Original article

Proximate composition, phytochemical screening and anti-hyperglycemic effect of elephant foot yam (*Amorphophallus paeoniifolius)* tuber on alloxan induced diabetic rats

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Summary. *Purpose:* This study's objectives were to explore the nutrient analysis of elephant foot yam tuber and observe the anti-hyperglycemic effect on both diabetic and non-diabetic rats. *Methods:* Healthy adult Long-Evans rats were grouped into four, and each group had six rats. Diabetes was induced intraperitoneally by injecting 140 mg/kg body weight of alloxan dose, and 500 mg/kg bd.wt of *Amorphophallus paeoniifolius* powder was administered orally for six weeks of both diabetic and non-diabetic experimental rats. Next three weeks, supplementary feeding diet was pulled out. *Results:* The proximate nutritional composition showed that elephant foot yam is a good source of carbohydrate, fiber, minerals viz. calcium, phosphorous, potassium, and zinc. This tuber was also rich with phytochemicals. About 17 (out of 25) antidiabetogenic phytochemicals were found in this tuber. Eight phytochemicals viz. anthraquinone, carotenoid, cardiac glycoside, glycoside, phlobotanin, phenol, saponin, and tannin were not found. Fasting blood glucose (FBG) level was reduced remarkably ($p<0.05$) in diabetic rats after 1st week and was highly significant ($p\leq0.001$) after 3rd weeks of experimental feeding. There was no significant change in the FBG levels of normoglycaemic animals. However, FBG was more sustainable even after withdrawn the supplementary diet. *Conclusion:* Elephant foot yam tuber could be used as a medicinal food to treat diabetes mellitus.

Keywords: Diabetes, Elephant foot yam tuber, nutrients, phytochemicals, rats.

Introduction

Diabetes mellitus (DM) is a major health problem for the people, and rapid prevalence puts a substantial burden on society and the public health sector (1). It is delimited by some synthetic drugs like insulin and oral hypoglycemic agents. However, they have some adverse effects at higher doses, and oral medications are not suitable for use during pregnancy. Many medicinal plants and foods still hold their unique place for the treatment of diabetes as alternative medicine, by showing few or no side effects (2, 3). About 800 plants have a potential therapeutic role on various non-communicable disorders like diabetes (4, 5). Among several plant sources, *Amorphophallus* species are supposed to have a significant effect on the management of diabetes. *Amorphophallus paeoniifolius* tuber is famous in many local names like 'Ol' in Bangladesh, 'Suran' or 'Jimmikand' in India, Elephant Foot Yam, or the White spot Giant Arum in English. *Amorphophallus* species are widely used in folk medicine, ethnomedicinal practices or ayurvedic preparations by different tribes for the treatment of many chronic, infectious and fatal diseases as antiinflammatory, antihaemorrhoidal, hepatoprotective, stomachic, analgesic, cytotoxic, antihelminthic, antifungal, antibacterial, antiprotease, and CNS depressant activities (6-8). However, excessive calcium oxalate present in the wild tubers can irritate the throat and mouth (9). Still, the therapeutic dose of *A. paeoniifolius* is considered a non-toxic up to 1.5 g/kg (10), and 2500 mg/kg in mice is regarded as an LD50 (11). The safe and effective dose (ED50) is 250 to 500 mg/kg bd.wt (12). Pramod et al. (2012) showed a therapeutic dose of up to 3000 mg/kg of both methanol and aqueous *A. paeoniifolius* tubers have no death (13). However, there is an insufficient study on nutritional composition (both quantitative and qualitative) of elephant foot yam tuber. The variability of nutrient contents depends on the traits, growth, and yield components. Ravi et al. (2009) found that these tubers are rich in calcium (50 mg g-1), phosphorus (34 mg g-1), and vitamin A (260 IU g-1) (14). Another study also showed that *A. paeoniifolius* tuber had an abundant amount of potassium (327.83 mg/100 g), followed by phosphorus (166.91 mg/100 g), calcium (161.08 mg/100 g), and iron (3.43 mg/100 g) (15). Thus, the aim of this study was to analyze the phytonutrients present in this tuber and also investigate the hypoglycemic effects on both diabetic and non-diabetic rats.

Materials and Methods

Sample collection:

Amorphophallus paeoniifolius tubers were collected from the local areas of the South-West region of Bangladesh from May to September.

