Original Article

Acute oral toxicity study of ethanol extract of *Oroxylum indicum* leaf in mice

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Abstract

The *Oroxylum indicum* plant is a herbal plant commonly eaten by the locals in Malaysia while the application of herbal remedies from the plant has been inherited and passed down through generations. However, there is a lack of toxicity profiling of the plant, hence this research aimed to investigate acute oral toxicity of ethanol extract of *O. indicum* in C57BL/6 male mice at different concentrations, to determine the LD_{50} of the plant extract. A total of twenty-five mice were randomly assigned into five experimental groups comprising the control (normal saline), vehicle (5% DMSO), low dose (1000 mg/kg bw), medium dose (2000 mg/kg bw) and high dose (5000 mg/kg bw). The extracts were administered in a single oral dose on day 1 and the mice were observed daily for mortality, physiological and behavioural changes throughout the 14 day study period. At the end of the study, vital organs and blood samples were collected to determine the effects of the extract on the relative organ weight, tissue changes and blood profile alterations. No mortality nor behavioural changes were recorded for 2 weeks. Results of the body weight, relative organ weight, haematological and serum biochemistry assessments showed no significant ($p>0.05$) changes. Nevertheless, there were significant differences in the mean corpuscular volume (MCV), urea and alanine transaminase (ALT) values but the levels were still within the normal range. Histopathological analysis of the liver and kidney tissues also revealed no striking lesions. In summary, this study indicates that *O. indicum* leaf ethanolic extract up to 5000 mg/kg bw did not cause any toxicological effects in the mice model and is safe to be used for therapeutic purposes.

Keywords: Acute oral toxicity study, mice, *Oroxylum indicum*, ethanol extract, LD_{50}, haemato-biochemistry

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Introduction

The use of herbal medicines is rapidly increasing across the world and people are opting for these products as an alternative treatment for many health conditions and diseases. The rising usage of herbal products has led an increase in the awareness of the potential adverse effect among consumers regardless that many herbal products are not tested, monitored or proven scientifically (Ekor, 2014). The efficacy and toxicity of most herbal plants relies solely on conventional practice and clinical experience (Zhang *et al*., 2015). The detection of the toxins and adverse effects of those herbal plants have become critical to ensuring the safety of herbal medicinal product application (Nurul *et al*., 2018).

Toxicology has been described as the study of the adverse effects of xenobiotics (Zhu *et al.* 2017). Modern and advanced toxicology evolves from analysing the adverse effects of exogenous agents on the molecular biology experiment using toxicants (Klaassen, 2013). In order to formulate drugs with therapeutic properties, a toxicological study should be conducted to ensure that the plant used is safe for consumption or application (Sajjaratul *et al.* 2016). The purpose of an oral toxicity study includes establishing the dose-dependent adverse effects, assessment of the lethal dose (LD_{50}) and provides information on the degree of safety of a pharmacological agent (Chinedu *et al*. 2013).

Oroxylum indicum is widely distributed throughout the Indian subcontinent and South East Asian countries, including Malaysia (Deka *et al*. 2013). In Malaysia, it is commonly known as "pucuk beka, bonglai or bolai kayu" and eaten raw as a salad or cooked with coconut milk. The leaves from the plant have previously been used among the older generation as herbal remedies and passed down to new generation to be applied. The boiled plant is usually drunk to relieve fever and the leaves are eaten as a supplement for women after childbirth. It is also believed that its fruit can help reduce blood pressure as well as in treating diabetes. Various scientific studies have reported that *O. indicum* possesses anticancer (Kamkaen *et al*., 2006), antioxidant and hepatoprotective (Tenpe *et al*., 2009), immunomodulaory properties (Zaveri *et al*., 2006) and gastro-protective properties (Zaveri and Jain, 2007). Despite the various claimed benefits of *O. indicum* leaf extract, toxicity profiling of this plant is still lacking and it has not been scientifically proven. Hence, this study aims to investigate the acute oral toxicity of *O. indicum* leaf ethanolic extract in mice and to determine the LD_{50} of the plant extract.

