EFFECT OF PADDY HUSK EXTRACTS ON GLUCOSE TOLERANCE IN GLUCOSE-INDUCED HYPERGLYCEMIC MICE

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ABSTRACT

Background. Paddy husks are the by-products produced in the paddy milling industry and are usually discarded or burnt. Yet, the husk is rich in compounds like ferulic, vanillic and p-coumaric acids, which have good antioxidant capacities. Diabetes mellitus is becoming almost endemic throughout the world, and oxidative stress has been said to play a major role in diabetes associated hyperglycemia and other complications. It was the objective of this study to evaluate the antihyperglycemic potential of various solvent extracts of paddy husk.

Methods. Oral glucose tolerance test (OGTT) was used to determine antihyperglycemic activity. Methanol (MEPH), ethanol (EEPH), and an alkali-soluble (ASPH) extract of paddy husks were evaluated.

Results. Administration of MEPH, EEPH, and ASPH led to dose-dependent and significant reductions in blood glucose levels in glucose-loaded mice. At doses of 50, 100, 200 and 400 mg per kg body weight, MEPH reduced blood glucose levels by 36.8, 41.0, 56.4, and 58.6%, respectively compared to control animals. At the same extract doses, EEPH reduced blood glucose levels by 21.9, 35.0, 42.4, and 51.8%. ASPH at the same doses reduced blood glucose levels by 20.9, 37.9, 43.2, and 50.8%. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 53.5%. Conclusion. Antihyperglycemic activities have not previously been reported for paddy husk. Since husks are widely available in Bangladesh, they can be a good source for isolation of bioactive compounds towards lowering blood sugar in diabetic patients.

Key words: Antihyperglycemic, paddy, husk, OGTT, Poaceae.
INTRODUCTION

Diabetes mellitus (DM) is a complicated disease characterized by presence of high amounts of glucose levels in blood and urine. The disease is primarily due to low insulin production by the pancreatic β-cells or due to development of resistance to insulin. The disease is reaching almost endemic proportions in the world, possibly through changes in dietary habits and a more sedentary style of living in human beings. Diabetes can lead to increased risks of cardiovascular disorders as well as diabetic nephropathy, neuropathy, and retinopathy. According to the World Health Organization (WHO), there were around 171 million people in the world with diabetes in the year 2000, which number is projected to increase to 366 million by the year 2030. \(^1\) Allopathic medicine does not offer a total cure for diabetes, but is merely symptomatic.

Both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of DM through apoptosis of pancreatic β-cells. As a result scientific research has been going on with plants or plant-derived compounds, which are antioxidants and can, as such, relieve oxidative stress. Various studies have also centered on plant extracts or plant-derived components that can reduce high blood glucose levels (hyperglycemia) or are both antihyperglycemic as well as antioxidants. Hydroalcoholic extracts of *Phyllanthus emblica* and *Curcuma longa*, as well as aqueous extract of *Tinospora cordifolia* has been shown to have protective effect against streptozotocin (STZ)-induced diabetes and oxidative stress, and which effect was mediated through their antioxidant and antiapoptotic actions. \(^2\) Alcoholic extract of stem bark of *Tamarindus indica* and *Cassia fistula* have also been shown to exert an antihyperglycemic and antioxidant effect in alloxan diabetic rats along with protective effects on renal complications associated with hyperglycemia. \(^3\) Although similar promising effects have been noted with other plant extracts (for instance *Clitoria ternatea* \(^4\), *Houttuynia cordata* in STZ-diabetic rats \(^5\), and *Tamarindus indica* and *Cajanus cajan* in alloxan diabetic mice \(^6\)), a problem can be limited availability of appropriate plant materials.

Paddy belongs to the grass family (Poaceae) and is scientifically known as *Oryza sativa* L. Paddy husks are the by-products produced in the paddy milling industry. The husks are said to contain bioactive compounds like tricin \(^7\), and other antioxidant compounds like ferulic, p-coumaric, and vanillic acids. \(^8\) Bangladesh is a rice producing country and the staple diet of the people is rice, which is obtained after de-husking paddy. The husks are usually discarded or used to cover floors of poultry farms to absorb poultry excreta. Paddy husk is
available in huge amounts and at very low costs and as such can a form a cheap and affordable source of bioactive compounds. Since husks are known to contain antioxidants, the objective of the present study was to evaluate various solvent extracts of paddy husks on glucose tolerance in glucose-loaded hyperglycemic mice.

METHODS

Paddy husk collection
Paddy husks were collected from a local rice selling shop in Dhaka city, Bangladesh during February 2014.

Preparation of methanolic extract of husks (MEPH)
Husks were washed thoroughly with de-ionized distilled water, air-dried in the shade, and 50g of dried and powdered husks were extracted with methanol (w:v ratio of 1:5, final weight of the extract 1.45g).

