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RESEARCH ARTICLE

Phytochemical screening and evaluation of antihyperglycemic and antihyperlipidimic activity of methanolic extracts of calophyllum inophyllum on Albino Wistar Rats

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Abstract

Calophyllum inophyllum L. has been used as folk medicine in the treatment of ocular burn and it has demonstrated potential to be an anti-diabetic agent. The aim of the study is to perform preliminary phytochemical screening, and to evaluate and compare the anti-inflammatory effect of methanolic extracts of leaf and stem bark of Calophyllum inophyllum. Calophyllum inophyllum leaves and stem bark were extracted using methanol as solvent by Soxhlet apparatus. The percentage yield of CILE was found to be 16.27% and percentage yield of CISBE was found to be 17.62%. Preliminary phytochemical screening revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, proteins, amino acids and steroids. Doses up to 2000mg/kg were found to be safe after acute toxicity tests. The results for alloxan induced diabetes in albino wistar rats depicted significant antihperglycemic and antihyperlipidimic activity .

Introduction

Diabetes is one of the most prevalence chronic diseases in the world. This is a chronic incurable condition due to insulin deficiency that affects 10% of the population. The number of diabetic people is expected to rise from present estimate of 150 million to 230 million in 2025. For a long time, diabetes has been treated with several medicinal plants or their extract based on the folklore medicine. [1].Hence there is ongoing research to develop safer and more effective herbal drugs for diabetic treatment. Calophyllum inophyllum Linn. (Guttiferae) is a medium to large tree distributed throughout Taiwan, India, and Australia. The active constituents of C. inophyllum is well known for containing xanthone, flavone, and terpene derivatives, some of which exhibit antitumor, and anti-HIV activities[2]. The oil of C. inophyllum enhanced the healing of Ocular burn . The present study was undertaken to evaluate the antihyperglycemic and antihyperlipidimic activity of leaf and Stem bark of C. inophyllum.

Materials and methods

Plant Material: The plant Calophyllum inophyllum was collected from "Sri Kotla Vijaybhaskar Reddy Botanical Garden", Hyderabad, India. The plant was identified by a taxonomist and voucher specimens representing Calophyllum inophyllum was deposited at the Department of Biology, Osmania University, Hyderabad, India.

Preperation of plant extracts :

Leaves and stem bark of Calophyllum inophyllum were washed carefully to make them free from dust and foreign material. Then they were dried under shade at room temperature. After seven days of drying, the leaves and stem bark were powdered by grinding and passed through a sieve. The powdered leaves and stem bark of Calophyllum inophyllum were stored in air tight container for further use.

Leaf Extraction

Leaf powder (60g) was subjected to Soxhlet extraction for 24 hours continuous extraction using pet ether. After the pet ether extraction was completed, the leaf powder was subjected to further extraction using methanol as solvent for 24 hours. The plant material to solvents ratio (pet ether and ethanol) were taken as 1:5. Extract was subjected to dried heat treatment on a hot plate and the final product was obtained. Percentage yield of the semisolid material obtained was calculated and was stored at 4^{o}C.

Stem Bark Extraction

Stem bark powder (123g) was subjected to Soxhlet extraction for 24 hours continuous extraction using methanol. The plant material and the solvent were taken in the ratio 1:5. Extract was subjected to dried heat treatment on a hot plate and the final product was obtained. Percentage yield of the semisolid material obtained was calculated and was stored at 4^{o}C. The methanol extracts were concentrated in a rotary evaporator. The concentrated methanol extracts were used for antdiabetic and antihyperlipidimic activity.

PHYTOCHEMICAL SCREENING

Phytochemical investigation of methanolic extracts of leaf and stem bark revealed the presence of flavonoids, alkaloids, glycosides, carbohydrates, tannins, steroids proteins and amino acids as major chemical constituents. Flavonoids isolated from different medicinal plants have been shown to possess anti-oxidant and anti-inflammatory activities and analgesic activities. These compounds may be responsible for the antihyperglycemic and antiglycemic acitivity.

