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Effect of Ethyl Acetate Leaf Extract of *Pterocarpus Santalinus* on Cholesterol and Triglycerides in Streptozotocin induced Diabetic Rats

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Abstract: *Pterocarpus santalinus* (PS) has been extensively used in traditional system of medicine for many diseases. The present study was directed at determining the variations in serum biochemical parameters after oral administration of *Pterocarpus santalinus* leaf ethyl acetate extract abbreviated as EE (500mg/kg b.wt) in streptozotocin (45mg/kg b.wt) induced diabetic rats. Diabetes was confirmed after 48 hours in rats showing blood glucose levels ≥ 250 mg/dl. The rats were randomly divided into five groups (n=6). Group 1 and 2 (normal and normal treated rats with plant extract), group 3 (Diabetic control), group 4 (diabetic rats fed with 500mg of PS EE), group 5 (diabetic rats fed with 20mg of glibenclamide). The oral administration of leaf extract and glibenclamide was continued for 21 days and then the animals were sacrificed and blood samples were collected for biochemical analysis. The results showed that the extract reduced serum cholesterol, triglycerides when compared to diabetic control. The HDL cholesterol and VLDL, LDL cholesterol levels are also controlled significantly ($P < 0.05$). From the above results it can be concluded that the extract has the ability to retain the altered biochemical parameters in diabetic rats like glibenclamide treated animals.

Keywords: *Pterocarpus santalinus*, Streptozotocin, Glibenclamide

I. INTRODUCTION

Diabetes mellitus is found in almost all Nations of the World, so it is called a global disease. Diabetes is characterized by a group of metabolic disorders including high blood sugar levels, diabetic ketoacidosis, and nonketotic hyper osmolar coma. Serious long term complications include cardiovascular diseases, kidney failure, foot ulcers and damage to eyes. The main reason for all these metabolic derangements in carbohydrates, proteins and fats is due to insufficient secretion or inefficient action of insulin (1). According to recent estimates the prevalence of diabetes is 4% worldwide and that indicates 143 million persons are affected which will increase to 300 million by the year 2025. The number of people with diabetes is expected to rise to 592 million by 2035. Thus diabetes is a potentially morbid condition with high prevalence world-wide being a major medical concern.

Plant based products have been popular all over the world from ancient times (2). Herbal remedies are widely used as they are effective and have minimal side effects. On the other hand there are 800 plants that are antidiabetic in nature which is known from literature (3) one of which is *Pterocarpus santalinus* that is widely available around southern part of India. There are various *Pterocarpus* species which are widely distributed throughout the world i.e. *Pterocarpus dalbergioides*, *Pterocarpus indicus*, *Pterocarpus marsupium* and *Pterocarpus santalinus* (4). It is a medium sized deciduous tree endemic to India with a very restricted range in the southern Eastern Ghats where it grows in dry, rocky ground at 150-900mts. The distribution of this tree in India is limited to several parts of which most can be found in Southern part of India (5). Ethno botanical reports shows that *P. santalinus* is being used to treat diabetes mellitus and related symptoms along with use of other diseases like skin infections, anthelmintic, aphrodisiac, alexiteric, vomiting, thirst, eye diseases, foot ulcers and blood diseases. The present study is aimed at studying the serum biochemical parameters including triglycerides, total cholesterol, HDL cholesterol, VLDL and LDL cholesterol.

II. MATERIALS AND METHODS

A. Collection of plant material

Leaf of *P. santalinus* was collected from the surroundings of Tirupati A. P., India. The leaves were dried in shade, powdered and the powder was used for the extraction process.

1) *Preparation of Extract:* The powdered plant material was extracted in soxhlet apparatus with ethyl acetate and then filtrate was concentrated in a rotary vacuum evaporator.

- 2) *Animal Model*: Male albino Wistar rats weighing 180-230 g were used for study. Rats were acclimatized to animal house conditions. The animals were fed with standard pellet diet and provided water ad libitum.
- 3) *Induction of Diabetes*: Diabetes was induced in 12hours fasted rats by single intraperitoneal injection with freshly prepared streptozotocin (50mg/kgbw) in ice cold citrate buffer (pH4.5) and fed with 5% glucose for 12hours(6). Fasting blood glucose levels were obtained on 2nd day after injection of STZ and FBG levels were assessed (Frode and Medeiros, 2008) by OneTouch Accu-check glucometer. Diabetic rats with FBG levels >250 mg/dl were selected for the study. Animal handling and their care was taken according to CPCSEA guidelines (1677/PO/Re/S/2012/CPCSEA/34)

B. Experimental Design

In the present study the animals were divided into five groups of six animals each, as given below.

