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Dates: Received: 06 December, 2016; **Accepted:** 22 December, 2016; **Published:** 23 December, 2016

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Keywords: *Spilanthes calva*; *Colocasia esculenta*; OGTT; Diabetes

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Research Article

Synergistic Action in Oral Glucose Tolerance Tests between *Spilanthes Calva* Aerial Parts and Tubers of *Colocasia Esculenta*

Abstract

Introduction: Diabetes is a debilitating disease characterized by elevated blood glucose levels, which is rapidly reaching endemic proportions throughout the world including Bangladesh. We had previously evaluated the antihyperglycemic potential of methanolic extract of aerial parts of *Spilanthes calva* and tubers of *Colocasia esculenta*. The objective of this study was to determine whether there is any synergistic antihyperglycemic activity between the methanolic extracts of the plant parts of the two plants.

Methods: Antihyperglycemic activity and synergistic potential were determined through oral glucose tolerance tests (OGTT) in mice.

Result: Administration of methanolic extract of aerial parts of *S. calva* (MESCA) at a dose of 400 mg per kg body weight led to lowering of blood glucose levels in glucose-loaded mice in OGTT by 41.6%. At the same dose, methanolic extract of tubers of *C. esculenta* (MECE) lowered blood glucose levels by 25.1%. At doses of 100, 200 and 400 mg each of (MESCA + MECE), blood glucose levels in mice were lowered, respectively, by 38.8, 43.0, and 49.5%. At the highest dose of the combination, the reductions in blood glucose were comparable to that of a standard antihyperglycemic drug, glibenclamide, which when administered at a dose of 10 mg per kg lowered blood glucose by 52.2%.

Conclusion: Synergistic antihyperglycemic activity was observed between aerial parts of *S. calva* and tubers of *C. esculenta* in oral glucose tolerance tests. The combination has the potential to be a substitute for glibenclamide.

Introduction

Diabetes is a disease characterized by elevated blood glucose levels and which if untreated can rapidly lead to more complicated disorders like disorders of the heart, kidney, eyes and brain. Recent years have seen a steady rise in the number of diabetic patients throughout the world, including Bangladesh, which possibly may be due to changes in food habits and lifestyle. Modern medicines cannot cure diabetes, but can be effective in lowering blood glucose.

More than 7% of males in both rural and urban areas of Bangladesh suffer from diabetes, as shown in a recent study [1]. What is also important is that antidiabetic drugs may not be readily available or affordable to the poorer segments of the population of Bangladesh who amount to a third of the population and whose income lies below the poverty level income of US\$ 1 per day. It is to be noted in this connection

that the average cost for just keeping diabetes under control in patients without any other serious complications has been estimated to be US\$ 314 per year in Bangladesh [2]. It is therefore necessary in a country like Bangladesh to seek alternate drugs for lowering elevated blood glucose in diabetic patients.

Traditional medicinal systems in Bangladesh like Ayurveda, Unani or folk and tribal medicines have always placed emphasis on plants for treatment of diabetes [3,4]. Since Bangladesh is rich in floral species, we had been over the last few years systematically screening various plant extracts and polyherbal formulations for their antihyperglycemic activities as determined through oral glucose tolerance tests in mice [5-12]. We had previously reported the antihyperglycemic activity of *Colocasia esculenta* (known locally as mankochu) stems [13] and *Spilanthes calva* (known locally as oshon shak) aerial parts [14].

S. calva is an erect annual herb with somewhat hairy stems and branches. It is usually found in the wild and fallow land areas of Bangladesh. Leaves of the plant are used in indigenous medicines for treatment of toothache, constipation, gout, gum disease, and paralysis in Sylhet and Moulvibazar districts of Bangladesh [15]. Manipuri tribal healers of Moulvibazar district, Bangladesh also use leaves of the plant for treatment of oral lesions [16]. *C. esculenta* is a fast growing plant with large-sized leaves that originate from a large corm (tuber). The plant can grow up to nearly 5 feet in height. The Garo tribal community of Netrakona district, Bangladesh use stems of the plant to stop bleeding from cuts and wounds [17].

