EFFECT OF *ORTHOSIPHON STAMINEUS* AND *TINOSPORA CRISPA* ON AMINOPYRINE METABOLISM IN RATS

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Summary

O. stamineus and *T.crispa* are widely used in Southeast Asia countries for treating several pathology conditions. However, the information with regard to the herb-drug interactions of *T. crispa* and *O. stamineus* extracts is still limited at present. The objective of the present study was to examine the possible herb-drug interactions of chloroform stem extract of *T. crispa* and methanol leaf extract of *O. stamineus* with hepatic aminopyrine metabolism mediated by CYP 3A4 in normal and STZ-induced diabetic female Sprague Dawley (SD) rats. The rate of hepatic aminopyrine metabolism was examined by monitoring the enzyme activity of aminopyrine *N*-demethylase using freshly isolated perfused hepatocytes. A significant increase in the aminopyrine N-demethylase activity was observed in the normal old female SD rats treated with 500 mg/kg ($P<0.05$) of *T. crispa* extract. *O. stamineus* extract did not affect the aminopyrine metabolism in SD rats. For conclusion, chloroform extract of *T. crispa* affected the metabolism of aminopyrine in aged female rats by increasing the activity of hepatic aminopyrine *N*-demethylase.

Keywords: Aminopyrine *N*-demethylase, Cytochrome P450, Hepatocytes, Herb-drug interactions.

Introduction

Orthosiphon Stamineus Benth (*Lamiaceae*), a medicinal plant native to tropical Asia, gets its common name as "cats whiskers" from its pale purple flowers with long wispy stamens shaped like cats whiskers. It is largely consumed by people as herbal tea by boiling the leaves. This plant is believed to be effective in treating urinary lithiasis, edema, eruptive fever, influenza, rheumatism, hepatitis and jaundice (1). Previously, methanol leaf extract revealed no adverse effect in male rats after treating up to 5 g/kg body weight for fourteen days, moreover it has an antioxidant property due to its polyphenolic compounds in the leaf extract (2).

Tinospora crispa Miers belongs to the family of Menispermaceae. The beneficial effect of *T. crispa* for the treatment of diabetes mellitus has been previously reported in animal models and the effect was probably due to its insulinotropic activity (3). However, the active compounds extracted from plant materials are incompletely characterised. The biological active compounds known as flavone and apigenin were identified and isolated from the stem of *T. crispa*.

Millions of people today use herbal therapies along with prescription and nonprescription medications. Although considered natural, many of these herbal therapies can interact with other medications, causing either potentially dangerous side effects and/or reduced benefits from the medications. Herb-drug interaction is also one of the key factors in causing hepatotoxicity in animals and in humans. One of the the mechanisms of pharmacokinetic interaction caused by herb-drug interaction is through the modulation of the activity of phase I and II drug metabolising enzymes in various organs. Phase I reaction is critical in the oxidative metabolism of xenobiotics mainly *via* cytochrome P450 (CYP), an enzyme family with multiple isoforms while phase II is involved in scavenging and conjugation (4). Any modulation of hepatic drug metabolising enzymes could affect the therapeutic effects of co-administrared drugs. Hence, the evaluation metabolism-based drug toxicity study is essential in preclinical safety testing to understand the adverse effects of drugs and ultimately the accurate prediction drug toxicity in humans (5).

The objectives of the present study were to examine the effect of oral administration of chloroform extract of *T. crispa* and methanol leaf extract of *O. stamineus* on the hepatic aminopyrine N-demethylase activity in female rats and to see the impact of age and diabetic condition on this herb-drug interaction study.

Materials and Methods

Chemicals: All chemicals used were of standard analytical purity grade. Aminopyrine, trypan blue, collagenase, potassium dihydrogen phosphate, streptozotocin (STZ) were supplied by Sigma Co., St Louis, MO, USA.

