TÜBİTAK

Turk J Biol
36 (2012) 574-579

doi:10.3906/biy-1112-11

Antihyperglycemic, antioxidant, and cytotoxic activities of *Alocasia macrorrhizos* (L.) rhizome extract

Md. Masudur RAHMAN[1], Md. Aslam HOSSAIN[2], Saiful Alam SIDDIQUE[1], Kaishar Parvej BIPLAB[1], Md. Helal UDDIN[1]

[1]Department of Pharmacy, International Islamic University Chittagong, Chittagong 4203 - BANGLADESH

[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka 1000 - BANGLADESH

Received: 06.12.2011 ● Accepted: 10.05.2012

Abstract: *Alocasia macrorrhizos* (L.) (Araceae), a coarse and erect plant that is widely cultivated to be eaten as a vegetable throughout Bangladesh, has been reported to possess a number of medicinal properties. The antihyperglycemic, antioxidant, and cytotoxic effects of the methanol extract of *A. macrorrhizos* rhizomes were investigated. The extract was given at a single dose (250 and 500 mg/kg) in alloxan-induced hyperglycemic mice. The extract at 500 mg/kg produced a significant ($P < 0.05$) decrease in the blood glucose level (55.49%) at 8 h of treatment when compared with the control and was comparable with the reference drug, metformin. The extract was also subjected to antioxidant potentiality and brine shrimp lethality bioassays. The IC_{50} value of the extract was 693.0 µg/mL and the LC_{50} was 188.14 µg/mL.

Key words: Antihyperglycemic, alloxan, *Alocasia macrorrhizos*, antioxidant activity, cytotoxicity, rhizome

Introduction

Diabetes mellitus is the seventh leading cause of death among high-income countries; it is characterized by high levels of blood glucose resulting from defects in insulin production (1). Statistical projection suggests that India, China, and the United States will have the highest number of people with diabetes (2) and Bangladesh will have the seventh highest number of diabetics in the world by 2030 (3,4). The increasing worldwide incidence of diabetes mellitus in adults constitutes a significant impact on the health, quality of life, and life expectancy of patients, as well as on the global public health care system. The long-term manifestation of this disease can result in the development of vascular disorders such as retinopathy, nephropathy, neuropathy, and angiopathy (5).

Alocasia macrorrhizos (L.) G.Don (synonym: *Alocasia indica*) is a very robust evergreen herb, stem caulescent, leaves petiolate, green, stout, leaf blade erect, broadly ovate, large, margin entire or subundulate, growing up to 1.2-1.8 m in height under favorable conditions. It is commonly known as Mankachu in Bengali and Giant Taro or Elephant Ear Taro in English, and it belongs to the family Araceae, distributed throughout Bangladesh and other parts of Asia. This plant contains flavonoids, cynogenetic glycosides, ascorbic acid, gallic acid, malic acid, oxalic acid, alocasin, amino acids, succinic acid, and β-lectins (6). The plant has been used as folk medicine in different districts of Bangladesh. The various parts are cooked and eaten as a vegetable due to being an enriched source of protein. It has been traditionally used (all parts) for the treatment of diabetes (7). Stem juice is applied to prevent edema,

pain, and bleeding from cuts and wounds (8). The leaves of *A. macrorrhizos* are also used to prevent iron deficiency and to enhance eye sight, while the whole plant is utilized for pus in the ears, jaundice, or constipation (9-11). It is experimentally reported that the leaf extract possesses antimicrobial, antifungal, antioxidant, hepatoprotective, antidiarrheal, and antiprotozoal activities (12-16). Alocasin, an antifungal protein, and a new ceramide from *A. macrorrhiza* have been isolated (17,18). The literature survey revealed that there are no scientific studies carried out regarding the antidiabetic, antioxidant, and cytotoxic activities of *A. macrorrhizos* rhizomes to substantiate its therapeutic claim. Hence, in the present study, the methanolic extract of the rhizomes was examined for its antihyperglycemic, antioxidant, and cytotoxic properties.

