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Absence of Toxicity of *Strobilanthes crispa* Juice in Acute Oral Toxicity Study in Sprague Dawley Rats

(Ketiadaan Ketoksikan Jus *Strobilanthes crispa* Bagi Ketoksikan Akut Secara Oral Pada Tikus Sprague Dawley)

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ABSTRACT

This study evaluated four different doses of Strobilanthes crispa *juice (700, 2100, 3500 and 4900 mg kg^{-1} of body weight) administered orally to normal female and male Sprague dawley rats on possible changes in various physical, behaviour, morphology and biochemical parameter. The rats were treated with a single dose of juice and observed for 14 days. No significant toxicity was observed with respect to clinical parameters and organ morphology. In addition, no significant changes were observed in the level of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine and albumin. The S. crispus juice was found to be safe at the maximum dose used in this study (4900 mg kg^{-1} of body weight).*

Keywords: Acute toxicity; clinical parameters; organ morphology; S. crispus *juice*

ABSTRAK

Kajian ini menilai empat dos yang berbeza bagi jus Strobilanthes crispa *(700, 2100, 3500 dan 4900 mg kg^{-1} berat badan) diberikan secara oral kepada tikus betina dan jantan Sprague Dawley ke atas perubahan dalam pelbagai fizikal, perilaku, morfologi dan parameter biokimia. Tikus diubati dengan dos tunggal jus dan diperhatikan selama 14 hari. Tidak ada ketoksikan signifikan yang didapati bagi parameter klinikal dan morfologi organ. Selain itu, tidak ada perubahan signifikan yang diamati pada tahap aspartat aminotransferase, alanin aminotransferase, alkali fosfatase, kreatinin dan albumin. Jus S. crispus didapati selamat pada dos maksimum yang digunakan dalam kajian ini (4900 mg kg^{-1} berat badan).*

Kata kunci: Jus S. crispus*; ketoksikan akut; morfologi organ; parameter klinikal*

INTRODUCTION

Acute toxicity testing, which measures the adverse effects that occur within a short time following administration of single doses of any herbal or chemical substance can be used to identify doses associated with target organ toxicity and lethality that may be relevant to humans. It is used to standardize biological products and can serve to establish dosing levels for repeated dose studies. Acute oral toxicity in the rats is also used to determine the level of lethality in terrestrial mammals (Rispin et al. 2002).

Herbal medicine has been traditionally used for a long time. A majority of people living in developing countries still rely on herbal medicines to meet their health needs. WHO supports the appropriate use of herbal medicines and encourages the use of remedies that have been proven to be safe and effective. A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore or improve health. The health claims of herbal medicine need to be verified scientifically, although the experience obtained from their traditional use over the years should not be ignored (WHO 1993). *Strobilanthes crispa* ZII 109 (L.) Bremek or *Saricocalyx crispa* ZII 109 (L.) Bremek (Acanthaceae) plant is native to countries from Madagascar to Indonesia, and is commonly known as *daun pecah beling* in Jakarta or *enyoh kelo*, *kecibeling*, or *kejibeling* in Java (Sunarto 1977). The plant is also known as *pecah kaca* or *jin batu* in Malaysia. It was first recorded by Thomas Anderson (1832–1870) who classified the plant under Spermatophyta (flowering plants and Gymnospermae) (Brummit & Powell 1992). The study showed that the leaves of *S. crispa* possess anti-AIDS and anti-leukemia activities (Kusumoto et al. 1992) as well as high antioxidant (Abu Bakar et al. 2004; Asmah et al. 2006a; Asmah et al. 2006b;) anticarcinogenic (Fauziah et al. 2005; Ismail et al. 2000; Mohd Fadzelly et al. 2006a; Suherman et al. 2004), antidiabetic (Mohd Fadzelly et al. 2006b; Norfarizan-Hanoon et al. 2009a) and wound healing (Norfarizan-Hanoon et al. 2009b). In this study, the acute toxicity effect of *S. crispus* juice in female and male Sprague Dawley rats was determined.

