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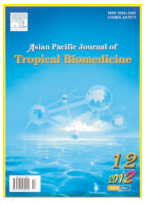

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Subacute oral toxicity study of ethanolic leaves extracts of *Strobilanthes crispus* in rats

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PEER REVIEW

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Comments

This is a good study in which the authors evaluated the oral toxicity of repeated in different doses of extracted *S. crispus* on liver and kidney functions of SD. The Results are interesting that 14-day oral administration of *S. crispus* ethanol leaves extract ranging from 150 mg/kg to 600 mg/kg was safe without affecting the liver and kidney function in female SD rats.
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ABSTRACT

Objective: To examine the oral toxicity of repeated dosing of *Strobilanthes crispus* (*S. crispus*) ethanol leaves extract on the liver and kidney functions in Sprague Dawley rats. **Methods:** Young female rats aged between 8 and 12 week-old were randomly assigned into four groups with five animals each group (n=5). The first group served as control, while the second, third and fourth groups were orally treated with a single dose daily with 150 mg/kg, 300 mg/kg, and 600 mg/kg of *S. crispus* ethanol leaves extract for 14 d consecutively. Cage-side observation was conducted for first 4 h after each dosing. The body weight changes, food consumptions and water intake were also recorded. Serum biochemical parameters, *i.e.*, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, creatinine and urea were determined at Day 15. All results were expressed as mean±SD and analysed using Dunnett's test. **Results:** It was obtained that 14-day oral administration of *S. crispus* ethanol leaves extract did not cause any adverse effects or lethality to the female Sprague Dawley rats. No significant changes in serum biochemical parameters, relative organs weights, body weights, food intake and water consumptions were observed between the treatment groups and control. **Conclusions:** In conclusion, 14-day oral administration of *S. crispus* ethanol leaves extract was safe to be consumed in female rats without affecting the liver and kidney functions.

KEYWORDS
Kidney, Liver, Oral toxicity, Serum enzymes, *Strobilanthes crispus*

1. Introduction

Strobilanthes crispus (Family: Acanthaceae) (*S. crispus*), or locally known as Pecah Kaca in Malaysia, has gained great attention due to its high medicinal values[1,2]. It is also known as "daun picah beling" in Jakarta and "enyoh kelo", "kecibeling", "ngokilo" in Java[3]. The leaves of this plant are oblong-lanceolate, rather obtuse, and shallowly crenate-crispate. The top surfaces of leaves of *S. crispus* are darker green in colour and less rough as compared to the under surfaces[3]. Moreover, both surfaces of the leaves are very scab rid and are covered with short hair[4]. Its flower is short, dense, and panicled spikes[4]. *S. crispus* only grows and attains the height of 2 m[5].

In Malaysia, *S. crispus* was traditionally used for the treatment of diabetes mellitus, as a diuretic and to treat high blood pressure[6]. Many scientific reports had also proven that *S. crispus* possessed anti-oxidative, anti-cancer, wound healing and anti-hyperglycemic property[6-9]. Phytochemical analyses revealed that the leaves of *S. crispus* containing minerals (potassium, calcium, sodium, iron, and phosphorus), vitamins (ascorbic acid, riboflavin,

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and thiamine), phenolic acids (*p*-hydroxybenzoic acid, *p*-coumaric acid, caffeic acid, vanillic acid, gentinic acid, ferulic acid and syryngic acid), caffeine, tannin, alkaloid, and catechin[10,11]. In addition to the chemicals stated above, *S. crispus* leaves also contained cystoliths of calcium carbonate in which the infusion was mildly alkaline[12].

The information with regards to the toxicity of *S. crispus* is still limited. In 2009, a scientific study reported LD_{50} of *S. crispus* juice was greater than 4 900 mg/kg[13]. In the repeated dose oral toxicity study, 140, 210 and 280 mg/kg of *S. crispus* juice were tested on both male and female normal and streptozotocin-induced diabetic rats showed no significant changes in general behaviour, body weight, and organ weight between the treatment and control groups. Moreover, the serum parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and albumin were significantly reduced[13].

