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# Evaluation of acute, subacute and subchronic oral toxicity of *Rhaphidophora decursiva* (Roxb.) Schott extract in male Sprague Dawley rats

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**Rhaphidophora decursiva (Roxb.) Schott has been used in some Chinese community to treat colon cancer. This study aims to evaluate the toxic effects of the plant extract after a single dose toxicity study (14-day acute toxicity study), as well as 28-day sub-acute and 90-day subchronic toxicity study in male Sprague Dawley rats. Seventy two rats were divided into 3 groups for the acute, sub-acute and sub-chronic toxicity evaluations. Each group also have its control group which received distilled water. For the acute toxicity study, the 3 treatment groups received a single oral dose of the plant extract at 700, 2800 or 3500 mg/kg. The rats were then sacrificed after 14 days. For the sub-acute toxicity study, the 3 treatment groups received a daily oral dose of the plant extract at 70, 140 or 210 mg/kg for 28 days. As no lethality was observed in the sub-acute toxicity study, similar doses were used for the 90-day sub-chronic toxicity study. The toxicity was evaluated by the incidence of lethality, cage-side observations, body weight measurements, hematological and serum biochemical results. No adverse effects were observed during the experimental periods in any of the studies. Behaviour, body weight, haematological and biochemical analysis also showed no significant changes in the three toxicity studies. Based on the results, we concluded that the methanol extract of R. decursiva did not cause any toxic effects in male Sprague Dawley rats. The lethal oral dose (LD50) of the extract was greater than 3500 mg/kg, while the noobserved-adverse-effect level (NOAEL) for the extract was 210 mg/kg when administered once per day for 90 days.**

**Key words:** *Rhaphidophora decursiva* (Roxb.) Schott extract, acute toxicity, sub-acute toxicity, sub-chronic toxicity

## INTRODUCTION

Plants and herbs have been used since ancient times to cure various ailments. By the middle of the nineteenth century, approximately 80% of all medicines were derived from herbs. *Rhaphidophora decursiva*, which is more

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commonly known as 'Climbing Dragon', is one of the most popular herbal remedies in the Chinese community in Malaysia. The decoction of the plant leaves has been widely used as a traditional herb for the treatment of colon cancer, and it is available without a prescription in most countries. *Rhaphidophora* is a member of the Araceae family, which is a large genus of climbing shrubs that is distributed throughout India, Sri Lanka, Cambodia, Venezuela, Malaysia, Australia and Indonesia (Kiritikar and Basu, 2001). The Araceae or aroids are mainly a tropical family and are distributed worldwide, chiefly in tropical and subtropical regions. This genus is one of the largest aroid genera represented in tropical Asia and has several nodes of diversity (Boyce, 2001). The species are found in various habitats ranging from swamps, ponds, lakes, rivers, rice fields and forests and include approximately 100 species. Approximately 50% of aroid species are medicinal plants and have been used since ancient times (Boyce and Bogner, 2000).

Zhang et al. (2001) reported that the leaf and stem extracts from *R. decursiva* Schott (Araceae), a perennial, evergreen, semi-succulent, epiphytic vine found in Cuc Phuong National Park (Nho Quan District, Ninh Binh Province, Vietnam), were shown to possess antimalarial activity against both the D6 and W2 clones of *Plasmodium falciparum* with no apparent toxicity. Other than *Rhaphidophora decursiva*, *Rhaphidophora hookeri* (Mao Gou Shan Long) is used as a treatment for fractures (Boyce, 2001), and *Rhaphidophora hongkongensis* (Shi Zi Wei) is used as a treatment for traumatic injuries, fractures, lumbago, rheumatism and internal fever (Boyce, 2000).

*R. decursiva* is also one of the herb species among many others used for medicinal baths among the traditional Yao communities of China. Medicinal baths are a valuable traditional means to avoid and heal common illnesses among the traditional Yao societies of China, thus becoming a cultural attribute of the Yao people. Among the herbs used for instance, the benefits of *R. decursiva* may include reducing stasis to activate blood circulation, injuries from falls, rheumatoid in waist and legs, carbuncle and skin ulcer (Li et al., 2006).

One of the reasons for the increasing interest in herbal medicines is the belief that because these medicines are natural and have been traditionally used, they are safe and harmless. Nevertheless, their natural origin is not a guarantee of safety, as many reports concerning the risks associated with the use of herbal products have noted (Chan, 1997; Ernst, 1998; Vaes and Chyka, 2000; Whiting et al., 2002). Hence, scientific information regarding the safety of this plant for use as an alternative medicine is very important before it is further developed into a new medicinal herbal therapy. Therefore, the objectives of the present study were to determine the acute toxicity, subacute toxicity and subchronic toxicity effects of *R. decursiva* (Roxb.) Schott extract *in vivo*.

## MATERIALS AND METHODS

### Sample preparation

The plant (*R. decursiva* (Roxb.) schott) was collected based on convenience sampling from residential areas of Bercham in the Ipoh city of Perak state, Peninsular Malaysia. Approximately 1 to 2 kg of the whole plant, including the stems and leaves, was collected. The leaves were separated from the stems and washed thoroughly with distilled water. The washed leaves were then cut into small pieces and freeze-dried overnight at -70°C. The freeze-dried samples were then stored at -20°C before extraction with methanol.

