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Research Article

Evaluation of antihyperglycemic and hypolipidemic activity of ethanolic extract of *Clerodendrum infortunatum* Linn. in experimental animals

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ABSTRACT

Background: Diabetes mellitus (DM) is the most common non-communicable disease of the modern world. The study of plants having antihyperglycemic and hypolipidemic activities may give a new approach in the treatment of DM. The study was intended to evaluate the antihyperglycemic and hypolipidemic activity of ethanolic extract of *Clerodendrum infortunatum* Linn. (EECL) in alloxan-induced diabetic albino rats.
Methods: Diabetes was induced in albino rats by administration of alloxan monohydrate (160 mg/kg, intraperitoneal). Rats were divided into six groups of six animals each. First group served as non-diabetic control, second group as diabetic control, third group as standard and was treated with 0.1 mg/kg/day of glibenclamide. Group 4, 5, and 6 received 200 and 400 mg/kg body weight of EECL. Blood samples were analyzed for blood glucose on day 1, 3, 7, 14, 21, and 28 and lipid profile on day 28.
Results: The EECL showed a significant reduction ($p<0.001$) in blood glucose level and serum lipid profile levels with 400 mg/kg body weight in alloxan induced diabetic rats as compared with control.
Conclusion: It is concluded that EECL is effective in controlling blood glucose levels and in improving lipid profile in diabetic rats.

Keywords: Ethanolic extract, Hypoglycemia, Hypolipidemia, *Clerodendrum infortunatum* Linn

INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable disease of the modern world. It is a chronic metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, and negative nitrogen balance.[1] It is associated with insulin resistance and impaired insulin release.

According to International Diabetes Federation Atlas update 2014, more than 387 million people in the world living with diabetes with prevalence of 8.3%, by 2035 this will rise to 592 million, there were 66.8 million cases of diabetes in India in 2014.[2]

DM is a progressive disease with associated complications of retinopathy, nephropathy, neuropathy, and DM has also been associated with an increased risk for premature arteriosclerosis due to increase in triglycerides level (TGL) and low-density lipoprotein (LDL) levels. About 70-80% of deaths in diabetic patients are due to vascular disease.[3]

Metabolic disorders that involve elevations in any lipoprotein components are termed as hyperlipidemia except high density lipoprotein-cholesterol (HDL-C).[4] Hyperlipidemia is major risk factor for atherosclerosis and atherosclerosis induced conditions like coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular diseases.[5] Atherosclerosis remains the major cause of death and premature disability in developed countries. Moreover, current predictions estimate that by the year 2020 cardiovascular diseases, notably atherosclerosis will become the leading cause of total disease burden.[6]

An ideal treatment for diabetes would be a drug that not only controls the glycemic level but also prevents the development of arteriosclerosis and other complications of diabetes.

Long before the use of insulin became common, indigenous remedies were used for the treatment of DM and hyperlipidemia. There has been an increasing demand from patients for the use of natural products with antidiabetic and

antihyperlipidemic activity. This is largely because insulin cannot be used orally and insulin injections are associated with the risk of hypoglycemia and impairment of hepatic and other body functions. The undesirable side effects and contraindications of synthetic drugs and the fact that they are not suitable for use during pregnancy, necessitates search for alternative hypoglycemic agents of plant origin. Many herbs and plant products have been shown to have antihyperglycemic and antihyperlipidemic action.[3]

Hence, there is increased demand for herbal and natural products with lesser side effects. In view of these reports, it is interesting to search for more efficient, safer, and cost effective antihyperglycemic and antihyperlipidemic agents.

