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EVALUATION OF ANTI-DIABETIC AND ANTI-HYPERLIPIDEMIC POTENTIAL OF ETHANOLIC EXTRACT OF *Calotropis procera* AGAINST STREPTOZOTOCIN INDUCED RATS.

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Abstract: The present study was aimed to evaluate the anti-diabetic and anti-hyperlipidemic activity potential of ethanolic extract of *Calotropis procera* against streptozotocin (STZ) induced male wistar rats. Ethanolic extract of *C. procera* was administered to male wistar rats. Glibenclamide was used as a standard drug. Blood glucose levels were determined on 0, 7th, 14th and 21st day after oral administration of a dose of different extract of *C. procera* (150, 300, 600 mg/kg b. wt) in diabetic groups. An ethanolic extract of *C. procera* was found to reduce blood sugar in streptozotocin induced diabetic rats. Reduction in blood sugar could be seen from 7th day after continuous administration of the extract. The effect of extracts of *C. procera* on serum lipid profile like total cholesterol, triglycerides (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and high density lipoprotein (HDL) were also measured in the diabetic and non-diabetic rats. There was significant reduction in total cholesterol, LDL cholesterol, VLDL cholesterol and improvement in HDL cholesterol in diabetic rats. These results indicated that *C. procera* possess an antidiabetic and antihyperlipidemic effect.

Keywords: Antidiabetic activity, Antihyperlipidemic, *Calotropis procera*, Streptozocin.

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INTRODUCTION

Diabetes mellitus is disease characterized by disordered metabolism and abnormally high blood sugar levels resulting from the body's inability to either produce or to use insulin properly [1]. Diabetes is occurs in several forms, approximately 10% of world population have diabetes mellitus type-I. It is an autoimmune disease that destroys insulin- producing β-cells in the pancreas leading to decrease in the concentration of insulin in the body and 90% world population have Diabetes mellitus type- II. Diabetes mellitus type -II is a metabolic disorder characterized by the progressive decreasing in insulin action [2]. The β cells normally compensate for insulin resistance by secreting the greater amount of insulin in order to maintain glucose homeostasis. In non-insulin dependent diabetes mellitus, this β cell function becomes impaired due to insulin resistance leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance [3, 4]. Diabetes mellitus is recognized as a major risk factor for cardiovascular diseases (CVD) such as atherosclerosis, heart attack, stroke, etc. About 75% of deaths among men with diabetes and 57% of deaths among women with diabetes are attributed to CVD [5]. Insulin and oral hypoglycemic agents are most widely used drugs for lowering blood sugar in diabetes but these drugs also have various side effects such as hypoglycemia, weight gain (sulfonylurea), lactic acidosis (biguanids). All of these drugs are responsible for liver and renal damage. Before the development of modern pharmaceutical treatments, doctors were completely dependent on the use of medicinal herbs for prevention and treatment of disease [6]. Ethnobotanical information also indicates that more than 900 medicinal plants are used as traditional drugs for the treatment of diabetes throughout the world [7]. Today still an unmet need for scientific proof of the hypoglycemic activity of medicinal plants and phytopharmaceuticals with a fewer or no side effects.

Calotropis procera (family Asclepiadaceae) commonly known as Aak is used in many Ayurvedic formulations like Arkelavana. The use of the best medicinal plants, its extracts, and pure active principles isolated from natural sources has always provided a foundation in modern pharmaceutical compounds [8, 9]. *Calotropis procera* is well known plant and has been traditionally used for diarrhoea, stomatic, diabetes, and skin disease, [10] and its leaves are for the treatment of jaundice. On the basis of many reports its usefulness, availability and literature survey no work has been carried out on the stem of this plant, which contains potentially useful ethno medicine. Therefore, the present work was undertaken to study the biochemical aspects of *Calotropis procera* and it's used in the hypoglycemic activity.

