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In vivo toxicity evaluation of a standardized extract of *Syzygium aqueum* leaf

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ABSTRACT

In this study, the acute and subchronic toxicity effect of the *Syzygium aqueum* leaf extract (SA) was evaluated. For the acute toxicity study, a single dose of 2000 mg/kg of the SA was given by oral-gavage to male Sprague-Dawley (SD) rats. The rats were observed for mortality and toxicity signs for 14 days. In the subchronic toxicity study the SA was administered orally at doses of 50, 100, and 200 mg/kg per day for 28 days to male SD rats. The animals were sacrificed at the end of the experiment. The parameters measured including food and water intake, body weight, absolute and relative organ weight, blood biochemical tests and histopathology observation. In both the acute and subchronic toxicity studies, SA did not show any visible signs of toxicity. There were also no significant differences between the control and SA treated rats in terms of their food and water intake, body weight, absolute and relative organ weight, biochemical parameters or gross and microscopic appearance of the organs. There were no acute or subchronic toxicity observed and our results indicate that this extract could be devoid of any toxic risk. This is the first *in vivo* study reported the safety and toxicity of SA.

1. Introduction

Syzygium aqueum (*Eugenia aquea*) is a medicinal plant, widely found in tropical regions such as Malaysia and Indonesia. Rumphius, the author of *The Ambonese Herbal*: Volume I has mentioned that *S. aqueum* was used in the traditional medicine of Ambon and the details of its isolated compound has appeared in NAPRALERT™ [1]. Various parts of this plant are used in traditional medicine; while the leaves has been shown to possess antibiotic activity and relieving child birth pains [2]. A decoction of the *S. aqueum*'s astringent bark is used for thrush [3]. In Malaysia, the powdered dried leaves have been used to treat mouth ulcers and a preparation of its roots has been used to relieve itching and reduce swelling [4].

Recently, the SA was shown to have antihyperglycemic properties [5]. The extract and its six bioactive compounds effectively enhanced adipogenesis, stimulated glucose uptake, increased adiponectin secretion and as well as being non-cytotoxic to 3T3-L1 cells [6]. The leaf extract has also been reported to have cosmeceutical properties, anti-tyrosinase, anticellulite, and lypolitic [7]. Other researchers have also established the free radical scavenging ability and high phenolic content of this plant extract [4,7,8].

Despite the increasing number of reports on the medicinal benefits of the *S. aqueum* leaf, the *in vivo* toxicological

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effect of the plant extract has yet to be reported. It is therefore deemed necessary to evaluate the acute and subchronic toxicity of the *S. aqueum* leaf extract (SA) in a rat model. The toxicity study would serve as a very important baseline for further studies in developing this plant as an herbal medicine.

2. Materials and methods

2.1. Experimental animals

Male Sprague-Dawley (SD) rats at 6-weeks of age (weighing approximately 160–200 g) were obtained from the Animal house, School of Science, Monash University, Bandar Sunway, Malaysia. The rats were acclimatized to the animal holding facility at the International Medical University (IMU) for 7 days before the start of the experiments. The rats were housed in individual polycarbonate cages and were given food rat chow (Gold Coin) *ad libitum* and had free access to distilled water. The rats were maintained at 23 ± 2 °C on a light/dark cycle of 12 h. All of the animal procedures were subjected to review and approval by the Research and Ethics Committee of the IMU and are in compliance with the National Institutes of Health (NIH) guidelines for humane treatment of laboratory animals.

2.2. Plant collection

The leaves were obtained from Kuala Lumpur, Malaysia. It was harvested and collected from March to June 2012. The leaves were authenticated at the Herbarium of the Forest Research Institute of Malaysia (FRIM) with the given sample number PID 210712-15. The leaves were thoroughly washed with copious amount of distilled water to remove dirt, following that the wet leaves were dried in an oven at 37 °C for one week. During this time, the leaves were periodically turned over to provide uniform drying. The dried leaves were ground to a fine powder using a Fritsch dry miller and the total powder obtained was weighed.

2.3. Extraction of SA

About 1 kg of the powdered leaves was extracted in 96% ethanol (1:10, w/v). The extraction was carried out at room temperature and was left for shaking for 24 h in an orbital shaker. The suspension thus obtained was filtered using a 114 Whatman filter paper and the filtrate collected. Ethanol filtrate was concentrated using a rotary evaporator at 40 °C. The dried crude extract was further concentrated by allowing it to stand overnight in an oven at 37 °C to remove trace of ethanol solvent. The dried extract was stored at 4 °C and its stability was monitored for 12 months.