Clerodendrum infortunatum Linn. of family verbenaceae. In English, it is called as glory tree and in Hindi as Bhant. It is an important and widely used medicinal plant, reported to contain active bitter substance like clerodin, has been widely used as a tonic and anthelmintic agent in the country sides of North India. Though, variously used in Ayurveda, Unani system of medicine and homeopathy in case of ailments like diarrhea, skin disorders, venereal and scrofulous complaints, wounds, post-natal complications, as external applications on tumors, etc., the plant needs thorough investigation for its specific medicinal activity. Traditionally, the plant is used as an antipyretic and anthelmintic. Leaves of the plant are prescribed for tumor, certain skin diseases and scorpion sting. The antioxidant, antimicrobial, anti-malaria, anthelmintic, and analgesic activities of the plant have further created an upsurge in the investigations on the plant.[7,8] This plant owes its antihyperglycemic and antihyperlipidemic properties to the presence of flavonoids, tannins, saponin in the leaves.[9]

METHODS

Plant material

The leaves of the *C. infortunatum* were collected from Amrutha Vana Centre for Herbal Gardens and Landscaping Services, Government of Karnataka, Bangalore in the month of August 2012. The plant identity was authenticated by botanist Prof. Jadimath and voucher specimen (voucher number: SNMC/Pharma 004), is kept in of Department Herbarium.

Preparation of the extract

The leaves of the *C. infortunatum* were dried under shade for a period of 4 weeks. The dried plant material was milled to a fine powder. The powder plant material was extracted with absolute ethyl alcohol using soxhlet extraction apparatus. Dried powder (300 g) was extracted in a soxhlet extractor with ethanol for about 8-9 hrs at 45°C. Extract was collected and dried using rotary flash evaporator at 40-45°C and the crude residue was collected. The solvent was completely removed under reduced pressure and semisolid mass was obtained. The yield was calculated as 30 g. The extract was stored in well-closed glass container at 5°C in a refrigerator for further study.[8,10,11] From this stock, fresh preparation was made whenever required.

Animals

Healthy Wistar albino rats of either sex weighing about 150-200 g were used. The animals were housed in polypropylene cages, maintained under standard conditions (12:12 hrs light: dark cycle; 25±2°C, 35-60% humidity). They were fed with standard rat pellet diet (Hindustan Lever Ltd. Mumbai, India) and water *ad libitum*. The Institutional Animal Ethical Committee of S. Nijalingappa Medical College (IAEC/SNMC-Reg. No.: 829/AC/04 CPCSEA), Bagalkot, Karnataka, India, approved the study protocol.

Phytochemical analysis

Extract was qualitatively analyzed for flavonoids, alkaloids, glycosides, saponins, tannins, proteins and amino acids, sterols and triterpenoids, carbohydrates, fixed oils, anthraquinone, steroids and resins.

Acute oral toxicity study

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (up and down procedure). All the five mice were administered 2000 mg/kg of ethanolic extract of leaves of *C. infortunatum* orally and observed continuously for a period of 14 days, every hourly for 24 hrs, and every day for 14 days for its movements, grooming activity, exploring activity, writing reflex, eye movements, and convulsion, etc.[12]

Induction of experimental diabetes

Diabetes was induced in overnight-fasted rats by a single intraperitoneal injection of freshly prepared 2% alloxan monohydrate (160 mg/kg dissolved in 0.9% normal saline). 72 hrs after the injection, blood was withdrawn from overnight fasted animals and blood glucose level was assessed by glucometer. The rats with a blood glucose level above 200 mg/dl were selected for the experiment as diabetic rats. Control animals were injected with normal saline only.

Sample collection

The experiment was conducted for a period of 28 days. Fasting blood glucose levels of rats was taken on 0, 7th, 14th, 21st, 28th day of post treatment and represented the results as mg/dl. At the end of the study, blood was withdrawn by retro-orbital plexuses from all animals of each group for estimation of serum lipid profile i.e., total cholesterol (TC), TGL, HDL, LDL, and very LDL (VLDL), respectively.

Experimental design

Rats fasted overnight for 12 hrs were randomly (simple random sampling technique) divided into six groups of six rats per group. Group 1 served as a normal control or non-diabetic group was treated with 0.5 ml of normal saline orally once daily. Group 2 served as untreated diabetic control received 0.5 ml of normal saline orally once daily.

Group 3 served as standard group and was treated with of glibenclamide 5 mg/kg body weight orally once daily.

Groups 4 and 5 were treated orally with 200, and 400 mg/kg of *C. infortunatum* Linn. orally once daily respectively, based on their acute oral toxicity study.