This study was aimed to examine the possible oral toxicity of repeated dosing (14-day treatment) of ethanol leaves extract of *S. crispus* on the liver and kidney functions in Sprague Dawley (SD) rats. The findings from the present study could provide essential information to ensure the safety and quality of the *S. crispus* based products in future. It also served as reference for other researchers to select appropriate dose for their pharmacological studies.

2. Materials and methods

2.1. Chemicals

Diethyl ether and ethanol (95%) were supplied from Chemolab Supplies Sdn. Bhd., Malaysia.

2.2. Plant materials and extraction

Dried leaves of *S. crispus* were supplied by a local supplier from Seremban, Negeri Sembilan. The leaves were then blended into fine pieces with the blender and macerated in 6 L of 95% ethanol at room temperature for 4 d[14]. Extraction was repeated until the last extract turned colourless. Then, the extract was then filtered through cotton wool. By the use of the rotary evaporator at 45 °C and reduced pressure, most of the ethanol solvent was removed and the concentrated *S. crispus* extract was obtained[14]. The crude extract was then dried in the oven at 60 °C until a constant weight was obtained[15]. The percentage yield was calculated. The extract was stored in a desiccator from where it was used when required.

2.3. Selection of experimental animals

Healthy female SD rats weighing (160±10) g body weight were used in this study. All rats were kept in the animal holding room at temperature of (25±2) °C with the lighting of 12 h light followed by 12 h dark[16]. All female rats were kept in their cages for at least 5 d prior to dosing as for the purpose of acclimatizing to the laboratory conditions[16]. Tap water and food pallet were provided *ad libitium*. However, food pallet was withheld over-night prior to dosing[16]. All rats were handled based on the guideline of the animal ethics, Faculty of Pharmaceutical Sciences, UCSI University.

2.4. Repeated (14-day) oral toxicity study

All experimental procedures of oral toxicity study were in accordance to Organisation for Economic Co-operation and Development (OECD) 423 guideline[16]. Based on the OECD 423 guideline, all the doses of *S. crispus* ethanol leaves extract must be prepared ffreshly prior to administration. All female SD rats were randomly assigned into four groups with five animals (n=5) each group. The first group served as control, while the second, third and fourth groups were orally treated with a single dose daily for 14 d with 150 mg/kg, 300 mg/kg, and 600 mg/kg of *S. crispus* ethanol leaves extracts, respectively. All animals were examined for signs of toxicity or mortality via cage-side observation after each dosing *S. crispus* ethanol extract[16]. Body weight changes, water intake and food consumptions were recorded on Day 0, Day 3, Day 7 and Day 14. On Day 15, blood samples were collected via cardiac puncture. The blood samples were then sent to UCSI University Pathology Laboratory within the day of collection for analysis. Several serum biochemical parameters, *i.e.*, AST, ALT, ALP, creatinine, and urea were determined[17]. Gross examination of the organs and the relative organ weight of heart, lungs, liver, spleen and kidneys were determined.

2.5. Statistical analysis

All data were presented as mean±SD and analysed using Dunnett's test (*post-hoc* analysis)[18]. The level of significant difference of this research study was set at $P<0.05$. Dunnett's test was used to compare the differences of the control group with other treatment groups.

3. Results

The percentage yields of ethanol extracts obtained from 1.2 kg powder of *S. crispus* leaves was found to be 58.3 g/kg. Based on the repeated oral toxicity, treatment of *S. crispus* ethanol leaves extract ranging from 150 mg/kg, 300 mg/kg, and 600 mg/kg did not cause any lethality, behavioural changes and toxic signs after each dosing throughout 14-day experimental duration. No significant changes were observed between all treatment groups and control group in term of body weight changes, water intake, food consumptions, serum biochemical analyses and relative organ weight (Tables 1-3).