### Sample extraction

Sample extraction was conducted according to a method developed by Othman et al. (2007), with slight modifications. Two grams of the freeze-dried samples was weighed and ground into powdered form. The ground freeze-dried samples were extracted using methanol (Merck) at a ratio of 0.2 g of sample to 40 ml of methanol. The mixture was placed in an orbital shaker (Heidolph Unimax 1010, German) at 200 rpm at room temperature for approximately 2 h. The mixture was then filtered twice using Whatman filter paper no. 4 until a clear solution was obtained. The cloudy solution was centrifuged at 500 rpm for 10 min and filtered again using the filter paper. The methanol was removed using a rotary evaporator (BUCHI Rotavapor R-200, Switzerland), and the extract powder was obtained after the solution was freeze-dried (The VIRTIS Company, USA).

### Animals and treatment

A total of 72 male Sprague Dawley rats, weighing approximately 200 to 220 g, were supplied by a local supplier (Chenur Supplier, Kuala Lumpur, Malaysia) for use in these studies. The rats were kept in the Animal House at the Faculty of Medicine and Health Sciences, University of Putra, Malaysia (UPM). The experiments were designed and conducted according to procedures that were approved by the Animal Care Use Committee (ACUC), Faculty of Medicine and Health Sciences, UPM. The animals were uniquely identified based on their body weights and kept in standard cages with three rats per cage. All rats were acclimatized at an ambient temperature of 22 ± 2°C, with a 12 h light-dark cycle, for at least five days prior to the start of the experiment. During the acclimatization and experimental periods, the rats were provided drinking water and normal rat chow *ad libitum*. The rats were then divided into 4 groups of 6 rats per group for the acute, sub-acute and sub-chronic toxicity studies. One control group and 3 treatment groups were used in each study. The rats were grouped based on different doses of the plant extract. The doses selected for the 14-day acute toxicity study were 0 (control group), 700, 2800 and 3500 mg/kg body weight of *R. decursiva* (Roxb.) Schott extract, according to the OECD guidelines for the testing of chemicals (OECD, 2001). For the 28-day subacute toxicity study and the 90-day subchronic toxicity study, the doses selected were 0 (control group), 70, 140 and 210 mg/kg body weight of the extract (OECD, 1998, 2008). Lower doses of the plant extract would have been used in the sub-chronic toxicity study if lethality had been observed

in the sub-acute toxicity study. The extract was administered orally for the sub-acute and sub-chronic toxicity studies, respectively. The toxicological effects of the extract were assessed on the basis of mortality within 14 days in the acute toxicity study, which was expressed as the median lethal dose (Lethal Dose or $LD_{50}$) (Miller and Tainter, 1944), and within 90 days in the subchronic toxicity study, which was expressed as the no-observed-adverse-effect level (NOAEL) using a gavage needle once for the acute toxicity study and once daily for 28 and 90 days.

#### Cage-side observations

The rats were observed individually and special attention was given to the treatment groups. The cage-side observations and mortality pattern assessments were performed daily throughout the study periods. The cage-side observations included the evaluation of the following: changes in skin, fur, and eyes; respiratory effects; autonomic effects, including salivation, diarrhea, and urination; central nervous system effects, including tremors and convulsions; changes in the level of activity, gait and posture; reactivity to handling or sensory stimuli; and altered strength (Kumarnsit et al., 2006; Demma et al., 2006; Mukinda and Syce, 2007; Obici et al., 2008).

#### Body weight measurements

Individual body weights were measured and recorded for two consecutive weeks prior to the administration of the extract and weekly throughout the experimental periods.

#### Hematological analyses

For the acute toxicity study, blood samples were collected prior to the beginning of (baseline) and at the end of the experimental period (Table 1). For the sub-acute toxicity study, blood was collected at baseline, day 15 and at the end of the treatment period, which was day 28 (Table 2). For the sub-chronic toxicity study, blood was collected at baseline, day 30, day 60 and at the end of the treatment period, which was day 90 (Table 3). All of the rats were anesthetized using diethyl ether and bled via cardiac puncture. The samples were collected in plastic test tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Hematological analyses were performed using an automated hematology analyzer (Sysmex KX21) with regard to the following parameters: red blood cell (RBC) or erythrocyte count, hemoglobin (Hgb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) or leukocyte count and platelet count (PLT) (Demma et al., 2006; Obici et al., 2008; Tan et al., 2008).

#### Serum biochemical analyses

Blood samples were collected at the same time points as described. The samples were collected in plastic test tubes without anticoagulant and allowed to stand for complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 15 min, after which point the serum samples were aspirated and stored at –20°C. The serum samples were analyzed to determine the levels of urea, creatinine (Crea), albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphate (ALP) using an automated biochemistry analyzer (ADVIA 2400, Japan) (Demma et al., 2006; Mukinda and Syce, 2007; Obici et al., 2008; Tan et al., 2008).