Methodology

The experiment was conducted for a period of 28 days. Fasting blood glucose levels of rats was taken on 0, 7th, 14th, 21st, 28th day of post-treatment and represented the results as mg/dl. At the end of the study, blood was withdrawn by retro-orbital plexuses from all animals of each group for estimation of serum lipid profile, i.e., TC, TGL, HDL, LDL, and VLDL, respectively.

On day 28, blood was collected from overnight fasted rats under anesthesia by retro-orbital plexus puncture method and was kept aside for 30 mins for clotting. By centrifuging the same sample at 6000 rpm for 20 mins, the serum was separated and analyzed for serum TC was estimated by cholesterol oxidase-phenol and aminophenazone method,[13] VLDL, LDL-C was estimated by Friedewald's formula, TG was estimated by glycerol-3-phosphate oxidase-peroxidase[14] method and HDL-cholesterol was estimated by phosphotungstic precipitation method.

Statistical analysis all the values were expressed as a mean±standard error of mean. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. $p<0.05$ was considered significant.

RESULTS

Phytochemical analysis

Phytochemical analysis of the extract showed the presence of flavonoids, alkaloids, glycosides, saponins, tannins, proteins and amino acids, sterols and triterpenoids, carbohydrates, fixed oils, anthraquinone, steroids and resins.[9]

Determination of lethal dose (LD_{50}) of ethanolic extract of C. infortunatum Linn. (EECL)

Administrations of a single dose of extract (500 mg/kg, b.w., and p.o.) did not produce any mortality. All five animals were alive, healthy, and active during the observation period of 14 days. AOT 425 software was used to obtain higher doses for LD_{50} determinations as per the OECD guidelines. In case of EECL, the computer program suggested doses 550, 1750, and 2000 mg/kg. Results indicate that the dose up to 2000 mg/kg were non-lethal. All the animals were found to be alive, healthy, and active during the observation period of 14 days post administration of highest dose. The computer program showed LD_{50} >2000 mg/kg.

Effect of EECL on diabetic rats

The antihyperglycemic activity of EECL on the fasting blood sugar level of diabetic rats is shown in Table 1. The treatment in the dose of 400 mg/kg b.w., in alloxan-induced diabetic rats showed a significant ($p<0.001$) decrease in the elevated blood glucose level as compared with the control. The extract in the dose of 200 mg/kg b.w. showed significant ($p<0.001$) antihyperglycemic activity on day 14 and the dose 400 mg/kg b.w., showed significant ($p<0.001$) result on day 3.

Other biochemical parameters since the reduction in elevated blood glucose were seen more significantly with the extract dose 400 mg/kg b.w. and the same dose of EECL when given in diabetic rats has shown a decrease in serum lipid profile ($p<0.01$) when compared with diabetic control and normal rats in Table 2.

DISCUSSION

The present study showed the antihyperglycemic and hypolipidemic effect of EECL in alloxan-induced diabetic rats. A marked rise in fasting blood glucose level observed in diabetic control compare to normal control rats. EECL (at 200 and 400 mg/kg) exhibited a dose-dependent significant antihyperglycemic activity. The extract dose of 200 mg/kg caused reduction in blood glucose level on day 14 and at 400 mg/kg on day 3 and the results were found statistically significant $p<0.001$, $p<0.001$, respectively. There were statistically significant differences in the serum cholesterol, TG, and HDL, LDL, VLDL, levels when diabetic rats received the EECL extract at a dose of 400 mg/kg b.w.

When compared with normal control group, showed a highly significant decrease in the levels of the total serum cholesterol 128.66±7.42 ($p<0.001$), serum TG 134.66±6.37 ($p<0.01$), serum VLDL 26.93±1.27 ($p<0.01$), serum LDL 72.40±8.48 ($p<0.001$), while there was highly significant increase in the level of the serum HDL 29343±6.37 ($p<0.01$). When compared to the normal control group i.e. EECL has got hypolipidemic activity.

Alloxan induces diabetes by destroying the insulin-producing beta cells of the pancreas. *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to induction of cell necrosis. This action is

Table 1: Effect of EECL on blood glucose level in alloxan (160 mg/kg. i.p.) induced diabetes rats.

