

Full Length Research Paper

Acute toxicity and safety assessment of oil palm (*Elaeis guineensis* Jacq.) leaf extract in rats

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Catechin-rich oil palm (*Elaeis guineensis*) leaf extract (OPLE) possesses good anti-diabetic, anti-hypertension, antioxidant, organ-protective, cardiovascular properties and other health benefits. Previous sub-chronic daily supplement of 500 mg kg⁻¹ body weight (bw) for 3 months in rats showed no significant adverse effects. The present investigation was carried out to evaluate the acute toxicity of the OPLE. The acute toxicity study was conducted by administering to the rats a single dose of either 2 or 5 g kg⁻¹ bw. General behavior, body weights, other adverse effects and mortality were monitored for up to 14 days. Total white blood cells (WBC), lymphocyte, red blood cell (RBC) counts, hemoglobin concentrations and packed cell volume (PCV) were determined at 0, 1, 3, 7 and 14 days. Liver and kidney function markers were monitored and the effects on the essential organs were examined histologically. The no-observed adverse effects (NOAE) were seen in the 2 g dose, while at the 5 g dose, behavioral changes, grooming, tachycardia, heavy breathing and weakness lasted for 2 h. The 5 g dose also caused significant ($P \leq 0.05$) reduction in RBC, hemoglobin and PCV although there was indifference in lymphocyte and WBC counts. Liver function markers and histology showed hepatocyte damage especially at day 7 in the 5 g dose, but there was partial recovery by the 14th day. The kidneys showed no significant changes. There was no death even at the 5 g dose, and the acute toxic injuries appeared reversible because the rats gradually recovered. In view of the NOAE dose of 2 g kg⁻¹, the OPLE at the normal dose levels may be considered relatively safe for use.

Key words: *Elaeis guineensis*, acute toxicity, hepatotoxicity, rats.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a perennial plant comprising of two species, *Dura* × *Tenera* hybrids of the Arecaceae family that grows in the lowlands of humid tropics, 15° N - 15° S having an evenly distributed rainfall of 1800 to 5000 mm/year. The planting material has planting density of 128 to 148 palms/ha, depending on planting material, soil and climate. Most common spacing is 9 × 9 m triangular (= 143 palms/ha). Oil palm adapts to a wide range of soils and to low pH (but sensitive to high pH above 7.5) and to stagnant water (Sundram et al.,

2003; Fairhurst and Mutert, 1999; von Uexkull and Fairhurst, 1991).

Oil palm leaf extract (OPLE) is not a regular food ingredient but has recently been shown to produce various beneficial health effects. The Oil palm leaf (OPL) contains higher total polyphenols content than green tea (particularly glycosylated flavonoids, carotenoids and catechins) (Runnie et al., 2003). The OPL phenolic compounds contains significant amount of green tea catechins, namely epigallocatechin (0.08%), catechin

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(0.30%), epicatechin (0.01%), epigallocatechin gallate (0.28%), epicatechingallate (0.05%) and their glycosides (Jaffri et al., 2011a), hence is a potential cheap source of green tea catechins. OPL is an under-utilized by-product of the oil palm industry, abundant in countries near the tropics such as South East Asia, Africa and South America. Traditionally, the palm leaves are used as woven bags for boiling rice, and the polyphenols are absorbed into the rice to give a desirable flavor. The OPLE have good phytoestrogenic properties (Namvar et al., 2009), valuable for postmenopausal women. The palm leaves have been consumed by ruminants for decades without any reported toxicity. It has excellent anti-hypercholesterol and antioxidant properties (Runnie et al., 2003; Salleh et al., 2002). The antioxidant content of OPL is higher than papaya shoot, green chilli and lemon grass *in vitro* (Abeywardena et al., 2002). The OPLE up-regulates the low density lipoprotein receptors '(LDL) *ex vivo*' (Salleh et al., 2002). It is anti-hypertensive (Jaffri et al., 2011b) with cardiovascular, liver, kidney (Jaffri et al., 2011a) and neuroprotective properties (Jaffri, 2008) under nitric oxide (NO) deficiency. The OPLE also showed cancer preventive and therapeutic effects on breast tumour (Namvar, 2009). Additionally, blood glucose and lipid oxidation in streptozotocin (STZ) induced rats are reduced by OPLE (Rosalina et al., 2011). In spite of the numerous benefits of OPLE for health management, there has not been previously reported on the safety and acute toxicity of OPLE which is the basis of this study. This study evaluates the acute toxicity of OPLE at a single dose of 2 to 5 g OPLE kg⁻¹ body weight.

