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Hypoglycaemic and Hypolipidemic Effects of Root Extracts of *Albizzia chevalieri* in Alloxan Induced Diabetic Rats.

Y. Saidu[1*], F.C. Nwachukwu[1], L.S. Bilbis[1], U.Z. Faruk[2] and A.Y. Abbas[1]

[1]Department of Biochemistry, [2]Department of Chemistry, Usmanu Danfodiyo University, P.M. B.2346, Sokoto, Nigeria.

[*Author of Correspondence: yusdab@yahoo.com]

ABSTRACT: The research investigated the hypoglycaemic and hypolipidaemic effects of aqueous and organic solvents extracts of *Albizzia chevalieri* root in alloxan-induced diabetic rats. The aqueous extract was administered at 100, 200 and 300mg/kg body weight while the organic solvent fractions of the aqueous extract were administered at 100mg/kg body weight. The 300mg/kg body weight of the aqueous crude extract caused a 24% reduction of serum glucose level of the diabetic rats. The chloroform and hexane fractions caused 25 and 24% reduction of serum glucose level of the diabetic rats. The results were comparable to 28% reduction obtained for treatment with 3.57mg/kg body weight of chlorpropamide. The petroleum ether fraction decreased serum total cholesterol, triaclglyceride, and very low density lipoprotein cholesterol levels significantly ($P<0.05$). chloroform fraction and last water extract fractions increased the high density lipoprotein cholesterol level of the diabetic rats. The results demonstrate that the aqueous root extracts of *Albizzia chevalieri* posses significant ($P<0.05$) hypoglycaemic and hypolipidemic effects in alloxan induced diabetic rats. The extract may be a potential source of lead compound(s) with anti-diabetic properties.

Key words: Hypoglycaemia, hypolipidaemia, *Albizzia chevalieri,* root extract, alloxan-induced diabetic rats.

INTRODUCTION

Diabetes mellitus (DM) is an important endocrine and metabolic disorder that is associated with considerable morbidity and mortality usually arising from its attendant long-term complications (Andreoli *et al*., 1990; Davis and Granner, 2001). It is characterized by persistent hyperglycaemia and disturbances of the metabolism of fuel compounds as a result of absolute or relative deficiency in insulin secretion or/and insulin action (WHO, 1994). It is a widespread metabolic disorder found in all population throughout the world (WHO, 1994). The prevalence of diabetes for all age groups worldwide was estimated at 2.8% in the year 2000 and this is projected to reach 4.4% (366 millions) of the world population by the year 2030 (Sarah *et al.*, 2004 and Wild *et al*. 2004). Diabetes mellitus is associated with increased risk of vascular, renal, retinal, and neuronal complications that may lead to premature disability and death (WHO, 1994).

Like many other metabolic diseases, diabetes has no known cure but is currently managed by the use of agents that exhibit hypoglycaemic effects. Insulin is the most popular and the most effective of such agents, especially for the management of type I or insulin dependent diabetes mellitus (IDDM). There are also a good number of oral hypoglycaemic agents which include sulphonylureas, biguanides, thiazolidinedione, insulin sensitizer, insulin secretagogues and α-glucosidase inhibitors (Evans, 1999). There use is however associated with drawbacks such as rigid and multiple dosing regimes, high cost, inaccessibility and untoward effects (Simoes, 1989; Kameswara *et al*., 1999; Jaouhari *et al.,* 2000; De Melo *et al*., 2002). These factors in the face of global economic recession have contributed to the recent increase in use of herbal products in the disease treatments (De Melo *et al*., 2002).

Accordingly WHO (1994) study group recommended among others, the need for the development and evaluation of better pharmacological agents for improving insulin secretion, enhancing insulin sensitivity, preventing beta-cell destruction, promoting beta–cell regeneration or repair interrupting pathways leading to various complications of diabetes.

These reports further recommended the evaluation of the efficacy of traditional medicine and non-pharmacological methods in use for the management of diabetes.

