

Antidiabetic activity of ethanol extract of *Colocasia esculenta* leaves in alloxan induced diabetic rats

Kumawat N. S., Chaudhari S. P., Wani N. S., *Deshmukh T. A., Patil V. R.

Department of Pharmacognosy, Tapi Valley Education Society's Hon'ble, Loksevak

Madhukarrao Chaudhari College of Pharmacy, North Maharashtra University,

Faizpur-425 503, India

*Corres.author: deshmukhta@rediffmail.com

Tel. No. : 02585 245574, Fax No.: 02585 245574

Abstract: *Colocasia esculenta* Linn (family: Araceae) commonly known as 'Alu and dasheen' widely used in traditional system of medicine for the treatment of diabetes mellitus. In the present study, ethanol extract of *C. esculenta* (EECE) leaves were subjected to phytochemical investigation and evaluated for antidiabetic activity on blood glucose level and on the body weight in alloxan induced diabetic rats. EECE (100, 200 and 400 mg/kg) and metformin (450 mg/kg) were administered orally in alloxan (120 mg/kg, i.p.) induced diabetic rats. In acute oral toxicity (AOT 425) study, administration of EECE no mortality upto 5000 mg/kg was observed. The onset of reduction of blood glucose was observed at 4 h (96 mg/dl), peak at 6 h (120 mg/dl) but antihyperglycaemic effect waned at 24 h. In subacute study, maximum reduction in blood glucose was observed (174.34 mg/dl) at the dose of 400 mg/kg on 14th day. EECE prevented further loss of body weight. EECE (400 mg/kg) was found to have significant ($p < 0.001$) blood glucose lowering effect. Preliminary Phytochemical investigation revealed the presence of alkaloids, flavonoids, saponins and tannins as the major constituents in the ethanol extract. These results suggest that EECE (400 mg/kg) showed antihyperglycaemic activity in alloxan induced diabetic rats.

Key words: *Colocasia esculenta*, alloxan, metformin, antidiabetic activity.

Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, abnormal lipid and protein metabolism along with specific long-term complications affecting the retina, kidney and nervous system.¹ Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus.² The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides.³ Unfortunately, apart from having number of side effects, none of the oral synthetic hypoglycemic agents has been successful in maintaining euglycaemia and controlling long-term microvascular and macrovascular complications.⁴ Dasheen is food crops in the Caribbean and it is use as traditional foods for the treatment of diabetes mellitus.⁵ The plant is useful for curing diabetes; it is anthelmintic, demulcent, helpful cough, sore throat and wounds.^{6,7} Dasheen extract or commercial linamarin

significantly increased the activities of intestinal disaccharides in streptozocin- induced diabetic rats.⁸ Mucilage isolated from *Colocasia esculenta* (L.) tubers was administered to experimental animal rats and the metabolism of lipids and lipoproteins was studied.⁹ However, the antihyperglycaemic activity of the *Colocasia esculenta* leaves has not been reported. Hence, the objective of the present study was to investigate the antihyperglycaemic activity of ethanol extract of *Colocasia esculenta* leaves in alloxan induced diabetic rats.

Material and Method

Collection of plant material:

The leaves of *Colocasia esculenta* were collected from the local area of Jalgaon in Maharashtra state, India and authenticated by Dr. Alka Chaturvedi, Department of Botany, Rashtrasant Tukadoji Maharaj, Nagpur

University, Nagpur. The voucher specimen (No. 9413) was deposited at that institute.

Preparation of EECE extract:

The leaves of *Colocasia esculenta* were shade dried and powdered in a grinder. The air-dried powder was subjected to hot continuous extraction with ethanol in a soxhlet extractor. The extract was concentrated under reduced pressure using rotary evaporator and the residue was dried in desiccators over anhydrous calcium chloride. The percentage yield of ethanol extract was 3.71 % w/w.

Chemicals:

Glucometer (Acucheck-Sensor) was purchased from Roche Diagnostics, Mumbai, India Metformin was obtained as gift sample from IPCA Laboratories, Mumbai, India. Alloxan monohydrate was purchased from Sigma, USA. Ethanol was purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India..