The objective of the present study was to determine whether any antihyperglycemic activity exists in *C. esculenta* tubers, and if so, to evaluate whether any synergistic antihyperglycemic activity is present in a combination of *S. calva* aerial parts and *C. esculenta* tubers.

Materials and Methods

Plant material collection

Aerial parts of *S. calva* were collected from Bandarban district in December 2015, and tubers (corms) of *C. esculenta* were collected in November 2015 from a local market in Dhaka city, Bangladesh. The plants were authenticated at the Bangladesh National Herbarium. The accession number for *S. calva* was 41,887. However, the Herbarium did not provide any accession number for *C. esculenta* as only tubers were taken there, but they did authenticate the tubers to belong to *C. esculenta*. The tubers were further authenticated by a qualified botanist of the University of Development Alternative. Notably, the tubers are very common and so easily recognized.

Preparation of methanolic extract of plant parts

The aerial parts of *S. calva* and tubers (non-peeled) of *C. esculenta* were sliced separately and dried in the shade and powdered. Powder was prepared with an electric grinder. 100g of each powder was stirred continuously in 500 ml methanol for 70 minutes. The mixture was left overnight followed by fresh stirring for 70 minutes the following morning. The mixture was again left overnight followed by filtration. All procedures were conducted at ambient temperature (25°C). Following extraction, methanol was evaporated at 50°C using a water bath. Final weight of the extract was 7.677g for *S. calva* and 3.986g for *C. esculenta*. The extracts were stored at -20°C till use.

Chemicals and drugs

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

Animals

Swiss albino mice of both sexes (male to female ratio::4:1), aged around 1.5 months and which weighed between 15-18g 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. During this time, the animals were housed in well-ventilated cages under hygienic conditions in the animal house of the University. The room temperature was maintained at 25°C with 12 hours each of light dark cycle. Animals were given standard mouse chow (obtained from ICDDR, B and water *ad libitum* till experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh (Approval No. 35/EC/2016/UODA).

Preliminary phytochemical screening

Preliminary phytochemical analysis of the two extracts for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before [18].

Oral glucose tolerance tests (OGTT) for evaluation of antihyperglycemic activity

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan [19]. with minor modifications. Briefly, fasted mice were grouped into seven 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). Group 3 received methanol extract of *S. calva* aerial parts (MESC) at a dose of 400 mg per kg body weight. Group 4 received methanol extract of *C. esculenta* tubers (MECE) at a dose of 400 mg per kg body weight. Groups 5-7 received (MESC + MECE) each at doses of 100, 200, and 400 mg per kg body weight, respectively. All substances were orally administered. Following a period of one hour, all mice were orally administered 2g 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 [20]. The percent lowering of blood glucose levels were calculated according to the formula described below.

$$\text{Percent lowering of blood glucose level} = (1 - W_e/W_c) \times 100,$$

where W_e and W_c represents the blood glucose concentration in glibenclamide or various extracts 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 [21].

Results

Preliminary phytochemical screening

Preliminary phytochemical screening of MESC showed the presence of alkaloids, flavonoids, saponins and tannins. Preliminary phytochemical screening of MECE showed the presence of alkaloids, flavonoids, and tannins.

Antihyperglycemic activity evaluation results

Administration of methanolic extract of aerial parts of *S. calva* (MESC) at a dose of 400 mg per kg body weight led to lowering of blood glucose levels in glucose-loaded mice in OGTT by 41.6%. At the same dose, methanolic extract of tubers of *C. esculenta* (MECE) lowered blood glucose levels by 25.1%. At doses of 100, 200 and 400 mg each of (MESC + MECE), blood glucose levels in mice were lowered, respectively, by 38.8, 43.0, and 49.5%. At the highest dose of the combination, the reductions in blood glucose were comparable to that of a standard antihyperglycemic drug, glibenclamide, which when administered at a dose of 10 mg per kg lowered blood glucose by 52.2%. The results are shown in Table 1 and demonstrate that not only the individual plant parts have antihyperglycemic activities (as demonstrated by improved glucose tolerance) but the two plant parts can provide a synergistic antihyperglycemic action (see Table 1 for significance values of MESC or MECE against different combinations of (MESC + MECE)).

