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Toxicity study of *Orthosiphon stamineus* Benth (Misai Kucing) on Sprague Dawley rats

Chin Jin Han[1*], Abas Hj Hussin[2], Sabariah Ismail[3]
[1*]School of Pharmacy, University College Sedaya International, 56000 Kuala Lumpur, Malaysia.
[2]School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia,
[3]Centre for Drug Research, Universiti Sains Malaysia, 11800 Penang, Malaysia
E-mail: jinhanchin@hotmail.com
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Abstract. *Orthosiphon stamineus* Benth (Family: Lamiaceae) or locally known as Misai Kucing has been widely used in Malaysia for treating kidney problems, gout, and diabetes. This study aims to evaluate the possible toxic effect after following fourteen days oral administration of methanol extract of *O. stamineus* in female Sprague Dawley (SD) rats. Control groups were treated orally with distilled water (vehicle) while the four test groups were treated up to fourteen days with 0.5 g/kg, 1 g/kg, 3 g/kg and 5 g/kg body weight of methanol extract of *O. stamineus* respectively. Toxicity of the methanol extract of *O. stamineus* was evaluated by the incident of lethality, side-cage observation and blood serum biochemical parameters. No lethality or adverse toxic signs were seen during the experimental period. A significant decrease in several serum biochemical parameters i.e. AST and ALT and increase in liver weight was observed in young female SD rat after being fed fourteen days with methanol extract of *O. stamineus*. No delayed toxic effect and lethality was observed in all rats during fourteen days of recovery period. In conclusion, methanol extract of *O. stamineus* within these range and treatment duration would not cause any severe toxic effects and organ damages in rats.

INTRODUCTION

Herbs have become more popular in Malaysia in recent years. However, herbal medicine is still poorly understood by the public and scientifically-proven research on these herbal products is needed. *Orthosiphon stamineus* Benth (Lamiaceae), a medicinal plant native to tropical Asia, gets its common name Misai kucing or cats whiskers from its pale purple flowers with long wispy stamens shaped like cats whiskers (Indubala & Ng, 2000). According to Winston (1992), there are three categories of herbs which are known as food herbs, medicine herbs and poison herbs. *Orthosiphon stamineus* has been used to treat urinary lithiasis, edema, eruptive fever, influenza, rheumatism, hepatitis and jaundice (Wiart, 2002). In Myanmar, the leaves of *O. stamineus* are used as antidiabetic drugs and used as a remedy to treat urinary tract and renal diseases (Bwin & Gwan, 1967). It is also consumed as a healthy java tea to facilitate body detoxification in Japan (Awale *et al.*, 2003). Although many scientific researches have been reported on *O. stamineus* Benth, however, the information of toxicology and the safe use of this herb is still limited and has not been extensively studied.

Numerous herbal products and herb-derived products could cause hepatic toxicity after use (Saad *et al.*, 2006). It is extremely important for herbalist or herbal products manufacturers to understand the correlation between pharmacological activities of the herbal active compounds involved, possibility of herbs interacting with other drugs when both are consumed concurrently. Some plants such as mushroom and stone fruit have been

documented by Fenton (2002) on its toxicity that causes death, potential toxins and the fatal dose. This information is limited but is very useful for human to avoid toxicity from excessively eating these plants or toxic compounds which may also be present in other types of plants. Toxicology studies contributed greatly to the pharmaceutical sciences for better understanding on human physiology and drug interactions within the body (Timbrell, 2000). LD_{50} value is an estimation of the potency of toxicant or agents to subjects which causes fifty percent of lethality and is normally expressed in mg/kg body weight (Salsburg, 1986). Other criteria such as organ damage, development of cancer caused by substances are not considered. There is uncertainty in the prediction of other outcomes such as organ damage, alteration of enzyme activity based on the basis of the LD_{50} value. Detailed analysis of blood serum tests such as liver and kidney function test, histology and hematology, food consumption and water intake, need to be carried out to examine the development of secondary stage of toxicity caused by chemical. Acute oral toxicity studies in animal are used as references for selecting starting dose for human phase I study. Toxicity study information will be utilized as a useful tool for choosing doses for repeated-dose study, providing preliminary identification of target organ toxicity or any adverse effects after used.

