Brine shrimp lethality and acute oral toxicity studies on *Swietenia mahagoni* (Linn.) Jacq. seed methanolic extract

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ABSTRACT

Background: The seeds of *Swietenia mahagoni* have been applied in folk medicine for the treatment of hypertension, diabetes, malaria, amoebiasis, cough, chest pain, and intestinal parasitism. Here we are the first to report on the toxicity of the *Swietenia mahagoni* crude methanolic (SMCM) seed extract. **Methods:** SMCM seed extract has been studied for its brine shrimp lethality and acute oral toxicity, in mice. **Results:** The brine shrimp lethality bioassay shows a moderate cytotoxicity at high concentration. The LC50 for the extract is 0.68 mg/ml at 24 hours of exposure. The LD50 of the SMCM seed extract for acute oral toxicity in mice is greater than 5000 mg/kg. **Conclusion:** This study demonstrates that *Swietenia mahagoni* crude methanolic seed extract may contain bioactive compounds of potential therapeutic significance which are relatively safe from toxic effects, and can compromise the medicinal use of this plant in folk medicine.

Key words: Acute oral toxicity, brine Shrimp, *Swietenia mahagoni* seeds

INTRODUCTION

Medicinal plants are natural resources yielding valuable phytochemical products, which are often used in the treatment of various diseases. A substantial part of the population in developing countries, use folk medicines for their daily healthcare.[1] Some of the traditional medicine involves the use of crude plant extracts, which may contain an extensive diversity of molecules, often with indefinite biological effects.[1] However, most of the information available to the consumer with regard to the medicinal herbs is not backed by credible scientific data. For this reason, research is carried out, to determine the toxicity of medicinal plants.

Swietenia mahagoni (Linn.) Jacq. (Meliaceae) is a large, deciduous, and economically important timber tree native to the West Indies. This timber tree is mainly cultivated in tropical zones, such as, India, Malaysia, and Southern China.[2] *Swietenia mahagoni* is a valuable timber tree closely related to the African genus Khaya, and one of the most popular traditional medicines in Africa. The decoction of the bark of these mahoganies is extensively used as febrifuge, and can be associated with its use as an antimalarial drug. *Swietenia mahagoni* seeds have been applied as folk medicine for the treatment of hypertension, diabetes, and malaria.[3] The seeds have also been reported to have medicinal value for treatment of cancer, amoebiasis, cough, chest pain, and intestinal parasitism. However the claim that *Swietenia mahagoni* seeds extract safe usage in folk medicine is unsubstantiated by scientific studies. Hence, the current study has been undertaken to investigate the toxicity of *Swietenia mahagoni* crude methanolic seed extract (SMCM) in laboratory animals and brine shrimp.

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MATERIALS AND METHODS

Plant materials

The *Swietenia mahagoni* seeds were collected in the state of Penang, Malaysia. The plant was identified by a botanist in the School of Biological Sciences of Universiti Sains Malaysia. The seeds were washed with running tap water to remove the dirt, prior to the drying process. The seeds were cut into small pieces and dried at 40°C for one week.

The seeds were powdered using a blender (New Deluhe, Suruchi).

Extract preparation

The powdered seeds were extracted with methanol by the maceration method for four days. The extract was filtered through Whatman filter papers and the filtrate was collected and concentrated in a rotary evaporator (RII0 Buchi) at 40°C. The concentrated extract was dried in an oven at 40°C for three days and stored under refrigeration until further use. The SMCM seed extract was dissolved in Prophyleneglycol/Tween 80/water (4:1:4) (to enhance the solubility) for further studies. All the test samples were prepared freshly on the day of the experimental studies.

