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Safety Assessment of Spray Dried *Strobilanthes crispus* Aqueous Extract on Liver, Kidney and Biochemical Profiles in Sprague-Dawley Rats

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ABSTRACT

Background: Nutraceutical products have been widely used nowadays to supplement for better health performance and scientific evidence on the safety level is important for consumption. **Objective:** Aim of this study was to evaluate the acute 14-days dose response toxicity of spray-dried Strobilanthes crispus aqueous extract supplementation on vital orga and important biochemical profiles. **Materials and methods:** Four different doses of spray-dried S. crispus (700, 2100, 3500 and 4900 mg /kg of body weight) were administered to 5 normal Sprague-Dawley rats to review and compare any possible changes in physical behaviour, biochemical parameters and organs morphology. Biochemical parameters tested include haematology, liver and kidney function tests. **Result:** Overall, there was no death and no significant toxicity was observed with respect to biochemical parameters and organ morphology compared to normal control group. However, dosage group of 2100 - 4900 mg/kg of body weight showed significant differences in sodium and chloride level as compared to control group. Abnormal morphological characteristics was found in liver tissues where the sinusoidal widening was found in dosage group of 3500 and 4900 mg/kg of body weight. **Conclusion:** In brief, findings on biochemical profile of spray-dried S. crispus aqueous extract verified that it is safe to be consumed at the highest dose tested in this study (4900 mg/kg of body weight) and histological observations provide support to less or negligible destructive nature on liver and kidney tissues.

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INTRODUCTION

Traditional herbal medicine has widely been used worldwide including Malaysia. Survey on the usage of traditional herbal medicine in Malaysia showed that 24.9% of the study population are using it, where 4.4% are using ginseng and 3.4% are believing in combination of traditional Chinese herbs [1]. There are approximately 2000 herbal medicinal plants species are reported from Malaysia, and 1200 of these species have been reported to have potential pharmaceutical value, some of which are being used as herbal medicines since a long time ago [2]. *Strobilanthes crispus* (Acanthaceae)or commonly known as *"pecah beling"* in Malaysia is one of the example. Since ancient time, the decoction filtrates is been used as antidiabetic, diuretic, antilytic and laxative [3]. Recently, the effectiveness of *S. crispus in vivo* and *in vitro* were actively been studied. Earlier study showed that the water extract of *S. crispus* leaves are able to possess anti-AIDS as well as anti-leukaemic property which inhibit the proliferation of retrovirus [4]. Then, Suherman *et al.* [5 found that supplementation of *S. crispus* (1, 2.5, 5 and 7.5 %) on diethylnitrosamine (DEN)/2-acetylaminofluorence (AAF) induced rats reduced the severity of hepatocarcinogenesis by reducing liver gamma-glutamyl traspeptidase (GGT) and alkaline phosphatase (ALP) as well as glutathione (GSH) in the early stages of hepatocarcinogenesis with the optimum dose of 5 % of *S. crispus* extract. Whereas, Fauziah *et al.* [6] had found that the animal treated with *S. crispus* extract caused a reduction in liver glutathione s-trasferase (GST) activity almost close to the control

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group (glycirrhizin). Cytotoxicity study of methanolic extracts of *S. crispus* showed the strongest effect on colon cancer, followed by human breast cancer hormone non-dependent and liver cancer with inhibitory concentration (IC_{50}) value of 22.3, 27.2 and 29.3 µg/ml, respectively [7]. Mohd-Fadzelly *et al.* [8] had reported that water extract of both fermented and unfermented *S. crispus* tea were able to reduce blood glucose and improve lipid profile in hyperglycaemic rats. The study was then supported by Norfarizan-Hanoon *et al.* [9] where they had revealed the significant decrease in serum glucose, cholesterol and trigliceride levels in diabetic and normal rats supplemented with *S. crispus* juice. Nofarizan-Hanoon *et al.* [10] also found a significant increase in the percentage of wound healing at day 3 and 7 in the group supplemented with 140 mg/kg body weight of *S. crispus* juice in diabetic rats as compared to control. On top of that, acute oral toxicity study also had been conducted on juice sample and the team had confirmed that there were absence of toxicity at the highest dose tested (4900 mg/kg body weight) [11]. Other than that, *S. crispus* is also able to inhibit peroxidation of membrane lipids and DNA damage thus giving a potential to treat or prevent degenerative diseases [12]. This health-giving property is mainly due to high antioxidant activity and phytochemical constituents especially minerals and vitamins content as well as other component such as catechins, caffeine and tannin [13,14,15,16,17]. Numerous research on plant medicine had urged Malaysian government to provide incentives and opportunities to the local manufacturers to produce better downstream products and thus contribute to a significant economic growth of the medicinal plants sector [2]. Spray-drying technique is widely been used in the industrial scale sector to produce powders from liquid foods [18]. Since the administration of a chemical substance to a biological system had a different types of interactions which will occur and result in a series of dose-related responses, this study were prompted to be conducted prior to human consumption.

