SUB-ACUTE ORAL TOXICITY STUDY OF *Orthosiphon stamineus* (LAMIACEAE) IN STZ-INDUCED DIABETIC RATS

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Summary

Orthosiphon stamineus is commonly used by Malaysians for the treatment of various diseases such as diabetes mellitus and hypertension. The objective of the present study was to evaluate the possible oral toxicity of 14 days repeated oral administration of methanol leave extract of *O. stamineus* in streptozotocin (STZ)-induced diabetic Sprague Dawley (SD) male rats. Healthy male rats weighing 200 ± 20g body weight were used in this study. Each treatment group consisted of five animals (n=5) and fed with the plant extract for consecutive fourteen days *via* oral. Control group was treated with distilled water as vehicle. Three doses of methanol leave extract of *O. stamineus*, i.e. 5 mg/kg, 125 mg/kg and 500 mg/kg were tested. Blood samples were collected through cardiac puncture and the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine were determined. Body weight changes, food intake and water consumption were measured at day-3, day-7

and day-14. From the results obtained, all the serum biochemical parameters obtained the treatment groups showed insignificant difference when compared to their respective control group. No any toxic signs and lethality were seen in all treatment groups. A significant increase in the body weight was observed in all *O. stamineus* treatment groups during experimental duration when compared to the control. In conclusion, fourteen days administration of *O. stamineus* did not affect the liver and kidney functions in diabetic male rats. The *O. stamineus* extract was safe to be consumed without causing any adverse effect.

Keywords: Diabetic, LD_{50}, *O. stamineus*, Serum Biochemical Parameters.

Introduction

Orthosiphon stamineus Bth. or commonly known as Cat whiskers belongs to the family of Lamiaceae (1). This plant is easily found along the roadside or garden for landscaping purpose. It has beautiful white flowers arranged in a terminal raceme. The leaves of this plant are believed to have antioxidant property and able to promote health. The leaves of this plant are commonly used to prepare herbal tea. It is believed beneficial for the treatment in diabetes mellitus, hepatitis, hypertension, rheumatism and urinary tract disorder (2-4).

Many scientific studies have been reported on the *Orthosiphon stamineus* using normal experimental rats. Previously, a few studies have reported the safe use of methanol leave extract of *O. stamineus* up to 5 g/kg in normal young rats (5-6). However, the diabetic animals are rarely used to evaluate the pharmacological activities and toxicity studies. Diabetes mellitus is one of the biological factors that believe to affect the pharmacokinetic and pharmacodynamic profiles of ingested test substance. To our best knowledge, there has been no any scientific report regarding the oral toxicity of *O. stamineus* leave extract in diabetic rats. This attracts our interest to further evaluate the possible oral toxicity of methanol leave extract of

O. stamineus using diabetic male rats. This study provides essential information to other researchers for better understanding about the impact of diabetes mellitus on the oral toxicity of *O. stamineus* in rats. In order to examine the possible toxic effect of *O. stamineus* on liver and kidney functions in STZ-induced diabetic rats, the serum biochemical liver and kidney parameters were determined after 14 days oral administration. Second objective was to determine the lethal dose, LD_{50} of *O. stamineus* in diabetic rats.

Materials and Methods

Chemicals: Streptozotocin was supplied from Sigma-Aldrich, St. Louis, MO, USA. Methanol, Sodium chloride (NaCl) was purchased from R & M Chemicals, UK.

Extraction of Plant Materials: Methanol leaves extract of *O. stamineus* was supplied from the Department of Pharmaceutical Chemistry, Universiti Sains Malaysia. The plant was extracted according to the method described by Akowuah *et al* (2004) (7). After the solvent was removed under reduced pressure, portion of the concentrated extract was spray-dried.

Selection of Experimental Animals: A total of 20 male Sprague Dawley (SD) rats bred in the Animal House Unit were used. SD male rats weighing 200±20 g were selected for the study. They were housed in the standard condition at 25^{o}C with 12-hour light: 12-hour dark cycle. Food pallet and tap water were provided *ad libitium*.