Materials and methods

Plant material

A. macrorrhizos rhizomes were collected from a local vegetable market in Chittagong, Bangladesh, in the month of August, and were authenticated by Dr Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh.

Preparation of extract

The rhizomes were sun-dried and ground. The ground rhizomes (250 g) were soaked in a sufficient amount of methanol for 1 week at room temperature, with occasional shaking and stirring, and were then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield a semisolid. The extract was then preserved in a refrigerator until further use.

Experimental animals

Male Swiss albino mice, about 25-30 g in weight and 4-6 weeks of age, were collected from the International Center for Diarrheal Diseases Research, Bangladesh (ICDDRB), and housed in polypropylene cages under controlled conditions. The animals were exposed to an alternating cycle of 12 h of light and 12 h of dark. The animals were allowed free access to drinking water and pellet diet, obtained from the ICDDRB. The mice were acclimatized for 7 days.

Drugs

Alloxan monohydrate (Sisco Research Laboratories Pvt. Ltd., India), metformin hydrochloride (Square Pharmaceuticals Ltd., Bangladesh), 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma Chemical Co., USA), and ascorbic acid (Fine Chem. Ltd., India) were used. All of the other chemicals and reagents used were of analytical grade.

Induction of diabetes and treatments

The alloxan was first weighed individually for each animal according to its weight and then solubilized with 0.2 mL of saline (154 mM NaCl) just prior to injection. Diabetes was induced by injecting the alloxan at a dose of 150 mg/kg body weight intraperitoneally after overnight fasting. After 48 h, animals with fasting blood glucose levels from 16 to 19 mmol/L were separated and included in the study.

Twenty-five male Swiss albino mice were used, divided randomly into 5 groups containing 5 mice each. Group 1 served as a control, which received a vehicle alone. In groups 2-5, each animal received a single dose of alloxan (150 mg/kg intraperitoneally) after overnight fasting. Group 1 received only dimethyl sulfoxide (DMSO) as the normal control group and group 2 was the diabetic control group, which did not receive either metformin or rhizome extract. Metformin (150 mg/kg body weight) was injected intraperitoneally into group 3, and extract at a dose of 250 mg/kg body weight and 500 mg/kg body weight was injected intraperitoneally into group 4 and group 5, respectively. Metformin and the extract were both dissolved in 0.2% DMSO vehicle. Blood samples were then analyzed for blood glucose content at 0, 2, 4, 8, and 16 h, respectively, using a glucometer kit (Accu-Check Active, Roche Diagnostic GmbH, Germany).

Antioxidant activity

DPPH free radical scavenging activity

DPPH scavenging activity was carried out using the method of Braca et al. (19). Different concentrations (500, 300, 100, 50, and 10 μg/mL) of *Alocasia macrorrhizos* extract were dissolved in methanol and placed in different test tubes, and 3 mL of a 0.004% w/v methanol solution of DPPH was added to each test tube. Absorbance at 517 nm was determined after 30 min against a blank, and the percent inhibition

activity was calculated from $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. Ascorbic acid was used as a reference standard and dissolved in methanol to make the stock solution with the same concentration. The control sample was prepared containing the same volume without any extract or reference drug. Methanol served as a blank. The inhibition curves were prepared and the half maximal inhibitory concentration (IC_{50}) values were calculated using linear regression analysis.

Reducing power capacity

The reducing power of the extract was evaluated using the method of Oyaizu (20). Different concentrations of rhizome extract of *A. macrorrhizos* (125, 250, 500, and 1000 µg/mL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1% w/v). The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid solution was added to each tube and the mixture was centrifuged at 3000 rpm for 10 min. Subsequently, 5 mL of the upper layer solution was mixed with 5 mL of distilled water and 1 mL of ferric chloride solution (0.1% w/v), and the absorbance was measured at 700 nm. The reducing power of the extract was linearly proportional to the concentration of the sample. Ascorbic acid was taken as a reference standard. Phosphate buffer (pH 6.6) was used as a blank solution.