Discussion

To our knowledge, the antihyperglycemic potential of *S. calva* aerial parts has not been reported previously except by us [14]. The ameliorative potential of *C. esculenta* tubers on renal and liver growths in streptozotocin diabetic rats has been reported [22]. The leaves of *C. esculenta* reportedly possess constituents capable of inhibiting aldose reductase and so having the potential for treatment of or reducing diabetes induced complications [23]. Thus various plant parts of *C. esculenta* have antidiabetic potential.

Our preliminary phytochemical screening of the two plant parts showed the presence of alkaloids, flavonoids and tannins in common. These classes of compounds have been shown in a number of reports to possess antihyperglycemic activity in crude extracts. For instance, phytochemical analysis of ethanolic extract of whole plant of *Tridax procumbens* demonstrating antidiabetic activity indicated the presence of alkaloids, tannins, flavonoids, saponins, and phenolic

Table 1: Effect of MESC, MECE and (MESC + MECE) 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	5.82 ± 0.25	-
Glibenclamide	10 mg	2.78 ± 0.15	52.2*
(MESC)	400 mg	3.40 ± 0.10	41.6*
(MECE)	400 mg	4.36 ± 0.11	25.1*
(MESC + MECE)	100 mg each	3.56 ± 0.11	38.8 ^{b*}
(MESC + MECE)	200 mg each	3.32 ± 0.09	43.0 ^{b*}
(MESC + MECE)	400 mg each	2.94 ± 0.07	49.5 ^{ab*}

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

^aSignificant compared to MESC only; P < 0.5

^bSignificant compared to MECE only; P < 0.05

compounds [24]. The antihyperglycemic activity of petroleum ether extract of leaves of *Ficus krishnae* has been reported; the crude extract was found to contain alkaloids, flavonoids, and tannins [25]. The aqueous extract of *Thymus serpyllum* containing alkaloids, flavonoids, and tannins also reportedly showed hypoglycemic activity [26]. Taken together, the classes of phytochemicals present in MESC and MECE can be potential sources of antihyperglycemic agents and as such show antihyperglycemic activity.

Thus the aerial parts of *S. calva* and tubers of *C. esculenta*, especially the latter, can potentially act as affordable and readily available sources of plant-based medications for reduction of blood glucose in diabetic patients and reducing diabetes-induced complications. That the two extracts can give synergistic antihyperglycemic effect in combination points to the importance of rapid identification of the relevant bio-active components.

Conclusion

The two plant parts at the highest dose tested (400 mg each) showed synergistic action and the resultant antihyperglycemic activity was comparable to that of glibenclamide. Thus the plants have the potential not only to act as substitutes for glibenclamide (an antidiabetic drug) but also merits further research towards discovery of possible new antidiabetic compounds.