Fourteen Days Oral Toxicity Study: After one week of acclimatization, a dose of 45 mg/kg of streptozotocin (STZ) was intravenously injected to the rat *via* tail's vein. Those rats with fasting blood glucose level above 13.5 mmol/L post-72-hour of STZ injection were selected for the oral toxicity study. Fixed dose procedure according to Organization for Economic Cooperation and Development (OECD) guideline 420, (2001d) was followed in this oral toxicity study (8). All diabetic rats were divided into four group with five animals per group (n=5).

First group of rats served as control group, treated with a single dose daily of distilled water while second, third and fourth treatment groups were orally fed with single dose daily of 5 mg/kg, 125 mg/kg and 500 mg/kg of methanol leave extract of *O. stamineus*, respectively for consecutive 14 days. Cage-side observation was conducted for first four hours after each treatment. All animals were overnight fasted after the last dose treatment. At day-15, blood samples of rats were collected through cardiac puncture. Serum samples were sent to the pathology laboratory for biochemical parameter analyses. Several parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine were measured.

Statistical Analysis: All data were presented as mean ± standard deviation and analysed using Dunnett's Test. $P<0.05$ was considered significant difference when compared to the control group.

Results

Oral administration of methanol leaves extract of *O. stamineus* up to 14 days significantly ($P<0.05$) prevented the body weight reduction in all diabetic rats (Figure 1). No significant change in the serum AST, ALT, ALP, urea and creatinine was observed between control group and *O. stamineus* treatment groups. Toxic signs and lethality was not seen in all treatment groups during the study.

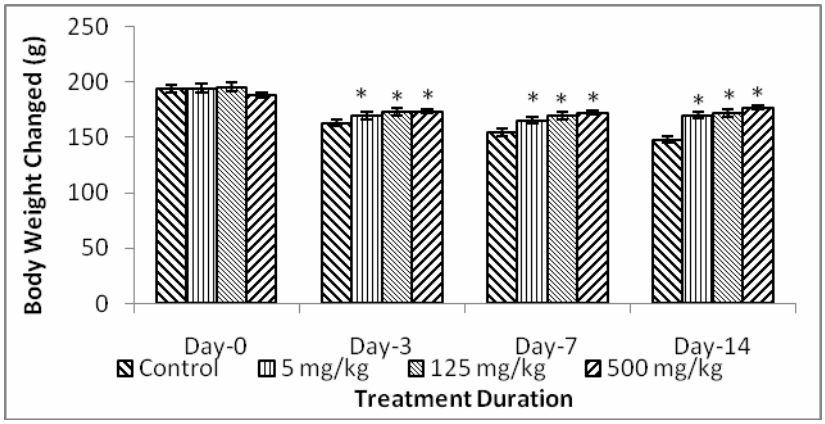

Figure 1. Effect of *O. stamineus* on Body weight in STZ-induced diabetic rats
N=5; Value=mean ± S.D; Analysed using Dunnett's Test;
*P<0.05 significant difference as compared to the control group.

Table 1. Effect of Methanol Leaves Extract of *Orthosiphon stamineus* (*O. s*) on Liver and Kidney Profiles in STZ-induced Diabetic Rats

	Control	5 mg/kg *O.s*	125 mg/kg *O. s*	500 mg/kg *O. s*
Liver Profile				
AST (U/L)	149.0±17.02	151.1 ± 56.93	132.6 ± 18.45	130.4 ± 15.34
ALT (U/L)	64.8 ± 9.83	70.2 ± 14.73	55.6 ± 6.43	50.2 ± 9.20
ALP (U/L)	112.4 ± 20.96	118.8 ± 39.56	136.4 ± 42.21	101.0 ± 27.61
Kidney Profile				
Urea (mmol/L)	37.8 ± 5.76	40.2 ± 3.56	38.0 ± 10.51	36.8 ± 5.76
Creatinine (μmol/L)	0.72 ± 0.08	0.76±0.1	0.72 ± 0.08	0.64 ± 0.13

N=5; Data = Mean ± standard deviation; Analysed using Dunnett's Test.

