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ANTIHYPERGLYCEMIC AND ANALGESIC ACTIVITY EVALUATION OF METHANOLIC EXTRACT OF *RAPHANUS SATIVUS* L. AERIAL PARTS

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ABSTRACT

Objective: The objective of the present study was to evaluate the ant hyperglycemic and analgesic potential of methanolic extract of *Raphanus sativus* aerial parts in Swiss albino mice. **Methods:** Extract was administered orally at doses of 50-400 mg/kg. Oral glucose tolerance test (OGTT) was used to evaluate ant hyperglycemic activity in glucose-loaded mice. Analgesic activity was assessed in intraperitoneally injected acetic acid-induced pain model. **Results:** The extract, at doses of 50, 100, 200, and 400 mg/kg, reduced blood glucose levels by 15.8, 27.6, 35.4, and 49.2%, respectively. By comparison, a standard ant hyperglycemic drug, glibenclamide (10 mg/kg) reduced blood glucose levels by 49.2%. In acetic acid-induced pain model, the extract reduced the number of abdominal constrictions by 31.0, 41.1, 44.8, and 51.7%, respectively, at doses of 50, 100, 200 and 400 mg/kg. A standard analgesic drug, aspirin (200 mg/kg) reduced abdominal constrictions by 34.5%. **Conclusion**: The extract possesses significant ant hyperglycemic and analgesic potential.

KEYWORDS: *Raphanus sativus*, Cruciferae, antihyperglycemic, analgesic, oral glucose tolerance, glibenclamide.

1. INTRODUCTION

Raphanus sativus L. belongs to the Cruciferae family of plants and is cultivated throughout the world, primarily for its edible roots, but also for its aerial parts, which are cooked or

consumed as salad in Bangladesh. There are numerous varieties and sub-species of the plant, and the root color can be red or white depending on the presence or absence of anthocyanins like pelargonidine and cyanidine. The red root variety of the plant is known as radish in English and 'lalmula' in Bangladesh, the word 'lal' signifying red. The most popular red variety in Bangladesh and the one used in the present study is the 'French Breakfast' variety with long cylindrical roots tapering at the end and with red skin color.

Chemical constituents reported in aerial parts include glucobrassicin, isobutyraldehyde, putrescine, and spermidine. [1] The leaves of the plant are rich in phenolic acid like ferulic acid, with small amounts of other phenolic acids like p-hydroxybenzoic, vanillic, salicylic and gentisic acid. [2] Ethnomedicinal uses have been reported for the plant or plant parts. The native people of Bargarh district of Orissa, India use seeds to treat sexual debility. [3] The tribals of Northeast Gujarat, India use the roots and leaves of the plant against urinary complaints. [4] The tribes of Pedabayalu Mandalam, Visakhapatnam district, Andhra Pradesh, India use roots against urinary trouble. [5] Local people and herbal practitioners of Dharmabad Taluka of Nanded district, Maharashtra, India use leaves of the plant to treat piles. [6]

Diabetes is a disease rapidly reaching endemic proportions throughout the world and is characterized by high blood sugar levels, frequent thirsts and frequent urges for urination. The disease can progressively lead to higher risks of cardiovascular disorders and cause diabetic retinopathy, neuropathy and nephropathy. It has been estimated that in 2009 alone, 3.4 million people died in the world from having high fasting blood sugar levels.[7] Prevalence of the disease is also on the rise in Bangladesh [8], possibly because of a change in dietary habits and a more sedentary lifestyle. Diabetes has no known total cure; modern medicine can alleviate some of its symptoms. On the other hand, such medicines are either not available or affordable to the generally rural population of Bangladesh, who mostly lacks access to qualified physicians and modern clinics or hospitals. Pain is usually described as physical suffering or discomfort arising from illness or injury and can affect millions of people throughout the world on a regular basis. While acute pain may result from injury, chronic pain can be an outcome of incurable illnesses like arthritis or cancer. Over-the-counter drugs like aspirin or paracetamol can provide temporary relief to pain, but adverse effects like gastric ulceration or hepatotoxicity can result from prolonged use or over-dosage of these drugs. Other opiod drugs like morphine are addictive. Thus there is a constant search

for newer and safer drugs against diabetes and pain, and scientists are searching the plant kingdom (among other sources) for lead compounds against these two afflictions. We had been systematically evaluating the plant resources of Bangladesh for their antihyperglycemic and analgesic potential. [9-15] the objective of the present study was to evaluate the antihyperglycemic and analgesic potential of methanol extract of aerial parts of *R. sativus*.

2. MATERIALS AND METHODS

2.1.Plant Material Collection

Aerial parts of *R. sativus* were collected during September 2013 from a vegetable market in Dhaka city, Bangladesh and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,620).

2.2.Preparation of Methanolic Extract of Aerial Parts

Aerial parts were sliced into small pieces, air-dried in the shade and grounded to a fine powder. 100g of the powder was extracted with 500 ml methanol at room temperature over 48 hours, following which the methanol was filtered and dried at 40^{o}C. The final weight of the methanol extract (MERS) was 8.65g.