MATERIALS AND METHODS

Plant material and extract preparation:

The fresh *S. crispus* leaves were obtained from University Agricultural Park, Universiti Putra Malaysia (UPM). The plant honorarium identification was proven by Abu Bakar *et al.* [8] with voucher number of AZ-6803. The matured leaves were plucked, washed under running tap water, rinsed with distilled water, blot dried, cut into small pieces and dried overnight in the oven at 50 °C. The dried samples were crushed into small pieces, homogenized and extracted with distilled water through decoction process [14]. Then, the extract were filtered with Whatman number 4 paper and were added with 20% (w/v) maltodextrin as a carrier in order to be processed into spray dried form. Spray-drying process was performed at Faculty of Food Technology (UPM) by using laboratory scale spray dryer (GEA Niro A/S Mobile Spray Dryer S80, Denmark).

Study protocol and design:

The ethic protocol and design was adopted according to the guideline No. 420 from Organisation for Economic Co-operation and Development [19] and study was conducted upon an approval from the Animal Care and Use Comittee (ACUC), Faculty of Medicine and Health Sciences, UPM. Total of 25 female Sprague-Dawley rats weighing approximately 200-250 g, supplied by Chenur Supplier Sdn. Bhd., Kuala Lumpur, Malaysia were housed in Faculty of Medicine and Health Sciences, UPM. Upon arrival, the animals were allowed for acclimatized for at least 7 days before the experimental studies. They were maintained on regular commercial diet (rat chow) and tap water *ad libitum*. The rats were divided into 5 groups (normal control, 700, 2100, 3500 and 4900 mg/kg body weight of spray dried *S. crispus* aqueous extract) with 5 rats in each group. The rats were force fed with 1 ml of the test substance, while normal control rats were force fed with normal saline. After the admission of test substance, the animals were observed for 1, 3 and 6 hours in post dose and continue once daily for 14 days. The growth and physiological changes of the rats were recorded based on the body weight, mortality, sign of gross toxicity, behaviour changes and physical condition.

Biochemical parameters:

Following 12 hours fast on the 15th day, the rats were anaesthetized and 5 ml blood was drawn and transferred into the heparin and EDTA tubes. Fresh blood of EDTA tubes were proceed for full blood count analysis including total red blood cell, white blood cell, haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), fibrinogen, lymphocyte and platelet volume using the automated haematology analyzer (KX-21N, Sysmex Corporation, Kobe, Japan). Whereas, blood in the heparin tubes were centrifuged to obtain plasma samples and were used to determine liver and kidney function tests, namely aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin, globulin, albumin/globulin ratio, urea, creatinine, uric acid, sodium, potassium and chloride using the automated analyzer (Hitachi 917, Roche Diagnostics, Indianapolis, Indiana).

Organ histology:

The complete autopsies of the rats were performed following overdose chloroform anaesthesia. Liver and kidney tissues were obtained from each rats, fixed in 10 % buffered formalin and underwent automated tissue processing for 24 hours using a tissue processer (Leica TP-1020, Germany). Organ sections were embedded in paraffin blocks, and processed for routine histological observation with the use of hematoxylin and eosin (H&E) stain under the light microscope at 40X magnification. The slides were then evaluated based on the presence of vacuolation, tissue degeneration, inflammation, necrosis, sinusoidal widening in liver tissue and; necrosis and tubular dilation in kidney tissues [20].

