

Antihyperglycemic and analgesic activity studies with boiled *Cicer arietinum* L. seeds

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ABSTRACT

In oral glucose tolerance tests, methanol extract of boiled seeds of *Cicer arietinum* (MECA) significantly and dose-dependently reduced blood glucose levels in glucose-loaded mice by 27.7, 31.9, 36.2, and 42.2%, respectively, at doses of 50, 100, 200 and 400 mg per kg in comparison to a standard antihyperglycemic drug, glibenclamide, which reduced blood glucose levels by 48.2% at a dose of 10 mg per kg. In acetic acid induced writhing tests in mice, MECA at the afore-mentioned doses reduced the number of writhings, respectively, by 21.4, 35.7, 39.3, and 46.4% versus the 42.9 and 53.6% reductions observed with a standard analgesic drug, aspirin, at doses of 200 and 400 mg per kg, respectively. MECA thus demonstrated considerable antihyperglycemic and analgesic properties and may be used to alleviate high blood glucose levels and provide relief from pain.

INTRODUCTION

Cicer arietinum L. is a legume belonging to the Fabaceae family. In English, it is known as Bengal gram, chick pea or garbanzo bean, while in Bangladesh it is known as boot dal or booter dal. The plant is cultivated widely in Bangladesh, because of its edible seeds, which are consumed in the boiled, fried and the cooked form. It is a non-expensive but popular food item in the country, being consumed both as a snack as well as a side dish with rice. Intake of chick peas has been recommended for humans suffering from type IIa hyperlipoproteinemia with altered lipid profile (Zulet *et al.*, 1999). In healthy middle-aged men and women, consumption of a chick pea meal led to substantially lower plasma glucose levels than the control group (Nestel *et al.*, 2004). It has been reported that vanadium-enriched chickpea sprout ameliorated hyperglycemia and impaired memory in streptozotocin-induced diabetes rats (Mao *et al.*, 2008). It has been shown that increased antioxidant and inhibitory potential of sprouted Bengal gram (chick pea) against

α -glucosidase and α -amylase makes them desirable for dietary management/prevention of diabetes (Prathapan *et al.*, 2011). Total saponins from chick pea reportedly demonstrated renal protection when administered to type 2 diabetes mellitus rats (Kaiser *et al.*, 2012). An antioxidant-rich extract of chick pea and its sprouts reportedly mitigated starch-induced postprandial glycemic spikes in rats (Tiwari *et al.*, 2013). Nine compounds have been isolated and identified from chick pea (seeds), namely, 3-hydroxy-olean-12-ene (1), biochanin A-7-O- β -D-glucoside (2), cerebroside (3), 1-ethyl- α -L-galactoside (4), uridine (5), adenosine (6), tryptophan (7), biochanin A (8), and fomononetin (9) (Tan *et al.*, 2007). Methanolic extract of chick pea grown in Pakistan reportedly inhibited arachidonic acid (AA) induced platelet aggregation (Ziaul-Haq *et al.*, 2012). Thus chick pea (seeds) has the potential for lowering blood glucose levels and alleviating inflammation.

Diabetes and pain are common problems in Bangladesh, and we have been investigating local plants and plant parts for their blood glucose lowering and pain alleviating effects (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; Akter *et al.*, 2014; Hossain *et al.*, 2014; Jahan *et al.*, 2014; Rahman *et al.*, 2014; Tazin *et al.*, 2014).

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Our study has mainly focused on commonly available plants or plant parts, which can be afforded by the population as a whole. The objective of the present study was to evaluate the antihyperglycemic and analgesic potential of methanolic extract of *C. arietinum* seeds (chick pea) that have been boiled by steaming.

MATERIALS AND METHODS

Plant material collection

Seeds of *C. arietinum* were collected during May 2015 from a local market in Dhaka City, Bangladesh.

Preparation of methanolic extract of boiled seeds

Dried seeds were steamed over boiling distilled water for 15 minutes, which is the average time used for cooking the seeds in Bangladesh. Steamed seeds were next dried, pulverized into a fine powder and 200g of powder was extracted with 1 liter methanol (final weight of the extract 9.986g). Extract was dissolved in Tween 20 prior to administration to mice by gavaging.

Chemicals and Drugs

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

Animals

Swiss albino mice, which weighed between 11-15g 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 six groups of five mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 20 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 boiled seed extract (MECA) dissolved in Tween 20 at doses of 50, 100, 200 and 400 mg per kg body weight. All substances were orally administered by gavaging. The amount of Tween 20 administered was same in both control and experimental mice. Following a period of one hour as described earlier (Haque *et al.*, 2013; Ghosh *et al.*, 2014), all mice were orally administered 2g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart following previously published procedures (Haque *et al.*, 2013; Ghosh *et al.*, 2014). 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 MECA administered mice (Groups 2-6), and control mice (Group 1), respectively.