Preparation of plant extract

***T. crispa* chloroform stem extract:** The stems of *T. crispa* were collected from the rain forest of Balik Pulau, Penang, Malaysia. The powdered stems of *T. crispa* (weight 2 kg) were extracted continuously with several volumes (5 x 1000 mL) of methanol at 45 ^{0}C for 5 days. The combined methanol extract was filtered and then evaporated to dryness under partial vacuum. The methanol residue (46.56 g) was defatted with *n*-hexane and then partitioned sequentially with a mixture of chloroform and *n*-butanol fractions, together with the n-hexane fractions were each evaporated to dryness and their percentage yield from starting material was calculated.

***O. stamineus* methanol leaf extract:** The leaves of *O. stamineus* 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 and water respectively, using soxhlet apparatus. After the solvent was removed under reduced pressure, the concentrated extract was spray-dried.

Experimental Animals: To study the influence of age and disease condition in drug metabolism, different ages of STZ-induced diabetic rats and normal rats were selected. The animals bred in the Animal House Unit, Universiti Sains Malaysia were used througout the experiments. The rats aged 14-week old (180 g ± 10 g body weight) and 54-week old (250 g ± 20 g body weight) were considered as adult rats and old female rats respectively. All rats were fed a normal laboratory chow (Gold Coin®) *ad libitum* and had free accessed to tap water for one week before the experiment began.

Induction of Diabetes: Streptozotocin (STZ) was freshly prepared by dissolving in 0.9% of NaCl before administration. A single dose of 50 mg/kg STZ was injected intravenously to the adult and old female rats through tail's vein. The blood glucose conconcentration was measured in blood after 72-hour of STZ injection. Only the rats with blood glucose concentration higher than 15.6 mmol/L at fasting state were selected for the experiment.

Aminopyrine *N*-demethylase Assay: All animal works carried out in the present study were approved by the Animal Ethic Committee of Universiti Sains Malaysia. All treatment rats were orally treated with a single dose daily of either methanol leaf extract of *O. stamineus* (5 mg/kg, 125 mg/kg and 500 mg/kg body weight) or chloroform extract of *T. crispa* (10 mg/kg, 100 mg/kg and 500 mg/kg body weight) up to 14 days *via* gavage. The control group was orally treated with distilled water. All rats were fasted overnight and sacrificed after twenty four hours of last dose treatment. The livers were perfused using Collagenase Perfusion Technique. The viability of the isolated hepatocytes was determined using Trypan Blue exclusion method. The isolated hepatocytes were used to analysis the activity of hepatic aminopyrine *N*-demethylase in rats. The activity of hepatic aminopyrine *N*-demethylase was measured using spectrophotometer at 415 nm (6).

Statistic analysis: All results were presented as mean ± standard deviation and analysed using Dunnett test. *$P<0.05$ and ** $P<0.01$ was considered as statistical significant when compared to the control group.

Results

From the results obtained, a significant herb-drug interaction effect was only seen in those normal old female rats treated with *T. crispa*. Daily administration of 500 mg/kg ($P<0.01$) of *T. crispa* chloroform stem extract for 14 consecutive days significantly increased the aminopyrine *N*-demethylase activity in normal old female rat hepatocytes when compared to the control group (Figure 1). There was no significant difference of the aminopyrine *N*-demethylase activity between the *O. stamineus* treatment groups and the control group (Figure 2). No any significant toxic sign or lethality was observed in all *O. stamineus* or *T. crispa* extract treatment groups during the experimental duration.