2.4. Standardization of SA against myricitrin

The SA was standardized against myricitrin [5] on an Agilent 1100UV HPLC system (Agilent, MO, USA), consisting of a quaternary gradient pump (model G1311A) with an on-line vacuum degasser (model G1322A), an automated sample injector (model G1313A), and a 1100-UV detector (model G1314A). The SA (10 mg/ml) was injected (10 μL) into the HPLC system with myricitrin (1 mg/ml) (Sigma Chemical, St. Louis, MO) used as the authentic marker. The standardization was carried out on a Chromolith C18 column (2.0 mm × 150 mm, 3 μm) (Merck, Darmstadt, Germany) with a Chromolith guard column (2.2 mm × 50 mm, 1 μm) (Merck, Darmstadt, Germany). The mobile phase consisted of solvent A: 0.1% formic acid in water and solvent B: 0.1% formic acid in acetonitrile. A gradient elution was performed at a flow rate of 0.5 ml/min as follows: 0–10 min, 10% B; 10–20 min, 70% B; 20–30 min, 100% B; and finally 5 min for washing and reconditioning the column with 100% B. The UV absorbance was monitored at 254 nm. Quantitative estimations were based on the area of peaks of sample (SA) and corresponding authentic marker (myricitrin) to ensure a standardized preparation of extract.

2.5. Toxicity evaluation

2.5.1. Acute toxicity study

The acute toxicity study was conducted to determine the adverse effects of SA feeding on the vital organs (liver, kidney, heart and pancreas) and the biochemical parameters (alanine aminotransferase, alkaline phosphate, creatinine, urea and total protein). This was carried out according to the Organisation of Economic Co-operation and Development (OECD) guideline 420 [9]. Prior to dosing with the extract, the rats were fasted overnight from food, but were allowed free access to water. A single dose of 2000 mg/kg of SA was orally gavaged in 5 male SD rats. The rats in the control group ($n = 5$) were given olive oil as vehicle. All of the experimental animals were maintained under close observation for any signs of toxicity and mortality immediately after dosing, at 4 h and at 24 h and intervals, and twice daily for 14 days. After overnight fasting, on day 15th, the rats were anaesthetized using ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively) and blood samples were collected *via* cardiac puncture. All the animals were sacrificed by cervical dislocation. The vital organs mainly liver, kidney, heart and pancreas was removed, cleaned with saline, weighed and preserved in 10% formalin for further histopathology observation. Relative organ weight was calculated as (weight of organ/body weight of rat on day of sacrifice) × 100% [10].

2.5.2. Subchronic toxicity study

The subchronic toxicity study was evaluated according to the OECD guideline 407 OECD [11] to determine the adverse effects of SA feeding for 28 days on the vital organs (liver, kidney, heart and pancreas) and the biochemical parameters (alanine aminotransferase, alkaline phosphate, creatinine, urea and total protein). Twenty male SD rats were divided randomly into four groups (5 rats per group). The experimental design was shown as below:

Group 1: Control rats (received olive oil as vehicle)
Group 2: 50 mg/kg (administered with 50 mg/kg of SA)
Group 3: 100 mg/kg (administered with 100 mg/kg of SA)

