Anti-diarrheal and hypoglycemic activities of methanol extract of Calamus rotang L. seed in rat

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Abstract

Background and objectives: Calamus rotang is used in traditional medicine. Regarding the previous phytochemical screening of methanol extract of Calamus rotang L. fruits (MCR), this study was performed to find out the antidiarrheal and hypoglycemic effects of the experimented extract in rodents. Methods: Castor oil induced diarrheal test was followed for screening anti-diarrheal effect of MCR. In order to explore the hypoglycemic effect, normoglycemic study, oral glucose tolerance test (OGTT) and study on alloxan-induced diabetic rats were carried out. In each experiment, 250 and 500 mg/kg body weight doses of MCR were used. Results: The plant extract showed pronounced, significant ($p < 0.01$) antidiarrheal activity on both doses in a dose-dependent manner. At higher dose, MCR showed almost similar antidiarrheal effect as standard loperamide. In case of normoglycemic test, a single dose of MCR caused a significant ($p<0.01$) reduction in blood glucose level over time compared to the control group. Although metformin reduced blood glucose more rapidly than MCR at both doses, its extent of reduction in blood glucose level was approximately the same as high dose. In OGTT, MCR was active and comparable to that of the glucose treated control group in both doses. For alloxan induced diabetic rodents, the study revealed that the MCR extracts could decrease the blood glucose level in both doses over a period of three days. Conclusion: Considering the results of the present study, further isolation of active components and establishment of the mechanism of action have to be continued in the future.

Keywords: alloxane, antidiarrheal effect, Calamus rotang, diabetes, hypoglycemic effect

Introduction

Utilization of medicinal herbs is terrifically mounting over the past decade as an option to develop the excellence of life and sustain an excellent health. Medicinal plants have been consumed for centuries as medications for human diseases [1,2]. In recent times, there has been budding attention in exploiting the biological activities of flora and fauna due to their natural source, cost effectiveness and minor side effects [3,4]. Herb-based natural ingredients can be obtained from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc [5]. Since medicinal plants are believed to be an important resource of novel chemical substances with potential remedial effects [6], they are used in Bangladesh for medical practice for the treatment of various diseases [7]. In fact for centuries in many cultures all over the world, plants have
been used in different diseases and should be considered as new sources of diarrheal and hypoglycemic therapeutic agents. *Calamus rotang* L. (rattan palm or climbing palm), is a common growing shrub in Bangladesh. *Calamus rotang* is a common growing shrub in Bangladesh which belongs to the family Arecaceae. It is a native plant of south-west Asia. The basal part of the plant grows vertically for ten meters and horizontally for about two hundred meters or more. Fruits can be consumed fresh or prepared into pickles and eaten with food. Tribal people use its tender shoots as antihelminthic agent [8]. The leaf sap is used for treating eye problems [9]. A saponin in the stem, an alkaloid in the leaves and a flavonoid in the root have been isolated from *C. rotang* which are used in convulsions and cramps [10].

Diarrhea ranks second to respiratory diseases as the cause of non-surgical pediatric admission and causes one fourth of the avoidable deaths in hospitalized children. It is one of the leading causes of morbidity and mortality in all age groups, particularly in infants and children under the age of three [11,12]. The leaves of *C. rotang* are used in traditional medicine to treat diarrhea[11,12].

Diabetes mellitus is a group of metabolic disorders resulting from defects in insulin secretion and/or reduced sensitivity of the tissues to insulin [13]. It is characterized through the chronic high blood glucose which causes the glycation of body protein and thus could lead to severe complications [14]. The number of people suffering from this disease is growing globally at a frightening speed. Drugs such as biguanides, sulphonylureas and insulin have been engaged for the dealing of diabetes; however nothing has been able to heal the ailment [15,16]. Furthermore, detrimental effects such as hypoglycaemia, anorexia nervosa, brain atrophy and fatty liver happen during the ingestion of oral synthetic hypoglycaemic drugs [17]. Inferior still, the cost of these medicaments is away from the reach of individuals in the squat earning group and particularly those living in the countryside areas. Hence, there is a requirement to search for new-fangled and reasonably priced remedies for diabetes. Lately, the search for antidiabetic agents has been focused on herbs because of their availability, affordability, and efficacy and also maybe due to their little side effects. However, few of these plants have received scientific or medical inspection and World Health Organization has suggested further assessment of traditional plants used for the management of diabetes [18].

The previous researches and phytochemical screening of the seed of *C. rotang* has inspired us to find antidiarrheal and hypoglycemic effects of its methanol extract in rat.