MATERIALS AND METHODS

Palm leaf extract preparation

Oil palm (*E. guineensis*) leaves (OPL) were harvested from the Universiti Putra Malaysia (UPM) campus, washed, chopped and dried at 40°C for 24 h. The dried leaves were macerated, and extracted with 50% aqueous ethanol under continuous agitation at room temperature for 2 h. The filtered solution was spray-dried under vacuum to a dark green OPLE powder, and stored sealed at -18°C until used.

Acute toxicity tests

Animals for acute toxicity study

Forty (40) male Sprague dawley rats (average weight: 130 g) were purchased from Sapphire Enterprise, Malaysia. The animals were acclimatized for about 1 week, and maintained in a standard environmental condition, temperature 22 ± 2°C and 12 h light/darkness cycle. The rats had free access to standard pelleted rat diet (Gold Coin Sdn. Bhd; Malaysia) and water.

Acute toxicity protocol

The rats were randomly assigned into clean plastic cages. All experimental protocols were carried out in accordance to the guidelines on the ethical use and care of laboratory animals by the

Faculty of Veterinary Medicine, University Putra Malaysia and UK animal act 1986. Measures were taken to minimize pain or discomfort. All experimental animals were fasted overnight approximately 18 h (4.00 pm to 10.00 am) before treatment. Groups of 12 animals were administered a single dose of either 2 or 5 g OPLE kg⁻¹ body weight by gavage (Fujii et al., 2008). The vehicle (refined palm oil) was administered to the control animals. Feeding was restarted 4 h after dosing. Treated animals were closely observed for clinical signs immediately after dosing at 1, 2, 3 and 4 h, and then subsequently, twice per day until 14 days. Abnormal behavior and body weights were recorded.

Three animals per group were sacrificed by an overdose of diethyl ether on days 1, 3, 7 and 14 and blood was collected via cardiac puncture (Mazzanti et al., 2009). Hematological parameters including total red blood cells (RBC), total white blood cells (WBC), hemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) were analyzed using hematological analyzer (CELL DYN 3700, USA). Differential WBC including lymphocytes, monocytes, neutrophils, eosinophils, and basophils were performed and the cells counted manually, using a microscope (NIKKON ECLIPSE 80i, Japan).

The blood samples were analyzed for liver and kidney function markers, namely plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen, creatinine and albumin, using commercial kits (Roche Diagnostics) with automated analyzer (Hitachi, Japan), following the instrument manufacturer's protocol.

Histopathological study

Liver and kidney of sacrificed rats were removed, weighed and fixed in 10% buffered formalin (Orafidiya et al., 2004). Tissue sections were embedded in paraffin wax, trimmed, deparaffinized and processed (Shandon, Citadel 2000 tissue processor, GMI, Inc. Ramsey, Minnesota, USA) for hematoxylin-eosin (H&E) staining. All images were captured using a light microscope (Olympus BX-51, Hamburg, Germany).

Statistical analysis

Results are presented as mean ± standard deviation (SD) and were analyzed by one way analysis of variance (ANOVA) followed by Duncan multiple range tests for significance ($P \leq 0.05$) (Lv et al., 2008).