Brine Shrimp Lethality Assay

Brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water prepared from commercial sea salt 38 g/L.[4] A lamp was placed above the open side of the tank to attract the hatched shrimps close to the tank wall. After 24 hours, the shrimps matured as nauplii (*Artemia salina)* and were ready for the assay. The brine shrimp lethality bioassay was carried out on the SMCM seed extract using the standard procedure.[5,6] Twenty milligrams of the extract was dissolved in 1 ml of Prophyleneglycol/Tween 80/water (4:1:4) to give a crude extract concentration of 20 mg/mL. A two-fold serial dilution was carried out with salt water to obtain a test solution in the range of 0.1 – 10 mg/mL. Each concentration was tested in triplicate. A test tube containing prophyleneglycol/Tween 80/water (4:1:4) in 5 ml of salt water was used as the negative control. Ten milligrams of potassium dichromate (as positive control) was dissolved in prophyleneglycol/Tween 80/water (4:1:4) and serially diluted, to obtain test concentrations ranging from 0.01 to 5 mg/ml. A suspension of larvae (0.1 ml), containing about 10 – 15 larvae, was added into each test tube and incubated for 24 hours. The test tubes were then examined, and the number of dead larvae in each bottle was counted after 6, 12, and 24 hours. The total number of shrimps in each bottle was counted and recorded. The death percentage (Equation 1) and lethal concentration (LC_{50}) were determined using statistical analysis.

Percentage of Death (%): (Total naupii – Alive naupii) x 100%/Total naupii

In vivo acute toxicity evaluation

Experimental animals

The animals were acclimatized in cages under standard environmental conditions of light/dark cycles (12 hours/12 hours) and temperature (23 ± 1°C). The animals had free access to tap water and a standard pellet diet, except for a short fasting period of four hours before and after the oral administration of single doses of the SMCM seed extract. The studies were approved by the Institutional Animal Ethics Committee (IAEC) of the School of Pharmaceutical Sciences, Universiti Sains Malaysia, and were performed in accordance with the wide-reaching established pattern of laboratory animal use and care.[7]

Acute oral toxicity

Acute oral toxicity study was carried out *in vivo*. The animals were divided into the control and five treated groups, each consisting of ten animals (five males and five females). All the animals were subjected to four hours of fasting prior to treatment. The control group received Prophyleneglycol/Tween 80/water (4:1:4) and the treatment groups received 25, 200, 2000, and 5000 mg/kg of SMCM seed extract, respectively. The animals were observed for one hour after treatment, and then intermittently for four hours, and thereafter the mice were further observed for up to 14 days following treatment. Clinical signs such as weakness or aggressiveness, food refusal, loss of weight, diarrhea, discharge from eyes and ears, noisy breathing, and the number of deaths in each treated group were monitored carefully. Subsequently, the animals were sacrificed by cervical dislocation. Vital organs including the liver, lung, spleen, kidneys, and heart were removed for macroscopic analysis, and weighed and preserved in 4% buffered formalin solution for a histology study.[8]

Relative organ weight

Body weight and weight of the organs from the control and the test groups were measured and recorded. The relative organ weight of each animal was then calculated as follows.[8] Relative organ weight: (Absolute organ weight x 100%)/Body weight of mice on the day of sacrifice.

Histopathology Analysis

All the samples were preserved in 4% buffered formaldehyde and subsequently embedded in paraffin. The paraffin-embedded samples were cut at eight microns using a rotary microtome and prepared for microscopic tissue slides. The tissue slides were stained with hematoxylin and eosin for histological examination. The histological sections were examined using Leica light microscope in the Microscopic Unit of the School of Biological Science, Universiti Sains Malaysia.[9]

Statistical analysis

The results were expressed as the mean value ± standard error of mean (S.E.M). Comparisons were performed within groups by the analysis of variance, using the ANOVA test. Significant differences between control and experimental groups were assessed by the SigmaStat software. A probability level $P < 0.05$ was considered to

indicate statistical significance.

RESULTS

The median lethal concentration of the brine shrimp lethality assay (LC_{50}) and the median lethal dose of the acute oral toxicity study (LD_{50}) for *Swietenia mahagoni* crude methanolic (SMCM) seed extract are given in Table 1. The result of the brine shrimp lethality bioassay [Figure 1] shows the extract to be moderately toxic to brine shrimp (LC_{50}: 1.1 mg/ml at 12 hours and 0.68 mg/ml at 24 hours).

