A preliminary study of the oral hypoglycaemic activity of the ethanol and water extracts of *Munronia pinnata* in the healthy Wistar rats

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Abstract

This study was conducted to determine the oral hypoglycaemic activity of different doses of ethanol and a single dose of water extracts of *Munronia pinnata* in healthy adult Wistar rats. The ethanol extract (MPEt) was prepared using soxhlet apparatus and water extraction (MPW) was prepared according to the conventional/ traditional method used in Sri Lanka using whole plants. Healthy adult male Wistar rats 8 weeks of age and weighing 175.0 -225.0 g of were used and they were divided randomly with six rats (n=6) in each group for these experimental studies. The selected doses of MPEt (50.0 mg, 100.0 mg and 200.0 mg/kg body weight) and a single dose (1.6 g/ kg body weight) of MPW were given orally via a Sondi needle. After an overnight fast, fasting serum glucose concentration was determined and a glucose challenge was performed for the selected doses of MPEt (50.0 mg, 100.0 mg and 200.0 mg/kg body weight) and a single dose of MPW. Blood was drawn after 90 minutes following glucose (3.0 g/kg body weight) administration. Serum glucose level was measured by the glucose-oxidase method. Both extractions (MPEt and MPW) exerted statistically significant hypoglycaemic effects. All three selected doses were elicited comparatively less mean serum glucose concentrations (5.2±0.24, 5.1±0.26 and 4.2±0.34 mmol/L) compared with the control group (5.4±0.22 mmol/L) after 2nd hour. The maximum hypoglycaeamic effect was recorded in 3rd hour of the 200.0 mg/kg dose (26.7%). The selected single dose of both extracts showed more effective hypoglycaemic activity (4.9± 0.1m mol/L of MPW and 4.6± 0.35 mmol/L of MPEt) when compared with the control group (5.1± 0.19 mmol/L) respectively. The reduction given by the MPEt was significantly lower when compared with the control in paired t test ($p \leq 0.001$). The MPW also showed a statistically significant ($p \leq 0.01$) effect compared with control group.

Introduction

Diabetes mellitus is the most common endocrine metabolic disorder. It has affected several millions in different populations all over the world. This clinical syndrome is characterized by hyperglycaemia due to a deficiency or diminished effectiveness of insulin. Although there are number of widely accepted synthetic allopathic drugs that are used to control this metabolic syndrome, some are ineffective in many patients. Further, the controls of Non Insulin Dependent Diabetes Mellitus patients (NIDDM) are managed with long term treatment of allopathic drugs which can produce serious side effects. There are many plant species which are known in traditional or folk medical systems in Sri Lanka which are used for the treatment of diabetic mellitus due to their hypoglycaemic property [1]. The different parts of the plants as well as whole plant have been used for the treatment of diabetes. There are various forms of medicine such as *kwatha* (decoctions), *vati* (pills), *churna* (powders), *kalka* (pastes) and *arishta/asva* (fermented preparations) with the combination of herbal or herb-mineral drug preparations have been utilized in the Sri Lankan ayurveda/ traditional medical system. The evaluation of these plants and their natural principles is a logic way of searching for new drugs to treat this disease. Though, Sri Lanka is the smallest island in the Indian Ocean today it is considered one of the most biodiversity areas in South Asia. The island's natural flora has been enriched with 1500 medicinal plant species including 180 endemic plant species. Many of these plants were introduced from places as far off as China, Ethiopia and Arabia by travelers who visited the country during a period of at least two thousand years [2]. The plant *Munronia pinnata* (Wall) Theob (Meliaceae) locally called "*Binkohomba*" is a valuable and rare medicinal herb in Sri Lanka is not an endemic plant but now it is a threatened plant due to over exploitation. About six species of this plant restricted to tropical Asia and subtropical China eastwards to Timor in drier forests up to about 900.0 m. Presently *M. pinnata* plant has been distributed in Sri Lanka, Southern and Northeastern India, China, Vietnam, Burma, Thailand and Timor etc. In Sri Lanka this plant can be identified naturally

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over 700.0 m to 900.0 meter hilly areas. Ayurvedic and traditional physicians use this plant as a substitute for *Swertia chirata*, Family - Gentianaceae which is named as *kiratha* or *kiratha thiktha* in Sanskrit.

This plant is not available in Sri Lanka and also is prohibited to be exported from India [3]. Further, *M. pinnata* has been extensively used for fever, dysentery, diabetes and skin diseases in the form of powder and decoction due to its bitter taste.

