

# Antihyperglycemic studies with boiled and non-boiled *Vigna mungo* seeds

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## ABSTRACT

*Vigna mungo* is a common leguminous crop cultivated in Bangladesh for its edible seeds, which are consumed following boiling or cooking. Since many lentil plants are known to have antihyperglycemic activity, it was of interest to determine the antihyperglycemic potential of seeds of *V. mungo* both in its boiled and non-boiled form. In oral glucose tolerance tests conducted in glucose-loaded Swiss albino mice, crude methanol extract of non-boiled seeds reduced blood glucose levels by 29.1, 36.5, 42.6, and 48.9%, respectively, at doses of 50, 100, 200, and 400 mg per kg body weight. At the afore-mentioned four doses, the percent reductions in blood glucose levels were, respectively, 24.1, 35.1, 39.4, and 46.5% with crude methanol extract of boiled seeds. Glibenclamide, a standard antihyperglycemic drug was observed to reduce blood glucose levels by 48.2%, when administered at a dose of 10 mg per kg. Thus the extracts of both non-boiled and boiled seeds demonstrated good antihyperglycemic potential comparable to glibenclamide and can be used as a readily available alternative to alleviate high blood glucose levels in diabetic patients.

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## INTRODUCTION

*Vigna mungo* (L.) Hepper is a legume crop cultivated in Bangladesh for its edible seeds, which are consumed following boiling in water and taken as lentil soup, or consumed in the cooked form with other vegetables, fish or meat. The plant belongs to the Fabaceae family and is known in English as black gram, black lentil or mungo bean and in Bangladesh as mashkalai dal. Seeds of the plant have been reported to have considerable hypolipidemic action (Indira and Kurup, 2003). Various solvent extracts of the seeds have been shown to demonstrate immunostimulatory activity (Dhumal *et al.*, 2013). In our ongoing program for antihyperglycemic and analgesic plants of Bangladesh (Morshed *et al.*, 2010; Rahmatullah *et al.*, 2010; Ahmed *et al.*, 2011; Shahreen *et al.*, 2012; Haque *et al.*, 2013; Haque *et al.*, 2014; Rahmatullah *et al.*, 2013a,b; Ghosh *et al.*, 2014; Hossain *et al.*, 2014; Jahan *et al.*, 2014; Rahman *et al.*, 2014; Tazin *et al.*, 2014), it was of interest to conduct such antihyperglycemic studies on *V. mungo* seeds. Moreover, since

the seeds are consumed in the boiled form, it was of further interest to evaluate the comparative antihyperglycemic potential of both non-boiled and boiled seeds. Antihyperglycemic activity evaluation was conducted through oral glucose tolerance tests (OGTT) in glucose-loaded Swiss albino mice.

## MATERIALS AND METHODS

### Plant material collection

Seeds of *V. mungo* were collected during May 2015 from a local market in Dhaka city, Bangladesh.

### Preparation of methanolic extract of non-boiled and boiled seeds

For preparation of extract of non-boiled seeds, seeds were de-husked, thoroughly dried and powdered and 150g of powdered seeds were extracted with methanol (w:v ratio of 1:5), final weight of the extract (6.686g). For preparation of extract of boiled seeds, de-husked and dried seeds were first steamed for 15 minutes over boiling water, then dried and powdered followed by extraction with methanol (w:v ratio of 1:5), final weight of the extract (5.069g).

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## 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 12-16g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, 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 (OGTT) were carried out as per the procedure previously described by Joy and Kuttan (1999) with minor modifications. Briefly, fasted mice were grouped into ten groups of five 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 methanolic non-boiled seed extract (MEVM) at doses of 50, 100, 200 and 400 mg per kg body weight. Groups 7-10 received boiled seed extract (MEVM) 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 2g 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 (Venkatesh *et al.*, 2004). The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level =  $(1 - W_e/W_c) \times 100$ , where  $W_e$  and  $W_c$  represents the blood glucose concentration in glibenclamide or MEVM administered mice (Groups 2-10), and control mice (Group 1), respectively.

## Statistical analysis

Experimental values are expressed as mean  $\pm$  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 (Hossain *et al.*, 2014).

