

Original Research Article

## Lipid profile and hepatoprotective effects of combined leaf extracts of *Azadirachta Indica* (Neem) and *Peristrophe bicalyculata* in alloxan-induced diabetic rats.

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### Abstract

Purpose of the study: This study was designed to investigate the protective efficacy of ethanolic extracts of leaves of *Azadirachta indica* (AI) and *Peristrophe bicalyculata* (PB) on the liver of alloxan-induced diabetic albino Wistar as well as their effect on serum lipid profile.

Main findings: A significant decrease ( $P < 0.05$ ) was observed in HDL, VLDL and triglyceride values while an increase ( $P < 0.05$ ) was observed in LDL and total cholesterol values in the diabetic control (DC) compared with the normal control (NC). Treatment with PB reversed the decrease in HDL levels comparable to the reversal by insulin. All the extracts caused an increase in the value of VLDL compared to DC. The values of LDL decreased significantly ( $p < 0.05$ ) in all the extract-treated rats relative to DC with the combine extracts showing a synergistic decrease. The decrease in TG was significantly ( $p < 0.05$ ) reversed by treatment with all the extracts and insulin while the increase in TC was reversed only by AI. There was an increase in AST and ALT and a decrease in ALP levels in DC, relative to NC. Only the combined extract resulted in a reduction in AST level, relative to DC, comparable to the Insulin value. There was a significant decrease ( $p < 0.05$ ) in ALT level in all treated animals compared to DC. Except for AI, no reversals were observed for ALP levels in all the treatment groups. Summary of findings: The results show that a combination of the two plant extracts will be most appropriate for a total normalization of the diabetes-induced hyperlipidemia and protection of the liver against diabetes-induced injury.

**Keywords:** Diabetes mellitus, liver enzymes, lipid profile, *Azadirachta indica* and *Peristrophe bicalyculata*.

### Introduction

Diabetes of all forms is prevalent in Nigeria and indeed the rest of Africa. Recent statistics showed that 75-90% of the rural populations of the world still rely on herbal medicines for their health care [1]. The use of plants derived natural compounds, as part of herbal preparations, as alternative sources of drugs continues to play a major role in chemotherapy especially in third world countries [2]. Several studies carried out in many laboratories have shown that traditional medicines could provide better glycemic controls than currently used conventional drugs [3,4]. Moreover, synthetic chemical drugs are not only known to elicit deleterious adverse effects, but also to be too expensive for rural and peasant populations in Sub-Sahara Africa.

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin [5]. It is a metabolic disease with severe socio-economic importance characterized by hyperglycemia and glycosuria due to absolute or relative lack of insulin [6]. In Nigeria about 2.5million people were estimated to be

diabetic in the year 2010 with a projection of over 4.8million diabetic by 2030. In 2010, an estimation of 1.5million people died from diabetes with almost 80% of deaths due to diabetes occurring in low and middle income countries [7].

*Azadirachta indica* (neem) has been reported to have beneficial effects in the management of diabetes mellitus and the amelioration of oxidative stress associated with the disease [8]. *Azadirachta indica*, also known as neem, belong to the family of Meliaceae [9]. It is one of the most useful medicinal plants in the world [10]. Extracts of neem have been shown to demonstrate anti-lipid peroxidation, anti-hyperglycemia and anti-hypercholesterolemia as well as the reduction of serum triglyceride levels in diabetic rats [11]. The leaf extract has been used traditionally in the control of diabetes mellitus in many countries including Nigeria. It has been reported that *Azadirachta indica* is included in a polyherbal antidiabetic drug currently undergoing controlled clinical trials in humans [12, 13].

*Peristrophe bicalyculata*, belonging to the family Acanthaceae, is another medicinal plant that has been used in South-West Nigeria in the treatment of hypertension and other cardiovascular related



diseases [14]. Recently, the plant was reported to have beneficial effects on plasma lipid profile by reducing lipid levels [14]. However, little or no research has been done to evaluate the efficacy of this plant in the treatment of diabetes mellitus. Thus this study is aimed at investigating the efficacy of these plants, singly and in combination, in alleviating diabetes mellitus and the protection of the liver during diabetes.

## Materials and Methods

### Source of Plant Materials

Matured fresh leaves of *Azadirachta indica* and *Peristrophe bicalyculata* were obtained from the premises of the University of Calabar, Nigeria. The plants were authenticated by Dr. M. Eko, a botanist in the Department of Botany, University of Calabar, Nigeria. The leaves were washed with clean tap water and rinsed with distilled water and a voucher specimen deposited in the herbarium unit of the Department of Botany, University of Calabar.