Group1–Normal control rats

Group 2 –Normal rats treated with 500mg/kgb.wt of ethyl acetate extract

Group3 – Diabetic control rats

Group4 – Diabetic rats treated with 500mg/kgb.wt of ethyl acetate extract

Group5 - Diabetic rats + 20 mg glibenclamide/kg b.wt

After 21 days of treatment the rats were sacrificed by cervical dislocation, whole blood was collected into plain tubes via cardiac puncture and allowed to clot the blood. Serum was collected and stored for biochemical analysis

C. Biochemical Analysis

- 1) Estimation of cholesterol by Zaks method (7)
- 2) Estimation of Triglycerides (8)
- 3) Estimation of HDL cholesterol (9)
- 4) Calculation of VLDL and LDL cholesterol (10)

III. RESULTS

The results of cholesterol, triglycerides in normal rats are normal but in diabetic groups the values increased. The increased values were brought to near normal in plant extract and glibenclamide treated groups of rats as shown in table 1. The table 2 shows the levels of LDL, VLDL cholesterol in different groups of rats. The values in normal treated rats in both the tables showed no significant difference when compared to normal group of rats.

Table 1; Effect of PSEA on serum cholesterol levels in normal and STZ induced diabetic rats

Group	Triglycerides(mg/dl)	Total cholesterol(mg/dl)	HDL cholesterol
Normal control	84.16±5.7	114.6±6.68	48.66±11.05
Normal rats treated with PS EA extract(500mg/kg b.wt)	85.16±6.5	117.1±8.01	50.66±8.28
Diabetic control	141.83±7.46	217±6.65	31.33±3.07
Diabetic rats treated with PS EA extract(500mg/kg b.wt)	111.5±4.80**	137.5±13.01**	40.66±2.06**
Diabetic rats treated with Glb extract(20mg/kg b.wt)	102.33±4.13**	126.1±14.03*	44.66±2.16*

*P values less than 0.05; significantly deviated when compared with diabetic control group

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Figure 1; Effect of PSEA on triglycerides in normal and STZ induced diabetic rats

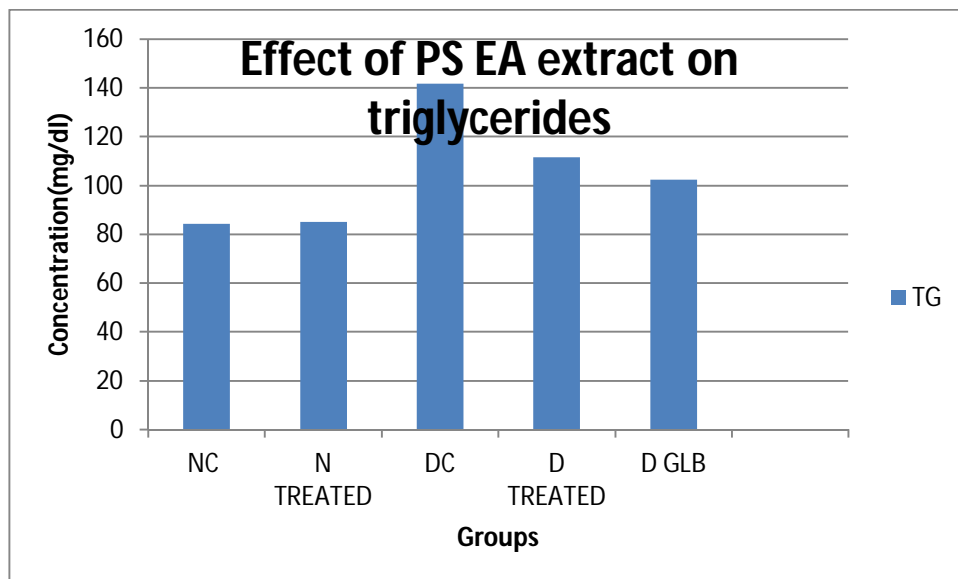


Figure 2; Effect of PSEA on total cholesterol in normal and STZ induced diabetic rats