There was no effect of lethality on any of the concentrations of the extract at six hours. The brine shrimp were still actively moving in the test materials. The SMCM seed extract was not toxic for the brine shrimp at 12 hours of exposure because the LC_{50} value was higher than 1 mg/ml. Nevertheless the extract showed a toxicity effect at 24 hours, with LC_{50} value at 0.68 mg/ml, for the extract. This suggested that the extract could contain compounds that are cytotoxic, as the LC_{50} value was low at 24 hours. [10] Potassium dichromate served as the positive control for this brine shrimp lethality assay. The LC_{50} values for the positive control at 6, 12, and 24 hours were 0.76, 0.56, and 0.28 mg/ml, respectively. There was a lethality effect in the brine shrimp two hours after exposure to the higher concentration of potassium dichromate (5, 2.5, and 1.25 mg/ml). Lagarto,[11] demonstrated that there was a good correlation ($r = 0.85$; $P < 0.05$) in the LC_{50} of the brine shrimp lethality assay and LD_{50} of the acute oral toxicity assay in mice. This confirmed that the brine shrimp lethality assay was useful for the screening of the plant extract, to predict the toxicity level.

Table 1: Median lethal concentration (LC_{50}) and Median lethal dosage (LD_{50}) of *Swietenia mahagoni* crude methanolic (SMCM) seed extract against brine shrimp lethality assay and acute oral toxicity evaluation

Assays		SMCM seed extract	Potassium dichromate
Brine Shrimp Lethality Assay (LC_{50})	6 hours	No death	0.76 mg/ml
	12 hours	1.107 mg/ml	0.56 mg/ml
	24 hours	0.68 mg/ml	0.28 mg/ml
Acute Oral Toxicity Study (LD_{50})	14 days	> 5000 mg/kg	ND

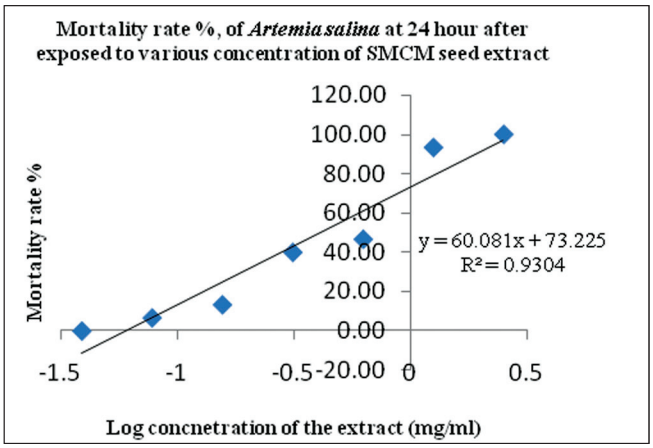

Figure 1: The graph shows the mortality rate % of Artemia salina at 24 hours, after being exposed to various concentrations of SMCM seed extract.