Phyto-constituents	**CILE**		**CISBE**
	Petroleum Ether	**Ethanol**	**Ethanol**
Alkaloids	-	+	+
Carbohydrates	+	+	+
Glycosides	-	+	+
Saponins	+	+	+
Tannins	-	+	+
Flavonoids	-	+	+
Proteins	+	+	+
Amino Acids	+	+	+
Steroids	+	+	+

Animals :

The healthy Adult Wistar albino rats of either sex, weighing 150-250g, were used for present investigation. Animals were housed under standard environmental conditions of temperature and humidity (25±2oC) and 12h light/dark cycle were utilized for studies. Rats were fed with standard pellet diet and water ad libitum

Acute oral toxicity studies

For toxicity studies, six Albino rats of either sex were administered orally with the test substance in the range of doses 5-2000mg/kg and the mortality rates were observed after 72h. The ethanol extracts of leaf and bark of calophyllum ionophyllum had shown no mortality at 2000 mg/kg. Therefore 2000mg/kg dose was considered as LD50 cut off dose (safe dose).

Hypoglycemic activity:

Albino rats of male weighing between 180-220 gms was categorized into five groups, each group consisting of 6 animals.

Group 1: Normal control (0.9% saline 10ml/kg)
Group 2: Control Glucose 2gm/kg
Group 3: Standard (glibenclamide 10mg/kg, p.o)
Group 4: Methanolic extract of Calophyllum ionophyllum leaf CILE (Low dose)
Group 5 : Methanolic extract of calophyllum ionophyllum leaf CILE(High dose)
Group 6: Methanolic extract of Calophyllum ionophyllum stem bark CISBE (low dose).
Group 7 : Methanolic exract of Calophyllum ionophyllum stem bark CISBE(high dose)

The oral glucose tolerance test was performed in overnight fasted (18 h) normal animals. Rats divided into 7 groups (n = 6) were administered vehicle, extract 1, extract 2 and glibenclamide (10 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the tail vein punture at 0, 30, 60, 90 and 120 min of extract administration. The fasting blood glucose levels were estimated by glucose oxidase–peroxidase reactive strips.

ALLOXAN INDUCED DIABETIC MODEL

Experimental induction of Diabetes in rats

The selected diabetic animals were divided into seven groups (n= 6) (Ghosh M.N, 2005)and one more group of normal non-alloxanised animals (Normal group) was also added to the study. Group 1 was kept as normal control (non-alloxanised rats) received only distilled water; Group 2 was kept as negative control, alloxan 150mg/kg induced and received only distilled water; Group 3,treated with glibenclamide 10mg/kg considered as standard 4, 5, 6 7 are diabetic induced and treated with 200mg/kg, 400mg/kg b.w. of leaf and stem bark methanolic extract respectively;. The treatment was continued for 14 consecutive days (p.o) at the end of 14 thday, the rats were fasted for 16h and blood glucose level was determined. The determination of blood glucose levels is done by tail tipping method using Accuchek-sensor glucometer .

STATISTICAL ANALYSIS

The values are expressed as mean ± SEM. $P<0.05$ was considered statistically significant and $P<0.01$ was considered statistically highly significant. Data obtained was analyzed by one-way ANOVA test (parametric ANOVA) followed by Dunnett's multiple comparisons post-hoc test using Graph pad Instat version 3.05, 32 bit for windows.

6. RESULTS

In the present study, the antihyperglycemic and antihyperlipidimic activity of methanol extracts of leaf and stem bark of calophyllum ionophyllum were assayed in Albino rats using alloxan induced diabetes method.

Effect of methanolic extract of Calophyllum ionophyllumon serum glucose level in alloxan induced diabetic rats.