## RESULTS AND DISCUSSION

In oral glucose tolerance tests conducted with glucose-loaded Swiss albino mice, crude methanol extract of non-boiled seeds reduced blood glucose levels by 29.1, 36.5, 42.6, and 48.9%, respectively, at doses of 50, 100, 200, and 400 mg per kg body weight. At the afore-mentioned four doses, the percent reductions in blood glucose levels were, respectively, 24.1, 35.1, 39.4, and 46.5% with crude methanol extract of boiled seeds.

Glibenclamide, a standard antihyperglycemic drug was observed to reduce blood glucose levels by 48.2%, when administered at a dose of 10 mg per kg. The results are shown in Table 1.

**Table 1:** Effect of crude methanol extract of non-boiled and boiled *V. mungo* seeds (MEVM) on blood glucose level in hyperglycemic mice following 120 minutes of glucose loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	5.64 $\pm$ 0.21	-
Glibenclamide	10 mg	2.92 $\pm$ 0.29	48.2*
(MEVM) non-boiled	50 mg	4.00 $\pm$ 0.48	29.1*
(MEVM) non-boiled	100 mg	3.58 $\pm$ 0.45	36.5*
(MEVM) non-boiled	200 mg	3.24 $\pm$ 0.45	42.6*
(MEVM) non-boiled	400 mg	2.88 $\pm$ 0.43	48.9*
(MEVM) boiled	50 mg	4.28 $\pm$ 0.28	24.1*
(MEVM) boiled	100 mg	3.66 $\pm$ 0.32	35.1*
(MEVM) boiled	200 mg	3.42 $\pm$ 0.24	39.4*
(MEVM) boiled	400 mg	3.02 $\pm$ 0.30	46.5*

All administrations were made orally. Values represented as mean  $\pm$  SEM, (n=5); \*P < 0.05; significant compared to hyperglycemic control animals.

The results indicate that MEVM (non-boiled) at the highest dose of 400 mg per kg was comparable in its blood glucose lowering efficacy to glibenclamide and can be substituted for the drug. MEVM (boiled) at all doses gave lower glucose lowering efficacy than the non-boiled extract, but the effects were only marginally lower. Thus at the highest dose of MEVM (non-boiled), the extract lowered blood glucose levels by 48.9%, while the extract (boiled) at the same dose, lowered blood glucose levels by 46.5%. Taken together, the results suggest that both non-boiled as well as boiled seed extract can be used for lowering blood glucose. Since the seeds of *V. mungo* are readily available and affordable in Bangladesh (*V. mungo* seeds are the lowest-priced lentil or pulse seed in Bangladesh) and are edible, the seeds can offer a cheaper alternative to diabetic patients for lowering blood glucose.

Legumes have been reported to lower glycemic responses by slowing down the absorption of carbohydrates or inhibiting its digestion. The causative agents behind these actions reportedly include dietary fiber and uronic acid in the legumes (Baer *et al.*, 1998). Since *V. mungo* is a leguminous crop, the above can explain the observed antihyperglycemic effect of the seeds, which contains high levels of fiber (Khan *et al.*, 2009) and so may have decreased absorption of glucose. This may also explain the somewhat lower glucose lowering efficacy of the seeds following steaming. Steaming may have partially digested some fibers and so enabled more glucose uptake.

Diets rich in legumes have been reported to lower the risk of diabetes and improve glycemic control (Ley *et al.*, 2014). Antioxidants in horse gram (a leguminous crop) have been postulated to reduce the risk of diabetes (Prasad and Singh, 2015). Dietary supplementation with fermented legumes has been shown to modulate hyperglycemia and acetylcholinesterase activities in streptozotocin-induced diabetes (Ademiluyi *et al.*, 2015). Thus *V. mungo* or black gram can also be a potential dietary factor in reducing the risk of diabetes incidences and lowering blood

glucose. As such, the seeds deserve scientific attention towards identifying the mechanism responsible for the observed antihyperglycemic effect.

## CONCLUSION

The results suggest that methanolic extract of *V. mungo* non-boiled as well as boiled seed extract can be used for lowering of blood glucose.

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The authors declare that they have no conflicts of interest.

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