Cytotoxicity and Acute Oral Toxicity Effects of Parkia Speciosa Seeds Extract in C57bl/6 Mice

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Parkia speciosa is frequently consumed as a raw salad due to the notion that the plant exhibits numerous pharmacological activities that could benefit health particularly among Asians. This study was aimed to investigate the cytotoxicity and acute oral toxicity consequences of Parkia speciosa seeds extract against 4T1 mouse mammary cancer cells on C57BL/6 female mice. The antiproliferative effect of aqueous and ethanolic extracts was studied using the in-vitro antiproliferative assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Meanwhile, for acute toxicity study, twenty female mice were categorized into 5 groups, each with three aqueous extract treatment groups: 50 mg/kg, 300 mg/kg, 2000 mg/kg, meanwhile one vehicle group (treated with sterile distilled water), and one control group (no treatment given). Changes in behavioural signs, mortality rate, relative body weight, haematology, serum biochemistry, and organ histological evaluation were observed. Based on the MTT assay, the aqueous extract of Parkia speciosa was weakly active (IC50 = 312.5 ± 1.20 ug/ml) against 4T1 mammary cancer cells. In the acute study, no mortality, behavioural and physical changes were observed in any of the mice groups throughout the 14-day experiment. The haematological and serum biochemistry results of the treated and control groups showed no alteration. The kidney and liver were histopathologically evaluated and found to have normal organ architectures. Analyzed results could conclude that aqueous extract of Parkia speciosa has weakly active against cancer cells but has no deleterious effects on C57BL/6 mice given at high doses up to 2000 mg/kg.

Keywords: Antiproliferative; Acute Toxicity Study; C57BL/6; Cytotoxicity; Parkia speciosa.

Cancer is the second greatest cause of mortality worldwide, according to the World Health Organization (WHO), with a reported nine million six hundred thousand deaths in 2018. Although many cancer medicines are in the investigation and most are in clinical trials, there is still a pressing need to seek medications that are considerably more effective and have fewer adverse effects.
Natural products are said to account for nearly half of all medicines in clinical trials, with many of them having the possibility to inflict programmed cell death in different cell lines\(^1\). Plants have been recognised as a source of safer and less expensive alternatives with beneficial qualities such as fewer side effects on the body and ecology\(^2\). The anticancer activities of some plant extracts and their bioactive components have been thoroughly investigated\(^3\). Some of the most clinically effective chemotherapeutic agents produced from natural substances include vincristine, podophyllotoxin, paclitaxel, and camptothecin\(^4\).

Furthermore, *Parkia speciosa* is a native leguminous tree in tropical countries\(^5\). *P. speciosa* is recognised as *petai* in Southeast Asian countries such as Indonesia, Malaysia, and Singapore. Meanwhile, it is known as *Sataw* or *Sator* and *u’pang* in the Thailand and Philippines, respectively\(^6\). Malaysians have been using the plant to cure various illnesses and complications, including diabetes, kidney failure, and headaches\(^6\). *P. speciosa* was reported to have hypoglycemic\(^7\), anticancer\(^8\), and angiogenesis inhibitor properties\(^9\). All components of the *Parkia speciosa* plant contain various bioactive compounds such as flavonoids\(^10\), alkaloids, terpenoids\(^11\), and phenolics\(^12\) that have a wide range of nutritional benefits\(^13\). Despite numerous fundamental findings regarding the plant’s biological activities, in vivo toxicological effects should be studied prior to any in vivo test. This is since the chemical composition of each natural herb substance varies depending on several factors\(^14\) mentioned that variabilities in pharmacological activity could be resulting in considerable pharmacological activity discrepancies, impacting both pharmacodynamic and pharmacokinetic issues. Regardless of the plant’s naturalness, some phytochemical compounds could pose a lethal threat to humans and animals when consumed\(^16\). The efficacy and potential toxicity of commonly consumed plant preparations are unidentified\(^16\). Even though many plant-based products are natural, however, they are not necessarily always safe to consume. In this study, the acute toxicity of *Parkia speciosa* seed extract was determined in C57BL/6 mice using an aqueous solvent. Acute toxicity is important for determining the clinical signs that appear in the consumer as a result of high doses of the test substance or drug, the time of onset and cessation of the signs, the potential determination of a minimum lethal dose, and the sequence and timing of effects leading to death or recovery\(^17\).