#### Statistical analyses

The data were represented as the mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) was performed to compare the differences between two or more means. A mean difference was considered significant when $p < 0.05$. Statistical analysis was performed using the statistical package for social science (SPSS) for Windows, version 20.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

The clinical appearance of all rats in the acute, sub-acute and sub-chronic toxicity studies, particularly the rats in the treatment groups, was normal or did not show any treatment-related adverse effects. Similar findings were observed when the body weights of the rats were analyzed (Figures 1, 2 and 3). No significant differences ($p > 0.05$) in the body weights of the treated rats was observed compared to the control rats in all three toxicity studies. The results of the hematological and serum biochemical analyses are summarized in Tables 1, 2 and 3. There were no apparent treatment-related adverse effects on the hematological parameters after exposure to *R. decursiva* (Roxb.) Schott extract. No statistically significant differences ($p > 0.05$) were observed between the groups in all studies. Similarly, serum biochemical results also showed no statistically significant ($p > 0.05$) differences between the groups in all studies.

## DISCUSSION

Herbal medicine is universally popular in primary healthcare, particularly in developing countries such as Malaysia. The wide usage of these so-called “naturalremedies” or “medicinal herbs" for self-medication is a result of the fact that the general public believes them to be safe and do not have any compromising health effects (Obici et al., 2008). However, because there has been a lack of scientific studies of the toxicity and adverse effects of these remedies, further investigations are vital for substances such as *R. decursiva* (Roxb.) Schott.

In the current study, the methanol extract has been used since the previous study conducted by Norhaizan and Phuah (2009) has shown that methanol extract of the leaves of *R. decursiva* possess higher total phenolic content and total antioxidant activity than the water extracts. The rats that received *R. decursiva* (Roxb.)

**Table 1.** Hematological and serum biochemical results from rats in the acute toxicity study.

| Parameter | 0 mg/kg (Control) | 700 mg/kg | 2800 mg/kg | 3500 mg/kg |
|---|---|---|---|---|
| **WBC ($10^9$/L)** | | | | |
| Baseline[a] | 10.97±1.35 | 13.18±0.45 | 11.07±0.75 | 12.65±1.09 |
| Final[b] | 9.18±2.42 | 12.08±2.47 | 13.37±0.91 | 10.52±1.06 |
| **RBC ($10^{12}$/L)** | | | | |
| Baseline[a] | 5.67±0.20 | 5.86±0.23 | 6.00±0.20 | 6.36±0.10 |
| Final[b] | 6.28±1.33 | 5.92±1.19 | 6.80±0.16 | 7.40±0.30 |
| **HGB (dL)** | | | | |
| Baseline[a] | 12.22±0.52 | 12.77±0.48 | 12.73±0.38 | 13.33±0.26 |
| Final[b] | 13.27±2.80 | 12.33±2.47 | 14.17±0.30 | 15.28±0.86 |
| **HCT (%)** | | | | |
| Baseline[a] | 37.38±1.53 | 38.87±1.36 | 39.30±1.24 | 40.98±0.54 |
| Final[b] | 40.93±8.60 | 37.45±7.49 | 44.05±0.75 | 46.80±2.42 |
| **MCV (fL)** | | | | |
| Baseline[a] | 65.93±0.72 | 66.40±0.71 | 65.55±0.99 | 64.48±0.51 |
| Final[b] | 54.45±10.90 | 52.70±10.56 | 64.93±1.08 | 63.16±0.75 |
| **MCH (pg)** | | | | |
| Baseline[a] | 21.53±0.30 | 21.78±0.25 | 21.23±0.29 | 20.98±0.15 |
| Final[b] | 17.63±3.53 | 17.37±3.49 | 20.88±0.43 | 20.62±0.34 |
| **MCHC (dl)** | | | | |
| Baseline[a] | 32.67±0.20 | 32.85±0.18 | 32.42±0.30 | 32.53±0.28 |
| Final[b] | 26.98±5.40 | 32.17±0.18 | 32.17±0.18 | 32.64±0.18 |
| **PLT ($10^9$/L)** | | | | |
| Baseline[a] | 873.50±178.72 | 941.17±177.36 | 1008.83±43.31 | 931.67±119.22 |
| Final[b] | 625.67±195.44 | 614.50±190.71 | 898.67±165.40 | 1045.20±52.37 |
| **ALT (U/L)** | | | | |
| Baseline[a] | 31.50±8.45 | 35.17±11.26 | 42.33±9.71 | 48.83±3.75 |
| Final[b] | 40.00±10.18 | 50.50±10.61 | 55.67±2.59 | 55.17±4.18 |
| **AST (U/L)** | | | | |
| Baseline[a] | 81.67±20.34 | 91.67±30.80 | 92.83±18.97 | 129.83±19.72 |
| Final[b] | 119.83±30.60 | 120.83±29.14 | 123.17±18.01 | 143.00±18.83 |
| **ALP (U/L)** | | | | |
| Baseline[a] | 288.83±72.55 | 315.33±101.62 | 373.00±84.94 | 402.83±27.20 |
| Final[b] | 256.00±57.71 | 291.17±61.37 | 376.17±35.80 | 344.67±22.46 |
| **Crea (µmol/L)** | | | | |
| Baseline[a] | 12.33±2.88 | 10.67±3.84 | 14.50±8.12 | 26.1667±16.36 |
| Final[b] | 20.67±10.69 | 23.83±18.16 | 21.67±1.48 | 19.50±2.09 |