Based on the acute oral toxicity study, it was concluded that a dose of 5000 mg/kg of SMCM seed extract, given orally, appeared to be preferably non-toxic [Table 1]. The LD50 of the SMCM seed extract for acute oral toxicity was greater than 5000 mg/kg. There was no mortality or any sign of behavioral change or toxicity observed after oral administration of SMCM seed extract at 25, 200 2000, and 5000 mg/kg body weight in mice. There were no changes in the character of the stool, urine or eye color of all the animals in the control and test groups. The relative body weights of the male mice decreased at 25 mg/kg and increased at 5000 mg/kg dose of administration. The female mice relative body weight was increased at 5000 mg/kg dose and it showed significant difference ($P < 0.05$) compared to the control group. There were no significant changes in the relative body weight in the female and male mice for the remaining groups ($P > 0.05$) [Table 2]. There were no significant changes in the relative organ weight of the liver, lung, heart, kidneys, and spleen for the tested groups in male mice, except at 2000 mg/kg, for the liver. There was a slight decrease in the relative organ weight of the liver. There were no significant changes for the female heart and spleen up to 5000 mg/kg of the dose. There were significant changes in the relative organ weight only at 5000 mg/kg for kidneys, lung, and liver. The relative weight of the kidney increased slightly, yet the relative organ weight of the lung and liver declined a little. The female mice showed a more sensitive response to oral administration of SMCM seed extract at tested doses. Further confirmation of this finding should be supported by histopathological analysis, to verify the toxicity of the test compound. Although there were significant differences in the relative body and organ weight, the histopathological analysis showed that the extract had not produced significant pathology in this acute oral toxicity evaluation. No alterations were observed in most of the organs of both sexes of control animals, as well as in animals treated with 5000 mg/kg SMCM seed extract. The photomicrographs of the vital organs of the control and 5000 mg/kg extract-treated groups, both male and female, had normal lobular architecture. The heart cross-section showed clear cardiac myocytes and its arrangement was

Table 2: Effects of oral acute treatment for *Swietenia mahagoni* crude methanolic seed extract on mice

Parameters	Swietenia mahagoni crude methanolic (SMCM) seed extract									
	Male					Female				
	0 mg/kg (control)	25 mg/kg	200 mg/kg	2000 mg/kg	5000 mg/kg	0 mg/kg (control)	25 mg/kg	200 mg/kg	2000 mg/kg	5000 mg/kg
Number of deaths	0	0	0	0	0	0	0	0	0	0
Body weight gain (g)	4.54 ± 0.27	1.1 ±0.35*	2.91 ±0.82	4.32 ± 0.55	8.05 ± 0.76*	2.55 ± 0.57	1.64 ± 0.44	1.45 ± 0.37	1.89 ± 0.22	4.26 ±0.32*
Relative organ weight (%)										
Liver	7.31±0.27	7.43±0.34	7.50±0.34	6.06±0.13*	6.42±0.01	5.44±0.14	6.94±0.12*	6.24±0.41*	5.86±0.09*	6.51±0.10*
Lungs	0.84±0.09	0.86±0.05	1.03±0.15	0.87±0.05	0.76±0.02	1.0±0.03	1.58±0.36	0.95±0.01	0.95±0.04	0.71±0.03*
Spleen	0.48±0.05	0.53±0.08	0.76±0.01	0.60±0.01	0.33±0.01	0.34±0.01	0.84±0.08*	0.65±0.09*	0.57±0.08	0.40±0.03
Kidneys	1.66±0.05	1.71±0.09	1.53±0.02	1.76±0.02	1.69±0.03	1.48±0.03	1.37±0.02	1.32±0.03	1.31±0.01	1.29±0.04*
Heart	0.51±0.01	0.53±0.02	0.51±0.01	0.56±0.02	0.51±0.02	0.47±0.03	0.41±0.01	0.52±0.01	0.47±0.01	0.46±0.02

The SMCM seed extract was administrated p.o as a single dose. Values are expressed as mean ± S.E.M of five animals. *Significant differences when compared to control group, $P < 0.05$. Control group was administrated with Proplyneglycol/Tween 80/ water (4:1:4).