Therefore, this study was undertaken to examine the possible toxicity effect of oral administration of methanol extract of *O. stamineus* in Sprague Dawley (SD) rats and hence to determine the LD_{50}, no-observable effect level (NOEL) and no-observable adverse effect level (NOAEL).

MATERIALS AND METHODS

Plant material

Plants were grown from cuttings using standard agronomic practices at Kepala Batas, Penang, Malaysia. The leaves were collected from 30-45-day-old white-flowered plants. The specimen was labeled, numbered and annotated with the date of collection and locality. Voucher specimen of the plant material was deposited at Bilik Herba, School of Pharmaceutical Sciences, Universiti Sains Malaysia. Plant leaves were ground to a homogeneous powder in a Wiley mill (no. 20 mesh) after drying in an oven (35 °C). The dried powdered leaves were extracted with methanol by using soxhlet apparatus. After the solvent was removed under reduced pressure, portion of the concentrated extract was spray-dried (Akowuah *et al.*, 2004).

Animals

Experimental animals used in this study were Sprague-Dawley (SD) rats obtained from the Animal House Unit of Universiti Sains Malaysia. Healthy young female rats (7 ± 1 weeks old) (100 ± 10 g) were used throughout this toxicity study. The animals were kept in the animal room (25 ± 2 °C) under 12 h –light/dark cycle and fed with standard diet of lever pellet diet and free access to distilled water prior to the start of the study. Food and water were not withheld before oral administration of the extracts to rats. The freeze-dried extract was dissolved in distilled water and vortexed prior to oral administration. Animals were maintained and handled according to the recommendations of the USM ethical committee which approved the design of the animal experiments.

Acute Oral toxicity studies (repeated dose 14 days)

Fixed dose procedures (FDP) (OECD guideline 420, 2001d) was followed in this subacute oral toxicity study. The usual starting dose for any unknown toxicity profile chemical will be 5, 50 and 500 mg/kg body weight. Sixty young female SD rats were randomly assigned into six groups (n =10). First group was served as negative control (untreated rat group). Group 2 (positive control group) was treated with vehicle i.e. distilled water only. Group 3-6 were treated once daily for 14 days consecutively with 0.5, 1.0, 3.0 and 5.0 g/kg body weight of methanol extract of *O. stamineus* respectively. The rats were observed closely at the first four hour to

examine any toxic symptoms caused by methanol extract of *O. stamineus* (Chan & Hayes, 1994). After the administration of last dose of methanol extract of *O. stamineus*, all treated rat and control rat groups were fasted overnight (at least for 16 h prior to blood collection). Blood was taken via cardiac puncture and used to prepare blood serum for clinical biochemical analyses (Levine, 1995). Half of the survived rats (n=5) were sacrificed to obtain relative weight of organs such as liver, kidney, heart, lung and spleen and examine for any abnormalities in the organ. Blood serum samples analysis was conducted by using Roche (Intergra 700®) machine. Serum biochemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), urea, creatinine, total cholesterol, triacylglycerol (TAG) and hematocrit were selected for the analysis.

Fourteen days recovery periods

Half of the survivaled rats (n=5) were then returned back to their own cage and kept for another 14 days observation period. During this period, rats had free access to commercial food pellets and water *ad libitum*. These groups of rats were observed two times daily to examine for any occurrence of delay toxic symptoms. Food consumption, water intake and body weight gained were recorded at day 7 and 14 during the recovery period. Survived rats were then sacrificed and necropsy was carried out to see any organ damage and to obtain relative organ weight of liver, kidneys, lungs, heart and spleen.

Data analysis

Analysis was done using Dunnett's multiple comparison test (Bürger *et al.*, 2005). The levels of significant were set at $P<0.05$ and $P<0.01$.

RESULTS

Subacute toxicity study (14 days treatment)

Oral administration of *O. stamineus* did not produce significant change in behavior, breathing, nervous responses in female rats. No significant change in the mean body weight, water intake and food consumption of female SD rat was observed when compared to their positive control group (Table 2). Fourteen days administration of 0.5, 1.0, 3.0 and 5 g/kg of methanol extract of *O. stamineus* to normal female SD rats had no significant change in the serum urea, creatinine and ALP level (Table 1). For the liver function tests, a significant decrease in serum AST (at 1.0 and 3.0 g/kg) ($P<0.05$) and ALT level (at 5g/kg) was observed in normal young female SD rats (Table 1). The effect of methanol extract of *O. stamineus* on rat hepatic system was further supported by the finding on the relative liver weight. Young female SD rats fed with 3 g/kg and 5 g/kg of methanol extract of *O. stamineus* showed increase in relative liver weight as compared to control group (Table 3). Relative organ weight of kidney, lung, heart and spleen in normal young female SD rats was not significantly changed as compared to positive control group (Table 3).