Sample Preparation:

Samples were cleaned, pilled, and chopped into small pieces followed by dried in a thermostatically controlled oven at 60°C for 24 h. The dried samples were converted into powder using a grinder. The powder was then packaged in lidded polyethylene containers until nutritional analysis and animal experiments.

Extraction:

The powdered *A. paeoniifolius* tuber was soaked in 70% methanol at room temperature for three days, and then filtered with Whatman No. 42 filter paper to collect the supernatant. The supernatant was then poured into a round-bottomed flask at low pressure (60 rpm at 37°C) to remove the excess methanol via evaporation. A dark-brown coloured, concentrated crude methanol extract was fractionated with hexane, and the hexane-insoluble residue was then mixed with chloroform. Ethyl acetate diluted with distilled water (1:1) was then poured into the chloroform-insoluble extract and subjected to evaporation to remove excess solvent. The sample was then kept in a refrigerator for further bioactivity assay (16).

Phytochemical analysis:

Qualitative assay of methanolic and aqueous extracts of *Amorphophallus paeoniifolius* was carried out for the presence of phytoconstituents using standard protocols as described by Aguzue et al. (2012) (17).

Physicochemical determination:

Nutritional composition of primary metabolites such as moisture, carbohydrate, protein, fat, fiber, ash, vitamin and mineral contents of the *A. paeoniifolius* tubers were determined by using the following protocols.

Animals:

Healthy male Long Evan rats (98-168 g) were used to assess anti-hyperglycemic activity. All animals were housed in the standard laboratory conditions temperature (24^{o}C±2) and humidity 45±5% with 12h day: 12h night cycle. The standard laboratory diet was

Nutrient	Methods	Nutrient	Methods
Moisture (g)	AOAC	Zinc (mg)	AAS
Carbohydrate (g)	AOAC	Magnesium (mg)	AAS
Crude fiber (g)	AOAC	Manganese (mg)	Flame AAS
Crude protein (g)	Kjeldahl	Sodium (mg)	AAS
Crude fat (g)	Bligh and Dyer (1959)	Potassium (mg)	AAS
Ash (g)	AOAC	Vitamin-C (mg)	Redox Titration
Energy value (Kcal)	Bomb Calorimeter	Total carotenoids (mg)	AOCS
Calcium (mg)	Trimetric/ AAS	Non protein nitrogen	AOAC (Kjeldahl)
Phosphorus (mg)	Spectophotometric (WL: 650 to 840 nm)	Protein solubility (mg/ml)	Kjeldahl
Iron (mg)	Spectophotometric Thiocyanate	Free fatty acid (FFA)	AOAC
Copper (mg)	AAS		

AOAC: Association of Official Analytical Chemists; AAS: Atomic absorption spectrometry

provided to the animals, and they were allowed to drink water ad libitum. Studies were carried out after the approval of the Institutional Animal Ethical Committee following institutional ethical guidelines for the care of laboratory animals of Applied Nutrition and Food Technology, Islamic University, Kushtia, Bangladesh.

Chemicals:

All the chemicals and reagents were the analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Induction of Diabetes and Study Design:

Alloxan monohydrate (stored at 4°C) was dissolved in normal saline at room temperature and was injected by intraperitoneal routes in overnight fasted rats. The rats were divided into four groups, and each group consisted of six rats: Group-I: Non-diabetic control; Group-II: Diabetic control; Group-III and IV: Non-diabetic and diabetic rats respectively administered *A. paeoniifolius* powder (500 mg/kg/day) orally for six weeks, and next three weeks supplementary feedings were pulled out. Diabetes was induced by injecting 140 mg/kg body weight of alloxan doses on randomly selected healthy adult Long-Evans rats. After 72 hours, fasting blood glucose (FBG) was determined from the tail vein by using Accu-Chek glucometer strips (Roche Diagnostics). Animals with FBG >250 mg/dl were considered diabetic and were included in this study. After that, FBG was recorded at days 2, 7, 14, 21, 28, 35 and 42 to determine fluctuations in FBG or auto-reversal of diabetes. Bodyweight, food and water consumption were also recorded regularly.

Statistical analysis:

Data were analyzed by using the SPSS (Statistical Package for the Social Sciences) software, version 15.0 SPSS Inc. Chicago, Illinois, USA. All results were expressed as the mean ± Standard Deviation. One-way analysis of variance (ANOVA) used and paired, or unpaired t-test was done to see any difference between groups.