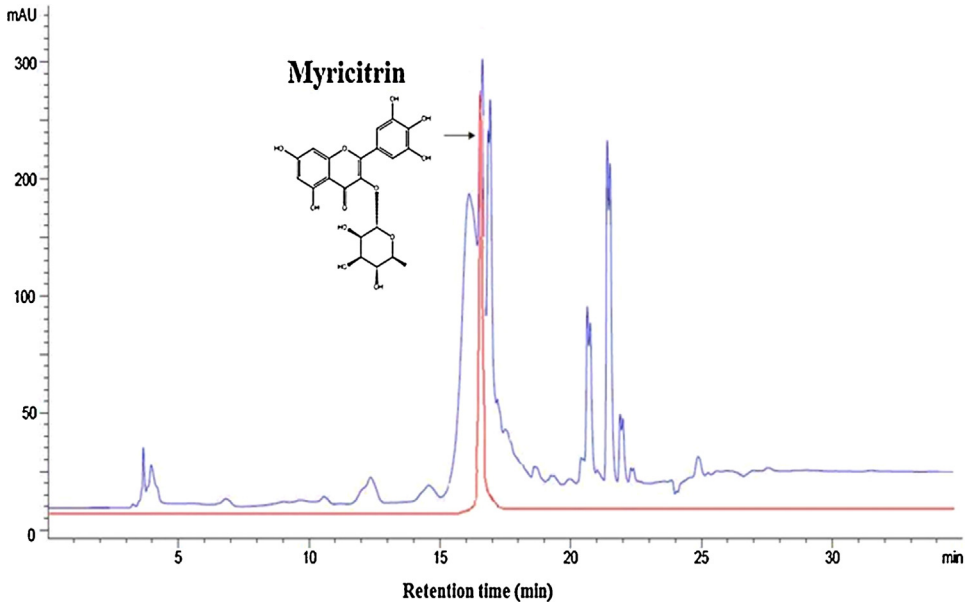

Fig. 1. Chromatogram of the standardized SA (blue line) against myricitrin (red line) on an analytical HPLC with detection wavelength at 254 nm. The arrow indicates the peak for myricitrin at retention time 17 min. The chemical structure of myricitrin is depicted in the chromatogram.

Group 4: 200 mg/kg (administered with 200 mg/kg of SA)

Toxic manifestations and mortality were monitored daily for 28 days. At the end of the experiment, all the rats were anaesthetized using ketamine and xylazine (50 mg/kg and 5 mg/kg, respectively) and blood samples were collected *via* cardiac puncture. Following this, the rats were sacrificed by cervical dislocation. Their vital organs liver, kidney, heart and pancreas were harvested, cleaned with saline, weighed and preserved in 10% formalin for histopathology analyses. Relative organ weight was calculated as described earlier.

2.6. Histopathology observation

Small blocks of tissues were taken from the organs harvested from the rats and processed using an automated tissue processor (Leica TP1020, Hi-tech Sdn. Bhd., Malaysia). After processing, the tissues were sectioned to a thickness of 5 μM using a rotary microtome and dried overnight in an oven at 37 °C. The sections were stained with hematoxylin and eosin (H&E) and examined microscopically for signs of toxicity.

2.7. Biochemical analysis

Blood samples collected *via* cardiac puncture into a serum separator tube (BD-Vacutainer, BiTA Lifescience, Malaysia) were left at room temperature for 30 min to allow clotting to take place. Serum was obtained from the clotted blood *via* centrifugation (5000 rpm for 15 min) and stored at −80 °C prior to analysis. The serum samples were analyzed for determination of alanine aminotransferase (ALT), alkaline phosphate (ALP), creatinine, urea and total protein.

2.8. Statistical analysis

All data are expressed as mean ± SD. Each value represents a minimum of five rats ($n = 5$) in a group. Data were analyzed using Student's paired *t*-test and one-way ANOVA (SPSS version 16). A $p < 0.05$ was considered statistically significant.

3. Results

3.1. Extraction and standardization

The extraction yield of SA was observed to be 10% (100 g yield in a total 1 kg powdered leaf extraction). To ensure the consistency of the SA preparation, chemical standardization was carried out using HPLC as described in Section 2.4. A standardized HPLC profile of the SA against myricitrin was established (Fig. 1). We had previously reported myricitrin to be one of the bioactive compounds found in the SA [5]. Hence, myricitrin was used as authentic marker in preparing a standardized extract. The amount of myricitrin in the SA was quantified to be 0.1 ± 0.02 mg/g extract.

3.2. Acute and subchronic toxicity evaluation

There were no signs of toxicity or mortality was observed in both the acute and subchronic toxicity studies. All the rats were in healthy condition, maintained normal behaviors and usual movement patterns until the end of the experimental period. Postulating that, SA is safe to be used as a medicinal plant with no toxic effects and the LD_{50} of this plant was therefore estimated to be above 2000 mg/kg b.wt.

Table 1
Absolute and relative organ weight of control and SA treated rats at a single dosage of 2000 mg/kg b.wt. in the acute toxicity study for 14 days.

Acute toxicity				
	Absolute organ weight (g)		Relative organ weight (%)	
	Control	SA 2000 mg/kg b.wt.	Control	SA 2000 mg/kg b.wt.
Liver	7.7 ± 0.5	7.9 ± 0.3	4.0 ± 0.2	4.2 ± 0.4
Kidney	1.2 ± 0.1	1.1 ± 0.2	0.6 ± 0.3	0.5 ± 0.2
Heart	0.5 ± 0.1	0.6 ± 0.2	0.4 ± 0.1	0.5 ± 0.1
Pancreas	0.9 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.3

Data indicate mean ± SD, $n = 5$. There are no significant ($p > 0.05$) differences between the control and SA treated rats in their absolute and relative organ weight.

