

## ACUTE TOXICOLOGICAL ASSESSMENT OF SEASHORE MANGOSTEEN (*Garcinia hombroniana*) AQUEOUS EXTRACT

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### SUMMARY

*Garcinia hombroniana* has been used in Malay traditional medicine to treat various disorders such as abdominal pain and gonorrhoea, and little is known about its toxicological properties. This study investigated the acute toxicological effects of the plant's leaves aqueous extract using theoral acute toxic class (ATC) method. Twenty-four female Sprague-Dawley rats were divided into T1, T2, T3 and control groups. The T1, T2 and T3 rats administered a single oral dose of 300, 2000 and 5000 mg/kg of *G. hombroniana* leaves aqueous extract, respectively. The animals were checked daily for abnormal clinical signs and mortality rate for 14 days. The body weight, daily food and water consumption were recorded. The haematological and biochemical parameters of blood as well as relative organ weight and tissues histology such as heart, kidney, liver and spleen were evaluated. Results have shown significantly decreased ( $p < 0.05$ ) food and water consumption in T2 and T3 rats, which returned to normal after 72 hr post administration. No significant differences ( $p \geq 0.05$ ) in the haematological and serum biochemical parameters in the treated rat groups, compared to the untreated control animals. An increase in the relative weight of spleen was noticed in T3 animals. Congestion of the splenic, hepatic, cardiac and renal tissues was seen in T2 and T3 rats. The oral LD<sub>50</sub> was higher than 5000 mg/kg of body weight. Thus, it can be concluded that *G. hombroniana* aqueous extract shows little toxicity in the laboratory rats and the therapeutic potentials should be further investigated.

Keywords: *Garcinia hombroniana*, acute toxicity, LD<sub>50</sub>, oral acute toxic class method

### INTRODUCTION

*Garcinia hombroniana* (Clusiaceae) is a small evergreen tree commonly known as "seashore mangosteen". The plant is native to the tropical rainforests of Southeast Asian countries such as Vietnam, Cambodia, Malaysia and Thailand (Stevens, 2007; Lim, 2012).

Species of the genus *Garcinia* have been used in traditional medicine for the treatment of a variety of ailments such as abdominal pain, purulence and gonorrhoea. *Garcinia hombroniana* is used in folk medicine to relieve itchiness and as an anti-infective agent following birth (Jamila *et al.*, 2014).

*Garcinia* species have been known to contain some xanthone compounds exhibiting antioxidant (Minami *et al.*, 1994), antiplasmodial (Mbwambo *et al.*, 2006; Elfita *et al.*, 2009), and antitumor activities (Aisha *et al.*, 2012; Chitchumroonchokchai *et al.*, 2012).

Extracts prepared from parts of seashore mangosteen were reported to have antioxidant and platelet aggregation inhibitory effects (Jantan *et al.*, 2011). The aqueous extract of *G. hombroniana* leaves exerted a potent antitrypanosomal activity *in vitro*, with an IC<sub>50</sub> of  $23.58 \pm 2.39$  µg/ml, and a lower toxicity level on mammalian.

Vero cells (CC<sub>50</sub> =  $14533.87 \pm 296.86$  µg/ml), scoring a selectivity index of 616.36 (Dyary *et al.*, 2014).

The aqueous extract has also shown *in vivo* antitrypanosomal effect against *Trypanosoma evansi* by increasing the longevity of experimentally infected Sprague-Dawley rats (Dyary *et al.*, 2015), which makes the plant extract a suitable candidate for the discovery of new antitrypanosomal compounds. However, little has been known about the toxicological properties of *G. hombroniana* on laboratory animals, especially its toxicity on the vital organs as liver and kidneys. The aim of this study is to investigate the acute toxicological effects of the plant's aqueous extract on the median lethal dose (LD<sub>50</sub>), blood and serological parameters on Sprague-Dawley rats. In addition, this study investigates the histopathological changes in the vital organs such as liver and kidneys caused by the plant extract.

### MATERIALS AND METHODS

#### Preparation of extract

Fresh leaves of *G. hombroniana* were collected from the Agricultural Conservatory Park at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM). The plant species was identified by Mr. Shamsul Khamis, and a voucher specimen (Voucher No. Acp 0188) was deposited at the Biodiversity Unit in IBS, UPM.

