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

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INTRODUCTION
An estimated 462 million individuals are affected by type 2 diabetes worldwide, corresponding to 6.28% of total world's population. Above 1 million deaths were attributed to Diabetes in 2017 alone, making it the ninth leading cause of mortality [1].

Drug therapy for metabolic control depends on adherence to both non-pharmacological and pharmacological treatment. Tight blood glucose level control can result in a reduction in diabetic complications. Commitment to prescribed medication is the key to achieving this control [2]. This elevated blood sugar ends up in long-term damage, dysfunction, and failure of vital organs. However, several hypoglycemic agents employed for the treatment of diabetes are reported to pose side effects, including liver dysfunction [3]. Synthetic drugs might pose deleterious side effects and therefore therapeutic intervention is aimed at herbal formulations in the management of diverse disorders [4]. In the history of humankind, various diseases, both infectious and non-infectious, have been efficiently treated using herbal medicinal products. It is well documented that many herbs with curing potentials were widely used in traditional medicines [5-8]. Phytochemical constituents such as saponins, phenols, and flavonoids studied in various plants such as Proteus vulgaris, Moringa oleifera, and Cassia glauca showed potential α-amylase inhibitors [9]. India is a country where there are abundant resources of medicinal herbs and plants with vivid medicinal uses in treating a variety of diseases [10-12].

However, researchers are always looking for more phytochemical constituents and medicinal efficacy of such plants [13,14]. The creeping wood sorrel (Oxalis corniculata Linn.) plant has recently gained more focus in India. As plants are considered to be rich source of medicines followed by animals and marine sources, therefore, the search for the bioactive molecule is an exhaustive one. Therefore, the authors have tried their best to present the existing knowledge base of this plant and its therapeutic applications while simultaneously emphasizing the need for more research and development on its multiple aspects for societal benefits. O. corniculata is also known as Anboti in Hindi, Amlapatrika in Sanskrit, and Indian sorrel in English Fig. 1 [15].

The phytochemical constituents of O. corniculata Linn had several oleic, linolenic, linoleic, and stearic acids with tannins and palmitic acid. The methanolic extracts also had other constituents such as carbohydrates, glycosides, phytosterols, phenolic compounds, flavonoids, amino acids, and volatile oil [16].

The herb O. corniculata Linn. is used traditionally as medicinal herb for curing gastric conditions, fever and dysentery [17]. In Assam, the herb juice is used as eye drops to treat conjunctivitis [18,19]. In the neighboring country Pakistan, skin diseases are treated using the leaves of the herb. Sensitive teeth are treated using the leaves of this plant [20,21]. The above-mentioned uses are believed due to the pharmacological activities such as wound healing, anti-diabetic, anti-amoebic, anti-ulcerative, anti-inflammatory, hepatoprotective, antifungal, and cardioprotective properties of the plant.

METHOD

Materials used
- Ethanolic extract of leaves of O. corniculata (ELOC)
- Glibenclamide
- Alloxan monohydrate
- Normal saline (as vehicle)
- Healthy albino rats (Rattus norvegicus)
- With IAEC permission.

Plant
Leaves of O. corniculata were collected and authentication was done by Dr. M. Islam, Professor at the Department of Life Science, at Dibrugarh University. A voucher specimen (No. DU/LS/214) was deposited at Dibrugarh University.

ABSTRACT
Objective: The aim of the study is to evaluate hypoglycemic action of Ethanol extract of leaves of Oxalis corniculata Linn. on normal and Alloxan-induced diabetic albino rats.

Methods: Hyperglycemia is induced by use of intraperitoneal injection of Alloxan and Adrenaline. After that test drug Ethanolic extract of leaves of O. corniculata (ELOC) and standard drug Glibenclamide is administered. The hypoglycemic effect of ELOC is compared with the standard drug and control.

Results: Significant hypoglycemic activity of ELOC was seen in Alloxan-induced hyperglycemia when blood glucose levels were estimated from different tissues. Furthermore, the significant hypoglycemic activity of ELOC was seen in Adrenaline-induced hyperglycemia.

Conclusion: ELOC possesses hypoglycemic activity.

Keywords: Hypoglycemic activity, Alloxan, Oxalis corniculata.

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Preparation of extract
Coarsely powdered leaves were extracted with ethanol water by maceration in a closed vessel for 72 h. At the interval of 24 h, the used ethanol was changed with fresh amount of ethanol. The vessel was shaken occasionally during the extraction period. The ethanolic extract of the leaves was filtered, pooled, and vacuum-concentrated using a rotary evaporator. The dark brown-colored sticky residue was collected after the complete removal of the solvent. ELOC thus obtained, was used for biological activity [22].