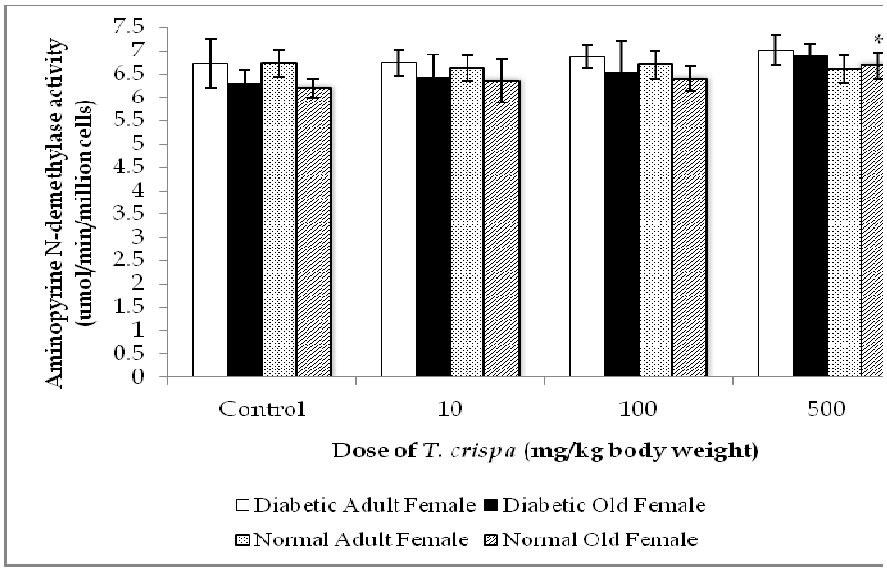

Figure 1. Effect of chloroform stem extract of *T. crispa* on the activity of aminopyrine N-demethylase in hepatocytes of SD rats. N=6; Values are presented as mean ± S.D; Results were analysed using Dunnett Test; *P<0.05 compared to control group.

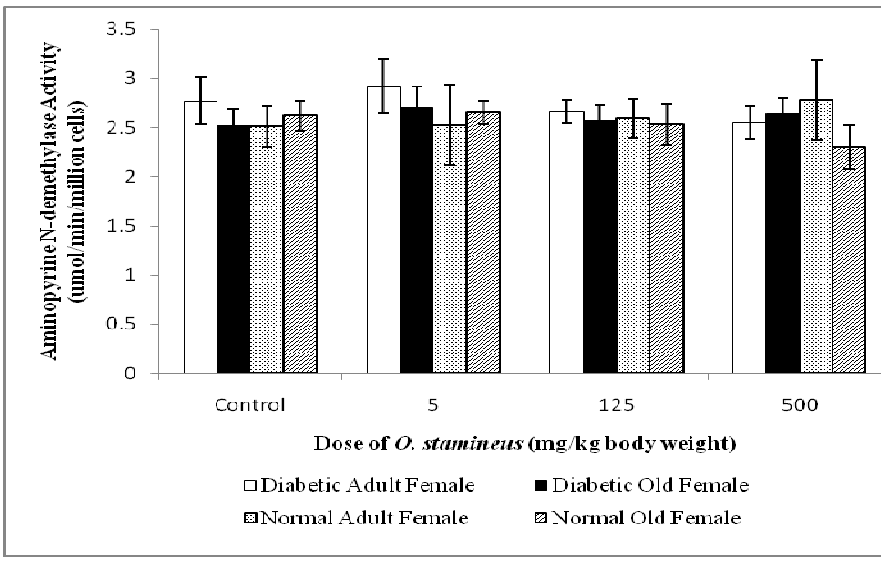

Figure 2. Effect of methanol leaves extract of *O. stamineus* on the activity of aminopyrine *N*-demethylase in hepatocytes of SD rats. N=6; Values were presented as mean ± S.D; Results were analysed using Dunnett Test.

Discussion

The cellular pathways leading to organ-specific toxicities are often ultifaceted, involving several cell types and biochemical networks of cell-cell and cell-matrix interactions. Drug bioactivation can be a key determinant of drug toxicity. The present study aimed to examine the modulation of hepatic cytochrome P450 system in causing herb-drug interaction. Aminopyrine ($C_{13}H_{17}N_3O$) is used as probe substrate to examine activity of CYP 3A in animal model. Aminopyrine is an analgesic and antipyretic drug with causes several adverse effects in humans (7). Aminopyrine is mainly *N*-demethylated by CYP 3A and 2B in the liver by initial hydroxylation at the α-carbon atom and subsequent breakdown of the carbinolamine intermediate yielding formaldehyde and monomethyl-antipyrene (8). CYP 3A is one of the cytochrome P450 of phase I enzymes, that is most abundant in the liver than other organs. More than 50% of the clinically used drugs by humans are metabolised by CYP 3A4 (9).