Groups	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28
Normal control	105.67±3.703	107.17±3.54	108.33±2.155	110.67±3.602	108.67±3.21	105.83±3.31
Diabetic control	264.67±6.401	265.67±6.15	270.17±6.57	273.5±3.98***	270.67±4.46***	274.67±4.18***
Diabetic standard	263.33±9.09	221.67±5.73***	196±3.27***	147±4.55***	128.83±2.45***	103±2.35***
200 mg	281.33±7.04	278.17±6.93	270±6.33	241.33±5.46***	213.83±3.188***	172.5±4.57***
400 mg	280.17±5.98	267.5±4.87***	246.83±5.32***	209.83±4.08***	163.67±3.19***	132±4.008***

Hypoglycemic activity of EECL *p<0.05, **p<0.01, ***p<0.001. All the values are expressed as mean±SEM (n=6), SEM: Standard error of mean, EECL: Ethanolic extract of *clerodendrum infortunatum* Linn

Table 2: Effect EECL on biochemical parameters in alloxan-induced diabetic rats.

Groups	Serum TC	Serum TG	Serum HDL	Serum VLDL	Serum LDL
Control	82.23±14.08	63.83±7.21	31.11±2.37	12.76±1.44	38.35±16.37
Diabetic control	153.30±4.93***	170.06±6.91***	20.66±2.41***	34.01±1.38***	98.62±6.85***
Diabetic standard	98.86±4.76***	121.96±8.6***	31.93±1.48***	24.39±1.73***	42.54±4.48***
400 mg	128.66±7.42***	134.66±6.37**	29.33±1.04**	26.93±1.27**	72.40±8.48***

Hypolipidemic activity of EECL *p<0.05, **p<0.01, ***p<0.001. All the values are expressed as mean±SEM (n=6), EECL: Ethanolic extract of *clerodendrum infortunatum* Linn, SEM: Standard error of mean, HDL: High-density lipoprotein, TC: Total cholesterol, LDL: Low density lipoprotein, VLDL: Very low density lipoprotein, TG: Triglyceride

mediated by reactive oxygen species with a simultaneous massive increase in calcium concentration leading to a rapid destruction of beta cells.[15] The use of lower dose alloxan (160 mg/kg b.w.) produced partial destruction of pancreatic beta cells even though the animals became permanently diabetic. Thus, these animals have surviving beta cells and regeneration is possible.

Glibenclamide, the second generation sulfonylurea is known to mediate the antihyperglycemic effect by stimulating insulin release from pancreatic beta cells, reducing the hepatic clearance, and suppressing the secretion of glucagon.[16,17] Sulfonylurea have been shown to suppress gluconeogenesis.

The antihyperglycemic effect of the ethanolic extract may be due to the enhanced secretion of insulin from the beta cells of the pancreas or may be due to increased tissue uptake of glucose by enhancement of insulin sensitivity.

Elevated plasma cholesterol and TG levels are major risk factors of cardiovascular disease. The existing antihyperglycemic agents allow a sharp control of blood glucose levels but insufficient correction of lipid abnormality, especially in hypertriglyceridemia. Diabetic rats showed elevated plasma cholesterol and TG levels due to hyperglycemia and insulin resistance. Ethanolic extract in the dose of 400 mg/kg b.w., reduced the TG and cholesterol levels along with a reduction in the blood glucose levels.

The active constituents responsible for antihyperglycemic and hypolipidemic activities are not known. Phytochemical analysis showed the presence of flavonoids, alkaloids, glycosides, saponins, tannins, proteins and amino acids, sterols and triterpenoids, carbohydrates, fixed oils, anthraquinone, steroids and resins.[9] The presence of any of these photo components might be responsible for the antihyperglycemic and hypolipidemic activity in diabetic rats. Some studies have also reported that these biological activities might be because of the presence of flavonoids, alkaloids, glycosides, saponins, tannins, proteins and amino acids, sterols and triterpenoids, carbohydrates, fixed oils, anthraquinone, steroids and resins in EECL.[9] Again these studies would require experimental validation.

