Journal of Pharmacology and Toxicology

ISSN 1816-496X

Academic Journals Inc.

www.academicjournals.com

Journal of Pharmacology and Toxicology 2 (6): 513-523, 2007
ISSN 1816-496X

Partial Purification and Elucidation of Mechanism of Hypoglycaemic Agent of Aqueous Leaf Extract of *Albizzia chevalieri* Harms (Leguminosae)

[1]Y. Saidu, [1]M. Lawal, [2]S.A. Isezuo, [1]R.A. Shehu, [1]D.M. Sahabi and [1]L.S. Bilbis
[1]Department of Biochemistry,
[2]Department of Medicine, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria

Abstract: This research studied the hypoglycaemic effect of aqueous leaf extract of *Albizzia chevalieri* in alloxan-induced diabetic albino rats, using activity-guided fractionation. Preliminary elucidation of the mechanism of hypoglycaemic activity was also studied. The crude aqueous leaf extract of the plant (100 mg kg^{-1} body weight) reduced blood glucose levels of both the diabetic and normal rats by about 30%. The hypoglycaemic agent(s) were fractionated in the hexane fraction of the aqueous extract and partitioned in the second elution fraction (H_2) by column chromatography. Thin layer chromatography of H_2 gave a single spot with water as the mobile phase. The preliminary results of the mechanism of action indicated that the extract did not affect the *in vivo* digestion of carbohydrate or intestinal absorption of glucose. It however caused significant ($p<0.05$) increase in the hepatic and extrahepatic glycogen store. Phytochemical screening of the crude extract indicated the presence of saponins, flavonoids, tannins, terpenes, steroids, balsams, glycosides and alkaloids. UV/visible spectral studies of H_2 indicated a λ_{max} of 320 nm. These results suggest that the hypoglycaemic effect of the extract is as a result of induction of glycogenesis.

Key words: Hypoglycaemia, purification, mechanism, glycogenesis, *Albizzia chevalieri*

INTRODUCTION

Diabetes mellitus is a heterogeneous group of disorders 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 populations throughout the world (WHO, 1994). The prevalence of diabetes for all age groups worldwide was estimated at 2.8% in the year 2000. This is projected to reach 366 million (4.4%) of the world population by the year 2030 (Sarah *et al*., 2004; Wild *et al*., 2004). The alarming increase in the prevalence may not be unconnected to life style changes associated with urbanisation and industrialization (Sobngwi *et al*., 2001). Diabetes mellitus is associated with increased risk of vascular, renal, retinal and neuropathic complications that may lead to premature disability and death (WHO, 1994).

Presently the disorder has no known cure but could be adequately controlled by the use of agents that exhibit hypoglycaemic effect. Insulin is the most popular and the most effective of such agents, especially for the management of type 1 or insulin dependent diabetes mellitus (IDDM). The mode of administration of insulin, which is currently parenteral, is difficult, inconvenient and expensive for most patients (Onoagbe *et al*., 1999). These make the use of insulin, in the management of diabetes mellitus unattractive and beyond the reach of most patients, especially in the developing countries including Nigeria (Onoagbe *et al*., 1999). A good number of oral hypoglycaemic agents are also

Corresponding Author: Y. Saidu, Department of Biochemistry, Usmanu Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria Tel: +234-803-613-1987 Fax: +234-60-235199

available in the market. These include sulphonylureas, biguanides, thiazolidinedione insulin sensitizer, insulin secretagogues and α-glucosidase inhibitors (Evans, 1999). Most of these agents are at disadvantage of either being too expensive or associated with some undesirable side effects (Jaouhari *et al*., 2000; Kameswara *et al*., 1999).

