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Research Article

# ANTI-DIABETIC ACTIVITY OF *EPIPREMNUM AUREUM.L* IN NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

**ABHINAYANI G*, NAGA KISHORE R, FATHIMA BENAZIR, POOJA AGARWAL**

**Department of Pharmacology, Geethanjali College of Pharmacy, Cheeryal, Keesara, Rangareddy - 501 301, Telangana, India.**
**Email: abhishivani.abhi@gmail.com**

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**ABSTRACT**

**Objective:** The study was carried out with the objective of phytochemical screening and to evaluate the anti-diabetic activity of aqueous and alcoholic extract of *E. aureum*.

**Methods:** The anti-diabetic activity was determined alloxan-induced diabetic rats. A total of 24 albino Wistar rats of either sex weighing 200-250 g were divided into 4 groups consisting of 4 rats in each group. Group-1 served as control, Group-2 received standard drug, Group-3 received test drug aqueous extract of E. aureum, and Group-4 received test drug alcoholic extract of E. aureum.

**Results:** Phytochemical investigation of aqueous and alcoholic extracts of E. aureum revealed the presence of alkaloids, tannins, saponins, terpenoids, and flavonoids as secondary metabolites. The both aqueous and alcoholic extracts of *E. aureum* showed a significant reduction in blood glucose levels due to the presence of phytochemicals such as flavonoids, terpenoids, and alkaloids in both extracts of *E. aureum*. The administration of drug (IP) was continued upto 15 days.

**Conclusion:** Extracts of *E. aureum* have shown the great potential of anti-diabetic activity in normal and alloxan-induced rats. Flavonoids might be producing hypoglycemic effect by a mechanism independent from insulin secretion, e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. This study *E. aureum* of both aqueous and alcoholic extracts was showed significant effect on alloxan-induced rats.

**Keywords:** *Epipremnum aureum*, Anti-diabetic activity, Alloxan-induced diabetic rats, Glucometer.

## INTRODUCTION

*Epipremnum aureum* (the secret life of plant crystals; Ivan Amato) belongs to a large Family Araceae, a family of monocotyledonous flowering plants, in which flowers are born on a type of inflorescence called a spadix. *E. aureum* commonly known as the Golden Pothos, Devil's Ivy, money plant, silver vine, taro vine, etc. The genus *Epipremnum* Linn. is represented by more than species in India, of which *E. aureum* and *E. pinnatum* are the most widely cultivated and best-known species among the other species.

Diabetes mellitus (DM) as a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, Insulin action, or both [1]. The presence of DM confers increased the risk of many devastating complications such as cardiovascular diseases, peripheral vascular diseases [2], complications such as coronary artery diseases, stroke, neuropathy, renal failure, retinopathy amputations, and blindness [3]. Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureas and new are available for the treatment of diabetes. However, none of these medications is ideal due to toxic side effects and in some cases domination of response after prolong use [4]. The main disadvantages of the currently available drugs are that they have to be given throughout the life and produce side effects [5]. Although several medicinal plants have gained importance for the treatment of DM, many remain to be scientifically investigated [6]. Many plants reported useful for the treatment of DM in ayurvedic system of medicine have been tested for hypoglycemic activity in experimental animals [7]. This study was done based on phytochemical constituents such as alkaloids, flavonoids, and terpenoids present in leaves, which are useful for the treatment of diabetes in normal and alloxan-induced diabetic rats.

## METHODS

### Drugs and chemicals

Drugs and chemicals used in this study were of analytical grade and highest purity procured from standard commercial sources in India.

### Experimental animals

Healthy adult albino Wistar rats, weighing 200-250 g of either sex, were selected for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of the experiment, after 72 hrs of fasting from the day of Alloxan introduction. Animals were housed within the departmental animal house, and the room temperature was maintained at 27°C. Animal studies had the approval of the Institutional Animal Ethics Committee (IAEC) of the committee for the purpose of control and supervision on experiments on animal [8] (CPCSEA) (1648/PO/a/12/CPCSEA/IAEC/06).