Since many anti-diabetic drugs do not correct dyslipidemia, the observed hypolipidemic effects of this plant extract in diabetic rat makes EECL quite important in the management of diabetes. Further investigations are needed to elucidate the mechanism of action, particularly the bioactivity-guided fractionation, isolation identification, and enzymatic study of constituents of the plant extract responsible for the observed pharmacologic activities. Since there is a strong, well-established link between DM, dyslipidemia, obesity, hypertension, and ischemic heart disease, effect of this plant extract on weight loss/gain and organ histopathologic studies need to be explored on scientific base.

In conclusion, this preliminary study has been able to demonstrate the antihyperglycemic, hypolipidemic potentials of EECL in diabetic rats. And further scientific evaluation is needed to derive its molecular level mode of action.

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Ethical approval: The study was approved by the Institutional Animal Ethics Committee

REFERENCES

1. Kasper DL, Braunwald E, Faucis AS, Hauser SL, Longo DL, Jamson JL, et al., editors. Harrison's Principles of Internal Medicine. 17th Edition. Vol. 2. New York: McGraw Hill; 2008: 2275.
2. Available at http://www.idf.org/diabetesatlas/5e/update2014. Accessed 15 January 2015.
3. Yadav JP, Sushila S, Kalia AN, Dangi AS. Hypoglycemic and hypolipidemic activity of ethanolic extract of *Salvadora oleoides* in normal and alloxan induced diabetic rats. Indian J Pharmacol. 2008; 40 (1): 23-7.
4. Malloy MJ, Kane JP. Agents used in dyslipidemia. In: Katzung BG, editor. Basic and Clinical Pharmacology. 12th Edition. New Delhi: Tata McGraw Hill; 2012: 619-33.
5. Bersot TP. Drug therapy for hypercholesterolemia and dyslipidemia. In: Bruton LL, editor. Goodman and Gilman's the Pharmacological Basis of Therapeutics. 12th Edition. New York: McGraw Hill; 2011: 877-908.
6. Libby P. The pathogenesis, prevention, treatment of atherosclerosis. In: Lango DL, Fauci AS, Kasper DL, editors. Harrison's Principle of Internal Medicine. 18th Edition. New York: McGraw Hill; 2012**:** 1983-92.
7. Bhattacharjee D, Das A, Das SK, Chakraborthy GS. *Clerodendrum infortunatum* Linn: a review. Adv Pharm Health Care Res. 2011;1(3):82-5.
8. Das S, Haldar PK, Pramanik G, Suresh RB. Evaluation of anti-inflammatory activity of *Clerodendron infortunatum* Linn. extract in rats. Glob J Pharm. 2010;4(1):48-50.
9. Baid SS. Evaluation of antihyperglycemic and hypolipidemic activities of *Clerodendrumn infortunatum* Linn. leaf extracts. Asian J Complement Altern Med. 2013;1(1):1-8.
10. Das S, Haldar PK, Pramanik G, Suresh RB. Anticonvulsant activity of methanolic extract of *Clerodendron infortunatum* Linn. in Swiss albino mice. Thai J Pharm Sci. 2010;34:129-33.
11. Girish G, Das K, Bhosle V, Einstein JW, Mathai KB. Evaluation of anticonvulsant activity of ethanolic leaves extract of *Desmodium triflorum* in mice. Rev Bras Farmacogn Braz J Pharmacogn. 2012;22(3):649-56.
12. Maniyar Y, Bhixavathimath P. Antihyperglycemic and hypoglycemic activities of aqueous extract of *Carica papaya* Linn. Leaves in alloxan-induced diabetic rats. J Ayurveda Integr Med. 2012;3(2):70-4.
13. Demacker PN, Hessels M, Toenhake-Dijkstra H, Baadenhuijsen H. Precipitation methods for high-density lipoprotein cholesterol measurement compared, and final evaluation under routine operating conditions of a method with a low sample-to-reagent ratio. Clin Chem. 1997;43(4):663-8.
14. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem. 1983;29(3):538-42.
15. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50(6):537-46.
16. Marshall A, Gingerich RL, Wright PH. Hepatic effect of sulfonylureas. Metabolism. 1970;19(12):1046-52.
17. Widén E. Does glibenclamide influence the clearance of insulin and glucose uptake in patients with type 2 diabetes mellitus? Scand J Clin Lab Invest. 1993;53(4):395-403.

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