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Anti-hyperglycemic & Anti-hyperlipidemic Activity of Leaves of *Centella asiatica* Linn. in Diabetic Rats

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ABSTRACT

Aim- The main aim of the study is to evaluate the Antidiabetic and antihyperlipidemic activity of *Centella asiatica* in diabetic animals. **Material & Methods-** Different extracts were prepared by successive solvent extraction methods. Diabetes was induced by single injection of STZ in normal animals and diabetes was confirmed by glucose oxidation methods. The treatments of different extracts were given from third day to 21st day and at the end of 22nd day, blood sample was withdrawn and different lipid level was determined. **Result-** Among all the extracts, dichloromethane extracts showed significantly activity in reducing blood glucose level and decreased the VLDL, LDL, TC, Triglycerides and significantly increased the HDL-C level. **Conclusion-** The results obtained in this study have shown that dichloromethane extract shown significant Antidiabetic and antihyperlipidemic activity. Further detailed studies are required to isolate the active phytoconstituents by bioactivity guided isolation techniques responsible for anti-diabetic and hypolipidemic activity. The present findings are significant for the development of alternative, inexpensive and safer therapy for the treatment of diabetes mellitus and hyperlipidemic.

Keywords: Hyperlipidemia, STZ induced diabetes, *Centella asiatica*, VLDL-C, LDL-C

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INTRODUCTION

Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients in Traditional Systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs[1].

Herbal extracts produce diverse range of natural products including isoflavonoids, indoles, phytosterols, polysaccharides, sesquiterpenes, alkaloids, glucans and tannins which exhibit complex pharmacological properties. Therefore, contrary to the allopathic medicines, herbal therapy has emerged as a promising alternative with least toxicities and fewer complications. There is an over growing interest in investigating different medicinal plants in order to identify their potential therapeutic applications[2,3].

As per the literature review, it has been observed that *Centella asiatica* Linn. (Leaves) is listed among the various medicinal plants widely been used as a antibacterial, demulcent, bitter tonic, laxative, carminative, refrigerant, and febrifuge, diuretic, useful in chronic cystitis, gonorrhea and cadiotonic, acute-chronic inflammatory conditions and in treatment of diabetes mellitus, hyperlipidemic conditions, liver diseases and as a antiulcer[4].

In the absence of any scientific evidence for their anti-diabetic and antihyperlipidemic activity in animals, so there is a need in scientifically establishing the anti-diabetic and antihyperlipidemic activity so that we are able to come up with a more effective and potent extracts fractions or bioactive phytoconstituents with fewer side effects in comparison with existing synthetic drugs.

MATERIAL & METHODS

Collection and authentication of the plant leaves-

The fresh leaves of *Centella asiatica* Linn. was collected from outfield during the month of September that shows the green color with rough surface. The plant leaves were washed thoroughly in tap water, dried in shade, finely powdered and used for successive extraction methods. Plant was identified by Dr. Anurag Titov, Professor, Department of Botany, Govt. Madhav Sciences, PG College, Dewas Road, Ujjain and herbarium specimen was submitted in Department of Botany for future references.

Extraction Method

The extraction was done by following continuous or extraction procedure. Powdered material (stem bark) was packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80°C) for about 30-35 complete cycles. Defatted material was subjected to further extraction process by dichloromethane, methanol and water. All the extracts were concentrated under vacuum. The yield values and other physical properties were observed[5].

The % Yield of the Petroleum ether, chloroform, Methanol, & Aqueous extract of was calculated by using the following formula.

$$\% \text{ Yield} = \frac{\text{Weight of Extract (gm)}}{\text{Weight of powder drug Taken for extraction}} \times 100$$

Qualitative Phytochemical Screening

Preliminary phytochemical screening was performed for presence of fatty acids, steroids, terpenoids, alkaloids, flavonoids, phenolic compounds, glycosides etc in all the extracts[6,7].

Antidiabetic study of different extracts

Animals

Wistar Albino rats of either sex (150 to 200 g) were purchased from the CPCSEA approved vendor New Delhi. Commercial pellet diet (MFD, by Nav Maharashtra Chakan Oil Mills ltd., New Delhi, India) and water were provided *ad libitum* throughout the course of study. All the experimental trial was carried out in agreement with the CPCSEA guidelines. The study designs were permitted by the Institutional Animal Ethical Committee of Oriental College of Pharmacy and Research, Oriental University, Indore (MP), India.