Brine shrimp lethality bioassay

The cytotoxicity assay was performed on brine shrimp (*Artemia salina*) nauplii using the Meyer method (21). The dried cysts of the brine shrimp were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) with strong aeration for a 48-h light/dark cycle to mature shrimp called nauplii. The test sample (extract) was prepared by dissolving extract in DMSO (not more than 50 µL in 5 mL of solution) plus sea water (3.8% NaCl in water) to attain concentrations of 10, 50, 100, 150, 200, and 400 µg/mL. A vial containing 50 µL of DMSO diluted to 5 mL was used as a control. Standard vincristine sulfate was used as the positive control. The matured shrimp were then applied to each of the experimental vials and the control vial. After 24 h, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial was counted. From these data, the percent (%) mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ mortality} = (N_t / N_0) \times 100,$$

where N_t = the number of killed nauplii after 24 h of incubation and N_0 = the number of total nauplii transferred, i.e. 10. The median lethal concentration (LC_{50}) was then determined.

Statistical analysis

The experimental data are presented as the means ± standard error of the mean (SEM). The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test using GraphPad Prism Version 5.0 for Windows (GraphPad Software, USA). $P < 0.05$ was considered significant.

Results and discussion

The methanol extract of *A. macrorrhizos* rhizomes (250 and 500 mg/kg) exhibited a dose-dependent antihyperglycemic activity. Although the reduction of blood glucose level (41.70%) at a dose of 250 mg/kg was not statistically significant, the extract at 500 mg/kg caused a significant ($P < 0.05$) reduction (55.49%) at 8 h of treatment compared to the diabetic control; when compared to the reference drug, metformin (150 mg/kg), it produced a significant reduction (66.13%) in blood glucose level. The results are shown in the Table and Figure 1.

A traditional plant, *Artemisia herba-alba*, was employed for the conventional therapy of diabetes mellitus, and this plant was reported to produce a significant ($P < 0.05$) hypoglycemic effect in both normal and diabetic rabbits (22). Studies in the literature indicate that the plant acts as a hypoglycemic agent, either by stimulating pancreatic β cells to release more insulin into the bloodstream, thereby increasing glycogen deposition in the liver and reducing glucose levels, or by increasing the number of insulin receptors (23-25). The *A. macrorrhizos* rhizomes might produce the same effect in terms of the prevention of hyperglycemia, and it significantly ($P < 0.05$) reduced the blood glucose levels in hyperglycemic mice.

Table. Antidiabetic effect of the rhizome extract of *A. macrorrhizos* on alloxan-induced diabetic mice.

Group	Treatment	Blood glucose level (mmol/L)				
		0 h	2 h	4 h	8 h	16 h
I	Normal control	6.39 ± 0.45	6.06 ± 0.19	6.17 ± 0.31	6.47 ± 0.82	6.50 ± 0.57
II	Diabetic control	18.08 ± 0.62	17.58 ± 0.24	17.70 ± 1.33	17.39 ± 0.86	16.91 ± 0.40
III	Metformin	16.96 ± 0.10	12.16 ± 0.53*	8.04 ± 1.54	5.89 ± 0.68*	6.70 ± 0.71*
IV	REAM** (250 mg/kg)	17.37 ± 0.76	15.57 ± 0.45*	12.75 ± 0.62*	10.14 ± 1.34	11.86 ± 0.84
V	REAM (500 mg/kg)	17.73 ± 0.83	14.12 ± 0.69*	10.23 ± 0.32*	7.74 ± 0.52*	9.69 ± 0.69

The results are expressed as mean ± SEM, n = 5. *P < 0.05 indicates significant activity compared to the diabetic control group.
**REAM: rhizome extract of *A. macrorrhizos*.