RESULTS

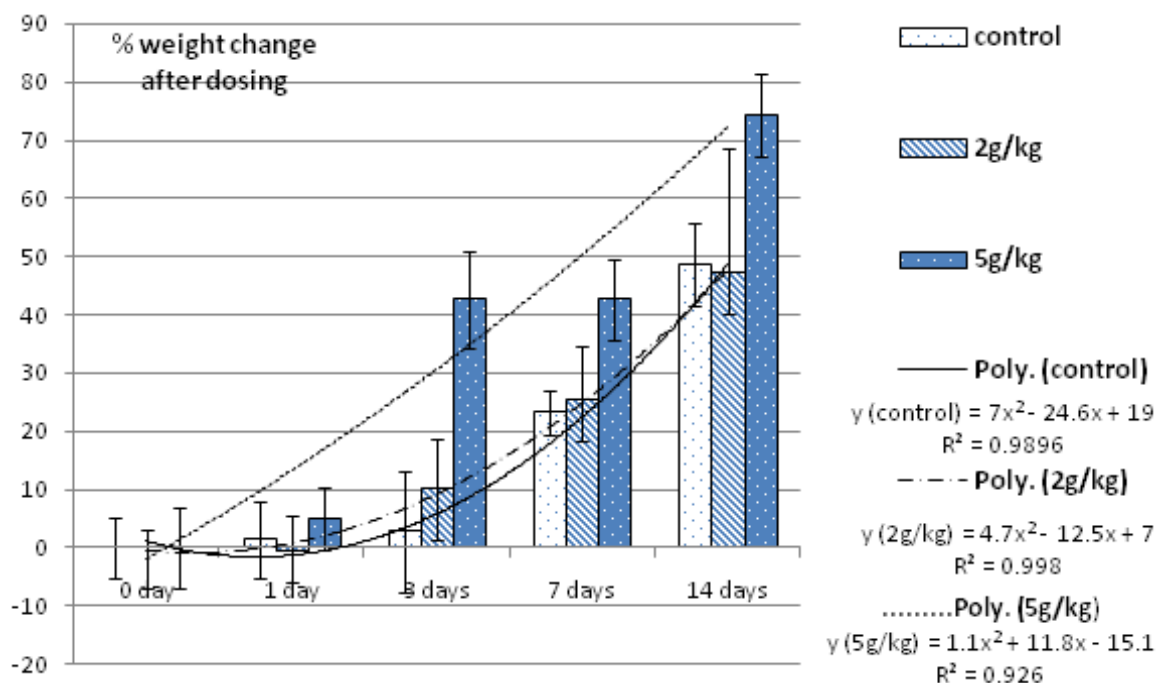
Acute toxicity of OPLE ethanol extract in rats

Symptoms, gross findings and mortality of animals after single oral dose administration of OPLE are shown in Table 1. Preliminary trial using low doses of OPLE between 1 and 2 g kg⁻¹ body weight were well tolerated by the animals. Neither death nor any strange behavioral symptoms occurred during the 14 days study, and they were similar to the control. Hence, only the 2 g kg⁻¹ body weight dose was repeated to compare with the acute toxic effects at 5 g kg⁻¹. At the high dose of 5 g kg⁻¹ body weight, observable symptoms include weakness, tachycardia, heavy breathing and grooming, starting 15 min after dosing and lasted for about 2 h following the treatment.

Table 1. Mortality and gross symptoms of toxicity observed after single dose administration of ethanol extract of OPLE in rats

Dose/group	Sex (M)	Nd/n	Duration D (h)	Symptoms of toxicity
Control oil	M	0/12	0	None
OPLE 2 g kg ⁻¹	M	0/12	0	None
OPLE 5 g kg ⁻¹	M	0/12	≤2	Grooming tachycardia, heavy breathing and weakness

Acute toxicity study – oral doses (2, 5 g kg⁻¹). Each dose extract was dissolved in the vehicle (bleached palm oil) and administered to groups of 12 rats by gavage. Number of treated rats (n = 12/group) were carefully examined for 14 days after dosing, for behavioral changes and mortality. M = Male; Nd/n = number of death/number of animals treated; (D) = length of time observations were monitored; none = no observable symptoms.

