



## RESEARCH PAPER

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## A study on antidiabetic activity of the leaf and stem of *Alocasia indica* L. in streptozotocin induced diabetic rats

Md. Rabiul Karim\*, Nasrin Ferdous, Narayan Roy, Subed Chandra Dev Sharma, M. G. Sarowar Jahan, Mohammad Shariar Shovon

Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh

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### Abstract

Medical and clinical investigations of plants have been making tremendous contributions of life sciences, health and medicine. Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager. The present study was aimed at evaluating the effect of *Alocasia indica* L on blood glucose level in normal and Streptozotocin-induced diabetic rats with stress on evaluation of probable mechanism of antidiabetic action. *In vivo*, antiheperglycemic activity of *Alocasia indica* leaves and stem extract showed activity against Streptozotocin induced diabetic rats. Diabetes was induced by single intraperitoneal injection of Streptozotocin (60 mg/kg) and doses of test extracts 200 and 400mg/kg were studied in Streptozotocin induced diabetic rats for the period of 21 days. Glibenclamide 10 mg/kg was used as standard drug. There was a significant increase in blood glucose level in diabetic rats when compared with normal control due to streptozotocin injection. In this study, daily oral administration of the leaves and stem extracts for the period of 21 days led to a dose dependent fall in blood glucose levels and showed significant ( $P < 0.05$ ) reduction in blood glucose levels compared to diabetic control group at dose 200 and 400 mg/kg. The test extracts also showed significant ( $P < 0.05$ ) weight gain of diabetic rats compared with the control group at the same dose. The present study reported that *Alocasia indica* L. may be very useful for the improvement of the complications of diabetes.

\*Corresponding Author: Md. Rabiul Karim ✉ [karim.robi@gmail.com](mailto:karim.robi@gmail.com)

## Introduction

Diabetes mellitus is a life threatening disease. These complications are arising day by day, specially in the developed country. Different types of treatments are being developed. Scientists are always trying to control diabetes mellitus by different treatments. Diabetes is a deficiency or absence of the hormone insulin, which is the main hormone responsible for the control of sugar in the blood. Research indicates that even moderately elevated blood sugar levels can increase the risk of cardiovascular disease, morbidity and mortality, even in non-diabetics. Research also shows that elevated blood sugar leads to increased oxidative stress and there is evidence that increased production of free radicals may be a contributing factor in the complications seen in diabetes. According to the National Institutes of Health (NIH), studies have shown that patients with diabetes appear to have decreased antioxidant defense capability with lower levels of specific antioxidants such as vitamin C and vitamin E, or reduced activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD) and glutathione peroxidase. Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is well documented that chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and eventually the failure of organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Diabetes mellitus is a chronic metabolic disorder involves the disturbances in metabolism of carbohydrate, fat and protein and characterized by hyperglycemia (Nelson *et al.*, 2005). Obesity and lack of exercise plays an important role in diabetes (Li WL *et al.*, 2004). From literature, statistics showed that Bangladesh will be the seventh diabetes affected country around 2030 (Rahman MM *et al.*, 2011). Increased production of superoxides and lowered antioxidant enzyme activities compromising with body antioxidants defence systems in hyperglycemia is associated with the pathogenesis of diabetic dyslipidaemia, micro and macro vascular complications (Brownlee M., 2001).