Result

Elephant foot yam tuber is popularly consumed throughout Bangladesh, mainly as vegetables. It is also used in traditional medicines for alleviating various diseases. But, scientific data are scarce about this tuber. The current study analyzed this tuber's nutrient contents and phytochemicals, as shown in Table 1 and Table 2.

The moisture content of elephant foot yam tuber (per 100 g powder) was 10.03 g, and dry matter was 89.97 g. Carbohydrate, protein, fat, fiber, and ash content were 71.71 g, 12.43 g, 0.26 g, 1.67 g, and 3.9 g, respectively, as shown in Table 1. The energy value (per 100 g) was 347.476 kcal. This tuber was a rich source of calcium (143.6 mg), iron (3.13 mg), potassium (263.74 mg), phosphorus (155.2 mg) and zinc (0.92 mg/100g). Other essential minerals such as copper, sodium, and manganese were found in trace amounts. Elephant foot yam tuber was a rich vitamin-C (5.57 mg), and protein solubility was found 86.90%. Free fatty acids and total carotenoids were 0.09 mg and 0.26 mg, respectively, whereas non-protein nitrogen (NPN) was detected but unable to quantify.

Table 2 represented the qualitative detection of phytochemicals in the extract of *A. paeoniifolius* tuber. In this study, the presence of 25 phytochemicals that

Table 1. Proximate macro and micronutrient composition of *Amorphophallus paeoniifolius* tuber per 100 g (dry basis)

Nutrient	(mean±SD)	Nutrient	(mean±SD)
Dry matter (g)	89.97±0.03	Copper (mg)	0.01±0.01
Moisture (g)	10.03±0.07	Zinc (mg)	0.92±0.01
Carbohydrate (g)	71.71±0.89	Magnesium (mg)	0.05±0.03
Crude fiber (g)	1.67±0.22	Manganese (mg)	0.25±0.07
Crude protein (g)	12.43±0.15	Sodium (mg)	0.06±0.02
Crude fat (g)	0.26±0.05	Potassium (mg)	263.74±0.17
Ash (g)	3.90±0.06	Vitamin-C (mg)	5.57±0.03
Energy value (kcal)	347.476±1.03	Total carotenoids (mg)	0.26±0.01
Calcium (mg)	143.6±0.02	Non protein nitrogen	Trace
Phosphorus (mg)	155.2±0.70	Protein solubility (mg/ml)	86.90±0.07
Iron (mg)	3.13±0.10	Free fatty acid (FFA)	0.09±0.22

Values are means of triplicates ± standard deviation.

Table 2. Phytochemicals investigated in *A. paeoniifollius* tubers extract

Phytochemicals	*Present (+) or Absent (-)*	Phytochemicals	*Present (+) or Absent (-)*
Alkaloid	+	Phlobotanin	-
Albuminoids	+	Phenol	-
Anthracene	+	Quercetin	+
Anthraquinone	-	Reducing compound	+
Betulinic acid	+	Rutin	+
Carotenoid	-	Saponin	-
Cardiac glycoside	-	β-sitosterol	+
Flavonoid	+	Steroid	+
Free anthraquinone	+	Sterols	+
Glucomannan	+	Stigmasterol	+
Glycoside	-	Tannin	-
Gums	+	Terpenoid	+
Lupeol	+		

have direct or indirect positive effects on diabetes was investigated. Among the investigated phytochemicals alkaloid, albuminoids, anthracene, betulinic acid, flavonoid, free anthraquinone, glucomannan, gums, lupeol, quercetin, reducing compound, rutin, steroid, sterols, β-sitosterol, stigmasterol, and terpenoid were present. While anthraquinone, carotenoid, cardiac glycoside, glycoside, tannin, phenol, phlobotanin, and saponin were not detected in elephant foot yam tuber.

Effects of elephant foot yam tuber on fasting blood glucose (FBG) level of non-diabetic and diabetic rats:

It is a long-lasting food taboo that diabetic people should avoid underground tuber-like vegetables such as elephant foot yam, considering they may further deteriorate diabetes condition. However, no scientific investigation report was found favouring the phenomenon. We are interested in justifying the existing misconception and investigate the diabetogenic or anti-diabetic effects, if any. An optimized dose (500 mg/kg/bd.wt./day) of elephant foot yam tuber was fed in addition to the regular diet. Fasting blood glucose (FBG) was measured every weekend before administering a supplementary therapeutic dose using a glucometer.