Statistical analysis:

All data were presented as mean ± standard deviation of mean using Statistical Package for Social Sciences (SPSS) version 20.0. One-way ANOVA following by post hoc analysis using Tukey's HSD were used for biochemical profile comparison to normal control group. The values were considered as statistically significant when $p<0.05$.

Results:

Clinical observation:

There was no notable treatment-related effect and mortality was found in 24 hours post dose and the study was prolong to 14 days observation. During 14 days of experimentation, all the rats were active and no apparent treatment related-behavioural changes were found. Body weight of the female Sprague-Dawley rats were monitored and the increasing trend was found to be similar for all groups (Figure 1). There were no mortality was observed throughout this study.

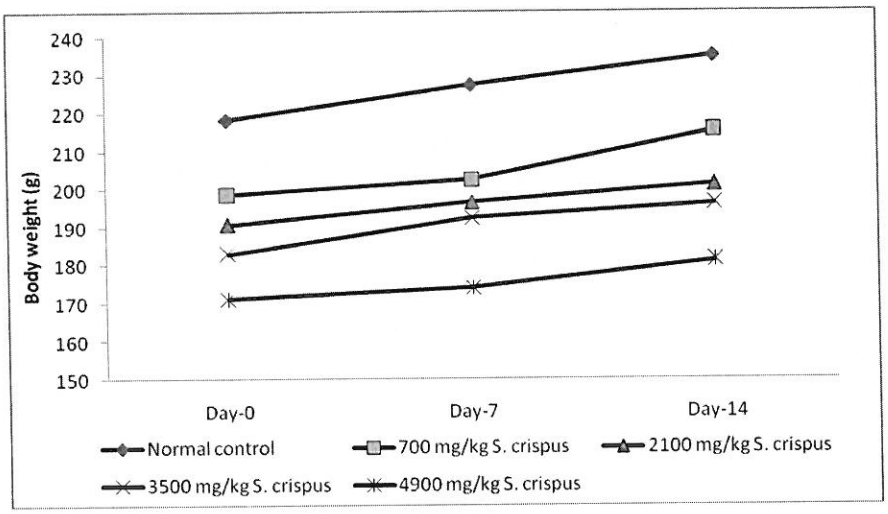


Fig. 1: Graph of similar trend of body weight progression for 14 days treatment.

Biochemical profiles:

Findings of liver and kidney function as well as haematological parameters were summarized in Table 1 and 2, respectively. Statistically, the results showed no significant differences in all the parameters when compared to the normal control groups. However, further analysis using Tukey's HSD had revealed that supplementation groups showed significant differences in sodium level (group of 2100 and 4900 mg/kg body weight) and chloride level (2100, 3500 and 4900 mg/kg body weight) when compared to normal control group. However, all the sodium and chloride values were still within the normal range of 132.4 to 168.3 mmol/L and 81.5 to 105.5 mmol/L, respectively. [21].

Table 1: Liver and kidney function parameters of the rats after day 14 of treatment

	Normal control	700 mg/kg	2100 mg/kg	3500 mg/kg	4900 mg/kg
ALP (U/L)	100.33 ± 19.86	127.00 ± 10.58	167.67 ± 54.00	136.33 ± 68.53	193.00 ± 110.37
AST (U/L)	152.00 ± 61.22	166.33 ± 75.18	88.67 ± 12.58	91.00 ± 13.00	139.67 ± 32.15
ALT (U/L)	59.67 ±4.16^{a,b}	72.00 ± 8.89^{b}	54.67 ± 6.66^{a,b}	44.33 ± 6.43^{a}	60.00 ± 6.56^{a,b}
Total protein (g/L)	71.67 ± 2.31	72.33 ± 5.86	71.33 ± 9.02	69.33 ± 4.93	68.33 ± 4.73
Albumin (g/L)	39.33 ± 1.53	39.00 ± 5.57	35.33 ± 6.35	35.67 ± 1.15	35.00 ± 4.58
Globulin (g/L)	32.33 ± 1.53	33.33 ± 2.89	36.00 ± 4.36	33.67 ± 3.79	33.33 ± 2.52
Albumin/	1.20 ± 0.10	1.17 ± 0.23	1.00 ± 0.20	1.07 ± 0.06	1.07 ± 0.21

