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The Hypolipidaemic Effects of the Partially Purified "Hypoglycaemic Agent" of Aqueous Leaf Extract of *Albizzia chevalieri* Harms.

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ABSTRACT
Aqueous leaf extract of *Albizzia chevalieri* and its fractions have been reported to possess significant hypoglycaemic effect. In the current work the effects of aqueous leaf extract of *Albizzia chevalieri* and the column chromatographic fractions, of the extract with hypoglycaemic effect, on serum lipid profile of alloxan induced diabetic rats were studied. The aqueous extract was fractionated with organic solvents and the fractions further fractionated using column chromatography. The results indicated that the aqueous leaf extract caused significant ($P<0.05$) decrease in triacylglycerol (14 %), LDL-cholesterol (16 %), VLDL-cholesterol (19 %) and atherogenic index (45 %). The hexane fraction of the aqueous extract which was reported to possess significant hypoglycaemic effect caused significant reduction in serum total cholesterol (57 %), triacylglycerol (29 %), LDL-cholesterol (75 %), VLDL-cholesterol (67 %) and atherogenic index (72 %) in the rats. The second elution fraction of the hexane fraction from the column, which is dark brown in colour and was earlier reported to contain the hypoglycaemic agent also reduced the serum levels of total cholesterol, triacylglycerol, LDL-cholesterol, VLDL-cholesterol and atherogenic index by 66, 54, 78, 80 and 76 % respectively. These results may be indicative of the fact that the aqueous leaf extract of *Albizzia chevalieri*, in addition to it hypoglycaemic activity, also possesses significant hypolipidaemic effect. It may be worthwhile therefore to study the nature and mechanism of action(s) of the agent(s) responsible for the observed effects.
Keywords: Lipid profile, *Albizzia chevalieri,* diabetes, rats

INTRODUCTION

Diabetes mellitus is a heterogeneous group of syndromes characterised by hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from relative or absolute deficiencies in insulin action and/or secretion [1]. The main biochemical manifestation of the syndrome, is hyperglycaemia, the disease is associated with significant abnormalities of serum lipid profile [2].

The heterogeneity of the aetiology of diabetic mellitus suggests that it is not a single entity. Accordingly, the WHO [1] classified diabetes mellitus based on clinical descriptive criteria, informed by the aetiology, clinical profile, natural history and response to insulin therapy. One of the clinical classes of diabetes mellitus is type 2, which constitutes the majority of cases in both developed and developing countries [3]. It is the commonest form of the disease among African diabetics [4]. The most important factors in the aetiology of type 2 are insulin resistance and hypeinsulinaemia. One of the major risks of type 2 diabetes is that it is closely related with metabolic syndrome, a cluster of cardiovascular risk factors [5]. The syndrome is made up of insulin resistance, hyperinsuinaemia, raised plasma triacyglycerol, low plasma high density lipoprotein among others [5]. Management of diabetes mellitus in general and type 2 diabetes in particular should therefore necessarily include reduction of cardiovascular risk factors.

Many laboratories of recent are studying the potentials of medicinal plants in the management of diabetes mellitus. Saidu *et al.*[6], for example reported that *Albizzia chevalieri,* which is use in Niger Republic and some part of Northern Nigeria possesses significant hypoglycaemic activity in alloxan induced diabetes rats. The aqueous leaf extract of the plant has also been reported to be relatively safe at therapeutic dose with LD_{50} of >3000 mg/kg body weight [7,8].

Albizzia is a large genus of trees, of the pea family (Fabaceae), native to warm regions of the Old

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World. The alternate, compound leaves are bipinnate (*i.e.,* the leaflets of the feather-formed leaves, in turn, bear leaflets). *Albizzia chevalieri* is a tree of acacia type native to tropical and subtropical regions including Nigeria and Niger Republic, with loose ball, of whitish fragrant flowers and flat brown pods. The small flowers are borne in globular or finger-shaped clusters. The fruit is a large, strap-shaped pod.

The current work report the effects of the aqueous leaf extract of *Albizzia chevalieri* and its hexane and column chromatogaphic fractions that possess hypoglycaemic effect on serum lipid profile of alloxan induced diabetic rats.

MATERIALS AND METHODS

Chemicals and Reagents: All the chemicals and reagents used for this work were of analytical grade. Alloxan monohydrate was purchased from Sigma-Aldrich. Lipid profile assay kits were purchased from Randox Laboratories Ltd, Antrim, United Kingdom.