### Preparation of Plant Extracts

3kg and 1kg of the fresh leaves of *Peristrophe bicalyculata* and *Azadirachta indica* were separately chopped into pieces and each separately blended in 2L of 80% ethanol. The extracts obtained were allowed to stand for 48hours in the refrigerator at 4°C. The extracts were then filtered with cheese cloth and again with Whatman No1 filter paper. The filtrates were concentrated under vacuum at 37°C to about one tenth of the original volumes, using a rotary evaporator. The concentrates were allowed to stand in the open in a water bath for complete dryness to give 34.8g (or 3.94%) and 66.4g (or 6.64%) of greenish brown and brown oily extracts for *Peristrophe bicalyculata* and *Azadirachta indica* respectively. The extracts were then refrigerated at below 8°C until required.

### Animals and Experimental Design

Thirty-six mature Wistar albino rats of both sexes weighing 150-200g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Nigeria and housed in cages under standard conditions (28±2°C, relative humidity 80±5% and a 12h light/ dark cycle). The rats were allowed to acclimatize, fed with standard rat pellets and given water *ad libitum*.

The animals were divided into 6 groups of 6 rats each. Group 1 (non-diabetic) served as normal control (NC), and Group 2 (Diabetic control). Groups 3, 4, 5 and 6 were diabetic. Diabetes was induced in the five diabetic groups by intraperitoneal injection of 100mg/kg bw Alloxan in normal saline solution. Diabetes was confirmed if the animal showed fasting blood glucose (FBG) levels of 200mg/dl, 3 days after diabetes induction. Groups 1 (NC) and 2 (DC) received (500mg/kg bw) normal saline solution throughout the experimental period. Group 3 received, by intraperitoneal injection, 5 iu/kg bw insulin per day. Group 4 (AI) received *Azadirachta indica*

extracts and Group 5 (PB) received *Peristrophe bicalyculata* extract (500mg/kg bw) respectively, twice daily. Group 6 received a combined AI and PB (500mg/kg bw) extract twice per day at a mixture of 1:1 ratio. After 14 days, the animals were sacrificed and whole blood collected for serum preparation which was used to assay for lipid profile and some liver enzymes activities.

### Biochemical Assays

Biochemical assays were carried using standard analytical kits and an AJ-semi auto-Biochemistry analyzer from Agappe Diagnostic Laboratories limited, Switzerland GmbH, Konnauerstrasse 54-6330 Cham. Alloxan was purchased from Sigma-Aldrich Inc, USA. Insulin was purchased from Actrapid-Insulin Human (rDNA), Novo-Nordisk A/S, Denmark.

### Data and Statistical Analysis

The results were analyzed for statistical significance by the one way Analysis of variance (ANOVA) using SPSS software. All data were expressed as Mean ± SEM and p values < 0.05 were considered significant.

## Results

A significant decrease ( $P<0.05$ ) was observed in HDL and VLDL values in DC compared to NC (Figure 1). The reverse was observed in LDL value. Treatment with PB reversed the decrease in HDL levels comparable to the reversal by insulin. There was no significant effect by treatment with AI or a combination of the two plants. All the extracts caused an increase in the value of VLDL compared to DC. No significant synergy was observed in the use of the combination of AI and PB. The values of LDL decreased significantly ( $p<0.05$ ) in all the extracts treated rats relative to DC (Figure 1). The combine extracts showed a significant ( $p<0.05$ ) synergistic decrease in LDL levels. There was a significant ( $p<0.05$ ) decrease in TG level in DC relative to NC (Figure 1). This decrease was significantly ( $p<0.05$ ) reversed (towards to NC values) by treatment with all the extracts and insulin. On the other hand there was a significant increase ( $p<0.05$ ) in TC levels in DC compared to NC which was reversed only by AI.

There was an increase in AST and ALT levels in DC, relative to NC, whereas there was a decrease in ALP level in DC (Figure 2). Treatment with extracts individually resulted in a further significant increase ( $p<0.05$ ) in AST level. A combination of the two extracts however significantly lowered ( $p<0.05$ ) the AST level relative to DC comparable to the level in the insulin treated rats. There was a significant decrease ( $p<0.05$ ) in ALT level with AI and PB compared to DC. The combined extracts also resulted in a significant decrease ( $p<0.05$ ) in ALT level although there was no synergy. Except for AI, no reversals were observed for ALP levels in all the treatment groups (Figure 2).