Materials and Methods

Plant Material and Preparation of S. Crispa Juice

The leaves of *S. crispa* were collected from the herbal garden of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor. The identity of the plant was verified by taxonomists from the Department of Botany, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. The voucher number of *S. crispa* was AZ-6803. *S. crispa* leaves were weighed, washed and cut into small pieces. Filtered water containing 0.1% (w/w) sodium metapbisulphite was added and the leaves blended in a mechanical grinder. The juice was then mixed with honey and 0.2% (w/w) xantham gum, homogenized using Homogenizer IKA II and pasteurized for 30 min using jacketed heater. The final product was hot-filled into sterilized glass bottles, cooled to room temperature under running water and kept chilled for analysis.

Experimental Animals and Study Design

Male (25) and female (25) Sprague Dawley albino white rats, weighing 150 to 200 g were used in this study. The rats were obtained from the Institute of Medical Research (IMR), Kuala Lumpur, Malaysia and housed in standard cage at an ambient temperature with 12-h-light/12-h-dark cycle of the Animal House, Faculty Medicine and Health Sciences, Universiti Putra Malaysia. They were fed commercial rat feed and tap water *ad libitum*. The rats were acclimatized to the laboratory conditions for 1 week on average before any experimental work was undertaken. The experiment was designed and conducted according to ethical norms as approved by the Animal Care and Use Committee (ACUC), Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor.

Study Design and Dosage

The rats were divided into 10 groups with 5 rats in each group as follow:

1. Female control (untreated),
2. Female fed with 700 mg/kg BW of *S. crispus* juice,
3. Female fed with 2100 mg/kg BW of *S. crispus* juice, 3 times higher than 700 mg/BW
4. Female fed with 3500 mg/kg BW of *S. crispus* juice, 5 times higher than 700 mg/BW
5. Female fed with 4900 mg/kg BW of *S. crispus* juice, 7 times higher than 700 mg/BW
6. Male control (untreated),
7. Male fed with 700 mg/kg BW of *S. crispus* juice,
8. Male fed with 2100 mg/kg BW of *S. crispus* juice,
9. Male fed with 3500 mg/kg BW of *S. crispus* juice,
10. Male fed with 4900 mg/kg BW of *S. crispus* juice.

Rats were fed a single dose of *S. crispa* juice orally by gavage for day 1. They were given commercial feed and plain water only for control (untreated) group.

The rats were observed twice daily for signs of physical and behavioural changes such as progressive dermatitis, rough hair coat, hunched posture, persistent recumbency, labored breathing, nasal discharge, jaundice or anemia, neurological signs, bleeding from any orifice, self-induced trauma, impaired eating or drinking, or excessive or prolonged hyperthermia or hypothermia. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, sleep and coma for 14 days after treatment. Body weights were recorded on days 0 and 14.

Gross Morphological Evaluations

The rats were euthanized by exsanguination from the abdominal aorta under ether anesthesia. Each rat was subjected to a full necropsy including examination of the external surface of the body, all orifices and the thoracic, abdominal and cranial cavities and their contents. The liver and kidneys were weighed wet immediately after dissection. Relative organ weight (organ to body weight ratio) for the liver and kidneys were calculated from the absolute organ weights and the terminal body weight of the rats.

Biochemical Analysis

On day 0 (baseline) and 14 days after treatment, the rats were anesthetized with diethyl ether following a 12-hours fast and 3 mL blood drawn via cardiac puncture and transferred into plain tube. Serum was prepared by centrifugation at 3000 rpm for 10 minutes at 4°C. Serum concentrations of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), albumin and creatinine were determined using Automated Clinical Chemistry Analyzer (Roche Diagnostic-Hitachi 902, USA).

Statistical Analysis

All data were presented as mean ± standard error of mean (S.E.M) using the SPSS version 13.0. The data for various biochemical parameters were analysed using ANOVA and the means were compared by Duncan's multiple range test. Values were considered statistically significant when $P<0.05$.

