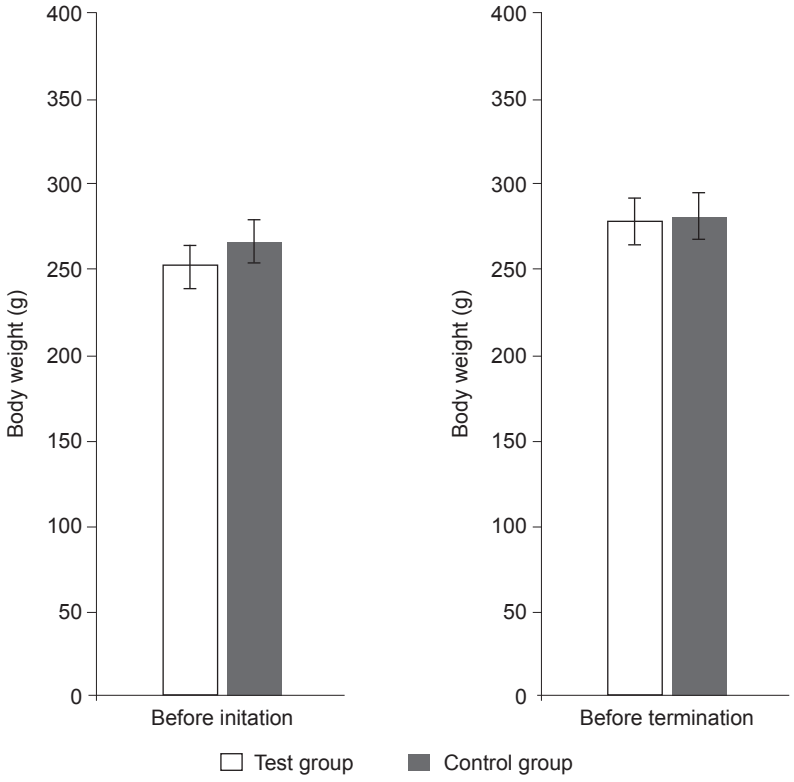
Limitations/Recommendations

The method for refining and processing of PMFO varies between factories. Apart from that, diversity, degree of palm fruit ripeness and geographical location of plantation could also contribute to variation in the carotene concentration (Loganathan *et al.*, 2017). In view of that, it is advisable to have a harmonised method and national standard specification for PMFO and refined PMFO to provide consistency in the nutritional properties.

TABLE 3. CLINICAL FEATURES OF THE TEST AND CONTROL ANIMALS DURING OBSERVATION PERIOD[1]

Observation-post dosing	Control group			Test group			Continuing test group (2000 mg/kg)		
	1	2	3	1	2	3	1	2	3
Sex	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**	**F**
Duration	**A-K**	**A-K**	**A-K**	**A-K**	**A-K**	**A-K**	**A-K**	**A-K**	**A-K**
30 min	0	0	0	0	0	0	0	0	0
1 hr	0	0	0	0	0	0	0	0	0
2 hr	0	0	0	0	0	0	0	0	0
3 hr	0	0	0	0	0	0	0	0	0
4 hr	0	0	0	0	0	0	0	0	0
6 hr	0	0	0	0	0	0	0	0	0
Day 2	0	0	0	0	0	0	0	0	0
Day 3	0	0	0	0	0	0			
Day4	0	0	0	0	0	0			
Day 5	0	0	0	0	0	0			
Day 6	0	0	0	0	0	0			
Day 7	0	0	0	0	0	0			
Day 8	0	0	0	0	0	0			
Day 9	0	0	0	0	0	0			
Day 10	0	0	0	0	0	0			
Day 11	0	0	0	0	0	0			
Day 12	0	0	0	0	0	0			
Day 13	0	0	0	0	0	0			
Day 14	0	0	0	0	0	0			

Note: [1]For Test Group and Control Group, animals were observed for morbidity and mortality at 30 min, hourly up to 4 hr, at 6 hr post dosing and once daily up to day 14. Continuing Test Group animals were observed at 30 min, hourly up to 4 hr, at 6 hr post dosing and at 24 hr. Alphabetical values (A - K) denote clinical changes observed and scored following treatment, with zero ("0") denoted as "normal" (A - fur and skin changes; B - eye changes; C - respiratory effect; D - mucous membrane; E - motor activity; F - tremor; G - convulsion; H - walking backwards or ataxia, inability to coordinate body movements; I - diarrhea; J - death; K - others).



