Hypoglycaemic effect of aqueous extracts of natural plants and callus cultures of *Munronia pinnata* (Wall.) W.Theob. in Wistar rats

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Received 25.04.14, revised 03.06.14

*Munronia pinnata* (MP) is one of the most demand herbs in the Sri Lankan drug market which has been used for various ailments in folk medical practice in Sri Lanka. There is an urgent need to adapt *ex situ* conservation methods via *in vitro* propagation techniques due to the high demand and overharvesting of this herb. The possible use of calli as a substitute for whole plants has been queried. The purpose of the present study was thus to compare the hypoglycaemic effect of aqueous extracts of natural plant (MPaq) and calli (MPCaq) of MP in healthy and alloxan induced diabetic rats. Natural plants and 3-months' old calli of MP were used for the preparation of aqueous extracts. Effect of graded doses of both extracts of MP on serum glucose (SGL) of normal rats was studied. In case of diabetic rats, the results were compared with the reference drug glibenclamide and safety assessment of extracts was evaluated following administration for three months. Both MPaq and MPCaq extracts were showed reduction of the serum glucose levels of normal and diabetic rats. The reduction of SGL by MPaq and MPCaq were showed higher (24% and 18%) as compared to that of glibenclamide (16%) in diabetic animals. This study has been able to demonstrate that both MP extracts exhibit statistically significant hypoglycaemic effect were compared to the reference drug and proves the scientific rationale for its use as a popular folk medicine for diabetes.

**Keywords**: *Munronia pinnata*, Hypoglycaemic, Anti diabetic, *In vitro* propagation, Callus cultures

**IPC Int. Cl.\(^a\)**: A61K 36/00, A01D 20/00, A01D 16/02, A01G

*Munronia pinnata* (Wall.) W. Theob. (Family Meliaceae) is one of the most demanded medicinal herbs in the Sri Lankan drug market. It has been used for fever, dysentery, diabetes, tuberculosis, cough, stomachache, sores, malaria and purification of blood and skin diseases as a substitute for *Swertia chirata* (Family Gentianaceae), which is not available in Sri Lanka. *Swertia chirata* is prohibited to be exported from India\(^1\). About six species of the genus *Munronia* are restricted to tropical Asia and subtropical China\(^2\). Phytochemical analysis and a few biological investigations have been carried out on *M. delavayi*, *M. henryi* and *M. sinica* \(^3\), which have been described in flora of China as synonyms of *M. pinnata* \(^4\).

Despite its long record of usage in folk medical practices in Sri Lanka, only a few scientific studies are recorded on the biological activities of *M. pinnata*. Due to the very high demand, plants are collected from natural forest for medicinal purposes and there has been no commercial cultivation. As a result of repeated harvesting from forests, the plant has become endangered and if protective measures are not taken, this plant faces the threat of becoming extinct in Sri Lanka. Seed production of *M. pinnata* is low and it is difficult to carry out large scale propagation through seeds. There is no practice of using stem cuttings. Previous studies have revealed the possibility of propagating *M. pinnata* through *in-vitro* hypocotyle callus culture\(^5\) and leaf callus culture\(^6\). No studies have been done on the biological activities of callus cultures of *M. pinnata*. Therefore, in the present study the hypoglycaemic activity of the natural plant was investigated in comparison with in the callus cultures of this threatened medicinal plant.

**Methodology**

**Plant material**

Three leaflet *Munronia pinnata* plants were collected from the medicinal plant nursery at
Haldummulla, Department of Ayurveda, Sri Lanka, between the periods of November – December 2009 and maintained in the greenhouse at the Department of Dravyaguna Vignana, Institute of Indigenous Medicine, Rajagiriya, University of Colombo. *M. pinnata* plant was taxonomically identified and authenticated by the National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka, where a voucher specimen was deposited (PDA/ MP 01). The established protocol of the development of callus cultures was used for the callus production from leaves. Air dried 1 yr old whole plant and 3 months old fresh calli were selected after washing with distilled water and used for the preparation of extractions.

**Experimental animals**

Healthy out-bred male Wistar rats (175.0 – 225.0 gm) 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*. All experimental protocols were approved by the Ethics Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura (No: 474/09). Animals were randomly selected and six rats were used per group in all experiment.

**Preparation of aqueous extracts**

The aqueous extracts of natural plants and calli of *M. pinnata* were prepared according to the conventional method used by traditional medical practitioners in Sri Lanka. The air dried coarsely powdered natural plant (MPeq) and calli (MPCaq) of *M. pinnata* (60.0 gm /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 (240.0 mL). [The dose is 240.0 mL per day (MPeq) for adult human].

