



RESEARCH ARTICLE

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Evaluation of Antihyperglycemic and Hypolipidemic Activities of *Clerodendrum infortunatum* Linn. Leaf Extracts

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ABSTRACT

Ethnopharmacological relevance: *Clerodendrum infortunatum* Linn. (Verbenaceae), commonly known as Bhant in Hindi, has been traditionally used in the Siddha system of medicine as a chief ingredient of many polyherbal formulations for the treatment of diarrhoea, rheumatic complaints, diabetes, fever and skin ailments. However, no scientific evidence is available to validate the folklore claim. The present study has been designed to evaluate its leaf extract for the antidiabetic and hypolipidemic activity.

Aim: To evaluate leaves of *Clerodendrum infortunatum* for the antihyperglycemic and hypolipidemic activity.

Materials and methods: Different leaf extracts of *Clerodendrum infortunatum* were evaluated for the polyphenol, flavonoid, steroid contents and *in-vitro* antihyperglycemic activity (alpha-glucosidase inhibition assay). On the basis of Quantitative estimation and *in vitro* alpha-glucosidase inhibition assay, chloroform extract (CECI) was screened for the antihyperglycemic activity by streptozotocin induced diabetic rats and biochemical parameters viz. total cholesterol, TG, HDL cholesterol and LDL cholesterol were also evaluated for hypolipidemic activity.

Results: Chloroform extract (CECI) at the doses of 200 and 400 mg/kg significantly ($P < 0.05$) and dose-dependently reduced and normalized blood glucose levels as compared to that of the STZ control group. Serum biochemical parameters were significantly ($P < 0.05$) restored towards normal levels in CECI-treated rats as compared to the STZ control. In order to assess the role of secondary metabolites in the relevant activity, polyphenolic, flavonoid and steroid contents were determined. The highest polyphenolic and flavonoid content were found in hydroalcoholic extract 58.78 $\mu\text{g}/\text{mg}$ (GAE mg/g of extract), 73.84 $\mu\text{g}/\text{mg}$ (rutin equivalent/g extract) respectively. The chloroform extract has shown highest steroid content 17.63 $\mu\text{g}/\text{mg}$ and α -glucosidase inhibition activity.

Conclusion: The present study demonstrated that the leaves of *C. infortunatum* had remarkable preclinical antihyperglycemic activity in STZ-induced diabetic rats. Our results contribute towards validation of the traditional use of *C. infortunatum* in the treatment of diabetes.

Keywords: Antihyperglycemic, *Clerodendrum infortunatum*, Streptozotocin, Metformin

1. INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder of carbohydrate, lipid, and protein metabolism. It is characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance, and sometimes ketonemia, due to insufficient or complete cessation of insulin synthesis, or secretion and/or peripheral resistance to insulin action. Diabetes has been known to medical sciences longer than any other hereditary metabolic diseases. The latest World Health Organization estimate (for the number of people worldwide, in 2000) is 177 million. This will increase to at least 300 million by the year 2025 (Soni, 2005). Nevertheless, the existing methods of treatment for this age old illness are not completely satisfactory. Since the treatment of patients with type-2 diabetes with oral hypoglycemic agents like sulfonylureas and biguanides, is always associated with side effects (Weidmann, 1993). Therefore, Researchers have shifted their research towards herbal drugs which are gaining popularity in the treatment of diabetes mellitus.

Clerodendrum infortunatum Linn. (Verbanaceae: Bhant in Hindi, Ghentu in Bengali) is a terrestrial shrub having square, blackish stem and simple, opposite, decussate, petiole, exstipulate, coriaceous, hairy leaves with a disagreeable odour (Anonymous, 1985; Kirtikar and Basu, 2003). The plant is common throughout the plains of India. Various parts of the plant have been used by tribes in colic, scorpion sting, snake bite, tumour and certain skin diseases (Nadkarni and Nadkarni, 2002; Chopra *et al.*, 1992). They are also used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy (Kapoor, 2001). Bhant has certain ethno-medicinal properties that are practiced by various tribes of our country. Extract of the leaves is given orally in fever and bowel troubles among the Kuki and Rongmai Naga tribes of North-East India. Also fresh leaf-juice is introduced in the rectum for removal of ascarides. Leaves and flowers are used to cure scorpion sting. Rabha, Rajbanshi, Polia and Lepcha tribes of North Bengal use fresh root-bark of bhant to cure diarrhea (Mitra and Mukherjee, 2010). Kachari, Hmar and Riang tribes of Barak Valley and North- Cachar hills use leaf extract in stomach pain and diabetes. Also a root paste is used as bandage in swelling (Barbhuiya *et al.*, 2009).