MATERIALS AND METHODS

Plant Material:

Calotropis procera plant was collected from Sikar (Rajasthan), with the help of field botanist. The plant was taxonomically authenticated by depositing voucher specimen of the plant (RUBL21092) has been deposited in the herbarium of the Botany Department at Rajasthan University, Jaipur. The whole plant was shade dried. It was preserved in a container and powdered as per requirements.

Preparations of its extracts:

The dried stem of plant was powdered using dry grinder and passed through sieve. About 150g of powder of stem was packed into soxhlet apparatus and extracted successively with petroleum ether, chloroform, and ethanol (yield 1.36%, 1.06%, 1.90%, respectively). The solvent was recovered by distillation under reduced pressure and extract was stored in desicators for the use of subsequent experiments [16].

Experimental animals:

Male wistar rats (150-180 g) were used to assess hypoglycemic activity. All animals were housed in standard laboratory conditions temperature (22.C± 2) and humidity (47±5) % with 12h day: 12h night cycle. The standard laboratory diet was provided to the animals and they were allowed to drink water ad libitum. Studies were carried out after the approval of Institutional Animal Ethical Committee in accordance with institutional ethical guidelines for the care of laboratory animals of Adina Institute of Pharmaceutical Science, Sagar, MP (approval no.1546/PO/E/S/11/CPCSEA).

Chemicals:

The estimation of biochemical parameters was carried out using commercially available kits (Primal Healthcare Limited, Lab Diagnostic Division, and Mumbai, India). STZ and other chemicals were procured from Himedia Laboratories, Mumbai, India.

Induction of Diabetes in rats:

STZ was dissolved in freshly prepared 0.1 M cold citrate buffer (pH 4.5) and administered by intraperitoneal route (45 mg/kg) to the overnight fasted rats 18. After 6h of STZ injection, rats were received 5% dextrose solution for the next 24h to prevent STZ induced fatal hypoglycemia as a result of releasing the massive pancreatic insulin after its administration. Diabetes was confirmed 72h after induction by measurement of tail vein blood glucose levels using glucose meter (Glucocard™ 01mini, Arkray Factory, Inc., Japan) by glucose oxidase-peroxidase method

using strips. Diabetic rats were kept 7 days under standard laboratory conditions for the stabilization of blood glucose levels 19. After 7 days induction of diabetes, blood glucose was again determined and animals with a blood glucose level greater than 240 mg/dL were selected for the study.

Phytochemical screening:

The preliminary phytochemical screening of the crude extract of *Calotropis procera* was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols [20].

Experimental design

The Streptozotocin-induced diabetic male wistar rats were randomly assigned into six groups (1-5) of five rats (n=6) each as Follows: viz.

Group 1 - Received normal saline 10 ml/kg of body weight orally

Group 2 - Diabetic control

Group 3 - Received glibenclamide 10 mg/kg of body weight orally

Group 4 - Received *C. procera* extract 300 mg/kg of body weight orally

Group 5 - Received *C. procera* extract 600 mg/kg of body weight orally

Determination of blood glucose levels:

Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination at intervals of 0, 7, 14 and 21 days by the glucose-oxidase principle using the one touch basic instrument and results were reported as mg/dl [21].

Biochemical parameters:

Triglycerides, cholesterol, HDL-cholesterol, and LDL-cholesterol were estimated from the serum by using standard kits [22-24].

Statistical analysis:

Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group. The values of $p<0.01$ were considered as significant [24]. The criterion for statistical significance was considered as P value <0.001. The difference between test and controls were evaluated by student's t-test.

RESULTS

Phytochemical analysis:

Freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents present in it. This revealed the presence of tannins, carbohydrate, saponins, flavonoids and alkaloids.

Antidiabetic effects:

Effect of ethanolic extract of *C. procera* on blood glucose levels in diabetic rats depicted in Fig-1. In animals treated with streptozotocin (45 mg/kg i.p) (Group II), a significant increase in blood glucose level was observed on 7th, 14th, 21st, and 28th day when compared with normal rats (Group I). Group III received glibenclamide (10 mg/kg p.o.) showed decrease in blood glucose level when compared with diabetic control rats. Group IV and V after the oral administration of ethanolic extract of *C. procera* in diabetic control rats, a significant reduction in blood glucose level was observed on the 7th, 14th, 21st, and 28th day compared with diabetic control rats.