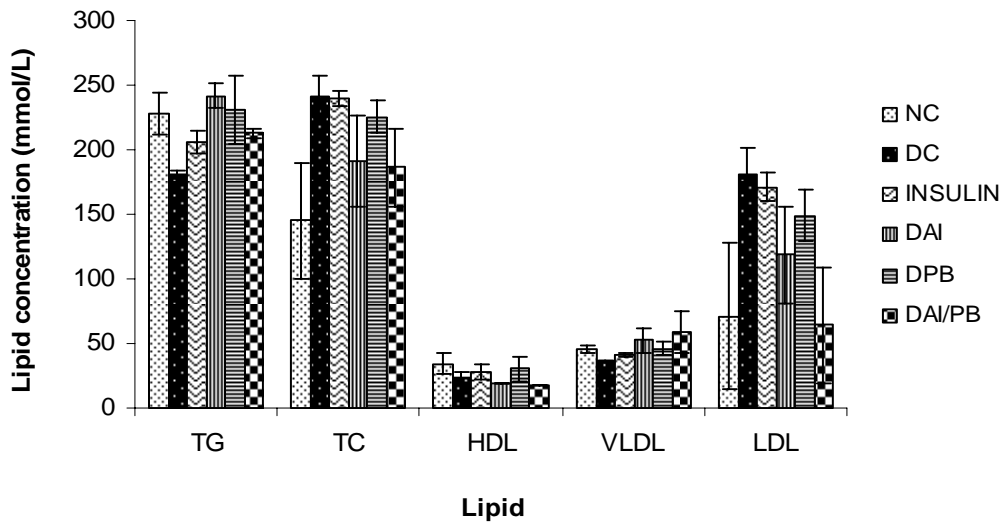


Figure 1: Effect of extracts of *Azadirachta indica* and *Peristrophe bicalyculata* on Lipid Profile of Alloxan-induced diabetes rats.

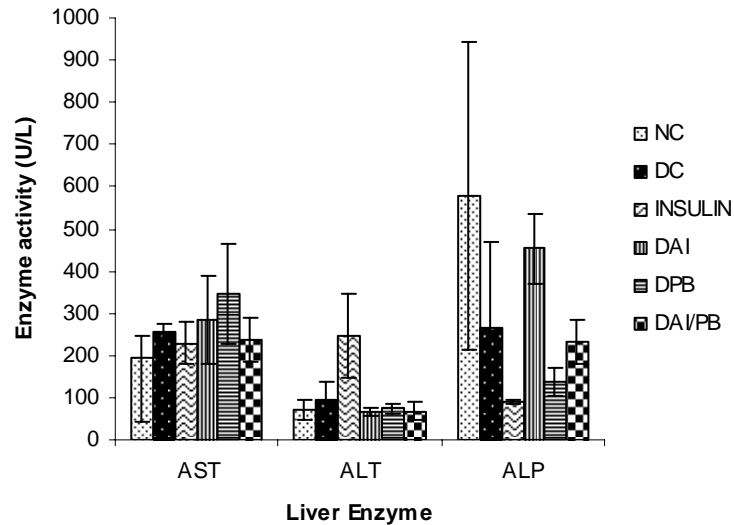


Figure 2: Effect of extracts of *Azadirachta indica* and *Peristrophe bicalyculata* on the activity of some Liver enzymes in the serum.

## Discussion

Diabetic lipid disorders are characterized by hypertriglyceridemia, increase in low density lipoprotein, decrease in high density lipoproteins and postprandial hyperlipidemia [15,16]. This is as a result of absolute or relative deficiency and subsequent disorders in the activity of lipid metabolising enzymes and transporters [15]. These observations are in agreement with those observed in our study except for the triglyceride and very low lipoproteins levels that was found to be lower in the diabetic control than the normal control. Synergy between the activities of the two plant extracts was only obtained in the lowering of the LDL levels. But considering that *Azadirachta indica* (AI) treatment reversed the

elevated TC levels and *Peristrophe bicalyculata* (PB) reversed the depressed HDL level in the diabetic rats, a combination of the two plant extracts will be appropriate for a total normalization of the diabetes-induced hyperlipidemia (lipid metabolising disorder) and thus prevent progression of atherosclerosis.

Serum enzymes in this case Alanine amino transferase (ALT), Aspartate amino transferase (AST), are the most commonly used biochemical parameters for the assessment of liver function. These enzymes are usually released from the hepatocytes and leak into circulation, during hepatocellular injury or inflammation of the biliary tract cells, causing increase in their activity in serum [17,18]. Activities of these enzymes in the serum are particularly high in acute hepatocellular damage caused by drug toxicity and

xenobiotics. Alkaline phosphatase (ALP) acts as markers of biliary function and cholestasis [18]. Report of increases [18] and no increases in ALP [17] with type 1 diabetes has been reported. In this study, there was conformity in the levels of these enzymes, in diabetic rats relative to normal control, with available literature [17,18,19,20]. The higher levels of AST and ALT observed in diabetic control may be attributed ostensibly to the alloxan-induced injury to the liver and pancreatic gland. The reversal of the AST increase by a combination of the two plant extracts (comparable to Insulin treatment), the reversal of the ALT increase in diabetic rats by the two individual plant extracts and the restoration of ALP to normal levels by AI suggests a hepatoprotective role for a combination of the two plant extracts.

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## Conclusion

Evidence from this study indicates a synergy in the activity of the combined extracts of *Azadirachta indica* and *Peristrophe bicalyculata*, over and above the individual plant extracts, in reversing the hyperlipidemia and liver injury associated with diabetes and makes the combination of the two plant extracts a potent antidiabetic remedy and a possible alternative to current drugs used for the management of diabetes.