**Experimental**

**Plant material**

In this work, the fresh fruits of *C. rotang* were collected from, Chittagong, Bangladesh in July, 2014. The collected plant was then identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen has been deposited (DACB: 36,704) for further reference. From the collected fruits, seeds were separated and dried for one week and pulverized into a coarse powder with a suitable grinder. The powder was stored in an airtight container, and was kept in a cool, dark and dry place for further analysis.

**Preparation of methanol extract of the plant seeds**

500 g dried powder was soaked in 500 mL of 95% methanol for 7 days in cold condition with occasional shaking and stirring. The whole mixture was successively filtered through a piece of clean, white cotton material and No. 1 Whatman filter paper. The methanol portion of the seed delivered a reddish brown gummy precipitate which was designated as MCR. The extract was transferred to a closed container for further use.

**Chemicals**

Alloxan (Fluka, Germany), Tween-80, castor oil
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(BDH Chemicals, UK), normal saline solution (Beximco Infusion Ltd., Bangladesh), metformin and loperamide (Square Pharmaceuticals Ltd., Bangladesh) were procured and used in the experiment. All chemicals in this investigation were of analytical grade.

Phytochemical analysis
The MCR extract was subjected to qualitative chemical screening for the identification of bioactive constituents (tannins, alkaloids, flavonoids and saponin) using standard procedures [19].

Animals
Young Long-Evans rats of either sex weighing about 80-120 g were used to conduct the research and were housed in stainless steel cages (34 cm × 47 cm × 18 cm) with soft wood shavings as bedding. The rats were procured from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (at 24.0±0 °C temperature, 55-65% relative humidity and 12 hours light/dark cycle) for two weeks for acclimation and fed ICDDR, B formulated rodent food and tap water ad libitum. All animals were fasted over night before tests while providing tap water ad libitum. The equipment usage and handling of the animals were performed in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals [20]. The protocols for the study were approved by the Departmental Ethics Committee. The set of rules followed for animal experiment were approved by the institutional animal ethical committee [21].

Antidiarrheal test
Castor oil induced diarrheal test described by Awouter et al. [22] was performed for checking anti-diarrheal effect of MCR extract. After weighing, twenty rats were fasted for 18 hours with free access to water and randomly separated into four groups with five rodents in each group. Group-I (control) received only normal saline (5 mL/kg bodyweight), while Group-II received the standard drug, loperamide (3 mg/kg body weight). Group-III and IV received experimented extract which was administered orally at doses of 250, and 500 mg/kg body weight, respectively. After one hour, diarrhoea was induced in each animal by oral administration of 1 mL castor oil by gavage. The animals were kept in separate metabolic cages to evaluate faecal matter consistency and frequency of defecation for 4 h. Faeces were collected on an absorbent sheet of paper placed below the cages. The total number of diarrheal faeces expelled was compared with the control group. The total score of diarrheal faeces for the control group was considered as 100%. The results were expressed as a percentage of inhibition of diarrhoea. The percent (% inhibition of defecation was calculated using the subsequent formula.

\[ \% \text{ Inhibition of defecation} = \frac{(A - B)}{A} \times 100 \]

A=Mean number of defecation produced by castor oil
B=Mean number of defecation produced by drug or extract

Hypoglycemic study
Effect of methanol extracts in normoglycemic rats
The rats were divided into four groups of 6 animals each. Group I served as control and received normal saline, group II served as standard control, taking metformin in the vehicle (110mg/kg/p.o.). Group III and IV received 250 and 500 mg/kg MCR orally, respectively. Blood glucose levels were determined at 0, 1, and 2 hr following treatment from withdrawing blood from tail vein.

Oral glucose tolerance test (OGTT)
OGTT was performed in overnight fasted normal rats which were uniformly divided into four groups each containing six rats. Normal control group received only vehicle (5 mL/kg normal
saline p.o.) and standard group received reference drug metformin in the vehicle (110 mg/kg p.o.), while groups 3 and 4 were administered with MCR at 250 and 500 mg/kg, p.o., respectively. Consequently 30 min post extract administration all rodents were fed with glucose (2 g/kg). Blood samples were withdrawn from tail vein prior to dosing and then at 30, 60, 90 and 120 min after glucose administration. The fasting blood glucose level was analysed using glucose-oxidase-peroxide reactive strips (Accu-chek, Roche Diagnostics, GmbH, Germany).