**Experimental protocol**

**Determination of Hypoglycaemic activity in healthy rats**

Initial screening of the extracts of MPaq and MPCaq was carried out to evaluate its glycaemic potential with a range of different doses. Rats were divided into eleven groups and all groups were fasted overnight with free access to water. A single dose of each extract was given as follows:

- **Group 1 to-5:** received 0.98, 1.96, 3.92, 7.84 and 11.76 gm/kg of MPaq extract
- **Group 6 to-10:** received 0.98, 1.96, 3.92, 7.84 and 11.76 gm/kg/day of MPCaq extract
- **Group 11:** received 2.5 mL of distilled water (DW)

Blood (0.1 mL) was drawn from the lateral tail vein of rats under light anaesthesia with diethyl ether for the estimation of fasting serum glucose (FSG) levels at 1, 2, and 3hrs after administering MPaq and MPCaq. Blood samples were centrifuged (3000 rpm x 20 min), serum was separated and the FSG level was measured. Effective dose of the extract was calculated by using linear regression analysis.

**Determination of Hypoglycaemic activity by Oral Glucose Tolerance Test (OGTT) in healthy rats**

Eleven groups of healthy rats were selected and treated in the same manner as described in the protocol above. After 30 minutes, a glucose load of 3.0 gm/ kg body weight was given and their glucose tolerance was studied up to 3hrs at regular interval of 1hr each.

**Induction of diabetes**

Diabetes was induced in male Wistar rats by a single intra venous injection of (40.0 mg/kg) alloxan monohydrate (Sigma, Aldrich) dissolved in sterile normal saline. Alloxan monohydrate induces diabetes by destruction of beta cells of Islets of Langerhan with consequent impairment of insulin secretion leading to hyperglycaemia. The animals were given 2.0 mL of 5% dextrose solution orally immediate after induction to overcome the drug induced hyperglycaemia. Fasting serum glucose level was measured using glucose - oxidase test (BIOLABO reagents, France) after 72 hrs and rats with serum glucose levels above 11.0 mmol/L were considered diabetic and selected for the experiment.

**Hypoglycaemic activity in diabetic rats**

The diabetic rats were divided into 4 groups of 6 rats each, and treated orally once a day for 3 months as follows:

- **Group 1:** treated with MPaq (2.0 gm/kg)
- **Group 2:** treated with MPCaq (2.0 gm/kg)
Group 3: served as a positive control which received glibenclamide 5.0 mg/kg\textsuperscript{12} (purchased from state pharmaceutical cooperation, Sri Lanka)
Group 4: served as negative control which treated with 2.5 mL of distilled water and all groups were subjected for glucose challenge to compare the efficacy of each extract.

**Determination of intestinal glucose absorption in healthy rats**

Healthy adult Wistar rats were divided into 3 groups; MPaq and MPCaq as test groups and distilled water (DW) as a control group. After an overnight fast, the rats were subjected to glucose challenge as above. Two hours after the administration of both extracts and distilled water, the animals were euthanized with diethyl ether and intestines harvested. Intestinal contents were flushed out with 50.0 mL of distilled water using a syringe and contents were collected in autoclaved containers and centrifuged. Blood was obtained by cardiac puncture for the determination of the serum glucose levels. The glucose level was determined in the supernatants of intestinal contents.

**Determination of effect on hepatic enzymes of diabetic rats**

At the end of 3 months, blood samples were collected for haematological and biochemical assays using commercial reagent kits (BIOLABO reagents, France). The serum levels of alanine transaminase (ALT), aspartate transaminase (AST), $\gamma$- Glutamyl transferase (GGT), alkaline phosphatase (ALP) and creatinine were estimated.

**Determination of LD\textsubscript{50}**

Toxicity of both extracts was also studied by LD\textsubscript{50} experiment. Three groups of healthy rats of both sex (3 males and 3 females) weighing about 175.0 – 225.0 gm were orally administered a dose of the selected highest dose from MPaq and MPCaq (11.76 gm/kg). Animals were then observed continuously for their behavioural changes and toxic effects up to 24 hrs using WHO guidelines\textsuperscript{13}. Food consumption, urine and faeces were also examined at 2 hrs and at 6 hrs intervals for 24 hrs.

**Statistical analysis**

Results were exhibited as mean ± SEM and significant differences between means of different groups were statistically analyzed by one way analysis of variance (ANOVA) followed by Turkey’s HSD multiple comparisons. Values were considered statistically significant at $p < 0.05$.