Preparation of ethanolic extract of husks (EEPH)
Husks were washed thoroughly with de-ionized distilled water, air-dried in the shade, and 50g of dried and powdered husks were extracted with ethanol (w:v ratio of 1:5, final weight of the extract 1.57g).

Preparation of alkali-soluble extract of husks (ASPH)
Husks were washed thoroughly with de-ionized distilled water, air-dried in the shade, and 100g of dried and powdered husks were boiled in 0.2N NaOH (w:v ratio of 1:5) for 1h at 95°C. The suspension was cooled to room temperature and the NaOH-soluble portion was separated from un-dissolved material by filtration. The filtrate was brought to acidic pH with the addition of HCl, and the resultant precipitate was filtered, washed once with distilled water and once with absolute ethanol briefly. Following washing, the precipitate was dried at 60°C (final weight of the extract 8.98g).

Chemicals and Drugs
Glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals
Swiss albino mice, which weighed between 14-19 g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research,
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Bangladesh (ICDDR,B). The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

**Oral glucose tolerance tests for evaluation of antihyperglycemic activity**

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999) \[^9\] with minor modifications. Briefly, fasted mice were grouped into fourteen groups of six mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received MEPH at doses of 50, 100, 200 and 400 mg per kg body weight. Groups 7-10 received EEPH at doses of 50, 100, 200 and 400 mg per kg body weight. Groups 11-14 received ASPH at doses of 50, 100, 200 and 400 mg per kg body weight. All substances were orally administered. Following a period of one hour, all mice were orally administered 2 g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method. \[^10\] The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = (1 – \(W_e/W_c\)) X 100, where \(W_e\) and \(W_c\) represents the blood glucose concentration in glibenclamide or MEPH, EEPH, ASPH administered mice (Groups 2-14), and control mice (Group 1), respectively.

**Acute toxicity test**

Acute toxicity test was conducted as previously described. \[^11\] Mice were divided into nine groups, each group consisting of six animals. Group 1 was given 1% Tween 80 in normal saline (2 ml per kg body weight). The other eight groups (Groups 2-9) were administered, respectively, 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg of MEPH per kg body weight. All animals were closely observed for the next 8 hours to notice any behavioral changes or mortality and were kept under close observation for the next two weeks. Similar experiments were conducted with mice administered EEPH and ASPH.

**Statistical analysis**

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases. \[^12\]
RESULTS
Administration of MEPH, EEPH, and ASPH led to dose-dependent and significant reductions in blood glucose levels in glucose-loaded mice. At doses of 50, 100, 200 and 400 mg per kg body weight, MEPH reduced blood glucose levels by 36.8, 41.0, 56.4, and 58.6%, respectively compared to control mice. At the same extract doses, EEPH reduced blood glucose levels by 21.9, 35.0, 42.4, and 51.8%. ASPH at the same doses reduced blood glucose levels by 20.9, 37.9, 43.2, and 50.8%. By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight, reduced blood glucose level by 53.5%. The results are shown in Table 1.

Toxicity evaluation
The crude extracts did not show any toxicity in mice even at the highest dose tested.

Table 1: Effect of various extracts of paddy husk on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg body weight)</th>
<th>Blood glucose level (mmol/l)</th>
<th>% lowering of blood glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10 ml</td>
<td>5.85 ± 0.20</td>
<td>-</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>10 mg</td>
<td>2.72 ± 0.32</td>
<td>53.5*</td>
</tr>
<tr>
<td>MEPH</td>
<td>50 mg</td>
<td>3.70 ± 0.11</td>
<td>36.8*</td>
</tr>
<tr>
<td>MEPH</td>
<td>100 mg</td>
<td>3.45 ± 0.25</td>
<td>41.0*</td>
</tr>
<tr>
<td>MEPH</td>
<td>200 mg</td>
<td>2.55 ± 0.10</td>
<td>56.4*</td>
</tr>
<tr>
<td>MEPH</td>
<td>400 mg</td>
<td>2.42 ± 0.22</td>
<td>58.6*</td>
</tr>
<tr>
<td>EEPH</td>
<td>50 mg</td>
<td>4.57 ± 0.29</td>
<td>21.9*</td>
</tr>
<tr>
<td>EEPH</td>
<td>100 mg</td>
<td>3.80 ± 0.18</td>
<td>35.0*</td>
</tr>
<tr>
<td>EEPH</td>
<td>200 mg</td>
<td>3.37 ± 0.33</td>
<td>42.4*</td>
</tr>
<tr>
<td>EEPH</td>
<td>400 mg</td>
<td>2.82 ± 0.23</td>
<td>51.8*</td>
</tr>
<tr>
<td>ASPH</td>
<td>50 mg</td>
<td>4.63 ± 0.42</td>
<td>20.9*</td>
</tr>
<tr>
<td>ASPH</td>
<td>100 mg</td>
<td>3.63 ± 0.20</td>
<td>37.9*</td>
</tr>
<tr>
<td>ASPH</td>
<td>200 mg</td>
<td>3.32 ± 0.21</td>
<td>43.2*</td>
</tr>
<tr>
<td>ASPH</td>
<td>400 mg</td>
<td>2.88 ± 0.13</td>
<td>50.8*</td>
</tr>
</tbody>
</table>

All administrations were made orally. Values represented as mean ± SEM, (n=6); *P < 0.05; significant compared to hyperglycemic control animals.