New approches in the design of antidiabetic drugs are emerging. Curently these include approaches aimed at reducing excessive glucose production by the liver, mechanisms to augment glucose-stimulated insulin secretion, specific molecular targets in the insulin signalling pathway and new approaches to obesity and altered lipid metabolism, which offer the prospect of net improvements in insulin action (or secretion). Natural products have been the single most productive source of lead compounds in the development of drugs including antidiabetic drugs (Harvey, 2008). Currently there are about 225 drugs based on natural products at different stages of drug developments (Harvey, 2008).

The aqueous leaf extract of *Albizzia chevalieri* has been reported to possess significant hypoglycaemic (Saidu *et al.,* 2007a) and hypolidaemic (Saidu *et al.,* 2009) effects in alloxan induced diabetics rats with LD_{50} greater than 3000mg/kg (Saidu *et al*., 2007b). The current study reports the hypoglycaemic effect and hypolipidemic properties of the aqueous root extract of *A. chevalieri.*

MATERIALS AND METHODS

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

Plant Material: Fresh *A. chevalieri* root was obtained from Sayinna, about 50km south of Sokoto, Nigeria. The plant material was identified by a Taxonomist in the Botany Unit of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto (UDUS) and a voucher specimen were prepared and deposited in the Herbarium of the same Department. The root was dried in a shade, pulverized into powder. The powdered root was kept in plastic bags in desiccators until required.

Preparation of Aqueous Crude Extract: The powdered plant material was soaked in cold distilled water for 48 hours, after which it was filtered using a piece of clean , sterile , white Muslin cloth to remove debris and filtered on a Whatman No. 1 filter paper. The filtrate was concentrated, the percent recovery calculated and the extract reconstituted in distilled water at 10% (w/v). The reconstituted extract was labelled crude aqueous extract and stored in small, capped plastic containers at +4^{o}C until required.

Fractionation of Aqueous Crude Extract: The 10% reconstituted extract was subjected to further fractionation by extracting with equal volume of different organic solvent successively starting with hexane, petroleum ether and chloroform in a separation funnel, vortexed vigorously and separated into two layers. The extracts were labelled hexane (HXN), petroleum ether (PTE) and chloroform (CHL) extracts respectively and the remaining aqueous extract was labelled last water extract (LWE). The extracts were evaporated in a rotary evaporator and reconstituted in distilled water at 10% (w/v) and stored at +4 ^{o}C until required. Each of the fractions was tested for hypoglycaemic and hypolipidaemic effects.

Experimental Animals: Seventy albino rats, weighing 100-130g, age 10-12 weeks were purchased from National Veterinary Research Institute Vom Jos, Nigeria. They were housed in metal cages in the Research Laboratory of Biochemistry Department, Usmanu Danfodiyo University, Sokoto. The rats were maintained on standard rat chow (Neimeth livestock feed, Ikeja, Nigeria) *ad libitum* and allowed free access to drinking water throughout the experimental period.

The Rats were then randomly divided into ten groups of seven (7) rats each:

1. Normal Untreated (NU)
2. Diabetic Untreated (DU)
3. Diabetic Treated with 100 mg/kg body weight of Aqueous Extract (DT_{100})
4. Diabetic Treated with 200 mg/kg body weight of Aqueous Extract (DT_{200})
5. Diabetic Treated with 300 mg/kg body weight of Aqueous Extract (DT_{300})
6. Diabetic Treated with 100 mg/kg body weight of HXN Extract (DTH)
7. Diabetic Treated with 100 mg/kg body weight of PTE Extract (DTP)
8. Diabetic Treated with 100 mg/kg body weight of CHL Extract (DTC)

9. Diabetic Treated with 100 mg/kg body weight of LWE Extract (DTL)
10. Diabetic Treated with 3.57mg/kg body weight of a sulphonylurea, Chlorpropamiade (DTS).

