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INDUCTION OF HEPATIC GLUTATHIONE-S-TRANSFERASE ACTIVITY BY *Orthosiphon stamineus,* BENTH IN STZ-INDUCED DIABETIC RATS

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The aim of this study was to investigate the acute (one-day treatment) effect of a methanol extract of Orthosiphon stamineus, *Benth on glutathione-S-transferase (GST) activity in streptozotocin (STZ)-induced diabetic young male and female Sprague Dawley (SD) rats. The methanol extract of* O. stamineus *was administered orally (5, 31.25, 125 and 500 mg/kg) to diabetic rats, and the effect on GST activity was measured by the method of Habig et al. (1974). No lethality and no significant changes in body weight and water intake were observed in the treated group as compared to the control group. A significant increase in the activity of GST was observed in the liver S-9 cytosolic fraction of diabetic male SD rats treated with 125 mg/kg ($P < 0.01$) and 500 mg/kg ($P < 0.01$) of the methanol extract* O. stamineus. *Administration of 500 mg/kg ($P < 0.01$) of the methanol extract of* O. stamineus *to diabetic female SD rats increased GST activity when compared to the control group. This study indicates that the methanol extract of* O. stamineus *could affect the activity of GST in rat liver and the effect seen was dose-dependent.*

Keywords: Orthosiphon stamineus, *GST, Diabetic, Liver S-9 cytosolic fraction*

INTRODUCTION

Orthosiphon stamineus, Benth, a folk-medicinal plant known as Misai Kucing in Malaysia, has been used as a remedy for diabetes mellitus, hypertension, and gout, to promote urination, and to treat bladder and kidney discomfort (Wiart 2002). A number of the active compounds in *O. stamineus,* including flavonoids, have been identified as contributors to the observed medicinal effects of the plant. Although it receives much attention from researchers, the modulatory effects of *O. stamineus* on hepatic drug metabolising enzymes has not yet been studied. It has become increasingly clear that alterations in catalytic activities of key hepatic drug metabolising enzymes are often at the heart of drug interactions leading to adverse drug reactions (ADR) (Bachmann, Ring and Wrighton 2003).

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Hepatic drug metabolising enzymes are composed of phase I and phase II enzymes. Phase I enzymes, mainly cytochrome P450, detoxify a variety of endogenous and exogenous chemicals and activate many carcinogens (Guengerich and Shimada 1991). Phase II enzymes, which include GST (E.C.2.5.1.18) and UDP-glucoronosyltransferase (UGT; E.C.2.4.1.17), catalyse the reduction or conjugation of phase I metabolites to various water-soluble molecules and accelerate the rate of metabolite excretion (Talalay *et al*. 1995). The *in vitro* or *in vivo* effects of *O. stamineus* methanol leaf extracts on aminopyrine N-demethylase and UGT activities in normal male and female SD rats have been previously reported (Chin and Hussin 2007; Chin *et al*. 2005). Hence, this study aims to investigate acute treatment (one-day treatment) with 5, 31.25, 125 or 500 mg/kg of an *O. stamineus* methanol leaf extract on another phase II detoxification enzyme known as GST in diabetic young male and female SD rats.

GST catalyses the conjugation of glutathione (GSH) with a variety of electrophilic xenobiotics and facilitates their excretion. GST is composed of six distinct gene families, including five cytosolic groups (α, μ, π, θ, and σ) and one microsomal form (κ) (van Lersel *et al*. 1999). The different gene families share a similar function, but differ in their substrate specificities. The class μ and θ GSTs are the major isoenzymes that participate in glutathione conjugation with benz[a]anthracene epoxides and polycyclic aromatic hydrocarbons (Dusinska *et al*. 2001). π GST is more effective in drug detoxification and detoxifies electrophilic α,β-unsaturated carbonyl compounds that are generated by radical reactions of lipids (Berhane *et al*. 1994).

METHODS

Chemicals

All of the chemicals used in this experiment were purchased from Sigma-Aldrich Chemical Company Inc., St. Louis, MO, USA. The chemicals were of analytical grade.

Experimental Animals

Young male and female SD rats, weighing 120 ± 10 g and 100 ± 10 g, respectively, bred in the animal housing unit of the School of Pharmaceutical Sciences, USM, were used throughout the experiments.

All animal experiments were approved by the ethical committee at USM. The animals were fed *ad libitium* with a normal laboratory pellet diet and water. Animals were kept at an environmental temperature between 25°C–27°C.

Plant Materials

Standardised methanol extracts of *O. stamineus* were supplied by Professor Zhari Ismail from the School of Pharmaceutical Sciences, USM. The leaves of the plant were collected in the late afternoon from 30–45-day-old white flowering plants. The leaves were chopped and dried at approximately 40°C for three days. A methanol extract of *O. stamineus* was prepared using a sample of 10 g of dried leaves in 100 ml of methanol by warming for 4 h at 40°C. The solution was filtered through filter paper (Whatman No. 1), concentrated and spray-dried to obtain the crude methanol extract

Induction of STZ

STZ, freshly prepared in 0.9% normal saline, was injected into experimental animals intravenously through the tail vein at a dose of 45 mg/kg body weight. Seventy-two hours after STZ administration, rats with moderate diabetes having hyperglycaemia (i.e., blood glucose more than 15.6 mmol/l) were chosen for the experiment.