Fresh juice of the leaves has been used as vermifuge and in treatment of malaria (Chopra *et al.*, 1992; Goswami *et al.*, 1998). *Clerodendrum infortunatum* leaves were reported to contain saponin, clerodin (a bitter diterpene), some enzymes (Saiprakash *et al.*, 2001), alkyl sterols and 2,-(3, 4-dehydroxyphenyl)

ethanol-1-O- α -2 rhamnopyranosyl (1 \rightarrow 3)- β -D-(4-O-caffeoyl)

glycolpyr-anoside (acteoside) (Prajapati *et al.*, 2001; Khatri *et al.*, 2005). Leaves also contain a fixed oil which consists of glycerides of lenoleic, oleic, stearic and lignoceric acid (Kapoor, 2001). Since no scientific validation on its folklore claim is available, the present study is designed to study its hypoglycemic as well as hypolipidemic activity.

2. MATERIALS AND METHODS

2.1. Animals

Adult male Wistar rats (200-250 g) obtained from animal house of our institute were used. They were housed under the controlled environment and temperature (22 \pm 5 $^{\circ}$ C, 35 to 60% humidity with 12-h of light/dark cycle). The animals were provided with regular rat chow and distilled water *ad libitum*. The animals were acclimatized to laboratory conditions for 1 week prior to the experiment. All experimental protocols were approved by Institutional Animal Ethics Committee, J.L. Chaturvedi College Pharmacy, Nagpur. **(648/02/CPCSEA)**

2.2. Chemicals and standard drugs

Streptozotocin (Calbichem Darmstadt Germany), metformin (Rubicon Research Pvt. Ltd., Mumbai). α -Glucosidase (from *Saccharomyces cerevisiae* type I) and 4-nitrophenyl α -D-glucopyranoside (PNPG) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The TG, HDL, LDL and VLDL estimation kits were purchased from Span Diagnostics, Surat, India. All other reagents used were of analytical grade and were obtained commercially.

2.3. Plant Material and preparation of extracts

The leaves of *Clerodendrum infortunatum* (CI) were collected locally from the areas of Chandrapur (Tadoba Forest), Maharashtra, India. The plant was authenticated by the Department of Botany, R. T. M. Nagpur University, Nagpur. A voucher specimen has been deposited in the Herbarium of Department of Botany (RA 9601). The leaves were shade dried at room temperature and pulverized to a coarse powder. The coarsely powdered material was defatted with pet ether (CIPE) (5.46 % w/w) and then extracted successively with the increasing order of polarity of solvent viz., chloroform (CECI) (4.31 % w/w), ethyl acetate (CIEA) (2.419 % w/w), acetone (CIA) (1.337 % w/w) and methanol (CIME) (7.04 % w/w). Finally, the marc was macerated with water and ethanol mixture (1:1) for 48 hrs (CIHA) (13.80 % w/w). The extracts were concentrated by using Rotary vacuum evaporator and subjected to phytochemical and pharmacological screening.

2.4. *Phytochemical screening* (Stahl, 1969; Harbone, 1976; Wagner *et al.*, 1984)

The various extracts were screened for the presence of tannins, saponins, unsaturated sterols, triterpenes, alkaloids, flavonoids, protein/amino acids and carbohydrates and/or glycosides with thin layer chromatography (TLC). Thin layer plates precoated with silica gel G (Merck, 0.25mm thickness) were used. Development was carried out with different solvent systems such as Benzene : Ethylacetate (9.5 : 0.5, v/v) for petroleum ether extract, Hexane : Ethylacetate (3 : 1, v/v) for Chloroform extract, Toluene : Methanol (5 : 1, v/v) for ethylacetate extract, Methanol : Chloroform : Ethyl acetate (2 : 2 : 2, v/v) for methanol extract. After development of chromatogram in the solvents the plates were dried and sprayed with $AlCl_3$, Dragendorff's, ninhydrin, hydroxylamine-ferric chloride, and antimony trichloride for the detection of flavonoids, alkaloids, protein/amino acids, lactones/esters, unsaturated sterols and triterpenes respectively. While detection of saponins, anthraquinones, tannins, carbohydrate and/or glycosides is carried out using anisaldehyde-sulphuric acid, reagent, KOH, ferric chloride and naphthoresorcinol reagent respectively and visualization is carried out under visible and UV light (λ :366 nm).

