

ORIGINAL ARTICLE

A Study on Antinociceptive and Anti-hyperglycemic Activity of Methanol Extract of *Brassica Juncea* (L.) Czern. Leaves in Mice

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ABSTRACT

Brassica juncea (L.) Czern. is widely cultivated in Bangladesh for both its edible green leaves and for its seeds from which oil is extracted and used for cooking. In folk medicinal practices, the oil is rubbed on the throat and chest for treatment of common colds with mucus. The objective of the present study was to investigate antinociceptive effects of methanol extract of leaves of the plant in acetic acid-induced gastric pain writhing models in mice, and anti-hyperglycemic effect in oral glucose tolerance tests in glucose-loaded mice. In antinociceptive tests, the methanol extract of leaves demonstrated dose-dependent and significant antinociceptive activity. At a dose of 200 mg leaf extract/kg body weight, writhing induced by acetic acid in Swiss albino mice was inhibited by 43.9%, which was the same as that observed with the standard drug aspirin when administered at a dose of 200 mg/kg body weight. Maximum inhibition of writhing was observed with an extract dose of 400 mg/kg body weight (75.7%). In oral glucose tolerance tests, the methanol extract of leaves also demonstrated significant and dose-dependent glucose lowering activity. The extract, when administered at a dose of 200 mg/kg body weight reduced serum glucose levels in glucose-loaded mice by 46.79% versus 73.40% obtained when mice were administered a standard hypoglycemic drug glibenclamide at a dose of 10 mg/kg body weight. Overall, the results suggest that the methanol extract of leaves contain constituents, which can prove to be valuable tools for treatment of pain as well as controlling of blood sugar in diabetic patients.

Key words:

Introduction

Brassica juncea (L.) Czern. also known as mustard greens, Indian mustard, Chinese mustard, and leaf mustard belongs to the family Cruciferae and is widely cultivated in Bangladesh for its edible green leaves and for its seeds from which an oil is extracted and used for cooking purposes. In the traditional medicinal systems of India, and particularly the Ayurveda, the plant has been reported to have anti-diabetic potential (Grover, J.K., 2002). In experiments conducted with rats the plant has been shown to have significant hypoglycemic action (Khan, B.A., 1995). The seeds of the plant when fed

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to rats along with a fructose-enriched diet reportedly prevented the development of insulin resistance (Yadav, S.P., 2004). Ethyl acetate fractions of leaves of the plant reportedly protected against oxidative stress in streptozotocin-induced diabetic animals (Yokozawa, T., 2003). The antioxidant effects of isorhamnetin 3,7-di-*O*- β -D-glucopyranoside, isolated from leaves of the plant in streptozotocin-induced diabetic rats has also been reported (Yokozawa, T., 2002).

Anti-hyperglycemic effect of aqueous extract of seeds of a related species, *Brassica nigra* (L.) Koch has been noted in streptozotocin-induced diabetic rats. The extract when fed for one month to diabetic animals brought down fasting serum glucose levels and reduced the levels of glycosylated hemoglobin (HbA1c) and serum lipids (Anand, P., 2007). Administration of aqueous extract of *Brassica nigra* to streptozotocin-induced diabetic rats for two months have been reported to decrease serum glucose, increase serum insulin, and increase release of insulin from pancreas, the latter being observed *in vitro* with isolated pancreas (Anand, P., 2009).

Several phytochemicals have been reported for the plant and plant parts. The whole plant contains 24-methylene-25-methylcholesterol, while the seeds contain allyl-isothiocyanate and crotonyl-isothiocyanate. A study on 17 leafy vegetables from *Brassica* species other than *Brassica oleracea* led to identification of 71 phenolic compounds consisting of kaempferol 3-*O*-diglucoside-7-*O*-glucoside derivatives, isorhamnetin 3-*O*-glucoside-7-*O*-glucoside hydroxycinnamoyl gentiobioses, hydroxycinnamoylmalic acids, and hydroxycinnamoylquinic acids (Lin, L.Z., and Harnly, J.M., 2010). An LC-MS profiling method for identification of components of three *Brassica* green leafy vegetables, collard greens, kale and Chinese broccoli led to the identification of 45 flavonoids and 13 hydroxycinnamic acid derivatives (Lin, L.Z., and Harnly, J.M., 2009). Considering the phytochemicals to be present in members of the *Brassica* species, it is not unlikely that some of the constituents present in *Brassica juncea* (even though they may be unidentified at the moment) may turn out to have good antinociceptive and anti-hyperglycemic properties. It was the objective of the present study to evaluate the antinociceptive and anti-hyperglycemic effects of methanol extract of the leaves, respectively, in acetic acid-induced gastric pain writhing model, and oral glucose tolerance tests in glucose-loaded mice.