Currently used oral hypoglycaemic agents for the

treatment of diabetes are stimulators of beta cells (sulfonylureas), inhibitors of gluconeogenesis (biguanides), inhibitors of intestinal  $\alpha$ -glucosidases (acarbose) and drugs which reduces insulin resistance (glitazones). It has been found that prolonged use of currently available oral hypoglycaemic agents such as sulphonylureas, biguanides etc. have side effects and failure of response (Pepato MT *et al.*, 2005). Therefore different type of hypoglycaemic agents are available along with insulin for the treatment of diabetes, there is a significant demand by patients to use natural products for the treatment of diabetic. Plant based medicines are showing prominence in the treatment of various metabolic diseases like diabetes (Venkatesh S *et al.*, 2003). Herbal medicines are popularized due to their effectiveness, easy availability, low cost and comparatively being devoid of toxic effect (M. Ali, 1998). Many plant species reported to have pharmacological properties as they are known to possess various secondary properties like glycosides, saponins, flavonoids, steroids, tannins, alkaloids which is therefore should be utilized to combat the disease causing pathogens (Hossain *et al.*, 2011). Many flavonoids containing plants serves as a hidden wealth of potentially useful natural product for diabetes control (Cazarolli LH *et al.*, 2008). *Alocasia indica* L. (Bangla-Maan kachu) is one of the most familiar medicinal plants which grow abundantly throughout the year all over Bangladesh. From literature, it is found that different parts of this plant are traditionally used as hepatoprotective (Mulla *et al.*, 2009), analgesic (Rahman *et al.*, 2011) antiarthritic, antitumour and antipyretic (Anonymous, 1952). It is also reported to use in the treatment of diabetes mellitus and piles (Pullaiah and Naidu, 2003). Alcoholic extract of leaves were evaluated for antimicrobial (Mulla *et al.*, 2010), antidiarrhoeal (Mulla *et al.*, 2011), antioxidant (Chapman and Hall, 1984), anti-inflammatory (Chapman and Hall, 1984) and anthelmintic (Mulla *et al.*, 2010) properties. It is evident that the plant has great potentials in treating various diseases where free radicals have been reported to be the major factors contributing to the disorders. It is claimed that these

products help regulate blood sugar levels (Projapati and Naidu, 2003). However literature indicates that there is no scientific data to support antidiabetic effect of leaves and stem of *Alocasia indica* L. in Bangladesh. Therefore, the present study investigates the effects of ethanolic extract of leaves and stem of *Alocasia indica* L. to ascertain the scientific basis for the use of this plant for treatment of diabetes. In this study, we made an attempt to investigate in comparative study of *Alocasia indica* L using leaves and stem available locally in Bangladesh.

## Materials and methods

### Plant materials collection

Giant taro or elephant ear is generally known as mankachu at Bangladesh and Botanical name is *Alocasia indica* L and family of Araceae. Fresh leaves and stem of *Alocasia indica* L used in this work were collected in the year 2012 from the experimental plot located at Rajshahi city, Rajshahi, Bangladesh. The authenticity of the *Alocasia indica* L was identified by Professor A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi, Bangladesh. The leaves and stem were separated manually from the tree and washed several times with water to remove the foreign materials. Afterward, the leaves and stem were dried in the sunlight for four consecutive days and again in an electric oven at 40°C until a constant weight were reached. The leaves and stem were ground to a fine powder, packed and stored in a refrigerator at 4°C prior to analysis.

### Extraction

For solvent extraction (Soxhlet method), 500g of leaves and stem were placed into a separate cellulose paper cone and extracted using ethanol in a 5-l Soxhlet extractor for 8 h (Pena *et al.*, 1992). By using rotary evaporator the extract was recovered and residual solvent was removed by drying in an oven at 60 °C for 1 h.

### Experimental animals

Healthy adult Wistar albino rats (*Rattus norvegicus*) of either sex, weighting between 180 -250 gm were used for the study. These rats were collected from

International Centre for Diarrheal Diseases Research, Bangladesh (ICDDR'B). The rats were housed in standard plastic cages at room temperature, 28-30°C and relative humidity, 50-55% for 4-6 days prior to the experimental work. During the experimental period, standard pellet diets were fed to the rats and *ad libitum* water was supplied. This study was done according to the guideline of the Institutional Animal Ethics Committee.

### Used chemicals

Streptozotocin was collected from Department of Biochemistry and Molecular Biology laboratory store. The streptozotocin solution was prepared by freshly dissolving in citrate buffer (0.1 M, pH 4.5). Glucose level was determined by Glucomiter. All other chemicals used here in the laboratory grade.

### Induction of Diabetes

A total of thirty five animals were equally divided into seven groups with five animals in each group. Leaving aside five rats for Normal Control Group, 30 rats were induced diabetes by streptozotocin.