In an alloxan induced diabetic rats (Control group) serum glucose level has significantly increased ($p<0.001$) in diabetic control rats when compared to normal groups.
Administration of CILE and CISBE 200 and 400 mg/kg and glibenclamide 10 mg/kg orally for 14 days treatment were reduced significantly serum glucose level on 7 days ($p<0.01$, $p<0.05$) and 14 days ($p<0.001$, $p<0.001$) as compared to control groups.

Effect of Methanolic extract of serum lipid profile level in alloxan induced diabetic rats.

In alloxan induced diabetic rats serum lipid profile such as total cholesterol, triglyceridesLDL (lowdensity lipids) VLDL (very low density lipids) levels were observed significantly increased ($p<0.001$) and HDL level in diabetic control group were seen significantly decreased ($p<0.001$) as compared to normal group. Administration of extracts of CILE and CISBE 200mg/kg 400mg/kg and glibenclamide 10 mg/kg on serum lipid profile.A decrease in the serum triglycerides, ($p<0.01$, $p<0.001$), total cholesterol ($p<0.001$), LDL($p<0.001$), and VLDL (very low density lipids) levels ($p<0.01$, $p<0.001$), and an increase in the HDL (high density lipids) cholesterol levels ($p<0.01$, $p<0.05$) were observed as compared to diabetic control group.

Table no.1: Effect of methanolic extract of *Calophyllum ionophyllum* on OGTT in rats.

Treated groups	Fasting blood glucose level			
	0 min	**30 min**	**60 min**	**120 min**
Normal	84.17±6.118	96.50±9.161	98.17±3.91	82.83±5.52
Control (2gm/kg glucose)	93.00±4.597	117.5±7.606	167.8±4.45a	142.7±6.27a
Standard (Glibenclamide 10mg/kg)	146.3±6.984	80.83±13.58	127.0±2.25***	77.50±4.55***
CILE (200mg/kg)	118.3±3.602	126.5±15.77	150.7±2.49*	96.67±5.57***
CILE (400mg/kg)	156.5±3.828	107.0±11.59	142.3±7.42**	73.17±4.68***
CISBE (200mg/kg)	112.2±3.206	121.5±15.77	141.1±2.49*	88.87±3.48***
CISBE (400mg/kg)	150.8±3.792	101.2±10.35	136.5±7.42**	67.39±3.88***

All the values are mean ± SEM,n=6, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test,*p<0.05,**p<0.01,***p< 0.001,as control group and ^{a}p<0.001, as compared normal

Table no.2 Effect of methanolic extract of *Calophyllum ionophyllum* on serum glucose level in alloxan induced diabetic rats

Treated groups	Serum glucose level		
	1st Day	7th Day	14th Day
Normal	78.61±2.762	86.83±1.74	85.04±5.05
Control (Alloxan 150mg/kg)	270.8±4.915	261.7±7.52a	239.7±9.04a
Standard (Glibenclamide 10mg/kg)	280.2±6.539	197.8±6.72**	158.7±5.46***
CILE (200mg/kg)	274.5±8.363	218.7±5.06*	203.7±5.66**
CILE (400mg/kg)	276.9±6.222	199.4±14.02*	182.4±9.01***
CISBE (200mg/kg)	268.3±7.477	212.3±4.01*	198.5±4.82**
CISBE (400mg/kg)	270.7±5.475	192.8±13.68*	177.2±8.03***