According to the World Health Organization (WHO), Malaysia is expected to have a total number of 2.48 millions diabetic patients in 2030 as compared to 0.94 million in 2000 (9). One of the reasons that *O. stamineus* gained high popularity among Malaysians was its high medicinal value towards diabetes mellitus. Based on the literature search, the leaves of *O. stamineus* contained high amount of highly oxygenated isopimarane-type diterpenes, phenolic compounds, rosmarinic acid and flavonoids (10). Phenolic compounds and flavonoids have been reported to possess anti-diabetic activity (11).

A variety of blood biochemical parameters can be used to examine the toxicity response of test substance. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are commonly used as marker to diagnose hepatocellular injury while alkaline phosphatase (ALP) is widely used for measuring hepatobiliary disease (12-13). Kidney functions could be diagnosed using serum urea and creatinine (14). An induction of these parameters in blood is commonly associated to the damages to the liver or kidney. From the serum biochemical analyses, 14 days repeated administration of 500 mg/kg of *O. stamineus* to diabetic rats was not causing any toxic effect to kidney and liver functions. The LD_{50} and no-observed-adverse-effect level (NOAEL) of *O. stamineus* was greater than 500 mg/kg body weight in diabetic rats because no lethality and adverse effect were observed during the 14 days oral administration period (15). These findings were similar to the oral toxicity using normal rats as reported in our previous study (5). In addition to that, the serum liver profile in rats treated with 500 mg/kg of *O. stamineus* was lower than the control group although insignificant results were shown. Lower serum liver profile indicates the possibility of liver protection rather than liver damaging. This information provides scientific data to prove the safe use of *O. stamineus* in diabetic animals. According to the WHO, fourteen days oral toxicity testing using experimental animals is equivalent to the expected clinical usage in human for single dose or repeated dose below one week (16). Acceptable daily intake (ADI) is calculated as NOAEL divide safety factor which is 100 (4). Hence, the ADI for the methanol

leave extract of *O. stamineus* tested in the present study is 500 mg/kg / 100 = 5 mg/kg body weight/day/lifetime.

According to Barrow (2000), rats weighing 0.15 kg are six times more efficient than humans weighing 60 kg to handle toxic effect (17). Thus, 500 mg/kg tested in rats the present study is equivalent to 83.3 mg/kg in humans. Hence, a 60 kg person is expected to consume 4998 mg (83.3 mg/kg X 60 kg) or approximately 5 g to have the same effect as seen in rats.

The reduction of body weight in control diabetic rats could be explained through the disruption of pancreatic islet cells by STZ. High blood glucose level increases the osmotic pressure which resulting large amount of urine to be excreted. This condition is called glycouria. Thirst and hunger are compensatory responses to the loss of fluid and the inability to utilize nutrients (18). However, the body weight of all *O. stamineus* treatment groups was gradually increased at day-3, day-7 and day-14 compared to control group. The prevention of body weight decrease in all treatment groups could be beneficial to the treatment of diabetes mellitus. *O. stamineus* aqueous extract has been reported to be effective for alleviating hyperglycaemic by reducing the plasma glucose level in diabetic rats (19-20). The mechanism that responsible for the anti-hyperglycaemic of *O. stamineus* was the direct stimulation of insulin secretion from the pancreatic beta cells (20).

Conclusion

LD_{50} of the methanol leave extract of *O. stamineus* could not be determined in diabetic rats but it was suggested to be higher than 500 mg/kg in the present study. Fourteen days oral administration of *O. stamineus* showed no toxic effects either on liver or kidney functions in STZ-induced diabetic rats. Further study needs to be carried out to confirm the long term of *O. stamineus* in diabetic rats.