in good order. Besides that, there were no hemorrhages, necrosis or inflammatory exudates in the cross-section of the heart [Figure 2]. The cross-sections of the glomeruli, distal, and proximal tubules in the kidney appeared to be normal in both male and female mice. There were also no interstitial and intraglomerular congestion tubular atrophies due to loss of epithelial arrangement and inflammatory infiltration. All the nucleoli in the kidney cells were clearly visible. There was no degeneration, bleeding or necrosis in the kidney cells [Figure 3]. There was no hepatocellular, central vein necrosis or sinusoidal congestion in the liver. The hepatocytes were still clearly visible and there were no lyses in the blood cells or cytoarchitectural distortions in the liver of any treated mice groups. The cross-section of the liver showed no neutrophil, lymphocyte or macrophage infiltration in the liver [Figure 4]. However there was fatty acid accumulation in the liver architectural at 5000 mg/kg of the extract, but it did not instigate any mortality in the tested group of mice. The morphology of lung tissue did not show any significant difference when compared with the control group. There was no bronchiole or alveoli collapse, no alveolar epithelial denaturation, and no inflammatory cell infiltration surrounding the bronchi of the treated groups [Figure 5]. From the cross-section of the spleen, the tissue structure of the spleen was normal. There was no hemorrhage and no pathological change in the spleen sinus [Figure 6]. The microscopic evaluations of the selected organs did not reveal abnormalities that could be attributed to the oral administration of SMCM seed extract to the mice.

DISCUSSION

Herbal medicines have received great interest as an alternative to clinical therapy, and the demand for these therapies has currently increased rapidly. The increase in the number of users as opposed to the scarcity of scientific evidences on the safety of medicinal plants, have raised concerns regarding the toxicity and detrimental effects of these remedies,[12] and similar concerns apply to the SMCM seed extract in this study. Hence in this study we report the safety evaluation of the SMCM seed extract. In this

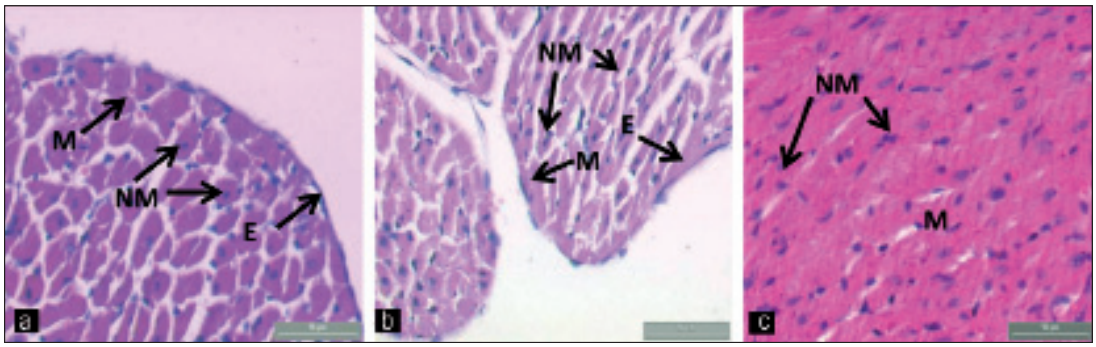

Figure 2: Shows the heart light microscope histological tissue slides of female and male 5000 mg/kg-treated mice following a single oral acute treatment with SMCM seed extract. (a) Heart of control mice (b) treated female mice heart, and (c) heart of treated male mice. M: Myocardium E: Endocardium NM: Nucleus of Myocytes.

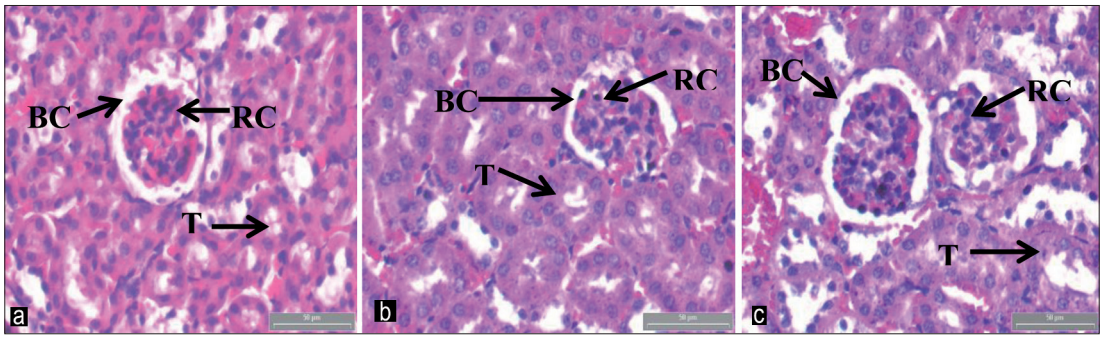

Figure 3: Shows the kidney light microscope histological tissue slides of female and male 5000 mg/kg-treated mice following a single oral acute treatment with SMCM seed extract. (a) Kidney of control mice (b) kidney of treated female mice, and (c) kidney of treated male mice. RC: Renal corpuscles T: renal tubules BC: Bowman's space.