Table 2
Absolute and relative organ weight of control and SA treated rats at doses of 50, 100, and 200 mg/kg b.wt. in the subchronic toxicity study for 28 days.

Subchronic toxicity								
	Absolute organ weight (g)				Relative organ weight (%)			
	Control	SA 50 mg/kg b.wt.	SA 100 mg/kg b.wt.	SA 200 mg/kg b.wt.	Control	SA 50 mg/kg b.wt.	SA 100 mg/kg b.wt.	SA 200 mg/kg b.wt.
Liver	7.3 ± 0.8	8.1 ± 0.2	7.8 ± 0.5	8.0 ± 0.4	3.8 ± 0.2	4.2 ± 0.4	3.9 ± 0.6	4.0 ± 0.5
Kidney	1.1 ± 0.1	1.0 ± 0.2	0.9 ± 0.2	1.2 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.3
Heart	0.8 ± 0.1	0.6 ± 0.2	0.8 ± 0.1	0.7 ± 0.3	0.9 ± 0.2	0.5 ± 0.1	0.8 ± 0.2	0.9 ± 0.2
Pancreas	0.9 ± 0.3	1.0 ± 0.2	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	0.8 ± 0.2	0.9 ± 0.3	1.1 ± 0.1

Data indicate mean ± SD, $n = 5$. There are no significant ($p > 0.05$) differences between the control and SA treated rats in their absolute and relative organ weight.

3.2.1. Body weight measurement

The body weight of rats were measured weekly until the end of acute and subchronic toxicity studies. There were no significant ($p > 0.05$) differences in the body weight changes between the control and SA treated rats (Fig. 2).

3.2.2. Food and water consumption

The amount of food and water consumed was measured daily from the quantity of food and water supplied and the amount remaining after 24 h (Fig. 3). These data showed no statistically significant ($p > 0.05$) differences between control rats and SA treated rats in both the acute and subchronic toxicity studies.

3.2.3. Absolute and relative organ weight

Tables 1 and 2 show the absolute and relative organs weight of control and SA treated rats from the acute and subchronic toxicity studies, respectively. There were no significant ($p > 0.05$) differences observed in the liver, kidney, heart and pancreas of control and SA treated rats in both acute and subchronic toxicity studies.

3.2.4. Biochemical analysis

Tables 3 and 4 show the results of various biochemical tests performed on the sera of control and SA treated rats from the acute and subchronic toxicity studies, respectively. There were no significant ($p > 0.05$) differences in the outcome of the biochemical tests analyzed between the control and SA treated rats in both acute and subchronic toxicity evaluation.

3.2.5. Histopathology observations

Histopathological examination of the vital organs; liver, kidney, heart and pancreas of animals treated with SA did not show any differences when compared with control in both the acute toxicity and subchronic toxicity studies. In the acute toxicity study, the liver showed normal architecture, clear lumen of central vein and no evidence of lesion (Fig. 4); the kidney showed adequate glomeruli and

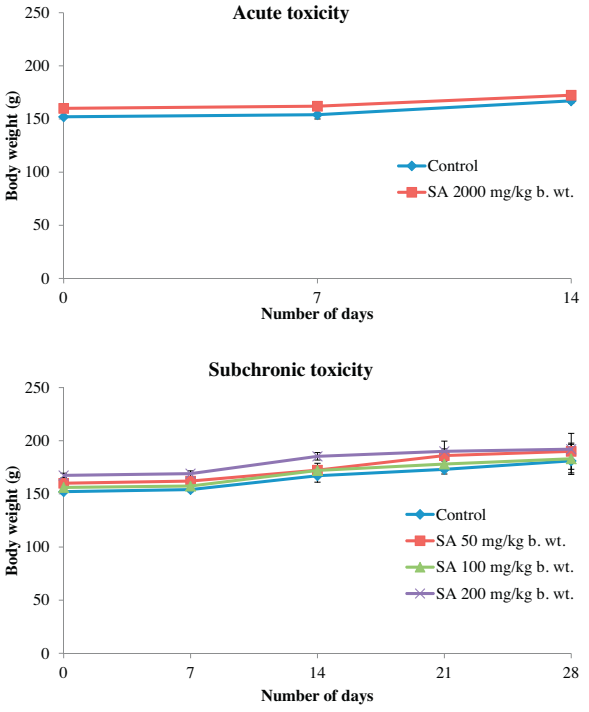

Fig. 2. Body weight of control and SA treated rats in the acute toxicity (for 14 days) and subchronic toxicity (for 28 days) studies. Data indicate mean ± SD, $n = 5$. There are no significant ($p > 0.05$) differences between the control and SA treated rats in their body weight.