**MATERIALS AND METHODS**

**Ethical approval**

Faculty of Veterinary Medicine, Universiti Malaysia Kelantan Animal Ethics Committee, granted animal ethics approval (UMK/FPV/ACUE/FYP/17/2019) in January 2020. 20 female C57BL/6 mice aged 7 to 18 weeks were bought from Animal Research and Service Centre Universiti Sains Malaysia (ARASC USM), Health Campus, Kubang Kerian, Kelantan.

**Housing and feeding condition**

Groups of four C57BL/6 mice were housed in the polycarbonate cage measuring 28.5cm x 16.5cm x 13cm in an experimental room with a temperature of 22 °C (± 3°C), relative humidity of 60% (± 5%), and a cycle of 12-hour light and 12-hour dark. The mice were fed ad libitum with conventional rodent laboratory diets and distilled water. The bedding was made of wood shavings, and it was changed daily. Mice were chosen at random and kept in a cage as a group of four for six days prior to dosing to allow them to adapt to the laboratory settings.

**Plant collection and Identification**

Seeds of *P. speciosa* were purchased from a market in Pengkalan Chepa, Kota Bahru, Kelantan. The seeds were certified with a deposited voucher specimen from the Botany Department Herbarium, Universiti Kebangsaan Malaysia (UKM), Bangi, Malaysia. The seeds were cleaned, rinsed with distilled water, then followed with oven drying for seven days.

**P. speciosa seeds extraction**

Clean seeds were pulverised in a heavy-duty grinding mill (High Power Smart Essence Blender, Model SSL - 766) to obtain the coarse particles. Afterwards, 100 g of tiny particles were mixed to 1000 ml of cleaned distilled water, in which the mixture was then boiled for 5 minutes. After the mixture was been cooled at room temperature, Filter paper (Whatman No.1) was used to separate the mixture from the blended
*P. speciosa* seeds. The filtered solution was evaporated using a rotary evaporator (Heidolph, Germany) under reduced pressure (72 mbar) with a water bath at 50-60°C, then freeze-dried to gain the dried extract. The final product of dried *P. speciosa* extracts was weighed and until further use, it was stored at -20°C in a tight and sterile container. Prior orally administered to mice, the final production of the extract was diluted with sterile distilled water, and doses of 50, 300 and 2000 mg/kg body weight (in sterile distilled water, 2 ml/kg body weight) was prepared.

**In vitro antiproliferative assay**

The 4T1 mouse mammary carcinoma cell was generously provided by Prof Dr. Nik Soriani Yaacob from the Department of Chemical Pathology, School of Medical Sciences, Universiti Sains Malaysia (USM). The cells were cultured in 10% RPMI 1640 media with L-glutamine, supplemented with 10% foetal bovine serum (FBS), and 1% antibiotic-antimycotic. Hundred microlitres of media containing 1×10⁴ cells (4T1 mammary cancer cells) were seeded into 96-well plates. This was followed by incubation of the plates at 37°C with 5% CO₂ overnight to allow cells attached to the bottom of the well plates. After overnight incubation, PSAE was dissolved in 1x PBS alone to obtain the stock solution for the extract with the concentration of 1mg/ml. Parkia speciosa of aqueous extract (PSAE) was serially diluted with concentrations ranging from 1000 ìg/mL to 15.63 ìg/mL and was added into the appropriate wells in three replicates for each concentration. For the untreated cells (0ìg/mL), it was used as the negative control in every plate. To get a consistent result for each concentration, triplicate in one plate, and the experiment was repeated three times. The plates were re-incubated for another 10 minutes with the same environment control. Followed by optical density (OD) measuring by using a spectrophotometer (BioTek ELx 808) at 490 nm. The cell viability in percentage (%) was calculated using the formula:

\[
\text{Cell viability (\%)} = \frac{\text{mean OD of treated cell} - \text{mean OD of blank}}{\text{mean OD of untreated cell} - \text{mean OD of blank}} \times 100\%
\]

