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p-Nitrophenol UDP-Glucuronosyltransferase Activity in Liver Microsome from Sprague Dawley Rats Fed with Methanol Extract of *Orthosiphon stamineus* (Misai Kucing)

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ABSTRACT This *in-vivo* (acute) study involved the use of twenty five normal old male Sprague Dawley rats (>52 weeks) weighing 350±50 g and each group (n=5) administered orally with a single dose of methanol extract of *Orthosiphon stamineus* (Misai Kucing) ranging from 5 mg/kg, 31.25 mg/kg, 125 mg/kg to 500 mg/kg. Controls were treated with the respective vehicles through the same route under similar conditions. After 24 hour of dose administration, rats were sacrificed. Microsomes were prepared by calcium precipitation method [1] and used for UDP-glucuronosyltransferase (UGT) activity study towards *p*-nitrophenol (*p*NP). The protein levels of microsomes were measured by the method of Lowry *et al* [2]. Results were expressed as nmol/min/mg protein and analyzed by Dunnett Test. The results showed that methanol extract of Misai Kucing increases *p*NP UGT activity significantly especially at 31.25 mg/kg ($P<0.01$), 125 mg/kg ($P<0.01$) and 500 mg/kg ($P<0.01$).

Keywords: *Orthosiphon stamineus*, microsome, UDP-glucuronosyltransferase

ABSTRAK Dalam kajian in-vivo (akut) ini, dua puluh lima ekor tikus jantan tua Sprague Dawley (>52 minggu) yang berat badannya 350±50 g dan setiap kumpulan (n=5) diadministrasikan secara oral dos tunggal ekstrak methanol *Orthosiphon stamineus* (Misai Kucing) berjulat daripada 5 mg/kg, 31.25 mg/kg, 125 mg/kg dan 500 mg/kg. Kumpulan kawalan dirawat dengan cara penyampaian dos yang sama dalam keadaan yang serupa. Selepas 24 jam administrasi, tikus akan dikorbankan. Mikrosom akan disediakan dengan kaedah pemendakan kalsium [1] dan akan digunakan untuk mengkaji aktiviti UDP-glukuronosiltransferase (UGT) terhadap *p*-nitrofenol (*p*NP). Tahap protein di dalam mikrosom akan ditentukan dengan kaedah Lowry *et al* [2]. Keputusan dinyatakan sebagai nmol/min/mg protein dan dianalisis dengan Ujian Dunnett. Keputusan kajian menunjukkan ekstrak methanol Misai Kucing telah meningkatkan aktiviti *p*NP UGT secara signifikan terutamanya pada kepekatan 31.25 mg/kg ($P<0.01$), 125 mg/kg ($P<0.01$) and 500 mg/kg ($P<0.01$).

INTRODUCTION

Orthosiphon stamineus Benth classified under family Lamiaceae (or Labiatea), is a herbaceous shrub native to tropical eastern Asia. It has purple flowers with long stamens, expanding and shaped like cat's whiskers. *O. stamineus* is believed to have diuretic properties and is used as remedy for kidney stones, diabetes and gout [3].

The methanol extract of *O. stamineus* had also been shown to exhibit cytotoxic activity against a highly liver metastatic colon carcinoma cells [4]. A mechanism that may be responsible for the anticarcinogenic potency of *O. stamineus* extract may be related to the modulation of drug metabolising enzymes involved in carcinogen activation and detoxification.

In general, drug metabolism or biotransformations can be divided into two phases: phase I and phase II, although the products of phase II may be further metabolised in what is sometimes called phase III reactions [5]. The chemical substances, drug, xenobiotic and endogenous compound undergo several types of phase I (functioning) and phase II (conjugation) reactions before changing into hydrophilic products, more suitable for excretion. Glucuronidation, catalyzed by UGT represents a major phase II metabolism reaction involved in the biotransformation of a wide variety of drugs,

environmental chemicals and numerous endogenous compounds [6].

The aim of this study is to assess the possibility of *O. stamineus* affecting the activity of a phase II drug metabolising enzyme, UDP-glucuronosyltransferase or UGT. *p*NP is chosen as the substrate to determine the activity of UGT under the influence of *O. stamineus* methanol extract.

MATERIALS AND METHODS

Materials

All the chemicals used were of standard analytical purity grade. Uridine diphosphate glucuronic acid (UDPGA) and *p*-nitrophenol (*p*NP) were supplied by Sigma Co, St Louis, MO, USA. *O. staminues* methanol extract (in powder form) was prepared by Professor Zhari Ismail from the School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang.

Animal

Sprague Dawley (SD) rats were bred in animal house, School of Pharmaceutical Sciences, Universiti Sains Malaysia. All rats were kept under normal condition and had free access to tap water. Normal old male SD rats (52-55 weeks old) (350±50 g) were used throughout this study. SD rats were divided into five groups and each group involved five rats (n=5). Control group was given distilled water as a vehicle while second, third, fourth and fifth group were given orally single dose of methanol extract of *O. stamineus*, 5 mg/kg, 31.25 mg/kg, 125 mg/kg and 500 mg/kg respectively. The rats were sacrificed 24 hours after *O. stamineus* administration and the livers were taken out for microsome preparation.