Elephant foot yam tuber could not significantly reduce FBG levels, but a gradual reduction of FBG levels ranging from 7.63 to 7.13 mmol/L was observed. However, FBG levels of diabetic rats were significantly ($p<0.05$) reduced from the 3rd weeks and continued the reduction until the supplementary feeding was withdrawn (6th week). In the 6th week, the FBG levels reached from 16.5 to 10.22 mmol/L, indicating that the tuber may gradually revert the damaged's pancreatic beta cells. This phenomenon was further confirmed when supplementary feeding of elephant foot yam tuber was withdrawn after the 6th week. FBG levels were gradually increased in diabetic (10.22 to 11.9 mmol/L) and non-diabetic (7.13 to 7.28 mmol/L) rats at the end of the experiments (9th week). The FBG levels' increments after the supplementary diet's withdrawal may be due to cessation of the

Table 3. Effect of *A. paeoniifollius* tuber (500 mg/kg bd.wt./day) on FBG (mmol/L) levels of normal and diabetic rats

Weeks	Non-diabetic		Diabetic	
	Control (Mean±SD)	*A.paeoniifollius* (Mean±SD)	Control (Mean±SD)	*A.paeoniifollius* (Mean±SD)
0 week	7.38±0.76	7.63±0.65	14.43±0.66	16.5±0.71
1st week	7.33±0.84	7.6±0.72	15.7±1.04	16.48±0.75
2nd week	7.4±0.79	7.55±0.73	17.68^{a**}±0.87	15.4^{a*b*}±0.51
3rd week	7.45±0.68	7.45±0.66	20.33^{a**}±0.55	14.86^{a*b**}±0.53
4th week	7.5±0.59	7.3±0.82	22.68^{a**}±1.28	12.18^{a**b**}±0.49
5th week	7.68±0.6	7.23±0.82	24.55^{a**}±0.82	10.92^{a**b**}±0.63
6th week	7.73±0.51	7.13±0.75	27.35^{a**}±0.48	10.22^{a**b**}±0.19
After withdrew supplementary feeding				
7th week	7.78±0.74	7.13±0.57	28.03 ±0.36	10.66 ±0.36
8th week	7.78±0.51	7.23±0.48	30.60 ±0.50	11.04 ±0.44
9th week	7.85±0.52	7.28±0.46	31.98^{c**}±0.72	11.9^{c*} ±0.33

**Significant values p≤0.05; **High significant values p≤0.001*

'a' indicates compare with initial day (0 week) in the same groups

'b' indicates compare with control group

'c' indicates compare with 6th week in the same group after omitting supplementary feeding

pancreatic beta-cell regeneration and the onset of the pancreatic damage's continuous alloxan injury. Fig. 1 also showed the suppressing ability of elephant foot yam tuber in normoglycemic rats.

Fig. 2 indicated the relative change of body weight gaining among diabetic and non-diabetic control and experimental rats. The initial average body weight (118-126 g) of all groups rose gradually, but the diabetic control group reduced body weight after 4th weeks compared to non-diabetic control rats. Whereas, experimental groups of both diabetic and non-diabetic rats administered (orally) with elephant foot yam tuber (500 mg/kg/day) increased body weight as like as control rats (Fig. 2). So, this tropical tuber has weight gaining properties.

Discussion

Elephant foot yam (*A. paeoniifolius*) is widely consumed as a vegetable throughout the Indian subcontinent, including Bangladesh. But diabetic patients avoid this tuber as a myth that it may worsen the hyperglycemic condition (18). Moreover, study reports regarding such misconception and nutritional values of elephant foot yam were not so available. The current study was designed to investigate the nutrient composition of this tuber, as well as antidiabetogenic effects on rats. Nutrient analysis (per 100 g) of elephant foot yam tuber was found that it is rich in carbohydrate (71.71 g), and minerals viz. calcium (143.6mg), phosphorus (155.2 mg), potassium (263.74 mg), iron (3.13 mg), zinc (0.92 mg), as well as vitamin C (5.57 mg). This result is accorded with the study of Singh and Neeraj (2014), who showed energy (236-566.70 KJ),