**Figure 1.** Mean body weight change (%) after administration of OPLE in rats.

Body weight after dose administration of OPLE ethanol extract in rats

Changes in body weight of control and treated groups are shown in Figure 1. Rats given 2 g OPLE kg⁻¹ body weight showed insignificantly different weight changes to that of control animals. All the animals gained weight with time as expected. The rats given 5 g OPLE kg⁻¹ body weight had significantly higher percent weight increases on and after day 3. The reasons for this were investigated through the histopathological observations.

Effect of OPLE on hematology in rats

Significant reductions were observed in WBC after day 1 of OPLE treatments at both OPLE doses, but the WBC were within the normal ranges by day 3 onwards.

Lymphocyte counts were insignificantly different from the control group although it appeared high in the OPLE treated groups on day 7. The RBC and haemoglobin levels of 5 g kg⁻¹ treated rats were significantly below that of the control rats. The packed cell volume of the 5 g OPLE kg⁻¹ body weight rats were significantly low on the 3rd day of treatment compared to the control. Plasma ALT, increased slightly $P \leq 0.05$ (although within the normal ranges), in the 5 g OPLE dose on day 1 but was not affected in subsequent days (Figure 5). The AST was not affected after dose administration. There were no significant changes in the kidney histology and kidney markers in all treated rats.