**Acute Toxicity Study**

The body weight of each animal was taken followed by dose calculation before dosing. The animals were fasted for 3-4 hours from food but not the water and aqueous seeds of *P. speciosa* extract were given *per os* in a single dose. According to OECD guideline, only up to 2 ml/100 g of body weight was been given. Single-dose of *P. speciosa* aqueous extract (PSAE) at a low dose (50 mg/kg), medium dose (300 mg/kg), and high dose (2000 mg/kg) was orally gavage to the treatment groups of mice, whereas the control groups were received with sterile distilled water (vehicle) and no treatment for the negative control group. After administration of the extract, food was withheld for a further 3 to 4 hrs.

**Observation Period**

Each mouse was observed once within the first 30 minutes, 1 hour, 2 hours, 4 hours, and 8 hours. Afterwards, daily observations were conducted for the next 14 days. They were excluded from the sample in case they were discovered dead. They were humanely slaughter for animal welfare purposes if they did not meet the research criteria. Physical and behavioural observations, autonomic effects, sensory responses and reflexes, respiratory abnormalities, and somatosensory activities were monitored and recorded.

**Necropsy**

On the termination day, all of the mice were sacrificed by carbon dioxide inhalation and decapitation by guillotine. The vital organs, primarily the liver, kidneys, lungs, and other organs such as the spleen and ovaries, were removed, cleaned, and weighed during the post-mortem. Subsequently, all of the organs were preserved in 10% formalin for further histopathological assessment. The relative weight of the organ was calculated during the necropsy using the following formula:
Haematological and Biochemical Analyses

The decapitation method was performed to harvest the blood and then deposited it in a heparin tube. The blood was then subjected to a complete blood count test, followed by biochemistry analyses. Packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell count, mean cell volume, mean corpuscular haemoglobin and platelet count were calculated. Furthermore, the biochemical analyses included alanine aminotransferase (ALT), creatinine, urea and total protein parameter measurements.

Histopathology examination

Vital organs such as kidneys and liver were preserved in 10% formalin for approximately 24 hours before the processed tissues were examined for toxicity-related abnormalities. A few parameters, for instance, granular cast, cellular cast, protein cast, necrosis (pyknotic nucleus), inflammation, and hydropic degeneration in the kidney, were included in histopathological evaluations. In addition, histopathological lesions in the liver included activated Kupffer cells, necrosis (karyolysis and eosinophilic cytoplasm), necrosis (pyknotic nucleus), degeneration (hydropic degeneration), and inflammation were observed.

RESULTS AND DISCUSSIONS

Qualitative phytochemical analysis of plant extract

Table 1 shows the finding of qualitative phytochemical screening of aqueous extract of *Parkia speciosa*. Among the phytochemical constituents that were screened for their presence in the extracts are phenolics, saponins, tannins, terpenoids, and flavonoids compounds. The result showed that *Parkia speciosa* seeds aqueous extract (PSAE) have all the phytochemical constituents except saponin and tannin.

**In vitro antiproliferative study**

Table 2 represents the inhibitory concentration (IC$_{50}$) of aqueous extract of *Parkia speciosa* on 4T1 mammary cancer cell lines.

The inhibitory concentrations (IC$_{50}$) of the extract against 4T1 mammary cancer cells were determined through the non-linear regression principle (Figure 1). The results demonstrated that aqueous extract of *Parkia speciosa* has a growth inhibitory effect on 4T1 mammary cancer cells with the IC$_{50}$ value of 312.5 ± 1.20 ug/ml.

**Acute toxicity study results**

**Mortality**

At 50, 300 nor 2000 mg/kg body weight of aqueous seed extract of *Parkia speciosa* demonstrated no fatality in female C57BL/6 mice for a single treatment in 14-days of study.

**Physical observation**

The physical parameters such as skin and fur colour in all treated mice have no deviations during the study as shown in Table 3.

**Behavioural observation**

There was no abnormal behavioural characteristic such as the central nervous system (CNS) excitation and depression observed in controls and treatment groups, respectively throughout 14 days of study. All animals showed normal behaviours and without changes in mood during the study after the administration of the extract. The animals also did not show any CNS stimulatory side effects for example tremors, twitches, convulsion, and CNS depressant effects included sedation, catatonia, and ataxia.