All the extracts were also quantified for presence of important secondary metabolites such as total polyphenol, flavonoid and steroid compounds using following spectroscopic methods.

2.4.1. *Determination of total polyphenol (TP), flavonoids and steroids*

Total polyphenol content was measured using Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965; Singlenton *et al.*, 1999). Gallic acid was used as a reference for constructing the standard curve (10–100 μ g/ml).

Flavonoid content was determined by the aluminum chloride method (Jin-Yuarn and Ching-Yin, 2007; Stanojević *et al.*, 2009). Rutin was used as a reference for constructing the standard curve (10–100 μ g/ml).

Estimation of total steroids was done by libermann burchard reaction (Brieskorn, 1953). Cholesterol was used as a reference for constructing the standard curve (10–100 μ g/ml). All the results were expressed as mg of gallic acid, rutin and cholesterol equivalents per g of extract respectively.

2.5. *In-vitro α -glucosidase inhibition assay* (Nishioka *et al.*, 1998)

Alpha-glucosidase activity was determined by a colorimetric assay which measured the release of p-nitrophenol from p-nitrophenyl- α -D-glucopyranoside at 405 nm. To a 96-well spectra plate, 45 μ l of α -glucosidase (0.2 U/mL) in acetate buffer (50 mM; pH

4.5), 10 μ l of testing sample, blanks or controls and 45 μ l of 4-nitrophenyl- α -D-glucopyranoside (2 mM) in acetate buffer (50 mM; pH 4.5) were added in triplicates. Acarbose was included at 2 mg/ml (50% ethanol/water) as the positive control. The plate was shaken in an orbital manner for 30 seconds. The plate was covered and incubated at 37°C (to mimic a mammalian system) for 30 minutes. Sodium carbonate 0.2M solution was added to stop the reaction. The plate was shaken again for 30 seconds. Absorbance was measured at 405 nm. A non-enzyme control plate was run simultaneously. The absorbance of the non-enzyme control was subtracted from the test enzyme absorbance. The percentage inhibition was calculated by:

$$\frac{A_{450nm} \text{ solvent control} - A_{450nm} \text{ test sample}}{A_{450nm} \text{ solvent control}} \times 100$$

On the basis of quantitative estimation, and alpha glucosidase method, chloroform extract was selected for sub-chronic diabetic study.

2.6. *Acute toxicity* (OECD guidelines 423, 2000)

Rats were divided into test and control groups (n = 6). The test group was given an increasing oral dose (1, 3 and 5g/kg) of CECI. The rats were allowed food and water *ad libitum* and were kept under regular observation for symptoms of mortality and behavioural changes for the period of 48 h.

2.7. *Oral glucose tolerance test (OGTT)*

OGTT for diabetic rats were performed according to the standard method in overnight fasted (18 h) normal animals (Du Vigneaud and Karr, 1925). Rats were divided into five groups, each comprising of six animals. Group I - received buffer solution; Group II - received only STZ; Group III & IV- received CECI extract in dose range of 200 and 400 mg/kg per oral respectively; Group V - received Metformin 100 mg/kg per oral. The rats of all groups were given glucose (2 gm/kg body weight, orally) 30 min after administration of test samples. Blood was withdrawn from tail-tip just prior to the drug administration (normal fasting) at 0, 30, 60 and 120 min of glucose loading. Blood glucose levels were estimated using glucose oxidase-peroxidase reactive strips and single touch glucometer (Accu-check, Roche Diagnostics, USA).

2.8. *Induction of experimental diabetes*

Diabetes was induced in overnight-fasted rats by a single intraperitoneal (i.p.) injection of streptozotocin 65 mg/kg dissolved in 0.1 M cold citrate buffer (pH 4.5). Seventy-two hours 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 250 mg/dl were selected for the experiment as diabetic rats. Control animals were injected with normal saline only.

