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In vivo toxicity study of *Cassia surattensis* flower extract

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ABSTRACT

Medicinal plant like *Cassia surattensis* is used in traditional healthcare as it carries variety of therapeutic properties. The chemical composition analysis of the crude extract revealed the components present in the extract are bioavailable. However, the safety of consuming this plant as medicinal source for long term usage still remains a doubt since lacking of toxicology data on this plant. Methanolic extract of *C. surattensis* flower was subjected to toxicity study in this experiment. The extract was found not toxic in brine shrimp assay with LC_{50} value of 3.32 mg/mL. In oral acute toxicity study mice were administrated orally with a single dose of 5000 mg/mL extract and observed for 14 days for any toxicity sign. Based on the body weight and histopathological examination *C. surattensis* flower extract was found to be non toxic as there were no significant differences in the body weight of the mice. Histopathology analysis on subjected organs did not reveal any pathological condition in the treatment group. Doses of *C. surattensis* flower up to 5000 mg/kg appear to be safe in a mouse model.

Keywords: *Cassia surattensis*, toxicity, oral acute toxicity, histopathology.

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INTRODUCTION

Medicinal plants got its popularity from our great old folks as plants were used commonly in folk medicine since Vedic period as it is rich with various capacities like antimicrobial, antioxidant, antitumor, antidiarrhoeal agent and more to be named [1]. Until today plants are used as a source of traditional medicine by most of the population in developing countries as it is passed on from generations to generations [2]. Most of the plants used in traditional medicine have solid scientific support with regard to their efficacy. There is little information available on the possible risks that the natural products may pose to health [3]. Therefore, evaluating the toxicological effects of any herbal extract intended to be used in animals or humans is a crucial part of its assessment for potential hazards.

Cassia surattensis plant is recognized as medicinal plants throughout in Asian countries. The root of this plant is commonly used to treat snake bites in India [4]. In Malaysia the leaves are boiled and consumed for cough and sore throat [5]. *C. surattensis* bark and leaves are commonly used to treat fungal skin diseases [6]. The assessment of medicinal plants in earlier days was based on their usage to ill various diseases over centuries. Plants must be used with caution because some have the ability to cause adverse or produce toxicity [7]. Toxicity refers to the potential of a substance to cause damage on organism up to any degree if safety precaution is not practiced. In an effort to provide some information on cytotoxic activity of *Cassia surattensis* flower to the quality of this potential therapeutic agent, this article will focus on primary brine shrimp assay and *in vivo* toxicity study.

MATERIALS AND METHODS

Plant Collection and Extraction

Fresh *Cassia surattensis* flowers were harvested in University Science Malaysia (USM), Penang. The flowers were washed thoroughly under the running tap water and dried in the oven for 2 days. Dried flowers were blend to a fine powder using an electronic blender. Plant sample was extracted by weighing 100 g of grounded plant material with 300 ml of methanol and macerated for 4 days with constant shaking. The extract was than filtrated using No.1 Whatman filter paper and concentrated using a rotary evaporator at 60 °C to obtain the final yield in a paste form. Crude extract was than stored at 4 °C until further use.

Gas chromatography–mass spectrometry (GC–MS) analysis

The GCMS analysis was done on a thermo gas chromatograph mass spectrometer (model Shimadzu 2010) equipped with DB-5 capillary column (30 m long., 0.25 mm i.d., film thickness 0.25 µm). The column temperature program was 50 °C for 6 min initially and increases 5 °C per min to reach 250 °C; which than maintained for 30 min. Helium was used as a carrier gas at a flow rate of 1 mL/min (splitless mode). The detector and injector temperatures were both maintained at 250 °C. The quadrupole mass spectrometer was

scanned over a range of 28–400 amu at 1 scan s^{-1}, with an ionising voltage of 70 eV, an ionisation current of 150 Ma and an ion source temperature of 200 °C. In order to determine the Kovats index of the components, a mixture of alkenes (C9–C24) was added to the crude extract before injecting in the GC–MS equipment and analysed under the same conditions as above. The compounds were identified by computer searches in commercial libraries of NIST (National Institute of Standard and Technology) and by their Kovats retention indexes.