**Respiratory effect**

Table 4 illustrates the respiratory system demonstrated no respiratory effects such as...
dyspnoea or apnoea. This indicating absents of respiratory distress in the mice by the single dose of PSAE.

**Relative body and organ weight**

The relative body weights and organ weight gain comparison of mice throughout 14 days’ experiment in an acute oral toxicity study of aqueous seed extracts of *P. speciosa* are presented in Table 5 and Table 6, respectively. Mice in medium dose (300 mg), high dose (2000 mg), and control groups were increased in body weight after a week of the study. However, mice in the low dose (50 mg) group showed a slight decline in their body weight, but it was increasing until the end of the study. Nevertheless, the minimal weight change in the treated groups was revealed no significant difference between the control groups’ body weight changes patterns throughout the experiment. The relative organ weights of C57BL/6 female mice are shown in Table 6. There was an insignificant difference (p > 0.05) in organs relative weights of liver, kidney, lungs, heart, brain, ovaries, and spleen which indicate that aqueous extract of *P. speciosa* seeds did not produce any demonstrable toxic effects on the organs in the mice.

**Haematological and serum biochemical analyses**

The results showed insignificant differences (p > 0.05) in the haematological parameters of treatment groups between the control group (Table 7).

Differential white blood cell count values showed an insignificant (p > 0.05) difference among treatment and control groups as shown in Table 8.

Vital parameter for serum biochemistry in acute toxicity study including both kidney and liver parameters (Table 9). For both parameters, there were insignificant differences (p > 0.05), suggest that both organs were unaffected by acute administration of *P. speciosa* seed extract.

**Histopathological analysis**

Histopathological evaluations of the

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**Table 3. Physical observations in C57BL/6 mice at all groups after a single administration of aqueous seed extract of *Parkia speciosa***

<table>
<thead>
<tr>
<th>Observations</th>
<th>0 - 30 minutes</th>
<th>1-2 hrs</th>
<th>4 – 8 hrs</th>
<th>24 hrs</th>
<th>7th day</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin colour</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Fur colour</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

*Note: N (Normal)*

**Table 4. Respiratory effects of aqueous seed extract of *P. speciosa* in C57BL/6 mice in all groups**

<table>
<thead>
<tr>
<th></th>
<th>0-30 minutes</th>
<th>1-2 hrs</th>
<th>4 – 8 hrs</th>
<th>24 hrs</th>
<th>7th day</th>
<th>14th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnoea</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Apnoea</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>

**Table 5. The effect of *P. speciosa* seed extracts on the body weight (mean ± SEM) (n = 4) of C57BL/6 mice**

<table>
<thead>
<tr>
<th>Day</th>
<th>Low Dose (50 mg)</th>
<th>Medium Dose (300 mg)</th>
<th>High Dose (2000 mg)</th>
<th>Vehicle</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>19.70 ± 0.61*</td>
<td>18.70 ± 0.81*</td>
<td>19.28 ± 0.77*</td>
<td>18.48 ± 0.88*</td>
<td>18.93 ± 0.41*</td>
</tr>
<tr>
<td>7</td>
<td>19.53 ± 0.54*</td>
<td>19.05 ± 0.56*</td>
<td>19.90 ± 0.61*</td>
<td>18.83 ± 0.50*</td>
<td>18.76 ± 0.63*</td>
</tr>
<tr>
<td>14</td>
<td>19.65 ± 0.60*</td>
<td>19.90 ± 0.38*</td>
<td>19.85 ± 0.47*</td>
<td>19.83 ± 0.50*</td>
<td>19.22 ± 0.37*</td>
</tr>
</tbody>
</table>

*Values in the same row with similar superscript letters do not significantly different from each other (p > 0.05).
kidney and liver of all the mice were conducted based on parameters such as granular cast, cellular cast, protein cast, necrosis (pyknotic nucleus), inflammation, and hydropic degeneration in the kidney. Meanwhile, in the liver, the histopathological lesions, for instance, activated Kupffer cells, necrosis (karyolysis and eosinophilic cytoplasm), necrosis (pyknotic nucleus), degeneration (hydropic degeneration) and inflammation were observed. There were no pathological changes observed in both kidney and liver tissues in the group of mice treated with extract of *P. speciosa*.