Materials and Methods

Preparation of ethanolic extract: The plant leaves were purchased from a local market in Pengkalan Chepa, Kelantan, in November 2019 (Fig 1). The leaflets were in 2-4 pairs, 7.0 x 3.5-8.0 cm, ovate-elliptic, acuminate, base obliquely rounded-obtuse. The leaves of *O. indicum* were then washed with distilled water and oven-dried at 40°C for 4 days. The dried leaves of *O. indicum* were ground into fine particle form and 150g of the pulverized leaves were soaked in 1.5 L of ethanol for 72 hours at room temperature. Then, the extract was filtered using Whatman filter paper (No. 1001-240, Grade 1) and was dried and evaporated using a rotary evaporator controlled in a water bath at 60°C. The final yield products were stored at under -20°C. The extract was tested for its sterility by inoculating 0.1g of the extract in 1ml of sterile nutrient broth. The extract was considered sterile with the absence of turbidity after being incubated at 37°C for 24 hours. The plant part was identified by a botanist from Herbarium, Universiti Kebangsaan Malaysia, Selangor, Malaysia. A voucher specimen (ID025/2020) was deposited at the herbarium for reference.

Figure 1 *Oroxylum indicum* leaf

Experimental animals and husbandry: A total of 25 male C57BL/6 mice aged between 17 to 18-weeks-old with a bodyweight of 25 to 30 g were obtained from the Animal Research and Service Centre, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan. The mice were placed in polycarbonate cages with optimum environmental conditions of temperature (24 ± 2°C), relative humidity (45 ± 5%) and lightning duration (12 hours daylight and 12 hours dark hours) were provided. Filtered tap water and commercial mice pellets were provided *ad libitum*. Wood shavings were provided as the bedding which was cleaned and changed daily. All mice underwent an acclimatization period for 5 days prior to the toxicity experiment. The research was conducted according to the guidelines and approval of the Institutional Animal Care and Use Committee (IACUC), Universiti Malaysia Kelantan (IACUC Ref No: UMK/FPV/ACUC/FYP/16/2020).

Acute oral toxicity study: The study was designed according to the Organisation for Economic Co-operation and Development Guideline Test No. 420 (OECD, 2001). The mice were randomly assigned into 5 experimental groups consisting of 5 mice per group. All the mice were fasted overnight from the feed but had free access to water before experimentation. The experimental groups were orally gavaged with a single dose of 1000 mg/kg bw, 2000 mg/kg bw and 5000

mg/kg bw of extracts accordingly, whereas the control and vehicle groups were treated with normal saline and 5% dimethyl sulfoxide (DMSO) respectively. No food was provided for the first 2 hours after *O. indicum* leaf extract administration. The conditions of all experimental mice were closely observed for the first 30 minutes to 6 hours, intermittently at 7 to 24 hours, and twice daily for 14 days for any abnormalities or signs of toxicity and mortality. The conditions which were assessed included changes in body weight, food and water intake, coat colour, mucous membrane, respiratory rate, neurological signs and behavioural changes.

Necropsy, gross organ examination and sample collection: At the end of the study, all surviving mice were sacrificed with inhalation of carbon dioxide and decapitation using a guillotine. The internal organs mainly the liver, kidneys, lungs, heart, brain and spleen were isolated, cleaned and weighed. The organs were examined for abnormalities and changes in the size, colour, consistency and texture. After that, all the organs were preserved in 10% formalin for further histopathologal evaluation. The relative organ weights were determined as follows: Relative organ weight = weight of organ/bodyweight of the mice on the sacrifice day x 100%.

Haematology and serum biochemistry analysis: Blood samples were also collected in heparin tubes using 25G needles via cardiac puncture. The serum samples were centrifuged at 5000 rpm for 30 seconds prior to analysis using an automatic analyser. The complete blood count tested were total white blood cell count and differentials count, total red blood cell count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration and platelet count. The serum samples were submitted for biochemical analysis for creatinine and blood urea nitrogen (BUN), alanine aminotransferase (ALT), and total protein, including albumin and globulin.

Histopathological evaluation: After 48 hours of formalin preservation, a small block of tissue was placed on the cassette and processed using an automated tissue processor. Then, the tissues were sectioned into a thickness of 5 µM using a rotary microtome and dried overnight in an oven at 37°C. Haematoxylin and Eosin (H&E) were used to stain the sectioned tissues for the examination of abnormalities and lesions. All the tissue sections were scored according to the established scoring method by Nurul *et al*. (2018).