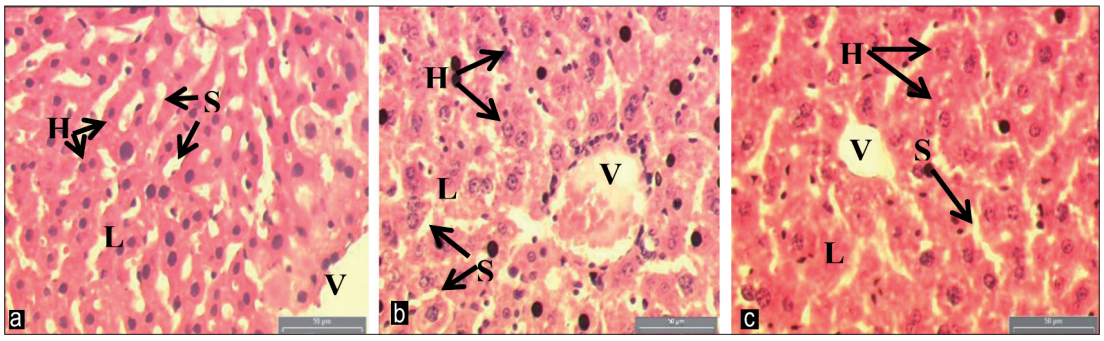

Figure 4: Shows the liver light microscope histological tissue slides of female and male 5000 mg/kg-treated mice following a single oral acute treatment with SMCM seed extract. (a) Liver of control mice (b) liver of treated female mice, and (c) liver of treated male mice. L: Liver lobules V: Central vein of lobule S: Sinusoids H: Hepatocytes.

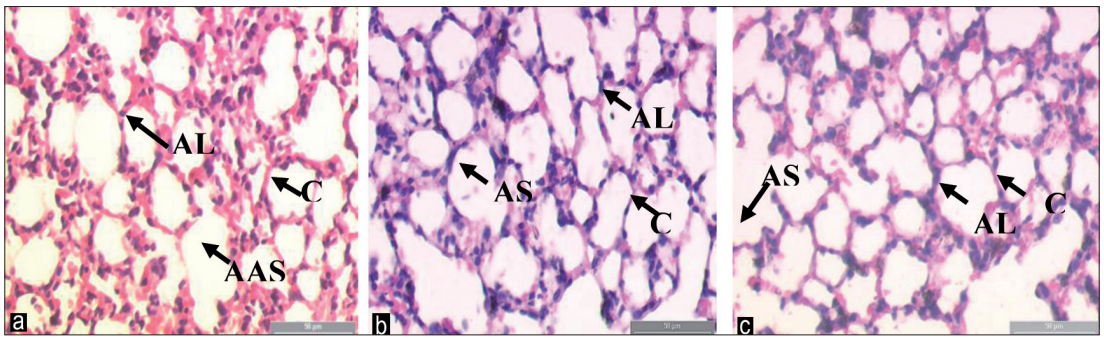

Figure 5: Shows the lung light microscope histological tissue slides of female and male 5000 mg/kg-treated mice following a single oral acute treatment with SMCM seed extract. (a) Lung of control mice (b) lung of treated female mice, and (c) lung of treated male mice. AS: Alveolar air space AL: Alveolar lining cells C: Alveolar capillary.