Selection of Dose

Acute oral toxicity test was carried out according to the OECD guideline No. 423. Wistar Albino Rats were kept for overnight fasting prior to drug administration. A total of three animals were used, which received a single oral dose in 2000 mg/kg, body weight of different extracts. The animals were observed for a period of 24 hr for the changes in behavior, hypersensitivity reactions etc. Mortality, if any, was determined over a period of 2 weeks. Hence in our studies we selected 1/10 and 1/5th dose i.e. 200 and 400 mg/kg dose.

Preparation of Doses

Doses equivalent to 200 mg and 400 mg of the crude drug per kilogram body weight were calculated, and suspended in 1% w/v tween 80 solutions for the experiment.

Streptozotocin (STZ) induced diabetes in rats

After fasting 18 hours, the rats were injected intraperitoneal injection through tail vein with a single dose of 40 mg/kg Streptozocin (Sigma, St. Louis, Mo, USA), freshly dissolved in citrate buffer (pH 4.5). After injection, the rats had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock. Diabetes in rats was observed by moderate Polydipsia and marked Polyuria. The diabetes was confirmed by estimating the blood glucose level after 3 days by glucometer based on glucose oxidation method. Rats having blood glucose level more than 250 mg/dl were selected for further study[9]. In order to assess the anti-diabetic activity, the animals were divided in eleven groups of six animals in each group.

Group 1: Normal control, 0.9% NaCl-treated animals

Group 2: Diabetic control, STZ -treated rats (40 mg/kg body weight)

Group 3: Treated with Pet. Ether extract of leaves of *CA* (200 mg/kg body weight)

Group 4: Treated with Pet. Ether extract of leaves of *CA* (400 mg/kg body weight)

Group 5: Treated with dichloromethane extract of leaves of *CA* (200 mg/kg body weight)

Group 6: Treated with dichloromethane extract of leaves of *CA* (400 mg/kg body weight)

Group 7: Treated with methanolic extract of leaves of *CA* (200 mg/kg body weight)

Group 8: Treated with methanolic extract of leaves of *CA* (400 mg/kg body weight)

Group 9: Treated with aqueous extract of leaves of *CA* (200 mg/kg body weight)

Group 10: Treated with aqueous extract of leaves of *CA* (400 mg/kg body weight)

Group 11: Standard drug, Glibenclamide-treated rats (5 mg/kg body weight)

The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes.

Blood collection and glucose level estimations in serum

On 22nd day, fasting blood samples were collected from the tail vein of all the groups of rats. Whole blood was collected for estimation of blood glucose by using the glucometer (Easy Gluco, Morepen Laboratories Ltd.; New Delhi)[10].

Determination of various lipids

Then serum samples were also used to analyze for serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C)[11].

Statistical Analysis

Data were expressed as the mean ± standard error of mean (S.E.M.) of the means and statistical analysis was carried out employing one-way ANOVA. Differences between the data were considered significant at $P < 0.05$.

RESULTS

Phytochemical Screening

Phytochemical screening of different extracts showed the presence of different phytoconstituents.

Table 1: Preliminary Phytochemical test for different extracts of *Centella asiatica*

S.No.	Test	Petroleum ether	Dichloromethane	Methanol	Aqueous
1.	Carbohydrate Molish test Felling test	 - -	 - -	 + +	 + +
2.	Glycosides Bronteger test	 -	 -	 +	 +
3.	Alkaloid Mayer test Hager test	 - -	 + +	 + +	 - -
4.	Phytosterol + Triterpinoids Salkowaski test	 -	 +	 +	 -
5.	Protein + Amino acid Biuret test Ninhydrin test	 - -	 - -	 - -	 - -
6.	Phenolic test Ferric test Lead acetate test	 - -	 + +	 + +	 - -
7.	Flavonoids Alkaline test	 -	 -	 +	 +
8.	Saponin Foam test	 -	 -	 +	 +

Note: (+) *ve* indicates positive result, whereas (–) *ve* indicates negative result

Antidiabetic study of leaves of *Centella asiatica* Linn.

Effect on different extracts on Blood glucose level in diabetic rats

The induction of diabetes with streptozotocin increases the blood glucose level significantly (p<0.001) in group II rats as compared to normal rats. In 21 day study glibenclamide the standard drug restored the blood glucose highly significantly with the p<0.001 in 14 days whereas dichloromethane extract (200 & 400 mg/kg) reduced the glucose level moderately and highly significant with p<0.01 & p<0.001. Among all the extracts aqueous extracts didn't show any significant decrease in glucose levels. The results are shown in Table No 2.