The *M. pinnata* whole plants are uprooted from the wild and dried for commercial purposes [4]. Sri Lanka is the only country where this plant is used for medicinal purposes. However, traditional physicians claim that *M. pinnata* has been used in folk medical practice in Sri Lanka for hundreds of years. But there are no reports of any experimental or clinical studies of the biological activities of this plant. Therefore, the present study was carried out as an analytical interventional study.

Materials and Methods

Plant material: *Munronia pinnata* whole plants were collected from the medicinal plant nursery at Haldummulla, Department of Ayurveda, Sri Lanka between the periods of November - December 2010. They were used for the preparation of extractions. *M. pinnata* plant was taxonomically identified and authenticated by the National Herbarium, Department of National Botanical Garden, Peradeniya, Sri Lanka where a voucher specimen was deposited (PDA/ MP 01). The air dried herb was coarsely powdered and used for the preparation of extractions.

Experimental animals and their care: Healthy out-bread male Wistar rats (175.0 g - 225.0 g) purchased from Medical Research Institute, Colombo, were used in this study. The study was conducted at the Department of Biochemistry and the Animal House of the Faculty of Medical Sciences, University of Sri Jayewardenepura. Rats were housed individually in rat cages in a well-ventilated room at an ambient temperature of 29 ± 2° C at the Animal House. The standard WHO recommended diet was given and water was supplied *ad libitum*. The experimental procedures and animal care were conducted according to international laws and guidelines [5].

Ethical clearance

A project protocol was submitted to the Ethical Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura and ethical approval was obtained (No: 474/09).

Preparation of extracts

The ethanol extract was prepared by using the Soxhlet extraction. The air dried coarse powder of *M. pinnata* (30.0 g) was run in Soxhlet apparatus using 300.0 ml of ethanol for 30 × 20 minute cycles. The ethanol was evaporated in rotary evaporator at 40° C and a sticky dark brown material was obtained and it was dissolved in 20.0 ml of distilled water. The supernatant was filtered and dried in a water bath. Finally, a brown colour material was obtained (MPEt) and was used for the hypoglycaemic study in rats.

The aqueous extract of *M. pinnata* was prepared according to the conventional/ traditional method used by traditional medical practitioners in Sri Lanka. The air dried coarsely powdered *M. pinnata* 60.0 g (12 *kalan*) was mixed with 8 parts/*patha* (1920.0 ml) of water in an earthen vessel and boiled over moderate heat and reduced to 1/8th part (240 ml). The dose is 240.0 mL per day (MPW) for adult human.

Dose response curve of the ethanol extract (MPEt) of *M. pinnata* on the blood glucose level in the healthy rats: Four groups of six rats (n=6) in each were divided according to weight and fasting serum glucose concentrations. All four groups were fasted overnight with free access to water. To detect the most effective dose, three doses of the ethanol extract (50.0 mg, 100.0 mg and 200.0 mg/kg body weight) were administered orally via Sondi needles to each animal in the three groups. The animal dose was corresponded to the normal therapeutic dose administered to adult humans as calculated on the basis of relative surface areas of humans and rats [6]. The control group was treated with 2.5 ml of distilled water. After 30 minutes, a glucose load of 3.0 g/ kg body weight was given. Blood (0.1 ml) was drawn from the lateral tail vein of rats under light anesthesia with diethyl ether 90 minutes after the glucose administration. Blood samples were centrifuged (3000 ppm × 20 min.) and serum was separated. The serum glucose concentration was measured by the glucose-oxidase method [7] using BIOLABO reagent kits.

Time course of the ethanol extract (MPEt) of *M. pinnata* on the blood glucose level in the healthy rats: To determine the optimal time of activity two groups of rats (n=6) were divided as test and control. After an overnight fast, fasting serum glucose concentration was determined and a glucose challenge was performed. The test group received 2.5 ml of MPEt as a single dose 200.0 mg/kg of and 2.5 ml each of distilled water was given to control group. After the administration of glucose (3.0 g/ kg), blood was drawn for the estimation of glucose at 1, 2, and 3hrs.

Comparison of the oral hypoglycaemic effect of a single dose of aqueous (MPW) and ethenol (MPEt) extracts of *M. pinnata* on the blood glucose level in the healthy rats: Healthy adult Wistar rats were divided in to three groups according to body weight. Fasting serum glucose levels were determined as above.