**Table 1.** Contd.

| | | | | |
|---|---|---|---|---|
| **Alb (g/L)** | | | | |
| Baseline[a] | 21.00±6.68 | 21.17±6.76 | 27.33±5.56 | 32.67±1.43 |
| Final[b] | 30.83±6.21 | 30.17±6.06 | 34.17±0.60 | 35.33±0.61 |
| **Urea (mmol/L)** | | | | |
| Baseline[a] | 3.82±0.94 | 3.87±1.27 | 4.17±0.90 | 5.88±0.42 |
| Final[b] | 6.15±1.37 | 5.22±1.09 | 6.05±0.22 | 7.23±0.34 |

The data are presented as the means±SEM. [a]Data obtained prior to the treatment period. [b]Data obtained at the end of the treatment period. All values were not significantly different from control at $p<0.05$.

**Table 2.** Hematological and blood serum biochemical results from rats in the subacute toxicity study.

| Parameter | 0 mg/kg (Control) | 70 mg/kg | 140 mg/kg | 210 mg/kg |
|---|---|---|---|---|
| **WBC ($10^9$/L)** | | | | |
| Baseline[a] | 11.23±1.12 | 10.77±1.22 | 8.55±0.73 | 10.62±1.33 |
| Final[b] | 12.22±1.61 | 12.02±1.65 | 19.13±8.84 | 14.94±5.35 |
| **RBC ($10^{12}$/L)** | | | | |
| Baseline[a] | 6.74±0.10 | 6.99±0.17 | 7.17±0.16 | 6.87±0.12 |
| Final[b] | 7.68±0.09 | 8.12±0.09 | 7.69±0.08 | 6.79±0.75 |
| **HGB (dl)** | | | | |
| Baseline[a] | 14.32±0.09 | 14.48±0.19 | 14.88±0.32 | 14.40±0.24 |
| Final[b] | 15.58±0.22 | 15.72±0.13 | 14.90±0.45 | 13.28±1.45 |
| **HCT (%)** | | | | |
| Baseline[a] | 44.07±0.32 | 43.93±0.41 | 45.33±0.86 | 43.73±0.74 |
| Final[b] | 47.34±0.65 | 48.28±0.37 | 45.87±0.70 | 40.58±4.59 |
| **MCV (fL)** | | | | |
| Baseline[a] | 65.38±0.58 | 64.17±1.00 | 63.23±0.64 | 63.67±0.77 |
| Final[b] | 61.66±0.61 | 59.50±0.88 | 59.63±1.32 | 59.68±0.24 |
| **MCH (pg)** | | | | |
| Baseline[a] | 21.50±0.28 | 21.12±0.28 | 20.77±0.25 | 20.97±0.27 |
| Final[b] | 20.28±0.13 | 19.38±0.31 | 19.37±0.74 | 19.56±0.07 |
| **MCHC (dl)** | | | | |
| Baseline[a] | 32.48±0.16 | 32.97±0.43 | 32.83±0.27 | 32.93±0.12 |
| Final[b] | 32.90±0.23 | 32.54±0.09 | 32.50±0.57 | 32.80±0.19 |
| **PLT ($10^9$/L)** | | | | |
| Baseline[a] | 1394.33±57.86 | 1163.50±98.95 | 1354.50±72.71 | 1327.00±101.43 |
| Final[b] | 893.80±86.18 | 850.60±114.29 | 991.00±99.51 | 614.20±172.27 |
| **ALT (U/L)** | | | | |
| Baseline[a] | 40.00±1.71 | 60.60±8.36 | 43.83±2.51 | 42.50±3.87 |

**Table 2.** Contd.

| | | | | |
|---|---|---|---|---|
| Final[b] | 41.75±3.97 | 67.33±19.21 | 61.67±1.20 | 47.75±8.34 |
| **AST (U/L)** | | | | |
| Baseline[a] | 131.67±6.50 | 130.50±7.37 | 133.50±12.75 | 143.83±13.70 |
| Final[b] | 103.00±17.82 | 131.20±9.27 | 126.00±22.27 | 162.67±41.25 |
| **ALP (U/L)** | | | | |
| Baseline[a] | 288.83±72.55 | 315.33±101.62 | 373.00±84.94 | 402.83±27.20 |
| Final[b] | 256.00±57.71 | 291.17±61.37 | 376.17±35.80 | 344.67±22.46 |
| **Crea (µmol/L)** | | | | |
| Baseline[a] | 24.67±1.36 | 26.67±1.65 | 20.50±2.23 | 34.33±12.02 |
| Final[b] | 28.75±3.40 | 28.00±1.58 | 30.67±2.91 | 27.75±1.70 |
| **Alb (g/L)** | | | | |
| Baseline[a] | 36.17±0.04 | 36.33±0.61 | 35.17±0.87 | 33.67±1.61 |
| Final[b] | 33.00±3.11 | 34.40±1.21 | 34.33±1.20 | 33.25±1.11 |
| **Urea (mmol/L)** | | | | |
| Baseline[a] | 6.10±0.86 | 6.37±0.30 | 5.02±0.30 | 6.20±0.48 |
| Final[b] | 4.93±0.23 | 6.30±0.35 | 6.03±0.35 | 6.10±0.37 |

The data are presented as the means ± SEM. [a]Data obtained prior to the treatment period. [b]Data obtained at the end of the treatment period.