Brine Shrimp Toxicity Assay

Preliminary toxicity activity of *C. surattensis* flower extract was monitored by the brine shrimp lethality test according to the protocol described previously by Meyer et al [8]. Brine shrimp eggs (*Artemia salina*) were hatched in artificial seawater (3.8% sodium chloride solution) under artificial light at 28 °C with full aeration. After incubating for 24 hour, the nauplii were attracted to one side of the beaker with a light source and collected with a Pasteur pipette. The test extract were initially dissolved in methanol and further diluted with artificial seawater to provide the required concentrations (0.195, 0.39, 0.78, 1.562, 3.156, 6.25, 12.5, 25, 50 and 100 mg/ml). Approximately 10-15 nauplii were added to each tube containing the sample. Potassium dichromate dissolved in methanol was used as a positive control. Twenty-four hours later, the number of survivors was counted and recorded. The percentage of mortality in each tube and control was determined using the equation:

% Mortality = (number of dead nauplii/initial number of live nauplii) x 100%

Lethality concentration fifties (LC_{50}) for the assay were determined from the percentage mortality against logarithm of concentration graph.

Acute Toxicity Study

Swiss albino mice, weighing between 30-35 g of both sexes were obtained from Universiti Sains Malaysia (USM), Penang animal house. Animals were kept in mice cages and maintained on standard pellet diet and water *ad libitum*. The ethics committee of USM granted permission for this animal experiment. Mice were divided to groups of six animals per sex each. One group served as a control received distilled water alone and the treatment group were treated with methanolic extract of *C. surattensis* flower at the dose of 5000 mg/kg according to the Organization of Economic Cooperation and Development (OECD) guidelines [9]. Test extract was administrated using a gavage in a volume of 10ml/kg [10]. All the animals were fasted overnight but still allowed for free access to water before the treatment was carried out. The animals were maintained for the next 14 days with daily observations. On the 15^{th} day the mice were sacrificed through cervical dislocation and the internal organs such as lung, heart, liver, spleen and kidney were removed, weighed and sent for histopathology examination.

Histopathological Examinations

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For histopathological analysis all the organ samples like lung, heart, liver, spleen and kidney were fixed in 10% neutral formalin for 48 hour in separate vials. Next, dehydration process was performed using few series of alcohol percentage and followed by dealcoholisation using xylene. The organ tissues were than embedded with paraffin wax and sectioned using a microtome. Thin tissue sections were than stained with hematoxylin and eosin (H&E) before the histopathological slides was read.

Statistical analysis

All values are mean±SD obtained from six animals. For statistical analysis, one-way ANOVA with Duncan's variance (SPSS 15) was used to compare the groups. In all the cases a difference was considered significant when p <0.05.

RESULTS AND DISCUSSION

GC–MS analysis

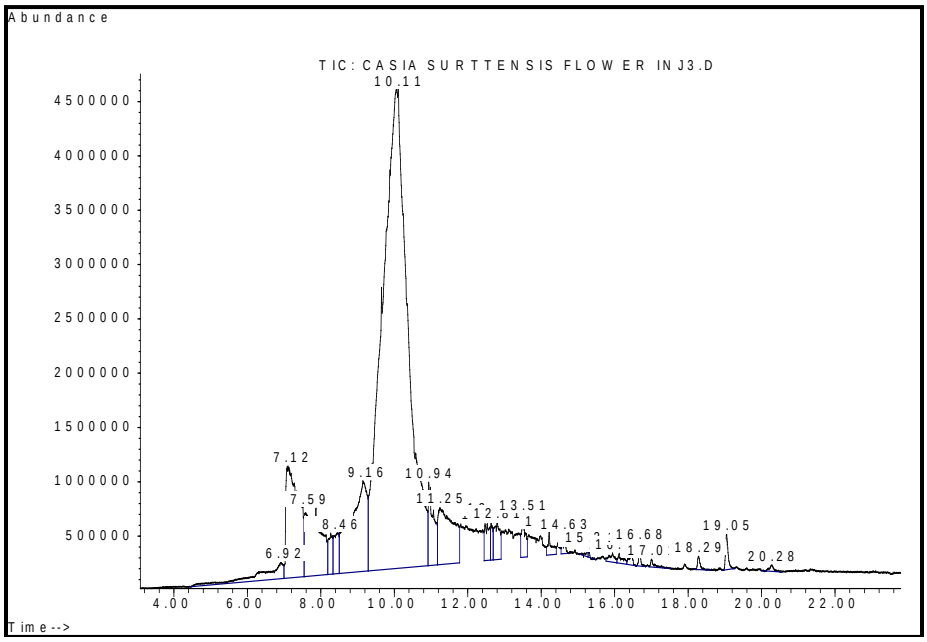

Fig. 1: GCMS chromatogram of crude extract from *Cassia surattensis* flower extract