Materials and Methods

Plant material and extraction

The leaves of *Brassica juncea* (L.) Czern. were collected from Satkhira district, Bangladesh in November, 2009. Notably, the plant is widely cultivated in the district both for its green leaves (consumed as vegetable) and for its seeds from which oil is extracted, which is used in cooking. The plant was taxonomically identified by Bangladesh National Herbarium at Dhaka (Accession Number 35,038). The leaves of *Brassica juncea* were air-dried in the shade for 120 hours, grounded into a fine powder, and were extracted with methanol at a ratio of 1:5 (w/v). After 24 hrs, the mixture was filtered; filtrate was collected and the residue was again extracted with methanol at a ratio of 1:3 (w/v) for 24 hrs. Filtrates were combined and evaporated to dryness. The initial weight of dried leaf powder used for extraction was 100g; the final weight of the extract was 5.2g.

Chemicals and Drugs

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin, glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals

In the present study, Swiss albino mice (male), which weighed between 20-25g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for one week prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Acetic acid-induced writhing method

Antinociceptive activity of methanol extract of *Brassica juncea* leaves was examined using previously described procedures of Deb *et al.*, (2010) with minor modifications. Pain was induced in mice in the writhing test through intraperitoneal administration of 1% acetic acid at a dose of 10 ml/kg body weight. Mice were separated into six groups of six mice each. Group-I served as control and was administered vehicle (1% Tween 80 in water, 10 ml/kg body weight). The standard drug, aspirin was administered to Group-II mice at a dose of 200 mg/kg body weight. Groups-III to VI received extract, respectively at 50, 100, 200, and 400 mg extract/kg body weight orally 30 min before acetic acid injection. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

Anti-hyperglycemic activity

Glucose tolerance property of *Brassica juncea* leaves was determined as per the procedure previously described by Joy and Kuttan (1999) with minor modifications. In brief, fasted mice were grouped into five groups of six mice each. The various groups received different treatments like Group-I received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, group-II received standard drug (glibenclamide, 10 mg/kg body weight) and the other three groups (III-V) received the methanol extract of *Brassica juncea* leaves at three different doses of 50, 100 and 200 mg/kg body weight. Each mouse was weighed and doses adjusted accordingly prior to administration of vehicle, standard drug, and test samples. 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 two hours after the glucose administration through puncturing heart. Serum glucose levels were measured by glucose oxidase method (Venkatesh, S., 2004).

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.

Results and Discussion

Antinociceptive activity

The methanol extract of leaves of *Brassica juncea* showed significant dose-dependent antinociceptive activity when administered to mice in acetic acid-induced gastric pain writhing tests. Maximum inhibition of writhing (75.7%) was observed at an extract dose of 400 mg/kg body weight. By comparison, the standard drug, aspirin, when administered at a dose of 200 mg/kg body weight inhibited writhings by 43.9%, which was the same percentage of inhibition noted with administration of methanol extract of leaves at a dose of 200 mg/kg body weight. The results, shown in Table 1 demonstrate that the methanolic extract of leaves of *Brassica juncea* is highly effective as a treatment for pain.