Leaves were cleaned from debris, dried at 40-45°C in a hot-air oven and ground to powder. A measured amount of the powder was soaked in sterilised water (0.1

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gml<sup>-1</sup>) in a tightly sealed container and put in a rotary incubator shaker for four hours. The mixture was then filtered using filter paper (Whatman No. 4) and the solvent was separated using freeze dryer. The extract powder was stored in a tightly-sealed container at -28°C until used.

#### *Acute toxicological study of Garcinia hombroniana extract*

The toxicological study of *G. hombroniana* was conducted using theoral acute toxic class (ATC) method that was followed by the Organization for Economic Co-operation and Development (OECD) in 2001 (OECD, 2001). All animals testing and handling were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Veterinary Medicine, Universiti Putra Malaysia, Malaysia (11R127).

#### *Laboratory animals*

Twenty-four female Sprague-Dawley rats, 8-10 weeks old and weighing 172.4 ± 15.3 g were used in the study. Animals were housed in plastic cages and food and water were served *ad libitum*. Twelve hours light/dark system was followed with the light turned off at 7 pm. Animals were acclimatized to the new environment for two weeks before commencement of the experiment.

Rats were divided into four groups of six rats each. Groups T1, T2 and T3 administered 300 mg/kg, 2000 mg/kg and 5000 mg/kg of body weight (BW) of *G. hombroniana* extract, respectively, and the control group administered sterile water (20 ml/kg of BW). The extract was prepared directly before administration to the rats by dissolving in water and was administered in a single oral dose using intra-gastric gavage.

The rats fasted four hours before administration of the extract and food and water were made available two hours following administration.

#### *Observation of mortality rate and behavioral changes*

The animals were continuously monitored for signs of toxicity for one hour after administration of the extract, and once every 30 min for the first 4 hr. For the next 24 hr, the rats were examined every 2 hr. Animals were monitored for scoring of mortality rate, food and water consumption, changes in body weight and behavioral changes such as respiration, lacrimation, salivation, and diarrhea. Nervous changes such as somatomotor function, convulsion, lethargy, and coma, as well as changes in mucous membrane, eyes, skin and fur, were also recorded. The rats were observed twice daily for the next 14 days following administration of the extract.

#### *Animal weight, food and water consumption*

The weight of the rats was measured directly before and after 1, 3, 5, 7, 9, 11, 13 and 14 days of extract administration. Food and water consumption was also recorded daily by measuring the amount supplied to the animal and the amount remaining on the following day.

#### *Post-mortem pathological changes*

Fourteen days after administration of the extract, rats were sacrificed by exsanguination under anesthesia using an intraperitoneal injection of 2 mg/kg xylazine and 75 mg/kg ketamine, given in the same syringe. Blood was collected for haematological and serum biochemical tests from the caudal vena cava after opening the abdominal cavity. Internal organs were examined for gross pathology and the rats' heart, liver, kidney and spleen were immediately collected, washed with normal saline and weighed. The organs were cut into 3–5 mm thick pieces and placed in 10% neutral buffered formalin for fixation.

#### *Haematological parameters*

Haematological parameters determined were erythrocyte count, haemoglobin (HB) concentration, packed cells volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) thrombocytes and total differential leukocytes counts. The parameters were measured using an automated haematology analyser (Abbott CELL-DYN® 3700, USA).

#### *Serum biochemical tests*

All biochemical analyses were conducted using an automatic chemistry analyser (Hitachi 902 Automatic Analyser) machine and standard reagents from Roche. Rats' sera were analysed for albumin, total bilirubin, cholesterol, creatinine, glucose, urea, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltranspeptidase (GGT) and lactate dehydrogenase (LDH) concentrations.

#### *Histopathological examination*

Tissue samples from liver, kidneys, spleen and heart were processed in an automatic tissue processor (Leica TP1020 Semi-enclosed Benchtop Tissue Processor). Processed tissues were embedded in paraffin blocks and sectioned into 3 µm-thick sections using a microtome (Leica Jung Multicut 2045). The tissue sections were placed on glass slides, stained with haematoxylin and eosin according to the protocol described by Luna (1968) and covered with coverslips.