Table 2: Effect of different extracts on glucose level in streptozotocin induced diabetic rats

Group No	Group	Blood Sugar level				
		Long Term Study (Days)				
		Before inducing Diabetes	3	7	14	21
I	Normal control	80.3 ± 3.22	81.4 ± 3.44	81.6 ± 2.47	82.9 ± 3.21	82.33 ± 2.55
II	Diabetic control	82.4 ±0.81	242.7 ± 3.33	274.2 ± 3.44***	269.3 ±3.37 ***	293.1 ± 4.31***
III	Pet. Ether extract (200 mg/kg)	80.4 ± 3.32	242.6 ± 3.56	237.2 ± 3.33**	235.3± 3.44**	225.6 ± 3.20**
IV	Pet. Ether extract (400 mg/kg)	83.77 ± 2.58	242.4 ± 3.74	224.3 ± 2.45**	216.3± 3.89**	205.4 ± 3.44**
V	DCM extract (200 mg/kg)	82.33 ± 3.39	243.4 ± 3.45	218.4 ± 2.32***	204.8±3.58***	197.5± 3.55***
VI	DCM extract (400 mg/kg)	81.33 ± 3.39	244.6 ± 3.44	206.3 ± 3.44***	193.6±3.88***	176.3± 2.82***
VII	Methanolic extract (200 mg/kg)	81.4 ± 2.33	244.6 ± 3.41	223.9 ± 3.77	217.2± 2.44**	214.6 ± 2.80**
VIII	Methanolic extract (400 mg/kg)	80.6 ± 3.44	242.6 ± 3.41	221.2 ± 3.99	212.8± 2.49**	209.2 ± 3.44**
IX	Aqueous extract (200 mg/kg)	82.4 ±2.93	240.7 ± 2.33	271.8 ± 2.35	271.3 ±3.66	283.1 ± 4.34
X	Aqueous extract (400 mg/kg)	82.1 ±3.81	241.7 ± 3.44	268.2± 4.33	265.3 ±3.15	262.1 ± 3.45
XI	Glibernclamide (5 mg/kg)	81.25 ± 3.44	242.1 ± 2.88	197.4 ± 3.51**	168.3± 2.19***	158.2± 3.99***

*Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups*

Effect of different extracts on lipid level

Untreated diabetic rats showed significant hyper-cholesterolemia, hyper triglyceridemia, elevated LDL-Cholesterol, VLDL-Cholesterol and decrease in HDL – Cholesterol in comparison to that of normal group. Dichloromethane or DCM extract of leaves showed a very good effect on lipid profile. It showed highly significant (p<0.001) effect on lipid profile in comparison to that of diabetic group. Dichloromethane extract also showed a highly significant effect on various lipids and also increased HDL level as compared to disease group or diabetic animals.