Fourteen days recovery period

No lethality and any delayed adverse effects occurred in any of the group (Table 4). Body weight, food consumption and water intake for normal young female SD rats in treatment groups was not significantly changed as compared to positive control group (Table 4). Gross necropsy findings for normal young female SD rats at the end of recovery period did not indicate any significant change in the external physical structure of the organs and their relative organ weight (Table 4).

DISCUSSION

LD_{50} describes only one end point i.e. death. Based on our acute toxicity findings, LD_{50} value could not be determined in this study. LD_{50} for *O. stamineus* was shown to be higher than 5 g/kg because no lethality was found in the normal young female SD rats. According to Ecobichon (1995), a test compound that causes no adverse effect at a dose exceeding 5 g/kg will be considered as 'practically non-toxic'. A significant

Table 1. The effect of methanol extract of *O. stamineus* on blood serum AST, ALT, ALP, urea, creatinine, total cholesterol, triacylglycerol and hematocrit in normal young female SD rats.

Dose of *O.s* (g/kg body weight)	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**Urea (mmol/L)**
Negative control	92.0± 4.2	37.3± 3.8	91.1±10.8	3.7±0.4
Positive control	107.8±32.65	41.0±13.4	116.0±21.2	4.0±1.0
0.5	68.5± 6.61	26.5± 2.7	89.9±13.5	3.6±0.5
1.0	63.3± 2.5*	30.0± 5.0	89.0±13.5	3.4±0.4
3.0	59.7± 9.5*	28.5± 5.3	94.5±20.5	3.8±1.3
5.0	68.3±11.1	23.8± 1.5*	105.0± 7.1	4.6±2.3
	TC (mmol/L)	**TAG (µmol/L)**	**Creatinine (µmol/L)**	**Hematocrit (%)**
Negative control	0.66±0.10	0.37±0.10	16.4±5.1	42.0±3.9
Positive control	0.68±0.12	0.35±0.14	18.0±8.8	44.2±4.0
0.5	0.72±0.08	0.35±0.03	11.5±1.7	42.1±4.8
1.0	0.66±0.13	0.32±0.06	13.5±6.8	44.6±3.4
3.0	0.71±0.25	0.40±0.15	12.0±6.1	43.8±1.9
5.0	0.69±0.45	0.46±0.19	15.0±5.1	42.9±4.1

n=10; Values are expressed as mean ± S.D. Analysed using Dunnett's test
* P<0.05 as compared to positive control. TC = total cholesterol; TAG = triacylglycerol
Negative control = untreated rats. Positive control = treated with distilled water only.

Table 2. The effect of methanol extract of *O. stamineus* on the body weight gained, food consumption and water intake in normal female SD rats

Dose of *O.s* (g/kg body weight)	**0 day**	**3rd day**	**7th day**	**14th day**
		Body weight (g)		
Negative control	101.1±1.5	105.7±2.4	108.7±2.0	125.5±1.8
Positive control	100.2±2.0	100.0±4.1	105.6±3.0	121.7±3.1
0.5	100.0±5.1	101.0±2.8	104.3±3.1	120.7±3.1
1.0	103.1±3.0	102.1±2.4	105.5±2.1	123.6±2.2
3.0	100.1±1.0	100.5±1.0	105.8±1.2	120.2±1.4
5.0	100.1±1.0	100.0±1.9	104.7±1.1	120.4±1.0
		Food consumption (g/rat/day)		
Negative control	7.8±0.8	8.1±0.7	10.2±0.6	15.0±0.5
Positive control	8.5±0.2	7.8±0.8	9.0±0.1	14.1±0.2
0.5	8.0±0.5	7.5±0.7	9.3±0.3	14.5±0.3
1.0	7.5±0.7	7.3±0.7	9.0±0.7	13.4±0.5
3.0	7.6±0.8	8.0±0.8	9.3±0.7	14.6±0.6
5.0	8.4±0.3	8.2±0.4	8.8±0.5	14.1±0.1
		Water intake (ml/rat/day)		
Negative control	10.0±1.0	11.5±0.4	12.0±0.8	16.0±0.4
Positive control	10.5±1.5	11.0±0.5	12.0±0.4	17.5±0.4
0.5	11.0±1.0	11.5±0.6	13.0±0.6	17.0±0.6
1.0	9.5±0.5	10.5±0.4	12.0±0.8	17.5±1.0
3.0	11.0±0.4	11.0±0.4	13.0±0.2	18.5±0.3
5.0	11.0±0.6	11.5±0.5	13.5±0.2	17.5±0.5