Plant Materials: *A. chevalieri* was obtained from a suburb, about 50km south of Sokoto, Nigeria and identified by a Taxonomist, Dr Awwal Argungu from the Botany unit of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. Voucher specimen (UDUS/VS/04/09) was prepared and deposited in the Herbarium of the same Department. The leaves were sun dried, ground using laboratory pestle and mortar and sieved with a $1mm^2$ sieve. The powdered leaf was kept in plastic bags in a desiccator until required.

Preparation of Crude Extracts: The powdered plant material was soaked in cold distilled water at 20 % (w/v) for 24 hr. The extract was filtered through several folded clean white muslin cloth to remove debris. The filtrate was then filtered through a Whatman No 1 filter paper. The final filtrate was concentrated in a Rotary Evaporator, percentage recovery calculated to be 18.75 % (w/w) and reconstituted in distilled water at 10% (w/v). The reconstituted extract, labelled crude aqueous extract was stored in small capped plastic containers at +4 ^oC until required. The extract was tested for it effect on the serum lipid profile of the rats.

Fractionation of the Extract: The crude water extract of the plant material was subjected to further fractionation with different solvents successively starting with hexane, then petroleum ether and chloroform. In this case a known amount of the dried water extract was reconstituted in distilled water and equal volume of hexane was added in a separation funnel. This was vortexed vigorously for about 10 minutes. The hexane fraction was carefully separated into a beaker and labelled hexane fraction. The extract was concentrated in a Rotary Evaporator, percentage recovery was calculated to be 5.18 % (w/w) and reconstituted in distilled water at 10 % (w/v). The effect of the extract on serum lipid profile was tested.

Column Chromatography: The hexane fraction was further subjected to separation and purification using column chromatography on silica gel mesh size 60-200 [9]. The fractions obtained were labelled H_1, H_2 and H_3. The subscript numbering was in order of elution from the column. The percentage recoveries of the fractions were calculated to be 1.47, 0.94 and 2.93 % (w/w) respectively. The H_2 fraction, which was demonstrated to exhibit hypoglycaemic effect [6], was reconstituted in distilled water at 10 % (w/v).

Animals: Albino rats of both sexes, aged 7-8weeks purchased from National Institute for Trypanosomiasis Research (NITR) Vom-Jos, Nigeria were allowed to acclimatise to the laboratory for a week. The research protocol was approved the Research and Ethics Committee of the Usmanu Danfodiyo University, Sokoto.

Preparation of Diabetic Rats: The Rats in the alloxan monohydrate induced diabetics group were injected with alloxan monohydrate, dissolved in sterile normal saline solution in a dose of 80mg/kg body weight/day for 3 consecutive days, intraperitoneally [2, 10-12]. After a week, from the last dose, animals with moderate hyperglycaemia with blood glucose range of 11-14 mM were considered diabetic. Serum glucose was estimated by the glucose oxidase method [13]

Treatment of the rats: There were four groups for each extract or fraction: alloxan induced diabetic treated (DT), alloxan induced diabetic untreated (DU), normal treated (NT) and normal untreated (NU). Each group was allocated five rats. The treated groups in all cases were

administered the extracts orally at 100 mg/kg body weight per day, in the morning hours (9 – 11 am), for one week. The untreated groups, in each case, were administered 0.4 ml distilled water through the same route for a week.

Twenty four hours after the last treatment, the animals were anaesthesized by dropping each of the animals in a transparent plastic jar saturated with chloroform vapour and blood samples collected through cardiac puncture, into labelled centrifuge tubes.

Serum Lipid Profile Determination: The blood samples were centrifuged in a bench-top centrifuge at 3000 rpm for 5 minutes and serum collected into labelled sample bottles and stored at -20 ^{o}C until required. Serum lipid profiles were determined within 72 hour of blood collection. Serum total cholesterol (TC) [14], high density lipoprotein cholesterol (HDL-C) [15] and triacylglycerols (TAGs) [14] were determined by enzymatic methods. Low density lipoprotein cholesterol (LDL-C) and very Low density lipoprotein cholesterol (VLDL-C) were calculated using Friedwald formulae [16]. Atherogenic index (Aix) which measures the risk of development of atherosclerosis, was calculated as he ratio of LDL-cholesterol to HDL-cholesterol [17].

