HYPOGLYCAEMIC ACTION OF ETHANOLIC EXTRACT OF LEAVES OF OXALIS CORNICULATA L. ON NORMAL AND ALLOXAN-INDUCED DIABETIC ALBINO RATS

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ABSTRACT

BACKGROUND

Since times immemorial, herbal drugs have been used in the treatment of many endocrinal disorders including diabetes mellitus. The alarming rise in the incidence of diabetes mellitus in India as well as the entire world has necessitated the development of safer and efficacious antidiabetic drugs.

The aim of the present study is to evaluate the hypoglycaemic action of ethanolic extract of leaves of Oxalis corniculata L. on normal and alloxan-induced diabetic albino rats.

MATERIALS AND METHODS

The ethanolic extract (500 mg/kg/d) was administered orally for two weeks to alloxan-induced diabetic rats. Blood glucose was estimated every week for two consecutive weeks along with bodyweight monitoring. The mechanism of action of the test drug was evaluated by estimating the glycogen content in liver, heart and skeletal muscle and also by the action of the test drug on adrenaline-induced hyperglycaemia in the albino rats. Phytochemical analysis of the extract was carried out. Antioxidant activity of the extract was analysed by undertaking serum level estimations of catalase and malondialdehyde in the albino rats.
Statistical Analysis- One-way ANOVA followed by Dunnett's multiple comparison test was used for statistical analysis. The body weights of the rats before and after drug administration were compared using Student's t-test (paired). Values of $p < 0.05$ were considered significant.

RESULTS

The test drug significantly ($p<0.05$) reduced the rise in blood glucose induced by alloxan. The test drug produced significant ($p<0.05$) increase in liver glycogen and also significantly ($p<0.05$) reduced adrenaline-induced hyperglycaemia. Significant ($p<0.05$) lowering of normal blood glucose was also found in the test drug group compared to the normal control group. Significant ($p<0.05$) increase in serum catalase levels and significant ($p<0.05$) reduction in malondialdehyde levels were seen in the test drug group and the diabetic standard group as compared to the diabetic control group.

CONCLUSION

Thus, the leaves of Oxalis corniculata has significant antidiabetic and hypoglycaemic activity.

KEYWORDS

Ethanolic Extract, Oxalis Corniculata, Alloxan, Diabetes Mellitus, Hypoglycaemic Effect.

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BACKGROUND

Diabetes mellitus is a chronic disease characterised by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism.

These metabolic abnormalities occur due to insulin deficiency, which either cause increased release of glucose into the circulation from the liver or due to reduced entry of glucose into the peripheral tissues.[1] The side effects associated with the use of insulin and other hypoglycaemic agents have led to an increased demand for the use of natural products with antidiabetic properties.[2] Researches conducted on several plants have revealed their effectiveness in the treatment of diabetes mellitus. However, in the perspective of an ever increasing incidence of diabetes mellitus, a lot of research still remains to be conducted in this field.[3]

Oxalis corniculata L., commonly known as Indian Sorrel or 'Chengeri tenga' is a common garden weed found throughout India. The leaves of the plant are considered cooling, antiscorbutic, astringent and useful in fever, biliousness, dysentery and prolapse of the rectum.[4] Preclinical studies have reported the plant to possess wound healing, antibacterial, antiepileptic, anxiolytic, antiamoebic, antifungal, anticancer, antidiabetic, antihyperlipidemic, cardioprotective, hepatoprotective, analgesic,

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antiinflammatory, abortifacient and antioxidant properties.[5] Literature reviews indicated that although studies on the antidiabetic potential of this plant in diabetic rats have been undertaken, not much studies on its blood glucose lowering activity in normal rats as well as its probable mechanism of antidiabetic action have so far been undertaken. In view of this, the present study (an experimental preclinical study) was aimed at evaluating the hypoglycaemic activity of ethanolic extract of leaves of Oxalis corniculata in normal and alloxan-induced diabetic albino rats and also to evaluate the probable mechanism of antidiabetic action.