### Plant material collection

The leaves of *E. aureum* were collected from the Geethanjali College in the month of December. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature, and coarsely powdered in a mixer. The powdered material was stored or taken up for extraction process.

### Preparation of plant extracts

#### *Preparation of aqueous extract*

Fresh leaves of *E. aureum* were collected and washed under tap water. The leaf extract used was prepared by taking 20 g of finely cut leaves

into 250 ml beaker containing 200 ml of distilled water. The contents were mixed well and then the mixture was boiled up to 80-100°C for 4-5 hrs. Further, the extract was filtered with Whatman filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature, and used for further experiment to check the activities [9].

### *Preparation of alcoholic extract*

Fresh leaves of *E. aureum* were collected and washed under tap water. The leaf extract used was prepared by taking 20 g of finely cut leaves into 250 ml beaker containing 200 ml of alcohol. The contents were mixed well and then the mixture was boiled up to 50-60°C for 4-5 hrs. Further, the extract was filtered with Whatman filter paper. The filtrate was boiled until the concentrated residue is formed. The concentrated product was sealed in sample covers and stored under room temperature and used for further experiment to check the activities [9].

### *Preliminary phytochemical analysis of the extracts*

Both the aqueous and alcoholic extracts of *E. aureum* were subjected to preliminary phytochemical screening [10].

**Selection of dose for animal study**

The dose considered for the experiment on rats was obtained from conversion of human dose of *E. aureum* (3-5 g/kg). The conversion factor of human dose (per 200 g body weight) is 0.018 for rats (Ghosh 1984). Hence, the calculated dose for the rats (considering human dose 5 g/kg) is 250 mg/kg. Thus, anti-diabetic activity was done at dose of 250 mg/kg body weight. Acute toxicity was done at dose of 2000 mg/kg body weight [11].

**Acute oral toxicity**

The acute oral toxicity of aqueous and alcoholic extracts of *E. aureum* was determined using Albino Wistar rats (200-250 g), which were maintained under standard conditions. The animals were fasted 12 hr before the experiment, up and down procedure OECD Guideline No. 4 [12].

**Assessment of anti-diabetic activity in normal and alloxan-induced rats**

### *Assessment of hypoglycemic activity on normal rats [13]*

Animals

A total of 24 albino Wistar rats of either sex weighing 200-250 g were divided into 4 groups consisting of 4 rats in each group. Group-1 served as control, Group-2 received standard drug, Group-3 received test drug aqueous extract of *E. aureum,* and Group-4 received test drug alcoholic extract of *E. aureum.*

Procedure

Animals were grouped and divided randomly into four groups of four, and each was fasted to overnight. The blood samples were withdrawn by tail vein at 0 hr, i.e., before intraperitoneal (IP) administration of extracts/standard/vehicle. Then, blood was collected at an interval of 1, 2, 4, and 8 hrs after the administration, and according to the procedure, blood glucose levels were measured by the glucometer (one touch glucometer). The administration of drug was continued up to 15 days.

**Oral glucose tolerance test (OGTT) in normal rats**

On the next day (16$^{th}$ day), after the assessment of hypoglycemic activity, OGTT was carried out in same normal animals.

### *Procedure*

All the animals in each group were administered 2 g/kg of glucose 1 hr after extract/glibenclamide/vehicle administration. The blood samples were collected by tail vein at 0 hr, 0.5 hr, 1 hr, 1.5 hrs, and 2 hrs after the administration of glucose load. Blood glucose levels were measured by the glucometer.