Statistical analysis: Data was analysed statistically using Statistical Package for Social Science (SPSS) software version 23. For different parameters of body weights, relative organ weights, haematology and serum biochemistry, the values were expressed in mean and standard error mean (SEM). Tests for Analysis of variance (ANOVA) was conducted to compare the data variations between and within groups. Post hoc analysis using the Duncan test was used to assess the level of statistical significance, which was set at $p<0.05$.

Results

Behavioural and physical observations: All toxicity signs including pain and stress and abnormal behavioural and physical changes were absent in all groups of mice (Table 1). Only one mouse from high dose (5000 mg/kg bw) and low dose (1000 mg/kg bw) extract treatment groups showed a sign of abdominal breathing in the first thirty minutes, which then subsided gradually. The sign observed was possibly due to stress during restraining for the oral gavage procedure.

The bodyweight of the mice: Body weights of the mice taken at the initial stage of experimentation (day 0), day 7 and day 14 of post extract administration did not show any significant ($p>0.05$) changes compared to the control groups (Table 2).

The relative organs' weight of the mice: There were no significant differences ($p>0.05$) observed in the organ's weights of the liver, kidneys, heart, lungs, testes and spleen (Table 3).

Mortality rate and determination of the LD_{50} value: Administration of the extract at different doses (1000, 2000, and 5000 mg/kg bw) resulted in no death of the mice within the 14 day period of the study and observation (Table 4). This finding indicates that the inoculation of *O. indicum* leaf ethanolic extract up to 5000 mg/kg bw is safe.

Haematological evaluation: There were no significant differences ($p>0.05$) in the hemogram parameters in all groups except in the mean corpuscular volume (MCV) values; however, the values were still within the normal range (Table 5). There were also no significant differences ($p > 0.05$) in the total white blood cell counts and differential leukocyte counts in both treatment and control groups (Table 6).

Serum biochemistry analysis of kidney, liver and protein: Results of a renal parameter of creatinine, total protein, albumin and globulin concentrations were normal in all groups. Whereas, there were significant differences ($p>0.05$) for the urea and alanine transaminase (ALT) in the study but the values were also within normal limits (Tables 7 and 8).

Organ gross examination and histopathological evaluation of the organs: No gross lesions were observed related to toxicity in both the treatment and control groups. The histopathological scoring revealed no abnormalities and lesions were noticed from the tissue sections of the liver and kidneys of mice in all treatments and control groups as shown in Figs 2 (a) to (j).

Table 1 Behavioural and physiological changes in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract in mice

Behaviour and physiology	Control (0-24h)	Vehicle (0-24h)	Low dose (1000 mg) (0-24h)	Medium dose (2000 mg) (0-24h)	High Dose (5000 mg) (0-24h)
Skin and fur colour	Normal	Normal	Normal	Normal	Normal
Increase movement	No	No	No	No	No
Dyspnea	No	No	No	No	No
Incoordination	No	No	No	No	No
Convulsion	No	No	No	No	No

Table 2 The body weight (mean ± SEM) in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract in mice

Day/Week	Control	Vehicle	Low dose (1000 mg)	Medium dose (2000 mg)	High dose (5000 mg)
Day 0	25.60 ± 1.62[a]	25.84 ± 0.50[a]	26.36 ± 0.24[a]	25.08 ± 0.59[a]	25.74 ± 0.76[a]
Day 7 (Week 1)	25.04 ± 1.48[a]	25.34 ± 0.27[a]	25.96 ± 0.35[a]	24.62 ± 0.86[a]	24.94 ± 0.66[a]
Day 14 (Week 2)	25.38 ± 1.33[a]	26.02 ± 0.37[a]	26.66 ± 0.30[a]	25.40 ± 0.59[a]	25.94 ± 0.41[a]

*Values in the same row with similar superscript letter were not significantly different ($p > 0.05$).