GCMS analyses of the crude extract of *C. surattensis* flower showed the presence of twenty five compounds with 7 major compounds (Fig. 1). Each of the peaks may represent one or more compounds present in the crude extract. Typically chromatographically separated peaks would contain a single major component. The major components was identified as 1,3-Dioxolane, 2-(1,1-dimethylethy l)-2-methyl (63.01%); Ethanone, 1-[4-(methylthio)phenyl] (1.85%); Resorcinol (7.46%); Molybdenum, (1-butylbenzocyclobute ne)-tricarbonyl (7.91%); N-Cyanobenzene carboximidamide (2.21%); 2,5-Di-O-acetyl-3,4,6-tri-O-methyl -D-mannonitrile (4.51 %); and Phenol, 2,4-bis(1-phenylethyl) (1.11%). This study began with the chemical

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composition analysis of the extract using GC-MS technique. The GC-MS analysis identified few potential bioactive components namely 1,3-Dioxolane, 2-(1,1-dimethylethy l)-2-methyl, Resorcinol and N-Cyanobenzene Carboximidamide in the crude extract. 1,3-Dioxolane, 2-(1,1-dimethylethy l)-2-methyl, Resorcinol and N-Cyanobenzene Carboximidamide have been tested previously and were reported to have a significant antimicrobial and antioxidant activities [11-13].

Brine Shrimp Toxicity Assay

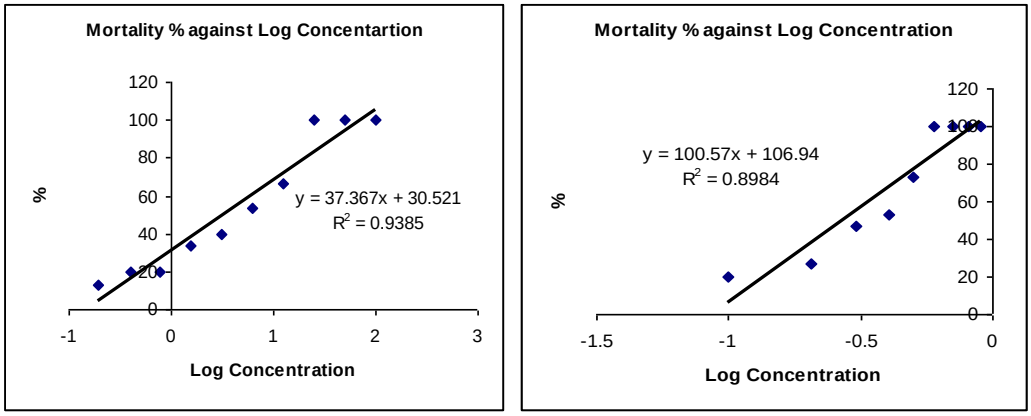

Fig. 2: (a) The toxicity effects of the *Cassia surattensis* flower extract using brine shrimp lethality assay after 24 hour (b) The toxicity effects of the potassium dichromate using brine shrimp lethality assay after 24 hour

The LC_{50} value obtained in the brine shrimp assay for the *C. surattensis* flower extract was 3.32 mg/mL (Fig. 2a). Potassium dichromate which was used as a positive control gave rise to LC_{50} value of 0.27 mg/mL from the mortality percentage against logarithm concentration graph (Fig. 2b). From the brine shrimp assay it was clear that *C. surattensis* flower extract does not posses any toxic effects as the LC_{50} value obtained for this assay was 3.32 mg/mL. Lethality Concentration at 50% value of 1.0 mg/mL is the cut-off point for detecting the toxicity level of any plant extract [14]. Brine shrimp assay is very useful to screen for toxicity activity of plant extracts as preliminary step since it is easy and fast, economical and lack of animal use. But this data it is not sufficient to support the claim that this extract is toxic free and can be taken in any dose. Despite, adequate interlaboratory validation is important and necessary before the plant extract is confirmed to be toxic free [15].