2.9. Experimental design and testing of fasting blood glucose level

The diabetic rats were fasted overnight and divided randomly into five groups (I-V) of six rats (n = 6) each as: Group I - Normal control received buffer solution (10 ml/kg; p.o.); Group II - Diabetic control received only STZ, Group III & IV- Diabetic rats received CECI extract in dose range of 200 and 400 mg/kg p.o.; Group V - Diabetic rats received Metformin 100 mg/kg p.o. for 28 days respectively.

Fasting blood glucose levels of rats were taken on 0, 7th, 14th, 21st, 28th day of post treatment and reported the results as mg/dl. At the end of the study, blood was withdrawn by tail-tip from all animals of each group for estimation of serum biochemical parameters like TG, HDL, LDL and VLDL respectively.

2.10. Body weight and estimation of serum biochemical parameters

Body weight of rats from each group was measured on 0, 7th, 14th, 21st, 28th day of post treatment by electronic balance. The biochemical parameters like TG, HDL, LDL and VLDL were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the commercially available reagent kits using Semi Autoanalyser (Chem-400 Semi Auto Chemistry Analyser, International Biological Laboratories, Ambala).

2.12. Statistical analysis

The experimental results were expressed as mean \pm standard error of mean (SEM). Statistical testing methods included one way analysis of variance (ANOVA) followed by Tukey-Kramer's test. P-values of ≤ 0.05 were considered to indicate statistical significance.

3. RESULTS:

3.1. Phytochemical screening

Investigations on the phytochemical screening of *Clerodendrum infortunatum* leaf extracts revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids. In qualitative screening of extracts; pet ether and chloroform extract was found to contain sterols and triterpenoids. Ethylacetate extract showed the presence of sterols, tannins and sugar. Acetone extract showed presence of alkaloids, tannins, flavonoids and sugar. Methanol and hydroalcoholic extract were found to contain tannins, flavonoids, sugars and saponins. These compounds are known to be biologically active and therefore aid the antihyperglycemic activities of *C. infortunatum*.

3.2. Determination of total polyphenol, flavonoid and steroids:

The total polyphenol content (mg/g) determined by Folin-Ciocalteu colorimetric method was found to be 35.4, 33.79, 54.17 and 58.78 mg (GAE mg/g of extract)

for CIEA, CIA, CIM and CIHA respectively. Polyphenol content was determined from linear regression equation of Gallic acid and expressed as GAE of extract ($y = 0.0035x - 0.0842$, $r^2 = 0.9917$) (Figure 1).

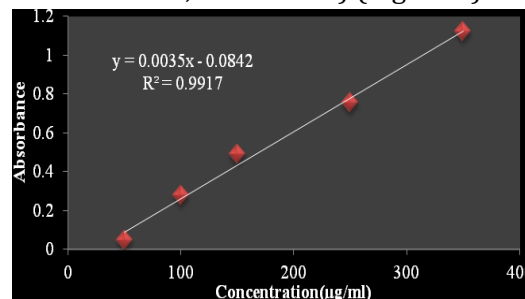


Figure-1: Calibration curve of gallic acid

The flavonoid content determined by aluminum chloride method was found to be 47.34, 67.12 and 73.84 mg/g of extracts for CIA, CIM and CIHA respectively. Flavonoid content was determined from linear regression equation of rutin ($y = 0.015x + 0.0604$, $r^2 = 0.9957$) (Figure 2).

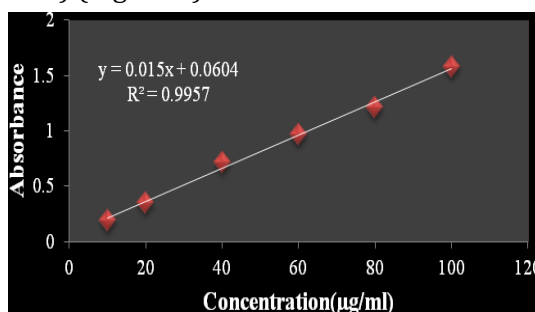


Figure-2: Calibration curve of rutin

The steroid content determined by libermann burchard reaction was found to be 14.95, 17.63 and 2.45 mg/g for CIPE, CECI and CIEA respectively. Steroid content was determined from linear regression equation of cholesterol ($y = 0.001x - 0.001$, $r^2 = 0.998$) (Figure 3).