Qualitative chemical tests:

Ethanol extract was tested to know the different constituents present in it by the standard procedures. The extract was tested for alkaloids, saponins,^{10, 11} flavonoids,¹² and tannins,¹³

Animals:

All the experiments on animal were conducted according to protocols that were approved by the Institutional Animal Ethics Committee (IAEC) of Hon'ble Madhukarrao Chaudhari College of Pharmacy, Faizpur, India. Wister albino rats (150–200 g) of either sex were purchased from Calcutta Fish Aquarium, Indore, India. They were maintained at a temperature of 25 ± 5 °C and relative humidity of 50% to 55%. The animals were allowed free access to food pellets (Amrut Feeds, Pune, India) and water throughout the study. Acute toxicity study was carried out using albino mice.

Acute toxicity study:

Albino mice of either sex weighing between 20-30 g were used. The animals were fasted over night. Acute toxicity study was performed according to OECD guidelines; method followed is according to number 420. It was found that tolerated dose was higher than 5,000 mg/kg body weight.¹⁴

Alloxan-induced diabetes:

The albino rats weighing 150-200 g of either sex were allowed to fast for 24 hours prior to experimentation and rendered diabetic by a single dose of intraperitoneal injection of alloxan 120 mg/kg body weight.¹⁵ After 18 hours of injection of alloxan, diabetes was confirmed by testing blood sugar level

more than 200 mg/dl were selected for the further study. Animals were maintained for four days in diabetic condition for well establishment of diabetes. They were divided into five groups. Group I: untreated but diabetes-induced animals served as a negative control; Group II: diabetes-induced animals and treated with standard drug metformin 450 mg/kg body wt./day orally; Group III: diabetic animals and treated with ethanol extract (100 mg/kg); Group IV: diabetic animals and treated with ethanol extract (200 mg/kg); Group V: diabetic animals and treated with ethanol extract (400 mg/kg). The ethanol extract was given orally.

The acute study involved estimation of blood glucose levels at 0, 2, 4, 6 and 24 hour after EECE and metformin administration. The animals had free access to feed and water after 6 h. The subacute study involved repeated administration of EECE and metformin for 14 days (once a day) at a prefixed times and blood glucose levels were estimated in samples withdrawn after 2 h on day 7 and 14.¹⁶ The animals had free access to feed and water during this period. Blood samples from the experimental rats were collected by the tail using pricking lancet. The collected blood samples were analyzed for blood glucose levels by the glucometer using strip technique and blood glucose levels were expressed in mg/dl. The data was represented as mean blood glucose level and standard error of mean (SEM).

During the study period of 14 days the rats were weighed daily and their body weights were recorded. From this data, mean change in body weight and SEM were calculated and tabulated.

Statistical analysis:

Data were expressed as mean \pm SEM and statistical analysis was carried out by one-way ANOVA with a post hoc Tukey's test performed using GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com. $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered to be significant.

Result and Discussion

In acute oral toxicity study, EECE was safe upto a dose level of 5000 mg/kg of body weight. No lethality or any toxic reactions were found upto the end of the study period.

In acute study, EECE (100,200 and 400 mg/kg) as well as metformin (450 mg/kg) showed significant reduction of blood glucose levels at 2, 4, and 6 h. The onset of reduction of blood glucose of EECE (100, 200 and 400 mg/kg) was observed at 2 h (17.17, 16.84 and 20.17 mg/dl respectively), peak effect at 6 h (93.5, 102.17 and 119.67 mg/dl respectively) but effect was waned at 24 h. The onset of antidiabetic effect of

metformin was at 2 h (36.17 mg/dl), the peak effect was at 6 h (150 mg/dl) (Table 1)

In the subacute study, repeated administration (once a day for 14 days) of the EECE as well as metformin causes significantly ($p < 0.001$) reduction in the blood glucose level as compared with negative control group. Maximum reduction in blood glucose level was observed (131.84, 155.67 and 174.34 mg/dl respectively) on 14th day in the diabetic rats treated with EECE (100, 200 and 400 mg/kg). Metformin treated animals showed maximum reduction in blood glucose level (203.5 mg/dl) on 14th day (Table 2).