References

- Zaman MM, Choudhury SR, Ahmed J, Talukder MH, Rahman AH (2016) Blood glucose and cholesterol levels in adult population of Bangladesh: Results from STEPS 2006 survey. *Indian Heart J* 68: 52-56. [Link: https://goo.gl/ny20EY](https://goo.gl/ny20EY)
- Afroz A, Chowdhury HA, Shahjahan M, Hafez MA, Hassan MN, et al. (2016) Association of good glycemic control and cost of diabetes care: Experience from a tertiary care hospital in Bangladesh. *Diabetes Res Clin Pract* 120: 142-148. [Link: https://goo.gl/ohE1wY](https://goo.gl/ohE1wY)
- Karim MS, Rahman MM, Shahid SB, Malek I, Rahman MA, et al. (2011) Medicinal plants used by the folk medicinal practitioners of Bangladesh: a randomized survey in a village of Narayanganj district. *Am-Eur J Sustain Agric* 5: 405-414. [Link: https://goo.gl/PjNQha](https://goo.gl/PjNQha)
- Rahmatullah M, Azam MNK, Khatun Z, Seraj S, Islam F, et al. (2012) Medicinal plants used for treatment of diabetes by the Marakh sect of the Garo tribe living in Mymensingh district, Bangladesh. *Afr J Tradit Complement Altern Med* 9: 380-385. [Link: https://goo.gl/9PmotD](https://goo.gl/9PmotD)
- Haque ME, Rahmatullah M (2014) *Elephantopus spicatus*: a plant with hitherto unreported antihyperglycemic and antinociceptive potential. *World J Pharm Pharmaceut Sci* 3: 71-80.
- Hasan MN, Ferdoushi A, Ara N, Rahman S, Hossain MS, et al. (2014) Preliminary phytochemical screening, toxicity, antihyperglycemic and analgesic activity studies with *Curcuma longa* leaves. *World J Pharm Pharmaceut Sci* 3: 81-91. [Link: https://goo.gl/8r3QHM](https://goo.gl/8r3QHM)
- Sultana S, Nandi JK, Rahman S, Jahan R, Rahmatullah M (2014) Preliminary antihyperglycemic and analgesic activity studies with *Angiopteris evecta* leaves in Swiss albino mice. *World J Pharm Pharmaceut Sci* 3: 1-12. [Link: https://goo.gl/zD56Bt](https://goo.gl/zD56Bt)
- Rahman KMH, Nandi JK, Sultana S, Rahman S, Hossain S, et al. (2014) Phytochemical screening, antihyperglycemic and analgesic activity