Statistical Analysis: The results are expressed as mean $\pm$ standard error of the mean. The results were analysed using analysis of variance (ANOVA). Post Hoc Tests Multiple Comparisons using LSD was utilized to identify differences in means. A P value < 0.05 was considered statistically significant. In all cases SPSS windows version 10 was employed for the analysis.

RESULTS

Treatment with Crude Aqueous Extract: The results of the effects of the aqueous leaf extract of *A. chevalieri* on lipid profile are presented in Figure 1.

Intraperitoneal injection of alloxan induced significant increase ($p < 0.05$) in serum levels of total cholesterol, LDL-cholesterol, VLDL-cholesterol and triacylglycerol in the experimental animals. The atherogenic index (ratio of LDL-cholesterol to HDL-cholesterol) was also increased as a result of alloxan treatment. HDL-cholesterol was however significantly decreased as a result of alloxan treatment. Treatment of the normal and alloxan-induced diabetic rats with aqueous extract of *A. chevalieri* caused a non significant ($p > 0.05$) decrease in the serum levels of total cholesterol and VLDL cholesterol. TAG of both groups decreased significantly ($p < 0.05$) upon treatment with *A. chevalieri* crude aqueous leaf extract. The HDL-cholesterol and atherogenic index of the diabetic animals also improved significantly ($p < 0.05$) when treated with the water extract of the plant.

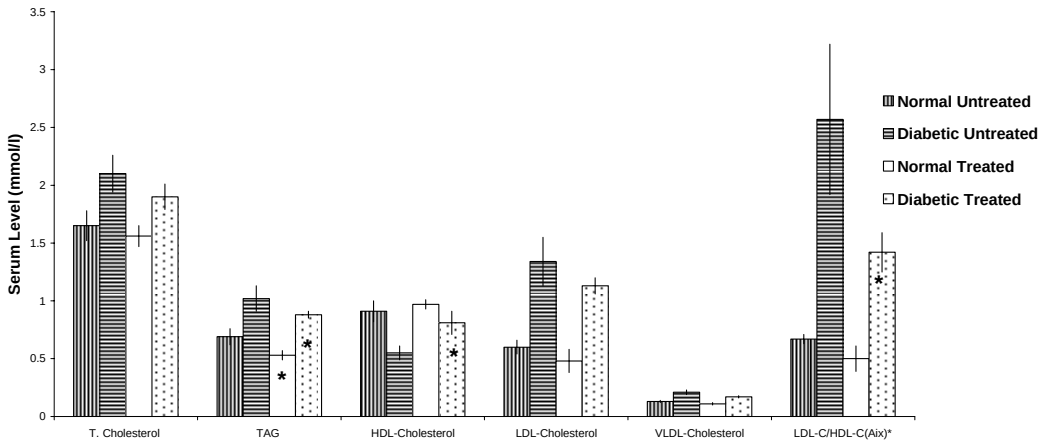

Figure 1: Serum Lipid profiles of Rat Treated with Crude Water Extract of *Albizzia chevalieri*
* (P<0.05) and ** (P<0.01): values differ significantly from the respective untreated group
n = 5 rats; T. Cholesterol = Total cholesterol; TAG = Triacylglycerol; HDL = High density lipoprotein
LDL = low density lipoprotein; VLDL = very low density lipoprotein; LDL-C/HDL-C (Aix) = Atherogenic index

Treatment with Hexane fraction: The result of the effect of the hexane fraction of the extract on serum lipid profiles of both alloxan induced diabetic and normal rats is presented in Figure 2. The results indicated that the hexane fraction induced significant hypocholesterolaemic in normal (P<0.05) and diabetic (P<0.01) rats. LDL – cholesterol (P<0.01), VLDL – cholesterol (P<0.05) and atherogenic index (P<0.01) of the diabetic rats treated with the hexane fraction were also significantly reduced.