Oral Acute Toxicity

Table 1: Effect of *Cassia surattensis* flower extract on organ body weight index (%) in mice

	Male		Female	
Organ	Control	Treatment	Control	Treatment
Lung	0.13 ± 0.02	0.12 ± 0.03	0.08 ± 0.01	0.12 ± 0.03
Liver	0.56 ± 0.0	0.73 ± 0.08	0.43 ± 0.02	0.51 ± 0.10
Spleen	0.07 ± 0.05	0.03 ± 0.00	0.08 ± 0.00	0.05 ± 0.01
Kidney	0.23 ± 0.03	0.21 ± 0.02	0.13 ± 0.02	0.09 ± 0.02

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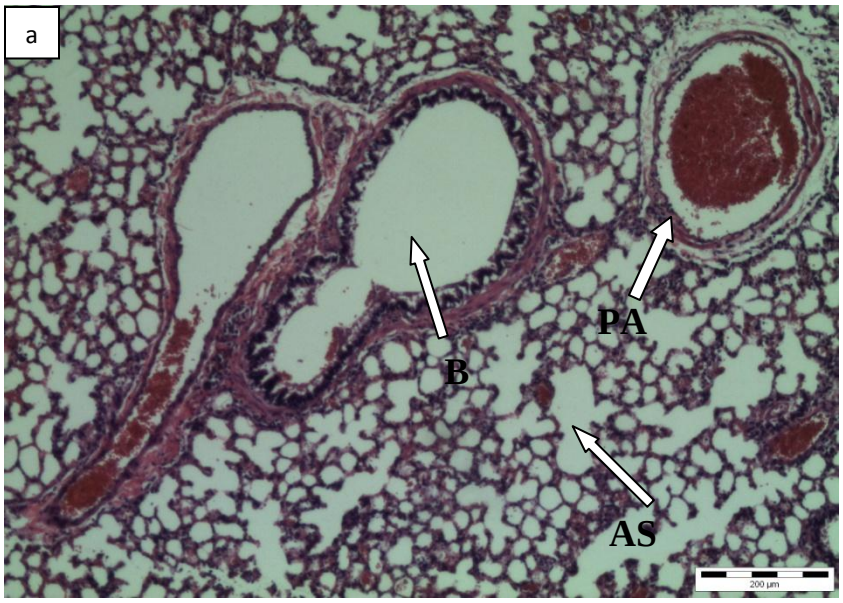
Heart	0.09 ± 0.02	0.07 ± 0.01	0.06 ± 0.00	0.03 ± 0.00
Body Weight (g)	34.50 ± 1.22	35.97 ± 2.47	28.13 ± 0.23	27.4 ± 2.26

Organ body weight index was calculated as (organ weight x 100) / body weight.
Values are mean ± S.D (n=6)
$p < 0.05$ (t-test)

All the Swiss albino mice were observed during 48 hour and morbidity and or mortality were recorded, if happens in the treatment group. But there were no deaths or hazardous signs were recorded during the study period in either control or treated groups. The vital organs and body weight for both the sexes in each group were recorded in table 1. There were no significant changes in the body weight or organ weight of control and treated group. In order to support the preliminary toxicity study and to choose the right dosage for pharmacology activity, *in vivo* toxicity study were conducted using mice as animal model [16]. According to OECD guidelines for testing of chemicals 420 (OECD 2001) only a single dose of 5000 mg/kg extract should be administrated to the animals in oral acute toxicity study. Generally laboratory animals are more sensitive to toxic effects of plant substances which help in justification of toxicity activity [15]. During the experimental period the weight was recorded constantly until all the mice was sacrificed as body weight is the main indicator in toxicity symptoms of any tested materials. Reduce in the body weight will reveal that there is some abnormalities in the animals. But there was not any significant difference in the body weight of treated group compared to control. This shows that *C. surattensis* flower extract is not toxic and to confirm this histopathology examination was performed.