Histological slides that were prepared from the liver, kidney, spleen and heart of the experimental animals were examined under a microscope for pathological changes. Histopathological evaluation was performed quantitatively by blind analysis, and lesion scorings were obtained by examination of ten randomly selected sections from each organ.

Scoring of liver lesions was based on the presence of vacuolisation in hepatocytes, the number of Kupffer cells and pyknotic hepatocytes, necrosis of hepatic tissue, the size of sinusoids and hemosiderosis (Stevens *et al.*, 2002; Wang *et al.*, 2011). The criteria for grading kidney lesions were presence or absence of degenerative changes in Bowman's capsule, proximal and distal tubules, the presence of interstitial edema, congestion of renal

vessels and hemosiderosis. In the spleen, fibrosis in the capsular or parenchymal areas, atrophy of the red and white pulps, vacuolisation in the splenic histiocytes, necrosis and the presence of hemosiderin were considered (Stevens *et al.*, 2002; Wang *et al.*, 2011). Scoring of heart tissue was based on presence or absence of congestion in the cardiac tissue, changes in the regular striation pattern of myofibrils, presence or absence necrotic cells and myocardial infarction (Stevens *et al.*, 2002; Wang *et al.*, 2011). Tissue lesions were graded as normal for the presence of a lesion in 0-25% of the organ tissues, mild for the presence of lesion involving of 26-50% of the organ, moderate for the involvement of 51-75% and severe in marked lesions involving > 75% of the organ tissues.

*Statistical analysis*

Data were analysed using one-way analysis of variance (ANOVA), followed by post hoc test (Duncan). A probability value less than 0.05 was considered statistically significant (p< 0.05).

**RESULTS**

*Observation of mortality rate and behavioral changes*

Animals of the T1 and control groups did not show any behavioral changes following administration of *G. hombroniana* extract. Animals in the Group T2 were reluctant to feed and drink following extract administration, which lasted for 3-5 hr, after which, food and water consumption returned gradually to normal. Rats of the T3 group were dull, reluctant to move with rapid respiration for 4-6 hr, however, following extract administration the animals' behavior slowly returned to normal.

Administration of 300 and 2000 mg/kg of *G. hombroniana* aqueous extract to rats of T1 and T2 groups, respectively, did not result in mortalities in these groups. Administration of 5000 mg/kg BW of the extract to group

T3 animals resulted in the death of one rat (16.6% of the animals). It wasn't possible to calculate the LD<sub>50</sub> since a dose higher than 5000 mg/kg, which is the maximum dose permitted for administration to a laboratory animal (OECD, 2001), should be given to the rats to score ≥ 50% mortality. An extract with the LD<sub>50</sub> value higher than 5000 mg/kg is considered very safe to laboratory rodents (OECD, 2001; Michael *et al.*, 2007).

*Animal weight, food and water consumption*

No significant differences in the animals' weights were reported among T1, T2 and control groups (Table 1). Rats received 5000 mg/kg of the extract in the Group T3 showed significantly lower body weights after 72 hr of extract administration than the other groups. However, no significant differences were observed in the weight of the animals in the following days.

Rats of T2 and T3 groups showed significantly reduced consumption of water after 24 and 48 hours of extract administration, compared to the control group (Table 2). Water consumption returned to normal 72 hr post-administration.

Rats received 5000 mg/kg of *G. hombroniana* extract showed significantly lower food consumption after 24 and 48 hours of administering the extract, while, in the next days, food consumption returned to normal, compared to the other groups (Table 3).

*Post-mortem pathological changes*

No significant differences in internal organ morphology were noticed between the control group and *G. hombroniana* extract-treated groups. The relative organ to the body weight of the liver, kidneys, brain, heart and lungs did not show significant differences among the control and treatment groups. Rats administered 5000mg/kg of the extract showed significantly larger spleen, compared to the untreated control group (Table 4).

