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Evaluation of Antidiabetic and Hypolipidemic Efficacy of Various Fractions of Heartwood of *Pterocarpus marsupium* (Fabaceae) on Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Authors VRB and BSN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author BSN managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The objective of the present work is to study the antidiabetic and hypolipidemic effect of different fractions of heart wood of *Pterocarpus marsupium* in alloxan induced diabetic rats. **Methodology:** Diabetes was induced by administering alloxan dissolved in saline (65 mg/kg body wt.), while the normal control group was given the vehicle (propylene glycol). After seven days of stabilization period, blood was drawn and the plasma was estimated for blood glucose. Animals which had more than 200 mg/dl of blood glucose were included in the study. Blood glucose estimation was done on Day 1, 15 and 30. Estimation of lipid profile was done on day 30 only.

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Diabetes induced animals were randomly assigned into different groups.
Results: Alloxan induced diabetic rats treated with various extracts (75 mg/kg body wt.) showed marked hypoglycemia, hypo-triglyceridemia and hypocholesterolemia at the end of the study period.
Conclusion: Thus the present study suggested the potential use of heart wood of *P. marsupium* in treating diabetes as well as related cardiovascular complications.

Keywords: Alloxan; antidiabetic; diabetes mellitus; hypolipidemic; P. marsupium.

1. INTRODUCTION

Diabetes mellitus is a complex metabolic disease characterized by chronic hyperglycemia. It is also an endocrine disorder affecting various systems within the body with biochemical and structural consequences. Initially, this metabolic disease commences with glucose insufficiency which leads to insulin resistance, as a result the body progressively fails to maintain glucose homeostasis resulting in glucose intolerance [1].

It has been constantly affecting the human race, irrespective of the socioeconomic profile and geographic location. Hence, it is pertinent to curtail the surge of diabetes and its complications so as to alleviate human suffering. Despite the remarkable advances in health sciences and medical care, there are many patients who are using alternative therapies to the prescribed medication. Herbal medicines have gained popularity in developing countries due to its lack of adverse effects [2] when compared to conventional anti-diabetic drugs [3].

The role of traditional medicines in the solution of health problems is invaluable on a global level. From ancient period, people are using medicinal plants for the treatment of diabetes and WHO estimates that 80% of the populations presently use herbal medicines for primary health care. Antidiabetic plants have the ability to restore the function of damaged pancreatic tissue by increasing the insulin or inhibiting the intestinal absorption of glucose [4].

The present study is more meaningful because of the added advantage of these plant extracts in being equally effective but without the untoward side effects of modern medicines. It is hoped that these studies will establish the therapeutic potentialities of these drugs. So this present study was taken up to study its beneficiary effects to a better mankind.

Currently available pharmatherapy for the treatment of diabetes mellitus include oral hypoglycemic agents and insulin. However these current drugs do not restore normal glucose homeostasis and they are not free from side effects. Moreover, due to high cost of allopathic drugs, it is difficult to provide modern medical health care especially in developing countries. It is therefore become necessary to make use of vast reserves of plant origins for medical purposes which will help to search effective as well as safer drug remedy for diabetes mellitus.

The aim of this study was to evaluate the antidiabetic and antihyperlipedemic effect of heart wood of *P. marsupium* in alloxan induced diabetic rats with the objective to focus on mechanism underlying the activity. The medicinal plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies [5].

2. MATERIALS AND METHODS

P. marsupium is a moderate-sized to large deciduous tree. The bark is grey, rough, longitudinally fissured and scaly. The heart wood is used as an astringent and in the treatment of inflammation and diabetes. It is good for dysentery, cough, diarrhea and greyness of hair. *P. marsupium* powder was obtained locally from a medicine shop. It was dried in open air and finely ground into powder.

2.1 Preparation of the Extract of Heart Wood of *Perocarpus marsupium*

About 3 kg of freshly procured finely dried powder was soaked in 95% ethanol for 4-5 days. The cold extract was decanted off and the soaked material was refluxed with 95% ethanol. Both the cold and warm extracts were boiled and concentrated under pressure till a thick syrupy consistency was obtained. It was evaporated to dryness and this was further fractionated using different solvents

2.2 Aqueous Extract

Finely dried powder was soaked in water for 1-2 hr. Refluxed for 2-3 hrs and decanted when it was hot. The contents were filtered using cotton plug. Concentrate the contents under reduced pressure (evaporate H_2O). Keep in water bath & heat till you attain a syrupy consistency. Weigh the extract and preserve it in an air tight container at room temperature. This research protocol was approved by the animal ethical committee of Manipal University.