DISCUSSION

Biochemical substances with LD₅₀ range of "500 to 5000

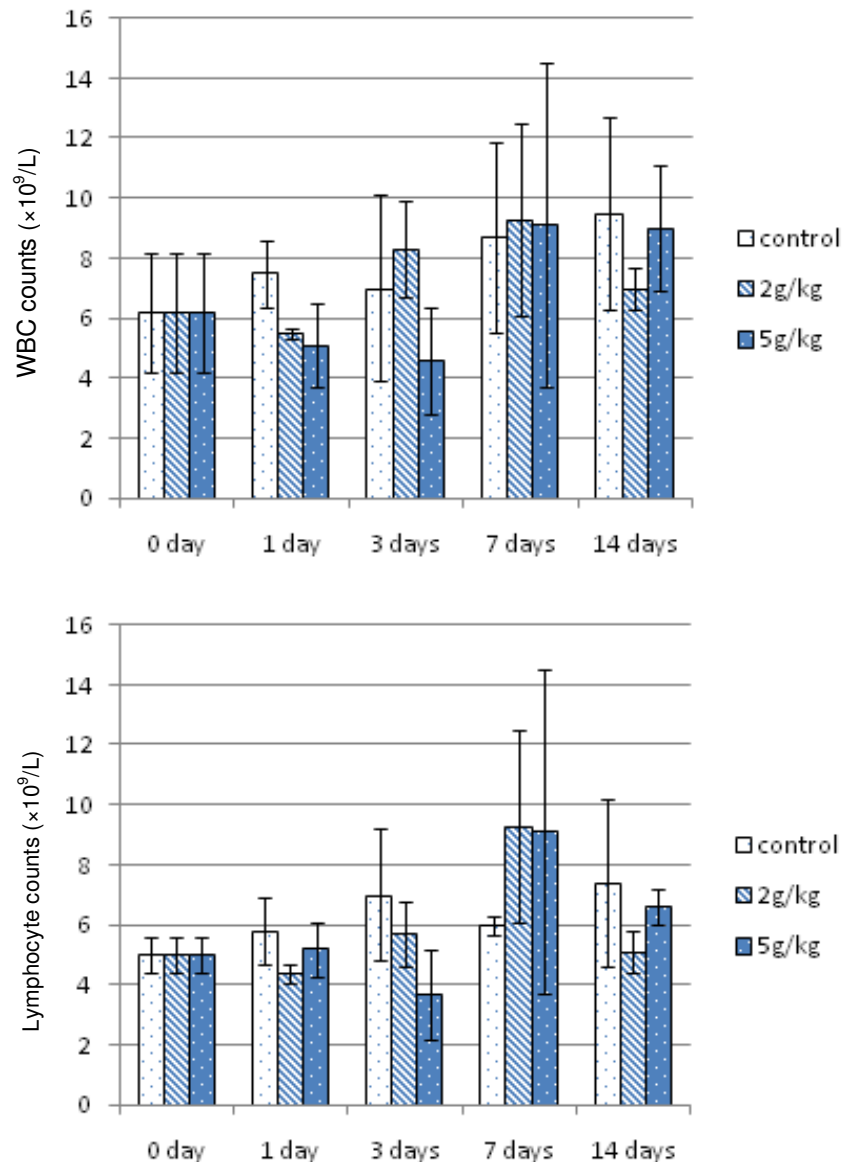


Figure 2. WBC counts ($\times 10^9/L$) and lymphocyte counts ($\times 10^9/L$) after administration of OPLE in rats.

and between 5000 and 15000 mg kg^{-1} body weight", are classified as non-toxic and fairly toxic, respectively (Anusuya et al., 2010; Loomis and Hays, 1996; Mukinda and Syce, 2007). Jaffri et al. (2011a) previously reported that a daily dose of 500 mg OPLE kg^{-1} body weight consumption for 3 months (sub-chronic toxicity evaluation) produced only minimal kidney tubules deviations in normal rats. Although a single acute dose of 2 g OPLE kg^{-1} body weight was well tolerated by the rats, the 5 g OPLE kg^{-1} body weight dose produced reversible adverse clinical and behavioral effects. Body weight gain, after a single 5 g OPLE kg^{-1} body weight dose progressively increased from day 1 through day 14; an indication that the OPLE can be categorized as of low toxicity (Figure 1).

There was no death at both doses indicating that the LD_{50} is greater than 5 g kg^{-1} body weight (Shim et al., 2008; Feres et al., 2006).

The 5 g OPLE kg^{-1} dose temporarily affected WBC counts (Figure 2), indicating leucopoiesis and altered bone marrow cell production (haematopoiesis). Hematological parameters, which include RBC, hemoglobin and hematocrit, were significantly reduced by the 5 g kg^{-1} body weight dose compared to the control and 2 g OPLE kg^{-1} dose, but recovered over time (Figures 3 and 4). The reductions in blood cells implied that the OPLE at the 5 g kg^{-1} dose temporarily interfered with the production of erythrocytes or caused haemolysis, leading to anemia and reduced oxygenated blood circulation capacity.