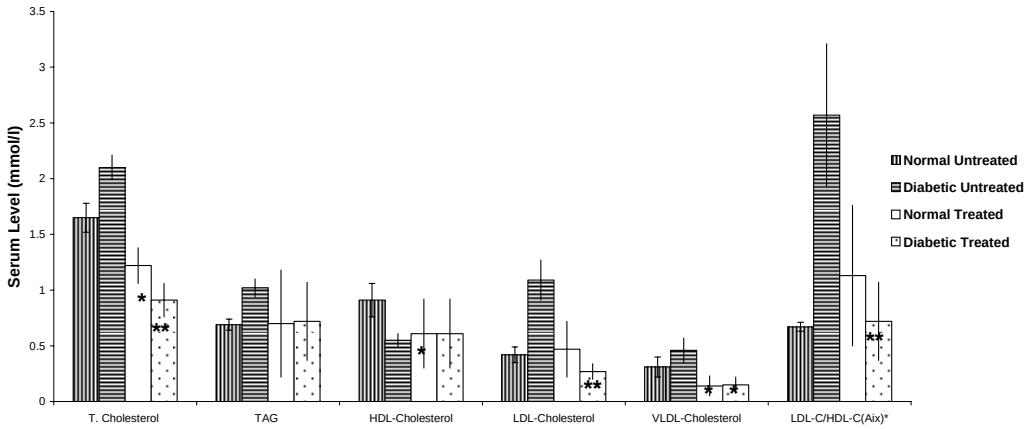

Figure 2: Serum Lipid Profile (mmol/l) of Albino Rats Treated with Hexane Fraction of the Aqueous Extract of *A. chevalieri*

* (P<0.05) and ** (P<0.01): values differ significantly from the respective untreated group

n = 5 rats; T. Cholesterol = Total cholesterol; TAG = Triacylglycerol; HDL = High density lipoprotein LDL = low density lipoprotein; VLDL = very low density lipoprotein; LDL-C/HDL-C (Aix) = Atherogenic index

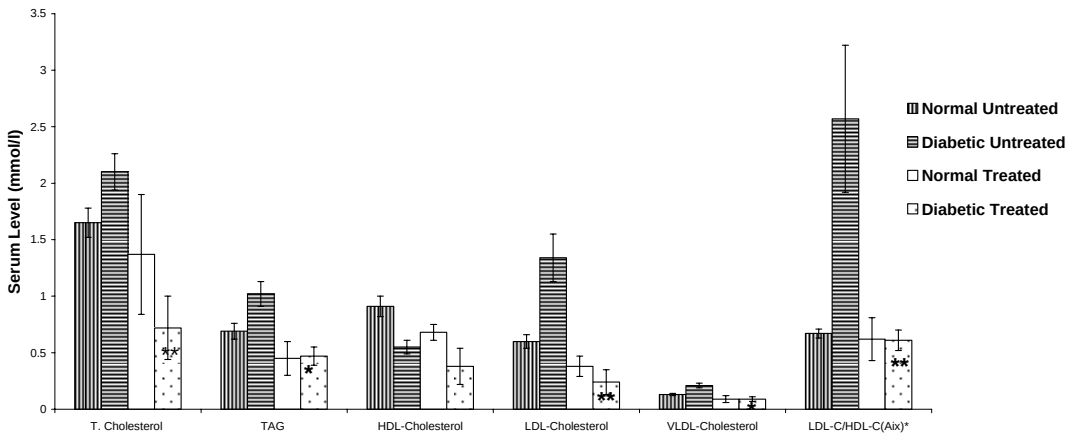

Figure 3: Serum Lipid profile (mmol/l) of albino rats treated with the second elution fraction of the Column Chromatography fractionated Hexane Fraction of the aqueous extract of *A. chevalieri.** (P<0.05) and ** (P<0.01): values differ significantly from the respective untreated group. n = 5 rats; T. Cholesterol = Total cholesterol; TAG = Triacylglycerol; HDL = High density lipoprotein; LDL = low density lipoprotein; VLDL = very low density lipoprotein; LDL-C/HDL-C (Aix) = Atherogenic index

Treatment with the Second Elution Fraction (H_2) from the Column of the Hexane Fraction: The effect of the second elution fraction (H_2) of the hexane fraction fractionated on silical gel in a column chromatography on lipid profile of albino rats is presented in Figure 3. The H_2 fraction which is dark brown in colour also showed significant hypocholesterolemic ($P<0.01$) and hypotriglyceridaemic ($P<0.05$) effects in diabetic rats. The Fraction also reduced the LDL- ($P<0.01$) and VLDL- ($P<0.05$) cholesterols significantly. The atherogenic index of the diabetic animals treated with the fraction approached the atherogenic index of normal untreated rats.