According to the results of dose curve 200.0 mg/kg of MPEt and 1.68g/kg of MPW (as determined previously) were given to the rats in the test group. The control group

was treated with 2.5 ml of distilled water and the glucose challenge test were performed as described above.

Statistical analysis

Statistical analysis was done by the help of student's t-test and presented as mean ± S.D and a p value of ≤ 0.05 was taken as significant.

Results and Discussion

The effects of different doses of ethanol extract of *M. pinnata* on the fasting serum glucose concentrations of healthy Wistar rats are shown in table 1. All three selected doses (50.0 mg, 100.0 mg and 200.0 mg/ kg body weight) were elicited comparatively less mean serum glucose concentrations (5.2±0.43, 5.1±0.26 and 4.2±0.34 mmol/L) compared with the control group (5.4±0.22 mmol/L) after 3rd hour. The dose 200.0 mg/kg was recorded the lowest mean serum glucose concentration (4.1±0.33) in 3rd hour after the administration of MPEt to the rats. Further, the maximum percentage of reduction of serum glucose concentration (26.7%) was also elicited from the same dose when compared with its control group ($p \leq 0.001$).

The table 2 describes the results of the effect of a single dose of water and ethanol extractions of *M. pinnata* on the serum glucose concentration in the healthy rats.

Accordingly both extracts of *M. pinnata* showed more effective hypoglycaemic activity when compared with the control groups (4.9± 0.04.6± 0.35 and 1± 0.19 m mol/L respectively).

The reduction given by the ethanol extract of *M. pinnata* was significantly lower when compared with the control group in paired t test ($p \leq 0.001$). The water extract of *M. pinnata* also showed a statistically significant ($p \leq 0.01$) effect compared with control group.

Table 1: Effect of different doses of (MPEt) on the blood glucose level in the healthy rats

Doses	*Serum blood glucose concentrations mmol/L (n=6)*			
	Time			
	Zero hour	*1st hour*	*2nd hour*	*3rd hour*
MPEt 50mg/kg	3.3± 0.21	5.7± 0.43 (12.5%)	5.2±0.24* (3 %)	4.9±0.07* (12.5%)
MPEt 100mg/kg	3.2±0.24	5.8±0.27* (10.7%)	5.1±0.26* (5 %)	4.7±0.14* (16.1%)
MPEt 200mg/kg	3.3±0.32	5.7±0.14* (12.3%)	4.2±0.34* (22.2 %)	4.1±0.13** (26.7%)
DW 2.5ml (Control group)	3.3±0.22	6.5± 0.41	5.4±0.22	5.6±0.13

Values are expressed as mean ± SEM (n=6). Asterisks denoted the significance levels in comparison to control values. *p ≤0.05 and **p≤0.0001.

MPEt: ethanol extract of *M. pinnata*; DW: distilled water.

Figures in parenthesis indicate reduction of the glucose percentage after administration of MPEt.

Table 2: Effect of the single dose of aqueous (MPW) and ethenolic (MPEt) extracts of *M. pinnata* on the serum glucose concentration in the healthy rats

Group (n=6)	*MPW*	*MPEt*	*Control*
Mean serum glucose concentration mmol/L	4.9± 0.04*	4.6± 0.12**	5.1± 0.07

Values are expressed as mean ± SEM (n=6). Asterisks denoted the significance levels in comparison to control values. *p≤0.01 and **p≤0.001.

MPW: water extract of *M. pinnata*; MPEt: ethanol extract of *M. pinnata*; control: 2.5 ml of distilled water

Conclusions

This is the preliminary experimental study of *Munronia pinnata* in healthy Wistar rats. Water and ethanol extracts of this plant exert a statistically significant oral hypoglycaemic effect in healthy rats. From the selected doses, the dose of 200.0 mg/ kg of ethanol extract markedly pronounced more oral hypoglycaemic effect than the other selected doses. Further, the maximum hypoglycaemic effect (26.7%) was elicited from same dose, 200.0 mg/ kg after 3rd hour from the administration of MPEt when compared with its control group ($p \leq 0.001$). The reduction given by the single dose of ethanol extract of *M. pinnata* was significantly lower when compared with the control in paired t test ($p \leq 0.001$). The water extract of *M. pinnata* (1.68 g/ kg) also showed a statistically significant ($p \leq 0.01$) effect compared with control group. Therefore, further experimental studies have to be carried out to determine the oral hypoglycaemic activity in diabetic rats.

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