globulin ratio					
Urea (mmol/L)	7.93 ± 0.96	8.17 ± 0.45	6.77 ± 0.85	7.50 ± 0.79	7.80 ± 0.50
Creatinine (mmol/L)	25.33 ± 2.08	25.33 ± 2.52	26.67 ± 6.66	28.67 ± 2.31	25.00 ± 2.00
Uric acid (mmol/L)	0.010 ± 0.00	0.010 ± 0.00	0.017 ± 0.01	0.023 ± 0.02	0.040 ± 0.03
Sodium (mmol/L)	132.67 ± 1.15^{a}	135.00 ± 1.00^{a,b}	138.33 ± 2.08^{b}	135.00 ± 1.00^{a,b}	137.00 ± 2.00^{b}
Potassium (mmol/L)	3.90 ± 0.36	3.80 ± 0.70	3.40 ± 0.40^{a}	3.50 ± 0.26	4.37 ± 0.67
Chloride (mmol/L)	89.33 ± 3.06^{a}	92.00 ± 1.00^{a,b}	101.00 ± 2.65^{c}	99.33 ± 2.08^{b,c}	97.67 ± 4.16^{b,c}

Data were expressed as mean ± standard deviation, n=5. Mean with different letter showed the differences between groups, yet not significantly different at the level of $p<0.05$.

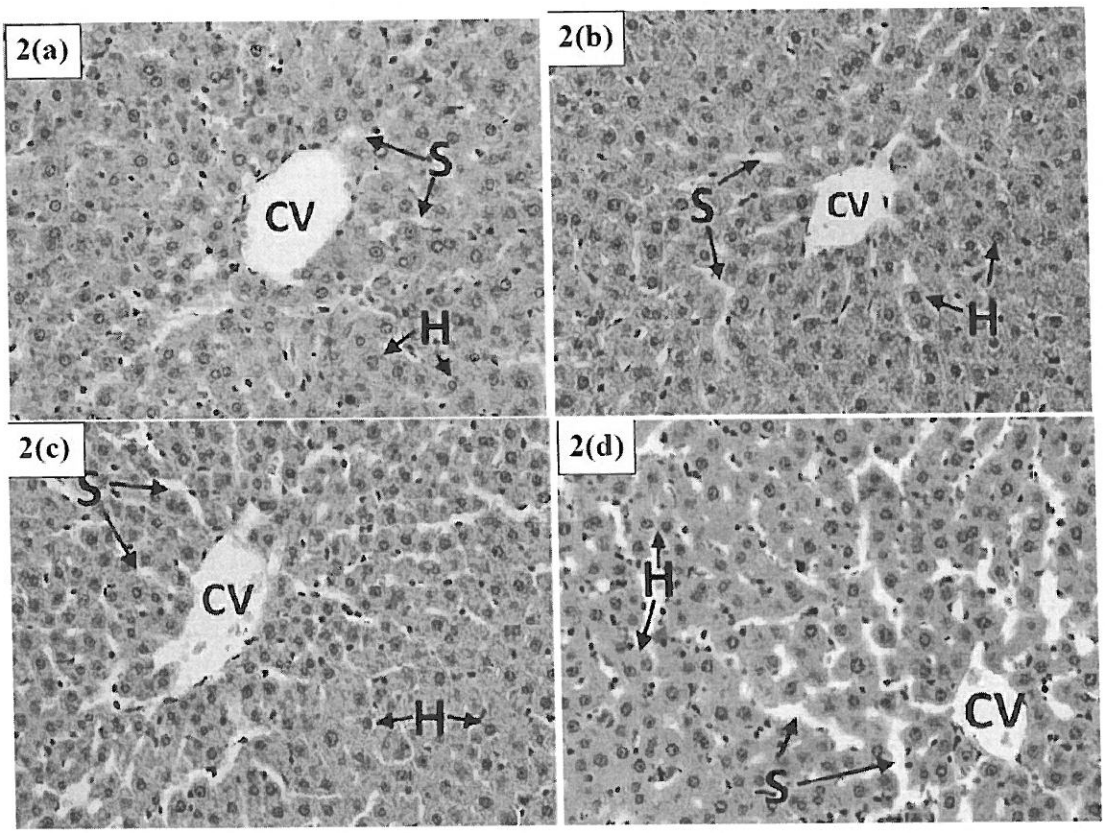
Table 2: Haematological parameters of the rats after day 14 of treatment