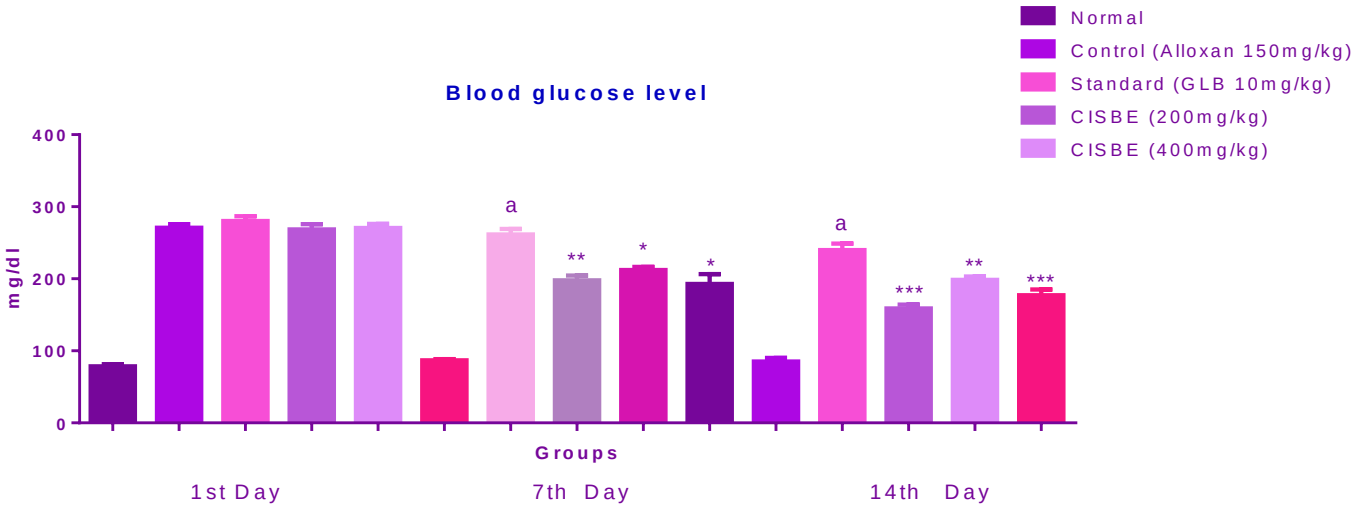
All the values are mean ± SEM ,n=6, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test,*****p<0.05**,****p<0.01,********p*< 0.001,as control group and [a]*p*<0.001, as compared to normal.

Table no 3 :Effect of methanolic extract of *Calophyllum ionophyllum* on serum lipid profile in alloxan induced diabetic rats.

TREATED GROUPS	TOTAL (mg/dl) CHOLESTEROL	HDL (mg/dl)	LDL(mg/dl)	TRIGLYCERIDES (mg/dl)	VLDL (mg/dl)
Normal	67.31±3.57	60.71±1.97	4.78±2.03	16.60±1.67	3.31±0.33
Control (Alloxan 150mg/kg)	128.4±3.18a	39.74±4.95a	81.80±6.94a	34.21±1.15a	6.84±0.23a
Standard(Glibenclamide 10mg/kg)	55.89±3.74***	52.48±1.79**	5.24±1.46***	7.48±1.19***	1.496±0.23***
CILE (200mg/kg)	66.03±4.11***	46.22±1.00*	6.99±2.99***	31.47±1.99**	6.09±0.29**
***CILE* (400mg/kg)**	61.66±1.99***	47.11±1.00*	8.23±2.94***	7.66±1.99***	1.89±0.99***
CISBE (200mg/kg)	62.09±3.31***	50.00±1.42*	6.65±2.23***	27.77±1.38**	5.49±0.27**
***CISBE* (400mg/kg)**	59.30±1.30***	50.86±1.44*	7.11±1.74***	6.59±1.09***	1.31±0.21***