**Table 1. Effect of *Garcinia hombroniana* aqueous extract on rat weights**

| Rat group | Rat weight (g) |                          |                         |           |           |           |           |           |           |
|-----------|----------------|--------------------------|-------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
|           | Day 0          | Day 1                    | Day 3                   | Day 5     | Day 7     | Day 9     | Day 11    | Day 13    | Day 14    |
| C         | 208.3±4.8      | 215.3 <sup>a</sup> ±6.2  | 215.4 <sup>a</sup> ±7.2 | 215.7±6.5 | 213.9±7.0 | 216.4±7.7 | 218.8±7.4 | 219.5±7.4 | 218.6±7.5 |
| T1        | 211.3±2.8      | 213.6 <sup>a</sup> ±2.3  | 214.8 <sup>a</sup> ±1.8 | 216.5±2.3 | 218.4±2.7 | 216.4±2.7 | 218.3±2.2 | 219.4±2.8 | 217.1±3.4 |
| T2        | 202.0±2.2      | 203.4 <sup>ab</sup> ±4.6 | 205.1 <sup>a</sup> ±4.4 | 207.8±5.4 | 208.7±4.6 | 209.3±3.9 | 212.1±1.8 | 215.0±3.4 | 215.5±2.5 |
| T3        | 201.0±3.1      | 196.0 <sup>b</sup> ±2.1  | 197.1 <sup>b</sup> ±2.8 | 200.8±4.2 | 206.3±5.3 | 210.2±5.4 | 209.9±5.1 | 209.7±5.3 | 208.2±4.0 |

C = Control; administered 20 mL/kg BW of sterilized water; T1: given 300 mg/kg of *G. hombroniana* extract; T2: given 2000 mg/kg of *G. hombroniana* extract; T3: administered 5000 mg/kg of *G. hombroniana* extract  
 Values are presented as mean of six rats per group ± SEM (T3 contains five rats). Test: one-way ANOVA (post hoc: Duncan). Different superscript letters within column denote statistical difference at p< 0.05

**Table 2. Effect of *Garcinia hombroniana* aqueous extract on rats' food consumption**

| Days after treatment | Food consumption (g)     |                           |                           |                          |
|----------------------|--------------------------|---------------------------|---------------------------|--------------------------|
|                      | Rat group                |                           |                           |                          |
|                      | Control                  | T1                        | T2                        | T3                       |
| 1                    | 14.16 <sup>b</sup> ±0.62 | 13.50 <sup>b</sup> ±0.33  | 12.11 <sup>b</sup> ±1.28  | 7.31 <sup>a</sup> ±0.46  |
| 2                    | 15.53 <sup>a</sup> ±1.22 | 13.50 <sup>ab</sup> ±0.60 | 13.07 <sup>ab</sup> ±0.68 | 11.27 <sup>b</sup> ±0.74 |
| 3                    | 15.45±1.42               | 15.43±0.43                | 14.67±0.58                | 13.70±0.81               |
| 4                    | 16.16±1.70               | 13.73±0.55                | 14.89±0.57                | 14.02±0.74               |
| 5                    | 14.38±0.72               | 14.90±1.02                | 14.57±0.81                | 13.90±0.75               |
| 6                    | 13.62±0.74               | 13.66±0.47                | 13.89±0.62                | 12.80±0.37               |
| 7                    | 14.46±1.14               | 14.86±0.66                | 14.31±0.93                | 14.45±0.50               |
| 8                    | 14.42±0.94               | 13.03±0.49                | 13.67±0.98                | 13.72±0.77               |
| 9                    | 13.76±0.63               | 14.46±0.42                | 13.79±0.67                | 13.96±0.83               |
| 10                   | 14.69±1.38               | 13.27±0.39                | 13.66±0.73                | 13.75±0.38               |
| 11                   | 14.09±0.62               | 14.47±0.60                | 14.34±0.81                | 13.27±0.96               |
| 12                   | 13.81±0.69               | 13.32±0.70                | 14.13±0.98                | 13.09±0.19               |
| 13                   | 13.81±0.59               | 14.15±0.49                | 14.31±0.94                | 12.20±0.73               |
| 14                   | 13.52±0.69               | 12.81±0.34                | 13.72±0.71                | 12.93±0.84               |