	Normal control	700 mg/kg *S. crispus*	2100 mg/kg *S. crispus*	3500 mg/kg *S. crispus*	4900 mg/kg *S. crispus*
WBC (x 10^3/µL)	11.47 ± 0.99	14.63 ± 1.72	15.23 ± 10.44	13.80 ± 2.52	13.40 ± 2.46
RBC (x 10^3/µL)	7.03 ± 0.67	7.08 ± 0.51	6.64 ± 0.53	6.65 ± 0.75	7.02 ± 0.54
Haemoglobin (g/dL)	13.40 ± 1.18	13.83 ± 0.49	13.60 ± 0.90	13.03 ± 0.64	13.43 ± 0.32
Haematocrit (%)	40.40 ± 3.48	41.83 ± 1.62	40.13 ± 3.17	39.00 ± 2.43	40.87 ± 1.25
MCV (fL)	57.53 ± 2.90	59.23 ± 3.03	60.47 ± 0.12	58.87 ± 3.61	58.33 ± 2.87
MCH (pg)	19.10 ± 1.35	19.57 ± 0.90	20.53 ± 0.32	19.70 ± 1.35	19.20 ± 1.11
MCHC (g/dL)	33.17 ± 0.70	33.07 ± 0.21	33.90 ± 0.52	33.43 ± 0.51	32.90 ± 0.36
Platelet (x 10^3/µL)	1304.33 ± 403.72^{a}	978.33 ± 57.74^{a,b}	1001.33 ± 182.32^{a,b}	1210.67 ± 192.26^{a}	1151.67 ± 189.95^{a}
Lymphocyte (x 10^3/µL)	8.70 ± 0.87	10.93 ± 0.95	10.43 ± 1.63	10.03 ± 2.34	9.97 ± 1.59

Data were expressed as mean ± standard deviation, n=5. There was no significant difference ($p<0.05$) found in all groups when compared to normal control group.

Histological findings:

Histological observation of the liver and kidney were shown in figure 2 and 3, respectively. The liver structure of the all groups were found to be normal except for 3500 and 4900 mg/kg body weight groups which had a slight sinusoidal widening as compared to others (Figure 2d and 2e). Normal kidney features were observed in the all the experimental groups.



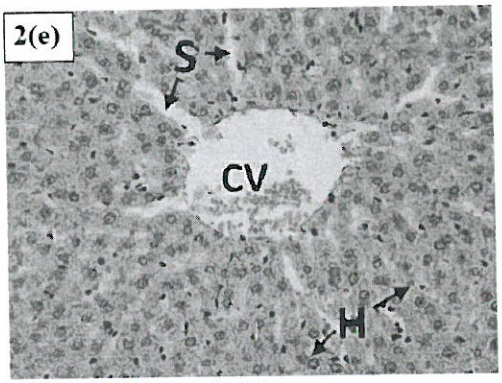


Fig. 2: Liver histology of the groups in acute toxicity studies after 14 days supplementation of spray-dried *S. crispus* extract.

2(a) is the liver structure of a female rat in normal control group; 2(b) is the liver structure of a female rat treated with 700 mg/kg body weight; 2(c) is the liver structure of a female rat treated with 2100 mg/kg body weight; 2(d) is the liver structure of a female rat treated with3500 mg/kg body weight; 2(e) is the liver structure of a female rat treated with 4900 mg/kg body weight (H&E, X40; CV is central vein; H is hepatocytes; S is Sinusoids). There were slight sinusoidal widening was found in figure 2(d) and 2(e).