Induction of diabetes in rats
Diabetes was created by a single dose (150 mg/kg body weight) subcutaneous injection of freshly prepared alloxan monohydrate dissolved in normal saline to overnight fasted rodents. Blood glucose level (BGL) was measured by using one-touch glucometer and diabetes was confirmed after 72 h of alloxan injection. Rats which showed hyperglycemia (BGL>10 mmol/L) were chosen for investigation.

Study on alloxan-induced diabetic rats
Rats which show hyperglycemia (BGL>10 mmol/L) were chosen for investigation. The experimental rodents were randomly divided into four groups consisting 6 rats in each. The groups were denoted as group-I, group-II, group-III, group- IV and group- V. Each group of rats received a specific treatment. Test samples at a dose of 250 mg/kg and 500 mg/kg body weight were used to evaluate the hypoglycemic activity. Standard metformin was used at a dose of 110mg/kg body weight. Before administering the drugs, each rat was weighed accurately and the doses were adjusted accordingly. In the evaluation of the hypoglycemic effect, the blood glucose level of the experimental animals were measured at 0 h by tail tipping method [23] using a glucometer (Bioland G-423S). Then the control, standard, MCR extract were administered orally to the experimental animals with the help of feeding needle. Blood samples were collected from tail vein prior to dosing (day 0) and then at regular intervals of day 1, 3 and 7.

Statistical analysis
All values in the test were expressed as mean ± standard error of the mean (SEM). The data were statistically analyzed by ANOVA and post-hoc Dunnett’s tests with the Statistical Package for Social Sciences (SPSS) program (SPSS 16.0, USA). Dissimilarity between the means of the various groups were measured significant at p<0.01.

Results and Discussion
The extract gave positive tests for tannins, alkaloids, saponin and flavonoids. In castor oil-induced anti-diarrhoeal study it was found that MCR at doses 250 and 500 mg/kg reduced the total number of faeces significantly (p<0.01) by approximately 57% and 66%, respectively compared to the control group. MCR at a dose of 500 mg/kg has been found to have the capacity to reduce the number of faeces to an extent similar to that achieved by the standard drug loperamide (table 1).

In the normoglycemic study it has been observed that administration of single dose of MCR at the two different doses in normal rats reduced the blood glucose level over a period of 2 hours compared to the control in a dose dependent manner. It was also noticed that MCR at a dose of 500mg/kg was effective as the standard drug metformin in reducing the blood glucose level over time. In oral glucose tolerance test performed on normal rats, MCR was found to be active in reducing blood glucose level slightly in a dose dependent manner.
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Table 2. Effect of MRC extract on blood glucose in normoglycemic rat

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose levels (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>N. saline (control)</td>
<td>-</td>
<td>7.21±0.16</td>
</tr>
<tr>
<td>Metformin</td>
<td>110</td>
<td>9.65±0.47</td>
</tr>
<tr>
<td>MCR-250</td>
<td>250</td>
<td>8.16±0.26</td>
</tr>
<tr>
<td>MCR-500</td>
<td>500</td>
<td>7.88±0.27</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SD (n=6); *p<0.01 significant compared to control

Table 3. Oral glucose tolerance test in rats for MCR

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Initial (mmol/L)</th>
<th>1 h (mmol/L)</th>
<th>2 h (mmol/L)</th>
<th>3 h (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>8.31±0.12</td>
<td>8.31±0.12</td>
<td>8.23±0.07</td>
<td>8.31±0.12</td>
</tr>
<tr>
<td>Metformin</td>
<td>110</td>
<td>9.47±0.18*</td>
<td>9.68±0.33*</td>
<td>8.72±0.37</td>
<td>8.07±0.37</td>
</tr>
<tr>
<td>MCR-250</td>
<td>250</td>
<td>10.18±0.19*</td>
<td>9.68±0.33*</td>
<td>8.72±0.37</td>
<td>8.07±0.37</td>
</tr>
<tr>
<td>MCR-500</td>
<td>500</td>
<td>9.87±0.36*</td>
<td>8.70±0.39</td>
<td>7.18±0.46*</td>
<td>6.30±0.42*</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SD (n=6); *p<0.01 significant compared to control

Table 4. Blood glucose level of alloxan induced diabetic rat after treatment with MCR

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Blood glucose levels (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day</td>
</tr>
<tr>
<td>Control (Non diabetic)</td>
<td>-</td>
<td>5.10±0.18</td>
</tr>
<tr>
<td>Control (diabetic)</td>
<td>-</td>
<td>19.12±0.91*</td>
</tr>
<tr>
<td>Standard (Metformin)</td>
<td>110</td>
<td>12.47±0.68*</td>
</tr>
<tr>
<td>MCR-250</td>
<td>250</td>
<td>18.33±0.42*</td>
</tr>
<tr>
<td>MCR-500</td>
<td>500</td>
<td>14.67±1.20*</td>
</tr>
</tbody>
</table>