Table 3 The relative organs weights (mean ± SEM) in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract in mice

Organs	Control	Vehicle	Low dose (1000 mg)	Medium dose (2000 mg)	High dose (5000 mg)
Liver	4.42 ± 0.30 [a]	4.24 ± 0.20[a]	4.12 ± 0.18[a]	3.91 ± 0.25[a]	4.29 ± 0.04[a]
Kidneys	1.30 ± 0.09 [a]	1.24 ± 0.03[a]	1.15 ± 0.06[a]	1.21 ± 0.07[a]	1.26 ± 0.02[a]
Lungs	0.71 ± 0.06 [a]	0.69 ± 0.06[a]	0.75 ± 0.06[a]	0.74 ± 0.08[a]	0.71 ± 0.04[a]
Heart	0.61 ± 0.06 [a]	0.53 ± 0.03[a]	0.50 ± 0.04[a]	0.52 ± 0.03[a]	0.62 ± 0.05[a]
Brain	1.66 ± 0.12 [a]	1.65 ± 0.05[a]	1.62 ± 0.02[a]	1.68 ± 0.06[a]	1.61 ± 0.06[a]
Testes	0.80 ± 0.08[a]	0.84 ± 0.05[a]	1.04 ± 0.16[a]	1.09 ± 0.20[a]	1.10 ± 0.23[a]
Spleen	0.29 ± 0.03[a]	0.30 ± 0.06[a]	0.25 ± 0.02[a]	0.30 ± 0.05[a]	0.24 ± 0.01[a]

*Values in the same row with similar superscript letter were not significantly different ($p > 0.05$).

Table 4 Mortality of mice in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract

Week/ Group	Control	Vehicle	Low dose (1000 mg)	Medium dose (2000mg)	High dose (5000mg)
Number of the animals used	5	5	5	5	5
Mortality at week 1	0	0	0	0	0
Mortality at week 2	0	0	0	0	0

Table 5 The hemograms and platelets evaluation (mean ± SEM) in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract in mice

Parameters	Control	Vehicle	Low dose (1000 mg)	Medium dose (2000 mg)	High dose (5000 mg)
RBC ($x10^{12}$/L)	7.34 ± 0.31[a]	7.75 ± 0.35[a]	7.24 ± 0.73[a]	8.094 ± 0.35[a]	7.31 ± 0.35[a]
Hb (g/L)	11.70 ± 0.66[a]	11.80 ± 0.44[a]	11.40 ± 1.51[a]	13.10 ± 0.61[a]	11.40 ± 0.42[a]
PCV(L/L)	33.16 ± 1.74[a]	34.64 ± 1.84[a]	32.80 ± 3.34[a]	36.44 ± 1.73[a]	31.92 ± 1.32[a]
MCV (Fl)	45.08 ± 0.60[ab]	44.62 ± 0.48[ab]	45.34 ± 0.52[b]	45.00 ± 0.45[ab]	43.70 ± 0.45[a]
MCHC(g/L)	35.28 ± 0.28[a]	34.32 ± 1.52[a]	34.14 ± 1.70[a]	35.98 ± 0.37[a]	35.76 ± 0.33[a]
Platelets (10^9/L)	77.0 ± 7.32[a]	110.00 ± 46.95[a]	108.20 ± 22.63[a]	88. 60 ± 15.15[a]	63.80 ± 4.14[a]

*Values in the same row with similar superscript letter were not significantly different ($p > 0.05$).

Table 6 Total white blood cells and differential leucocyte counts (mean ± SEM) in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract in mice

Parameters	Control	Vehicle	Low dose (1000 mg)	Medium dose (2000 mg)	High dose (5000 mg)
WBC(x10^9/L)	3.42 ± 0.38^a	2.82 ± 0.32^a	2.86 ± 0.52^a	2.84 ± 0.57^a	2.16 ± 0.24^a
Neutrophils (x10^9/L)	0.23± 0.04^a	0.17 ± 0.08^a	0.12 ± 0.57^a	0.13 ± 0.03^a	0.11 ± 0.04^a
Lymphocytes (x10^9/L)	2.68 ± 0.47^a	2.61 ± 0.30^a	2.70 ± 0.49^a	2.70 ± 0.55^a	2.00 ± 0.21^a
Monocytes (x10^9/L)	0.03 ± 0.002^a	0.02 ± 0.01^a	0.03 ± 0.02^a	0.01 ± 0.005^a	0.03 ± 0.02^a
Eosinophils (x10^9/L)	0.02 ± 0.02^a	0.03 ± 0.03^a	0.01 ± 0.01^a	0.00 ± 0.00^a	0.03 ± 0.02^a
Basophils (x10^9/L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*Values in the same row with similar superscript letter were not significantly different ($p > 0.05$).