MATERIALS AND METHODS

Collection of Plant Material and Extraction- The leaves of Oxalis corniculata were collected from the local market, Dibrugarh, Assam, in the months of January-April and the plant material was authenticated by Dr. L.R. Saikia, Associate Professor, Department of Life Sciences, Dibrugarh University, Dibrugarh. A voucher specimen (No. DU/LS/206) was deposited at the Department of Life Sciences, Dibrugarh University.

The leaves were air dried, powdered (500 g) and ethanolic extract was prepared using 90% ethanol by percolation method.[6] The extract was evaporated to dryness under vacuum and dried in a vacuum desiccator to obtain a final yield of 39.5 g of the extract (7.9% w/w).

Preliminary Phytochemical Analysis- The ethanolic extract of leaves of Oxalis corniculata Linn was subjected to phytochemical analysis as per the standard methods.[7,8]

Animals- The study was carried out in healthy adult albino rats (Rattus norvegicus) of either sex (100-200 g each). They were procured from Chakraborty Enterprises, Kolkata, and were housed in clean polypropylene cages with food pellets and tap water provided ad libitum. Permission from the Institutional Animal Ethical Committee for laboratory use of animals (Registration No: 634/02/a/CPCSEA; dated 19/05/2002) was duly obtained and the animals were taken care of as per the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Toxicity Study- Acute oral toxicity test for the ethanolic Extract of Leaves of Oxalis Corniculata (ELOC) was carried out as per OECD Guidelines 425.[9] Previous studies reported the leaf extract of Oxalis corniculata to be relatively nontoxic when administered orally.[10] Hence, the limit test at 2000 mg/kg was performed. One fourth of the maximum dose tested was decided to be considered for the studies.

Chemicals Used- Aventis Pharma Ltd., Goa, provided the crude powder of glibenclamide. The glucose kit for blood glucose estimation was purchased from Sigma Diagnostic (India) Pvt. Ltd., Baroda. Alloxan monohydrate, thiobarbituric acid, hydrogen peroxide, ferric chloride, chloroform, ethanol, potassium sodium tartrate, copper sulphate and all other chemicals and solvents of analytical grade used for antioxidant and phytochemical analysis were obtained from Sigma-Aldrich India, Bangalore.

Study of Hypoglycaemic Effect in Normal Rats[11] - Three groups of animals (six in each) were divided as follows-

- Group A- Normal Control. Received normal saline, 10 mL/kg/d orally.
- Group B- Test Drug. Received ELOC 500 mg/kg/d orally.
- Group C- Standard Drug. Received glibenclamide[12] 0.5 mg/kg/d orally.[13]

All the rats were kept fasting for 18 hours with free access to water before the experiment. Blood samples were collected from the orbital sinus of rats for glucose estimation at '0' min. before drug administration and also at '120' mins. after the above treatment. Blood glucose estimation was done by glucose oxidase method[14] using a glucose kit.

Experimental Design for Antidiabetic Study[2] - A total of 30 animals were equally divided into four groups with six animals in each group-

- Group A- Normal control. Received normal saline, 10 mL/kg/d.
- Group B- Diabetic control. Received normal saline, 10 mL/kg/d.
- Group C- Diabetic test. Received ELOC, 500 mg/kg/d.
- Group D- Diabetic standard. Received glibenclamide 0.5 mg/kg/d.

The above drugs were administered orally once daily for two weeks.

Induction of Diabetes- Leaving aside six rats for normal control group, 24 rats were induced diabetes by a single intraperitoneal injection of alloxan monohydrate in the dose of 150 mg/kg bodyweight. The fasting blood glucose was determined after 72 hours, i.e. on day '1'. Only 18 rats showing blood glucose level greater than 200 mg/100 mL were taken for the study.[15] Blood glucose was estimated every week for two consecutive weeks. Blood glucose estimation was done by glucose oxidase method. During the experimental period, the rats were weighed on day '0' and day '15' of the experiment and the change in bodyweights was compared.[16]

Biochemical Estimations for Antioxidant Activity of Plant Extracts- The antioxidant activity of the plant extract was carried out by estimating the activity of antioxidant enzyme catalase in serum and also the serum level of malondialdehyde as an indicator of oxidative stress.