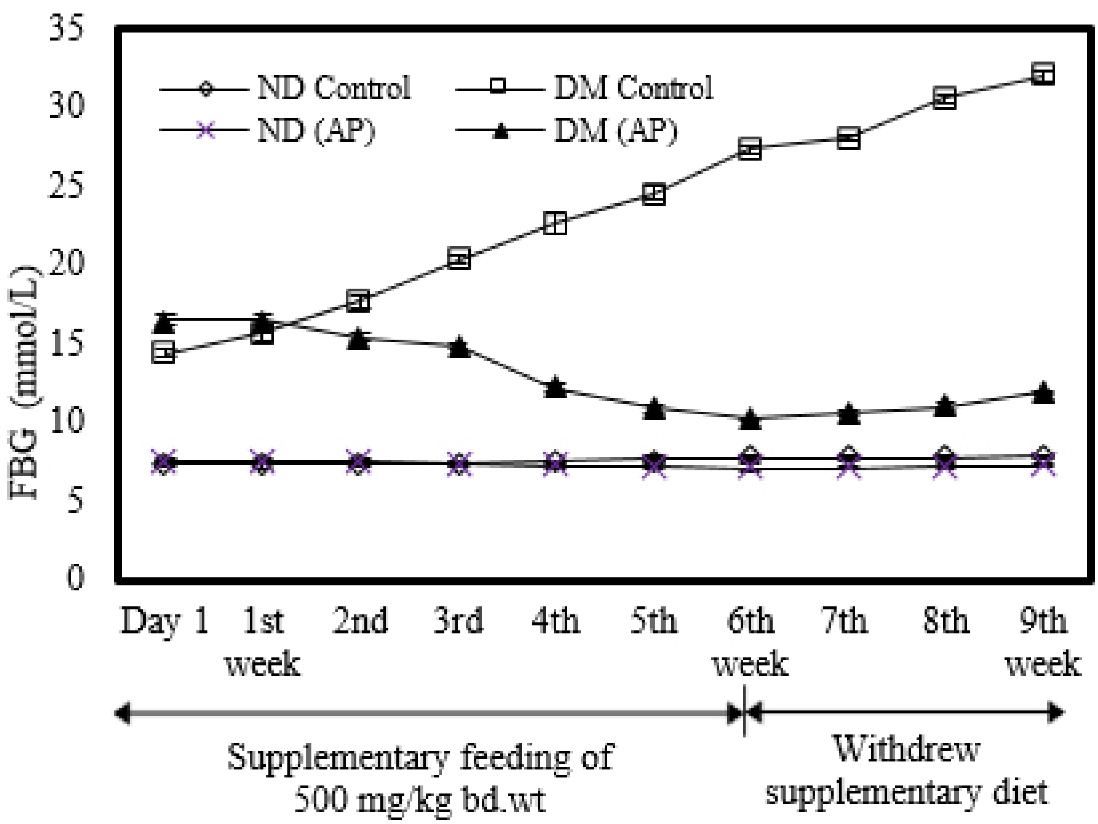

Fig. 1. Fasting blood glucose (FBG) levels of normal and diabetic rats with/ without *A. paeoniifollius* tuber powder.