Table 7 Renal and hepatic evaluation in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract in mice

Parameters	Control	Vehicle	Low dose (1000 mg)	Medium dose (2000 mg)	High dose (5000 mg)
Urea (mmol/L)	25.80 ± 0.80^{ab}	28.40 ± 2.06^b	23.2 ± 6.01^{ab}	15.20 ± 4.87^a	26.00 ± 3.20^{ab}
Creatinine (µmol/L)	0.28 ± 0.07^a	0.24 ± 0.02^a	0.18 ± 0.05^a	0.16 ± 0.04^a	0.26 ± 0.04^a
ALT (U/L)	124.20 ± 4.35^b	102.80 ± 8.71^{ab}	47.00 ± 16.51^a	99.20 ± 29.88^{ab}	117.60 ± 28.16^b

*Values in the same row with similar superscript letter were not significantly different ($p > 0.05$).

Table 8 Protein evaluation in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract in mice

Parameter	Control	Vehicle	Low dose (1000 mg)	Medium dose (2000 mg)	High dose (5000 mg)
Total protein (g/L)	6.28 ± 0.20^a	6.44 ± 0.26^a	6.56 ±0.60^a	6.68 ± 0.12^a	7.02 ± 0.29^a
Albumin (g/L)	2.98 ± 0.14^a	3.06 ± 0.11^a	2.78 ±0.10^a	2.70 ± 0.20^a	2.90 ± 0.29^a
Globulin (g/L)	3.26 ± 0.10^a	3.38 ± 0.12^a	3.78 ± 0.61^a	3.98 ± 0.23^a	4.08 ± 0.29^a

*Values in the same row with similar superscript letter were not significantly different ($p > 0.05$).

Discussion

Herbal products prepared from medicinal plants are commonly claimed to be safe and do not produce side effects to general health and wellbeing. They are typically self-prescribed by consumers who lack dosage control. Therefore, the toxicological study of medicinal plant extracts planned for use in the medical and veterinary fields is significant to determine its potentially harmful effects (Zahi *et al*., 2015; Nurul *et al*., 2018). In this study, the acute oral toxicity of *O. indicum* leaf ethanolic extract was assessed successfully before further evaluation of its potential as a natural herbal supplement.