Results and Discussion

All rats showed normal growth and appeared to be active and healthy with no signs of gross toxicity, adverse pharmacological effect or abnormal behaviour during the 14 day post-treatment observation period. The changes of body weight were not significantly different in either

TABLE 1. Body weight (g) in female and male rats

Groups	DAY 0	DAY 14
Female control	201.14 ± 5.00	201.23 ± 4.09
Female + SCJ 700 mg/kg	199.02 ± 7.27	205.64 ± 5.49
Female + SCJ 2100 mg/kg	198.71 ± 3.49	199.28 ± 4.57
Female + SCJ 3500 mg/kg	210.59 ± 2.68	211.42 ± 2.02
Female + SCJ 4900 mg/kg	205.65 ± 2.28	210.74 ± 1.19
Male control	234.21 ± 7.54	250.64 ± 9.11
Male + SCJ 700 mg/kg	250.52 ± 15.04	251.39 ± 7.63
Male + SCJ 2100 mg/kg	252.27 ± 4.77	255.56 ± 11.92
Male + SCJ 3500 mg/kg	271.52 ± 18.40	279.68 ± 16.87
Male + SCJ 4900 mg/kg	252.07 ± 12.25	256.21 ± 5.13

Note. Data are expressed as mean ± SEM, n=5. No significant difference ($p < 0.05$) found.
SCJ = *S. crispus* juice

TABLE 2. Liver weight and relative liver weight (liver weight per body weight ratio) in female and male rats

Groups	Liver weight (g)	Liver/BW ratio
Female control	8.77 ± 0.77	0.043 ± 0.0032
Female + SCJ 700 mg/kg	7.51 ± 0.57	0.037 ± 0.0019
Female + SCJ 2100 mg/kg	7.23 ± 0.35	0.036 ± 0.0018
Female + SCJ 3500 mg/kg	7.37 ± 0.54	0.035 ± 0.0023
Female + SCJ 4900 mg/kg	6.57 ± 0.57	0.031 ± 0.0028
Male control	7.84 ± 0.15	0.030 ± 0.0001
Male + SCJ 700 mg/kg	8.80 ± 0.89	0.035 ± 0.0020
Male + SCJ 2100 mg/kg	8.52 ± 1.21	0.033 ± 0.0030
Male + SCJ 3500 mg/kg	9.53 ± 0.15	0.034 ± 0.0020
Male + SCJ 4900 mg/kg	8.87 ± 0.34	0.035 ± 0.0010

Note. Data are expressed as mean ± SEM, n=5. No significant difference ($p < 0.05$) found.
SCJ = *S. crispus* juice

TABLE 3. Kidney weight and relative kidney weight (kidney weight per body weight) in female and male rats

Groups	Kidney weight (g)	Kidney/Body weight ratio
Female control	1.57 ± 0.08	0.008 ± 0.00028
Female + SCJ 700 mg/kg	1.61 ± 0.07	0.008 ± 0.00028
Female + SCJ 2100 mg/kg	1.71 ± 0.08	0.008 ± 0.00028
Female + SCJ 3500 mg/kg	1.69 ± 0.07	0.008 ± 0.00032
Female + SCJ 4900 mg/kg	1.59 ± 0.08	0.007 ± 0.00039
Male control	2.51 ± 0.08	0.009 ± 0.00015
Male + SCJ 700 mg/kg	2.17 ± 0.07	0.008 ± 0.00026
Male + SCJ 2100 mg/kg	2.10 ± 0.13	0.008 ± 0.00031
Male + SCJ 3500 mg/kg	2.10 ± 0.13	0.007 ± 0.00028
Male + SCJ 4900 mg/kg	1.99 ± 0.12	0.008 ± 0.00039

Note. Data are expressed as mean ± SEM, n=5. No significant difference ($p<0.05$) found.
SCJ = *S. crispus* juice

male or female rats or between treatment and control groups (Table 1). No changes were observed on the gross morphology of the liver and kidney. There was no significant difference in the weight of the liver and kidney and the relative organ weight/body weight in all treated groups compared to controls (Tables 2 and 3).

There was no significant difference in the AST activity of all treated groups compared to controls (Table 4). In female rats the level of ALT was significantly ($P<0.05$) reduced in animal fed with *S. crispa* juice 700, 2100, 3500 and 4900 mg/kg BW compared to control group and at the start of the study (zero time). In male rats, the activity of ALT was not significantly different in all groups fed with *S. crispa* juice when compared to the control group and zero time (Table 5). The activity of ALP was not significantly different in all groups fed with *S. crispus* juice when compared to the control groups in female and male rats (Table 6). Not significantly different of serum albumin (Table 7) and creatinine (Table 8) level in male and female groups fed with *S. crispa* juice compared to control group.