Group-I (control) received normal saline (5 mL/kg body weight, p.o.). Group-II (control-diabetic) received only alloxane. Group- III (standard) received metformin (10 mg/kg body weight, p.o.). Group- IV and Group-V were treated with 250 and 500 mg/kg body weight of the extracts (p.o.), respectively. Values are mean ±SEM, (n=6); *p < 0.01, Dunnett t-test as compared to control. MCR: methanol extracts of Calamus rotang seeds

MCR at a dose of 500mg/kg achieves an anti-hyperglycemic effect approximately half of that achieved by the control and the standard drug. Table 4 illustrates the effect of two doses of MCR on blood glucose level of hyperglycemic rats. MCR reduced the blood glucose level more than half its initial concentration over the period of 7 days (p<0.01); however, the standard drug was approximately twice as much active than MCR-250 in reducing blood glucose level in diabetic rats.

The phytochemical screening showed that the plant extract gave positive results for tannins, alkaloids, saponin and flavonoids. These compounds have been reported to elicit a wide range of biological activities such as insulin-like effects, anti-hypercholesterol, hypotensive and anti-diarrhoal activity [24-29]. In the current study, we have evaluated antidiarrheal and hypoglycaemic effects of methanol extract of seeds of C. rotang in rodents at doses of 250 and 500 mg/kg. Although medicinal plants are used as antidiarrheal agents in folk medicine, there are few scientific studies to explain their action and effectiveness as antidiarrheal agents. The treatment of acute diarrhea with the World Health Organization (WHO) standard oral rehydration solution (ORS) provides effective rehydration but does not reduce the severity of diarrhea [30]. In the castor oil-induced anti-diarrhoeal study it was found that MCR reduced the total number of faeces significantly (p < 0.01) compared to the control group and at a dose of 500mg/kg its antidiarrheal action was as effective as the...
standard drug loperamide. The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretions which are altered in this intestinal condition [28,29]. *In vitro* and *in vivo* experiments have shown that flavonoids are able to inhibit the intestinal secretory response induced by prostaglandins E₂ [31]. In addition, flavonoids present antioxidant properties [32] which are presumed to be responsible for the inhibitory effects exerted upon several enzymes including those involved in the arachidonic acid metabolism [33]. These constituents may be responsible for the anti-diarrheal activity of the MCR.

In the current research blood glucose levels were continuously reduced in OGTT and normoglycemic tests. The uninterrupted post-treatment for seven days with the MCR showed prospective hypoglycemic activity in antidiabetic activity in alloxan induced rat models. OGTT and normoglycemic rats and antidiabetic activity in alloxan-induced rat models. Here, alloxan was preferred to generate diabetic condition in rats because alloxan is a specific toxin that destroys the pancreatic β cells, provoking a state of primary deficiency of insulin without upsetting other islet types [34,35]. In other words, alloxan promotes an immense decline in insulin discharge by damaging β cells of the islets of Langerhans and thus stimulates hyperglycemia. Several plants were found to possess hypoglycemic effects and the possible means suggested for such hypoglycemic actions could be through the increased insulin secretion from β cells of islets of Langerhans or its release from bound insulin [36]. So, such hypoglycemic effects of plant extracts could also be due to their insulin like actions [37,38]. It is assumed that MCR exerts it hypoglycemic activity in the same approach. So the probable mechanism of action of MCR could be correlated with the reminiscent effect of the hypoglycemic sulphonylureas, which encourage insulin-secretion channels, membrane depolarization, and stimulation of Ca²⁺ influx, a preliminary key step in insulin emission [36]. Preliminary phytochemical investigation revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids and tannins in the plant extracts. On the basis of the above results, we speculate that MCRS may also have brought about antihyperglycemic action through stimulation of β-cells of islets of langerhans to release more insulin and this effect may be due to its constituents like saponins, flavonoids and glycosides [39]. Besides, flavonoid and terpenes isolated from other antidiabetic medicinal plants have been found to stimulate secretion or possess an insulin like-effect [40].

Though the crude extract of the investigated plant exhibited potent antidiabetic and antidiarrheal activities, we still don’t identify which of the components have the above possessions. Advanced studies are obligatory before it can be suggested for use as a nutritional supplement, health food and adjuvant in the management of diabetes and diarrheal disorders.

**Declaration of interest**

The author declares that there is no conflict of interest. The author alone is responsible for the content of the paper.

**References**


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