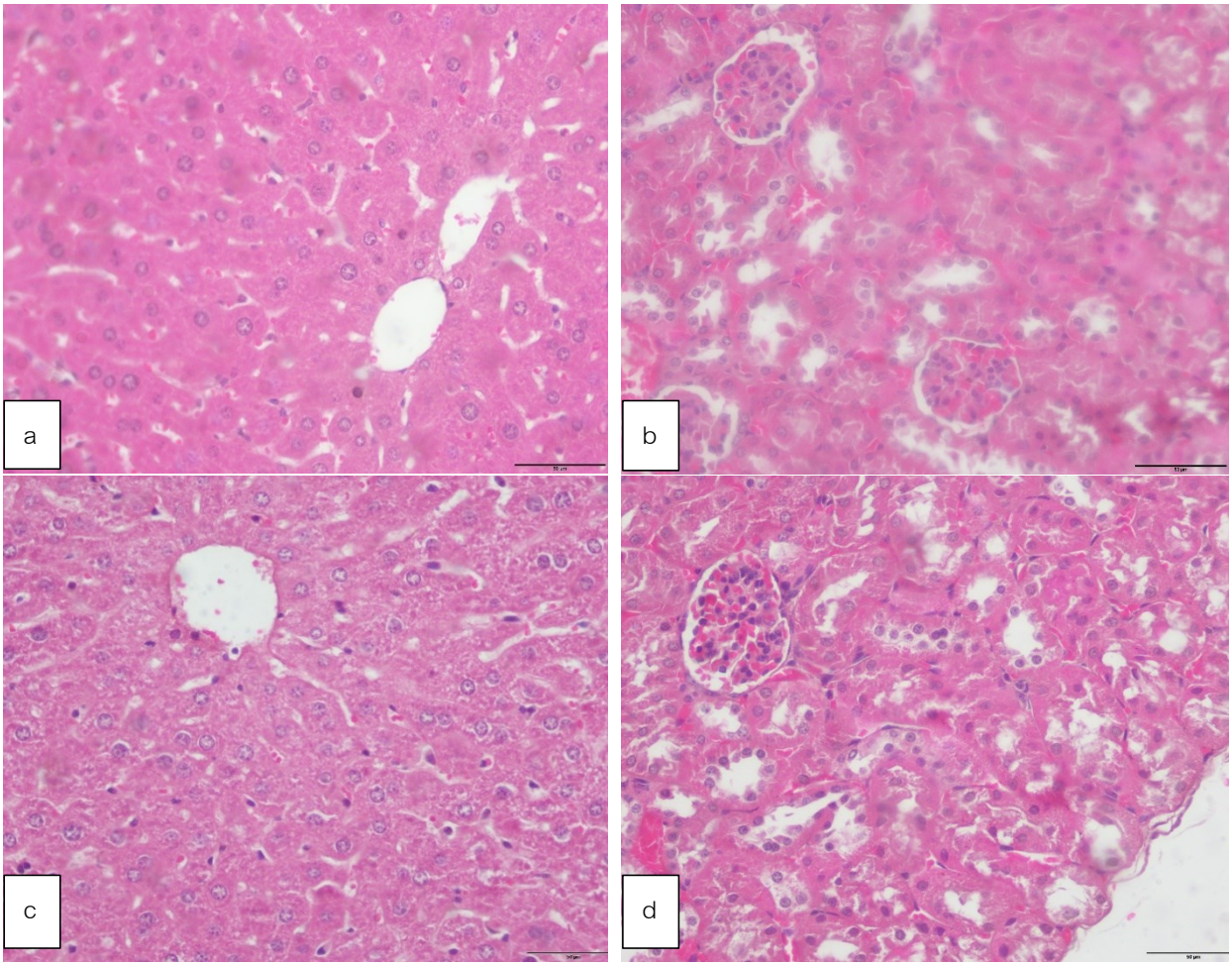
Observation of mortality rates and changes in behaviour are crucial in toxicity research. This is because the administration of extract will produce either mortality or behavioural changes if the plant is toxic at specific doses. In the present study, all groups treated with extract at different doses displayed an absence of mortality and normal behaviour. In addition, the LD_{50} of the *O. indicum* extract was up to 5000 mg/kg bw which suggests a wide margin of safety for therapeutic doses. The findings were similar to a study conducted on acute oral toxicity evaluation of *Cassia fistula* which demonstrated no signs of mortality, toxicity or behavioural changes at the highest dose of 5000 mg/kg bw in mice (Jothy *et al*., 2011).

One criterion used to determine the health status of laboratory animals is body weight. Changes in body weight due to exposure to potentially toxic substances can indicate an effect of toxicity (Reduan *et al*., 2020). Herbal extracts can suppress animal appetite which contributes to a decrease in animal body weight. In this current study, weekly body weights reported the absence of any significant difference in all groups which suggests that the extract is safe for oral administration. Similar findings were reported by Li *et al*., (2017) who revealed no significant alteration found in body weights of mice up to 3000 mg/kg bw dose of *Eriobotrya japonica* leaf extract. In addition, relative organ weight is important in assessing the level of injury to the organs. The organs may undergo cellular changes such as atrophy, hypotrophy, hypertrophy, hyperplasia and hypoplasia due to the direct effects of toxicants on the cells which may alter the organ weights. The primary organs affected by the metabolic processes caused by toxic substances are the heart, lungs, liver, kidney and spleen (Mohamed *et al*., 2011). In the current analysis, the relative organ weight ratio to body weight showed no significant changes in all groups of mice indicating that the extract did not induce significant cellular injury to the vital organs. Whereas, another study using a higher dose of sesamol at 2000 mg/kg bw caused a significant decrease in liver weight as compared to the control mice (Khan *et al.* 2016).