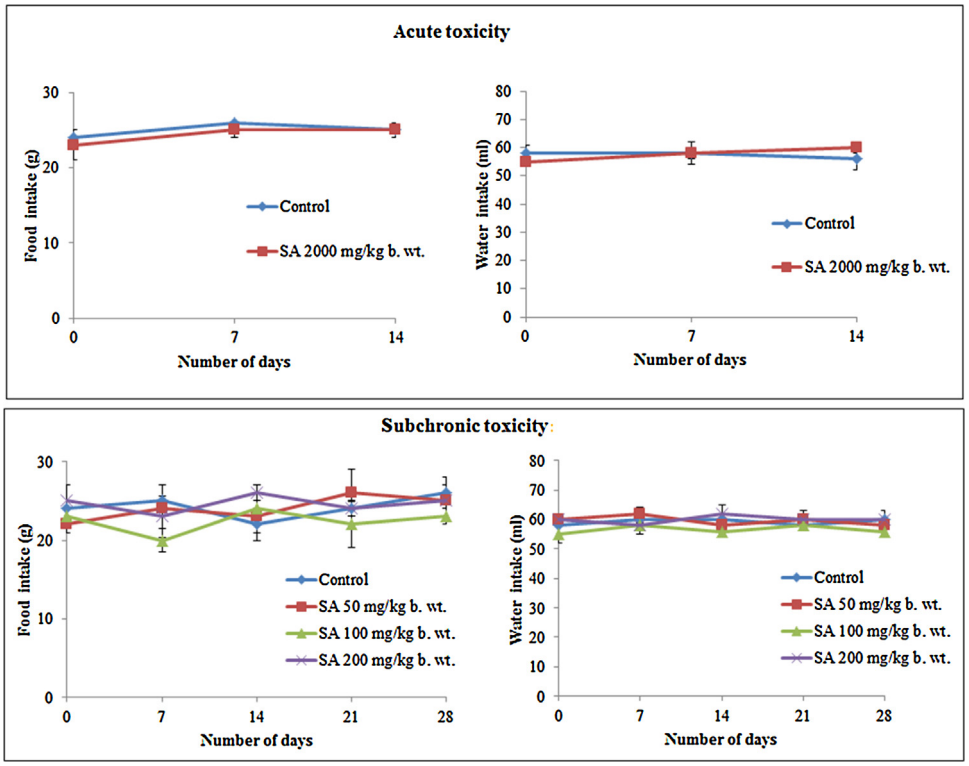

Fig. 3. Consumption of food and water by the control and SA treated rats during the acute toxicity (for 14 days) and subchronic toxicity (for 28 days) studies. Data indicate mean ± SD, $n = 5$. There are no significant ($p > 0.05$) differences between the control and SA treated rats in their food and water intake.

Table 3
Results of biochemical tests performed on the serum of control and SA treated rats at a single dosage of 2000 mg/kg b.wt. in the acute toxicity study for 14 days.

Acute toxicity		
Biochemical parameters	Control	SA 2000 mg/kg b.wt.
ALT (U/L)	65 ± 2	63 ± 2
ALP (U/L)	281 ± 2	280 ± 1
Creatinine (μmol/L)	58 ± 3	56 ± 2
Urea (mmol/L)	7 ± 2	6 ± 1
Total protein (g/L)	57 ± 1	58 ± 2

Data indicate mean ± SD, $n = 5$. There are no significant ($p > 0.05$) differences between the control and SA treated rats in their biochemical parameters tested.

normal tubules, with no trace of focal intestinal edema and lesion (Fig. 4); the heart showed normal architecture of the myocardium (images not shown); and the pancreas showed normal arrangement of acini, with adequate islets (images not shown). Similar results were observed in the subchronic tocity study where the liver, kidney, heart and pancreas of animals treated with SA (50, 100, and 200 mg/kg b.wt.) did not show any differences when compared with control. The photomicrographs of liver and kidney of animals treated with SA (50, 100, and 200 mg/kg b.wt.) in subchronic toxicity study was shown in Fig. 5, while the heart and pancreas was not shown. In conclusion, no lesions or pathological changes attributable to the administration of SA were found in the organs of rats in the experimental groups. Indicating that SA up to a maximum concentration of 2000 mg/kg b.wt. is safe to be used without any adverse toxicological effects.