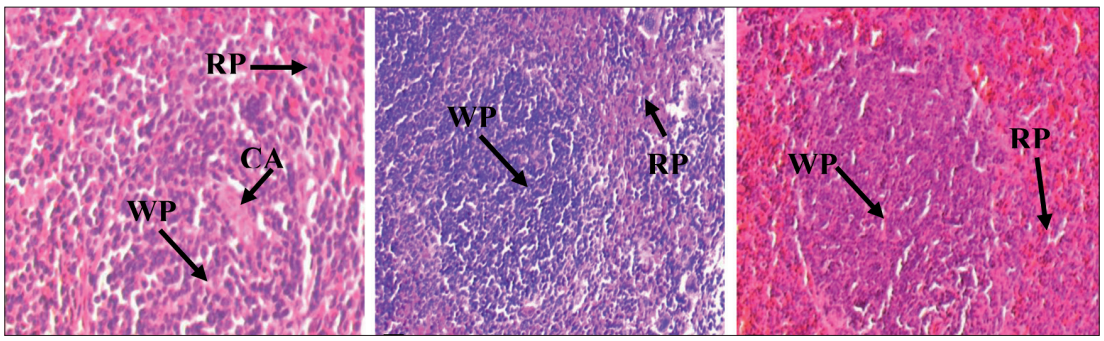

Figure 6: Shows the spleen light microscope histological tissue slides of female and male 5000 mg/kg-treated mice following a single oral acute treatment with SMCM seed extract. (a) Spleen of control mice (b) spleen of treated female mice, and (c) spleen of treated male mice. RP: Red pulp WP: White Pulp CA: Central Artery.

study the SMCM seed extract's LC_{50} was 0.68 mg/ml and it possessed a mild toxicity effect.

It is suggested that the plant extract has a high amount of bioactive substances and may contain compounds that possess cytotoxicity effects. Lagarto,[11] demonstrated that there is a good correlation ($r = 0.85$; $P < 0.05$) between the LC_{50} of the brine shrimp lethality assay and the LD_{50} of the acute oral toxicity assay in mice. Based on the Lagarto[11] correlation result; the brine shrimp $LC_{50} < 10$ µg/ml possesses LD_{50} between 100 and 1000 mg/kg; $LC_{50} < 20$ µg/ml possesses LD_{50} between 1000 and 2500 mg/kg, and $LC_{50} > 25$ µg/ml possesses LD_{50} between 2500 and 8000 mg/kg. We can assume that the LD_{50} of oral acute toxicity for SMCM seed extract also will be more than 2500 mg/kg, because the LC_{50} of brine shrimp lethality assay is 680 µg/ml. Hence, this finding indicates that the extract could be developed as an anticancer agent with further detailed study.

The oral LD_{50} value in this study suggests that the SMCM seed extract is a relatively nontoxic plant. The chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the Organization for Economic Co-operation and Development (OECD, Paris, France)[13] are as follows: very toxic ≤ 5 mg/kg; 5 > toxic ≤ 50 mg/kg; 50 > harmful ≤ 500 mg/kg; and 500 > no label ≤ 2000 mg/kg.[14] Therefore, an LD_{50} of more than 5000 mg/kg of SMCM seed extract is an indication that the extract is safe and has no adverse effect. The results of the current study concur with the use of this plant by traditional healers as traditional medicine. A Word Health Organization survey indicated that about 70 – 80% of the world's population rely on non-conventional medicine, mainly of herbal source, in their primary healthcare.[15] Our present study shows that SMCM seed extract does not exhibit any apparent toxicity and may be used as an antimicrobial or antioxidant agent in known dosages, especially in rural communities, where conventional drugs are unaffordable. Studies of this type are needed before a phytotherapeutic agent can be generally recommended for use.

CONCLUSION

This study presents valuable data on the acute oral toxicity of the SMCM seed extract, which should be very useful for any future *in vivo* or clinical study of this seed extract. However, further toxicity studies are needed to determine the effects of this plant on chronic oral toxicity, on animal fetus, pregnant animals, and their reproductive capacity, to complete the safety profile of this extract.

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