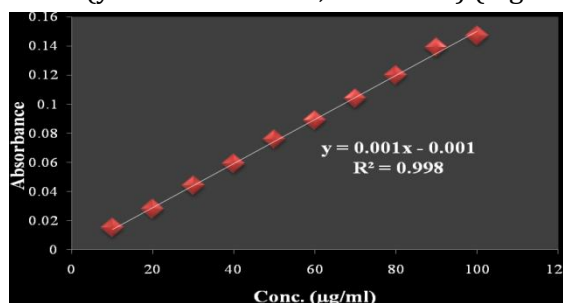


Figure-3: Calibration curve of cholesterol

3.2. In-vitro assay: alpha-glucosidase inhibition assay

The hypoglycemic potential of various extracts were evaluated by the α -glucosidase inhibition assay. The optimal concentration of extract required for the 50 % inhibition (IC_{50}) against α -glucosidase was 1.3 μ g /ml. Acarbose was used as positive control with IC_{50} value of 2.5 μ g /ml. This assay presented dose dependent inhibition and all the experiments were carried out in triplicates. The chloroform extract showed highest

inhibition among all extracts and hence was used for further pharmacological studies (Figure 4).

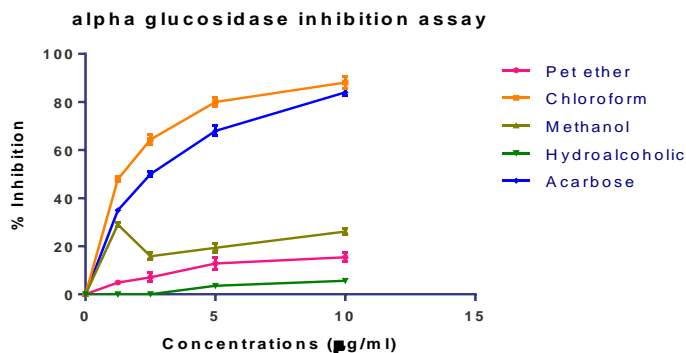


Fig 4: α-glucosidase inhibition assay

3.3. Antihyperglycemic effect

Acute toxicity studies revealed the non-toxic nature of CECI. There were no lethality or toxic reactions found at any doses selected. OGTT reveals how quickly glucose is metabolized from the bloodstream for use by cells as an energy source. The CECI in dose range 200 and 400 mg/kg showed promising results and blood glucose level decreased significantly ($p < 0.05$) at 120 min (Figure 5).

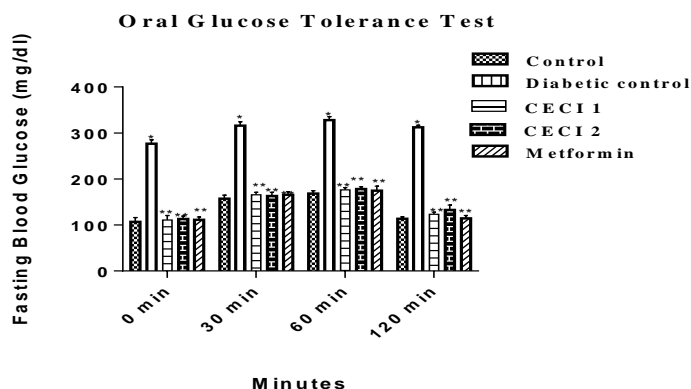


Figure 5: Effect of CECI on blood glucose level in rats in OGTT (Mean ±S.E.M, n=6):

* indicates value is significant at $p < 0.05$ when STZ induced diabetic control is compared with normal control; ** indicates value is significant at $p < 0.05$ when treated group is compared with STZ induced diabetic control.

STZ at the dose of 65 mg/kg produced marked hyperglycemia as evident from a significant ($P < 0.05$) elevation in fasting blood glucose level in the STZ control group as compared to the normal control group. Progressive decrease in blood glucose level was found in all three treatment group during study. Administration of CECI in STZ-induced diabetic rats at the doses of 200 and 400 mg/kg b.w. produced a significant ($P < 0.05$) and dose-dependent decrease in

blood glucose levels when compared with the STZ control group (Figure 6).