Control: administered 20 ml/kg BW of sterilized water; T1: given 300 mg/kg of *G. hombroniana* extract; T2: given 2000 mg/kg of *G. hombroniana* extract; T3: administered 5000 mg/kg of *G. hombroniana* extract  
 Values are presented as mean of six rats per group ± SEM (T3 contains five rats). Test: one-way ANOVA (post hoc: Duncan). <sup>a,b</sup>Means with different superscript within are statistically different at p<0.05

**Table 3. Effect of *Garcinia hombroniana* aqueous extract on rats' water consumption**

| Days after treatment | Water consumption (mL)   |                          |                          |                          |
|----------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                      | Treatment group          |                          |                          |                          |
|                      | Control                  | T1                       | T2                       | T3                       |
| 1                    | 23.21 <sup>a</sup> ±0.28 | 20.64 <sup>a</sup> ±0.48 | 14.70 <sup>b</sup> ±0.72 | 11.42 <sup>c</sup> ±1.77 |
| 2                    | 26.57 <sup>a</sup> ±0.77 | 25.96 <sup>a</sup> ±0.43 | 22.77 <sup>b</sup> ±0.53 | 22.02 <sup>b</sup> ±0.96 |
| 3                    | 25.11±1.14               | 26.82±0.80               | 27.10±0.97               | 27.26±0.31               |
| 4                    | 30.65±1.12               | 27.61±0.45               | 30.37±1.50               | 28.30±0.52               |
| 5                    | 30.36±1.01               | 29.20±1.01               | 28.53±0.65               | 30.68±0.79               |
| 6                    | 27.50±1.44               | 29.23±0.69               | 26.69±0.43               | 27.0±0.58                |
| 7                    | 28.42±1.29               | 30.20±1.52               | 28.69±0.78               | 29.17±1.03               |
| 8                    | 29.17±0.92               | 27.37±0.84               | 28.98±0.53               | 28.40±0.71               |
| 9                    | 28.57±1.06               | 29.97±0.92               | 28.48±0.45               | 27.35±0.83               |
| 10                   | 30.04±1.19               | 28.89±0.70               | 28.80±0.90               | 28.33±0.64               |
| 11                   | 30.36±0.80               | 29.25±0.82               | 29.81±0.66               | 28.37±0.69               |
| 12                   | 28.30±1.04               | 28.47±1.15               | 28.50±0.39               | 27.31±0.68               |
| 13                   | 30.52±1.32               | 30.80±1.10               | 28.31±0.45               | 28.14±0.35               |
| 14                   | 30.04±0.89               | 29.68±0.56               | 29.14±0.54               | 29.07±0.71               |

Control: administered 20 ml/kg BW of sterilized water; T1: given 300 mg/kg of *G. hombroniana* extract; T2: given 2000 mg/kg of *G. hombroniana* extract; T3: administered 5000 mg/kg of *G. hombroniana* extract  
 Values are presented as mean of six rats per group ± SEM (T3 contains five rats). Test: one-way ANOVA (post hoc: Duncan). <sup>a,b,c</sup>denotes statistical difference within the row at p<0.05

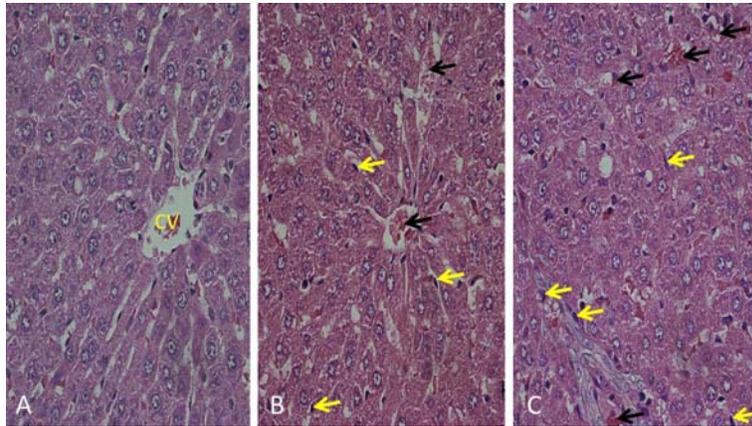
**Table 4. Effect of aqueous *Garcinia hombroniana* extract on spleen weight of rats**

| Group | Rat weight (g) | Spleen     |                          |
|-------|----------------|------------|--------------------------|
|       |                | Weight (g) | Relative weight (%)      |
| C     | 218.60±7.50    | 0.51±0.04  | 0.23 <sup>a</sup> ±0.01  |
| T1    | 217.10±3.40    | 0.57±0.04  | 0.26 <sup>ab</sup> ±0.02 |
| T2    | 215.50±2.50    | 0.55±0.05  | 0.25 <sup>ab</sup> ±0.02 |
| T3    | 208.20±4.00    | 0.59±0.04  | 0.34 <sup>b</sup> ±0.06  |