2.3 Animals

In-house bred albino Wistar strain male rats weighing 120-200 g were used in the study. All the animals were maintained under 12 hr. day light environment. The animals were kept in a hygienic environment and the bed was changed every day. All the animals were provided with water and food ad libitum. The standard rat pellet food was supplied by Gold Mohur Lipton India Ltd. The normal control group was injected with 0.9% saline intraperitonially. Alloxan was dissolved in saline (0.9%). Injection of either saline or alloxan (65 mg/kg body wt.) was carried out on rats that had been deprived of food for about 24 hours. After about 30 minutes of injection, food was provided to animals. After seven days of stabilization period blood samples were obtained from animals fasted overnight. Blood was drawn from intra orbital plexus by inserting a “mucap” capillary. About 1 – 1.5 ml of blood was drawn into a test tube having sodium fluoride and oxalate mixture. After an hour the sample of blood was centrifuged (R&C centrifuge) at 2000 rpm for about 15 minutes and the plasma was used for the assay. Blood sugar was estimated with the plasma sample obtained. The alloxan injected group which had more than 200 mg % of blood glucose was included in the study. The diabetes induced animals were randomly assigned into different groups with eight animals in each group. The day of confirmation of diabetes was taken as day 1 for further course of treatment as per the group. Blood sugar estimation: Day 1, 15 & 30. Estimation of lipid profile: Cholesterol, triglyceride, HDL, LDL and VLDL on Day 30 only. Another blood sample was also collected to get the required serum samples and was used for other biochemical assays mentioned below.

2.4 Treatment Schedule

The treatment schedule of the various groups is as detailed. On 15th and 30th day of treatment, blood from animals fasted overnight was drawn and the biochemical parameters were assessed. NC – Normal Control – Vehicle (propylene glycol), DC – Diabetic Control – Vehicle (propylene glycol), IN – Insulin treated – 6 units/kg body weight, Aqueous extract – 75 mg/kg bodyweight (decoction of heart wood), EE – Ethanol extract treated – 75 mg/kg body weight, PE – Petroleum ether extract – 75 mg/kg body weight, DE – Diethyl ether extract – 75 mg/kg body weight and EA – Ethyl acetate extract – 75 mg/kg body weight. The various extracts were dissolved in propylene glycol and the extract was administered orally.

Normal control and diabetic control groups were also given the vehicle as per their body weight. The insulin group was injected with insulin intra peritoneal with a dose of 6 unit/kg body weight.

Blood glucose was estimated by glucose oxidase–peroxidase method. (kit supplied by Point scientific Inc.USA) Triglyceride, cholesterol and HDL, were estimated using enzymatic methods (kits were supplied by Pointe Scientific Inc. USA). The LDL and VLDL cholesterol were determined by calculation method. (Standard formula for calculation was used)

2.5 Statistical Analysis

All data obtained were analyzed using SPSS and was expressed as mean ± SE. (commonly used software version 16) The significance of difference among the groups was assessed using Kruskal Wallis test followed by Mann Whitney ‘U’ test. Significant value was set to $p<0.05$. Comparison of data within the group at different intervals was assessed by paired ‘t’ test. Anova (One way analysis of variance) and Tukey HSD test was used for inter comparison between the groups. Bonferroni test and Anova was used for inter comparison between the time periods.

3. RESULTS

A significant decrease in the blood glucose level was observed which may be an indication of the progressive metabolic control of the extract (Fig. 1). Petroleum ether and ethyl acetate extracts were most effective in lowering blood glucose, cholesterol and triglyceride levels (Figs. 2 and 3). There was a highly statistical significance in EA, aqueous and PE treated groups when compared to DC. In the present study, there was statistical difference when DC was compared with alcohol treated group in

which DC had an increase in TG level when compared to EE treated group. However, decrease in cholesterol and triglyceride indicates that the plant extract is more useful in the treatment of diabetes as it has hypolipidemic effect. In our present study there was no significant change in HDL levels (Fig. 4) which indicates a low risk factor for atherosclerosis.