2.3.Chemicals and Drugs

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

2.4.Animals

Swiss albino mice, which weighed between 18-22g 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 in the laboratory prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

2.5.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) [16] 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 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 aerial part extract (MERS) 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. [17] The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = $(1 – W_e/W_c) X 100$,

WhereW_e and W_c represents the blood glucose concentration in glibenclamide or MERS administered mice (Groups 2-6), and control mice (Group 1), respectively.

2.6.Antinociceptive Activity Evaluation Through Acetic Acid-Induced Abdominal Writhing Test

Antinociceptive activity of MERS was examined as previously described by Shanmugasundaram and Venkataraman. [18] Mice were divided into six groups of five mice each. Group 1 served as control and was administered vehicle only. Group 2 was orally administered the standard antinociceptive drug aspirin at a dose of 200 mg per kg body weight. Groups 3-6 were administered MERS 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 MERS, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. Intraperitoneal administration of acetic acid results in sensation of pain, which is manifested by abdominal constrictions, also known as writhing's. A period of 5 minutes was given to each animal to ensure bioavailability and onset of chemically induced irritation of acetic acid [19], 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.

Percent inhibition = $(1 – W_e/W_c) X 100$

Where W_e and W_c represents the number of writhings in aspirin or MERS administered mice (Groups 2-6), and control mice (Group 1), respectively.

2.7.Acute Toxicity Test

Acute toxicity test was conducted as previously described. [20] 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 MERS 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.

2.8.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. [13]

2.9.Preliminary Phytochemical Screening

Preliminary phytochemical analysis of MERS for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before. [21]

3. RESULTS

Preliminary phytochemical screening of the extract indicated the presence of alkaloids, flavonoids, saponins, and tannins. The crude extract did not show any toxicity in mice even at the highest dose tested. There were no changes in behavioral pattern and any mortality was not observed.

In oral glucose tolerance tests, MERS, when administered at doses of 50, 100, 200, and 400 mg/kg to glucose-loaded Swiss albino mice, was found to dose-dependently reduce blood glucose levels, respectively, by 15.8, 27.6, 35.4, and 49.2%. The results were statistically significant at the three higher doses (p value < 0.05 in all cases). By comparison, a standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg/kg, reduced blood glucose level by 49.2%. Thus at the highest dose of the extract (400 mg/kg), the percent reduction in blood glucose levels was the same as that seen with glibenclamide and suggests that the extract has strong antihyperglycemic activity. The results are shown in Table 1.In acetic acid-induced abdominal writhing test for analgesic activity, MERS, at doses of 50, 100, 200 and 400 mg/kg both dose-dependently and significantly reduced the number of abdominal constrictions. At these doses, the percent reductions in abdominal constrictions were, respectively, 31.0, 41.4, 44.8, and 51.7. A standard analgesic drug, aspirin, when administered at a dose of 200 mg/kg reduced the number of constrictions by 34.5%. Thus MERS exhibited more potent analgesic activity than aspirin, at least at the three higher doses, and as such can be considered to be a potential candidate for isolation of pain-relieving compound(s). The results are shown in Table 2.

Table 1: Effect of crude methanol extract of *R. sativus* aerial parts (MERS) 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	5.94 ± 0.33	-
Glibenclamide	10 mg	3.02 ± 0.16	49.2*
(MERS)	50 mg	5.00 ± 0.22	15.8
(MERS)	100 mg	4.30 ± 0.26	27.6*
(MERS)	200 mg	3.84 ± 0.25	35.4*
(MERS)	400 mg	3.02 ± 0.24	49.2*

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

Table 2: Antinociceptive effect of crude methanol extract of *R. sativus* aerial parts (MERS) in acetic acid-induced pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control	10 ml	5.8 ± 0.37	-
Aspirin	200 mg	3.8 ± 0.73	34.5*
(MERS)	50 mg	4.0 ± 0.32	31.0*
(MERS)	100 mg	3.4 ± 0.51	41.4*
(MERS)	200 mg	3.2 ± 0.58	44.8*
(MERS)	400 mg	2.8 ± 0.20	51.7*

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

4. DISCUSSION

The results indicate that the methanolic extract of aerial parts of *R. sativus* (red variety) possess significant ant hyperglycemic and analgesic activities. The component(s) responsible for the observed pharmacological effects or their mechanism of action has not been determined in this preliminary study, which mainly was an attempt to screen Bangladeshi plants for their ant hyperglycemic and analgesic potentials. From that view point, the attempt was successful, more so, because neither of the observed pharmacological activities have been reported for aerial parts of this plant before. Root juice of *R. sativus* has been previously shown to reduce blood glucose levels in STZ-induced sub- and mild-diabetic rats in a previous study [22], but not aerial parts. The observed reductions in blood glucose as a consequence of administration of the extract can be due to the extract's ability to increase insulin secretion from pancreas, or through extra-pancreatic mechanisms like increasing