Schott extract showed no mortality and did not exhibit any clinical signs of toxicity in the acute, sub-acute, and sub-chronic toxicity studies. Based on the acute toxicity findings, an $LD_{50}$ value could not be determined, as the $LD_{50}$ describes only one end point, that is death. Thus the results obtained in the study suggested that the $LD_{50}$ of *R. decursiva* (Roxb.) Schott extract is higher than 3,500 mg/kg, as no lethality was found in the rats exposed to that concentration of *R. decursiva* (Roxb.) Schott throughout the experimental period of the study. Meanwhile, the no-observed-adverse-effect level (NOAEL) for this plant extract was determined to be 210 mg/kg for 90 days, as no lethality was observed in the sub-chronic toxicity study.

Apart from the mortality rate, cage-side functional observations, including the evaluation of changes in skin, fur, and eyes as well as respiratory effects, autonomic effects and nervous system effects, are very important in toxicity studies. Eaton and Klaassen (1996) suggested that animals given high doses of plant extracts or chemicals might show slight changes in behavior as a consequence of the metabolism of the plant extracts or chemicals. However, the signs are quickly reversible (Eaton and Klaassen, 1996). The rats in the present toxicity studies showed neither signs of behavioral changes nor abnormalities in the parameters mentioned.

In general, increases or decreases in the body weights of animals can be used as an indicator of adverse effects of drugs and chemicals (Teo et al., 2002). However, Harizal et al. (2010) reported that increases in the body weights of animals are more closely related to body fat accumulation rather than to the toxic effects of drugs or chemicals. Rhiouani et al. (2008) suggested that reductions in the body weights of animals in toxicity studies may be associated with normal physiological adaptation responses to the plant extracts or compounds, which lead to low appetite and, hence, lower caloric intake by the animal. High doses of plant extracts or compounds might also induce stress in the animals, thereby reducing their food intake, which may lead to reductions in their body weights (Harris et al., 1998). In the present toxicity studies, *R. decursiva* (Roxb.) Schott extract did not appear to affect the body weights of the rats at any dose throughout the treatment periods.

The hematopoietic system is one of the most sensitive targets of toxic compounds and is an important index of physiological and pathological status in humans and animals (Mukinda and Syce, 2007). Blood parameters are relevant indicators for risk evaluation. Olson et al. (2000) reported that changes in the hematological system have a higher predictive value for human toxicity when the data are extrapolated from animal studies.

**Table 3.** Hematological and blood serum biochemical results from rats in the subchronic toxicity study.

| Parameter | 0 mg/kg (Control) | 70 mg/kg | 140 mg/kg | 210 mg /kg |
|---|---|---|---|---|
| **WBC ($10^9$/L)** | | | | |
| Baseline[a] | 12.37±1.56 | 10.53±1.32 | 8.78±1.57 | 11.57±0.96 |
| Final[b] | 24.65±2.09 | 20.76±3.49 | 21.15±2.62 | 15.33±2.11 |
| **RBC ($10^{12}$/L)** | | | | |
| Baseline[a] | 6.83±0.19 | 6.76±0.11 | 7.27±0.14 | 7.31±0.27 |
| Final[b] | 7.80±0.26 | 7.96±0.12 | 7.66±0.23 | 7.29±0.90 |
| **HGB (g/L)** | | | | |
| Baseline[a] | 145.7±0.38 | 142.0±0.30 | 151.2±0.38 | 150.7±0.50 |
| Final[b] | 150.5±0.82 | 145.4±0.41 | 147.8±0.27 | 148.3±0.23 |
| **HCT (%)** | | | | |
| Baseline[a] | 44.70±1.43 | 43.33±1.02 | 45.48±0.96 | 45.07±1.47 |
| Final[b] | 45.10±1.85 | 44.94±0.98 | 43.55±1.11 | 40.43±5.07 |
| **MCV (fL)** | | | | |
| Baseline[a] | 65.43±0.71 | 64.17±1.23 | 62.50±0.40 | 61.70±0.40 |
| Final[b] | 57.78±0.68 | 56.50±0.85 | 56.88±0.24 | 55.43±0.31 |
| **MCH (pg)** | | | | |
| Baseline[a] | 21.33±0.33 | 21.02±0.37 | 20.75±0.26 | 20.63±0.23 |
| Final[b] | 19.23±0.61 | 18.28±0.38 | 19.33±0.34 | 21.73±3.67 |
| **MCHC (g/L)** | | | | |
| Baseline[a] | 326.2±0.31 | 327.7±0.31 | 332.2±0.31 | 334.0±0.20 |
| Final[b] | 333.3±0.71 | 323.4±0.34 | 339.5±0.46 | 392.3±6.84 |
| **PLT ($10^9$/L)** | | | | |
| Baseline[a] | 1233.33±81.03 | 1249.83±55.08 | 1433.17±84.26 | 1305.00±176.42 |
| Final[b] | 1161.00±109.48 | 946.60±108.12 | 1150.75±61.11 | 815.25±253.98 |
| **ALT (U/L)** | | | | |
| Baseline[a] | 38.50±2.36 | 46.50±3.53 | 37.33±3.96 | 43.00±2.00 |
| Final[b] | 62.75±4.15 | 60.80±5.14 | 68.50±2.66 | 69.00±6.98 |
| **AST (U/L)** | | | | |
| Baseline[a] | 111.33±6.78 | 128.17±7.35 | 118.50±14.48 | 122.17±8.15 |
| Final[b] | 79.50±6.25 | 109.80±1.93 | 115.75±5.12 | 136.50±25.41 |
| **ALP (U/L)** | | | | |
| Baseline[a] | 340.33±21.37 | 367.17±41.26 | 250.17±26.36 | 225.83±14.11 |
| Final[b] | 174.17±19.17 | 170.20±35.36 | 190.50±17.00 | 161.00±18.00 |
| **Crea (µmol/L)** | | | | |
| Baseline[a] | 22.83±0.95 | 24.67±1.65 | 18.83±3.51 | 23.50±1.61 |
| Final[b] | 36.00±3.03 | 34.60±1.96 | 29.25±0.63 | 28.75±2.87 |