Preparation of diabetic rats: Diabetic was induced by injecting alloxan monohydrate dissolved in sterile normal saline solutions intraperitoneally to the rats at a dose of 80mg/kg body weight/day for 3 consecutive days (Stanely *et al.,* 2000). One week, after the last dose, serum glucose levels of the rats were assayed using glucose oxidase (Trinder, 1969) based glucometer and rats with moderate hyperglycaemia of 9-11 mM were considered diabetic and employed in the study.

Treatment of Rats: The treated groups were administered the extracts orally at appropriate doses per day, in the morning hours, for three weeks. The untreated groups were also administered 0.4ml of distilled water through the same route for three weeks. Weight changes of the rats were monitored throughout the experimental period. After the last dose, the rats were fasted for eight hours, anaesthesized in a chloroform vapor and blood samples collected from the animals through cardiac puncture, into labelled centrifuge tubes. The samples were allowed to clot and centrifuged in a bench top centrifuge at 3000 rpm for 5 minutes and serum collected into labelled sample bottles.

Serum Glucose and Lipid Profile Determinations: Serum glucose levels were estimated by the glucose oxidase method (Trinder, 1969) using Randox glucose oxidase kits in which glucose oxidase catalysed the oxidation of glucose to hydrogen peroxide (H_2O_2) and gluconic acid. The H_2O_2 is broken down by perosidase and the oxygen released was reacted with 4-aminophenazone (4-aminoantipyrine) and phenol to produce a pink coloured quinoneimine complex that was measured spectrophotometrically at 500 nm (Trinder, 1969).

Serum level of cholesterol was determined by the method of Zlatkis *et al. (*1953) in which cholesterol esters in serum are hydrolysed by cholesterol ester hydrolase. The total cholesterol was then oxidized by cholesterol oxidase to the corresponding ketone, with a shift in the location of a double bond. The H_2O_2 formed is decomposed by peroxidase in the presence of 4-aminoantipyrine and phenol to yield a quinoneimine dye. The absorbance of the dye was measured spectrophotometrically at 500 nm. Serum HDL-C by the method of Lopes-Virella., *et al., (*1977) in which LDL and VLDL cholesterols were precipitated using phosphotungstate reagent and magnesium chloride. The triacylglycerols are estimated after an enzymatic hydrolysis with lipases (Trinder, 1969). The indicator is quinoneimine formed from H_2O_2, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase (POD. The absorbance was read spectrophotometrically at 500 nm. Low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) were calculated using Friedwald formula (Friedwald *et al.,* 1972). The ratio of LDL-cholesterol to HDL cholesterol, atherogenic index (Aix), which measures the risk of development of atherosclerosis was also calculated (Murray *et al*., 1996).

Statistical Analysis: Data were expressed as mean ± standard deviation of the mean of seven animals. The results were analyzed using one way analysis of variance (ANOVA). Post Hoc test was also conducted to determine the level of significance between the groups using Turkey-Kramer Multiple Comparisons Test. Values with P-values less than 5% were considered significant.

RESULTS

Weight changes: Table 1 show the weight changes of the alloxan-induced diabetic rats treated with aqueous root extract of *A. chevalieri* for three weeks. The result indicated that alloxan injection decreased body weight of the animals. Treatment with the extract however reversed the decrease in weights of the rats as a result of alloxan injection.

Table 1: Weight of Rats (g) during the experimental Period.

Group	Before Alloxan Injection	After Alloxan Injection			
		Day Zero	1st week	2nd week	3rd week
DU	132±5.4	130±20.1	130±4.03	129±20.89	131±27.65
NU	132±0.96	133±13.45	134±11.93	136±11.62	144±26.13
DT_{300}	132±20.89	130±13.45	130±15.59	134±29.80	138±8.98
DT_{200}	132±2958	130±24.35	132±11.73	134±24.35	136±22.03
DT_{100}	132±14.79	130±14.00	130±40.30	134±6.50	134±24.17
DTS	132±8.98	130±14.00	131±11.76	131±11.75	136±40.30
DTH	132±24.35	130±40.30	133±14.79	130±24.35	140±6.50
DTP	132±25.46	130±6.50	131±22.03	132±20.89	138±24.17
DTC	132±4.03	130±24.17	130±24.35	130±13.35	138±24.35
DTL	132±20.89	130±5.72	130±8.98	134±29.80	138±24.35