The streptozotocin diabetic rat model was performed by the method of Kandur and Goyal. Rats were injected intraperitoneally with 60 mg/kg body weight. The rats were then kept on 10% glucose solution for next 24 hours, in their cages, to prevent hypoglycemia. Streptozotocin dissolved in 0.1 M cold citrate buffer (pH 4.5). Forty eight hours later of streptozotocin administration, blood samples were drawn from tail and glucose levels was determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels in the range 275 and 300mg/100ml were selected for the studies (Kandur SV *et al.*, 2005). Two doses, 200 and 400 mg/kg were selected for the present study to evaluate antidiabetic activity (OECD, 2000). The above drugs were administered orally, once daily for 21 days.

### Experimental design for antidiabetic study

All the diabetic animals were randomly divided into seven groups with five animals each and treated once a day for 21 days as follows.

Group I (normal control) Received only normal diet  
 Group II (diabetic control) Received only normal diet  
 Group III and Group IV (extract treated) received ethanolic extract of *Alocasia indica* leaves at dose of 200 and 400 mg/kg b.w. for 21 days.  
 Group V and Group VI (extract treated) received ethanolic extract of *Alocasia indica* stem at dose of 200 and 400 mg/kg b.w. for 21 days.  
 Group VII (standard) treated with Glibenclamide 10 mg/kg b.w. for 21 days (Gagandeep K., 2011).

### Collection and estimation of blood glucose level

Blood of the rats were collected from their tail (by cutting the edge of tails) at the 1st, 7th and 14th days of the experiment. After 21 days of treatment, finally rats were sacrificed and their blood was collected for the estimation of blood parameters. After every week of oral administration, rats were kept fasting for overnight, and the fasting blood was collected. Serum was obtained immediately by centrifugation (15min at 4000 r.p.m), which was used for the measurement of various biochemical parameters. All analyses were carried out within 24 hrs of blood collection. Blood glucose was estimated by single touch glucometer (one touch ultra, Johnson & Johnson Ltd.).

### Statistical analysis

The results are expressed as the mean of standard deviation (S.D.) of triplicate analyses. All statistical comparisons were performed using a one-way analysis of variance (ANOVA) followed by a multiple two-tailed t-test. Differences between the data were considered significant at  $P < 0.05$ .

## Results and discussion

### Effect on body weight

The results in Table 1 show the effects of body weight of normal rats, diabetic rats, leaves and stem extracts treated and glibenclamide treated rats at 21 days study. The body weight of diabetic control group significantly decreased compared with normal controls and was about 196.51 and 212.73 respectively. The results of leaves extracts treatment on body weight in diabetic rats were 214.05 and 224.1

respectively in 200mg/kg and 400mg/kg doses. During regular observation of the *Alocasia indica* leaves extract treated diabetic rats there were significant ( $P < 0.05$ ) weight gains on 21 day relative to initial day. On the other hand stem extract treated diabetic rats there were significant weight gains on 21 day relative to initial day and was about 221.4 and 227.9 respectively in 200mg/kg and 400mg/kg doses.

### Effect on blood glucose level

The results in Table 2 show the effects of blood glucose level of normal rats, diabetic rats, leaves and stem extracts treated and glibenclamide treated rats at 21 days study. There was a significant increase in blood glucose level in diabetic rats when compared with normal control due to Streptozotocin injection. In the study, daily oral administration of the test extracts for the period of 21 days led to a dose dependent fall in blood glucose levels and showed significant ( $P < 0.05$ ) reduction in blood glucose levels compared to diabetic control. At the end of experiment blood glucose level were 136.84 and 109.63 mg/dl at the doses of 200 and 400 mg/ kg of ethanolic extract of *Alocasia indica* L. leaves respectively. Before the treatment blood glucose level were 329.15 and 324.23 mg/dl. Besides, blood glucose levels were 138.31 and 111.26 mg/dl at the doses of 200 and 400 mg/ kg of ethanolic extract of *Alocasia indica* L. stem respectively. Before the treatment blood glucose level were 334.12 and 328.58 mg/dl.