**Results**

**Effect on fasting serum glucose levels of healthy rats**

The effects of graded doses of the aqueous extracts of natural plants and calli of *M. pinnata* on the fasting serum glucose levels of healthy Wistar rats are shown in Table 1. All selected doses (0.98, 1.96, 3.92, 7.84 and 11.76 gm/kg/day) caused a reduction in the mean blood glucose concentrations compared with the control group in a dose dependent manner. Rats treated with 11.76 gm/kg showed the highest percentage reduction (MPaq 42%, and MPCaq 40%) in the serum glucose level at 2nd hrs of

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Dose (gm/L/kg)</th>
<th>Mean serum glucose concentration (mmol/L)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 hr</td>
<td>1 hr</td>
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<tr>
<td>MPaq</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.98</td>
<td>3.64±0.43</td>
<td>5.6±0.4</td>
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<td>1.96</td>
<td>3.86±0.65</td>
<td>5.94±0.34</td>
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<tr>
<td>3.92</td>
<td>3.8±0.42</td>
<td>6.2±0.47*</td>
</tr>
<tr>
<td>7.84</td>
<td>3.9±0.51</td>
<td>6.5±0.52*</td>
</tr>
<tr>
<td>11.76</td>
<td>3.7±0.43</td>
<td>6.8±0.41</td>
</tr>
<tr>
<td>MPCaq</td>
<td></td>
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<tr>
<td>0.98</td>
<td>3.2±0.23</td>
<td>6.46±0.5</td>
</tr>
<tr>
<td>1.96</td>
<td>3.5±0.6</td>
<td>4.84±0.23</td>
</tr>
<tr>
<td>3.92</td>
<td>3.4±0.32</td>
<td>5.2±0.32*</td>
</tr>
<tr>
<td>7.84</td>
<td>3.5±0.11</td>
<td>5.5±0.42*</td>
</tr>
<tr>
<td>11.76</td>
<td>3.2±0.33</td>
<td>5.8±0.41</td>
</tr>
<tr>
<td>DW (control)</td>
<td>3.4±0.46</td>
<td>5.8±0.52</td>
</tr>
</tbody>
</table>

Mean ± SEM. (n=6). Asterisks denoted the significance levels in comparison to control values. *$p< 0.05$ and **$p< 0.001$. DW: distilled water. Figures in parenthesis indicate reduction of the glucose percentage after administration of MPaq and MPCaq.
oral administration of both extracts. The effective dose for the reduction of serum glucose level by MPaq and MPCaq was 2.0 mg/kg at 2nd hrs. These aqueous extracts exerted statistically significant oral hypoglycaemic effects in dose dependent manner in healthy rats ($p < 0.001$ and $p < 0.05$, respectively).

**Effect on glucose tolerance of healthy rats**

Fig. 1a & b describe the hypoglycaemic effect of a single dose administration of the selected doses of MPaq and MPCaq on oral glucose tolerance test (OGTT) of healthy rats. The maximum hypoglycaemic effect was recorded at the 2nd hrs from the dose of 11.76 gm/L of both extracts and the reduction in the serum glucose of test groups were statistically significant ($p < 0.01$) compared with the control group.

**Effect on glucose tolerance of alloxan induced diabetic rats**

All the tested groups were showed a significant reduction ($p < 0.05$) in the serum glucose concentration levels compared with negative control (DW). There were 24% and 18% reductions in the serum glucose level of diabetic rats of MPaq and MPCaq, respectively compared to the control group given DW. The reference drug glibenclamide group showed a reduction of 16 % ($p < 0.05$) in glucose levels compared to the DW (Fig. 2).

**Effect on intestinal glucose absorption of healthy rats**

The test groups (MPaq and MPCaq) exhibited significant reductions ($p < 0.05$) in intestinal glucose concentration as well as serum glucose levels when compared to the control group after glucose challenge (Fig. 3).

**Effect on hepatic enzymes of diabetic rats**

After continuous daily oral administration of both MPaq and MPCaq extracts for three months in diabetic rats, blood samples were taken for the investigation of the key hepatic enzymes. The results presented in Table 2 indicate that, oral administration of both

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**Fig. 1a—Hypoglycaemic effect of graded doses of aqueous extract of natural plants of *M. pinnata* on OGTT of healthy Wistar rats** Each values indicated in mean ± S.E.M. (n=6). Treatment groups of the aqueous extracts of natural plants with different doses: MPaq1 (0.98 gm/kg), MPaq2 (1.96 gm/kg), MPaq3 (3.92 gm/kg), MPaq4 (7.84 gm/kg) and MPaq5 (11.76 gm/kg). Control group (distilled water): DW. *p< 0.05 and **p< 0.001, significance levels compared to the control group at the corresponding time.

**Fig. 1b—Hypoglycaemic effect of graded doses of aqueous extract of callus cultures of *M. pinnata* on OGTT of healthy Wistar rats.** Each values indicate the mean ± S.E.M. (n=6). Treatment groups of the aqueous extracts of callus cultures with different doses: MPCaq1 (0.98 gm/kg), MPCaq2 (1.96 gm/kg), MPCaq3 (3.92 gm/kg), MPCaq4 (7.84 gm/kg) and MPCaq5 (11.76 gm/kg). Control group (distilled water): DW. *p< 0.05 and **p< 0.001, significance levels compared to the control group at the corresponding time.