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References

1. Suresh A, Yasuhiro T, Arjun H, Banskota S, Shimoji KT, Shigetoshi K. Norstamine and isopimarane-type diterpenes of *O. stamineus* from Okinawa. Tetrahedron 2002; 58: 5503-12.
2. Wiart C. *Orthosiphon stamineus* Benth. In: Wong FK, ed. Medicinal Plants of Southeast Asia, Kuala Lumpur: Prentice Hall, 2002: 265.
3. Eisai PT. Indonesia Medicinal Herb index in Indonesia, 2nd ed. Godjah Mada University Press, 1995.
4. WHO (World Health Organization). Principles for the safety assessment of food additives and contaminations in food. Vol. 70. IPCS Environmental Health Criteria, 1987: 39-59.
5. Chin JH, Hussin AH, Ismail S. Toxicity study of *Orthosiphon stamineus* Benth (Misai Kucing) on Sprague Dawley rats. Tropical Biomedicine 2008; 25: 9-16.
6. Abdullah NR, Ismail Z, Ismail Z. Acute toxicity of *Orthosiphon stamineus* Benth Standardised Extract in Sprague Dawley rats. Phytomedecine 2009; 16: 222-6.
7. Akowuah GA, Zhari I, Norhayati I, Sadikun A, Khamsah SM. Sinensitin, eupatorin, 3'-hydroxy-5,6,7,4'-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of *Orthosiphon stamineus* from Malaysia. Food Chemistry 2004; 82: 559-66.
8. OECD (Organization for Economic Cooperation and Development). (2001d) OECD Test Guideline 410: Acute Oral Toxicity-Fixed dose method. Organization for Economic Cooperation and Development, Paris.
9. Mafauzy M. Diabetes mellitus in Malaysia. [Homepage of the internet]. [Cited 2007 June 21]. From: www.mma.org.my/mjm/4_diabetes_06.htm.
10. Olah NK, Radu L, Mogosan C, Hanganu D, Gocan S. Phytochemical and pharmacological studies on *Orthosiphon stamineus* Benth. (Lamiaceae) hydroalcoholic extracts. Journal of Pharmaceutical and Biomedical Analysis 2003; 33: 117-23.

11. Hsu FL, Chen YC, Cheng JT. Caffeic acid as active principles from the fruit of Xanthium strumanium to lower plasma glucose in diabetic rats. Planta medica 2000; 66: 228-30.

12. Burger C, Fischer DR, Cordenuzzi DA, Batschauer APB, Filho VC, Soares ARS. Acute and subacute toxicity of the hydroalcoholic extract from Wedelia paludosa (Acmela brasiliensis) (Asteraceae) in mice. Journal Pharmaceutical Sciences 2005; 8(2): 370-3.

13. Evans GO. General introduction. In: Evans GO, ed. Animal Clinical Chemistry. London: Tylor & Francis, 1996:1-10.

14. Tortora GJ, Derrickson B. Principles of anatomy and physiology. 11th ed. USA: John Wiley and Sons, Inc., 2006.

15. Copplestone JF. The development of the WHO recommended classification of pesticides by hazard. Bulletin of the World Health Organization 1988; 66: 545-51.

16. Zhang X. General guideline for methodologies on research and evaluation of traditional medicine. Geneva: World Health Organisation (WHO), 2000.

17. Barrow P. Reproduction and development of toxicology safety studies. In: Krinke GJ, ed. The Handbook of Experimental Animals: the laboratory rats. United Kingdom: Academic Press, 2000: 11-27.

18. Lawrence JC. Insulin and oral hypoglycemic agents. In: Brondy TM, Larner J, Minneman KP, eds. Human Pharmacology Molecular to Clinical. Missouri: Mosby-Year Book, 1998: 541-3.

19. Mariam A, Asmawi MZ, Sadikun A. Hypoglycemic activity of the aqueous extract of *Orthosiphon stamineus*. Fitoterapia 1996; 67: 465-8.

20. Sriplang K, Adisakwattana S, Rungsipipat A, Yibchok-Anun S. Effects of *Orthosiphon stamineus* Aqueous extract on plasma glucose concentration and lipid profile in normal and streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 2007; 109(3): 510-4.