Note: [1] Results are expressed as mean ± SE of at least three independent experiments.
* No significant difference (p>0.05) was observed as compared to Control Group.

Figure 2. Mean body weight of Test Group and Control Group measured at two intervals of the study period.

CONCLUSION

Our toxicological assessment provides a preliminary finding on the safety usage of refined PMFO as a food component. Refined PMFO shows no cytotoxicity and did not cause any DNA damage in V79-4 cells. Acute toxicity animal testing has also shown no significant difference between groups treated. Based on toxicological study, refined PMFO showed no-observed-adverse-effect level (NOAEL) up to 2000 mg/kg body weight of the animal. Further analysis may be needed to conduct more in-depth analysis on various aspects in relation to impact to health.

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REFERENCES

Abd Majid, R; Mohammad, A W and Choo, Y M (2012). Properties of residual palm pressed fibre oil. *J. Oil Palm Res., 24*: 1310-1317.

Ainie, K; Siew, W; Tan, Y A; Nor Aini, I; Mohtar, Y; Tang, T and Nuzul, A (2005). *MPOB Test Methods*. MPOB, Bangi.

Ara, A and Usmani, J A (2015). Lead toxicity: A review. *Interdiscip. Toxicol., 8*: 55-64.

Awang, N; Ahlam, S N F and Chan, K M (2015). Cytotoxicity and genotoxicity assessments of Batik industrial wastewater on V79 cells. *Toxicol Inter., 22(3)*: 76-82.

Ayeleso, A; Brooks, N and Oguntibeju, O (2014). Impact of dietary red palm oil (*Elaeis guineensis*) on liver architecture and antioxidant status in the blood and liver of male Wistar rats: Peered reviewed original articles. *Med. Tech. SA., 27(2)*: 18-23.

Chang, S K; Hamajima, H; Amin, I; Yanagita, T; Mohd Esa, N and Baharuldin, M T H (2014). Cytotoxicity effect of oil palm (*Elaeis guineensis*) kernel protein hydrolysates. *Int. Food Res. J., 21(3)*: 909-914.

Choo, Y M; Yap, S C; Ooi, C K; Ma, A N; Goh, S H and Ong, A S H (1996). Recovered oil from palm-pressed fibre: A good source of natural carotenoids, vitamin E, and sterols. *J. Am. Oil Chem. Soc., 73(5)*: 599-602.

Fu, J Y; Che, H L; Tan, D M Y and Teng, K T (2014). Bioavailabilities of tocotrienols: Evidence in human studies. *Nutr. Metab. (Lond.), 11(1)*: 5.

Hasliyati, A; Rusnani, A M; Wan Hasamuddin, W H; Ng, M H; Nor Faizah, J and Rohaya, M H (2021). The effects of recylcing palm pressed fibre oil on crude palm oil quality. *J. Oil Palm Res.* DOI: 10.21894/jopr.2021.0016.

Lau, H L N; Choo, Y M; Ma, A N and Chuah, C H (2006). Quality of residual oil from palm-pressed mesocarp fibre (*Elaeis guineensis*) using supercritical CO_2 with and without ethanol. *J. Am. Oil Chem. Soc., 83(10)*: 893-898.

Loganathan Jr, R; Selvaduray, K; Nesaretnam, K and Radhakrishnan, A K (2010). Health promoting effects of phytonutrients found in palm oil. *Malays. J. Nutr., 16(2)*: 309-322.

Loganathan, R; Subramaniam, K M; Radhakrishnan, A K; Choo, Y M and Teng, K T (2017). Health-promoting effects of red palm oil: Evidence from animal and human studies. *Nutr. Rev., 75(2)*: 98-113.

Manorama, R and Rukmini, C (1991). Nutritional evaluation of crude palm oil in rats. *Am. J. Clin. Nutr., 53(4)*: 1031S-1033S.