Until today, limited research studies on herb-drug interactions are conducted using diabetic animals, moreover in aged female rats. The present study provides important information about the influence of age and diabetic condition on herb-drug interactions of *O. stamineus* and *T. crispa* in animals. Several biological factors such as age and diabetic are reported to affect the expression and the activity of hepatic phase I and phase II drug metabolising enzymes in animals and in man. Based on the results obtained, there was no significant interaction between *O. stamineus* and CYP 3A-mediated metabolism of aminopyrine in the livers of normal and STZ-induced diabetic adult and old female SD rats. The findings are similar with the *in vitro* study of *O. stamineus* reported earlier (10). The influence of age and diabetic factor in affecting the herb-drug interaction of *O. stamineus* with hepatic aminopyrine metabolism could be ruled out. Rosmarinic acids, sinensitin and eupatorin are the major active ingredients in the methanol extract of *O. stamineus*. These ingredients are known to induce the CYP P450 system. However, this effect was not seen in those rats fed with *O. stamineus* extract. This could be explained through the poor absorption and distribution of *O. stamineus* extract in experimental animals. Rosmaric acids, sinensitin and eupatorin are reported to have poor absorption in gastrointestinal tract in rats and typically have poor miscible with oils and lipids (11-12). The amounts of these ingredients that enter to the

hepatic system are not sufficient to induce the activity of aminopyrine N-demethylase in rat hepatocytes.

On the other hand, caution should be focused to the *T. crispa* extract treatment especially involved those aged female SD rats. Results have demonstrated that *T. crispa* extract could increase the hepatic metabolism of aminopyrine mediated by CYP3A in rats. The active compounds that responsible for this interaction remain unknown and need to be elucidated. Increased activity of aminopyrine *N*-demethylase is able to cause pharmacokinetic interaction. This condition reduces the bioavailability of aminopyrine in blood circulation due to higher metabolism rate in hepatocytes. Therefore, precaution should be given to other drugs such as erythromycin, morphine, midazolam which are mainly metabolised by CYP 3A4 (13). They may have the same possibility to interact with *T. crispa* if both are consumed simultaneously. As mentioned by Gibson and Skett (1994), an increase in CYP P450 activity may arise by three possible mechanisms: the synthesis of new protein, the conversion of an inactive protein to an active enzyme and changes in activity with enzyme phosphorylation (8). To our knowledge, the pathway metabolism and pharmacokinetic characteristics of *T. crispa* and *O. stamineus* extracts have not been fully investigated. A more extensive work needs to be carried out to confirm the possible mechanisms.

The present *in vivo* findings are not accordance with the *in vitro* study that have suggested the inhibitory effect of *T. crispa* on CYP 3A4 with IC_{50} of 428 μg/ml (15). It is suggested that the difference between *in vitro* and *in vivo* effect of chloroform extract of *T. crispa* on hepatic drug metabolising enzyme activity is dependent on the pharmacokinetic profile such as absorption of chloroform extract of *T. crispa* and the duration of treatment. Theoretically, the effect seen in the *in vitro* study could be reproducible in the *in vivo* system if same total amount of chloroform extract of *T. crispa* enters hepatic system. However, this ideal concept is not achievable. The absorption and distribution of the chloroform extract of *T. crispa* may be varied from one species to another species. Some of the hepatic metabolising enzyme changes could be seen rapidly and other hepatic enzyme may require more time for induction and metabolism of the drug. Prolonging the treatment duration could increase the amount of

chloroform extract of *T. crispa* to the hepatic system and may elicit similar responses as in the high dose during *in vitro* study.

There are some documented reports to interpret data from one species to another according to their life span, metabolic rate, and total body surface area (16). The equivalent surface area of the rat's body weight with 150 g to human of 60 kg is about 1/6. Therefore, to convert a dose of 500 mg/kg in the rat to an equivalent dose in human and assuming equivalence on the basic of body weight/surface area, multiply 500 mg/kg X 1/6 = 83.3 mg/kg in human. This information is important for scientist to estimate the possible toxic effects occur in another species.

Conclusion

Chloroform stem extract of *T. crispa* increased the metabolism of aminopyrine in female rat hepatocytes by increasing the activity of aminopyrine N-demethylase.

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