Table 1
Effect of 14 d treatment of *S. crispus* ethanolic leaves extract on body weights, water and food consumptions in young female SD rats.

	Groups (mg/kg)	Day 0	Day 3	Day 7	Day 14
Body weight (g/rat per day)	Control	158.10±14.21	162.5±13.30	170.40±14.27	179.10±13.57
	150	157.60±16.48	163.8±16.35	169.00±16.74	181.20±17.45
	300	159.60±14.06	166.2±18.02	174.20±16.54	188.20±17.69
	600	162.20±10.05	165.40±9.58	169.70±8.56	179.60±10.36
Water consumption (mL/rat per day)	Control	27.70±0.47	26.30±2.36	27.70±0.47	27.00±4.24
	150	27.20±4.01	28.40±7.19	28.70±1.89	33.30±1.89
	300	22.60±1.29	26.50±3.54	25.00±1.41	28.00±2.83
	600	26.30±5.30	25.10±3.66	26.70±1.89	33.00±9.90
Food consumption (g/rat per day)	Control	15.00±2.51	12.40±1.76	13.30±1.64	14.60±2.99
	150	12.90±1.36	11.90±0.58	12.00±0.41	15.50±0.96
	300	12.40±0.54	12.70±0.89	12.10±3.03	22.50±11.37
	600	11.70±0.90	12.70±1.74	10.60±1.65	13.20±1.38

Values are expressed in mean±SD (n=5).

Table 2
Effect of 14-day treatment of *S. crispus* ethanolic leaves extract on serum biochemical parameters in young female SD rats.

Groups (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L) (mmol/L)	Urea	Creatinine (μmol/L)
Control	234.60±34.39	66.20±4.60	134.00±17.41	7.50±1.24	56.20±3.35
150	193.80±32.89	62.00±10.00	158.80±35.97	6.90±1.31	50.00±4.18
300	222.40±39.46	62.00±5.75	141.00±13.66	7.10±1.95	51.40±4.83
600	192.40±15.99	64.40±5.68	136.20±37.77	7.10±1.06	49.80±2.39

Values are expressed in mean±SD (n=5).

Table 3
Effect of 14-day treatment of *S. crispus* ethanolic leaves extract on relative organ weight in young female SD rats.

Groups (mg/kg)	Liver	Kidney	Heart	Lung	Spleen
Control	3.30±0.29	0.69±0.10	0.45±0.09	0.74±0.08	0.21±0.03
150	2.90±0.21	0.67±0.07	0.38±0.05	0.69±0.03	0.20±0.03
300	3.50±0.51	0.76±0.12	0.43±0.03	0.88±0.14	0.22±0.02
600	3.10±0.09	0.64±0.06	0.40±0.05	0.83±0.12	0.19±0.01

Values are expressed in mean±SD (n=5); relative organ weight (g/100 g body weight).

4. Discussion

Median lethal oral dose (LD_{50}) is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route[16]. According to the findings of repeated oral toxicity study, *S. crispus* ethanol leaves extract ranging from 150 mg/kg to 600 mg/kg bodyweight caused neither toxic signs nor lethality in female SD rats after 24-hour administration. Thus, LD_{50} of the *S. crispus* ethanol leaves extract in female SD rats was greater than 600 mg/kg.

According to the OECD 423 guideline, animals need to be observed individually for at least once during the first 30 min after each dosing, and special attention need to be given during the first 4 h daily for a total of 14 d[17]. According to Chan and Hayes, any toxic signs observed in the toxicity study would reflect indirectly which organs, tissues, or systems were most likely to be affected by the tested substance[19]. In the repeated dose (14-day) oral toxicity study, the adverse effects of *S. crispus* on the central nervous system, respiratory system, cardiovascular system, and gastrointestinal system could be ruled out due to the absence of unusual aggressive behaviour, tremor, twitch, ataxia, paralysis, tachycardia, bradycardia, diarrhoea, discolouration of stool, and gasping of air after each dosing throughout 14 days experimental duration.