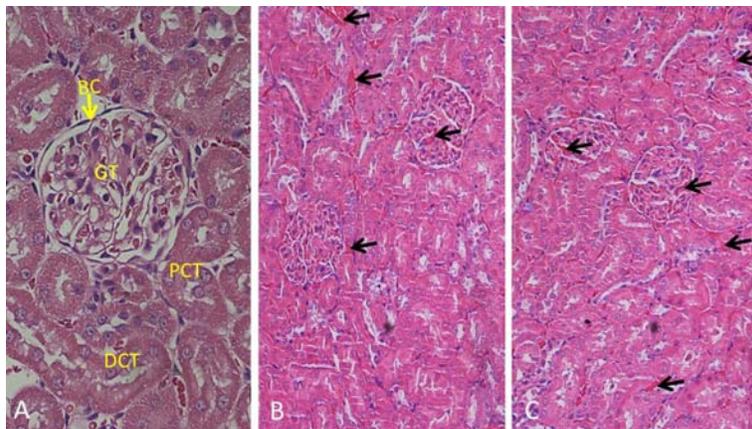
C = control rats; T1, T2, and T3 are rats treated with 300, 2000 and 5000 mg/kg BW of aqueous *G. hombroniana* extract, respectively. Relative weight = relative organ weight to rat's body weight Values are presented as mean of six rats per group ± SEM (T3 contains five rats)  
<sup>a,b</sup>Means with different superscript within a column are significantly different at p<0.05. Test: one-way ANOVA (post hoc: Duncan)

*Effect of Garcinia hombroniana extract on liver histology*

Normal hepatocytes and liver architecture were observed in the control and T1 group animals (Figure 1A). Rats received 2000 mg/kg BW *G. hombroniana* aqueous extract (T2) showed mild congestion of central veins with a slight increase in the number of Kupffer cells (Figure 1B). While rats receiving 5000 mg/kg BW of *G. hombroniana* aqueous extract (T3) exhibited a moderate increase in the number of Kupffer cells, congestion of blood vessels, increased necrotic cells and narrowing of liver sinusoids (Figure 1C).



**Figure 1. Liver tissue sections: A.** from a rat of the control group showing the central vein (CV), distribution of hepatic Kupffer cells and radially arranged hepatic sinusoids; **B.** from a rat administered *G. hombroniana* (2000 mg/kg BW) showing mild congestion (black arrow) and slight increase in the number of Kupffer cells (yellow arrow); **C.** from a rat administered 5000 mg/kg BW of *G. hombroniana* extract which shows increased Kupffer cells and congestion of liver tissue. Haematoxylin & Eosin. Magnification: 400X



**Figure 2. Kidney tissue sections: A.** from a rat of the control group (G: glomerulus, PCT: proximal convoluted tubule, BC: Bowman's capsule, DCT: distal convoluted tubule); **B.** rat administered 2000 mg/kg BW of *G. hombroniana* extract showing mild congestion (black arrow) of the renal blood vessels; **C.** rat administered 5000 mg/kg of the plant extract showing mild to moderate congestion (black arrow) of the renal blood vessels with edema in the interstitial tissue. Haematoxylin & Eosin. Magnification: 400X (plate A) and 200X (plate B and C)

*Effect of Garcinia hombroniana extraction the spleen*

Rats from the T1, T2 and control groups showed normal splenic parenchyma with normally distributed white and red pulps (Figure 3A) while the T3 group showed mild lesions including congestion and fibrotic splenic parenchyma (Figure 3B).