peripheral utilization of glucose or decreasing glucose absorption from gut. Ferulic acid is known to be present in aerial parts of the plant. [2]In streptozotocin (STZ)-induced diabetic rats, ferulic acid has been shown to normalize diabetes-induced elevated blood glucose levels. [23] The hypoglycemic effect of ferulic acid has also been reported in STZ-induced diabetic mice and KK-Ay mice. [24] An ethereal fraction of ethanol extract of *Syzygium cumini* seeds was found to lower blood glucose in STZ-diabetic male rats; the bioactive component in the fraction was identified to be ferulic acid.[25] Significant reductions in blood glucose levels were observed in STZ-diabetic rats with ferulic acid alone, or in combination with antidiabetic drugs like metformin. [26]The protective action of ferulic acid has been reported in alloxan-diabetic mice.[27] Thus ferulic acid can be at least one of the component(s) responsible for the observed antihyperglycemic effect, although this does not negate the possible antihyperglycemic influences of other phytoconstituents present in the extract.

Preliminary screening of the extract indicated presence of alkaloids, flavonoids, saponins, and tannins. These groups of compounds, individually or in combination, have been shown to possess both antihyperglycemic and analgesic potentials. Antidiabetic activity (lowering of blood glucose levels) has been reported with methanolic root bark extract of *Afzelia africana* in alloxan diabetic mice. The extract was found to contain alkaloids, flavonoids, saponins and tannins. [28] Hypoglycemic effects of aqueous extract of seeds of *Persea americana* have been reported in OGTT tests in alloxan diabetic Wistar rats. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins and tannins with glycosides.[29] Hypoglycemic effect of ethanolic extract of whole plant of *Tridax procumbens* has been observed in STZ-diabetic Wistar rats; phytochemical analysis showed the presence of alkaloids, flavonoids, phenolic compounds, saponins, and tannins.[30] Alkaloids, flavonoids, saponins and tannins were also present in stem bark extract of *Tamarindus indica*, which lowered blood glucose levels in OGTT tests in hyperglycemic and normoglycemicWistar rats.[31]

Various solvent extracts of *Vernonia condensata* containing alkaloids, phenolic compounds, flavonoids, tannins and saponins reportedly exhibited analgesic activity as demonstrated by writhing tests. [32] Crude ethanol extract of leaves of *Ageratum conyzoides* containing alkaloids, flavonoids, saponins and tannins demonstrated antinociceptive activity in acetic acid-induced writhing tests in mice. [33] Analgesic activity has also been reported in acetic acid-induced writhing tests in mice for ethanol extract of *Sida cordifolia* roots; reducing sugar, alkaloids, steroids and saponins were found in the extract. [34] Thus alkaloids,

flavonoids, saponins and tannins can also account for the observed analgesic activity seen in the present study. It may also be noted that ferulic and salicylic acids have been reported to be present in aerial parts of the plant.[2] It has been reported that ferulic acid can produce analgesia in mice in thermal hyperalgesia and mechanical allodynia tests.[35] Salicylic acid can also produce a pain-relieving effect (to be noted is that a standard pain-relieving drug, aspirin is acetyl salicylic acid). Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritant principles like acetic acid in mice. [36]Such pain can arise from increased synthesis of prostaglandins (PGE2 and PGF2□) and lipooxygenase products mediated through the cyclooxygenase/lipooxygenase pathway. [37] Thus the extract or any of its phytochemical constituents may be producing the observed analgesic effects through inhibition of the cyclooxygenase/lipooxygenase pathways.

We have previously conducted antihyperglycemic and antinociceptive studies with methanolic extract of aerial parts of *R. sativus* var. *hortensis* (white variety of *R. sativus*, known in English as daikon) in Swiss albino mice.[15] In that study, at the highest dose of 400 mg/kg, the extract reduced blood glucose levels by 51.5% and abdominal constrictions by 55.6%, versus the 49.2% and 51.7%, respectively, as observed in the present study. Thus the results of both white and red varieties of *R. sativus* were nearly equivalent with the white variety demonstrating slightly more antihyperglycemic and antinociceptive/analgesic effect than the red variety. Both varieties are cultivated in Bangladesh. Since *R. sativus* is cultivated in rural areas of Bangladesh and the aerial parts more easily available and affordable (because urban affluent population mostly consumes the roots), the plant can serve as a viable alternative for reducing blood sugar and alleviating pain in diabetic patients and patients with painful injuries or diseases. Since the aerial parts can be eaten as salad, there is also no possibility of any bioactive constituents being destroyed through cooking processes. Also, the plant has no report, anecdotal or otherwise, of causing adverse effects in the body. As such, since the plant is widely cultivated throughout the world, the plant and any of its isolated bioactive constituents can become an effective source for controlling diabetic sugar and relieve painful conditions.

5. CONCLUSION

The results suggest that methanolic extract of *R. sativus* (red variety) aerial parts can be used for lowering of blood glucose and for alleviating pain.

6. Conflicts of Interest

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

7. ACKNOWLEDGEMENTS

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