**Fig. 2—Effect of aqueous extracts of natural plants and calli of *M. pinnata* on intestinal glucose absorption in healthy rats** *P < 0.05 compared to the control
aqueous extracts of *M. pinnata* for three months had no statistically significant changes (*p* > 0.05) in haematological and biochemical parameters of test groups compared to the control groups of both healthy and diabetic animals. All results were included within reference values for the strain and age of the animals used. These results revealed that both aqueous extracts of *M. pinnata* do not exert any adverse effects as assessed by the parameters studied.

**Determination of LD₅₀**

All the tested groups showed neither mortality nor changes in general behaviour compared with control groups during the period of 3 months.

**Discussion**

Many plant species are used in various parts of the world to manage diabetes. Different varieties of locally available medicinal plants are used as substitutes for unavailable medicinal plants in different drug preparations in traditional and ayurvedic physicians in Sri Lanka. The plant *Munronia pinnata* is used for the drug preparations in the traditional and ayurvedic medicine as substitutes for *Swertia chirata* (Family Gentianaceae), which is not available in Sri Lanka. The hypoglycaemic activities of *S. chirata* and *A. paniculata* have been studied extensively in healthy and diabetic rats.

The increase in demand for *M. pinnata* is lead to its absolute exploitation from the natural resources. This situation could be overcome only by establishing a proper cultivation technique. *In vitro* propagation techniques have been applied as an attempt to meet the increasing demand of this plant. There have been only a few scientific studies on this herb. Further, biological activities of the calli of *M. pinnata* have not been investigated.

In the present study, an attempt has been made to evaluate the hypoglycaemic activity of aqueous extracts of natural plants and calli of *M. pinnata* in alloxan induced rats. All the doses of MPaq at 2nd hrs, reduced serum glucose levels significantly compared with the control group. Only two doses (7.84 and 11.76 gm/L) of MPCaq elicited a significant (*p* < 0.05) serum glucose reduction compared to the control group. The dose 11.76 gm/L of both extracts (MPaq and MPCaq) showed the most significant reduction (31% and 24%, respectively) at 2nd hrs, *p* < 0.05 and *p* < 0.001, respectively. Though the serum glucose levels were reduced at 3rd hrs compared with the control group, the percentage of inhibition was lower than the 2nd hrs of both extracts. The most effective dose (2.0 gm/kg) of MPaq and MPCaq was detected by using linear regression analysis. Moreover, these results imply that callus culture of aqueous extract of *M. pinnata* possesses a significant hypoglycaemic activity which is similar to that of the aqueous extract of natural plant.

There was no statistically significant difference between both extract (MPaq and MPCaq) and the tested standard anti diabetic drug being compared. These results have been able to demonstrate that the long term administration of *M. pinnata* exhibits statistically significant hypoglycaemic effect (*p*<0.05) comparable to anti diabetic drug (glibenclamide 5.0 mg/kg) and proves the scientific rationale for its use as a popular folk medicine for diabetes.

Terpenoids and other chemical constituents present in *M. pinnata* may in part be responsible for the observed significant activity of this extract either
singly or in synergy with one another. In this study, however the mechanisms of the both extracts of *M. pinnata* on the inhibition of the intestinal glucose absorption were evaluated in vivo to examine whether the extracts could inhibit glucose absorption. The lower concentration of glucose in the intestinal contents of the test groups revealed that the uptake in the intestinal brush border was increased by *M. pinnata*. However, the concentrations of glucose in the serum of the test groups were showed also significantly lower than that of the control. These results indicated that the both extracts may lower the serum glucose concentration by increasing glucose utilization of peripheral tissues. Similar mode of action has also been demonstrated by *A. paniculata* and *Radix ginseng palva*. Although *M. pinnata* has been used in treatment of different ailments in the traditional medicine in Sri Lanka for a long time, there has been a paucity of research concerning its safety effects. In the present study, results of key hepatic enzyme assays after 3 months regular treatment with *M. pinnata* was exhibited no statistically significant changes in haematological and biochemical parameters of test groups compared to the control group of diabetic animals. This is an important in the case of hypoglycaemic drugs, which have to be administered over a relatively long period of time in the therapeutic practice in Ayurvedic as well as traditional medical systems. Moreover, further studies will be required to investigate the pharmacological effect of this plant and also to carry out isolation of the active chemical constituents of these extracts.

In conclusion, this study has been able to demonstrate the hypoglycaemic potentials of *M. pinnata* in healthy and diabetic rats and may prove the scientific rationale for its popular folk medicine as hypoglycaemic agent.

Acknowledgement

This work was financially supported by the University Grant Commission, Sri Lanka, (Research Grants -2008 UGC/ICD/045).

References