**DISCUSSION**

Herbs or plants nowadays are used to cure various ailments and problems, including acute and chronic conditions, for instance, cardiovascular disease, prostate problems, depression, and inflammation. Unfortunately, certain phytochemical compounds in plants, when consumed, might pose a lethal threat to humans and animals\(^\text{16}\). Data on *P. speciosa*, like many other commonly consumed plants, are scant, especially on their efficacy and possible toxicity\(^\text{16}\).
As a result, determining the toxicological effects of any plant extract intended for use in animals or human medicine is an important aspect of risk assessment, and the extract should not be harmful to the consumers. Consequently, assessing the toxicological effects of any plant extract intended for use in animals or human medicine is a crucial part of risk assessment, and it should have no toxic effects or severe interfering effects on consumers. The cytotoxic effect of PSAE and was measured based on the minimum extract concentration that provides at least 50% of the cancer cell survivability (IC$_{50}$). According to the United State National Cancer Institute (USNCI) plant cytotoxic classification, there are four extract categories: very active (IC$_{50}$ ≤ 20 ìg/mL), moderately active (IC$_{50}$ > 20–100 ìg/mL), weakly active (IC$_{50}$ > 100–1000 ìg/mL), and inactive (IC$_{50}$ > 1000 ìg/mL). The MTT assay revealed that the aqueous extract of *Parkia speciosa* was weakly active (IC$_{50}$ = 312.5 ± 1.20 ìg/ml) against 4T1 mammary cancer cells. The presence of phenolic, flavonoid and terpenoid compounds was discovered in a quantitative phytochemical analysis of an aqueous extract of *Parkia speciosa* seeds. Terpenoids compounds such as ß-sitosterol, stigmasterol, lupeol, campesterol, and squalene were detected in *Parkia speciosa* seeds using gas chromatography in a previous study. Breast cancer, ovarian cancer, lung cancer, stomach cancer, leukaemia, prostate cancer, and colon cancer are only a few cancers that these compounds have demonstrated anticancer properties against in both humans and animals.

The previous study has researched botanical compounds, such as ß-sitosterol, for use in chemotherapy as potential future therapies, in which it expressed variable action mechanisms in cell culture, including cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, and inflammation. Besides, lupeol,

### Table 7. The haematology values (mean ± SEM) (n = 4) of C57BL/6 mice in acute oral toxicity of aqueous extract of *P. speciosa* seeds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low Dose (50 mg)</th>
<th>Medium Dose (300 mg)</th>
<th>Groups High Dose (2000 mg)</th>
<th>Vehicle</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>8.30 ± 0.81 a</td>
<td>7.73 ± 0.50 a</td>
<td>8.25 ± 0.33 a</td>
<td>7.93 ± 0.22 a</td>
<td>7.80 ± 0.12 a</td>
</tr>
<tr>
<td>Hb</td>
<td>13.33 ± 0.27 b</td>
<td>11.05 ± 0.49 a</td>
<td>13.48 ± 0.36 b</td>
<td>13.55 ± 0.46 b</td>
<td>12.73 ± 0.23 b</td>
</tr>
<tr>
<td>PCV</td>
<td>37.00 ± 0.82 a</td>
<td>35.03 ± 2.89 a</td>
<td>37.55 ± 1.16 a</td>
<td>37.98 ± 1.28 a</td>
<td>35.78 ± 0.84 a</td>
</tr>
<tr>
<td>MCV</td>
<td>44.65 ± 0.51 a</td>
<td>45.35 ± 0.82 a</td>
<td>45.68 ± 0.73 a</td>
<td>47.83 ± 0.37 b</td>
<td>45.45 ± 0.45 a</td>
</tr>
<tr>
<td>MCHC</td>
<td>36.50 ± 0.58 a</td>
<td>29.63 ± 5.98 a</td>
<td>35.93 ± 0.47 a</td>
<td>35.70 ± 0.29 a</td>
<td>35.60 ± 0.23 a</td>
</tr>
<tr>
<td>Platelet</td>
<td>45.75 ± 1.93 a</td>
<td>82.00 ± 39.10 a</td>
<td>80.00 ± 12.43 a</td>
<td>166 ± 90.68 a</td>
<td>65.50 ± 13.77 a</td>
</tr>
</tbody>
</table>

*Values in the same row with similar superscript letters do not significantly different from each other (p > 0.05).