TABLE 4. Aspartate aminotransferase (AST) activity (μkat/L) in female and male rat

Groups	DAY 0	DAY 14
Female control	2.52 ± 0.23	2.57 ± 0.76
Female + SCJ 700 mg/kg	2.43 ± 0.22	2.47 ± 0.18
Female + SCJ 2100 mg/kg	2.37 ± 0.14	2.33 ± 0.17
Female + SCJ 3500 mg/kg	2.42 ± 0.24	2.03 ± 0.19
Female + SCJ 4900 mg/kg	2.48 ± 0.41	2.62 ± 0.55
Male control	2.92 ± 0.28	2.10 ± 0.16
Male + SCJ 700 mg/kg	2.75 ± 0.47	2.37± 0.39
Male + SCJ 2100 mg/kg	3.03 ± 0.24	2.33 ± 0.29
Male + SCJ 3500 mg/kg	2.94 ± 0.24	2.10 ± 0.32
Male + SCJ 4900 mg/kg	2.80 ± 0.12	2.52 ± 0.31

Note. Data are expressed as mean ± SEM, n=5. No significant difference ($p<0.05$) found.
SCJ = *S. crispus* juice

TABLE 5. Alanine aminotransferase (ALT) activity (μkat/L) in female and male rats

Groups	DAY 0	DAY 14
Female control	1.08 ± 0.08	1.15 ± 0.16
Female + SCJ 700 mg/kg	1.05 ± 0.12	0.65 ± 0.05 *
Female + SCJ 2100 mg/kg	1.02 ± 0.06	0.73 ± 0.08 *
Female + SCJ 3500 mg/kg	1.13 ± 0.12	0.78 ± 0.08 *
Female + SCJ 4900 mg/kg	1.32 ± 0.20	0.80 ± 0.15 *
Male control	1.53 ±0.08	1.28 ± 0.13
Male + SCJ 700 mg/kg	1.41 ± 0.21	0.97 ± 0.09
Male + SCJ 2100 mg/kg	1.51 ± 0.14	1.37 ± 0.28
Male + SCJ 3500 mg/kg	1.62 ± 0.13	1.50 ± 0.10
Male + SCJ 4900 mg/kg	1.63 ± 0.14	1.32 ± 0.09

Note. Data are expressed as mean ± SEM, n=5. * Significant reduction ($p<0.05$) compared with control group.
SCJ = *S. crispus* juice

TABLE 6. Alkaline phosphatase (ALP) activity (μg/L) in female and male rats

Groups	DAY 0	DAY 14
Female control	204.33 ±18.19	99.33 ± 18.08
Female + SCJ 700 mg/kg	200.67 ± 9.08	81.17 ± 9.83
Female + SCJ 2100 mg/kg	232.67 ± 26.45	118.83 ± 22.49
Female + SCJ 3500 mg/kg	220.66 ±14.19	97.83 ± 10.78
Female + SCJ 4900 mg/kg	208.67 ± 12.30	100.60 ± 22.82
Male control	238.16 ± 28.03	198.17 ± 25.20
Male + SCJ 700 mg/kg	274.00 ± 30.97	206.00 ± 19.08
Male + SCJ 2100 mg/kg	280.17 ± 24.97	240.00 ± 37.45
Male + SCJ 3500 mg/kg	222.40 ± 17.19	193.33 ± 23.38
Male + SCJ 4900 mg/kg	288.50 ± 32.08	239.00 ± 25.89

Note. Data are expressed as mean ± SEM, n=5. No significant difference ($p<0.05$) found.
SCJ = *S. crispus* juice