Blood parameters analysis is also vital in the evaluation of the toxic effects of medicinal plants as the blood cells are first exposed to toxicants (Aliyu *et al*., 2020). The toxicants may directly damage the mature cells in the blood circulation or affect the hematopoietic tissue in the bone marrow. This may result in a

reduction of blood cell count and affect the health status of the animal (Reduan *et al*. 2020). In addition, the severity of toxicity can be predicted from the significant alteration of the blood parameters (Nurul *et al*., 2018). In this study, there were no significant alterations in all groups for all haemogram parameters except in mean corpuscular volume. However, the values were still within the normal range. These findings suggest that the extract administrated even at the 5000 mg/kg bw dose did not affect the haematopoietic system of the mice. In another study conducted by Khan *et al*. (2016), there were significant differences with an increment of red blood cells, haemoglobin, haematocrit, mean platelet volume and lymphocyte and decreased granulocyte in treatment mice administered with 2000 mg/kg bw sesamol extract.

On the other hand, serum biochemistry analyses are carried out to assess the severity of organ injury preceding the extract administration. Liver enzymes of alanine transaminase (ALT) and nitrogenous wastes marker of the kidneys such as blood urea nitrogen (BUN) and creatinine are typically used as markers for the liver and kidney toxicity as they are directly involved in detoxification of metabolites (Dina *et al*, 2011; Sajjaratul *et al*., 2016; Aliyu *et al*. 2020). The albumin is synthesized in the liver and liver toxicity and disease may impair albumin production. In this study, *O. indicum* leaf ethanolic extract did not induce significant changes in serum biochemical parameters of creatinine and protein concentrations of albumin and globulin. Even though significant differences were observed in the urea and alanine transaminase parameters, the values were within normal limits. Hence, the present study indicates the absence of systemic toxicity induced following oral administration of the extract on the mice at a dosage of up to 5000 mg/kg bw. These findings were similar to another acute toxicity study of *Rhaphidophora decursiva* which demonstrated no apparent treatment-related adverse effects on the liver and kidney parameters on rats up to 3500 mg/kg bw dose of the extract (Arsad *et al*., 2013). In contrast, previous studies demonstrated increments in ALT (Ugwah-Oguejiofor *et al*., 2019) and blood urea nitrogen (Teshome *et al*., 2008) which suggest the toxic potential of the plant extracts.