4. Discussion

Natural therapeutic medicine has become universally popular in primary healthcare, particularly in developing

Table 4
Results of biochemical tests performed on the serum of control and SA treated rats at doses of 50, 100, and 200 mg/kg b.wt. in the subchronic toxicity study for 28 days.

Subchronic toxicity				
Biochemical parameters	Control	SA 50 mg/kg b.wt.	SA 100 mg/kg b.wt.	SA 200 mg/kg b.wt.
ALT (U/L)	63 ± 1	64 ± 1	62 ± 2	64 ± 2
ALP (U/L)	275 ± 2	277 ± 4	274 ± 4	276 ± 1
Creatinine (μmol/L)	60 ± 3	57 ± 3	56 ± 1	58 ± 3
Urea (mmol/L)	8 ± 2	6 ± 1	8 ± 1	7 ± 2
Total protein (g/L)	53 ± 1	54 ± 1	55 ± 3	56 ± 2

Data indicate mean ± SD, $n = 5$. There are no significant ($p > 0.05$) differences between the control and SA treated rats in their biochemical parameters tested.

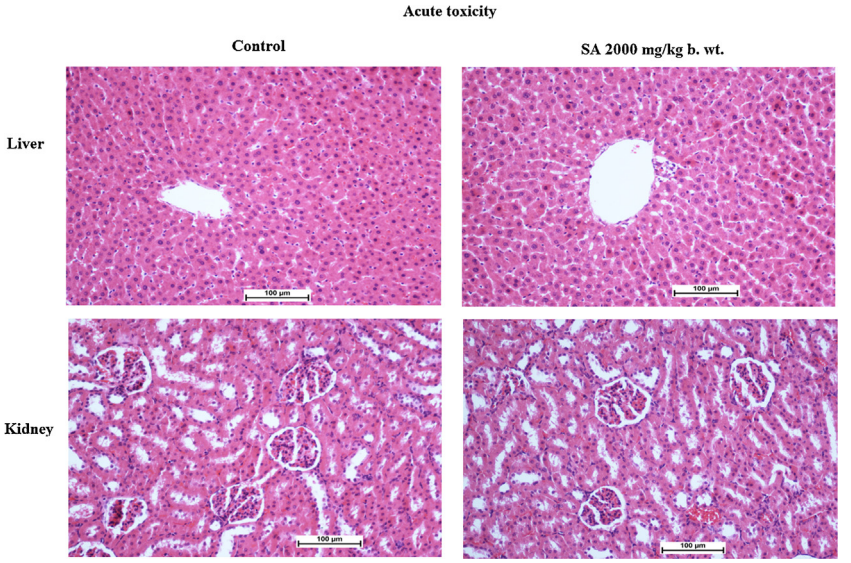

Fig. 4. Photomicrographs from the liver and kidney of control and SA treated rats at a single dosage of 2000 mg/kg b.wt. in the acute toxicity study for 14 days. The liver showing hepatocytes arranged around central veins with no evidence of necrosis, lesion or any pathological damage (H&E, 20×), and the kidney, showing adequate glomeruli and normal tubules, with no evidence of glomerular damage or lumen casts, (H&E, 20×). There are no treatment related microscopic changes in both the liver and kidney of SA treated rats compared to the control rats.

countries such as Malaysia. Medicinal plants are presumed to be safe without any compromising health effects [12,13]. Appropriate use of medicinal plants in dietary supplementation is very important in the maintenance of health [14]. Many studies have reported the harmful effects from improper use of medicinal plants [15–17]. Therefore, evaluating the toxicological effects of any medicinal plant extract intended to be used in animals or humans is a crucial part of its assessment for potential toxic effects.