TABLE 7. Serum albumin level (g/L) in female and male rat

Groups	DAY 0	DAY 14
Female control	56.38 ± 2.17	37.75 ± 4.55
Female + SCJ 700 mg/kg	58.78 ± 1.02	42.08 ± 4.83
Female + SCJ 2100 mg/kg	58.88 ± 1.34	38.57 ± 2.85
Female + SCJ 3500 mg/kg	58.43 ± 4.80	34.90 ± 1.79
Female + SCJ 4900 mg/kg	57.38 ± 2.30	32.72 ± 6.68
Male control	36.75 ± 1.64	50.40 ± 4.08
Male + SCJ 700 mg/kg	34.18 ± 4.76	38.53 ± 4.85
Male + SCJ 2100 mg/kg	36.93 ± 2.23	45.05 ± 5.38
Male + SCJ 3500 mg/kg	39.44 ± 2.54	43.03 ± 5.52
Male + SCJ 4900 mg/kg	36.77 ± 2.35	49.02 ± 2.90

Note. Data are expressed as mean ± SEM, n=5. No significant difference ($p<0.05$) found.
SCJ = *S. crispus* juice

TABLE 8. Creatinine level (mg/dL) in female and male rats

Groups	DAY 0	DAY 14
Female control	0.47 ± 0.03	0.39 ± 0.06
Female + SCJ 700 mg/kg	0.45 ± 0.02	0.39 ± 0.05
Female + SCJ 2100 mg/kg	0.41 ± 0.02	0.34 ± 0.04
Female + SCJ 3500 mg/kg	0.43 ± 0.06	0.27 ± 0.02
Female + SCJ 4900 mg/kg	0.45 ± 0.04	0.31 ± 0.08
Male control	0.23 ± 0.01	0.32 ± 0.05
Male + SCJ 700 mg/kg	0.20 ± 0.06	0.23 ± 0.05
Male + SCJ 2100 mg/kg	0.25 ± 0.02	0.32 ± 0.03
Male + SCJ 3500 mg/kg	0.28 ± 0.03	0.19 ± 0.04
Male + SCJ 4900 mg/kg	0.22 ± 0.01	0.23 ± 0.03

Note. Data are expressed as mean ± SEM, n=5. No significant difference ($p<0.05$) found.
SCJ = *S. crispus* juice

Investigation of acute toxicity is the first step in the toxicological analysis of herbal drugs. Rats that were administered with the highest dose of *S. crispa* juice (4900 mg/kg body weight) did not show any toxicity for the 14-day experimental period. All rats survived without any physical or behavioural changes.

Consumption of *S. crispa* juice did not cause morphological or biochemical changes to the liver or kidney. These organs perform vital functions for the maintenance of health. The liver primarily detoxifies harmful substances, secretes bile into the intestine, synthesizes and stores important molecules, among other things. The kidney helps in maintaining homeostasis of the body by reabsorbing important materials and excreting waste products (Wurochekke et al. 2008). Liver function tests, which include determination of liver enzymes, are designed to provide information about the state of the liver.

Enzyme activities in the serum and tissues are often used as 'markers' to ascertain early toxic effects of administered foreign compounds to experimental animals (Coodley 1970). ALP is a membrane bound enzyme (Wright & Plummer 1974) while ALT and AST are cytosolic enzymes (Christen & Metzler 1985). These enzymes are highly concentrated in the liver and kidney and are only found in serum in significant quantities when the cell membrane becomes leaky or when cells are completely ruptured (Cotran et al. 1989: Ngaha 1981). A rise in serum level or decrease in tissue level of these intracellular enzymes is an index of damage to liver and kidney cells (Moss & Rosalki 1996). Seven days after treatment, no significant differences were observed in the aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and albumin levels, except in female rats, where the level of ALT was significantly reduced compared to control group. A reducing ALT level indicate that no inflammatory stage of liver cells. These result indicated that the juice did not bring about pronounced cellular damage in the liver and kidney of the rats during the experimental period.

There was no significant difference in the serum levels of creatinine of the treated animals, indicating that the juice is not nephrotoxic. Creatinine a waste product formed in the muscles via creatine metabolism, which then enters into the blood stream to be removed by the kidneys. Elevation of these waste products in the blood (serum) is an indication of renal function impairment (Cameron & Greger 1998; Orth & Ritz 1998).

Conclusion

S. crispa juice does not have any toxicity signs in normal Sprague Dawley rats. No significant changes were observed in the physical, morphological and biochemical although a significant reduction of ALT was observed in female rats. The *S. crispus* juice was found to be safe at the maximum dose used in this study (4900 mg/kg BW.).

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