Enzymatic Essay of Catalase- Catalase activity was measured in blood on day '1' and day '15' of the experiment by continuous spectrophotometric rate determination by Beers and Sizer method.[17] The values were expressed as μmol of H_2O_2/min./mL.

Estimation of Serum Malondialdehyde- Malondialdehyde (MDA) level was measured on day '1' and day '15' of the experiment by colorimeter using thiobarbituric acid reactive substance as described by Satoh K.[18] The results were expressed in nmol/mL.

Probable Mechanism of Antidiabetic- Action- Glycogen Estimation of Liver, Skeletal Muscle and Cardiac Muscle[19] - Out of 30 rats, diabetes was induced in 24 rats by intraperitoneal administration of alloxan monohydrate 150 mg/kg bodyweight. Of them, 18 rats having blood glucose level >200 mg/100 mL after 72 hours of diabetes induction were considered for the study.
Four groups of rats with six in each were taken as follows-

- Group A- Normal control (normal saline, 10 mL/kg/d).
- Group B- Diabetic control (normal saline, 10 mL/kg/d + alloxan).
- Group C- Diabetic test (ELOC, 500 mg/kg/d + alloxan).
- Group D- Diabetic standard (glibenclamide, 0.5 mg/kg/d + alloxan).

Two hours after drug administration, all the 24 rats were decapitated and their liver, leg muscle and heart tissues were taken out. Extraction of the tissue was done by homogenisation with Trichloroacetic Acid (TCA) solution using a tissue homogeniser. Alcohol was used to precipitate the glycogen from the extract. The glycogen content of the tissues was then determined by direct treatment of the precipitated glycogen with anthrone reagent.[19]

Effect on Adrenaline-Induced Hyperglycaemia-[20] For this study, three groups of rats with 6 in each were taken.

- Group A- Normal control (normal saline 10 mL/kg/d orally).
- Group B- Test drug (ELOC, 500 mg/kg/d orally).
- Group C- Standard drug (glibenclamide 0.5 mg/kg/d orally).

Before administration of the above drugs, fasting blood samples were drawn from the rats. One hour after drug administration, adrenaline hydrochloride 100 µg was administered intraperitoneally to all the rats. Half an hour after adrenaline administration, blood samples were again collected.

Statistical Analysis- The data was statistically analysed using one-way ANOVA followed by Dunnett's multiple comparison test. The bodyweights of the rats before (on '0' day) and after (on 15th day) drug administration were compared using Student's t-test (paired). The statistical analysis was done using computerised GraphPad Prism Software Version 5.00. Values of $p < 0.05$ were considered significant.

RESULTS

Acute Toxicity Test- None of the three rats, which were consecutively administered 2000 mg/kg of the extract, died. Therefore, LD50 of the extract was considered to be more than 2000 mg/kg. One fourth of the maximum dose tested was selected for antidiabetic studies.

Phytochemical Analysis- Preliminary phytochemical analysis indicated the presence of flavonoids, saponins, tannins, glycosides, carbohydrates, proteins, fatty acids and phytosterols.

Effect on Fasting Blood Glucose Level- The statistical methods used for analysing the data were one way ANOVA and Dunnett's multiple comparison test.

Normal Rats- A significant ($p < 0.05$) lowering of normal blood glucose was found in test drug and standard drug groups when compared to normal control group after 120 mins. of drug administration (Table 1).

Mean Blood Glucose Level in mg/100 mL			
Groups		**'0 min.'**	**'120 mins.'**
Normal control		107 ± 0.97	104 ± 0.93
Test drug		108 ± 1.24	93 ± 1.46[a]
Standard drug		108 ± 1.53	87 ± 0.86[a]
ANOVA	F	0.2083	59.73
	Df	2, 15	2, 15
	P	>0.05	<0.05

Table 1. Effects on Blood Glucose Level of Normal Rats

- Values are expressed as mean ± SEM; n=6 rats in each group.
- One-way ANOVA followed by Dunnett's multiple comparison test done.
- [a]$p < 0.05$ when compared to normal control group.