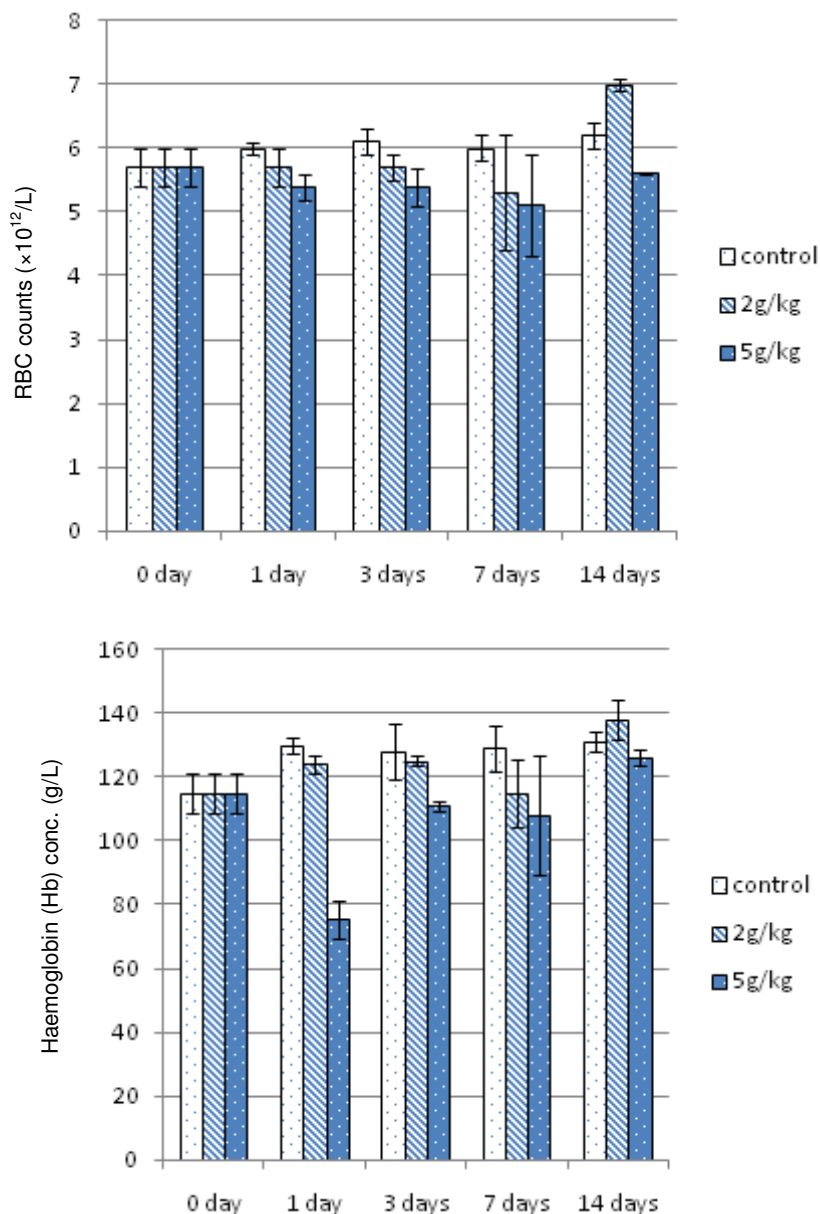


Figure 3. Total RBC counts ($\times 10^{12}/L$) and Haemoglobin (Hb) conc. (g/L) after single dose administration of OPLE in rats.

Polyphenols with catechol groups (for example, catechins, as present in OPLE), can form stable chelates with ferric ions, to inhibit non-heme iron absorption even in humans (Hurrell et al., 1999).

Liver hepatocytes injury were observed at day 7 (Figure 6), but by day 14 the liver showed recovery from the damages. Increased plasma ALT, which is mainly present in the hepatocytes are indicators of liver cell membrane leakages or injury. This was observed at the 5 g OPLE kg^{-1} body weight dose. Excess polyphenols can have pro-oxidant effects *in vivo*, reducing iron(III) to iron(II) to generate hydroxyl radicals through the Fenton reaction. The catechol groups can oxidize to quinone *in*

vivo and generate free radicals through redox cycling, but their formation in endogenous tissues are limited by conjugating enzymes such as quinine reductase, and catechol-O-methyltransferase (Scalbert et al., 2005).

Green tea catechins were demonstrated to prevent inflammation, cancer, hypercholesterol, angiogenesis, arthritis, oxidative stress, and neurodegeneration (Naghma and Hasan, 2007; Hirao et al., 2010). However, in an inflammatory milieu, pro-inflammatory mediators overwhelm the anti-inflammatory signals leading to oxidative stress. Oxidative stress decreases glutathione activity, arachidonic acid and metabolites activation and Ca^{2+} influx (Rao et al., 1993; Li et al., 1997). It may alter