Histopathology Analysis



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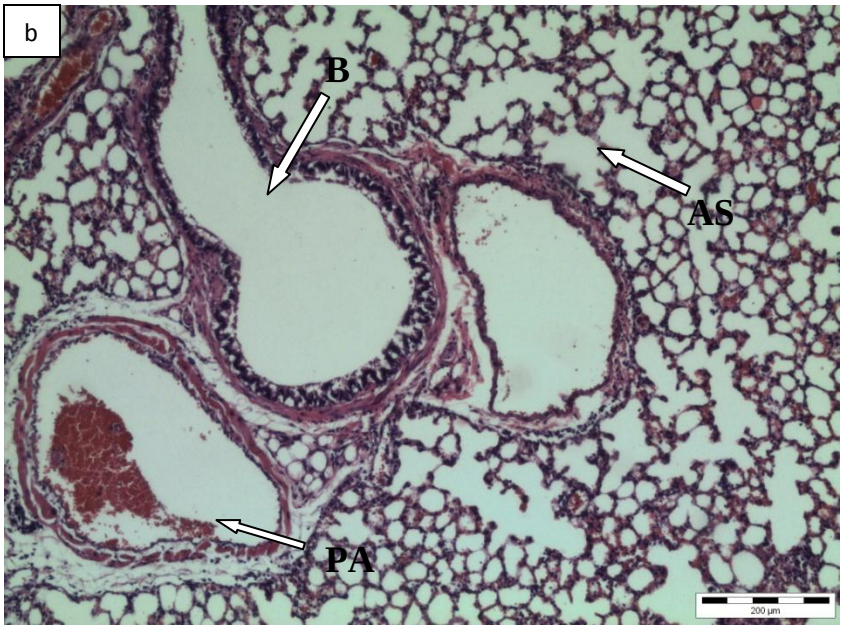
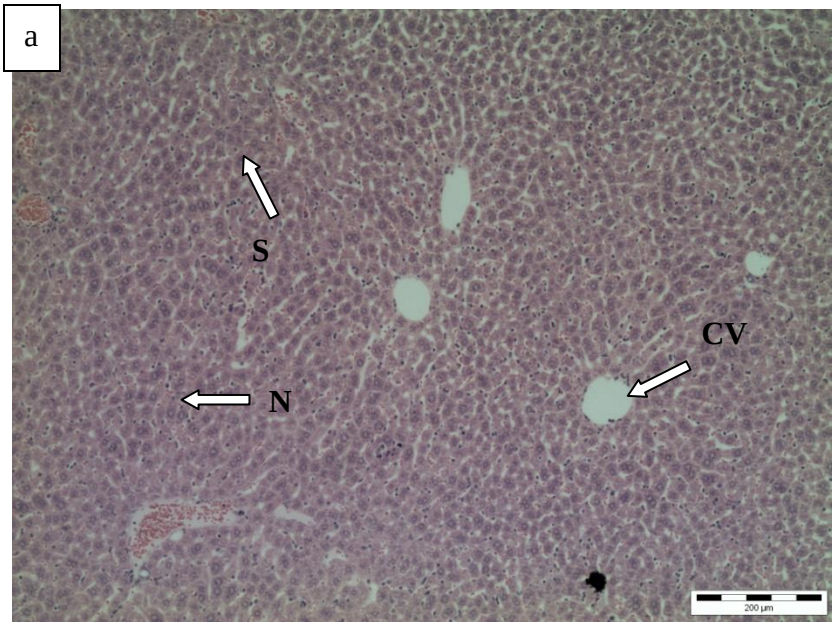


Fig. 3: Photomicrograph of a section of lung from control group (a) and treatment group (b) at 10X (H&E).