Values are expressed as mean ± standard deviation of n=5; NU = Normal Untreated; DU = Diabetic Untreated; DT_{100} = Diabetic Treated with 100 mg/kg body weight of Aqueous Extract; DT_{200}=Diabetic Treated with 200 mg/kg body weight of Aqueous Extract; DT_{300}= Diabetic Treated with 300 mg/kg body weight of Aqueous Extract DTH= Diabetic Treated with 100 mg/kg body weight of HXN Extract ; DTP = Diabetic Treated with 100 mg/kg body weight of PTE Extract; DTC =Diabetic Treated with 100 mg/kg body weight of CHL Extract; DTL= Diabetic Treated with 100 mg/kg body weight of LWE Extract; DTS = Diabetic Treated with 3.57mg/kg body weight of a sulphonylurea, Chlorpropamiade.

Hypoglycaemic activity: The aqueous crude and organic solvent fraction of the plant material caused significant (P<0.05) decrease in serum glucose level of the alloxan induced diabetic rats (Table 2). The results of serum glucose of alloxan induced diabetic rats indicated that the antihyperglycaemia caused by aqueous root extract of *A. chevalieri* is dose dependent. The organic solvent fractions of the extract, equally caused decrease in serum glucose level with Chloroform fraction having the most hypoglycaemic effect (6.88mmol/L) (Table 2) which was comparable to that of the rats treated with a sulphonylurea, chlorpropamide (6.60mmol/L) (Table 2).

Effect of extract on lipid profile: Table 3 showed the serum levels of total cholesterol (TC), triacylglyceride (TAG), HDL-cholesterol , LDL-cholesterol and VLDL-cholesterol levels of rats treated with the organic solvent extracts of the root extract *A. chevalieri*. The HXN and PTE fractions caused significant decrease (P<0.05) in TC and TAG. The results were comparable with those of the rats treated With chlorpropamide (Table 3). The result of the rats treated with CHL and LWE indicated that the extracts increased significantly (P<0.05) the serum level of HDL-C (Table 3).

Table 2: Serum glucose levels (mmol/L) of rats treated with root extracts of *A. chevalieri*.

Group	Glucose	% Blood Glucose Reduction
DU	9.16± 0.06[a]	
NU	5.11± 0.15	
DT_{300}	6.96±0.3**	24.08
DT_{200}	7.86±0.2*	14.19
DT_{100}	8.27. ±0.2	10.91
DTH	6.98 ± 0.78**	23.80
DTP	7.50±0.01*	10.91
DTC	6.88±0.11**	24.89
DTL	7.71±0.14*	15.89
DTS	6.60 ±0.30**	27.95

Values are express as mean ± standard deviation of n=5; Values bearing *, ** and *** are significantly different from the DU at P<0.05, 0.01 and 0.001 respectively; [a] indicates a significant difference (P<0.001) from NU

Table 3: Serum lipid profile (mg/dl) of albino rats treated with organic solvent fractions of *A. chevalieri*.