Most of the orally consumed drugs undergo metabolism and excretion via liver and kidney, respectively[20]. Hence, liver and kidney are always the targeted organs in the oral toxicity study. In this research, toxic effect of *S. crispus* ethanol leaves extract on liver and kidney were examined based on the blood serum biochemical parameters. Liver is responsible for the metabolism of most of the drugs, mainly due to its large organ size and its high drug metabolising enzymes concentration relative to other organs[20]. In addition, liver is also highly perfused by blood containing high drugs concentration absorbed from the gut via entero-hapatic circulation[20]. In the present study, three serum hepatic biochemical parameters, namely, ALT, AST, and ALP were analysed. Any damages to the liver by hepatotoxin would cause these hepatic enzymes to leak into the circulation and rise in the serum levels[21]. ALT and AST were the common hetapatocellular markers used in the liver function test. However, ALT served to be the more specific hepatocellular indicator as compared to AST because the concentration of ALT in the liver was far exceeded in any other organs[18]. On the other hands, ALP was an useful hepatobiliary marker for the liver function test. The elevation of serum ALP level would explain the presence of cholestasis[21]. According to the results, all doses of *S. crispus* ethanol leaves extract showed no significant effect on the AST, ALT, and ALP between the treatment groups and the control group. Moreover, the relative organ weight and gross examination of liver of all treatment groups were found to be normal and not significant as compared to the control group. Hence, *S. crispus* ethanol leaves extract did not produce any significant toxic effect to the liver.

Kidney function test was generally examined based on the capability of kidneys to remove excess fluids and wastes from the blood stream[22]. In this research, serum urea and creatinine levels were examined as the indicators for kidney function test. Urea is generally derived from the metabolism of excessive protein in the liver while creatinine was derived from the break-down product of creatine phosphate in muscle tissue. Any increment of the level of these waste products in the blood would indicate the decline function of the kidney filtration[20]. Based on the result, all doses of the *S. crispus* ethanol leaves extract showed no significant effect on serum urea and creatinine between the treatment groups and the control group. Furthermore, the relative organ weight and gross examination of kidney of all treatment groups were also found to be normal and not significant as compared to the control group. Hence, *S. crispus* ethanol leaves extract did not cause any significant toxic effect to the kidney.

By evaluating every aspect of the results obtained, no-observed-effect-level and no-observed-adverse-effect-level (NOAEL) of *S. crispus* could be determined[19]. Repeated oral dosing with *S. crispus* ethanol leaves extract showed no significant effect in all parameters examined when compared to the control group. Hence 600 mg/kg per day of

S. crispus ethanol leaves extract was known to be the no-observed-effect-level and NOAEL in female SD rats in repeated dose (14-day) oral toxicity study. Acceptable daily intake (ADI) was defined as an estimated maximum amount of an agent, expressed on a body mass basis, to which individuals in a (sub) population may be exposed daily over their lifetimes without appreciable health risk[23]. ADI was generally derived from NOAEL (mg/kg per day) multiplied by an uncertainty factor of 100 to obtain the level that is harmless to humans[24]. In this research, the NOAEL of *S. cripus* ethanol leaves extract was 600 mg/kg per day, and thus the ADI of *S. cripus* ethanol leaves extract was 6 mg/kg per day.

The interchange of dosage used in the toxicity study from one species to another can be done by multiply its dosage with the equivalent surface area dosage conversion factor of the other species[25]. According to Freireich *et al.*, the equivalent surface area dosage conversion factor from rat to humans was 1/6[25]. Assuming 600 mg/kg of *S. crispus* ethanol leaves extract was orally administered to a SD rat, thus, the equivalent oral dose of *S. crispus* ethanol leaves extract for human usage was 100 mg/kg. According to WHO research guidelines, two weeks to one month of repeated oral dosing in pre-clinical study was equivalent to a single administration or repeated oral dosing less than one week in clinical study[26]. Hence, single dose or repeated oral dosing less than one week of 100 mg/kg of *S. crispus* ethanol leaves extract used in humans was expected to have the same effects as observed in this 14-day repeated dosing of 600 mg/kg of *S. crispus* ethanol leaves extract in female SD rats.