*Effect of Garcinia hombroniana extract on the heart tissue*

Rats of T1, T2 and control groups exhibited normal cardiac muscle fiber patterns with regular striation and

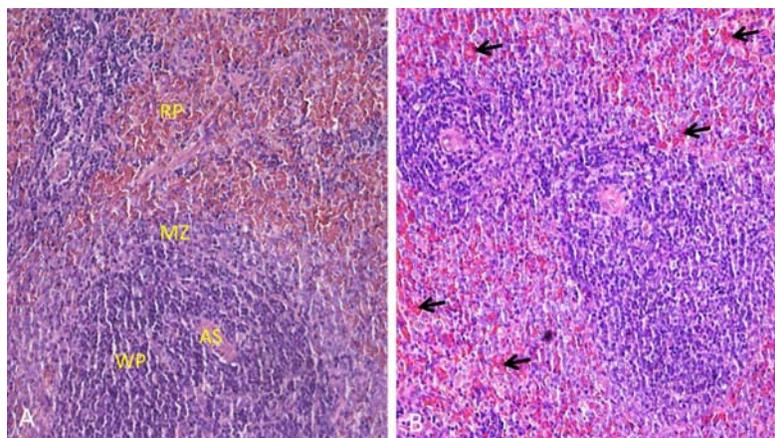
*Effect of administration of Garcinia hombroniana on kidney tissue*

Rats from T1 and control groups had normal renal histology (Figure 2A). Rats of the T2 group showed mild renal vascular congestion and a few necrotic cells (Figure 2B) while the T3 group rats showed mild to moderate vascular congestion, mild interstitial edema and a few necrotic cells (Figure 2C).

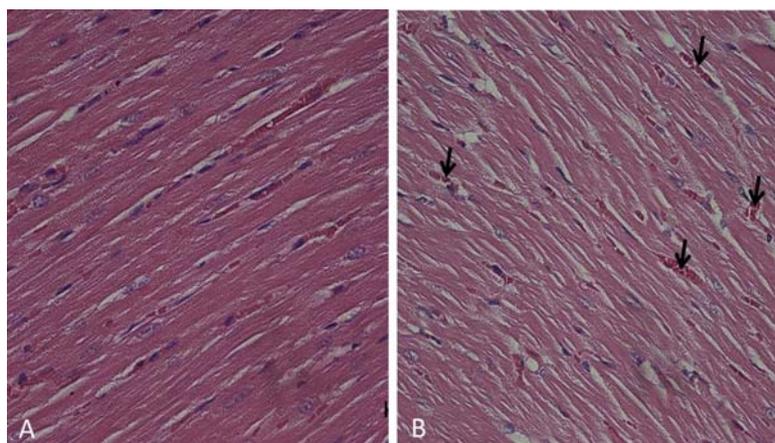
intact nuclei (Figure 4A). Rats of the T3 group showed mild congestion of the blood vessels and a few necrotic cells that were dissolved with acidophilic cytoplasm (Figure 4B).

*Haematological parameters*

Comparison of haematological parameters among the different groups did not show statistical differences in the values of PCV, MCV, MCHC, thrombocyte count and total differential leukocyte count.



**Figure 3.** Tissue section from spleen. A. A section from spleen of a of the control group; B. A section from spleen of a rat administered 5000 mg/kg of *Garcinia hombroniana* extract showing mild congestion of red pulp (black arrow). WP: white pulp, RP: red pulp, CA: central arteriole. Haematoxylin & Eosin. Magnification: 200X



**Figure 4.** Heart tissue sections: A. A section from the heart of a control group rat; B. A section from the heart of a rat administered *G. hombroniana* extract (5000 mg/kg BW). The section shows mild congestion of cardiac blood vessels (black arrow). Haematoxylin & Eosin. Magnification: 400X

#### Serum biochemical tests

No statistical differences were observed in serum albumin, total bilirubin, cholesterol, creatinine, glucose, urea, ALT, AST, GGT and LDH concentrations among the different groups.

#### DISCUSSION

*Garcinia hombroniana* has been used in traditional medicine for the treatment of a number of ailments and little is known about its toxicological properties. The present study demonstrated that the acute oral toxicity of *G. hombroniana* aqueous extract in Sprague-Dawley rats as an effort to determine the potential acute effects of this plant on the vital organs such as the liver, kidneys, heart and spleen.