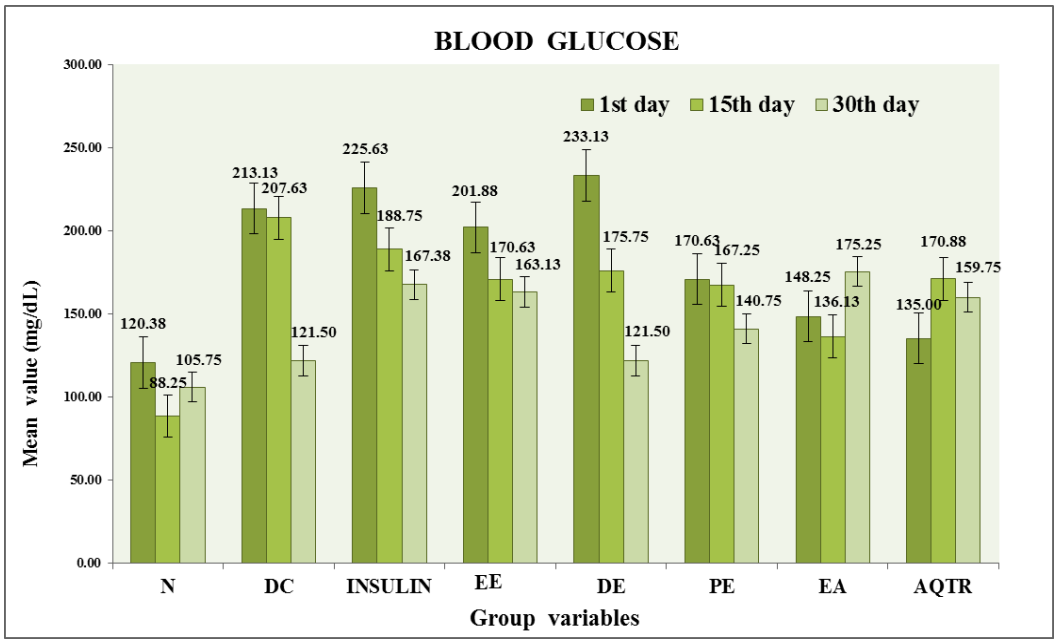

Fig. 1. Blood glucose levels in various groups

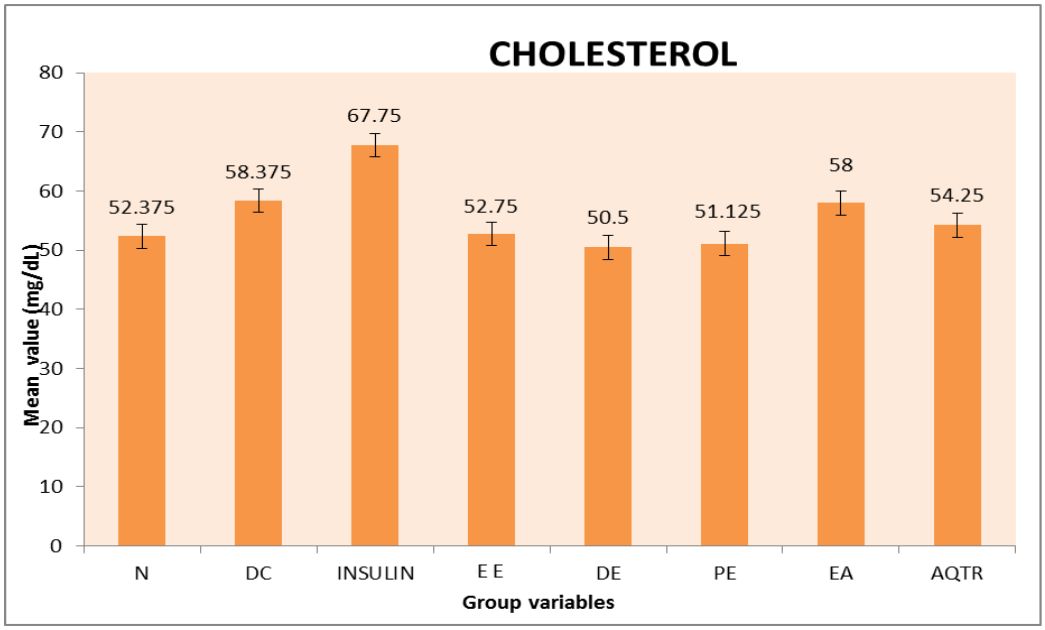

Fig. 2. Blood cholesterol levels in various groups

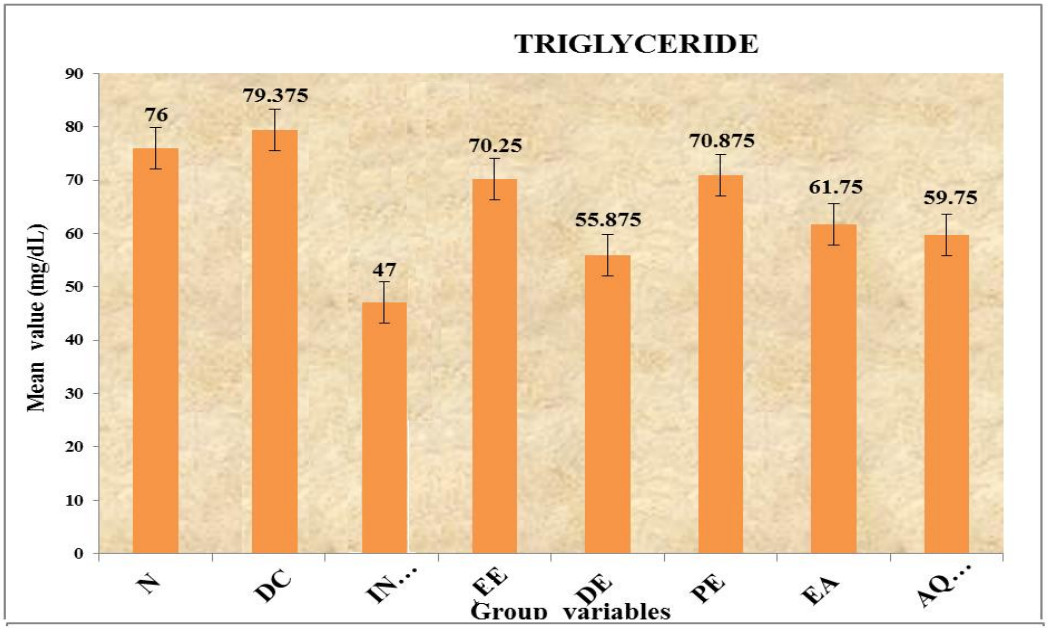

Fig. 3. Triglyceride levels in various groups

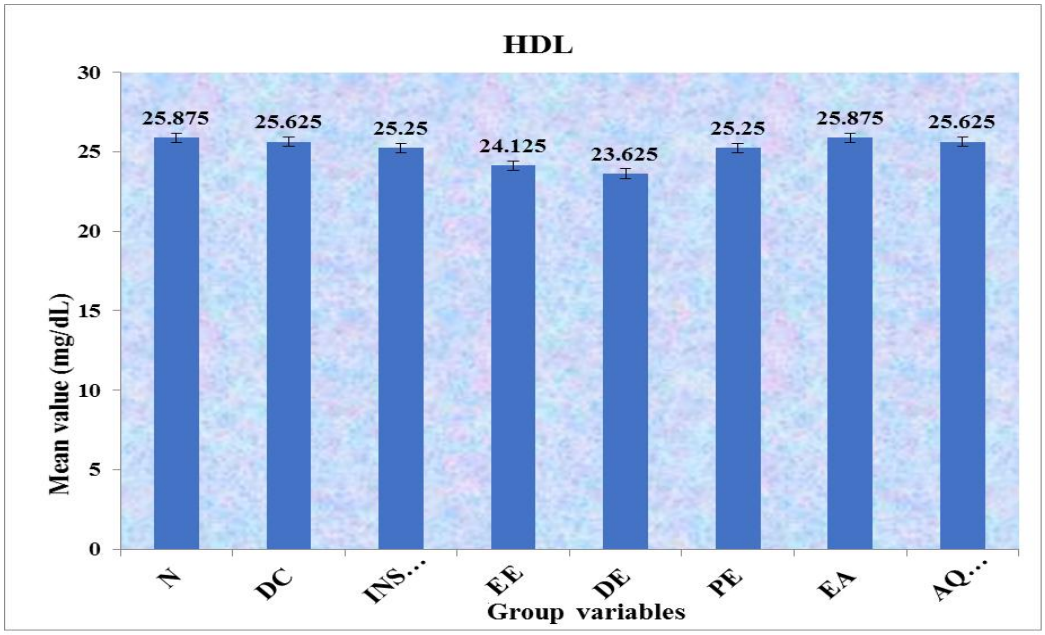

Fig. 4. HDL cholesterol levels in various groups

4. DISCUSSION

4.1 Blood Glucose

Oral administration of the *P. marsupium* extract showed significant decrease in the blood glucose level, decline in total cholesterol and triglyceride which may be an indication of the progressive metabolic control of the extract on mechanisms involved in the elimination of lipids and glucose from the body. A reduction in triglyceride levels may be due to decreased lipogenesis and increased lipolytic activation of the hormone sensitive lipase or lipogenic enzymes and

activation of lipoprotein lipase [6-8]. Similar hypolipedmic properties have been confirmed in many plant species and plant products in medicinal use [9-11]. It is likely that the plant extract produces its hypoglycemic effect by acting as an analog of insulin and it also mimics some of the actions of insulin on glucose metabolism such as enhancing uptake of glucose absorption in the intestine as well as acting as antimetabolites that are capable of blocking the pathway of fatty acid oxidation [12]. The results of the present study is in accordance with the previous study which demonstrated that the aqueous extract of *Mangifera indica* leaf at a dose of 400mg/kg body weight