Anti-hyperlipidaemic activity:

The lipid profiles in control and experimental rats are depicted in Table 2 in STZ induced diabetic rats. The diabetic control rats (Group II) showed significant increase in blood triglycerides, Total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) when compared with normal (Group I). Standard glibenclamide (Group III) also reduced triglycerides, Total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and increased high density lipoproteins (HDL) when compared with normal (Group I).The ethanolic extract showed significant decrease ($p<0.001$) in Total cholesterol, LDL, VLDL, Triglycerides and significant increase ($p<0.001$) in HDL when compared with diabetic control group (Group II). All these effects were observed on Day 14th, 21st, and 28th. The present experimental result indicated that ethanolic extracts exhibited antihyperlipidemic activity in STZ diabetic rats.

Fig.1.Effect of *C. procera* on reduction of blood glucose levels (mg/dl) in streptozotocin-induced diabetic male wister rats treated by various doses of ethanolic extract (sub acute study)

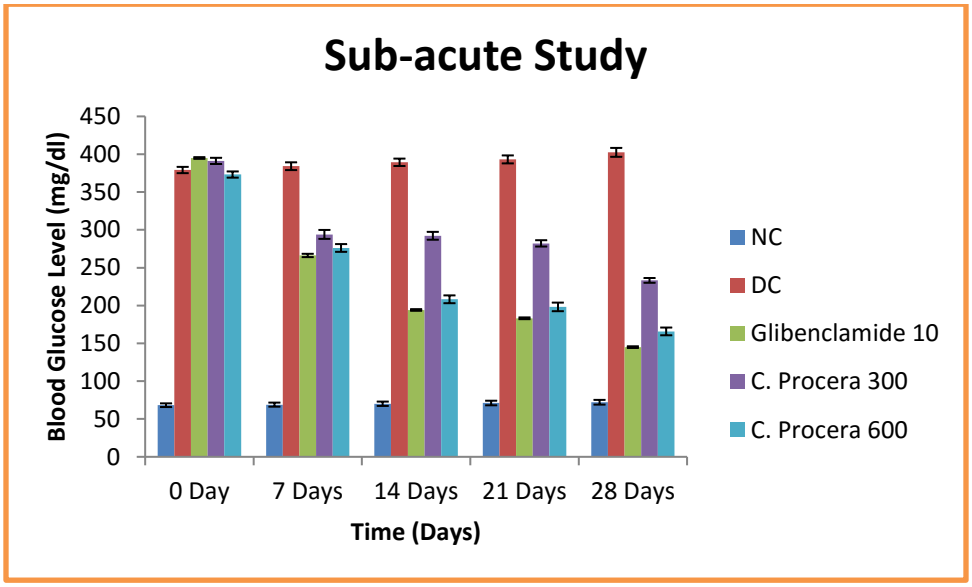
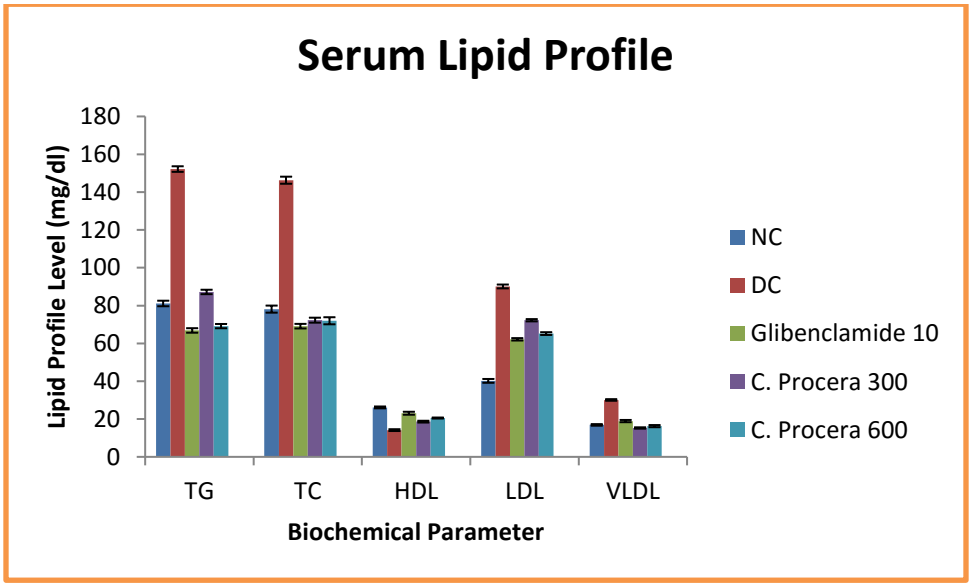