### Table 8. The differential white blood cell (WBC) count (mean ± SEM) (n = 4) of C57BL/6 mice in acute oral toxicity of aqueous extract of *Parkia speciosa* seeds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low Dose (50 mg)</th>
<th>Medium Dose (300 mg)</th>
<th>Groups High Dose (2000 mg)</th>
<th>Vehicle</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total White Blood Cells</td>
<td>4.25 ± 0.58 a</td>
<td>3.45 ± 0.42 a</td>
<td>6.0 ± 0.65 a</td>
<td>5.05 ± 1.12 a</td>
<td>3.95 ± 0.58 a</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.25 ± 0.10 a</td>
<td>0.17 ± 0.06 a</td>
<td>0.27 ± 0.11 a</td>
<td>0.18 ± 0.05 a</td>
<td>0.22 ± 0.06 a</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3.27 ± 1.10 a</td>
<td>3.10 ± 0.34 a</td>
<td>5.14 ± 0.39 a</td>
<td>0.18 ± 0.05 b</td>
<td>0.22 ± 0.06 a</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.09 ± 0.6 a</td>
<td>0.05 ± 0.03 a</td>
<td>0.07 ± 0.04 a</td>
<td>0.19 ± 0.17 a</td>
<td>0.03 ± 0.006 a</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.00 ± 0.00 a</td>
<td>0.03 ± 0.02 a</td>
<td>0.04 ± 0.02 a</td>
<td>0.03 ± 0.02 a</td>
<td>0.00 ± 0.00 a</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.00 ± 0.00 a</td>
<td>0.00 ± 0.00 a</td>
<td>0.00 ± 0.00 a</td>
<td>0.00 ± 0.00 a</td>
<td>0.00 ± 0.00 a</td>
</tr>
</tbody>
</table>

*Values in the same row with similar superscript letters don’t significantly different from each other (p > 0.05)
and stigmasterol are anti-angiogenic compounds that inhibit cancer formation by downregulating inflammatory cytokine production, macrophage recruitment, and tumour angiogenesis\textsuperscript{20}. Lupeol is also a pharmacologically active triterpenoid that accounts for 0.71% of the total fatty acid content of \textit{Parkia speciosa}. It has antinociceptive, anti-inflammatory, and anti-mutagenic properties and inhibits nitric oxide production, iNOS and COX-2 expression\textsuperscript{21}. Furthermore, the squalene found in \textit{Parkia speciosa} is known for its antioxidant, tumour reducing and anticancer properties\textsuperscript{22}. Lastly, thiazolidine-4-carboxylic acid found in \textit{Parkia speciosa} seed has been reported to have antiproliferative effects against cancer cells as it inhibits the endogenous formation of carcinogenic N-nitroso compounds\textsuperscript{23}.

### Table 9. The serum biochemistry analyses (mean ± SEM) (n = 4) of C57BL/6 mice in acute oral toxicity of aqueous extract of \textit{P. speciosa} seeds