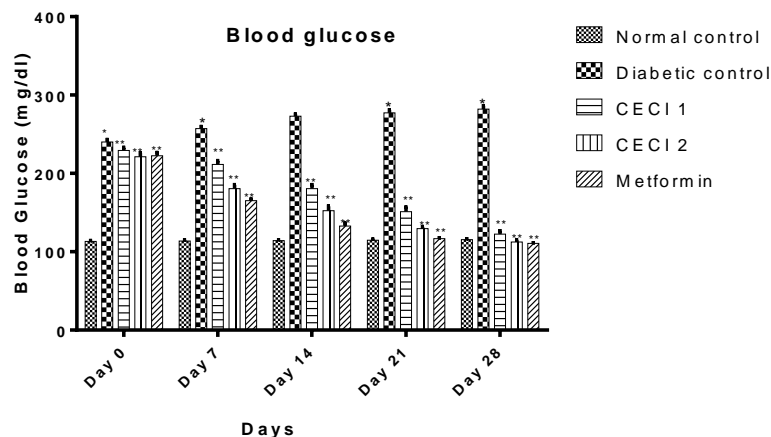


Figure 6: Effect of CECI on blood glucose level in rats (Mean ±S.E.M, n=6):

* indicates value is significant at $p < 0.05$ when STZ induced diabetic control is compared with normal control; ** indicates value is significant at $p < 0.05$ when treated group is compared with STZ induced diabetic control.

Body weight of animals in all groups was recorded at 0, 7th, 14th, 21st and 28th day of studies. The change in % body weight was also mentioned. CECI at 200 mg/kg and 400 mg/kg b.w. significantly ($p < 0.05$) increased body weight towards normal levels in a dose-dependent manner compared to the STZ control group. Data of present study shows that treatment with CECI prevents loss of body weight in diabetic condition but statistically it was not significant when compared with normal untreated groups and was significant when compared with control diabetic group (Figure 7).

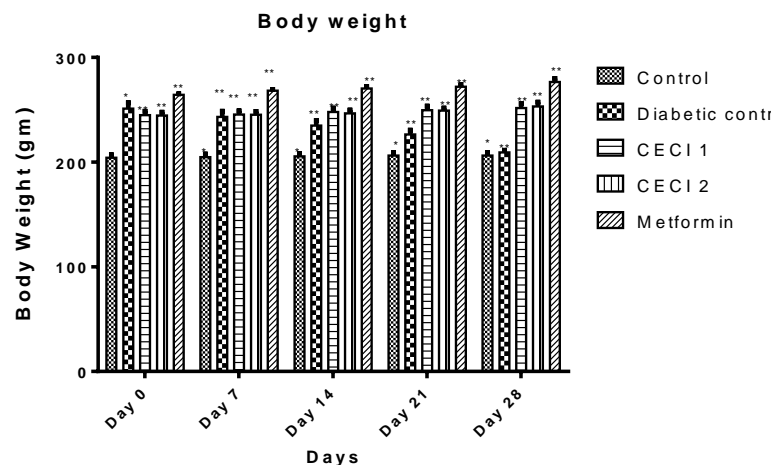


Figure 7: Effect of CECI on body weight in rats (g) (Mean ± SEM, n=6):

* indicates value is significant at $p < 0.05$ when STZ induced diabetic control is compared with normal control; ** indicates value is significant at $p < 0.05$ when treated group is compared with STZ induced diabetic control.

In diabetic control group, there was marked increase in total cholesterol, LDL cholesterol, and TG, while significant decrease in HDL cholesterol level was found. Treatment with CECl at the doses of 200 and 400 mg/kg b.w. significantly ($P < 0.05$) decreased total cholesterol, triglyceride, and LDL cholesterol. In 200 mg/kg of CECl and 400 mg/kg of CECl treatment group, HDL cholesterol increased significantly ($P < 0.05$) (Figure 8).

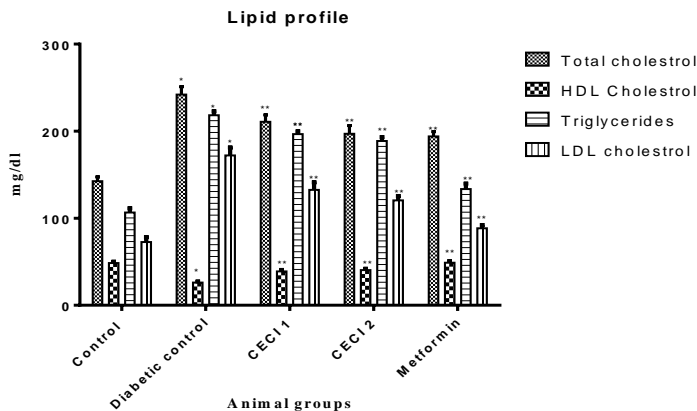


Figure 8: Effect of CECl on serum lipid profiles in rats (Mean \pm S.E.M, n=6):

* indicates value is significant at $p < 0.05$ when STZ induced diabetic control is compared with normal control; ** indicates value is significant at $p < 0.05$ when treated group is compared with STZ induced diabetic control.