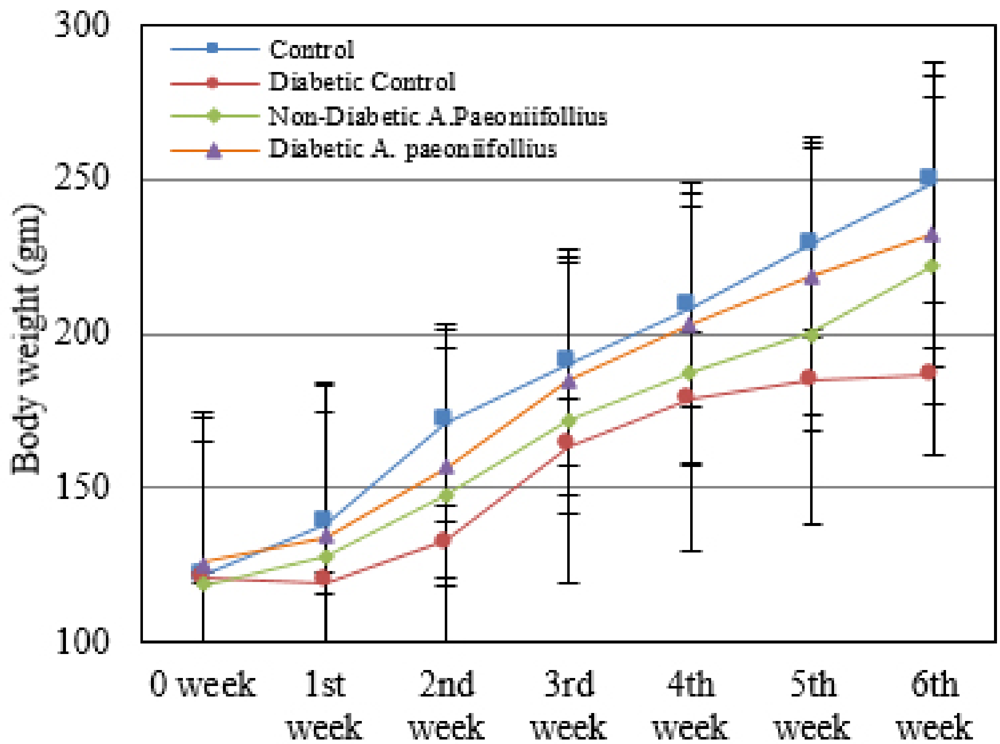

Fig. 2. Graphical presentation of the effect of *A. paeoniifollius* tuber on body weight of Long Evans rat.

starch (11-28%), sugar (0.7-1.7%), protein (0.8-2.60%), fat (0.07-0.40%) and minerals viz. calcium (131-247 mg), potassium (230-417mg), phosphorus (120-247 mg), iron (1.97-5.56 mg), zinc (0.12-1.92 mg), manganese (0.19-0.65 mg), and soluble oxalate (6.65-18.50 mg) (19).

Arup et al. (2009) reported that elephant foot yam tuber is rich with potassium (327.83 mg), followed by phosphorus (166.91 mg), calcium (161.08 mg), and iron (3.43 mg) (15). Recent data strongly indicated that complex carbohydrates, fiber, minerals, and important phytochemicals could reduce blood glucose levels by regenerating the damaged pancreatic β-cells or facilitating glucose uptake by the tissues (20, 21). Zinc, lupeol, glycosides, and saponin have a significant role in regenerating the β-cells (22). Hence, this study also confirmed the qualitative test of twenty-five phytochemicals that are believed to have antidiabetic roles either directly or indirectly. Among them, seventeen phytochemicals viz. alkaloid, albuminoids, anthracene, betulinic acid, flavonoid, free anthraquinone, glucomannan, gums, lupeol, quercetin, reducing compound, rutin, steroid, sterols, β-sitosterol, stigmasterol, and terpenoid were found in the elephant foot yam tuber. Eight phytochemicals viz. anthraquinone, carotenoid, cardiac glycoside, glycoside, phlobotanin, phenols, saponin, and tannin were not found in this tuber.

Phytochemicals like betulinic acid, stigmasterol, and β-sitosterol inhibit α-glucosidase & α-amylase (23), anthraquinone sensitizes insulin receptors, alkaloids and tannin prevent β-cell destruction and enhance glucose uptake (24). Thus, elephant foot yam tuber might be beneficial for diabetic patients. The probability was investigated using the Long-Evans rat model, as mention in the methodology. When elephant foot yam tuber was fed as supplementary diet (500mg/kg bd. wt.) to the non-diabetic rats, no significant reduction of the fasting blood glucose (FBG) level was found as shown in Fig. 1. In contrast, the FBG level of diabetic rats showed significant ($p<0.001$) reduction after 3rd weeks and continued until the supplementary feeding was withdrawn (6th week). However, elephant foot yam tuber was unable to revert the diabetic rats into normoglycemic condition by the end of the 6th weeks of feeding trial. The reasons behind this difference may be due to the lower amount of fiber content as well as the absence of several phytochemicals such as- anthracene, carotenoids, glycoside, saponin & tannin in elephant foot yam. The phenomenon was more clearly shown when the supplementary feeding diet was withdrawn after 6th weeks, as shown in Table 3 and Fig. 1. During this session (7th to 9th weeks), the FBG level of diabetic rats was slightly raised. This study also found that elephant foot yam tuber also had weight gaining properties in both diabetic and non-diabetic rats (Fig. 2).

Conclusion

Traditional and ethnomedicinal literature revealed that *A. paeoniifolius* tuber is safe and effective for the treatment of various acute and chronic diseases like piles, enlargement of spleen, gastrointestinal disorders, arthritis etc. This study confirmed that this tuber appreciably reduced serum blood glucose level and gently increased bodyweight like a normoglycemic patient. This compiled data may be helpful for a diabetic person to build up a new food habit in their daily life and well aware of the local food taboos about this tuber. This study also focused on the priority areas of research to discover the natural drugs for a hyperglycemic patient.

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***Conflicts of Interest*:** The author(s) declare(s) that there is no conflict of interest regarding the publication of this manuscript.

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