**Assessment of anti-diabetic activity in alloxan-induced diabetic rats [14-16]**

### *Animals*

About 24 albino Wistar rats of either sex weighing 150-200 g were divided into 4 groups consisting of 4 rats in each group. Group-1 served as control, Group-2 received standard drug, Group-3 received test drug aqueous extract of *E. aureum,* and Group-4 received test drug alcoholic extract of *E. aureum.*

**Induction of diabetes**

DM was induced by single IP dose of 10 mg/kg of alloxan dissolved in 2 ml of distilled water were administered into 12 hrs fasted rats. On the 2$^{nd}$ day of alloxan-injection, the rats were fasted for 72 hrs, and blood was taken from tail vein of the rats. Rats with moderate diabetes having hyperglycemia (that is, with blood glucose of 250-400 mg/dl) were taken for the experiment. The diabetic rats were then divided randomly in the different groups.

### *Effect of aqueous and alcoholic extracts of E. aureum on blood glucose levels in alloxan-induced diabetic rats*

All the animals of above groups were administered as per treatment protocol mentioned above. The blood samples were collected by tail vein at 0, 1, 2, 4, and 8 hrs after the administration. The treatment was continued for next 22 days. Again blood samples were collected on 4$^{th}$, 7$^{th}$, 14$^{th}$, and 21$^{st}$ day after 1 hr administration for subacute study. According to the procedure, blood glucose levels were measured by the glucometer (one touch glucometer). The administration of the drug was continued up to 15 days.

### *OGTT in alloxan-induced diabetic rats*

On the 8$^{th}$, 15$^{th}$, and 22$^{nd}$ day, OGTT was carried out on the same alloxan-induced diabetic animals used for assessment of anti-diabetic activity studies.

### *Procedure*

All the animals in each group were administered 2 g/kg of glucose 1 hr after extract/glibenclamide/vehicle administration. The blood samples were collected by tail vein at 0 hr, 0.5 hr, 1 hrs, 1.5 hrs, and 2 hrs after administration of the glucose load. Blood glucose levels were measured by glucometer.

## RESULTS

**Preliminary phytochemical analysis of the extracts**

Phytochemical investigation of aqueous and alcoholic extracts of *E. aureum* revealed the presence of alkaloids, tannins, saponins, terpenoids, and flavonoids as secondary metabolites.

**Acute toxicity testing**

Acute toxicity studies revealed that the aqueous and alcoholic extracts of *E.aureum* were safe up to 2000 mg/kg of body weight, and approximate LD50 is more than 2000 mg/kg. No lethality or any toxic reactions were observed up to the end of the study period. This is not surprising as *E. aureum* is used as a decoration plant.

**Table 1: Effect of extracts of *E. aureum* on fasting blood glucose level (FBGL) in normal rats**

| Treatment | Dose (mg/kg) | Blood glucose level (mg/dl) | | | |
|---|---|---|---|---|---|
| | | 0 | 5$^{th}$ day | 10$^{th}$ day | 15$^{th}$ day |
| Normal control | - | 86±1 | 89±1 | 88±1 | 91±2 |
| Glibenclamide | 10 | 85±2 | 73±2 | 58±2 | 56±2 |
| AQEEa | 200 | 77±2 | 95±3 | 82±2 | 69±2 |
| ALEEa | 200 | 92±3 | 91±2 | 81±2 | 67±2 |

Values are expressed as mean±S.E.M. n=3. Significant values were compared with p<0.01, normal control versus all groups. Parenthesis indicates % reduction in BGL

**Table 2: Effect of extracts of *E. aureum* on 16th day in normal rats**

| Treatment | Dose (mg/kg) | Blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | 0 hour | 0.5 hour | 1 hour | 1.5 hour | 2 hour |
| Normal control | - | 89±1 | 145±2 | 121±2 | 103±2 | 101±2 |
| Std (Glibenclamide) | 10 | 76±1 | 66±1 | 62±2 | 58±2 | 57±1 |
| AQEEa | 200 | 130±2 | 118±2 | 121±2 | 114±2 | 120±2 |
| ALEEa | 200 | 164±2 | 185±2 | 108±2 | 130±2 | 101±2 |