Microsome Preparation

Microsomes were prepared using Calcium Precipitation technique [1]. Microsomal protein concentration was determined by the method of Lowry [2]. The microsome was kept frozen at -80°C with 20% (v/v) glycerol added.

Enzyme Assay

UGT activity towards *p*-nitrophenol (*p*NP), a prototype planar phenolic substrate, was assayed following slight modifications of the method by previous workers [7, 8, 9].

The absorbance was read at 405 nm on a Powerwave X340® microplate reader. The absorbance difference between the control sample (without *O. stamineus* treatment) and the sample incubated in the presence of *O. stamineus* represents the difference amount of *p*NP consumed through glucuronide formation.

Statistical Analysis

Results were expressed as μmol *p*NP conjugated/min/mg protein and as percentages of *p*NP-UGT activity as compared with the control. Means and standard deviations were calculated and the comparison between the means of treated group and the control group was done using the Dunnett test at a significant level of $P<0.05$ and $P<0.01$.

RESULTS AND DISCUSSION

Methanol extract of *O. stamineus* had no effect on rat's body weight as compared with the control group (data not shown). In addition, methanol extract of *O. stamineus* did not cause any significant change in the ratio of liver weight to rat's body weight as compared with the control group (Table 1).

In this study, methanol extract of *O. stamineus* caused a significant increase in *p*NP-UGT activity in the liver as compared to the control. Methanol extract of *O. stamineus* at 31.25 mg/kg, 125 mg/kg and 500 mg/kg showed significant increase in *p*NP-UGT activity of 71.3%, 142.6% and 175.4% respectively (Table 2).

Drug metabolising enzymes like cytochrome P450, UGT and glutathione S-transferase (GST) are known to be responsive to the inductive and inhibitory effects of many endogenous and exogenous factors, such as hormone, growth factor and nutrition. Induction and/or modulation of the activity of these enzymes may change the pharmacological and toxicological effects of xenobiotics in human and result in serious drug-drug interactions.

This finding demonstrates that methanol extract of *O. stamineus* can alter the activity of UGT, a major phase II drug metabolising enzyme. It suggests that there is a possibility for *O. stamineus* to affect the glucuronidation pathway. It is however difficult to extrapolate this information directly from rat to human due to species differences. Further subchronic or chronic studies have to be continued to investigate the long term effect of methanol extract of *O. stamineus* on liver metabolising enzymes.

Table 1. Effect of Orthosiphon stamineus methanol extract on relative liver weight.

Dose	Liver weight (g/g body weight)
Control	0.024±0.0033
5 mg/kg	0.022±0.0025
31.25 mg/kg	0.022±0.0026
125 mg/kg	0.028±0.0050
500 mg/kg	0.028±0.0042

Value = mean ± S.D; n=5
* P<0.05 against control
** P<0.01 against control

Table 2. Effect of Orthosiphon stamineus methanol extract on pNP-UGT activity in rats liver

Dose	μmol *p*NP consumed/min/ mg protein	% *p*NP-UGT activity
Control	30.5±1.6	100±5.4
5 mg/kg	27.1±2.2	89.1±8.1
31.25 mg/kg	**52.2±6.9****	**171.3±13.2****
125 mg/kg	**73.9±6.8****	**242.6±9.2****
500 mg/kg	**83.9±8.6****	**275.4±10.3****

Value = mean ± S.D, n=5
* P<0.05 against control
* P<0.05 against control

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REFERENCES

1. Gibson, G. & Skett, P. (1994). *Introduction to drug metabolism.* 2nd ed. United Kingdom: Blackie Academic & Professional.
2. Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). *Journal of Biological Chemistry* **193**, 265-275.
3. Indubala, J. & Ng, L.T. (2000) *Herbs: The Green Pharmacy of Malaysia.* Kuala Lumpur: Vinpress Sdn. Bhd.
4. Ohnishi, Y. *et al.* (1997). *Tumor Biol* **18**, 113-122.
5. Timbrell, J. (2000) *Principles of Biochemical Toxicology.* 3rded. United kingdom: T.J. International Ltd.
6. Embola, C.W., Sohn, O.S., Fiala, E.S. & Weisburger, J.H. (2002). *Food and Chemical Toxicology* **40**, 841-844.
7. Bock *et al.* (1983). *Biochemical Pharmacology* **32**, 953-955.
8. Guo *et al.* (1992). *Carcinogenesis* **13**, 2205-2210.
9. Isselbacher, K.J., Chrabas, M.F. & Quinn, R.C. (1962). *Journal of Chemistry* **237**, 3033-3036.