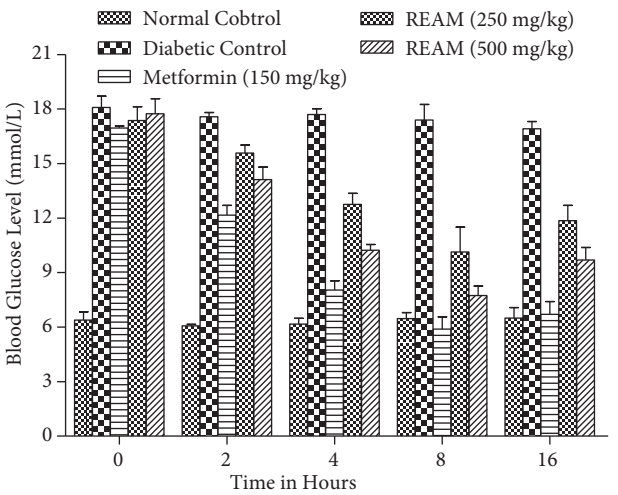

Figure 1. Effect of the rhizome extract of *A. macrorrhizos* in lowering the blood glucose level in mice. REAM: rhizome extract of *A. macrorrhizos*.

The DPPH method is economic, simple, rapid, and widely used to determine the antioxidant activity of phenolic compounds in natural products. In the DPPH radical scavenging assay, the extract showed a dose-dependent moderate DPPH radical scavenging activity, and the maximum scavenging activity of 38.42% was found at 500 µg/mL. The extract of the plant and the standard showed antioxidant activity with an IC_{50} value of 693.0 µg/mL and of 48.38 µg/mL, respectively (Figure 2). The reducing capacity of the compound may serve as a significant indicator of its potential antioxidant activity (26). The extract exhibited mild reducing power activities compared to ascorbic acid, which proportionally increased with the increasing concentration of the extracts (Figure 3). Reactive oxygen species, including free radicals and nonfree radicals along with various forms of active oxygen, are involved in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, aging, hepatic damage, neurodegenerative and cardiovascular complications, etc. (27,28).

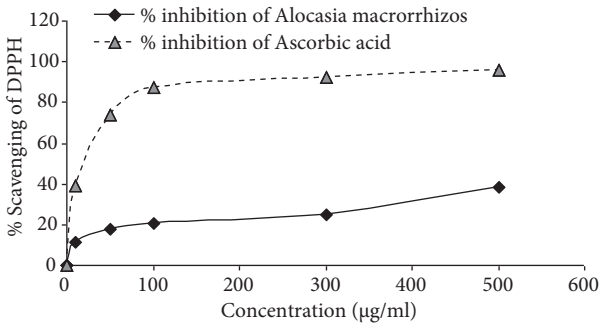

Figure 2. DPPH radical scavenging activity of *A. macrorrhizos* rhizomes.

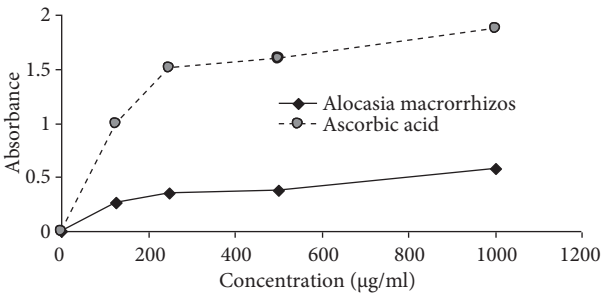

Figure 3. Reducing power of *A. macrorrhizos* rhizomes.

Clinical and experimental evidence suggests that an increase in free radical production can lead to a hyperglycemia-induced enhancement in glucose autoxidation and protein glycation (23). The aforementioned studies indicate that the antioxidant properties of the extract might also potentiate the antihyperglycemic activity in the mice.