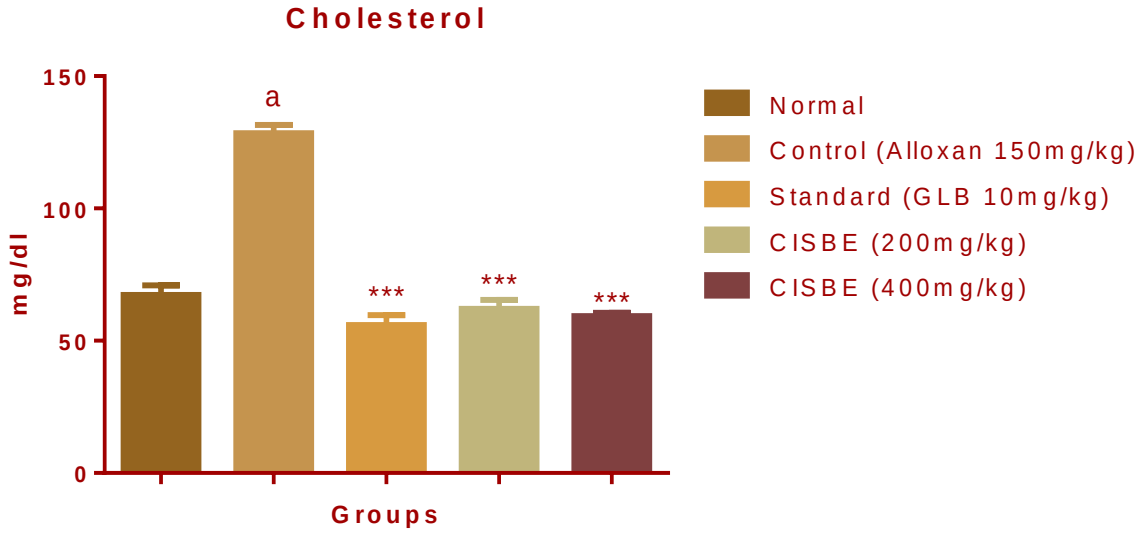
All the values are mean ± SEM ,n=6, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test,*****p<0.05, **p<0.01**, *****p< 0.001 vs. control group and [a]p<0.001, vsnormal group.

Graph no 1: Effect of methanolic extract of *Calophyllum ionophyllum* on serum glucose level in alloxan induced diabetic rats.



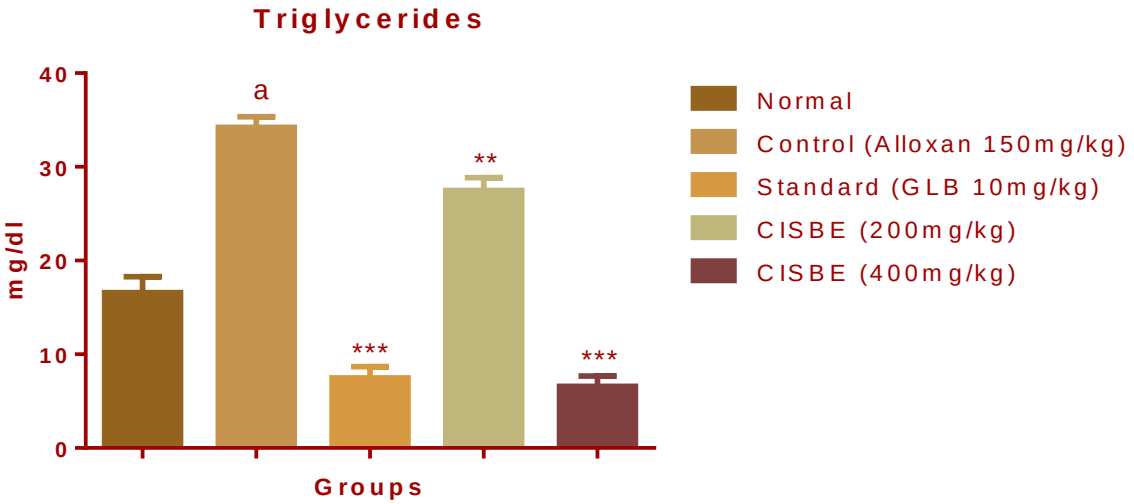
All the values are mean ± SEM , n=6, One way Analysis of Variance (ANOVA) followed by Dunett's multiple comparison test,*p<0.05,**p<0.01,***p< 0.001,as control group and ^{a}p<0.001, as compared to normal.

Graph.no. 2: Effect of methanolic extract of *Calophyllum ionophyllum* cholesterol in diabetic rats.