People with Type I diabetes must take insulin because their bodies do not make enough of it, whereas people with Type II diabetes benefit by reducing blood sugar levels through exercise and a healthy diet. However, it is not uncommon for people with Type II diabetes to require medication to stimulate the pancreas to produce more insulin, decrease the amount of glucose made by the liver, slow the absorption of starches in the diet, or take a combination of medications to control blood sugar. But the management of diabetes without any side effects is still a challenge and has increased the demand for research on natural products with antidiabetic activity.

**Table 1.** Effect of *Alocasia indica* L. leaves and stem extracts on body weights in diabetic rats.

Groups	Treatment	Body Weight (gm)			
		Initial Day	7th Day	14th Day	21st Day
I	Normal control	192±2.96	197.4±3.56	205.84±3.54	212.73±4.11
II	Diabetic control	213.50±2.48	207.62±4.87	203.6±2.38	196.51±3.96
III	LEAI +Diabetic (200 mg/ kg)	215.25±3.01	212.6±3.98	213.09±3.24	214.05±4.23
IV	LEAI +Diabetic (400 mg/kg)	219.9±3.55	221.5±2.92	223. 9±4.55	224.1±3.02
V	SEAI +Diabetic (200 mg/kg)	218.25±3.01	217.52±3.96	219.02±3.25	221.4±3.9
VI	SEAI+Diabetic (400 mg/kg)	221.2±3.55	223.21±2.78	226.68±4.22	227.9±2.65
VII	Glibenclamide+Diabetic (10 mg/kg)	213.30±3.95	215.23±3.25	217.9±3.52	219.86±3.39

LEAI - Leave extract of *Alocasia indica* , SEAL- Stem extract of *Alocasia indica*. All values represent means ± S.D. n = 5. P < 0.05 indicates significant activity compared to the diabetic control group.

**Table 2.** Effect of *Alocasia indica* L. leaves and stem extracts on body glucose level in diabetic rats.

Groups	Treatment	Body Glucose Level (mg/dl)			
		Initial Day	7 <sup>th</sup> Day	14th Day	21st Day
I	Normal control	95.12±1.29	95. 32±1.61	96.05±2.11	95.85±2.34
II	Diabetic control	296.20±2.85	335.63±1.96	398.71±3.79	423.11±3.63
III	LEAI+Diabetic (200mg/kg)	329.15±2.12	258±1.32	168.69±1.02	136.84±2.69
IV	LEAI(400mg/kg)	324.23±2.9	274.69±1.77	188.40±2.87	109.63±2.32
V	SEAI+Diabetic (200mg/kg)	334.12±3.96	263.74±1.98	173.62±3.09	138.31±3.56
VI	SEAI+Diabetic (400mg/kg)	328.58±2.09	242.7±2.98	153.38±2.03	111.26±1.87
VII	Glibenclamide+Diabetic (10 mg/kg)	323.30±3.65	273.3±2.01	138.4±2.34	96.23±1.92

LEAI - Leave extract of *Alocasia indica* , SEAL- Stem extract of *Alocasia indica*. All values represent means ± S.D. n = 5. P < 0.05 indicates significant activity compared to the diabetic control group.

The pancreas secretes two important hormones, insulin and glucagon which are concerned with the control of the blood sugar level. Virtually all forms of diabetes mellitus are due to either a decrease in the circulating concentration of insulin (insulin deficiency) or a decrease in the response of peripheral tissues to insulin (insulin resistance) in association with an excess of hormones with actions opposite to those of insulin (glucagon, growth hormone, cortisol and catecholamines).

Historically, insulin has been associated with "blood sugar", and true enough insulin has profound effects on carbohydrate metabolism. Yet it is abnormalities of fat metabolism, causing such conditions as acidosis and arteriosclerosis, which are the usual causes of death of a diabetic patient. Also, in patients with prolonged diabetes, diminished ability to synthesize proteins leads to wasting of the tissues as well as many cellular function disorders. Therefore, it is clear that

insulin affects fat and protein metabolism almost as much as it does carbohydrate metabolism.