S. aqueum leaf has been used as medicinal plant in tropical regions and there are an increasing number of studies reporting on its health benefits [4,5,7]. Recently *S. aqueum*

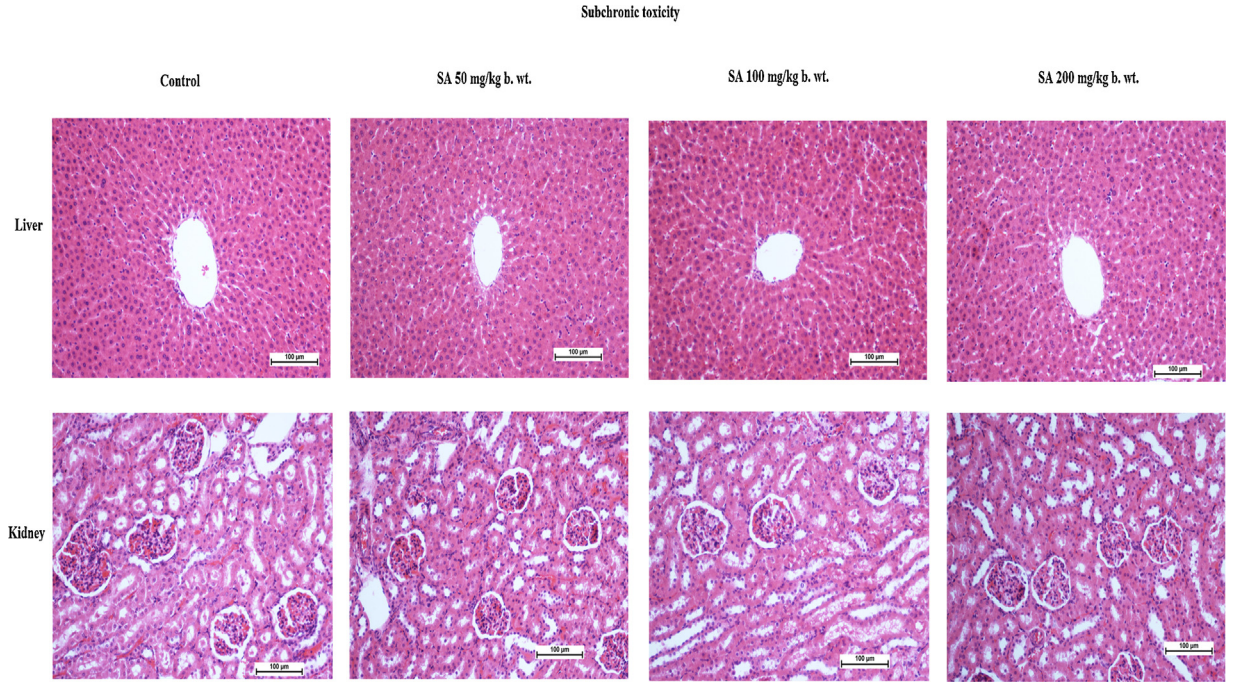

Fig. 5. Photomicrographs from the liver and kidney of control and SA treated rats at doses of 50, 100 and 200 mg/kg b.wt. in the subchronic toxicity study for 28 days. The liver showing hepatocytes arranged around central veins with no evidence of necrosis, lesion or any pathological damage (H&E, 20×), and the kidney, showing adequate glomeruli and normal tubules, with no evidence of glomerular damage or lumen casts, (H&E, 20×). There are no treatment related microscopic changes in both the liver and kidney of SA treated rats compared to the control rats.

leaf extract (SA) and its bioactive compound, myricitrin have been established as a potent antihyperglycemic agent [6,8]. Although the SA has been reported to have heavy metals far below the permissible levels for nutraceutical use [18], there is a lack of proven scientific studies on the toxicity effect of SA. It is deemed important to evaluate the toxicity effect of a medicinal plant extract in order to increase the confidence in their safety to humans, particularly for use in the development of nutraceuticals and pharmaceuticals. There exist many studies on the acute and subchronic toxicity of plant extracts in rat models [12,19–24]. Therefore, the present study was carried out to evaluate the acute and subchronic toxicity effects of SA using an *in vivo* model. To our best knowledge, this is the first study reported the toxicity effects of SA in SD rats.

In the acute toxicity study, a maximum dose of 2000 mg/kg b.wt. of the SA caused neither signs of toxicity nor mortality during the 14 days of experiment. Throughout the 14 day periods all animals were found to be healthy with no changes in their skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system and as well as somatomotor activity and behavioral patterns. Therefore, it is safe to propose that its oral LD_{50} value should be greater than 2000 mg/kg. Subsequently, the subchronic toxicity study was advocated as a fundamental test to assess safety of SA. In this 28-day subchronic toxicity evaluation, rats were given SA at doses of 50, 100, and 200 mg/kg b.wt. showed no mortality or any symptoms of toxicity.