Values are expressed as mean±S.E.M. n=3. Significant values were compared with p<0.01. Normal control versus all groups. Parenthesis indicates % reduction in BGL

**Hypoglycemic activity in normal rats**

Fasting blood glucose levels (FBGLs) were within the range of 90-105 mg/dl in all the groups at 0 day. Repeated treatment with the doses of aqueous and alcoholic extract (200 mg/kg) significantly decrease the blood glucose level on 5th, 10th, and 15th day indicating that the extract produces significant hypoglycemic activity after repeated administration. Glibenclamide (10 mg/kg) also significantly reduced FBGL after repeated administration as compared to normal control group. Changes in FBGL in different groups after repeated dose administration are summarized in Table 1.

Repeated administration of both aqueous and alcoholic extracts had significantly (p<0.01) reduced the FBGL on 5th, 10th, and 15th day indicating these extracts can produce hypoglycemia on repeated administration. However, hypoglycemic activity was more significant on 5th, 10th, and 15th day for glibenclamide treated as compared with other groups. The results suggest that the both aqueous and alcoholic extracts possess significant hypoglycemic activity after repeated dose administration. The detailed results are summarized in Table 1.

**OGTT on 16th day in normal rats**

Both the AQEEa and ALEEa (200 mg/kg) significantly (p<0.005) suppress the rise in FBGL after glucose load (2 g/kg) in rats, at first ½ hr and up to 2 hrs period as compared with other groups extract glibenclamide on 16th day, while AQEEa and ALEEa produced significant reduction in FBGL. Glibenclamide (10 mg/kg) showed (p<0.005) significant suppression in FBGL rise at first ½ hr, 1 hr, and normalized FBGL within 2 hrs. The detailed results are summarized in Table 2.

**Anti-diabetic activity in alloxan-induced diabetic rats**

FBGLs in normal rats were in the range of 90-100 mg/dl. Treatment with alloxan (120 mg/kg, IP) had increased the FBGL to range of 252-266 mg/dl after 72 hrs. These values on subsequent days got stabilized by day seven on an average between 255 mg/dl.

Changes in the FBGLs in different groups are tabulated in Table 3. These data shown that blood glucose level of normal control animals has maintained throughout the study period.

The Group-1, which is the diabetic control group, has shown a significant increase in FBGLs during this 21st day study period.

The Group-2 glibenclamide (10 mg/kg) treated group has shown (p<0.01) a significant decrease in FBGL during 7th, 14th, and 21st day of the study period.

**Effect of *E. aureum* extracts on anti-diabetic activity in alloxan-induced diabetic rats**

The animals treated with 200 mg/kg of AQEEa and ALEEa shown a significant decrease (p<0.01) in FBGL on 7th, 14th, and 21st day of treatment when compared to other groups of animals. The aqueous extract has reduced more (%) in FBGL when compared to alcoholic extracts except standard group. The detailed results are summarized in Table 3.

**OGTT on 16th day**

Both the AQEEa and ALEEa (200 mg/kg) significantly (p<0.01) suppress the rise in FBGL after glucose load (2 g/kg) in rats, at first ½ hr and up to 2 hrs period as compared with other groups extract glibenclamide on 15th day, while AQEEa and ALEEa produced significant reduction in FBGL. Glibenclamide (10 mg/kg) showed (p<0.01) significant suppression in FBGL rise at first ½ hr, 1 hr, and normalized FBGL within 2 hrs. The detailed results are summarized in Table 4.