Experimental Design

In the experiment, a total of 30 rats (young males and females) were used. The rats were divided into five groups. Each group consisted of six animals (n = 6). Group 1: Diabetic control rats fed orally with distilled water; the 2nd, 3rd, 4th and 5th groups of diabetic rats were fed orally with a single dose of 5, 31.25, 125 and 500 mg/kg of *O. stamineus* extract, respectively. Rats were observed for the first four hours after treatment for the presence of toxic symptoms and lethality. Each rat's body weight and water intake was also recorded. All rats were sacrificed 24 h post-administration of *O. stamineus* treatment. Livers were removed, weighed and used to prepare the S-9 cytosolic fraction.

Preparation of Liver S-9 Cytosolic Fraction

Livers were removed from the sacrificed animals, washed with ice-cold KCl and then homogenised in three volumes of the same solution in a Potter-Elvehjem homogeniser using a Teflon pestle. The homogenates were centrifuged at 9000 x *g* for 20 min at 4°C. The supernatants (S9) were kept at –70°C until use (Gibson and Skett 1994). The protein concentration of the S9 fractions was determined by Lowry's method using bovine serum albumin as a standard (Lowry *et al*. 1951).

Determination of GST Activity

A convenient spectrophotometric method has been developed by Habig *et al.* (1974) for the analysis of GST activity based on the enzyme-catalysed condensation of glutathione with the model substrate, 1-choloro, 2,4-dinitrobenzene. The product formed (2,4-dinitrophenyl-glutathione) absorbs light at 340 nm with an extinction coefficient of 9.6 mM/cm, which facilitates the analysis of enzyme activity based on product formation.

Data Analysis

Results were compared with the respective controls and means and standard deviation were calculated. Data was analysed using the Dunnett test. The levels of significance were set at $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

Table 1 shows the body weight, water intake and lethality in the experimental and control groups. Treatment with *O. stamineus* did not cause a significant change in body weight and water intake when compared to the control group. As compared to three days following STZ injection and following *O. stamineus* treatment with STZ injection the day before, the body weights of rats were significantly decreased, whereas an increase in water intake was generally observed in each group. The effect seen above was caused by the STZ, which causes autoimmune destruction of pancreatic β cells (Thulesen *et al*. 1997). The degeneration of pancreatic β cells, which produce insulin, is called type I diabetes

Table 1: The changes of body weight and water intake of male and female rats during experimentation.

Young female SD rats							
Doses of *O. stamineus*	Body weight (g)			Water consumption (ml/rat/day)			Lethality
(mg/kg)	a	b	c	a	b	c	
Control	102 ± 1.0	93.5 ± 0.9*	88.4 ± 0.5*	13.0 ± 1.0	17.5 ± 0.5*	20.6 ± 0.1*	0/6
5	103.1 ± 2.0	92.7 ± 1.4*	90.2 ± 1.3*	12.5 ± 0.5	16.0 ± 0.6*	19.4 ± 0.4*	0/6
31.25	104.3 ± 3.1	92.2 ± 2.6*	90.3 ± 2.0*	13.5 ± 0.6	16.5 ± 0.8*	19.0 ± 0.5*	0/6
125	106.1 ± 3.2	94.3 ± 2.5*	92.0 ± 2.5*	14.0 ± 0.4	17.0 ± 0.4*	19.0 ± 0.8*	0/6
500	105.8 ± 1.3	93.2 ± 1.3*	91.0 ± 1.2*	13.0 ± 0.8	17.4 ± 1.0*	19.5 ± 0.9*	0/6

Young male SD rats							
Doses of *O. stamineus*	Body weight (g)			Water consumption (ml/rat/day)			Lethality
(mg/kg)	a	b	c	a	b	c	
Control	119 ± 2.0	105.5 ± 1.9*	100.4 ± 1.5*	18.0 ± 0.0	22.5 ± 0.5*	24.6 ± 0.2*	0/6
5	123.0 ± 3.0	107.7 ± 1.4*	102.5 ± 1.2*	18.5 ± 1.0	23.0 ± 0.6*	24.4 ± 0.4*	0/6
31.25	122.1 ± 1.1	106.6 ± 2.6*	103.0 ± 2.2*	18.5 ± 0.8	25.5 ± 0.8*	25.8 ± 1.0*	0/6
125	120.1 ± 1.2	106.3 ± 2.5*	104.0 ± 1.4*	17.4 ± 1.4	24.0 ± 0.4*	24.5 ± 0.2*	0/6
500	122.8 ± 1.3	108.2 ± 1.3*	105.8 ± 1.0*	18.6 ± 0.6	23.4 ± 1.0*	23.5 ± 0.6*	0/6