4. Discussion:

In the mid -1960s streptozotocin was found to be selectively toxic to the β -cells of the pancreatic islets, the cells that normally regulate blood glucose levels by producing the hormone insulin. This suggested the drug's use as an animal model of type I diabetes. It is well established that biguanides like metformin produce hypoglycemia by increasing the secretion of insulin from the pancreas and these compounds are active in mild Streptozotocin-induced diabetes whereas they are inactive in intense streptozotocin diabetes (nearly all β -cells have been destroyed). However, since our results showed that metformin reduced the blood glucose levels in hyperglycemic rats, the state of diabetes is not severe. Streptozotocin-treated animals receiving the chloroform extract of *C. infortunatum* showed rapid normalization of blood glucose levels in comparison to the control and this could be due to the possibility that some β -cells are still surviving to exert their insulin releasing effect. No histological studies were carried out to prove this and it is not possible to explain the detailed mechanism of antidiabetic action of the chloroform extract of *C. infortunatum*. Alpha-glucosidase is one of the glucosidases located in the brush-border surface membrane of intestinal cells, and is a key enzyme in carbohydrate digestion (Lebovitz, 1997). The main benefits attributable to glucosidase

inhibitors are reductions in the postprandial and total range of glucose levels. These results of *in-vitro* studies related to α -glucosidase inhibition assay suggest that CECl may interfere with transit, digestion or absorption of sugar in the gastrointestinal tract (Figure 4). Furthermore, CECl markedly inhibited increase in plasma glucose levels.

The active principles in the *C. infortunatum*, which may be responsible for the hypoglycemic and hypolipidemic actions, are unknown. This may be due to the presence of hypoglycemic steroids and triterpenoids. Other probable mechanisms by which the extracts of *C. infortunatum* lowered blood glucose levels in diabetic rats might be by increasing glycogenesis, inhibiting gluconeogenesis in the liver, or inhibiting the absorption of glucose from the intestine or these might have improved insulin resistance. Further experiments are needed to determine the actual mechanism of action of the active constituents of the relative plant extracts. It is considered (Lebovitz, 2001) that postprandial hyperglycemia contributes significantly to the development of the chronic complications of type-2 diabetes. The plant may help to prevent or delay the onset of the complications caused by diabetes, such as increased levels of total cholesterol, triglycerides and LDL. In the present study, there was a significant reduction in the levels of total cholesterol and triglycerides. Further investigations are warranted to identify the hypolipidemic mechanism of the active principles in *C. infortunatum*.

5. Conclusion:

Plant medicines (phytotherapies) have a long history as treatment for diabetes. With a disturbing rise in the prevalence of this metabolic disease and associated healthcare costs, interest in alternative or complementary therapies has grown. In the present research work, attempts were made to study the probable antidiabetic activity of leaf extract of *C. infortunatum* Linn. Work has clearly proved that the chloroform extract have considerable antidiabetic and hypolipidemic activity. In qualitative screening of extracts, chloroform extract was found to contain steroids and triterpenoids. Thus, the pharmacological activities of chloroform extract of leaves of CI may be due to the presence of steroids, triterpenoids. Intraperitoneal administration of STZ produced cardinal symptoms such as hyperglycemia, loss of body weight. In conclusion, our data suggest chloroform extract of leaves of CI possess potential antidiabetic activity as it lowers serum glucose level and significantly increases glucose tolerance. *Clerodendrum infortunatum* also possess significant antihyperlipidemic activity as it lowers serum cholesterol, LDL cholesterol and triglycerides levels and

increase HDL cholesterol level. Present study indicated that 400 mg/kg of CECI was more effective than 200 mg/kg CECI for antidiabetic activity. Overall the work presents that chloroform extract of CI as a new formulation for achieving an antidiabetic activity and hypolipidemic agent. Hence it might help in preventing disease complications and may serve as a good alternative in the present armamentarium of antidiabetic drugs.

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