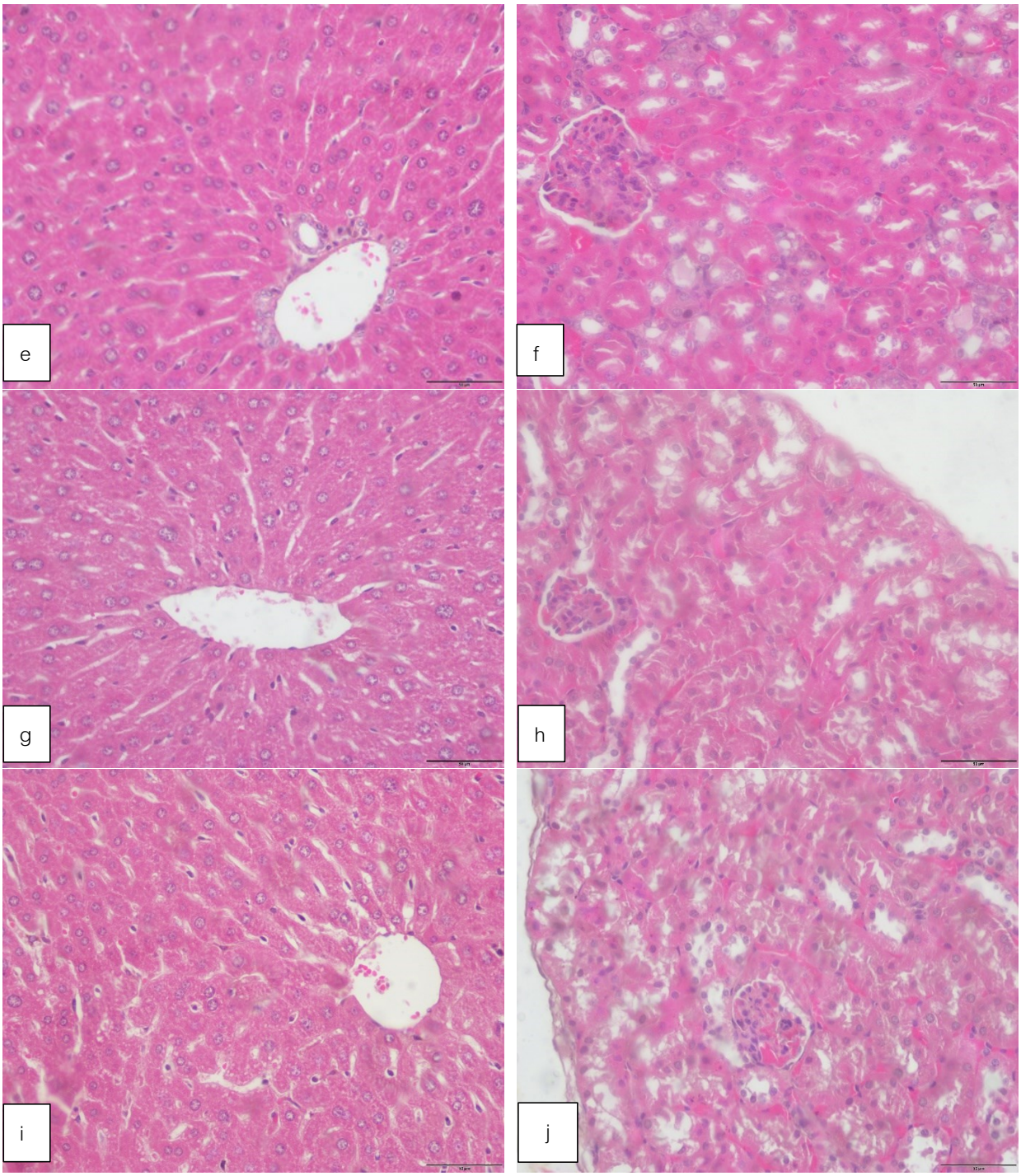


Figure 2 Liver and kidney sections in acute oral toxicity study of *Oroxylum indicum* leaf ethanolic extract. There were no histopathological changes observed in these organs in both treatment and control groups. Liver (a) and kidney (b) sections of the control group mice; Liver (c) and kidney (d) sections of the vehicle group mice; Liver (e) and kidney (f) sections of the low dose, 1000 mg/kg of group mice; Liver (g) and kidney (h) sections of the medium dose, 2000 mg/kg of group mice; Liver (i) and kidney (j) sections of the high dose, 5000 mg/kg of group mice (H&E, 40x magnification).

The histopathological evaluation provides supporting evidence for the biochemical and haematological results, as stated in OECD guidelines 420 of 2001. It is also an important tool for assessing pathological changes in the organs due to systemic toxicity (Arsad *et al*., 2014; Sajjaratul *et al*., 2016; Aliyu *et al*. 2020). The histopathological scoring in the present study revealed the absence of lesions observed in the kidneys and liver in all groups. These findings were supported with normal ranges of haemogram, serum biochemistry parameters and normal gross findings of the organs. The findings in this study were in accordance with a study conducted by Yuet Ping *et al*., (2013) who reported the normal structure of the liver and kidneys after administration of 5000 mg/kg bw of *Euphorbia hirta* extract and there was an absence of gross pathological lesion in the organs. In contrast, histological assessments of liver and kidneys of mice orally induced with 800 mg/kg bw methanolic extract of *Hibiscus rosa-sinensis* in mice showed dilated sinusoids, apoptotic nuclei and inflammatory infiltrate in the sinusoid of the liver. Additionally, the

disorganisation of tubules and glomeruli and enlarged interstitial spaces could also be observed in the kidneys compared to the control group (Nath and Yadav, 2015). Meanwhile, another study showed significant differences with mild to moderate lesions of hepatic necrosis, very mild hepatic degeneration and hepatitis in Sprague Dawley rats inoculated with high doses of *Mariposa christia vespertilionis* at 1480 mg/kg bw (Nurul *et al*. 2018).

In conclusion, results in this study suggest the oral administration of *Oroxylum indicum* leaf extract up to 5000 mg/kg bw once at the initial stage of experiment did not produce any toxicological effects in mice. *Oroxylum indicum* leaves can be recommended and indicated safe for medicinal use in humans as they do not cause systemic toxic reactions in mice.

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