Advances in Natural and Applied Sciences, 7(1): 67-73, 2013
ISSN 1995-0772

This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLE

Medicinal potential of pteridophytes – an antihyperglycemic and antinociceptive activity evaluation of methanolic extract of whole plants of *Christella dentata*

Rabiea Tanzin, Shiblur Rahman, Md. Shakhawat Hossain, Bipasha Agarwala, Zubaida Khatun, Sharmin Jahan, Md. Moshiur Rahman, Sadia Moin Mou, Mohammed Rahmatullah

Faculty of Life Sciences, University of Development Alternative, Dhanmondi, Dhaka-1205, Bangladesh.

Rabiea Tanzin, Shiblur Rahman, Md. Shakhawat Hossain, Bipasha Agarwala, Zubaida Khatun, Sharmin Jahan, Md. Moshiur Rahman, Sadia Moin Mou, Mohammed Rahmatullah; Medicinal potential of pteridophytes – an antihyperglycemic and antinociceptive activity evaluation of methanolic extract of whole plants of *Christella dentata*

ABSTRACT

Not much has been reported on the medicinal values of pteridophytes (fern and fern allies). Yet, a number of ferns are used in folk medicinal system and by various tribes of the Indian sub-continent (inclusive of Bangladesh) for medicinal purposes. *Christella dentata* is one such fern used by the folk medicinal practitioners of Bangladesh for treatment of diabetes (to lower high blood sugar levels) and for treatment of pain. It was of interest to determine whether folk medicinal uses of this fern species can be validated through modern scientific methods. Accordingly, antihyperglycemic activity evaluation of methanolic extract of whole fern was carried out through oral glucose tolerance tests in glucose-loaded Swiss albino mice. In parallel, antinociceptive activity of methanolic extract of whole ferns was evaluated in gastric pain model Swiss albino mice, where gastric pain was induced by intraperitoneal administration of acetic acid. The methanolic extract, in antihyperglycemic activity evaluation experiments, demonstrated dose-dependent significant lowering of blood sugar levels when orally administered to glucose-challenged mice at doses of 100, 200 and 400 mg per kg body weight. At these doses, the extract significantly lowered blood sugar levels by 48.02, 49.44 and 54.52%, respectively, when compared to control mice (i.e. mice administered vehicle only). The results obtained from the extract compare favorably with the result obtained with a standard antihyperglycemic drug, glibenclamide, which when administered orally at a dose of 10 mg per kg body weight, lowered blood sugar levels by 52.40%. In antinociceptive activity tests, the extract at doses of 50, 100, 200 and 400 mg per kg body weight lowered the number of gastric pain-induced writhings in mice by 42.84, 47.00, 48.96 and 51.04%, respectively. The reduction in the number of gastric writhings was both dose-dependent and statistically significant. By comparison, a standard antinociceptive drug, aspirin, reduced the number of writhings in mice by 51.04 and 67.32%, respectively, when orally administered at doses of 200 and 400 mg per kg body weight. The results not only validates the folk medicinal use of this plant for lowering of blood sugar and alleviation of pain, but also suggests that fern species should not be overlooked in the quest for discovery of newer and more efficacious drugs.

Key words: *Christella dentata*, antihyperglycemic, antinociceptive, Thelypteridaceae

Introduction

Christella dentata (Forssk.) Brownsey et Jermy (Family: Thelypteridaceae, English: soft fern, Bengali: dheki shak) is a common fern species of Bangladesh and can be found throughout the country. It is occasionally cooked and eaten by the rural poor families and often serves as a famine food. A number of tribal practitioners as well as folk medicinal practitioners (Kavirajes) of the mainstream Bengali-speaking population use whole plants for lowering of blood sugar (in diabetic patients) and for alleviation of pain.

Pteridophytes (fern and fern allies) comprise more than 10,000 species throughout the world and are roughly distributed among 305 genera. Although reports on traditional medicinal uses of fern species are scant, within the Indian sub-continent and especially India (which hosts about 1,000 fern species), there are a few reports on use of various fern species in folk and tribal medicine. To cite a few instances, *Adiantum capillus-veneris* L. (Adiantaceae) leaf extract is used for fever, cough and bronchial disorders (Dixit, 1974). The plant extract of *Adiantum incisum* Forsk. (Adiantaceae) is used in cough, diabetes, and skin diseases. The Bheel tribe,

Corresponding Author: Professor Dr. Mohammed Rahmatullah, Pro-Vice Chancellor and Dean, Faculty of Life Sciences University of Development Alternative, House No. 78, Road No. 11A (new) Dhanmondi, Dhaka-1205, Bangladesh
Tele: +88-01715032621 Fax: +88-02-815739 E-mail: rahamatm@hotmail.com

residing in Mt. Abu area of India, uses the juice of leaves in skin diseases. The Garasia tribe of India mixes the dry leaves of the fern with tobacco and smoke the mixture to curb internal burning of the body (Sharma and Vyas, 1985). A further fern species, *Adiantum lunulatum* Burm.f. (Adiantaceae), is recognized by the classical traditional medicinal system of India – Ayurveda to be pungent, alexiteric (obviating the effects of poison), and used for indigestion. The decoction of leaves is said to be useful in dysentery, diseases of the blood, ulcers, and erysipelas – a type of skin infection (Caius, 1935). *Asplenium pumilum* var. *hymenophylloides* Fee (Aspleniaceae) is used as a depurative and sedative as well as in sores and ulcers (Singh, 1999). The medicinal properties of a number of such fern species from Rajasthan, India has been reviewed (Parihar and Parihar, 2006).