Diabetic Rats- On repeated administration of the extract and glibenclamide for two weeks, a significant ($p < 0.05$) decrease in blood glucose was found in diabetic test group and diabetic standard group respectively as compared to diabetic control group, which showed a significant ($p < 0.05$) rise in blood glucose as compared to normal control group. However, both the drugs failed to restore the blood glucose level to that of the normal control group (Table 2).

Mean Blood Glucose Level in mg/100 mL					
Groups		**'0 Day' (Baseline)**	**'1st day' (After 72 Hours)**	**'8th Day'**	**'15th Day'**
Normal control		109 ± 0.82	112 ± 0.93	109 ± 1.34	111 ± 0.86
Diabetic control		108 ± 1.07	296 ± 2.32[a]	330 ± 1.00[a]	372 ± 1.34[a]
Diabetic test		108 ± 1.10	310 ± 0.68[a]	251 ± 1.29[b]	142 ± 0.93[b]
Diabetic standard		111 ± 2.00	279 ± 1.07[a]	184 ± 0.77[b]	135 ± 2.15[b]
	F	1.143	4339	7018	7449
ANOVA	Df	3, 20	3, 20	3, 20	3, 20
	P	>0.05	<0.05	<0.05	<0.05

Table 2. Effects on Blood Glucose Level of Alloxan-Induced Diabetic Rats

- Results are expressed as Mean ± SEM; n=6 rats in each group.
- One-way ANOVA together with Dunnett's multiple comparison test was done.
- [a]p <0.05 when compared to normal control group. [b]p <0.05 when compared to diabetic control group.

Effect on serum catalase and Malondialdehyde (MDA) levels in diabetic rats- One-way ANOVA followed by Dunnett's multiple comparison test was used for the statistical analysis of the results. On Day 1 of the experiment, there was significant increase (p <0.05) in serum levels of MDA and significant decrease (p <0.05) in serum levels of catalase in the diabetic rats as compared to the normal control group. However, on administration of ELOC and glibenclamide consecutively for two weeks, there was significant increase (p<0.05) in catalase levels and significant decrease (p<0.05) in MDA levels in test drug and standard drug groups respectively as compared to the diabetic control group (Table 3).

Groups		Catalase (µmol/min./mL)		Malondialdehyde (MDA) (nmol/mL)	
		Day 1	Day 15	Day 1	Day 15
Normal control		199 ± 0.95	200 ± 2.08	3.5 ± 0.43	3.3 ± 0.76
Diabetic control		176.8 ± 2.09[a]	139.5 ± 2.16[a]	5 ± 0.95[a]	6.3 ±0.88[a]
Diabetic test		177.8 ± 2.44[a]	190.8 ± 1.35[b]	4.8 ± 0.68[a]	3 ± 0.58[b]
Diabetic standard		178.8 ± 1.70[a]	198.6 ± 3.18[b]	4.6 ± 0.37[a]	2.8 ± 0.60[b]
	F	32.44	158.4	3.91	5.33
ANOVA	Df	3, 20	3, 20	3, 20	3, 20
	P	<0.05	<0.05	<0.05	<0.05

Table 3. Effects on Antioxidant Levels in Alloxan-Induced Diabetic Rats

Each value represents Mean ± SEM; n=6 rats in each group. One-way ANOVA and Dunnett's multiple comparison test done. [a]p <0.05 when compared to normal control group. [b]p <0.05 when compared to diabetic control group.

Effect on Changes in Body Weight- The bodyweights of the rats before (on '0' day) and after (on 15th day) drug administration were compared using Student's t-test (paired). The final bodyweight showed significant (p<0.05) increase from the initial bodyweight in all the groups except in diabetic control group, in which there was significant (p<0.05) decrease in bodyweight compared to the initial bodyweight (Table 4). However, the percentage increase in bodyweights in the diabetic test group and the diabetic standard group respectively was relatively less than the normal control group (Table 4).

