

ANTIHYPERGLYCEMIC ACTIVITY STUDIES WITH NON-BOILED AND BOILED *CAJANUS CAJAN* (L.) MILLSP. SEEDS

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ABSTRACT

Background. *Cajanus cajan* is a leguminous plant with edible seeds. Since diabetes, which causes elevated blood glucose levels and leads to often fatal complications, is reaching alarming proportions throughout the world, it was of interest to evaluate various plants of Bangladesh for their potential antihyperglycemic activity. The objective of this study was to evaluate the antihyperglycemic potential of boiled and non-boiled seeds of *Cajanus cajan*. **Methods.** Oral glucose tolerance test was done to evaluate antihyperglycemic potential. **Results.** In oral glucose tolerance tests, methanol extract of non-boiled seeds of *Cajanus cajan* (MECCNB) significantly and dose-dependently reduced blood glucose levels in glucose-loaded mice by 19.0, 31.4, 33.3, and 41.2%, respectively, at doses of 50, 100, 200 and 400 mg per kg body weight in mice. At the afore-mentioned four doses, methanolic extract

of boiled seeds (MECCB) dose-dependently and significantly reduced blood glucose levels in glucose loaded mice, respectively, by 12.7, 21.6, 34.3, and 34.3%. By comparison, a standard antihyperglycemic drug, glibenclamide, reduced blood glucose levels by 43.8% at a dose of 10 mg per kg. **Conclusion.** Since the seeds form a common pulse item consumed by all sections of the population in Bangladesh, both non-boiled and boiled seeds, particularly the latter (since they are consumed frequently) can form an affordable means of lowering blood glucose in diabetic patients of the country.

KEYWORDS: Antihyperglycemic, *Cajanus cajan*, Arhar, Fabaceae.

BACKGROUND

Cajanus cajan (L.) Millsp. (Fabaceae) is a leguminous crop known in English as pigeon pea and in Bangladesh as arhar dal. The plant is cultivated in Bangladesh for its seeds, which are cooked and taken with rice as a pulse dish. The seeds are mostly consumed by the poorer sections of the population because of its ready availability and cheapness of price and offer a much needed protein source to the population who cannot afford fish or meat.

Acetone extract of germinated seeds of the plant has been reported to reduce blood glucose levels in alloxan-induced diabetic rats.^[1] Methanol extract of leaves has been shown to lower elevated blood glucose levels in alloxan-induced diabetic rats.^[2] Antihyperglycemic activity of various solvent extracts of leaves of the plant in alloxan-diabetic mice has also been reported.^[3]

Diabetes is a common disorder throughout the world and the number of diabetic patients (characterized primarily by elevated blood glucose levels) is rising throughout the world. Available antihyperglycemic drugs or insulin injections are not always available or affordable particularly to the rural people of Bangladesh, who, however, also suffer considerably from diabetes.^[4] To provide the diabetic population with easily affordable and available drugs, we had been experimenting with various local plants and formulations for their blood glucose lowering effects.^[5-17]

Since *C. cajan* is a commonly available legume crop of Bangladesh with edible seeds, the objective of the present study was to evaluate the antihyperglycemic potential of methanolic extract of *C. cajan* seeds (both non-boiled and those that have been boiled by steaming).

Methods

Plant material collection

Seeds of *C. cajan* were collected during January 2016 from a local market in Dhaka City, Bangladesh.

Preparation of methanolic extract of non-boiled and boiled seeds

For preparation of methanol extract of non-boiled seeds (MECCNB), seeds were thoroughly dried and pulverized into a fine powder. 100g of the powder was extracted with 500 ml methanol over 48 hours. Methanol was evaporated at 50°C and the extract was dissolved in

Tween 20 prior to administration to mice by gavaging. For preparation of methanol extract of boiled seeds (MECCB), dried seeds were steamed over boiling distilled water for 20 minutes, which is the average time used for cooking the seeds in Bangladesh. Steamed seeds were next dried, pulverized into a fine powder and 100g of powder was extracted with 500 ml methanol (rest of the procedure same as described for preparation of MECCNB). The final weight of the extract was around 12g for both MECCNB and MECCB.

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 12-15g 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.^[18] 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 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 MECCNB dissolved in Tween 20 at doses of 50, 100, 200 and 400 mg per kg body weight. Groups 7-10 received MECCB 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,^[8, 14] 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.^[8, 14] Blood glucose levels were measured by glucose oxidase method^[19]. 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 MECCNB or MECCB administered mice (Groups 2-10), and control mice (Group 1), respectively. Experiments were conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

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.^[14]

RESULTS

In oral glucose tolerance tests, methanol extract of non-boiled seeds of *Cajanus cajan* (MECCNB) significantly and dose-dependently reduced blood glucose levels in glucose-loaded mice by 19.0, 31.4, 33.3, and 41.2%, respectively, at doses of 50, 100, 200 and 400 mg per kg body weight in mice. At the afore-mentioned four doses, methanolic extract of boiled seeds (MECCB) dose-dependently and significantly reduced blood glucose levels in glucose loaded mice, respectively, by 12.7, 21.6, 34.3, and 34.3%. By comparison, a standard antihyperglycemic drug, glibenclamide, reduced blood glucose levels by 43.8% at a dose of 10 mg per kg. The results are shown in Table 1. There are two interesting features within the results. The first is that extract of *C. cajan* seeds can lower blood glucose levels. The second feature is that this antihyperglycemic property of the seeds, even though a little lower following steaming, still persists suggesting that cooked seeds possibly can also be used for lowering blood glucose levels. Thus dietary intake of an edible pulse can be an affordable and readily available substitute for allopathic drugs. This not only can reduce medical costs but also permit daily diet to result in diabetic therapy in a natural manner.

Table 1: Effect of crude methanol extract of *C. cajan* non-boiled (MECCNB) and boiled (MECCB) seeds 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	6.12 ± 0.16	-
Glibenclamide	10 mg	3.44 ± 0.22	43.8*
(MECCNB)	50 mg	4.96 ± 0.28	19.0*
(MECCNB)	100 mg	4.20 ± 0.35	31.4*
(MECCNB)	200 mg	4.08 ± 0.21	33.3*
(MECCNB)	400 mg	3.60 ± 0.13	41.2*

(MECCB)	50 mg	5.34 ± 0.34	12.7*
(MECCB)	100 mg	4.80 ± 0.31	21.6*
(MECCB)	200 mg	4.02 ± 0.22	34.3*
(MECCB)	400 mg	4.02 ± 0.31	34.3*

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

DISCUSSION

An important question is how can *C. cajan* seeds reduce blood glucose? It may be noted that legumes are naturally high in dietary fiber and so can slow absorption of carbohydrates from the intestine. As such, legumes can be used for both prevention and control of diabetes.^[20] Interestingly, low glycemic indexes of several boiled legumes consumed in Nigeria have been reported.^[21]

The methanolic extract of roots of *C. cajan* has also been reported to reduce blood glucose levels in alloxan diabetic mice.^[22] Thus it appears that leaves, seeds and roots of the plant all have antihyperglycemic activities ^[2, 22, this study]. The plant can, therefore, in totality serve as a good source of antidiabetic agents and merits further research towards possible discovery of novel antidiabetic compounds.

CONCLUSION

The results suggest that methanolic extract of *C. cajan* boiled and non-boiled seeds 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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Author's contributions

HA, MK and MHF collected the seeds, did the extraction, and performed the experiments under the supervision of MR. MR wrote the manuscript draft, which was read and edited by all authors. All authors read and approved the final version of the manuscript.

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