Analgesic activity evaluation through abdominal writhing test

Analgesic activity of methanolic extract of boiled seeds (MECA) was examined as previously described (Shanmugasundaram and Venkataraman, 2005). Mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MECA at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or MECA, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 15 minutes was given to each animal to ensure bioavailability and onset of chemically induced irritation of acetic acid after a slight modification of procedure described earlier (Akter *et al.*, 2014), following which period, the number of abdominal constrictions (writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

$$\text{Percent inhibition} = (1 - W_e/W_c) \times 100$$

Where W_e and W_c represents the number of writhings in aspirin or MECA administered mice (Groups 2-7), 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

MECA, when administered to mice at doses of 50, 100, 200, and 400 mg per kg body weight, caused dose-dependent and significant reductions in blood glucose levels in mice. At these four doses, the percent reductions in blood glucose levels were, respectively, 27.7, 31.9, 36.2, and 42.2%. In comparison, a standard antihyperglycemic drug, glibenclamide, when administered to mice at a dose of 10 mg per kg, caused a significant drop in blood glucose level by 48.2%. The results are shown in Table 1 and suggest that at the highest dose, MECA has blood glucose lowering activity almost comparable to that of glibenclamide. The results are interesting in the sense that MECA can be an affordable and readily available substitute for costlier blood glucose lowering allopathic drugs, which moreover may not be readily available in rural areas of Bangladesh. In analgesic

activity tests, MECA at doses of 50, 100, 200, and 400 mg per kg, also caused dose-dependent reductions in the number of writhings caused by intraperitoneal administration of acetic acid in mice. At these four doses, the percent reductions in the number of writhings were, respectively, 21.4, 35.7, 39.3, and 46.4%. While the result with the dose of 50 mg per kg was not significant, the higher doses produced statistically significant reductions in the number of writhings. A standard analgesic drug, aspirin, when administered at doses of 200 and 400 mg kg, inhibited the number of writhings by 42.9 and 53.6%, respectively. The results are shown in Table 2. Intraperitoneal administration of acetic acid causes pain sensation in mice, which is demonstrated by the number of abdominal constrictions or writhings. It is apparent from the results that the highest dose of MECA was comparatively better in relieving pain than 200 mg per kg aspirin.

Table 1: Effect of crude methanol extract of *C. arietinum* boiled seeds (MECA) 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 ± 0.21	-
Glibenclamide	10 mg	2.92 ± 0.29	48.2*
(MECA)	50 mg	4.08 ± 0.34	27.7*
(MECA)	100 mg	3.84 ± 0.08	31.9*
(MECA)	200 mg	3.60 ± 0.20	36.2*
(MECA)	400 mg	3.26 ± 0.17	42.2*

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

What is interesting in the results that methanolic extract of boiled (steamed) seeds of *C. arietinum* caused both lowering of blood glucose and alleviation of pain in glucose-challenged and acetic acid administered mice, respectively. The percent yield of the extract from boiled seeds was roughly 5%. Thus 400 mg per kg of the extract roughly translates to 8g of boiled seeds per kg. The results thus suggest that boiled seeds of *C. arietinum*, when incorporated into diet, can play a significant role in lowering blood glucose in diabetic patients and also can provide pain relief to persons suffering from chronic or acute pain. It is therefore important to continue trials on human volunteers to see whether the same effects can be observed in humans.

Legumes like *C. arietinum* have high fiber content, which can slow absorption of carbohydrates from the intestine. As such, legumes have been reviewed as a potential functional food for both prevention and management of diabetes (Venn and Mann, 2004). Low glycemic indexes of several boiled legumes consumed in Nigeria have been reported (Oboh *et al.*, 2010). Since the staple food of the people of countries of South and Southeast Asia is rice, addition of legumes like *C. arietinum* seeds to diet can therefore be of considerable benefit to diabetic patients as confirmed in the present study. It is also interesting to note in this context that previous studies have also pointed out the efficacy of *C. arietinum* seeds in lowering blood glucose levels in streptozotocin as well as alloxan diabetic rodent models (Yadav *et al.*, 2009; Prabha *et al.*, 2012; Tiwari *et al.*, 2013). Thus the results obtained in the present

study on antihyperglycemic activity of *C. arietinum* seeds are in agreement with previous studies.

The anti-inflammatory activity of *C. arietinum* seed extracts has been reported (Zia-ul-Haq *et al.*, 2012). However, to our knowledge, this is the first report on analgesic activity evaluation in boiled seeds of *C. arietinum*. The exact nature of the phytochemical(s) responsible for the observed antihyperglycemic and analgesic effects were not determined in this study and is now currently under investigation in our laboratory.

Table 2: Analgesic effect of crude methanol extract of *C. arietinum* boiled seeds (MECA) in acetic acid-induced pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control	10 ml	5.6 ± 0.24	-
Aspirin	200 mg	3.2 ± 0.58	42.9*
Aspirin	400 mg	2.6 ± 0.40	53.6*
(MECA)	50 mg	4.4 ± 0.60	21.4
(MECA)	100 mg	3.6 ± 0.40	35.7*
(MECA)	200 mg	3.4 ± 0.24	39.3*
(MECA)	400 mg	3.0 ± 0.45	46.4*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); **P* < 0.05; significant compared to control.

CONCLUSION

The results suggest that methanolic extract of *C. arietinum* boiled seeds can be used for lowering of blood glucose and for alleviating pain.

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