Body Weights in Grams (g)						
Groups		Initial ('0' Day)	Final (15th Day)	Change	% of Increase	% of Decrease
Normal control		125 ± 1.57	160 ± 1.77[a]	35 ± 1.57	28.00	
Diabetic control		127 ± 0.77	82 ± 1.00[a]	45 ± 0.93		35.00
Diabetic test		126 ± 1.59	149 ± 1.07[a]	23 ± 0.83	18.00	
Diabetic standard		123 ± 0.82	153 ± 0.97[a]	30 ± 2.18	24.00	
	F	1.862	849.5	38.56		
ANOVA	Df	3, 20	3, 20	3, 20		
	P	>0.05	<0.05	<0.05		

Table 4. Effects on Bodyweights in Alloxan-Induced Diabetic Rats

Values expressed as Mean ± SEM; n=6 rats in each group. One-way ANOVA and Student's t-test (paired) test done. [a]p <0.05 when compared to the initial bodyweight.

Effect on Glycogen Estimation- The data was statistically analysed using one-way ANOVA followed by Dunnett's multiple comparison test.

There was a significant (p <0.05) increase in the glycogen content of liver, skeletal muscle and cardiac muscle in diabetic test group and diabetic standard group as compared to diabetic control group, which showed a significant (p <0.05) reduction in glycogen content in the above tissues as compared to normal control group (Table 5).

Groups		Glycogen Concentration (mg/100 g)		
		Liver	Skeletal Muscle	Cardiac Muscle
Normal control		45 ± 1.00	38 ± 0.68	32 ± 0.89
Diabetic control		6 ± 1.07[a]	5 ± 0.93[a]	3 ± 0.58[a]
Diabetic test		42 ± 1.77[b]	27 ± 1.34[b]	16 ± 1.00[b]
Diabetic standard		40 ± 1.46[b]	32 ± 1.32[b]	27 ± 0.86[b]
	F	180.7	170.1	231.2
ANOVA	Df	3, 20	3, 20	3, 20
	P	<0.05	<0.05	<0.05

Table 5. Effect on Glycogen Concentration in Liver, Skeletal Muscle and Cardiac Muscle

Values are expressed as Mean ± SEM; n=6 rats in each group. One-way ANOVA followed by Dunnett's multiple comparison test was done. [a]p <0.05 when compared to normal control group. [b]p <0.05 when compared to diabetic control group.

Effect on Adrenaline-Induced Hyperglycaemia- The test drug and the standard drug significantly (p <0.05) reduced hyperglycaemia induced by adrenaline. The percentage reduction of blood glucose by ELOC was 20.31% and that caused by glibenclamide was 50% (Table 6).

Groups		Blood Glucose Level (mg/100 mL)				
		'0 hour' Fasting	½ hour after Adrenaline	Change	% of Increase	% of Decrease
Normal control		130 ± 0.82	222 ± 0.58	92 ± 0.73	70.76	
Test drug		133 ± 0.77	208 ± 1.48a	75 ± 1.07a	56.39	20.31
Standard drug		130 ± 1.24	176 ± 1.92a	46 ± 1.21a	35.38	50.00
	F	3.214	269.0	518.0		
ANOVA	Df	2, 15	2, 15	2, 15		
	P	>0.05	<0.05	<0.05		

Table 6. Effect of ELOC on Adrenaline-Induced Hyperglycaemia in Albino Rats

Each value represents Mean ± SEM. One-way ANOVA and Dunnett's multiple comparison test done. [a]p <0.05 when compared to the normal control group.

DISCUSSION

From the study, it was seen that ELOC significantly lowered the blood glucose level in diabetic rats. The ELOC also significantly (p <0.05) lowered the normal blood glucose level at 120 minutes after drug administration. The antidiabetic and hypoglycaemic action of the leaves of Oxalis corniculata may be due to the presence of saponins, tannins and flavonoids in them. In the present study, the phytochemical analysis of ELOC revealed the presence in it of flavonoids, saponins, tannins, glycosides, carbohydrates, proteins, fatty acids and phytosterols.