The determination of the LD<sub>50</sub> is usually the first step in the evaluation of the toxic properties of medicinal plants (Yuet *et al.*, 2013). The ATC method that was followed by OECD (OECD, 2001) was used in the study. This method is considered as an alternative to the classical method for the determination of LD<sub>50</sub>. The ATC method has the advantage that fewer animals are used as the animals are tested in a stepwise manner. That is, three animals are given the same dose in each step, and when 0-1 animals died, another 3 animals will be used to administer the same dose of the extract. When less than

half of the animals (less than 3 rats out of 6 tested) died due to the administration of the extract, another 3 rats were administered a higher dose of the extract. Hence, fewer animals are utilised in the determination of the LD<sub>50</sub>.

In the current study, the administration of 5000 mg/kg BW (the highest permitted dose) of the extract resulted in 16.6% mortality. According to the chemical categorisation advocated by the OECD, the aqueous extract of *G. hombroniana* falls under class 5 compounds, which have the lowest toxicity on laboratory rodents (LD<sub>50</sub> higher than 5000 mg/kg) (OECD, 2001).

Internal organs such as liver, kidneys, spleen and heart are sensitive to predict the toxicity of chemicals and changes in the physiologic functions of the animal (Michael *et al.*, 2007). Following administration of *G. hombroniana* extract with the highest permitted dose (5000 mg/kg BW), no significant changes in the weights of liver, kidneys and heart were observed. These results also suggest the low toxic effects of this extract. However, the histopathological changes observed mainly in the liver and kidney of group T2 and T3 rats can be justified as drugs and other xenobiotics are concentrated in these organs, making these organs more prone to the appearance of toxic signs. The liver and kidneys are highly supplied with blood vessels and their function is to metabolise and filtrate xenobiotics present in the blood (Navarro and Senior, 2006; Farkas and Farkas, 2009).

Hepatotoxicity, which is defined as injury to the liver caused by a noninfectious agent, is often associated with defective liver function, which can be caused by many drugs and xenobiotics (Navarro and Senior, 2006). Laboratory evaluation of liver diseases can be categorised into three groups of tests to investigate (1) hepatocellular integrity, such as AST and ALT, (2) to screen biliary excretory function, such as serum bilirubin and GGT and (3) to monitor hepatocyte function, such as measuring of serum albumin, a plasma protein produced by hepatocytes (Navarro and Senior, 2006; Farkas and Farkas, 2009). The results of serum biochemical analyses exhibited no significant differences in these values among the control and treatment groups. The histopathological lesions observed in the liver of T2 and T3 animals did not result in the alteration of the organ's function based on the biochemical tests. The reason could be attributed to the enormous capacity of the liver to regeneration and detoxification of toxics and other xenobiotics.

The levels of urea and creatinine in the serum are usually measured concomitantly to assess the renal function, especially in acute renal injury as both creatinine and urea levels rise in the serum (Trombetta and Foote, 2009). In the acute toxicity study of *G. hombroniana* aqueous extract, the effect of the extract on serum levels of creatinine and urea was assessed and the results revealed no significant differences among the groups. Hence, the administration of *G. hombroniana* aqueous extract did not cause toxic effects on the renal function of the rats used in the study. The LDH is a widely used parameter in toxicological studies to diagnose tissue and organ damage, as a higher level of this enzyme is a marker for example, of hepatic injury or hemolysis (Kato *et al.*, 2006). No significant differences in the level of LDH were observed, which could imply that no alterations in the functions of the different organs and tissues were caused by the administration of the plant extract.

## CONCLUSION

The results of the current study suggest the low level of toxicity of *G. hombroniana* leaves aqueous extract. This plant exerts many medicinal properties and, hence, it can be considered as a good candidate to analyse and study its chemical constituents.

## ACKNOWLEDGEMENTS

The authors thankfully acknowledge the Faculty of Veterinary Medicine, Universiti Putra Malaysia for supplying the facility to conduct the study.

## FUNDING

This study was financially funded by the Ministry of Higher Education, Malaysia through grant number 01-11-08-642FR.

## CONFLICTS OF INTEREST

None of the authors of this paper has any financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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