DISCUSSION
The various extracts at the doses tested gave comparable results, with the methanol extract (MEPH) showing more antihyperglycemic activity than EEPH or ASPH. The percent lowering of blood sugar at the two highest doses of MEPH (200 and 400 mg) was greater than that obtained with glibenclamide and suggests that the extract can be used for lowering
blood sugar in hyperglycemic patients. However, at the highest doses, i.e. 400 mg, the other extracts (EEPH and ASPH) also showed antihyperglycemic activity, which was nearly equal to that obtained with glibenclamide.

Paddy husk reportedly contains bioactive compounds like tricin, p-coumaric acid, ferulic acid, and vanillic acid, which are also good antioxidants. The antioxidant potential of tricin has been previously reported. Antioxidant and inhibition of porcine pancreatic amylase activity has been reported for clonal oregano (Origanum vulgare) extracts, and which activities have been attributed to a number of phenolic compounds, including p-coumaric acid. Aqueous solutions of cranberry powder containing significant amounts of p-coumaric acid has been shown to exhibit antioxidant properties and inhibit α-glucosidase activity, and as such can be used for alleviation of hyperglycemia. Ferulic and p-coumaric acids present in Sambucus nigra flowers have been shown to increase glucose uptake in primary porcine myotube cultures and reduce fat accumulation in Caenorhabditis elegans, and so can be potential antihyperglycemic agents.

Ferulic acid has been shown to demonstrate antioxidant activity and reduce blood glucose levels in STZ-induced diabetic mice as well as KK-Ay mice, a model of non-insulin dependent diabetes mellitus (NIDDM). STZ-induced diabetic rats exhibited significant elevation in fasting blood glucose level and in the activity of hepatic glucose-6-phosphatase. Glycogen levels were diminished in liver and skeletal muscle along with diminution in the activities of hepatic glucose-6-phosphate dehydrogenase, catalase and peroxidase in diabetic rats when compared with controls. Hepatic levels of thiobarbituric acid reactive substance (TBARS) and conjugated dienes (CD) were elevated in respect to control. Administration of an ethereal fraction of ethanolic extract of Syzygium cumini was found to reverse these effects. Further studies revealed that the fraction contained ferulic acid. Thus ferulic acid demonstrated both antioxidant and antihyperglycemic effects. In obese diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats, ferulic acid has been shown to significantly decrease blood glucose levels and exert a protective action against diabetic nephropathy. Antihyperglycemic and antioxidant effects of a combination of ferulic acid and resveratrol have been shown in alloxan-induced diabetic mice. Treatment of ferulic acid of STZ-diabetic rats significantly improved blood glucose, serum total cholesterol, triglycerides, creatinine, urea, and albumin levels toward normal. At the same time, the compound showed antioxidant effect and reduced STZ-induced apoptosis of pancreatic β-cells.
It is interesting to note that besides acting alone, ferulic and vanillic acids can produce a synergistic or additive effect when combined with existing allopathic drugs against diabetes. For instance, ferulic acid has been shown to act synergistically with drugs like metformin or Thiazolidinedione to lower blood glucose levels and improve liver and kidney functions in STZ-diabetic rats. [22] Vanillic acid showed an additive effect with 2,4-thiazolidinedione in enhancing 2-deoxyglucose uptake in 3T3-L1 adipocytes. [23] Thus these compounds may play a beneficial role in reducing the amount of antidiabetic drugs taken by a patient and so reduce the drug’s any adverse side-effects.

Although the exact component(s) responsible for the observed antihyperglycemic or oral glucose tolerance effects observed in the present study has not been elucidated, all the reported compounds in paddy husk, namely tricin, ferulic acid, vanillic acid, and p-coumaric acid could have acted alone or in combination to produce the observed effects. Studies are ongoing in our laboratory to identify the responsible components and find out the mechanisms behind the antihyperglycemic effect. Paddy husk can be obtained cheaply in Bangladesh. As such, the husks can be an excellent source for isolation of these compounds. It is important that ferulic, vanillic, and p-coumaric acid have both reported antioxidant and antihyperglycemic properties. From that view point, paddy husk can play a role in not only alleviating high glucose levels in diabetic patients and alleviating diabetes-induced complications, as well as lower dependency on costly allopathic medicines, but also play a beneficial role in other diseases involving oxidative stress like cardiovascular and neurodegenerative disorders.

CONCLUSION

The results suggest that the various extracts of paddy husk can be used for lowering of blood glucose.

Conflicts of interest

The author(s) declare that they have no competing interests.

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