Group	Cholesterol	Triacylglyceride	HDL-C	LDL-C	VLDL-C	A i x
DU	115.96±3.90	98.33±4.04*	43.37±11.12**	27.28±1.06	19.67±0.8*	0.63±0.05
NU	86.06± 1.20*	72.60±10.21	71.48±6.40**	16.03±6.54*	14.52±2.04	0.25±0.02
DTS	54.79±2.77***	61.41±9.30**	30.44±6.02***	13.85±2.52**	12.28±1.94*	0.45±0.04
DTH	70.05±1.57**	53.72±9.70***	46.00±0.61	12.81±0.32**	10.74±1.94*	0.27±0.05
DTP	89.45±4.15*	60.80±12.52**	41.11±9.94	41.62±2.83**	12.16± 2.50*	1.01±0.14***
DTC	107.15±1.38	82.03±7.91	74.67±6.30**	10.53±3.00**	16.41±1.58	0.14±0.08
DTL	99.39 ±2.27	85.58 ±9.69	71.48± 6.4**	14.36± 0.67**	17.12±1.94	0.20±0.06

Values are expressed as mean ± Standard deviation of n=5.; *p<0.05; ** p<0.01; ***p<0.001; HDL-C = High density lipoprotein cholesterol; LDL-C = Low density lipoprotein cholesterol; VLDL-C = Very low density lipoprotein cholesterol;and Aix=Atherogenic Index

Discussion

Insulin, which is produced by the β-cell of the pancreas is known to stimulate lipogenesis and protein synthesis. As a result of the destruction of the β-cells by alloxan, insulin/glucagon ratio decreased. This effect has lipolytic activity and causes diminished entry of amino acid into muscle cell, which may explain the observed weight loss. The weight of the animals improved during the treatment, with the extract administration at the second and third week. Highest improvement was recorded at the third week (Table 1).

The result of the current study showed that the aqueous crude root extract of *A. chevalieri* lowered fasting serum glucose level of treated rats in a dose dependent manner. The organic fractions equally lowered the fasting serum glucose in treated rats with chloroform fraction been the most potent (Table 2) and this is comparable to 300mg/kg body weight of aqueous organic solvent extract and Chlorpropamide (3.57mg/kg). These suggest that *A. chevalieri* root may possess an active hypoglycaemic principle(s). The aqueous extract of *A. chevalieri* was partitioned between different organic solvents and the extract obtained were used for hypoglycaemic and hypolipidaemic testing. The results of the effects of these extracts on serum glucose and lipid profiles of both alloxan induced and normal rats as presented in Tables 2 and 3 showed that the root extracts of *A. chevalieri* possesses significant hypoglycaemic and hypolipidaemic effects and that the agent(s) may be partitioned in the hexane and petroleum ether fractions. It is a well established fact that 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 (Harborne, 1973; Hostettmann *et al.,* 1995). A number of medicinal plants have been reported to possess antidiabetic properties. These include *A. hypogaea (*Bilbis *et al.,* 2002), *M. charantia* (Akhtar *et al.,* 1981), *M. cymbalaria* (Kameswara *et al.,* 1999), *P. graatum* (Jafri *et al.,* 2000), *Z. gaetulum* (Jaouhari, *et al.,* 2000), *C. colocynthis (*Abdel-Hassan *et al.,* 2000) and *T. foenum* (Ajabnoor *et al.,* 1988).

Leaf extract of *A. chevalieri* has been reported to possess hypoglycaemic effect and stimulate glycogen synthesis (Saidu *et al.,* 2007a). The hypoglycaemic effects of root extract of *A. chevalieri* recorded in the present study may be due to one or more phytochemicals present in the root extract.

The chloroform fraction lowered T-cholesterol and LDL-cholesterol but increased HDL-cholesterol in the group treated which will be good in the management of cardiovascular disease. Hexane fraction caused decrease in serum Total Cholesterol and LDL-cholesterol, which may be promising in reducing the risk of cardiovascular disease. According to Mayne (1996) there is a positive correlation between the risks of developing ischemic heart disease and raised plasma total cholesterol and LDL-cholesterol concentrations. One of the risk factors of diabetes mellitus is metabolic syndrome, which includes among others hpercholesterolaemia and hypertriglyceridaemia. The ability of the extract to reduced not only serum glucose but also total cholesterol and triacylglyceride may therefore be of significant importance. The work thus, also underscores the potential of the root extract

of *A. chevalieri* as a source of lead compound(s) in the management of diabetes mellitus.

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