Animal
Healthy Albino rats (Rattus norvegicus) weighing from 150 to 250 g of either sex were taken from Central Animal House, Assam Medical College (registration no. 654/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages and maintained at normal room temperature. The rats were maintained on a standard animal diet of Bengal gram, wheat, maize and carrot in sufficient quantity for the entire period of the experiment. Water was given ad libitum during the entire period of the experiment.

Acute toxicity study
An acute toxicity test was done for the ethanolic extract of *O. corniculata* following OECD 425 guidelines [23]. The extract was found safe at doses more than 2000 mg/kg without any sign of toxicity or mortality, so an arbitrary dose of 500 mg/kg was selected for the study.

Study on normal rats
- Normal Control: Normal saline (10 mL/kg body weight)
- Test drug: ELOC (500 mg/kg body weight)
- Standard drug: Glibenclamide (0.5 mg/kg body weight)
- Fasting blood glucose is estimated at “0” and “120” min.

Study on diabetic rats
- Normal Control: Normal saline (10 mL/kg/day)
- Diabetic Control: Normal saline (10 mL/kg/day)
- Diabetic test: ELOC (500 mg/kg/day)
- Diabetic standard: Glibenclamide (0.5 mg/kg/day)

Drugs were administered orally for 2 weeks. Blood glucose is estimated every week for 2 consecutive weeks [24].
Induction of diabetes
Twenty-four rats were administered intraperitoneally injection of Alloxan monohydrate (150 mg/kg body weight). Fasting blood glucose was taken after 72 h. 18 rats with blood glucose >200 mg/100 ml were included in the study. Body weight was measured on day “0” and day “15” [25].

Glycogen estimation of the liver, skeletal muscle, and cardiac muscle
- Normal saline -- Normal saline (10 mL/kg body weight)
- Diabetic control -- Normal saline (10 mL/kg body weight+Alloxan)
- Diabetic test -- ELOC (500 mg/kg body weight)+Alloxan
- Diabetic standard -- Glibenclamide (0.5 mg/kg body weight)+Alloxan

After 24 h of drug administration, animals were killed. Glycogen content of liver, leg, and heart muscle was estimated by use of Anthrone reagent [26].

Effect on adrenaline-induced hyperglycemia
- Group - I -- Normal saline (10 mg/kg body weight)
- Group - II -- ELOC (500 mg/kg body weight)
- Group III -- Glibenclamide (0.5 mg/kg body weight).

Adrenaline 100 µg was administered i.p. 1 h after. Blood samples were collected half an hour later [27].

Data analysis was done using “One way ANOVA” followed by Dunnett’s and Student’s t-test (paired).

RESULTS AND OBSERVATION
Results and observation are shown in Figs. 1-6.

DISCUSSION
Alloxan, a cytotoxic agent, induces chemical diabetes/alloxan diabetes in a variety of animal species through damage to insulin-secreting cells known as beta cells found in the islets of Langerhans of the pancreas. The cytotoxic action of this diabetogenic agent is mediated by reactive oxygen species (ROS) with the formation of superoxide radicals. Oxidative stress that leads to an increased production of ROS, and finally cellular lipid peroxidation has been found to play an important role in the development of diabetes mellitus and its complications [28].
Adrenaline-induced hyperglycaemia has been described as a useful experimental model to study the activity of anti-diabetic agents as adrenaline is responsible for elevated glucose label by producing glycogenolysis.

In this study, ELOC 500 mg/kg was effective in normalizing the elevated levels of blood sugar as shown in Figs. 2 and 3. Results are comparable with that of the standard drug Glibenclamide. Glycogen levels in liver, skeletal muscle, and cardiac muscle is also decreased significantly as shown in Fig. 4.

ELOC is also effective in reducing blood glucose levels in Adrenaline-induced hyperglycaemia as shown in Fig. 5. However, the effect of ELOC on the weight of the experimental rats is not significant as seen in Fig. 6.

By the mechanisms shown in Fig. 7, it is seen that ELOC possesses hypoglycemic action in diabetes-induced albino rats. It may be due to the presence of some phytoconstituents, which have got insulin-like action (Fig. 7a) or induce insulin secretion from the beta cells (Fig. 7b) or due to enhanced transport of blood glucose to peripheral tissues (Fig. 7c) or there is increased transcription of GLUT 4 to the cell membranes (Fig. 7d).

That is why, its traditional use in various conditions is justified. However, further studies and development of more purified products of leaves of O. corniculata Linn are required for proper clinical use.

CONCLUSION

O. corniculata has hypoglycemic action and anti-diabetic action. It has a positive influence on glycogen metabolism and it also has insulin-releasing action.

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AUTHORS CONTRIBUTION

All the authors contributed equally to conducting the experiment and drafting of the manuscript.

CONFLICTS OF INTEREST

The authors declare that there is no any conflict of interest as the present work is solely done by the authors.

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