**Table 3.** Contd.

| | | | | |
|---|---|---|---|---|
| **Alb (g/L)** | | | | |
| Baseline[a] | 37.50±0.67 | 35.17±0.95 | 34.17±0.70 | 34.67±0.33 |
| Final[b] | 36.80±0.86 | 31.40±2.93 | 35.75±0.75 | 34.00±0.45 |
| **Urea (mmol/L)** | | | | |
| Baseline[a] | 5.98±0.66 | 6.75±1.04 | 6.12±0.91 | 7.42±0.80 |
| Final[b] | 6.35±0.39 | 7.53±0.86 | 7.98±0.45 | 6.80±0.36 |

The data are presented as the means ± SEM. [a]Data obtained prior to the treatment period. [b]Data obtained at the end of the treatment period. All values were not significantly different from control at $p<0.05$.

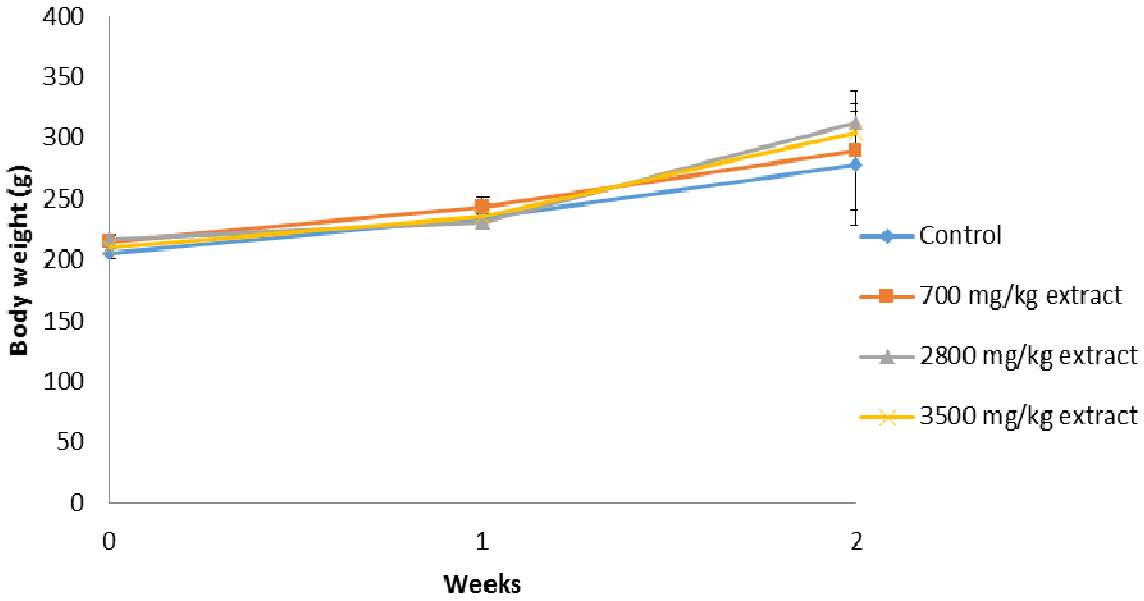


**Figure 1.** Body weight changes in rats in the acute toxicity study.

Administration of *R. decursiva* (Roxb.) Schott extract to the rats in all three toxicity studies caused no abnormalities in the hematological parameters, indicating that this plant extract would not likely cause any hematological abnormalities in humans, including bleeding, anemia or bone marrow suppression. Marked decreases in the numbers of RBC, WBC and platelets, as well as packed cell volumes (PCV) and hemoglobin concentrations, were observed in rats that received a high dose of the chemical *N*-Methyl-*N*-Nitrosourea (MNU). These rats also showed clinical signs of acute toxicity, including ruffled fur, bloody diarrhea, blindness and high mortality rates within 14 days after administration of the chemical. Necropsy findings in these animals revealed marked subcutaneous hemorrhages and hemorrhagic gastroenteritis, which were consistent with acute toxicity or poisoning (Hazilawati et al., 2009a). Meanwhile, reductions in the numbers of RBC (anemia), WBC (leukopenia) and platelets (thrombocytopenia) that are not accompanied by clinical signs of acute toxicity are usually associated with bone marrow suppression, as observed in cattle grazing on small quantities of bracken fern over several months (Knight and Walter, 2001).