Cytotoxicity screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work (29). The brine shrimp lethality of the extract is shown in Figure 4. The rhizome extract of *A. macrorrhizos* showed mild toxicity with a LC_{50} value of 188.14 µg/mL as compared to vincristine sulfate, which served as the positive control with a LC_{50} value of 11.32 µg/mL. No mortality was found in the control group. An approximate linear correlation was observed when the concentration of the extract versus the percentage of mortality was plotted on graph paper.

A. macrorrhizos is a popular dietary plant enriched with minerals and proteins. The present investigation revealed that the extract possesses significant ($P < 0.05$) antihyperglycemic activity and mild antioxidant and cytotoxic activity when compared to the reference drug. More details about phytopharmacological and toxicological studies remain to be investigated.

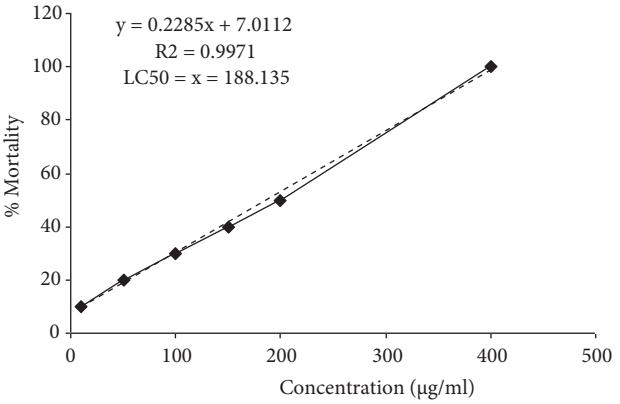

Figure 4. Determination of the LC_{50} value of the rhizome extract of *A. macrorrhizos* from linear correlation between concentrations versus percent mortality.

Acknowledgments

The authors wish to thank the management of the International Islamic University Chittagong, Chittagong, Bangladesh, for their encouragement and for providing research facilities.

Corresponding author:
Md. Masudur RAHMAN
Department of Pharmacy,
International Islamic University Chittagong,
Chittagong 4203 - BANGLADESH
E-mail: mamun2001@hotmail.com

References

1. World Health Organization. Top Ten Causes of Death. Fact Sheet No. 310. WHO. Geneva; 2007.
2. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 21: 1414-1431, 1998.
3. Wild S, Roglic G, Green A et al. Global prevalence of diabetes: estimates for 2000 and projections for 2030. Diabetes Care 27: 1047-1053, 2004.
4. Rahman MM, Islam MS, Ali MS et al. Antidiabetic and cytotoxic activities of methanolic extract of *Tabernaemontana divaricata* (L.) flowers. Int J Drug Dev & Res 3: 270-276, 2011.
5. Pirart J. Diabetes mellitus and its degenerative complications: a prospective study of 4400 patients observed between 1947 and 1973. Diabetes Care 3: 97-107, 1977.
6. Prajapati ND. A Handbook of Medicinal Plants. Agrobios India. Jodhpur; 2003: p. 32.
7. Mollik MAH, Jilani MA, Hasan MM et al. Plants used by traditional health practitioners of Natore and Naogaon districts, Bangladesh to treat diabetes mellitus. American Society of Tropical Medicine and Hygiene 59th Annual Meeting; 2010.
8. Rahmatullah M, Khatun MA, Morshed N et al. A randomized survey of medicinal plants used by folk medicinal healers of Sylhet Division, Bangladesh. Adv Nat Appl Sci 4: 52-62, 2010.
9. Rahmatullah M, Haque MR, Islam KS et al. A survey on the use of medicinal plants by folk medicinal practitioners in three areas of Pirojpur District, Bangladesh. Am Eurasian J Sustain Agric 4: 247-259, 2010.
10. Rahmatullah M, Al-Mahmud A, Rahman MA et al. An ethnomedicinal survey conducted amongst folk medicinal practitioners in the two southern districts of Noakhali and Feni, Bangladesh. Am Eurasian J Sustain Agric 5: 115-131, 2011.