Notes: Result = mean ± S.D; analysed by Dunnett's test; n = 6
a = before STZ injection
b = 72 h post-STZ injection
c = 24 h post-*O. stamineus* treatment
* $P < 0.05$ as compared to a (before STZ injection)

mellitus. The most common form of STZ-induced diabetes mellitus in experimental animals is an excess level of sugar in the blood (hyperglycaemia) due to an insufficient amount of insulin caused by disrupted pancreatic cells. The decrease in body weight is a hallmark of untreated diabetes and the increased water consumption of the diabetic rats in our laboratory could be explained by the fact that the higher sugar level in the blood causes an increase in osmotic diuresis, resulting in large quantities of urine to be passed. Thirst and hunger are compensatory responses to the loss of fluid and the inability to utilize nutrients (Lawrence 1998).

Table 2 shows the effects of the methanol extract of *O. stamineus* on GST activity in diabetic male and female SD rats. GST activity significantly increased in diabetic male rats treated with 125 mg/kg ($P < 0.01$) and 500 mg/kg ($P < 0.01$) of the *O. stamineus* extract, whereas oral administration with 500 mg/kg ($P < 0.01$) of the *O. stamineus* extract to diabetic female SD rats significantly increased the activity of GST when compared to the respective control group. The effect was shown to be dose-dependent.

The STZ diabetic rat serves as an excellent model to study the molecular, cellular and morphological changes in the liver induced by stress during diabetes (Aragno *et al*. 2000). GSH acts as an antioxidant and was found to decrease in diabetes mellitus under *in vivo* conditions (Rotruck *et al*. 1973). The depletion of GSH content may also lower GST activity. If the toxic xenobiotics were not conjugated to glutathione, they would be free to combine with DNA, RNA or cell proteins, and could thus lead to serious cellular damage (Murray 1998). On the other hand, a higher tissue level of phase II detoxification enzymes results in a lower susceptibility to carcinogenic insult. For example, oltipraz has been demonstrated to elevate GST levels in human peripheral lymphocytes at single doses as low as 125 mg, and is used in clinical trials in Qidong, China as a chemopreventive agent for aflatoxin-induced hepatocellular carcinoma (Kensler *et al*. 1996).

Table 2: The effects of oral administration of methanol leaf extracts of *O. stamineus,* Benth on GST activity in diabetic male and female liver S-9 cytosolic fraction.

	GST activity (nmol/min/mg protein)	
Dose of *O. stamineus*	Diabetic young male SD rats	Diabetic young female SD rats
Control	17.21 ± 3.21	19.45 ± 2.41
5	18.03 ± 1.13	20.14 ± 1.42
31.25	20.02 ± 2.14	22.16 ± 2.33
125	22.53 ± 1.18**	23.47 ± 3.15
500	23.02 ± 2.16**	25.52 ± 1.88**

Notes: Results are analysed using the Dunnett test; n=6; Value = mean ± S.D
**P < 0.01 as compared to respective control group

Strategies to induce the expression and activity of phase II enzymes have been shown to protect against carcinogens in a variety of organs and across several species (Kensler 1997). In experimental animals, GST enzymes have been demonstrated to be induced by phenobarbital-type inducers and Ah receptor ligands. However, the level of induction rarely appears to be greater than two- to three-fold (Hayes *et al.* 1996). From the results obtained, the increased GST activity in diabetic rats fed with *O. stamineus* extract could protect cellular proteins against oxidation and detoxify reactive oxygen species generated from exposure to STZ. It has been shown that the free radical species responsible for STZ toxicity is hydroxyl radical, formed via the metal-catalysed Haber-weiss reaction or the Fenton reaction (Nistico *et al*. 1992).

O. stamineus has been reported to be rich in flavonoids and alkaloids, well-known antioxidants, which could scavenge the free radicals generated during diabetes. In addition, Hodek *et al*. (2002) showed that natural active compounds derived from plants such as flavonone and flavone can increase the activities of GST in rat liver. Any compound, natural or synthetic, with antioxidant properties that might contribute to the partial or total alleviation of this damage may have a significant role in the treatment of diabetes mellitus (Pari and Latha 2004). Since the study of the induction of phase II detoxification enzymes is considered to be a reliable marker for evaluating the antioxidant efficacy of the medicinal plant, this finding suggests a possible antioxidant role played by *O. stamineus* methanol extracts in addition to its hypoglycaemic effect.

CONCLUSION

Treatment of diabetic rats with *O. stamineus* extract significantly increased hepatic GST activity. Hypothetically, an increase in phase II drug metabolising enzymes might be beneficial as this could enhance the conjugation and excretion of toxins such as free radicals and carcinogens. Further studies need be carried out to confirm this theory.

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