n=10; Results are expressed as mean ± S.D. Analysed using Dunnett's test
Negative control = untreated rats. Positive control = treated with distilled water only.

Table 3. The effect of methanol extract of *O. stamineus* on the relative organ weight and lethality in normal young female SD rats

Dose of *O.s* (g/kg body weight)	Relative organ weight (g/100g body weight)			
	Liver	**Heart**	**Kidney**	**Lung**
Negative control	2.60±0.08	0.30±0.01	0.46±0.02	0.52±0.01
Positive control	2.61±0.10	0.30±0.02	0.46±0.02	0.51±0.03
0.5	2.64±0.08	0.31±0.02	0.47±0.03	0.52±0.02
1.0	2.68±0.06	0.32±0.02	0.48±0.04	0.54±0.01
3.0	2.86±0.07*	0.34±0.01	0.49±0.05	0.55±0.03
5.0	2.94±0.10*	0.32±0.02	0.49±0.04	0.54±0.02

Dose of *O.s* (g/kg body weight)	Spleen	% Lethality
Negative control	0.32±0.01	0/10
Positive control	0.32±0.01	0/10
0.5	0.31±0.01	0/10
1.0	0.30±0.02	0/10
3.0	0.33±0.01	0/10
5.0	0.33±0.02	0/10

n=5; Results are expressed as mean ± S.D.
Analysed using Dunnett's test; * P<0.05 as compared to positive control
Negative control = untreated rats. Positive control = treated with distilled water only.

Table 4. The effect of methanol extract of *O. stamineus* on body weight, water intake and food consumption in normal young female SD rats after fourteen days recovery period

Dose of *O.s* (g /kg body weight)	Body weight (g)		Food consumption (g/rat/day)	
	7th day	**14th day**	**7th day**	**14th day**
Negative control	130.8±3.1	136.8±2.8	17.0±0.5	19.8±0.6
Positive control	127.0±2.1	134.0±2.0	16.3±1.0	19.0±0.8
0.5	126.8±2.6	133.2±2.3	16.0±0.3	20.0±0.3
1.0	128.6±1.7	136.7±2.0	17.2±0.6	20.4±1.0
3.0	125.8±3.1	133.8±2.5	15.6±0.8	18.8±1.0
5.0	127.1±2.5	137.2±2.4	17.4±1.2	19.0±1.0

	Water intake (ml/rat/day)		Lethality
Negative control	18.0±1.2	21.2±0.8	0/5
Positive control	18.4±0.6	22.0±0.4	0/5
0.5	19.0±0.8	22.4±0.8	0/5
1.0	19.2±1.0	23.0±1.1	0/5
3.0	18.8±0.4	21.6±0.6	0/5
5.0	18.0±0.4	21.8±0.6	0/5

	Relative organ weight (g/100g body weight)			
	Liver	**Heart**	**Kidney**	**Lung**
Negative control	2.76±0.06	0.34±0.02	0.50±0.04	0.52±0.01
Positive control	2.74±0.05	0.32±0.02	0.48±0.02	0.51±0.01
0.5	2.68±0.04	0.36±0.03	0.47±0.04	0.50±0.01
1.0	2.72±0.03	0.32±0.02	0.46±0.04	0.53±0.01
3.0	2.79±0.06	0.33±0.03	0.48±0.03	0.52±0.01
5.0	2.75±0.03	0.34±0.01	0.49±0.01	0.53±0.01

n=5; Results are expressed as mean ± S.D
Analysed using Dunnett's test; * P<0.05 as compared to positive control
Negative control = untreated rats. Positive control = treated with distilled water only.