Central and peripheral analgesia can be detected by acetic acid-induced writhing test (Shanmugasundaram, P., 2005). Pain and inflammation involves a mechanism where prostaglandins, mainly prostacyclines (PGI₂) and prostaglandin-E (PG-E) are produced, which have been shown to be responsible for excitation of the Ad-nerve fibers, leading to sensation of pain (Reynolds, J.E.F., 1982; Rang, H.P. and Dale, M.M., 1993). Intraperitoneal administration of acetic acid induces pain in mice leading to gastric writhings, a process possibly involving the formation of prostacyclines and prostaglandins. Analgesia will be demonstrated then by any agent that lowers the number of writhing by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. Leaf extract of *Brassica juncea* significantly caused reduction in the number of abdominal constrictions as well as stretching of hind limbs induced by the intraperitoneal injection of acetic acid, in fact much more so than the standard drug aspirin, when the extract was used at a dose of 400 mg/kg body weight. The results suggest that the methanolic extract of leaves may contain components, which when administered leads to inhibition of lipooxygenase and/or cyclooxygenase thereby reducing prostaglandin PGE₂ synthesis

Table 1: Antinociceptive effect of crude methanol extract of *Brassica juncea* leaves in the acetic acid-induced gastric pain model mice.

Groups	Dose (mg/kg body weight)	Mean no. of writhing	Inhibition (%)
Control (vehicle)	10 ml	11.00 ± 1.29	-
Aspirin	200 mg	6.17 ± 0.95	43.9*
<i>Brassica juncea</i>	50 mg	7.17 ± 0.60	34.8*
<i>Brassica juncea</i>	100 mg	6.67 ± 0.84	39.4*
<i>Brassica juncea</i>	200 mg	6.17 ± 1.01	43.9*
<i>Brassica juncea</i>	400 mg	2.67 ± 0.88	75.7*

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

Table 2: Effect of methanol extract of *Brassica juncea* leaves on serum glucose level in hyperglycemic mice.

Treatment	Dose (mg/kg body weight)	Serum glucose level (mg/dl)	% lowering of serum glucose level
Control	10 ml	84.50 ± 8.63	-
Glibenclamide	10 mg	22.48 ± 2.86	73.40*
<i>Brassica juncea</i>	50 mg	61.63 ± 5.50	27.07*
<i>Brassica juncea</i>	100 mg	58.53 ± 4.13	30.74*
<i>Brassica juncea</i>	200 mg	44.96 ± 3.48	46.79*

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

and causes decrease or cessation of pain through interfering with the mechanism of transduction in primary afferent nociceptor. A similar mechanism has been proposed for antinociceptive activity of *Ficus deltoidea* aqueous extract in acetic acid-induced gastric pain model (Sulaiman, M.R., 2008). Studies are ongoing in our laboratory to identify the antinociceptive components of *Brassica juncea* leaves in the methanol extract.

Anti-hyperglycemic effect

The results from the present study showed that the methanol extract of *Brassica juncea* leaves exhibited dose-dependent and significant anti-hyperglycemic activity in glucose-induced hyperglycemic mice. Even at the lowest dose of the extract tested (50 mg/kg body weight) serum glucose levels were lowered by 27.07%. The maximum serum glucose lowering effect was found with the dose of 200 mg extract/kg body weight (46.79%). The standard drug, glibenclamide at a dose of 10 mg/kg body weight lowered serum glucose level by 73.40% (Table 2). The anti-hyperglycemic effect observed following administration of methanolic leaf extract suggests that the extract may potentiate pancreatic secretion of insulin or increase the glucose uptake (Nyunai, N., 2009; Farjou, I.B., 1987) or may inhibit glucose absorption in gut (Bhowmik, A., 2009). The exact mechanism of action needs to be elucidated and can form the basis for further experiments.

The present study demonstrates that the leaves of *Brassica juncea* contains components, which when administered, can lead to antinociceptive and anti-hyperglycemic effects in mice models. Pain can be both a primary and secondary symptom, which can arise from multiple causes and diseases. At the same time, diabetes (where level of blood sugar is raised) is a debilitating disease affecting millions of people throughout the world against which allopathic medicine has no known cure. It is therefore important to conduct further studies with *Brassica juncea* towards finding suitable lead compounds, which can be used for treatment of pain as well as diabetes.

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