11. Rahmatullah M, Das AK, Mollik MAH et al. An ethnomedicinal survey of Dhamrai Sub-district in Dhaka District, Bangladesh. Am Eurasian J Sustain Agric 3: 881-888, 2009.

12. Wahid AM, Prafull BS, Ajinkya PM et al. Evaluation of antimicrobial activity of leaves of *Alocasia indica* Linn. Int J PharmTech Res 2: 327-333, 2010.

13. Mulla W, Kuchekar S, Thorat V et al. Antioxidant, Antinociceptive and anti-inflammatory activities of ethanolic extract of leaves of *Alocasia indica* (Schott.). J Young Pharm 2: 137-143, 2010.

14. Mulla WA, Salunkhe VR, Kuchekar SB et al. Free radical scavenging activity of leaves of *Alocasia indica* (Linn). Indian J Pharm Sci 71: 303-307, 2009.

15. Patil BR, Bamane SH, Khadsare UR. *In vitro* protection of hepatocytes by *Alocasia macrorrhiza* leaf juice against CCL_4 and Tylenol mediated hepatic injury. Int J Pharm Appl 2: 122-127, 2011.

16. Mulla WA, Chopade AR, Bhise SB et al. Evaluation of antidiarrheal and in vitro antiprotozoal activities of extracts of leaves of *Alocasia indica*. Pharm Biol 49: 354-361, 2011.

17. Tien NQ, Ngoc PH, Minh PH et al. New ceramide from *Alocasia macrorrhiza*. Arch Pharm Res 27: 1020-1022, 2004.

18. Wang HX, Ng TB. Alocasin, an anti-fungal protein from rhizomes of the giant taro *Alocasia macrorrhiza*. Protein Expr Purif 28: 9-14, 2003.

19. Braca A, Tommasi ND, Bari LD et al. Antioxidant principles from *Bauhinia terapotensis*. J Nat Prod 64: 892-895, 2001.

20. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Nutr 44: 307-315, 1986.

21. Meyer BN, Ferrigni NR, Putnam JE et al. Brine shrimp: a convenient general bioassay for active plants constituents. J Med Plant Res 45: 31-34, 1982.

22. Iriadam M, Musa D, Gümüşhan H et al. Effect of two Turkish medicinal plants *Artemisia herba-alba* and *Teucrium polium* on blood glucose levels and other biochemical parameters in rabbits. J Cell Mol Biol 5: 19-24, 2006.

23. Naeem F, Khan SH. Evaluation of the hypoglycemic and hypolipidemic activity of *Butea monosperma* fruit in diabetic human subjects. Turk J Biol 34: 189-197, 2010.

24. Pari L, Saravanan G. Antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetes mellitus. Comp Biochem Physiol Toxicol 131: 19-25, 2002.

25. Saravanan G, Pari L. Effect of Cogent db, an herbal drug on serum and tissue lipids metabolism in experimental hyperglycemic rats. Diabetes Obes Metab 5: 156-162, 2003.

26. Liu J, Head E, Gharib AM et al. Memory loss in old rats is associated with brain mitochondrial decay and RNA/DNA oxidation: partial reversal by feeding acetyl-L-carnitine and/or R-α-lipoic acid. Proc Natl Acad Sci USA 99: 2356-2361, 2002.

27. Tanaka M, Kuie CW, Nagashima Y et al. Applications of antioxidative Maillard reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkaishi 54: 1409-1414, 1988.

28. Mavi A, Yiğit N, Yiğit D et al. Antioxidant and antimicrobial activity of Turkish endemic *Sonchus erzincanicus* extracts. Turk J Biol 35: 243-250, 2011.

29. Cardellina JH, Fuller RW, Gamble WR et al. Evolving strategies for the selection dereplication and prioritization of antitumor and HIV-inhibitory natural products extracts. In: Bohlin L, Bruhn JG. eds. Bioassay Methods in Natural Product Research and Development. Kluwer Academic; 1999: pp. 25-37.