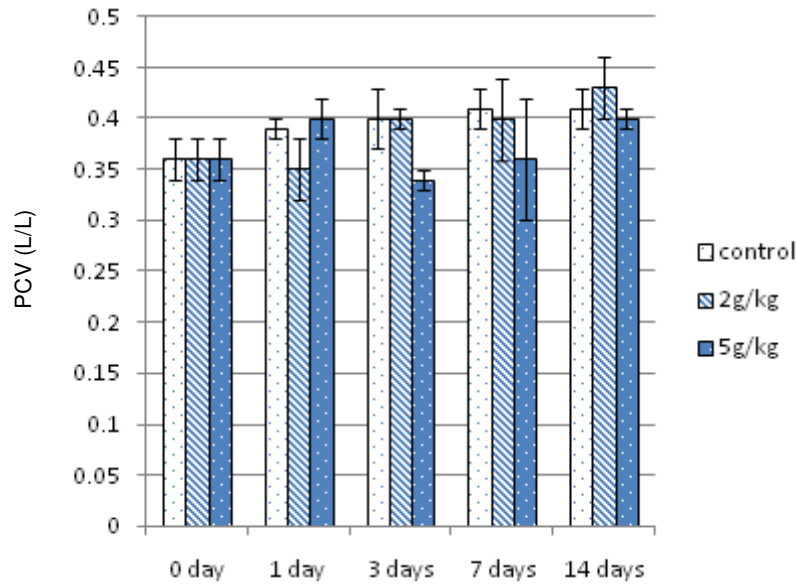


Figure 4. PCV (L/L) after a single dose administration of OPLE in rats

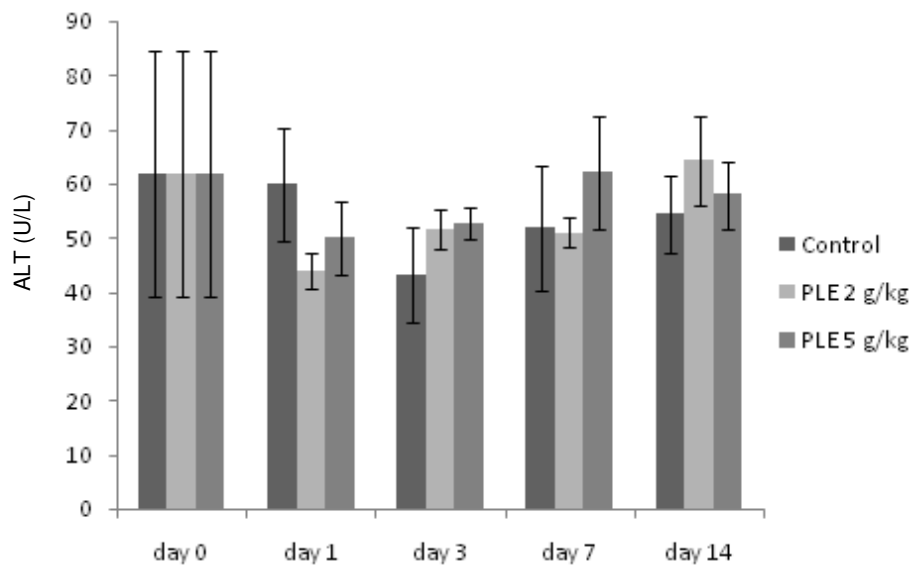


Figure 5. ALT (U/L) after dose administration of OPLE in rats.

cell and nucleoli membrane permeability, and mitochondrial activity, causing cell cycle arrest (Neal and Halpert, 1982), and the resultant toxicity. Catechins consumption is generally beneficial to the tissues (Moore et al., 2009) and other intracellular signaling pathways (Li et al., 2006). However, acutely high concentrations may produce adverse effects. Flavonoids such as genistein, daidzein, quercetin, kaempferol, vitexin or naringenin have goitrogenic or anti-thyroid effects by irreversibly inhibiting thyroid peroxidase. This may result in thyroid hypertrophy (goiter) and decrease plasma thyroid levels especially under iodine deficiency, (Doerge and Chang,

2002). High polyphenol intake generally protects the cardiovascular system, but excessive consumption could increase homocysteinemia formation and enhance cardiovascular diseases risk (Olthof et al., 2001). Acute polyphenols toxicity was reported in animals (Clifford and Scalbert, 2000). Some polyphenols exhibit pro-carcinogenic effect in cultured cells and animal models of pancreatic, colon or hormone dependent cancers (Scalbert et al., 2005; Ju et al., 2001). Excess phytoestrogens adversely affected the reproductive functions of certain animals, causing infertility in sheep, persistent estrus phase in females and sexual behavior deficits in