DISCUSSION

The crude leaf extract of *A. chevalieri* has been reported to possess significant hypoglycaemic properties in experiment rats [6]. In the current work, the extract was observed to cause significant hypotriglyceridaemia. It also reduced serum cholesterol level though non-significantly ($P>0.05$) and caused significant ($P<0.05$) improvement in the HDL-cholesterol and atherogenic index. The hypoglycaemic agent of the extract has earlier been reported to be fractionated in the hexane fraction [6]. The current work also demonstrated that the hexane fraction possesses significant ($P<0.05$) hypocholesterolaemic and hypotriglyceridaemic effects. Although the crude extract decreased serum cholesterol non-significantly only, when fractionated, the hexane fraction caused 26 and 57 % decrease in the serum level of the parameter in normal and diabetic animals respectively. It could be possible that though, the concentration of the hypocholesterolaemic agent of the extract is low in the crude extract, fractionating with the organic solvent might have increased the effective concentration of the agent. This assertion may also explain the observed improvement in the hypotriglyceridaemic property of the extract from 13 % in the crude extract to 29 % in hexane fraction. Low density lipoprotein -, VLDL- cholesterols and atherogenic index were also significantly ($p<0.05$) reduced in diabetic animals treated with the hexane fraction. The pattern of the increased hypolipidaemic activities as a result of fractionation was maintained for all the parameters. The LDL- and VLDL-cholesterols, for example were decreased by up to 75 and 67 % by the hexane fraction as against 15 and 19 % respectively by the crude aqueous extract. The solubility of plant constituents differ significantly in different solvents. This observation is exploited in the fractionation and purification of organic compounds from plant extracts [9, 20].

The second fraction (H_2) of the hexane fraction showed significant hypocholesterolemic and hypotriglyceridaemic effects. Fraction 2 of the hexane fraction also reduced the LDL and VLDL cholesterols significantly ($p<0.05$). The atherogenic index of the diabetic animals treated with the fraction approached the atherogenic index of normal untreated animals. Mahpara *et al.*[21] has also reported that jaman fruit extract may have a hypolipidaemic effect in type 2 diabetes. Ethanolic extract of *Gongronema latifolium* has also been shown to possess significant hypotriglyceridaemia and hypocholesterolaemia [22]

The mechanism of the cholesterol lowering effects of the extracts of *A. chevalieri* in the current study though not clear, may involve increased stimulation of cholesterol excretion into the intestine and its oxidation into bile acid [17]. It may also involve a shift in the distribution of cholesterol from the plasma into the tissues which may be as a result of increased catabolic rate of LDL. This is largely due to up-regulation of LDL-receptors [17]. Agents that interrupt the enterohepatic circulation of bile acid may be used for the control of hypercholesterolaemia. These agents may block the intestinal reabsorption of the bile acid, thus releasing the feed back inhibitory effects of bile acid on cholesterol conversion to bile. The ultimate effect of this is an increase in conversion of cholesterol into bile acid. Sitosterol is one such agent [17]. The effect of the aqueous extract of *A. chevalieri* at various levels of purification on serum cholesterol level indicated that the agent may have similar mechanism of action with these drugs. Some of these drugs may have the disadvantage of aggravating hypertriglyceridaemia [23]. The extract of the plant used in the current work, in addition to its hypoglycaemic effect [6] also possess hypocholesterolaemic and hypotriglyceridaemic activity. Other Hypolipidaemic drugs include Clofibrate derivatives which are effective in reducing TAG and increasing HDL. The drug may also decrease LDL and improves glycaemic control [23]. HMG reductase inhibitors also reduce LDL-cholesterol but do not affect glycaemia [23]. Nicotinic acid is

another drug that reduces both TAG and LDL and increase HDL. Its main disadvantage is that it aggravates insulin resistance, hyperglycaemia and hyperuricaemia [23]. Biguanide also exerts a beneficial effect on dyslipidaemia in diabetes. It has been reported to produce 10 -20 % reduction in plasma TAG in non-hypertriglyceridaemic subjects and up to 50 % in the hypertriglyceridaemic patients. The decrease is attributable to decrease hepatic synthesis of VLDL-cholesterol. Total cholesterol and LDL-cholesterol were also reported to be reduced significantly by metformin [24]. Metformin also has a beneficial effect on the HDL-cholesterol [24]. The results of the current study may be an indication of the hypolipidaemic effect of *A. chevalieri* in addition to its reported hypoglycaemic effect [6]. This may necessitate the need to conduct an indebt study into the nature and the mechanism of actions of the agent(s) responsible for these effects, as it (they) may be useful in the management of type 2 diabetes mellitus.

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