Modern or allopathic medicine, despite its remarkable advancements made in the treatment of diseases, is still unable to cure diseases like diabetes or rheumatoid arthritis. Moreover, many modern drugs have serious side-effects or have developed drug-resistant vectors. Even treatment of a common affliction like pain, for which there are hundreds of over the counter (OTC) remedies including aspirin and paracetamol suffers from the problem of developing gastric ulceration or hepatotoxicity from over dosage or overuse. As a result, there is a constant need to develop better and more efficacious drugs not only to counter emerging diseases but also to improve on existing diseases. Traditional medicinal practices, which mainly rely on various medicinal plants for treatment, can be one of the ways through which many drugs can be discovered. This has been borne out by the discovery of modern drugs like atropine, artemisinin, quinine, vincristine, and vinblastine, to name only a few, from close observations of indigenous medicinal practices (Balick and Cox, 1996; Cotton, 1996; Gilani and Rahman, 2005). In fact, in recent years, there has been a resurgence of scientific interest in medicinal plants and observations of indigenous medicinal practices.

We have been conducting a two-pronged scientific approach over a number of years within the general scope of natural products and drug discovery. The first part of this approach involves ethnomedicinal surveys among traditional medicinal practitioners of both the mainstream community as well as various tribes of Bangladesh (Rahmatullah *et al*., 2009a-c; Rahmatullah *et al*., 2010a-g; Rahmatullah *et al*., 2011a,b; Rahmatullah *et al*., 2012a-d). In the second phase of this multi-dimensional study, we are screening various medicinal plants obtained from our ethnomedicinal survey data and screening whole plants or plant parts for various pharmacological activities. Primarily, our screening has concentrated on anti-diabetic, anti-cancer and antinociceptive effects in these plants (Anwar *et al*., 2010; Jahan *et al*., 2010; Khan *et al*., 2010; Mannan *et al*., 2010; Rahman *et al*., 2010; Rahmatullah *et al*., 2010h; Shoha *et al*., 2010; Ali *et al*., 2011; Barman *et al*., 2011; Hossan *et al*., 2011; Jahan *et al*., 2011; Rahman *et al*., 2011; Sutradhar *et al*., 2011). As part of this systematic screening of medicinal plants, this study was conducted to determine whether the folk medicinal uses of *Christella dentata* for alleviation of pain and lowering of blood sugar can be validated or not in carefully conducted laboratory experiments.

Materials and Methods

Whole plants of *Christella dentata* were collected from Dhaka district, Bangladesh during January, 2011. The plant was taxonomically identified at the Bangladesh National Herbarium at Dhaka (Voucher specimen No. 35,329). The sliced and air-dried whole plants of *Christella dentata* were grounded into a fine powder and 100g of the powder was extracted with methanol (1:5, w/v) for 48 hours. The extract was evaporated to dryness. The final weight of the extract was 3.72g.

Chemicals:

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin, glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh.

Animals:

In the present study, Swiss albino mice (male), which weighed between 15-22 g 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 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.

Antihyperglycemic activity:

Glucose tolerance property of methanol extract of *Christella dentata* whole plants was determined as per the procedure previously described by Joy and Kuttan (1999) with minor modifications. In brief, fasted mice

were grouped into six groups of six 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 methanol extract of *Christella dentata* whole plants at doses of 50, 100, 200 and 400 mg per 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 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method (Venkatesh *et al*., 2004).

Antinociceptive activity:

Antinociceptive activity of the methanol extract of *Christella dentata* whole plants was examined using previously described procedures (Shanmugasundaram and Venkataraman, 2005). Briefly, mice were divided into seven groups of six mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard antinociceptive drug aspirin at a dose of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered methanolic whole plant extract of *Christella dentata* 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 extract, 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 bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

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.

Results and Discussion

In antihyperglycemic activity tests, the methanolic extract was observed to produce a dose-dependent and statistically significant lowering of glucose concentrations in blood of glucose-loaded mice. The percent reductions in blood glucose concentrations when the extract was administered at doses of 100, 200 and 400 mg per kg body weight of mice were, respectively, 48.02, 49.44 and 54.52 as compared to control mice, which did not receive any extract or antihyperglycemic drug. The extract, at a dose of 50 mg per kg body weight, reduced blood glucose level by only 14.12%, which was not statistically significant. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg per kg body weight to mice, was observed to lower blood sugar by 52.40%, when compared to control mice (i.e. mice administered vehicle only). Thus the antihyperglycemic activity of the extract can be considered to be comaparable to glibenclamide, at least at the highest dose of the extract tested, i.e. 400 mg per kg body weight, although reductions in blood sugar level were not much when compared between the extract dose of 100 mg per kg body weight and 400 mg per kg body weight. The results are shown in Table 1. It may be added that the observed results validate the folk medicinal use of the leaves for lowering blood sugar levels.