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Low Dose (50 mg)</th>
<th>Medium Dose (300 mg)</th>
<th>High Dose (2000 mg)</th>
<th>Vehicle</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>23.50 ± 0.29\textsuperscript{ab}</td>
<td>22.50 ± 2.60\textsuperscript{ab}</td>
<td>18.75 ± 3.33\textsuperscript{a}</td>
<td>21.75 ± 1.31\textsuperscript{ab}</td>
<td>22.10 ± 0.85\textsuperscript{ab}</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.23 ± 0.03\textsuperscript{a}</td>
<td>0.20 ± 0.00\textsuperscript{a}</td>
<td>0.25 ± 0.05\textsuperscript{a}</td>
<td>0.23 ± 0.03\textsuperscript{a}</td>
<td>0.33 ± 0.08\textsuperscript{a}</td>
</tr>
<tr>
<td>ALT</td>
<td>134.75 ± 9.04\textsuperscript{b}</td>
<td>98.00 ± 18.89\textsuperscript{ab}</td>
<td>136.75 ± 13.10\textsuperscript{b}</td>
<td>101.07 ± 10.91\textsuperscript{a}</td>
<td>128.75 ± 5.12\textsuperscript{b}</td>
</tr>
<tr>
<td>TP</td>
<td>8.25 ± 1.33\textsuperscript{a}</td>
<td>7.13 ± 1.64\textsuperscript{a}</td>
<td>5.63 ± 0.61\textsuperscript{a}</td>
<td>6.90 ± 0.48\textsuperscript{a}</td>
<td>6.40 ± 0.23\textsuperscript{a}</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.03 ± 0.03\textsuperscript{ab}</td>
<td>2.70 ± 0.21\textsuperscript{ab}</td>
<td>2.53 ± 0.45\textsuperscript{a}</td>
<td>3.55 ± 0.44\textsuperscript{b}</td>
<td>3.10 ± 0.13\textsuperscript{ab}</td>
</tr>
<tr>
<td>Globulin</td>
<td>5.30 ± 1.35\textsuperscript{a}</td>
<td>4.45 ± 1.46\textsuperscript{a}</td>
<td>3.10 ± 0.18\textsuperscript{a}</td>
<td>3.38 ± 0.10\textsuperscript{a}</td>
<td>3.23 ± 0.13\textsuperscript{a}</td>
</tr>
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\*Values in the same row with similar superscript letters don’t significantly different from each other (p > 0.05).

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**Fig. 2.** Photomicrograph of kidney sections in (A) low dose (50 mg/kg), (B) medium dose (300 mg/kg), (C) high dose (2000 mg/kg) and (D) control groups of single-dose oral toxicity study of seeds extract of \textit{P. speciosa} (H&E stain, ×400). G denote glomerular, GC denote granular cast, T denote renal tubule.
PSAE revealed the intense colour as per the flavonoid qualitative test. According to another study, kaempferol is the most common flavonol in the diet and has some therapeutic qualities as a breast cancer chemotherapy treatment. It mentioned that kaempferol not only had in vitro anti-breast cancer activity, but it also produced in vivo anti-cancer effects specifically by inhibiting cell proliferation, inhibiting cell migration, and triggering apoptosis. According to ethanol extract of empty Parkia speciosa pod had higher total flavonoid and total phenolic contents as compared to aqueous extract of Parkia speciosa. Gallic acid, catechin, ellagic acid, and quercetin were the most abundant phenolic constituents in Parkia speciosa extract. Flavonoids such as quercetin, myricetin, luteolin, kaempferol, and apigenin were also found in the ethanol extract of P. speciosa seeds. Even though the aqueous extraction of Parkia speciosa seeds exhibited lesser bioactive compounds than other solvents, the extract nevertheless was mild-weakly active (IC$_{50}$ = 312.5 ± 1.20 µg/ml) against 4T1 mammary cancer cells. Further in vivo research is needed to better understand the action mechanism of PSAE with the involvements of the animal immune system and microenvironment in breast cancer. Normal cell line cytotoxicity studies can be performed on the selectivity index (SI) to identify selective anti-cancer activity in the aqueous extract of Parkia speciosa seeds.

The aqueous extract of P. speciosa seed was relatively non-toxic at a single dose of 2000 mg/kg in the current study. Upon administering aqueous extract at a dose of 2000 mg/kg, all-female mice displayed neither mortality nor behavioural changes such as mood, central nervous system excitation, or depression throughout the entire 14-day observation timeframe. In line with the Globally Harmonised System of Classification and Labelling of Chemicals, PSAE belonged to Category 5, and its LD$_{50}$ value was more than 2000 mg/kg. This LD$_{50}$ value was similar to other...
In this study, all the treated groups' pharmacological responses to therapeutic drugs used in analysing pathogenic processes or herbal plants such as Atropa belladonna, Hyoscyamus niger, and Cannabis indica demonstrated potential haematoxicity. The haematological indices, including RBC, haemoglobin concentration, MCV, MCHC, and platelet counts, showed insignificant differences compared to the control group. There were also insignificant differences in the WBC count and differential white blood cell count in leukocyte parameters. A study on aqueous extract of P. biglobosa seeds, on the other hand, found deviations in PCV, MCH, and MCHC, as well as a significant decrease in platelet and WBC levels.