According to Ref. [25], after some exposure to potentially toxic substances, there will be a slight reduction in body weight gain. In our acute and subchronic toxicity studies, all rats at each dosage group continued to gain weight throughout the experimental periods (Fig. 2). This suggests that administration of the SA did not affect the body weight of the rats. In addition, no significant changes in the food and water consumption of the SA treated rats compared to the control rats in both the acute and subchronic toxicity studies (Fig. 3). Utilization of food and water exhibited normal metabolism in the animals [26] and this suggests that the administration of the SA did not retard the growth of the rats.

We also observed no changes in the absolute weight of the liver, kidney, heart and pancreas of the rats in the acute toxicity (Table 1) and subchronic toxicity (Table 2) studies. This suggests no gross toxic effect from the SA to the vital organs. Demma et al. [27] reported that relative organ weight is more indicative of toxicity than absolute organ weight. Our results revealed, there were also no significant changes in the relative organ weight of rats treated with SA compared to the control rats in the acute toxicity (Table 1) and subchronic toxicity (Table 2) studies. The gross and microscopic observations conducted in all the above mentioned organs further suggested no toxic effect of the extract at the concentrations studied.

The liver and kidney are very sensitive organs to toxic substances and can serve as an important index of the physiological and pathological status of both the animals and humans [10,28]. Therefore, we decided to focus only on the liver function test and kidney function test as biochemical parameters. The alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are enzymes mainly found in the liver and kidneys. The total protein; albumin and globulin are mainly found in the blood. An increase in blood ALT, ALP and total protein levels indicates impaired liver and kidney function and chances of developing an infection [23]. Creatinine and urea are waste products excreted in urine by kidneys. As the kidneys become impaired, the creatinine and urea levels in the blood will rise due to poor clearance by the kidneys [14].

In this study, biochemical parameters in particular, ALT, ALP, creatinine, urea and total protein were tested and the analysis showed there were no significant differences in ALT, ALP, creatinine, urea and total protein levels of the rats treated with SA compared to the control in both the acute toxicity (Table 3) and subchronic toxicity (Table 4) studies. Although there is a slight decrease or increase in the level of biochemical parameters in SA treated rats compared to the control, these values were still within the normal range. The biochemical findings suggested that the administration of SA did not cause any toxicological effect.

Gross and microscopic examination on the vital organs, liver, kidney, heart and pancreas as well revealed no treatment-related changes due to the administration of SA in the animals. Only the liver and kidney were explored more for histopathology observation because of their primary function to expel toxins that results from body's metabolism of food, water, drug or any other substances that consumed. In both the acute toxicity (Fig. 4) and subchronic toxicity (Fig. 5) studies, the rats treated with the SA showed; normal architecture of the liver, and adequate glomeruli and normal tubules in the kidney. There is no evidence of lesion due to toxic effect of SA in the liver and kidney. There were also no microscopic changes was observed in heart and pancreas of the SA treated rats compared to the control rats (images not shown). In conclusion, the acute and subchronic toxicity studies showed that the SA did not produce any signs of toxicity. It is safe to be used as a medicinal plant. However, further studies to determine the long-term toxicity effects of SA on animals are needed to establish the safety and toxicity of this plant extract. Apart from that current toxicology research are focusing on the usage of female rats to test the adverse effect of medicinal plant extracts. Female rats are more sensitive to toxic substances and their pathological changes are can be easily revealed [28]. Therefore, further acute and subchronic toxicity studies on female SD rats are deemed important to be carried out to ensure a complete safety profile of SA.

5. Conclusion

In this study, the SA was established to have a LD_{50} value greater than 2000 mg/kg. Administration of the SA (50, 100, and 200 mg/kg b.wt.) to male SD rats for 28 days did not result in death or cause adverse effects in any of the experimental animals. There were no significant differences observed in the absolute or relative weight of liver, kidney, heart and pancreas. In addition, there were no gross or microscopic abnormalities observed in any of these organs. Furthermore, all of the biochemical tests performed on the sera of the experimental animals were in the normal range of the control rats. Thus no acute or subchronic

toxicity effects were observed. Although the cytotoxicity of SA was established before, this is the first *in vivo* study report that the SA is devoid of any toxic risk. However, further long-term toxicity studies using SA may therefore be warranted before this extract can be further developed as a nutraceutical or pharmaceutical product.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.toxrep.2014.09.006.

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