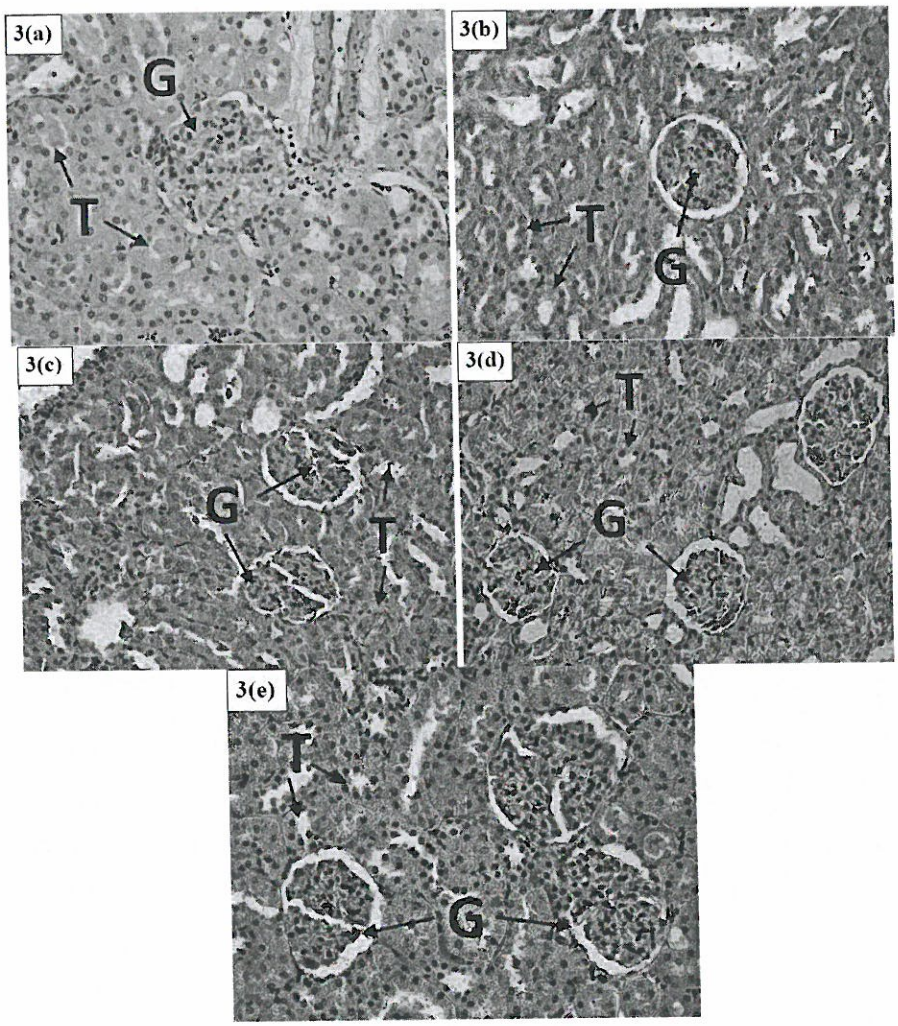


Fig. 3: Kidney histology of the groups in acute toxicity study after 14 days supplementation of spray-dried *S. crispus* extract.

3(a) is the kidney structure of a female rat in normal control group; 3(b) is the kidney structure of a female rat treated with 700 mg/kg body weight; 3(c) is the kidney structure of a female rat treated with 2100 mg/kg body weight; 3(d) is the kidney structure of a female rat treated with 3500 mg/kg body weight; 3(e) is the kidney structure of a female rat treated with 4900 mg/kg body weight (H&E, X40; T is the tubule; G is the gromerulus).

Discussion:

The best and most convenient method of herb preparation is a formulation which feasible to human consumption. Spray dried *S. crispus* aqueous extract can be one of the potential products to be commercialized in a big scale production in form of tablet and capsule for easy and convenient consumption. The safety of the nutraceutical products is an important criteria prior commercialization. There are many reasons that lead to curiosity on the product's safety including the direct toxic effect of the product or ingredient compounds, effect of the contaminant during production and handling as well as any possible interaction with other drugs or other products [22]. Safety evaluation of drugs and plant products is widely performed in animals *in vivo*. There was significant correlation has been reported between toxicological effects in rats and humans [23] and thus healthy Sprague-Dawley rats were used in this study.