No generally accepted methodologies for extrapolating the *in vivo* evaluation in animal models to human are currently available although many statistical methods have been attempted[27]. The poor reliability is reported mainly due to the interspecies differences[27]. Additional information needs to be considered in designing animal studies in order to predict the similar effects might be anticipated in human. Thus, it is important to consider the correlation of the pharmacokinetic variables such as absorption, metabolism and elimination to the toxicokinetic variables such as potency and duration of effect. The mechanisms of *S. crispus* uptake following oral administration remain to be characterised. Metabolic aspect and quantitative structure-activity relationships in pharmacokinetics could enhance the understanding of the mechanisms underlying gastrointestinal absorption, bioavailability, membrane permeability and first-pass extraction of *S. crispus*[28]. As mentioned by Derelanko *et al.*, the lowest dose level used in the animals should not be less than the pharmacokinetically equivalent human exposure and should produce no toxicological effects if a toxicity study conducted in animal is used for risk assessment of human exposure[19].

It should be noticed that acute toxicity study serves as preliminary reference for dose selection. The present oral toxicity data cannot be used to predict the adverse effects after long-term exposure since the duration of oral toxicity was only 14-day studied by using female rats. Several biological variations such as age and gender which are generally known to affect the sensitivity of animals to test agents are not included in this research. Hence, chronic oral toxicity study will be conducted in future to ascertain the biological effect after long-term exposure of *S. crispus* leaves extracts in different animal models.

In conclusion, 14-day oral administration of *S. crispus* ethanol leaves extract ranging from 150 mg/kg to 600 mg/kg was safe without affecting the liver and kidney functions in female SD rats.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

S. crispus was traditionally used for the treatment of diabetes mellitus, as a diuretic and to treat high blood pressure and possessed anti-oxidative, anti-cancer, wound healing and anti-hyperglycemic property. However, the information with regard of the toxicity of *S. crispus* was still limited. Therefore, these studies were conducted by investigating the oral toxicity of repeated dosing of *S. crispus* ethanol extraction on the liver and kidney function in SD rats. Results showed that 14-day oral administration of *S. crispus* ethanol leaves extracted was safe to be consumed in female rats without affecting the liver and kidney functions.

Research frontiers

Studies are being performed in order to determine the serum biochemical parameters; AST, ALT, ALP, creatinine, and urea in four groups of rats were orally treated with a single dose daily with 150 mg/kg, 300 mg/kg, and 600 mg/kg of *S. crispus* ethanol leaves extract for 14 d consecutively.

Related reports

The percentage yields of ethanol extracts obtained from 1.2 kg powder of *S. crispus* leaves was found to be 58.3 g/kg did not cause any lethality, behavioural change and toxic sign after dosing throughout 14-day experimental duration. These data are in agreement with recent reported by Mohamed *et al.*, (2011).

Innovations and breakthroughs

Data regarding toxicity examination of *S. crispus* hasshowed the additional information for the needs to be

considered in designinganimal studies in order to predict the effect might be anticipated in humans.

Applications

Correlation of the pharmacokinetic variables to the toxicokinetic variables and understanding of the mechanism gastrointestinal absorption, bioavailability, membrane permeability and first-pass extraction of *S. crispus*.

Peer review

This is a good study in which the authors evaluated the oral toxicity of repeated in different doses of extracted *S. crispus* on liver and kidney functions of SD. The Results are interesting that 14-day oral administration of *S. crispus* ethanol leaves extract ranging from 150 mg/kg to 600 mg/kg was safe without affecting the liver and kidney function in female SD rats.

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