decrease in serum AST & ALT levels showed beneficial effects to the respective organs rather than adverse effects. Therefore, fourteen days treatment of methanol extract of *O. stamineus* until 5 g/kg could be the no-observable adverse effect level (NOAEL) value for normal young female SD rats. Interestingly, among the tested dose of methanol extract of *O. stamineus* either one day or repeated adminstration on normal young female SD rats, only at dose level 0.5 g/kg of methanol extract of *O. stamineus* did not produce any significant elevation in serum biochemical analyses, relative organ weight, necropsy findings and even on the body weight, food consumption and water intake. Hence, 0.5 g/kg of methanol extract of *O. stamineus* could be determined as no-observable effect level (NOEL). However, further investigation such as histological and morphological experiments need to be carried out to confirm the chronic effect of methanol extract of *O. stamineus*.

Serum urea and creatinine were examined as indicators for kidney function tests (Williams, 1999) while lipid metabolism profiles were mainly represented by serum cholesterol and triacylglycerol (Alpers *et al.*, 1993). Based on the results obtained after analysing serum urea, creatinine, total cholesterol and triacylglycerol, it has demonstrated that repeated administration of methanol extract of *O. stamineus* had no direct adverse effect on kidney function and also lipid metabolism in normal young female SD rats.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are the most widely used markers for measuring hepatocellular injury (Bürger *et al.*, 2005). Other parameters such as alkaline phosphatase (ALP) and γ-glutamyl transaminase (GGT) are also useful in diagnosing hepatobiliary diseases (Evans, 1996). In general, liver damage can be divided into direct destruction of hepatocytes or impairment of bile flow. In the early stage of liver damage, cytoplasmic enzymes in hepatocytes may leak from cells into blood whose membrane permeability has been increased (Sallie *et al.*, 1991). Liver damage often leads to fat accumulation in hepatocytes (Lombardi, 1966). However, based on our results, the increase in the relative liver weight and associated with the decrease in serum liver function tests such as ALP and AST was not in agreement with the general liver damage action by hepatotoxin (Klaunig & Kolaja, 1998). This observation could indicate that liver function is protected by oral administration of *O. stamineus*. Liver growth is frequently encountered in laboratory animals exposed to drugs or environmental pollutants (Schulte-Hermann, 1979). The increase of macromolecules protein synthesis within hepatocytes and the proliferation of smooth endoplasmic reticulum could result in the increase of the liver weight. According to Schulte-Hermann (1979), the increase in liver mass in short-term experiments cannot usually be attributed to pathologic or regenerative changes but appears to be due to a combination of hypertrophy and hyperplasia as shown by increase of total DNA content, parenchymal DNA synthesis and mitotic activity. Enhancement of the activity of enzymes which degrade drugs or other lipophilic substrate is the alteration most frequently encountered (Schulte-Hermann, 1974). This was supported by our previous findings in which the oral administration of methanol extract of *O. stamineus* was able to increase phase II metabolizing enzyme, UDP-glucoronosyltransferase (UGT) activity in rat liver microsomes (Chin *et al.*, 2005). Relative liver weight in normal young female SD rats that showed increment when continuously fed with methanol extract of *O. stamineus* was abolished during recovery period. Hence, the effect of methanol extract of *O. stamineus* on rat liver is reversible. The doses examined throughout this study were several times higher than those used in other pharmacological studies of *O. stamineus* such as decrease of blood glucose levels in streptozotocin-induced diabetic rats (0.2-1 g/kg) (Sriplang *et al.*, 2007) and antioxidant activity in rats (0.125, 0.25, 0.5 and 1 g/kg of leaf extract) (Yam *et al.*, 2007). Our study demonstrated that methanol extract of *O. stamineus* seems to be destitute of toxic effects which could be compromised the

medicinal use of this plant in herbal medicine. Methanol extract of *O. stamineus* in doses from 0.5, 1.0, 3.0 and 5 g/kg is safe to be used in young female SD rats. Fourteen days oral administration of methanol extract of *O. stamineus* in rats did not produce any death or cause any adverse effects on body weight, food consumption, water intake and relative organ weight.

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