Fig.2.Various biochemical parameters of serum lipid profile (mg/dl) in streptozotocin induced diabetic rats after 28 Days treatment by various doses of ethanolic extract of *C. procera.*



DISCUSSION

Diabetes mellitus is one of the leading causes of death, illness and economic loss all over the world. Insulin-dependent (Type I, IDDM) diabetes is characterized by juvenile onset and by absolute insulin deficiency. Non-insulin-dependent (Type II, NIDDM) diabetes is characterized

by mature onset, by varying basal insulin levels and a frequent association with obesity. It was observed that the elevated blood glucose concentration accompanied by increase in Total cholesterol, Triglycerides, LDL,VLDL and decrease in HDL cholesterol in streptozotocin induced diabetic rats as compared to control animals. Oral administration of ethanolic extract of *C. procera* normalized the levels of blood glucose. The potent antidiabetic effect of the plant extract suggests that the presence of potent antidiabetic active principles, which produced antihyperglycemic effect in diabetic rats.

In recent years, considerable interest is being taken towards the investigation of plasma lipids and lipoproteins pattern in Diabetes mellitus due to the fact that abnormal lipid level leads to the development of coronary artery disease in diabetic patients [26]. Reduced insulin secretion and defect in insulin function results in enhanced metabolism of lipids from adipose tissue to the plasma. Impairment in insulin sensitivity due to high concentration of lipids in the cells is responsible for the elevated cardiovascular risk in Diabetes mellitus. Thus, the altered lipid and lipoprotein pattern observed in diabetic rats could be due to defective secretion of insulin and/or action. Accumulation of cholesterol and phospholipids in liver due to elevated plasma free fatty acids have been reported in diabetic rats.

In the present study, ethanolic extract of *C. procera* had significantly decreased Total Cholesterol, Triglycerides, VLDL, and LDL with increase in HDL which is having a protective function for the heart.

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REFERENCES

1. Babu V, Gangagadevi T, Subramaniam A: Diabetes induced by STZ in rats. Ind J of Pharmacol. 2003; 35: 290-96.

2. Bajpay A. Ecological Studies of Boerhaavia verticillata poir with special reference to phytochemical and therapeutic importance. Dissertation. Varanasi: Banaras Hindu University; 1993.167p.

3. Bhattaram VA, Ceraefe M, Kohlest C, Vest M, Deundorf H. Pharmacokinetics and bioavailability of herbal medicinal products. Phytomed. 2002; 9: 1-36.

4. Chopra RN, Ghosh S, Dey P, Ghosh BN. Pharmacology and therapeutics of Calotropis procera .Indian Medical Gazette. 1923; 68: 203-208.

5. Dhar ML, Dhar MM, Dhawan BN. Screening of Indian plants for biological activity. Indian Journal of Experimental Biology. 1968; 6: 232– 247.

6. EL-Hazmi MAF, Warsy AS. Obesity, over weight and type II diabetes in Saudi adult patients. Saudi Med J. 1999; 20: 167-172.