Acute oral toxicity test is the basis of safety determination of products. This study showed that rats supplemented with spray dried *S. crispus* aqueous extract at the highest dosage of 4900 mg/kg did not posed any mortality or any observable signs of gross toxicity. Hence, in general, it indicates that 14 days supplementation of *S. crispus* aqueous extract at the highest dose could be the no-observable adverse effect level (NOAEL) for the female Sprague-Dawley rats. This finding is in line with the 14 days acute toxicity study done on *S. crispus* juice done by Norfarizan-Hanoon *et al*. [11].

Gradually increasing body weights of the supplemented rats in this study were in line with the growth rate of the rats. Observation on the similar increasing trend as compared to normal control group indicated that they were grown healthily. The same findings were observed in the acute toxicity study of *Orthosiphon stamineus* standardized extract in male Sprague-Dawley rats [24] as well as *O. stamineus* crude extract in female Sprague-Dawley rats [25]. Liver and kidney tissues were selected in this study as they perform significant functions for the health survival of the body such as to secrete, synthesize and store important enzymes and molecules as well as help to reabsorb important substances and detoxifies harmful substance [26].

This study revealed that supplementation of spray dried *S. crispus* aqueous extract at various concentrations up to 4900 mg/kg body weight of the rats did not produce any significant difference in liver function markers when compared to the normal control group. The findings indicated that the sample did not cause any cellular damage to the liver of healthy rats during the experimental period. The finding was supported by previous study done using *S. crispus* juice, where there were also no significant differences found in AST, ALT, ALP and albumin levels when compared to its respective normal control groups [11]. Similar findings were also seen in the acute toxicity study of other herbal plant such as *Orthosiphon stamineus* and *Xemenia americana* at their highest treatment dose level [24, 25, 26]. Kidney function tests for 14 days supplementation groups also revealed no significant difference when compared to the normal control group except for the sodium and chloride parameters even though the values were still within the normal range [21]. Sodium and chloride are the important electrolytes in body system where they regulates the total amount of water in the body and play an important roles in the transmission of electrical signals in body system process. Hence, too much or too little of the electrolytes can cause cells to malfunction [27]. Absence of significant variation in haematological parameters indicated the ability of *S. crispus* supplementation product to maintain the blood cellular components in the body. Haematological markers provides information about quantity and quality of different types of the blood cells. Abnormal high or low levels of haematological parameters indicate many possible medical conditions. The normal parameters also represent a healthy bone marrow environment as it is produced in the bone marrow [28].

Histology of the liver and kidney were investigated to further confirm the effect of spray dried *S. crispus* aqueous extract apart of biochemical markers. Minor sinusoidal widening around the central vein area was detected in liver of 3500 and 4900 mg/kg body weight groups. Hepatic sinusoidal dilation can be encountered in three different situations; in vicinity of hepatic tumours, clinicopathological entity non-cirrhotic intrahepatic portal hypertension which consists of various types of architectural alterations and idiopathic role of oral contraceptives which is still unclear [29]. Hence, the possible causal of the sinusoidal widening in the present study might be due to the architectural alterations by the sample or might due to poor histological handling. Similar findings was also reported in the study of *Rhaphidophora decursiva* extract where sinusoidal dilation was found in moderate and highest dose (2100 and 3500 mg/kg body weight) of treatment groups with no significant changes of overall liver lesion score[30].

Conclusion:

In brief, spray dried *S. crispus* aqueous extract has no observable acute effect on the experimental animals at the highest dose tested (4900 mg/kg body weight) and considered non-toxic for consumption. Histological

findings provide support to less or negligible destructive nature on liver and kidney tissues in this study. Further dose response toxicity assessment are recommended to offer a scientific evidence of herbal product development as a safety proof for longer biological consumption. Apart from that, more studies are needed to clarify if spray dried *S. crispus* aqueous extract are efficient in bioavailability of various types of cells to confirm the absorption of the nutrient in the body system.

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