The kidneys and liver are two major organs that play roles in detoxification. A number of cases of renal and hepatic toxicity have been reported following the use of phytotherapeutic products (Corns, 2003; Hilaly et al., 2004; Isnard et al., 2004; Saad et al., 2006). Measurements of urea and creatinine levels in the blood are usually performed to evaluate kidney function

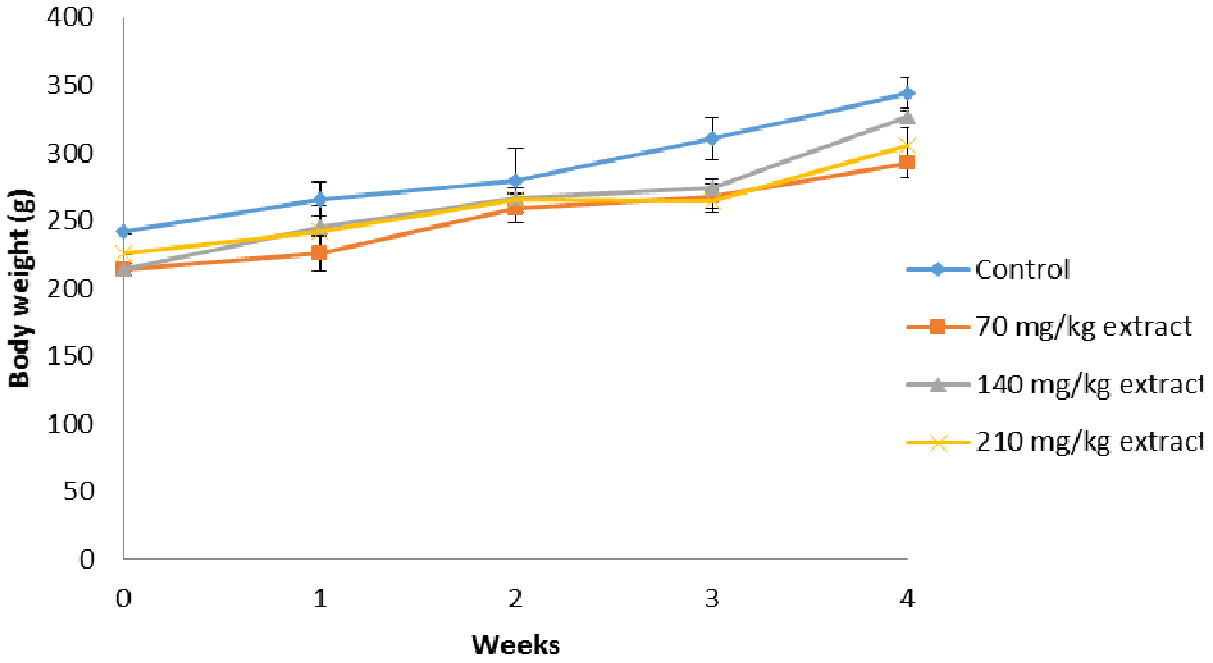


**Figure 2.** Body weight changes in rats in the sub-acute toxicity study.

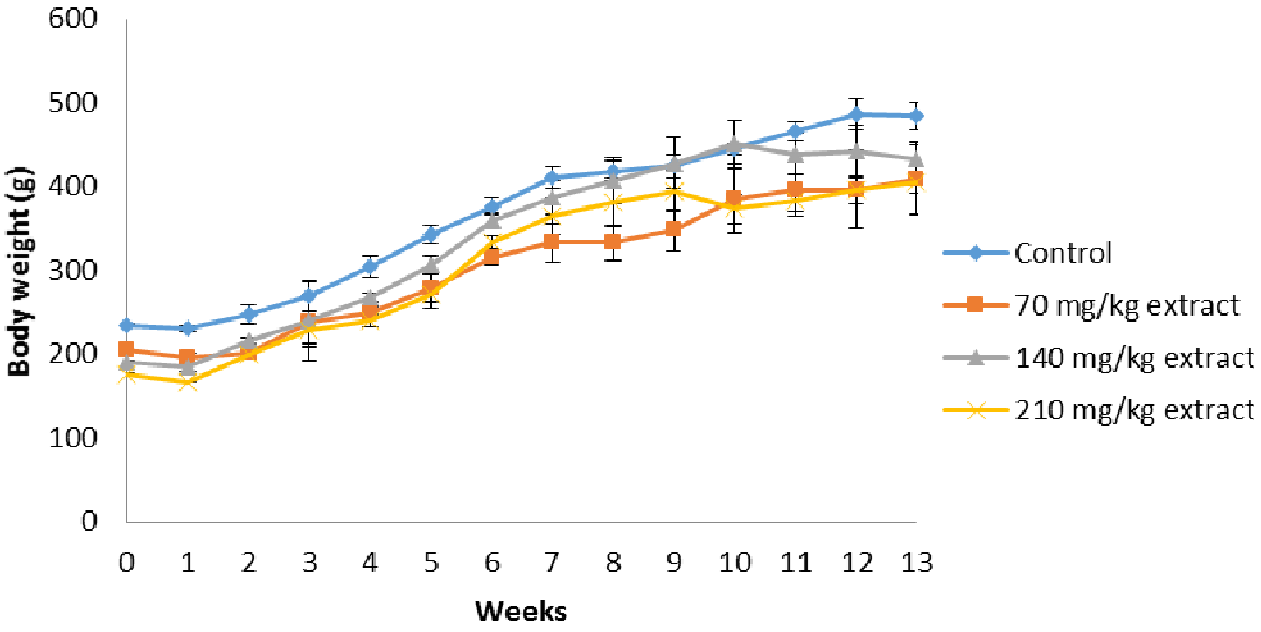


**Figure 3.** Body weight changes in rats in the sub-chronic toxicity study.

(Newman and Price, 1999). In cases of acute or chronic renal toxicity, these two parameters are usually markedly increased to four or five times higher than the normal values in control animals. In the present toxicity studies, all of the rats that had been orally administered various doses of *R. decursiva* (Roxb.) Schott extract had urea and creatinine levels ($p > 0.05$) similar to those of control animals, which indicated that the extract has no toxic

effects on the kidneys. Further examination of the microscopic morphology of the kidneys revealed no abnormalities (data not shown). Rats with acute kidney injury due to overdoses of gentamicin showed marked elevations in urea and creatinine levels in the blood when compared to the levels in control animals (Hazilawati et al., 2009b). Microscopic examination of the renal tissues revealed numerous protein casts in the renal tubules, which is one of the hallmarks of acute renal toxicity (Hazilawati et al., 2009b). Meanwhile, rats fed an adenine diet daily for 90 days also had high blood urea and creatinine levels compared to control animals, and the microscopic morphology of the kidneys of the adenine fed animals revealed lesions that are typical in chronic renal toxicity, including interstitial fibrosis, chronic inflammation and dilatation of the renal tubules (Farah et al., 2011). No such lesions were observed in any of the three toxicity studies presented here.

The most common parameters used to assess liver function are ALT, AST and ALP (Tolman and Rej, 1999; Hilaly et al., 2004). AST is an enzyme found in the cytoplasm and mitochondria in different tissues, including the heart and skeletal muscles, liver, kidneys, pancreas, and erythrocytes (Chaves and Silva, 1998; Aniagu et al., 2004). The concentration of AST in the blood is higher than that of ALT because the cells of the body contain more AST than ALT (Mayne, 1996). This enzyme is used as a marker of liver injury in certain species of animals, including cattle, goats, sheep, horses and pigs. Hence, the increases in AST without any concomitant increases in other enzymes, including CK and ALP, that were observed in these animals suggest that liver injury occurred. ALT is a liver-specific enzyme in dogs, cats, rabbits, rats and primates (Farah et al., 2011). It is localized primarily in the cytosol of hepatocytes and is considered to be a sensitive marker of hepatocellular damage in these animal species when compared to the levels of AST. It can provide a quantitative assessment of the degree of damage sustained by the liver (Al-Mamary et al., 2002).