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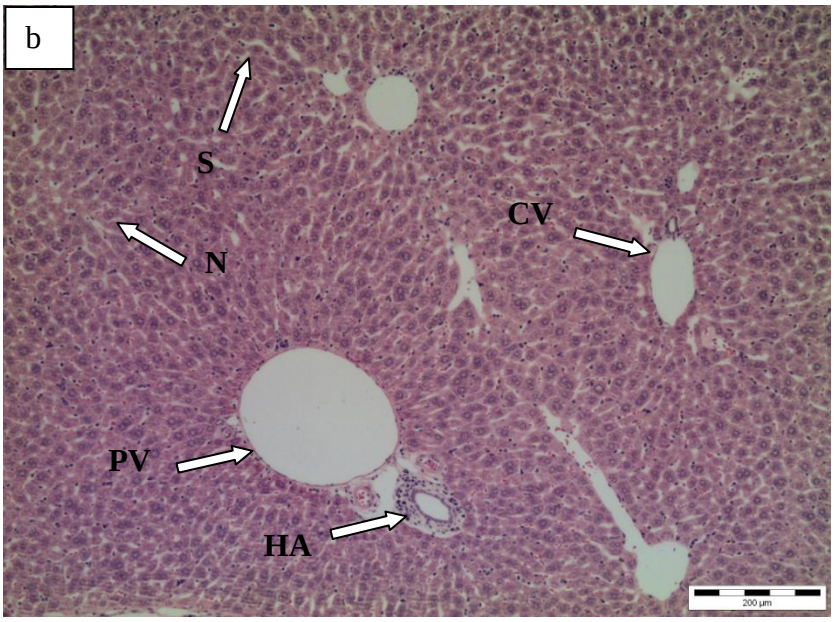
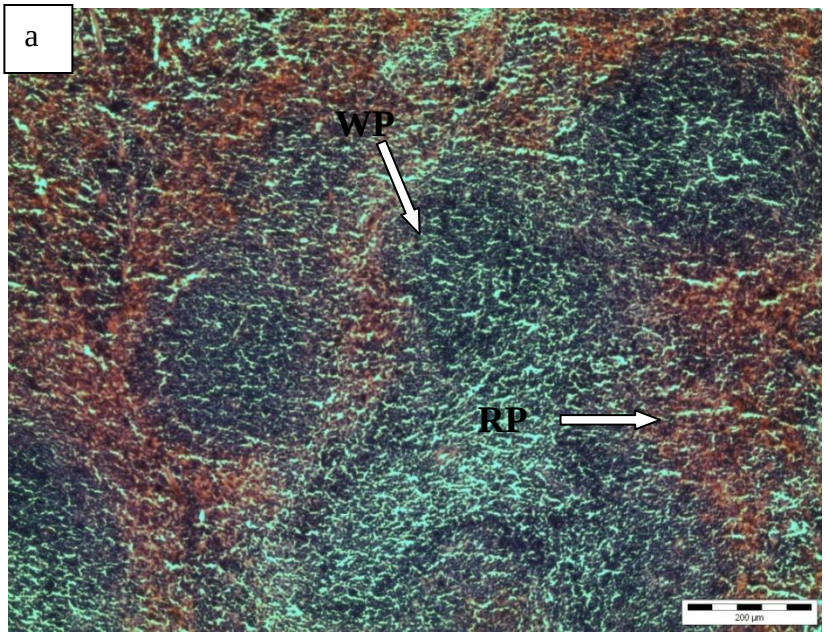


Fig. 5: Photomicrograph of a section of liver from control group (a) and treatment group (b) at 10X (H&E).

[B- bronchiole; AS- alveolar sac; PA- pulmonary artery]



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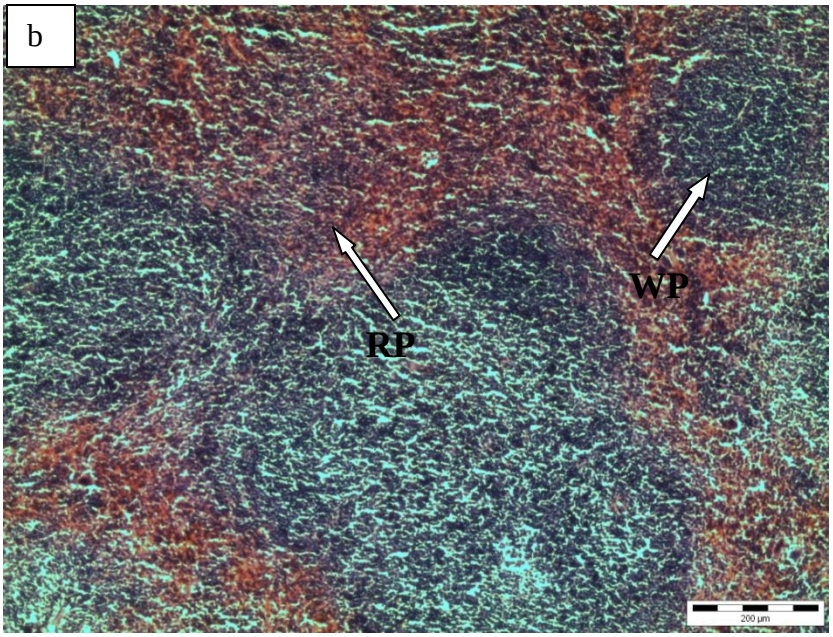
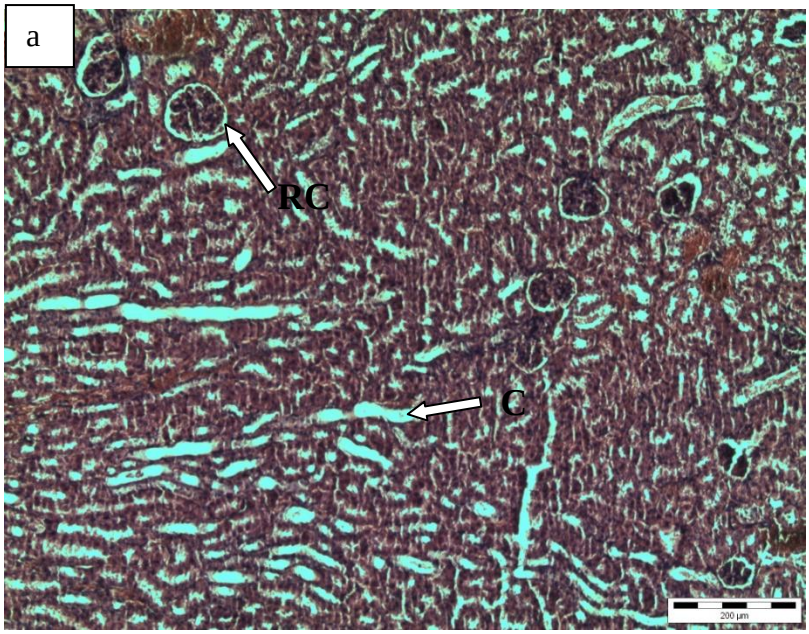