Table 1: Effect of methanol extract of *Christella dentata* whole plants 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 (Group 1)	10 ml	7.08 ± 0.40	-
Glibenclamide (Group 2)	10 mg	3.37 ± 0.40	52.40*
Christella dentata (Group 3)	50 mg	6.08 ± 0.44	14.12
Christella dentata (Group 4)	100 mg	3.68 ± 0.29	48.02*
Christella dentata (Group 5)	200 mg	3.58 ± 0.17	49.44*
Christella dentata (Group 6)	400 mg	3.22 ± 0.17	54.52*

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

The observed reduction of blood sugar by the extract can be attributed to any of several possible mechanisms or a combination of the mechanisms. Any bio-active compound or compounds may lower blood sugar either by potentiating the pancreatic secretion of insulin or increasing the glucose uptake, as has been observed in studies with *Artemisia* extract or extract of *Ageratum conyzoides* L. (Asteraceae) (Farjou *et al*., 1987; Nyunai *et al*., 2009). Alternately, a compound or compounds may inhibit glucose absorption in gut, as observed with *Mangifera indica* L. (Anacardiaceae) stem-barks (Bhowmik *et al*., 2009). A further mechanism can possibly be increase of peripheral glucose consumption induced by the extract, as has been seen with

ethanolic extract of *Sapindus trifoliatus* L. (Sapindaceae) (Sahoo *et al*., 2010). In either of these mechanisms or a combination of these mechanisms, the resultant effect will be reduction of sugar levels in the blood. In this preliminary screening on the antihyperglycemic activity of methanolic extract of *Christella dentata* whole plants, we have not explored the actual mechanism behind the reduction of blood sugar in glucose-loaded mice, but further experiments are on the way to elucidate the actual mechanism(s).

Table 2: Antinociceptive effect of crude methanol extract of *Christella dentata* whole plants in the acetic acid-induced gastric pain model mice.

Treatment	Dose (mg/kg body weight)	Mean number of writhings	% inhibition
Control (Group 1)	10 ml	8.17 ± 0.73	-
Aspirin (Group 2)	200 mg	4.00 ± 0.53	51.04*
Aspirin (Group 3)	400 mg	2.67 ± 0.82	67.32*
Christella dentata (Group 4)	50 mg	4.67 ± 0.52	42.84*
Christella dentata (Group 5)	100 mg	4.33 ± 0.88	47.00*
Christella dentata (Group 6)	200 mg	4.17 ± 0.61	48.96*
Christella dentata (Group 7)	400 mg	4.00 ± 0.76	51.04*

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

In antinociceptive activity evaluation experiments, the methanolic extract of whole plants was observed to dose-dependently and significantly reduce the number of writhings in mice arising from gastric pain. At extract doses of 50, 100, 200 and 400 mg per kg body weight, the percent reductions in the number of writhings were, respectively, 42.84, 47.00, 48.96 and 51.04. By comparison, the percent reductions in the number of writhings observed on administration of a standard antinociceptive drug aspirin at doses of 200 and 400 mg per kg body weight were, respectively, 51.04 and 67.32. The results are shown in Table 2. Thus the highest dose of the extract tested, namely 400 mg per kg body weight showed similar antinociceptive activity when compared to the standard antinociceptive drug tested, namely that of aspirin at 200 mg per kg body weight. Our observed results not only validates the folk medicinal use of the leaves for treatment of pain, but also suggests the presence of component(s) within the leaves with fairly strong antinociceptive potential, and which further merits isolation and identification of such components.

Pain (analgesia) can be central or peripheral, and both central and peripheral analgesia can be detected with the acetic acid-induced writhing test (Shanmugasundaram and Venkataraman, 2005), as has been done in the present study. Production of prostaglandins [mainly prostacyclines (PGI_2) and prostaglandin- (PG-E)] has been shown to be responsible for excitation of Adelta-nerve fibers, leading to the sensation of pain (Reynolds, 1982; Rang and Dale, 2003). As such, the antinociceptive activity exhibited by crude methanolic extract of the leaves may be due to the extract's ability to block any synthesis of prostaglandins, which may be mediated through inhibition of cyclooxygenase and/or lipooxygenase activities. It is to be noted that a similar mechanism has been proposed for antinociceptive activity of *Ficus deltoidea* Jack (Moraceae) aqueous extract in acetic acid-induced gastric pain model (Sulaiman *et al*., 2008), and this may also be the mechanism operating in the present study.

The phytochemical constituents of *Christella dentata* and their pharmacological activities are yet to be studied. Since, as is clear from our observations, the plant has significant antihyperglycemic and antinociceptive potential, it would be of interest to isolate the responsible phytochemicals and study the mechanism of action responsible behind the observed effects. Our next course of studies will be precisely to isolate and identify the phytochemicals responsible for antihyperglycemic and antinociceptive effects from this plant and determine the exact mechanism(s) behind the observed effects.

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