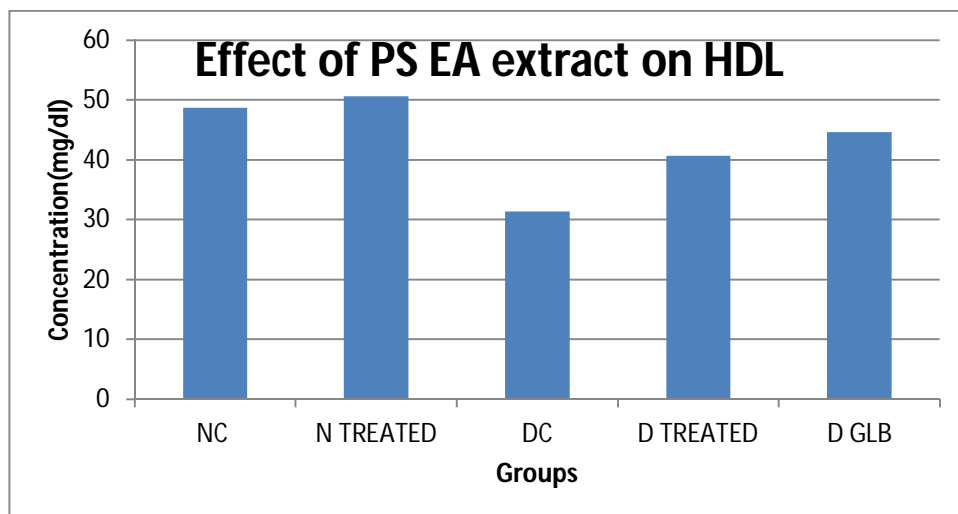
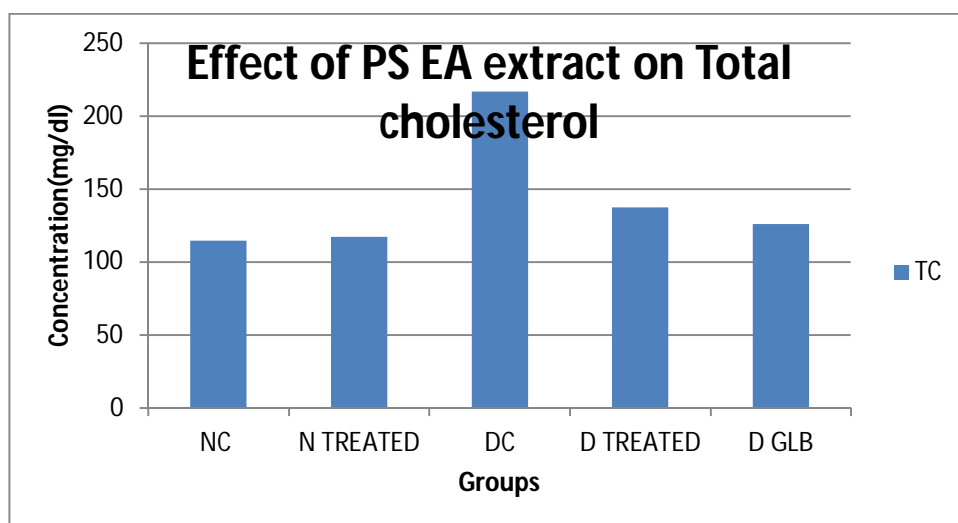


Figure 3; Effect of PSEA on HDL cholesterol in normal and STZ induced diabetic rats

Table 2; Effect of PS EA on VLDL and LDL cholesterol in normal and STZ induced diabetic rats

Group	VLDL cholesterol(mg/dl)	LDL cholesterol(mg/dl)
Normal control	16.832	49.108
Normal rats treated with PS EA extract(500mg/kg b.wt)	17.0	157.304
Diabetic control	28.3	157.304
Diabetic rats treated with PS EA extract(500mg/kg b.wt)	23.1	74.54
Diabetic rats treated with Glb extract(20mg/kg b.wt)	20.46	60.974

IV. DISCUSSION

Diabetes is a chronic disorder affecting major metabolisms in the body including mobilization of free fatty acids from adipose tissues. This results in the variations of normal levels of cholesterol, triglycerides, HDL, LDL and VLDL in diabetic rats. These altered values results in increased cardiovascular complications that results in mortality of diabetic individuals (11).The decrease in triglycerides and VLDL cholesterol indicates their utilization in synthesis of HDL cholesterol. The values in plant extract treated rats showed significant difference when compared to diabetic group of rats ($p < 0.05$). Similar results were observed in Glb treated rats and normal levels remain unchanged in PS EA treated normal rats.

V. CONCLUSION

The present study demonstrated the treatment of *Pterocarpus santalinus* extract showed the protecting activity on serum cholesterol levels. Further experiments are planned on identification and isolation of components which are capable of influencing cholesterol levels.

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