Manorama, R; Chinnasamy, N and Rukmini, C (1993a). Effect of red palm oil on some hepatic drug-metabolizing enzymes in rats. *Food Chem. Toxicol., 31(8)*: 583-588.

Manorama, R; Chinnasamy, N and Rukmini, C (1993b). Multigeneration studies on red palm oil, and on hydrogenated vegetable oil containing mahua oil. *Food Chem Toxicol., 31(5)*: 369-375.

Manorama, R; Harishankar, N; Polasa, K and Rukmini, C (1989). Mutagenicity studies on repeatedly heated crude and refined palm oil. *J. Oil Tech. Assoc. India., 21(2)*: 29-31.

Mosmann, T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods., 65(1-2)*: 55-63.

Nang Lau, H L ; Puah, C W; Choo, Y M; Ma, A N and Chuah, C H (2005). Simultaneous quantification of free fatty acids, free sterols, squalene, and acylglycerol molecular species in palm oil by high-temperature gas chromatography-flame ionization detection. *Lipids, 40(5)*: 523-528.

Neoh, B K; Thang, Y M; Zain, M Z M and Junaidi, A (2011). Palm pressed fibre oil: A new opportunity for premium hardstock? *Int. Food Res. J., 18(2)*: 769-773.

Noorshamsiana, A W; Astimar, A A; Iberahim, N I; Nor Faizah, J; Anis, M; Hamid, F A and Kamarudin, H (2017). The quality of oil extracted from palm pressed fibre using aqueous enzymatic treatment. *J. Oil Palm Res., 29(4)*: 588-593.

Obibuzor, J U; Okogbenin, E A and Abigor, R D (2012). Oil recovery from palm fruits and palm kernel. *Palm Oil.* p. 299-328.

OECD (2002). Test No. 423: Acute oral toxicity - Acute toxic class method. *OECD Guidelines for the Testing of Chemicals*. OECD Publishing, Paris. https://www.oecd-ilibrary.org/environment/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en.

Putra, N R; Wibobo, A G; Machmudah, S and Winardi, S (2019). Recovery of valuable compounds from palm-pressed fibre by using supercritical CO_2 assisted by ethanol: Modeling and optimization. *Sep. Sci. Technol., 55(17)*: 3126-3139.

Siew, E L; Farris, A F; Rashid, N; Chan, K M and Rajab, N F (2020). *In vitro* toxicological assessment of gadolinium (III) chloride in V79-4 fibroblasts for classification of health hazard. *Genes Environ., 42*: 22.

Subramaniam, V; Menon, N R; Sin, H and May, C Y (2013). The development of a residual oil recovery system to increase the revenue of a palm oil mill. *J. Oil Palm Res., 25(1)*: 116-122.

Sulihatimarsyila, A N; Lau, H L N; Nabilah, K M and Azreena, I N (2019). Refining process for production of refined palm-pressed fibre oil. *Ind. Crops Prod., 129*: 488-494.

Sulihatimarsyila, A N; Lau, H L N; Nabilah, K and Azreena, I N (2020). Production of refined red palm-pressed fibre oil from physical refining pilot plant. *Case Stud. Chem. Environ. Eng., 2*: 100035.

Teh, S S; Lau, H L N and Mah, S H (2019). Palm-pressed mesocarp fibre oil as an alternative carrier oil in emulsion. *J. Oleo Sci., 68(8)*: 803-808.

Teh, S S and Lau, H L N (2021). Quality assessment of refined red palm-pressed mesocarp olein. *Food Chem., 340*: 127912.

Teh, S S; Mah, S H; Lau, H L N; Teng, K T and Loganathan, R (2021). Antioxidant potential of red palm-pressed mesocarp olein. *J. Oleo Sci., 70(12)*: 1719-1729.

Tice, R R; Agurell, E; Anderson, D; Burlinson, B; Hartmann, A; Kobayashi, H; Miyamae, Y; Rojas, E; Ryu, J C and Sasaki, Y F (2000). Single cell gel/comet assay: Guidelines for *in vitro* and *in vivo* genetic toxicology testing. *Environ. Mol. Mutagen., 35(3)*: 206-221.