**Table 3: Effect of extracts of *E. aureum* on fasting blood glucose level (FBGL) in alloxan-induced diabetic rats**

| Treatment | Dose (mg/kg) | Blood glucose level (mg/kg) | | | |
|---|---|---|---|---|---|
| | | 0 day | 7th day | 14th day | 21st day |
| Normal control | - | 100±1 | 100±1 | 96±1 | 97±2 |
| Diabetic control | 10 | 253±2 | 256±3 | 264±2 | 271±1 |
| Glibenclamide | 10 | 98±1 | 96±3 | 80±1 | 72±2 |
| AQEEa | 200 | 117±2 | 104±1 | 89±2 | 76±1 |
| ALEEa | 200 | 103±2 | 98±2 | 86±2 | 77±1 |

## DISCUSSION

The phytoconstituents are known to play an important role in bioactivity of medicinal plants. In qualitative phytochemical analysis reveals the presence of alkaloids, flavonoids, tannins, terpenoids, and saponins have associated with various degrees of anti-microbial, anti-bacterial, anti-fungal, anti-oxidant, and anti-termites. Therefore, the anti-diabetic activity was observed in this study may be due to the presence of chemical constituents in both aqueous and alcoholic extracts of *E. aureum.*

This study was aimed at discovering the anti-diabetic activity of aqueous and alcoholic extracts of *E. aureum* at a dose of 200 mg/kg showed a significant effect on glucose tolerance and the extracts also showed reduction in FBGLs in normal and alloxan-induced diabetic rats. These findings indicate that the extracts might be producing hypoglycemic effect by a mechanism independent from the insulin secretion, e.g., by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. Alloxan monohydrate is one of the chemical agents used to induce DM in animals. It induces diabetes by dose-dependent destruction of β-cells of islets of Langerhans. It is a generator of free radicals of oxygen which cause extensive DNA damage. It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state. As the hyperglycemia induced by alloxan falls under category of mild diabetes and may reverse after a few weeks, the hypoglycemic effect of the plant in hyperglycemic rats was studied during 21 days treatment. The difference observed between the initial and final fasting serum glucose levels of extract-treated hyperglycemic rats revealed anti-hyperglycemic effect of *E. aureum* throughout the period of study. The effect of the extracts was compared to that of reference standard, glibenclamide and was found to be significant.

## CONCLUSION

*E.aureum is* a natural plant and it has anti-termites, anti-bacterial, anti-microbial, anti-fungal, and anti-oxidant activities. The phytochemical constituents present in the leaves of two different extracts of *E. aureum*

**Table 4: Effect of extracts of *E. aureum* on 15th day in normal rats**

| Treatment | Dose (mg/kg) | Blood glucose level (mg/dl) | | | | |
|---|---|---|---|---|---|---|
| | | 0 hour | 0.5 hour | 1 hour | 1.5 hour | 2 hour |
| Normal control | - | 101±2 | 142±4 | 132±2 | 118±2 | 98±2 |
| Diabetic control | 10 | 259±3 | 383±5 | 339±2 | 290±2 | 281±2 |
| Std (Glibenclamide) | 10 | 107±2 | 125±2 | 115±2 | 112±1 | 88±1 |
| AQEEa | 200 | 156±1 | 102±4 | 122±2 | 102±1 | 94±1 |
| ALEEa | 200 | 158±2 | 148±3 | 145±1 | 111±4 | 75±2 |

Values are expressed as mean±S.E.M. n=3. Significant values were compared with p<0.01. Normal control versus all groups. Parenthesis indicates % reduction in BGL

may vary. Among these studies, it could be concluded that leaves of *E. aureum* have shown the great potential of anti-diabetic activity in normal and alloxan-induced rats. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific drugs. The pharmacological activities of the present studies support the folkloric usage of plant and suggest that *E. aureum* extracts may also possess anti-psychotic, hypolipidemia, anti-convulsants, etc., can be studied further.

Based on the phytochemical screening, it has been concluding that both the aqueous and alcoholic extracts had hypoglycemic activity because the presence of flavonoids, which are rich in the treatment of hypoglycemia with fewer side effects. Flavonoids might be producing hypoglycemic effect by a mechanism independent from insulin secretion, e.g. by the inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. This study *E. aureum* of both aqueous and alcoholic extracts was showed significant effect on glucose tolerance and also showed reduction in FBGLs in normal diabetic rats.