- studies with methanol extract of *Trevesia palmata* leaves. *World J Pharm Pharmaceut Sci* 3: 91-101. [Link: https://goo.gl/ddgtOR](https://goo.gl/ddgtOR)
9. Syeda S, Rahman S, Afsana NA, Mahal MJ, Swarna A, et al. (2014) Antihyperglycemic activity evaluation of a formulation consisting of *Phyllanthus emblica*, *Terminalia bellirica* and *Terminalia chebula* fruits and *Trigonella foenum graecum* seeds. *Adv Nat Appl Sci* 8: 12-15. [Link: https://goo.gl/q43aTZ](https://goo.gl/q43aTZ)
 10. Monalisa MN, Rahmatullah M (2015) Antihyperglycemic, analgesic activity, and acute toxicity studies with methanol extract of *Foeniculum vulgare* seeds. *World J Pharm Pharmaceut Sci* 4: 198-206. [Link: https://goo.gl/c306qc](https://goo.gl/c306qc)
 11. Parvin S, Marzan M, Rahman S, Das AK, Haque S, et al. (2015) Preliminary phytochemical screening, antihyperglycemic, analgesic and toxicity studies on methanolic extract of aerial parts of *Corchorus olitorius* L. *J Appl Pharmaceut Sci* 5: 68-71. [Link: https://goo.gl/klhkwy](https://goo.gl/klhkwy)
 12. Akther M, Islam E, Islam MT, Das PR, Haque ME, et al. (2016) A preliminary study on significant antihyperglycemic activity as determined through oral glucose tolerance tests of three common plants belonging to the Brassicaceae family. *World J Pharm Pharmaceut Sci* 5: 159-172. [Link: https://goo.gl/Kx08hk](https://goo.gl/Kx08hk)
 13. Saha S, Rahmatullah M (2013) Antihyperglycemic and antinociceptive activities of methanolic extract of *Colocasia esculenta* (L.) Schott stems: a preliminary study. *Adv Nat Appl Sci* 7: 232-237. [Link: https://goo.gl/6kztvP](https://goo.gl/6kztvP)
 14. Nahar UJ, Bhuiyan MMR, Rahmatullah M (2016) Antihyperglycemic activity of methanolic extract of *Spilanthes calva* aerial parts. *World J Pharm Pharmaceut Sci* 5: 1648-1654. [Link: https://goo.gl/ra9SLt](https://goo.gl/ra9SLt)
 15. Akter S, Khairuzzaman M, Saleem SM, Sattar F, Rahman I, et al. (2015) Documentation of some folk medicinal practices in Sylhet and Moulvibazar districts, Bangladesh. *World J Pharm Pharmaceut Sci* 4: 176-186. [Link: https://goo.gl/CGddoX](https://goo.gl/CGddoX)
 16. Nandi JK, Molla MF, Mishu MK, Hossain M, Razia MS, et al. (2015) Phyto- and zoo-therapeutic practices of a Manipuri tribal healer in Moulvibazar district, Bangladesh. *J Chem Pharmaceut Res* 7: 722-726. [Link: https://goo.gl/VsZi9d](https://goo.gl/VsZi9d)
 17. Rahmatullah M, Mukti IJ, Haque AKMF, Mollik MAH, Parvin K, et al. (2009) An ethnobotanical survey and pharmacological evaluation of medicinal plants used by the Garo tribal community living in Netrakona district, Bangladesh. *Adv Nat Appl Sci* 3: 402-418. [Link: https://goo.gl/MJTYZR](https://goo.gl/MJTYZR)
 18. Kumar C, Kumar R, Nehar S (2013) Phytochemical properties, total antioxidant status of acetone and methanol extract of *Terminalia arjuna* Roxb. bark and its hypoglycemic effect on Type-II diabetic albino rats. *J Pharmacogn Phytochem* 2: 199-208. [Link: https://goo.gl/Jzb8gs](https://goo.gl/Jzb8gs)
 19. Joy KL, Kuttan RJ (1999) Anti-diabetic activity of *Picrorrhiza kurroa* extract. *J Ethnopharmacol* 67: 143-148. [Link: https://goo.gl/9yIbDn](https://goo.gl/9yIbDn)
 20. Trinder P (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol* 22: 158-161. [Link: https://goo.gl/vFfNGg](https://goo.gl/vFfNGg)
 21. Hossain AI, Faisal M, Rahman S, Jahan R, Rahmatullah M (2014) A preliminary evaluation of antihyperglycemic and analgesic activity of *Alternanthera sessilis* aerial parts. *BMC Complement Alternat Med* 14: 169. [Link: https://goo.gl/Tp3lr9](https://goo.gl/Tp3lr9)
 22. Eleazu CO, Iroaganachi M, Eleazu KC (2013) Ameliorative potentials of cocoyam (*Colocasia esculenta* L.) and unripe plantain (*Musa paradisiacal* L.) on renal and liver growths in streptozotocin induced diabetic rats. *J Acute Dis* 140-147. [Link: https://goo.gl/34yhm6](https://goo.gl/34yhm6)
 23. Li HM, Hwang SH, Kang BG, Hong JS, Lim SS (2014) Inhibitory effects of *Colocasia esculenta* (L.) Schott constituents on aldose reductase. *Molecules* 19: 13212-13224. [Link: https://goo.gl/SZk0WW](https://goo.gl/SZk0WW)
 24. Petchi RR, Parasuraman S, Vijaya C (2013) Antidiabetic and antihyperlipidemic effects of an ethanolic extract of the whole plant of *Tridax procumbens* (Linn.) in streptozotocin-induced diabetic rats. *J Basic Clin Pharm* 4: 88-92. [Link: https://goo.gl/GpG2D5](https://goo.gl/GpG2D5)
 25. Sidhu MC, Sharma T (2014) Antihyperglycemic Activity of Petroleum Ether Leaf Extract of *Ficus krishnae* L. on Alloxan-induced Diabetic Rats. *Indian J Pharm Sci* 76: 323-331. [Link: https://goo.gl/1MroVx](https://goo.gl/1MroVx)
 26. Alamgeer, Mushtaq MN, Bashir S, Ullah I, Karim S, et al. (2016) Comparative hypoglycemic activity of different fractions of *Thymus serpyllum* L. in alloxan induced diabetic rabbits. *Pak J Pharm Sci* 29: 1483-1488. [Link: https://goo.gl/us9VGw](https://goo.gl/us9VGw)