Pancreas is the main organ which involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose; insulin is secreted (Dietrich L., 1983). Streptozotocin causes a massive reduction in insulin release by the destruction of  $\beta$ -cells of the islets of Langerhans. An insufficient release of insulin, that leads high blood glucose called hyperglycemia. Insulin deficiency causes various metabolic alterations such as increased blood glucose, increased cholesterol and transaminases. The mechanism of hyperglycemia in diabetes mellitus involves overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. The majority of the experiments confirmed the benefits of medicinal plants with hypoglycaemic effects in the management of diabetes mellitus. Numerous mechanisms of actions have been proposed for this plant extracts. Some hypotheses relate to their effects on the activity of pancreatic  $\beta$  cells (synthesis, release, cell regeneration/revitalization) or the increase in the protective/inhibitory effect against insulinase and the increase of the insulin sensitivity or the insulin-like activity of the plant extracts. Other mechanisms may involve improved glucose homeostasis (increase of peripheral utilization of glucose, increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis acting on enzymes, inhibition of intestinal glucose absorption, reduction of glycaemic index of carbohydrates, reduction of the effect of glutathione. All of these actions may be responsible for the reduction and or abolition of diabetic complications. In the present study the antidiabetic activity of ethanolic extract of *Alocasia indica* leaves and stem was evaluated in streptozotocin induced diabetic rats. The activity exhibited was compared with the standard antidiabetic drug Glibenclamide. Daily treatment with the test extracts for a period of 21 days showed a significant ( $P < 0.05$ ) decrease in the serum glucose level at 7th, 14th and 21st days and maximum reduction occurred at 21st day in diabetic rats. It is

evident from these investigations that the ethanolic extract is effective in maintaining the serum glucose levels in streptozotocin induced diabetic rats. During the 21-day experimental period the body weight was reduced in diabetic rats, whereas there was a significant ( $P < 0.05$ ) gain of body weight in treated rats. The administration of test extract checks the loss in body weight and restored these levels significantly towards normal. The ability of the test extract to restore body weight seems to be a result of its ability to reduce diabetes by increased glucose metabolism. This may also be due to the protective effect of the extract in controlling muscle wasting i.e. reversal of gluconeogenesis.

These results have shown that the *Alocasia indica* leaves and stem extract possess blood glucose lowering effect in streptozotocin induced hyperglycemic rats. Thus, the folk use of *Alocasia indica* leaves extract for the control of diabetes may be validated by this study. Streptozotocin treated animals receiving the leaves and stem extracts of *Alocasia indica* showed significant ( $P < 0.05$ ) reduction of blood glucose levels in comparison to the control and this could be due to the possibility that some  $\beta$ -cells were still surviving to exert their insulin releasing effect by ethanol extract. This suggests that the mode of action of the active constituents of *Alocasia indica* leaves and stem extract was probably mediated by an enhanced secretion of insulin, like sulphonylureas. It is also possible that the leaves extracts of *Alocasia indica* L. might be taken up by the liver cells and the process of gluconeogenesis was inhibited or these might have improved insulin resistance. The improvement of liver function and subsequent increase in uptake of blood glucose and its utilization may be another mechanism of action of the extract. Other possible mechanism includes the stimulation of  $\beta$ -cells and subsequent release of insulin and activation of the insulin receptors. Estimation of insulin level and insulin receptor may give more insight into the mechanism of the antidiabetic activity exhibited by the extract. The studies also reveal that steroids and flavonoids present in the plant extract known to possess



antidiabetic activity (Shanmugasundaram K R *et al.*, 1983).

On the basis of the results, we concluded that ethanolic extract of *Alocasia indica* leaves and stem have beneficial effects on serum glucose levels and other metabolic aberrations as it lowers blood glucose level in diabetic rats. Therefore, *Alocasia indica* L. is considered to be effective and alternative treatment for diabetes. Further more these result suggest that both the leaves and stem extracts contain antidiabetic active principles, which would reduce the sugar level in Steptozotocin induced diabetic rats. Further study using the purified actives principal from leaves and stem extracts may reveal the role of the respective preparations as hypoglycemic agents in diabetes management.

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