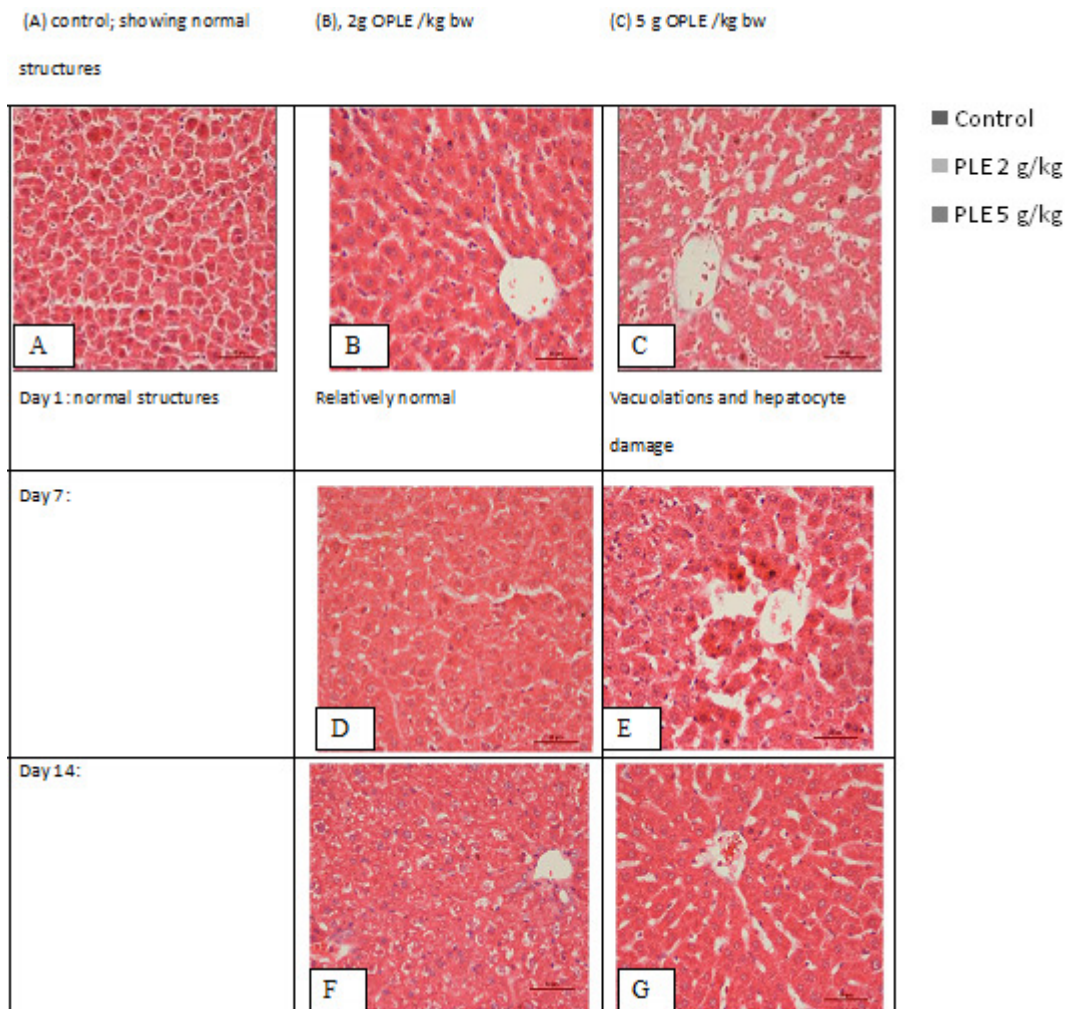


Figure 6. Photomicrograph of liver of rat. (Magnification x 40) showing liver damage in 5 g kg⁻¹ bw dosed rats and recovery after 14 days.

males (Whitten et al., 1995).

In conclusion, the present investigation shows that the acute single dose of 5 g OPLE kg⁻¹ body weight produced and reversible toxic effects in rats. Hence, the OPLE can be classified as of low toxicity.

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