Flavonoids have been found to stimulate insulin secretion or possess an insulin-like effect.[21] Saponins are also known to lower blood glucose levels.[22] Raised blood glucose level is the principal stimulus for insulin secretion.[23] The fact that the ethanolic extract of leaves of Oxalis corniculata has lowered normal blood glucose level, asserts the presence in it of some constituents with insulin-like action. The constituents of leaves of Oxalis corniculata might cause hypoglycaemia by exerting a direct insulin-like insulin-independent effect on glucose transport and glucose metabolism.

Induction of diabetes with alloxan is associated with a characteristic loss of bodyweight,[3] which is due to increased lipolysis and increased muscle wasting and loss of tissue proteins caused by insulin deficiency.[24] Alloxan exerts its diabetogenic effect by its cytotoxic action against insulin producing beta cells of pancreas.[25] ELOC, when administered to diabetic rats caused an increase in bodyweight on the 15th day when compared to the initial bodyweight on the 0th day probably by ameliorating the extent of insulin deficiency and thereby preventing lipolysis and proteolysis.

Diabetes is associated with an imbalance between the pro-oxidant and antioxidant status of the living tissues.[26] In the present study, the ability of the extract (ELOC) to reduce the raised levels of malondialdehyde and increase the reduced levels of catalase in diabetic rats might be because of its antioxidant and free radical scavenging property. This has also been stated by Borah A et al.[27] The presence of flavonoids and saponins in the ethanolic extract of Oxalis corniculata (as revealed from the phytochemical analysis done in this study) is mainly responsible for its antioxidant property. Flavonoids acts as antioxidants by increasing the function of endogenous antioxidants, directly scavenging free radicals, directly inhibiting lipid peroxidation and inhibiting release of peroxidise, which inhibits the generation of reactive oxygen species.[28]

Glycogen metabolism in the liver regulates the blood glucose. The control of the synthesis and breakdown of glycogen in the liver is central to the regulation of blood glucose level.[29] In this study, it was seen that ELOC caused increase in glycogen concentration of the liver and this correlates well with the fall in blood glucose level seen in normal rats at 120 minutes (2 hours) after ELOC administration. The rise in liver glycogen maybe due to the activation of glycogen synthase system[30] by insulin or insulin-like substances. Also, the increase in glycogen content in skeletal muscles indicate enhanced peripheral uptake of glucose together with stimulation of muscle glycogen synthase enzyme,[23] which may be due to insulin-like action of ingredients present in the leaves of Oxalis corniculata.

Adrenaline produces hyperglycaemia by inhibiting insulin secretion, stimulating glycogenolysis in muscle and thus providing substrate in the form of lactate for hepatic gluconeogenesis, stimulating glucagon secretion and stimulating ACTH secretion, which in turn stimulates glucocorticoid secretion from the adrenal cortex.[31] The test drug significantly (p <0.05) reduced the adrenaline-induced hyperglycaemia probably by inhibiting adrenaline-induced stimulation of α_2 2 receptors in β-cells of pancreas and thus promoting further insulin release.[32]

Thus, from this study, it can be said that the hypoglycaemic and antidiabetic effect of leaves of Oxalis corniculata may be at least partly due to its positive effect on glycogen synthesis in liver, skeletal muscle and heart along with its stimulatory action on insulin release by blocking the α_2 2 receptors in β-cells of pancreas.

However, the present study failed to encompass a few other studies, which were necessary to confirm the probable mechanism of antidiabetic action of leaves of Oxalis corniculata. Studies on serum insulin assay and insulin secretagogue activity of the components of the leaves of Oxalis corniculata are necessary to confirm their insulin

releasing action. Also, studies for isolating and elucidating the structure of the active principles responsible for hypoglycaemia that are present in the leaves of Oxalis corniculata can be undertaken.

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