Subacute treatment for 14 days with the EECE in the treated doses brought about improvement in body weights, indicating its beneficial effect in preventing loss of body weight in diabetic rats. The ability of EECE to prevent body weight loss seems to be due to its ability to reduced hyperglycaemia (Table 3).

EECE (100, 200 and 400 mg/kg) showed significant $p < 0.001$ decrease in blood glucose level at 2, 4 and 6 h. Continuous treatment with EECE (100, 200 and 400 mg/kg) for a period of 14 days showed a significant ($p < 0.001$) decrease in the blood glucose level in diabetic rat. Maximum reduction of blood glucose

level in acute and subacute occurred at the dose of 400 mg/kg. p. o. The EECE showed short onset and short duration of antihyperglycaemic action.

Subacute treatment for 14 days with the EECE in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic rat.¹⁷ The ability of EECE to prevent body weight loss seems to be due to its ability to reduced hyperglycemia.

Flavonoids are potent antioxidant and known to modulate the activities of various enzymes due to their interaction with various biomolecules.^{18, 19} reported that flavonoids, alkaloids, tannins and phenolics as bioactive antidiabetic principles.¹⁹

The plants of *C. esculenta* have been reported to contain alkaloids, flavonoids, saponin and tannins.²⁰ Preliminary phytochemical analysis indicated that, the ethanol extract of *Colocasia esculenta* leaves contain sterols, flavonoids, glycosides, tannins, carbohydrates and Vitamin A and C.

The antihyperglycaemic activity of EECE may probably be due to the presence of several bioactive antidiabetic principals. It is thus apparent that EECE possesses antihyperglycaemic activity.

Table1. Effect of EECE on blood glucose level (mg/dl) in alloxan-induced diabetic rats (acute study).

Treatment (mg/kg. p. o)	Blood Glucose Level (mg/dl)				
	0 hr	2 hr	4 hr	6 hr	24 hr
Negative control	276±12.31	279±5.28	283±12.28	295.33±14.44	299.16±12.44
Metformin (450)	347.83±25.11	311.66±8.80	228.66±12.57*	197.83±13.95**	237.33±12.9*
EECE (100)	320±15.55	302.83±12.58	247.66±10.31	226.5± 17.40 *	250.66±14.13
EECE (200)	33.5±17.50	2306.66±10.94	244±10.41	221.33±19.41*	247±12.96
EECE (400)	330±16.37	309.83±10.15	234±8.95*	210.33±18 **	245.83±13.92*

Values are mean ± S.E.M., n = 6, data was analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared with negative control group (Distilled water, 10 ml/kg).

Table 2. Effect of EECE on blood glucose level (mg/ dl) in alloxan-induced diabetic rats (Subacute study).

Treatment (mg/kg. p. o)	Blood Glucose Level (mg/dl)		
	0 day	7day	14 day
Negative control	276±12.31	316.33±15.09	332.16±11.21
Metformin (450)	347.83±25.11	199±11.20***	144.33±12.27***
EECE (100)	320±15.55	242±18.45*	188.16±9.43***
EECE (200)	323.5±17.50	223.66±17.69**	167.83±15.00***
EECE (400)	330±16.37	220.5±18.14***	155.66±12.84***

Values are mean ± S.E.M., n = 6, data was analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared with negative control group (Distilled water, 10 ml/kg).

Table3. Effect of EECE on body weight (g) in alloxan-induced diabetic rats.

Treatment (mg/kg. p. o)	Mean body weight (g)		
	0 day	7 day	14 day
Negative control	175.5±1.97	172.66±1.90	170.5±1.97
Metformin (450)	181±2.03	183.5±2.15*	186.33±1.85**
EECE (100)	179±1.23	178.5±1.38	176±1.31
EECE (200)	179.16±2.62	178.5±2.70	177.16±2.93
EECE (400)	180.16±2.71	182.16±2.71*	184.16±2.71**

Values are mean ± S.E.M., n = 6, data was analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software *P<0.05, **P<0.01, ***P<0.001 as compared with negative control group (Distilled water, 10 ml/kg).

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