Based on the phytochemical screening, it has been concluding that both the aqueous and alcoholic extracts had anti-diabetic activity due to the presence of alkaloids, terpenoids, and flavonoids. The decreasing effect of plant extracts in blood glucose levels justified the use of the plant as an anti-diabetic agent. Based on this study, we conclude that both aqueous and alcoholic extracts having good anti-diabetic activity. Alloxan monohydrate is one of the chemical agents used to induce DM in animals. It induces diabetes by dose-dependent destruction of β-cells of islets of Langerhans. It is a generator of free radicals of oxygen which cause extensive DNA damage. It was observed that single intravenous dose of alloxan exhibited significant hyperglycemia. Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissues is the fundamental mechanism underlying hyperglycemia in the diabetic state.

## REFERENCES

1. Amos AF, Mc Carty DJ, Zimmet P. The rising global burden of diabetes and its complications: Estimates and projections to the year 2010. Diabet Med 1997;14 Suppl 5:S1-5.
2. Bajaj JS, Madan R. Diabetes in tropics and developing countries. IDF Bull 1995;38(2):5-6.
3. Nathan DM. The pathophysiology of diabetic complications: How much does the glucose hypothesis explain? Ann Intern Med 1996;124:86-9.
4. Dixit VP, Joshi SC. Antiatherosclerotic effects of alfalfa meal ingestion in chicks: A biochemical evaluation. Indian J Physiol Pharmacol 1985;29(1):47-50.
5. Eshrat MH. Effect of *Coccinia indica* (L.) and *Abroma augusta* (L.) on glycemia, lipid profile and on indicators of end-organ damage in streptozotocin induced diabetic rats. Indian J Clin Biochem 2003;18(2):54-63.
6. Punitha R, Vasudevan K, Manoharan S. Effect of *Pogamia pinnata* flowers on blood glucose and oxidative stress in alloxan induced diabetic rats. Indian J Pharmacol 2006;38(1):62-3.
7. Chopra RN, Chopra IL, Honda KL, Kapur LD. Indigenous Drugs of India. 2nd ed. Calcutta: U.N. Dhar and Sons Ltd.; 1958. p. 300-29.
8. Abhinayani G, Shanker VS, Benazir F. Evaluation of antidiabetic activity of alcoholic extract of *Aloe barbadensis*, *Momardica Charantia*, *Trigonella Foenum-Graecum* and their combinations used in alloxan induced diabetic rats. World J Pharm Pharm Sci 2015;4(11):1380-9.
9. Macwan CP, Patel MA. Antioxidant potential of dried powder of *Capparis zeylanica* Linn. Int J Pharm Pharm Sci 2010;2(3):58-60.
10. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Pune: Nirali Prakashan; 2003. p. 149-56.
11. Chanda S, Dave R, Kaneria M, Shukla V. Acute oral toxicity of *Polyalthia longifolia* var. Pendula leaf extract in Wistar albino rats. Pharm Biol 2012;50(11):1408-15.
12. OECD. Guideline for Testing of Chemicals. Acute Oral Toxicity-Up and Down Procedure. Paris: OECD; 2001. p. 425.
13. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti-hyperglycemic potential of *Tridax procumbens* (Linn.). BMC Complement Altern Med 2009;9:48.
14. Vats V, Grover JK, Rathi SS. Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. J Ethnopharmacol 2002;79(1):95-100.
15. Khosla P, Gupta DD, Nagpal RK. Effect of *Trigonella foenum graecum* (Fenugreek) on blood glucose in normal and diabetic rats. Indian J Physiol Pharmacol 1995;39(2):173-4.
16. Abdel-Barry JA, Abdel-Hassan IA, Al-Hakiem MH. Hypoglycaemic and antihyperglycaemic effects of *Trigonella foenum-graecum* leaf in normal and alloxan induced diabetic rats. J Ethnopharmacol 1997;58(3):149-55.