In plant toxicological studies, the use of blood serum or plasma enzymes as a marker to detect organ damage in the kidneys and liver, specifically cell destruction, enzyme induction, activation, or inhibition of enzymes. Numerous blood tests could be used to assess levels of tissue injury, identify potential target organs and evaluate organ function degradation. Besides, the kidneys and liver are major organs with a critical role in the body’s metabolic detoxification.

Nephrotoxicity occurs if the kidneys cannot excrete urine and body waste materials, commonly due to any drugs or toxins with a nephrotoxic effect. The total protein, albumin, globulin, urea, and creatinine levels were used to evaluate the kidney function, and elevation of these markers can indicate nephrotoxicity. There were no significant differences in all kidney function parameters of the treated and control groups. In comparison, in plant-extract-induced nephrotoxicity, elevated creatinine level and presence of significant kidney lesions in an acute toxicity study of Aquilaria malaccensis leaf extract and a significant decrease of urea level in the serum post administration of aqueous extract of Myrsine africana seeds.

Hepatotoxicity is liver damage caused by chemicals, drugs, herbs, or dietary supplements. The clinical symptoms of the liver injury include abdominal discomfort, nausea, vomiting, changes in urine and stool colour, jaundice, hive, recurrent weariness, weakness, exhaustion, and pyrexia. Hepatotoxicity can be increased ALT, AST, ALP, and bilirubin levels in the case of liver injury, but a decrease of albumin level in the serum biochemistry analyses of the animal. Liver function testing in this study revealed no significant differences compared to the control and P. speciosa treatment groups. With all the treated groups' haematology results, including RBC, haemoglobin concentration, MCV, MCHC, and platelet counts, showed insignificant differences compared to the control group. There were also insignificant differences in the WBC count and differential white blood cell count in leukocyte parameters. A study on aqueous extract of P. biglobosa seeds, on the other hand, found deviations in PCV, MCH, and MCHC, as well as a significant decrease in platelet and WBC levels.

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of the justifications, it was possible to conclude that PSAE is non-hepatotoxic at a dosage of 2000 mg/kg. In addition to behavioural and haematological analyses for toxicity research, histopathological analysis provides supporting data for biochemical and haematological assessments. Besides kidneys, the liver is the essential organ in detoxification and metabolism since it excretes most toxicants and metabolic wastes. There was no evidence of toxicity in the kidney and liver tissues, supported by insignificant results from the haematological parameters of treated mice throughout the 14-day experiment. This implies that at a dose up to 2000 mg/kg, PSAE is likely to be non-toxic to the kidneys and liver. After oral administration of extracts at doses of 430 mg, 700 mg, and 1480 mg, groups treated with *M. christia vespertilionis* extract demonstrated a significant difference (p < 0.05) in hepatic lesion characterized by mild to moderate necrosis. Mild hepatic degeneration and necrosis were also significant (p < 0.05) despite no significant difference (p > 0.05) in liver function tests (total protein, albumin, globulin, and alanine aminotransferase).

**CONCLUSION**

In conclusion, the findings of in vitro and in vivo studies conveyed that an aqueous extract of *Parkia speciosa* seeds was safe; there were no deleterious effects on C57BL/6 mice even at doses up to 2000 mg/kg for 14 days after a single-dose treatment of PSAE. Additionally, PSAE is weakly active cytotoxic to the 4T1 mammary cancer cells, with a minimum inhibitory concentration (IC₅₀) of 312.5 ± 1.20 µg/ml. Sub-acute and sub-chronic PSAE should be performed in more mouse models to determine the safety and possible toxicity effects of a higher dose and long-term intake.

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**Conflict of interest**

The authors declare that there is no conflict of interest in this research.

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