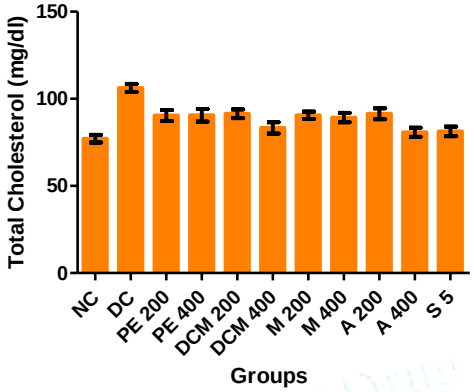

Figure 1: Effect of different extracts on Total Cholesterol level

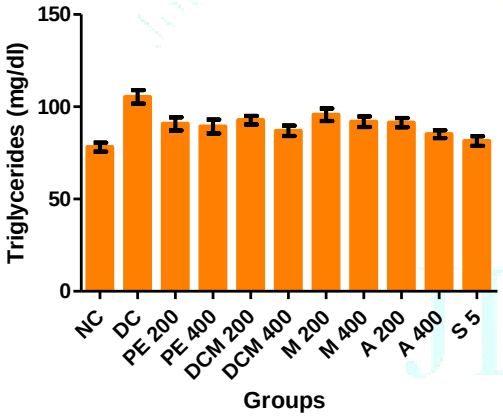

Figure 2: Effect of different extracts on Triglycerides level

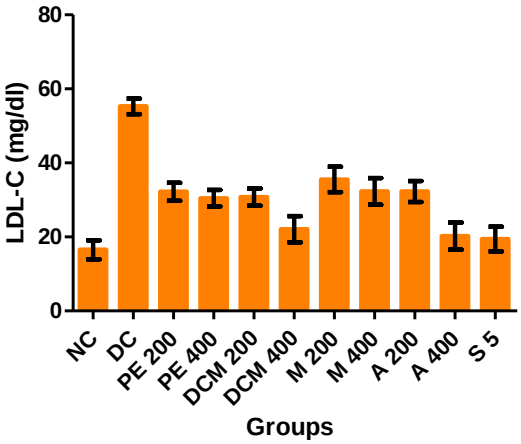

Figure 3: Effect of different extracts on LDL-C level

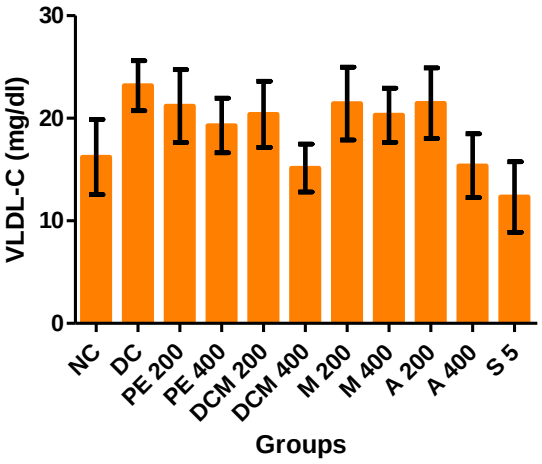

Figure 4: Effect of different extracts on VLDL-C level

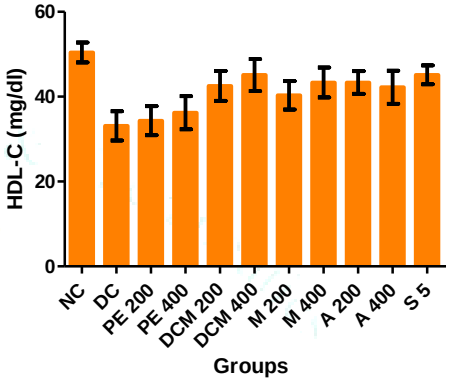

Figure 5: Effect of different extracts on HDL-C level

DISCUSSION

Preliminary phytochemical evaluation reported that existence of triterpenoids, steroids and fatty acids, saponins, phytosterols, flavonoids, phenols, alkaloids, and glycosides.

Toxicity study of a new compound must be done accurately for the selection of the dose, used for its pharmacological screening.

In this study, all the extracts at the dose of 2000mg/kg indexed neither visible signs of toxicity nor mortality and observations did not point out any proofs of substance related toxicity. Based on the LD50 value, 1/5th and 1/10th (200 & 400 mg/kg) of its value was chosen for pharmacological studies.

The islet β-cells are susceptible to damage caused by oxygen free radicals [12,13] since the antioxidant defense system is weak under diabetic condition. The levels of antioxidant defense system are altered in streptozotocin-induced diabetic rats, which are in good correlation with the present observation. Non protein thiols like glutathione are one of the important primary defenses that counteract the oxidative stress. Decreased levels of serum glutathione in streptozotocin diabetic rats, which is in consistent with earlier reports[14].

The dichloromethane extract of *Centella asiatica* produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin-diabetic rats after 21 days treatment. The Antidiabetic effect may be due to increased release of insulin from the existing β-cells of

pancreas similar to that observed after glibenclamide administration.

From the previous reported literature, Triterpenoids, flavonoids and phenolic compounds are responsible for anti-diabetic & hyperlipidemic effect. So probably, overall effect of plant may be due to presence of terpenoids in dichloromethane extract.

STZ-diabetic rats showed increase in plasma cholesterol and triglyceride concentrations[15] which may contribute to the development and progression of micro and macro-vascular complications It has been known that hyperlipidemia induced with hyperglycemia is an important determinant of cardiovascular mortality and is linked to diabetes mellitus. Hence, attenuation of hyperglycemia or glycation of lipoproteins, enzymes and receptors involved in lipid metabolism can decrease the risk of cardiovascular death in diabetic patients[16].

The marked hyperlipidemia that characterizes the diabetic state may, therefore, be regarded as a consequence of uninhibited actions of lipolytic hormones on the fat deposits. Studies on STZ-induced diabetes in experimental animals have suggested that an increase in circulatory VLDL and their associated triglycerides are largely due to defective clearance of these particles from the circulation.

Normally circulating LDL-C undergoes reuptake in the liver via specific receptors and gets cleared from the circulation (Lusis, 2000). HDL-C is protective by reversing cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogenic effects of oxidized LDL-C.

In diabetic rats treated with dichloromethane extract showed an elevation in HDL-C and reduction in LDL-C and VLDL-C.

CONCLUSION

The results obtained in this study have shown that dichloromethane extract shown significant Antidiabetic and antihyperlipidemic activity. Further detailed studies are required to isolate the active phytoconstituents by bioactivity guided isolation techniques responsible for anti-diabetic and hypolipidemic activity. The present findings are significant for the development of alternative, inexpensive and safer therapy for the treatment of diabetes mellitus and hyperlipidemic.

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