The ALT, AST and ALP levels were normal in all of the rats from the 14-day acute toxicity study, 28-day sub-acute toxicity study and the 90-day sub-chronic toxicity study. This was also confirmed by the absence of histopathological changes in the liver (data not shown). In other studies, elevated liver enzymes always showed concurrent changes in the liver microscopically. For instance, centrilobular degenerative changes, steatosis or necrosis always accompanied elevated liver enzymes (Ishak and Zimmerman, 1995). These changes were not observed in the livers of the rats from our study (data not shown); this result corroborates the normal levels of ALT observed in those rats. Similar findings were observed in other rats in these studies, suggesting that *R. decursiva* extract causes no hepatotoxicity.

## Conclusion

The present studies demonstrate that *R. decursiva* (Roxb.) Schott extract lacks the toxic effects that could compromise the medicinal use of this plant as an herbal medicine. No mortality was found 14 days after the administration of a single oral dose of the extract. This indicates that the extract has a high $LD_{50}$ value and suggests a wide margin of safety for therapeutic doses. In the repeated-dose oral toxicity studies (28 and 90 days), there were no toxic effects observed with regard to the behavior, body weight, hematological and biochemical parameters of the rats; hence, the NOAEL for this extract was determined to be 210 mg/kg body weight for 90 days. The present findings show that *R. decursiva* (Roxb.) Schott extract is not likely to produce any toxicological effects and suggest that the extract is safe for medicinal use.

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## ABBREVIATIONS

**RBC,** Red blood cell or erythrocyte; **Hgb,** hemoglobin; **HCT,** hematocrit; **MCV,** mean corpuscular volume; **MCH,** mean corpuscular hemoglobin; **MCHC,** mean corpuscular hemoglobin concentration; **WBC,** leukocyte count; **PLT,** platelet count; **Crea,** creatinine; **Alb,** albumin; **AST,** aspartate aminotransferase; **ALT,** alanine aminotransferase; **ALP,** alkaline phosphate; **CK,** creatinine kinase; **PCV,** packed cell volume; **SD,** Sprague Dawley; **ACUC,** Animal Care Use Committee; **OECD,** Organization of Economic Cooperation and Development; **EDTA,** ethylenediaminetetraacetic acid.

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