Fig. 6: Photomicrograph of a section of spleen from control group (a) and treatment group (b) at 10X (H&E).

[WP- white pulp; RP- red pulp]



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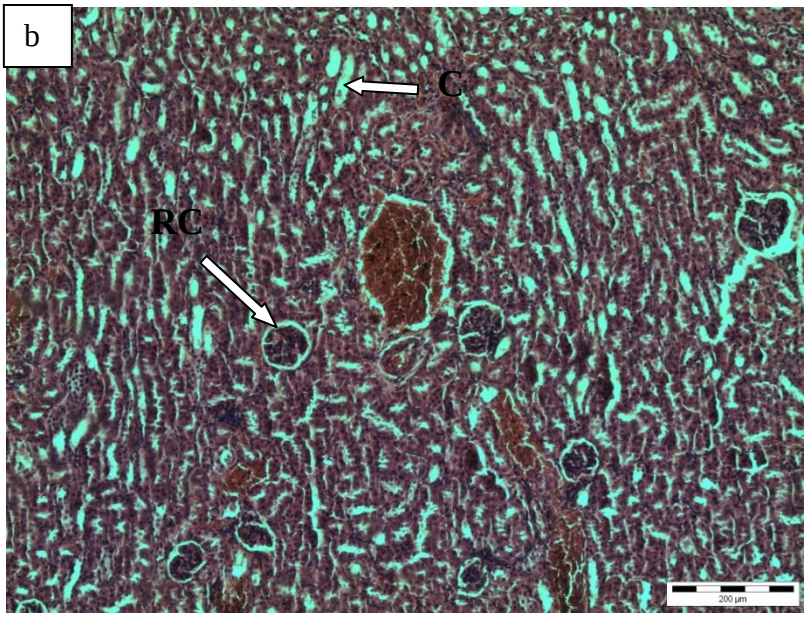

Fig. 7: Photomicrograph of a section of kidney from control group (a) and treatment group (b) at 10X (H&E).

Microscopy examination did not suggest any histological alterations in the lung, heart, liver, spleen and kidney (Fig. 3-7). *C. surattenis* flower extract did not cause any abnormal alterations or structural damage of the organs in the histopathology analysis of the treatment group. Generally major structural modifications were not observed in the treatment as well as in the control group. The histopathology analysis of the lung, heart, liver, spleen and kidney of treatment was free from any damages as those in control group because *C. surattensis* flower extract was not toxic to the animal tissue. This suggests that *C. surattensis* flower extract can be used as a potential drug to treat diseases due to its various pharmacological properties. Liver is the largest organ in the body. Liver is the main principle site of body metabolism and is the first target of acute toxicity as all the substances like carbohydrates, lipids and proteins take place are metabolized in this organ [17-18]. Thus, if the extract was toxic the liver cells will definitely have some modification on the cell structure but the treated histopathology tissue was normal. Basically this study successfully discovered *C. surattensis* flower extract free of toxic substances.

CONCLUSION

In short, the methanolic flower extract of *Cassia surattensis* did not show any toxicity signs based on the brine shrimp assay and oral acute toxicity findings. However, further investigation on the full safety usage of this extract will be necessary before it becomes a potential drug to treat various pathology conditions due to toxic free.

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