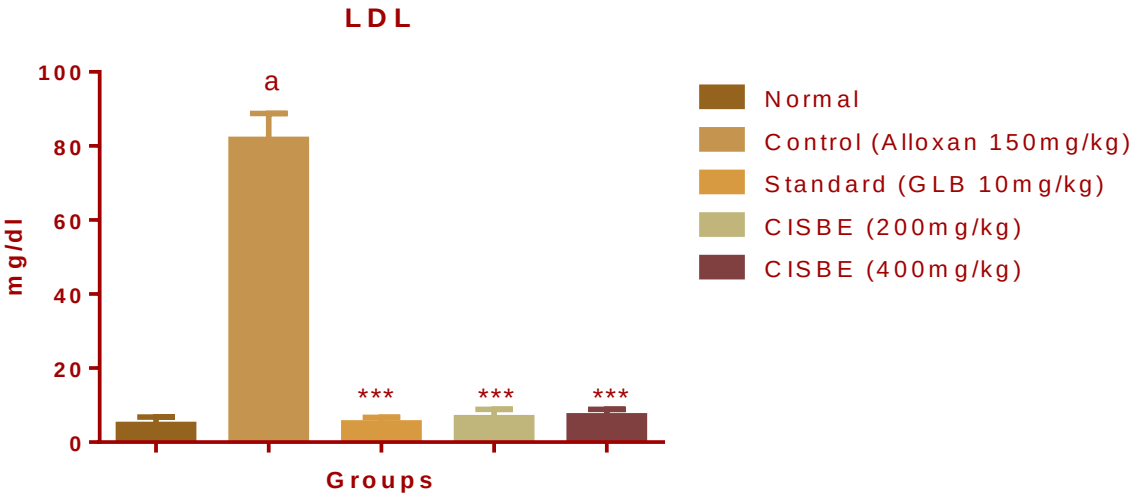
All the values are mean ± SEM ,n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, ***p< 0.001,as control group and ^{a}p<0.001, as comparednormal.

Graph.no. 3: Effect of methanolic extract of *Calophyllum ionophyllum on triglycerides* in alloxan induced diabetic rats.



All the values are mean ± SEM ,n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test,**p<0.01,***p< 0.001,as control group and [a]p<0.001, as comparednormal.

Graph.no.4: Effect of methanolic extract of *Calophyllum ionophyllum* LDL in alloxan induced diabetic rats.



All the values are mean ± SEM ,n=6, One way Analysis of Variance (ANOVA) followed by Dunetts multiple comparison test, ***p< 0.001,as control group and [a]p<0.001, as comparednormal.

DISCUSSION

Antihyperglycemic and antihyperlipidimic activity of of methanol extracts of leaf and stem bark of *Calophyllum inophyllum* were investigated in alloxan induced diabetes albino wistar rats. It is known that alloxan monohydrate[16] induces diabetes mellitus in rats by selective necrotic action on the beta cells of pancreas leading to insulin deficiency. Insulin deficiency leads to various metabolic aberrations in animals like increased blood glucose level, increased levels of cholesterol and triglyceride .As expected in alloxan treated rats, there was significant increase in blood glucose, cholesterol (CHL) and triglyceride (TGL) levels. The diabetic animals showed significant decrease in blood glucose level after 14 days treatment.This study showed that the methanolic extracts of leaf and stem bark of *Calophyllum inophyllum* possess antidiabetic and antihyperlipidimic activities. In addition,methanol extracts of bark showed more antihyperglycemic activity compared to methanol extracts of stem bark. More of the active principle component(s) responsible for the antihyperglycemic activity might be present in higher concentration in the stem bark extract.

CONCLUSION :

We can conclude from the above results that , ethanol leaf and stem bark extracts of *Calophyllum inophyllum* does possess significant antihyperglycemic and antihyperlipidimic activity with high doses of the extracts being more active. In addition, the stem bark extract of *Calophyllum inophyllum* shows significantly antihyperglycemic and antihyperlipidimic activity compared to methanolic leaf extract. The results, thus, might support the use of the plant for antidiabetic activity.

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