reduced the blood glucose level significantly in alloxan induced diabetic rats which indicates that alloxan induces diabetes by completely destroying the pancreatic islet of beta cells which produces insulin. Presence of flavonoids could also be a probable cause for producing hypoglycemia as supported by previous studies [13,14].

4.2 Serum Cholesterol and Triglyceride

The hypercholesterolemia observed in diabetics generally might be due to increased intestinal cholesterogenesis, resulting from increased activity of β-OH- β methyl glutaryl COA in the intestine of alloxan induced diabetic rats [15] and partly from the increased availability of acetyl-CoA as a result of increased oxidation of fatty acids in diabetes mellitus. The fall in the serum cholesterol level of diabetic rats that received the plant extract further supports the hypoglycemic effect of *P. marsupium.* In the present study there was a decrease in the cholesterol level in DE and PE treated groups indicating that, the hypocholestermic effect may be an indication of progressive metabolic control of the plant extract. The fall in the cholesterol level of alloxan induced diabetic rats that received plant extract further supports the hypocholestermic effect of the heart wood of *P. marsupium*. Similar hypolipidemic properties have been confirmed in many plant species and plant products in medicinal use [9]. In the present study, it was found that the diabetic control group was able to register hypotriglyceridemia even though there could not be any significant increase or decrease in other extract treated groups. A reduction in TG levels may be due to decreased lipogenesis and increased lipolytic activation of the hormone sensitive lipase, lipogenic enzymes and activation of lipoprotein lipase (6-8), as is observed in *Mormodica charantia* [16].

As many antidiabetic drugs do not correct dyslipidemia the observed hypo-cholesterolemic and hypotriglyceridaemic effects of the extract in alloxan induced diabetic rats unveils the potential effects of *P. marsupium* in the management of diabetes. Administration of ethanol extract of the leaf of *Gynura procumbens* [17] seeds of *Eruca sativa* leaves of *Averrhoea hilimbi* etc. have also demonstrated the hypotriglyceridemic effects in diabetes.

4.3 HDL and LDL Cholesterol

Diabetic dyslipidaemia is mainly due to decreased removal of triglycerides from the fat depots and the increase in the plasma concentration of LDL-cholesterol. However, in the present study there was no significant change in HDL levels in any extract treated groups compared to DC which indicates a low risk factor for atherosclerosis. Earlier studies with human beings showed higher concentration of LDL cholesterol and lower concentration of HDL cholesterol in diabetic patients [18].

5. CONCLUSION

P. marsupium extract exhibited hypolglycemic and hypolidemic effect therefore our study suggested the potential use of heart wood of *P. marsupium* to lower glucose as well as lipids.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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