7. Frayn KN, editor. Insulin resistance, impaired postprandial lipid metabolism and abdominal obesity. Med Princ Pract. 2002; 11: 31-40.

8. Ghosh, MN. Fundamentals of Experimental pharmacology, 2nd ed. Scientific book agency, Kolkata 1994,159-158.

9. Granner DK, Granner RK, Mayes PA., Rodwell VW. Hormones of the Pancreas and Gastrointestinal Tract. In: Murray. Harper's Biochemistry, Appleton and Lange, Connecticut, USA, 1996; 24: 586587.

10. Hardman JG, Limberd LE. Insulin, Oral Hypoglycemic Agents and the Pharmacology of the endocrine pancreas. In Good- man and Gilman's: The Pharmacological basis of Therapeutics. Mcgraw-Hill Company Limited, USA, 2001; 10: 1383-1399.

11. Hou, Z, Zhang Z, Wu H. Effect of Sanguis draxonis (a Chinese traditional herb) on the formation of insulin resistance in rats. Diabetes Res. Clin. Pract. 2005; 68: 3-11.

12. Huang TH, Kota BP, Razmovski V, Roufogalis BD. Herbal or natural medicines as modulators of peroxisome proliferator-activated receptors and related nuclear receptors for therapy of metabolic syndrome. Basic Clin. Pharmacol. Toxicol.2005 ; 96: 3-14.

13. Lopes-Virella, Maria F, Stone P, Ellis S, Colwell JA. Cholesterol determination in HDLs separated by three different methods. Clin Chem. 1977; 23: 882-4.

14. Lowry OH, Rosenborough NT, Farr AL, Randall JR. Protein measurements with the folin phenol reagent. J Biol Chem. 1951; 193: 265-75.

15. Nandkaran KM.The Indian Materia Medica. 3 rd ed, Bombay Popular Prakashan, Mumbai 1996b,209.

16. Kokate, C.K., Practical Pharmacognosy, 1st ed, Vallabh Prakashan, New Delhi 1986b, 1: 1530.

17. Ramachandran S, Asok KK, Ravi TK. Investigation of antidiabetic, antihyperlipidemic, and in vivo antioxidant properties of Sphaeranthus indicus Linn. in type 1 diabetic rat: an identification of possible biomarkers. Evid Based Complement Alter. Med. 2011; 2011: 571-721.

18. Tanko Y, Yerima M, Mahdi MA, Yaro AH, Musa KY, Mohammed A. Hypoglycemic activity of methanolic stem bark of Adansonnia digitata extract on blood glucose levels of streptozotocin-induced diabetic Wistar rats. Int J Appl Res Nat Prod. 2008 ; 1: 32-36.

19. Trease GE., Evans MC. Textbook of Pharmacognosy, London Bailliere, Tindall, 1983, 683-684.

20. Beach EF, and Turner JJ. An enzymatic method for glucose determination uptake in body fluids. Clin Chem. 1958; 4:462-468.

21. Oliver-Bever B.Oral hypoglycemic action of medicinal plants in tropical West Africa. Cambridge University Press, London, 1986: 245-267.

22. Rai MK. A review on some antidiabetic plants of India. Ancient Science of Life, 1995; 14: 42-54.

23. Sarti C, Gallagher J. The metabolic syndrome: prevalence, CHD risk, and treatment. J Diabetes Complications. 2006 ; 20:121-132.

24. Duncan RC, Knapp RG, Miller MC. Test of hypothesis in population. In: Introductory Biostatistics for the health sciences. John Wiley and Sons Inc. NY. 1977, 71-96p

25. Scheen, JA. Drug treatment of non-insulin dependent diabetes mellitus in the 1990s. Achievement and future development drugs, 1997; 54: 355-368.

26. Shukla R, Sharma SB, Puri D, Pabhu KM, Murthy PS. Medicinal plants for treatment of diabetes mellitus. Indian J. Clinical. Biochem. 2006; 15: 169-177.