Based on the problems associated with the available therapeutic agents for the management of diabetes mellitus, WHO Study Groups (WHO, 1994) recommend 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 and interrupting pathways leading to the various complications of diabetes. These reports further recommend the evaluation of the efficacy of traditional medicine and non-pharmacological methods in use for the management of diabetes. These recommendations and the cost and side effects of most hypoglycaemic agents, stimulated an increase demand for natural products with antidiabetic activity and fewer side effects (Kameswara *et al*., 1999). The most promising of such products are of plant origin (WHO, 1994). Indigenous remedies for the management of diabetes mellitus have been in use as far back as the 6th century (Kameswara *et al*., 1999). A number of plants with acclaimed anti-diabetic properties are being studied in different laboratories throughout the world, especially in developing countries. Many recent reports have demonstrated hypoglycaemic effects of some plant extracts (Kato and Miura, 1994; Kameswara *et al*., 1999; Joy and Kuttan, 1999; Pari and Maheswri, 1999; Jaouhari *et al*., 2000; Stanely *et al*., 2000; Jafri *et al*., 2000; Perez *et al*., 2000; Alarcon-Aguilar *et al*., 2000). These extracts are therefore potential sources of anti-diabetic agents.

Albizzia chevalieri Harms belongs to the large genus of trees, *Fabaceae*, native to warm regions of the Old World. The alternate, compound leaves are bipinnate. The leaf of *A. chevalieri* is used, either as cold-water decoction or dried, ground and mixed with pap, for the management of diabetes mellitus by traditional medical practitioners in some part of Niger Republic and Sokoto, Nigeria (Moussa, 2004; Personal communication).

The current study reports the hypoglycaemic effect of the aqueous leaf extract of *A. chivalieri*. It also reports partial purification of the hypoglycaemic agent(s) and mechanism of hypoglycaemic effect of the aqueous leaf extract of the plant.

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 Materials

A. chivalieri was obtained from a suburb, about 50 km south of Sokoto, Nigeria in February 2004 and identified by a Taxonomist from the Botany unit of the Department of Biological Sciences, Usmanu Danfodiyo University Sokoto, Nigeria. Voucher specimen was prepared and deposited in the Herbarium of the same Department with voucher No. UDUS/VS/04/09. The leaves were sun dried, ground using laboratory pestle and mortar and sieved with a 1 mm^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 for 24 h. 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 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°C until required. This was used for the preliminary hypoglycaemic activity and mechanism of action studies.

Extraction, Separation and Partial Purification of Hypoglycaemic Agent(s)

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 using separation funnel. The fractions were labelled hexane, petroleum ether and chloroform fractions respectively and the remaining aqueous extract was labelled last remaining water extract. These extracts were concentrated in a Rotary Evaporator and reconstituted in distilled water at 10% (w/v).

The extract with the highest relative hypoglycaemic activity was subjected to further separation and purification using column chromatography on silica gel mesh size 60-200 (Harborne, 1973). The fractions obtained were labelled H_1, H_2 and H_3 for the hexane fractions. The subscript numbering was in order of elution from the column.

Animals

Apparently healthy albino rats of both sexes, purchased from National Institute for Trypanosomiasis Research (NITR) Vom-Jos, Nigeria, allowed to acclimatise to the laboratory environment for a week during which they were allowed free access to clean tap-water and chow *ad libitum* throughout the experimental period. For each set of hypoglycaemic test, the rats were divided into two groups: normal and alloxan monohydrate induced diabetic rats.

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 80 mg kg^{-1} body weight/day for 3 consecutive days, intraperitoneally (Kato and Miura, 1994; Pari and Maheswari, 1999; Kamaswara *et al*., 1999; Stanely *et al*., 2000). After a week, from the last dose, animals with moderate hyperglycaemia with blood glucose range of 11-14 mM were considered diabetic.

Testing for Hypoglycaemic Effect of Extracts

There were four groups for each extract: alloxan induced diabetic treated, alloxan-induced diabetic untreated, normal treated and normal untreated. Each group was allocated five rats. The treated groups in all cases were administered the extracts orally at 100 mg kg^{-1} body weight per day, in the morning hours, for one week. The untreated groups, in each case, were administered 0.4 mL distilled water through the same route for a week. The rats were weighed before the commencement of alloxan injection, before the commencement of treatment and 24 h after the last treatment. Twenty four hours after the last treatment, fasted rats were anaesthesized in a chloroform vapour and blood samples collected from the animals through cardiac puncture, into labelled centrifuge tubes.

Serum Glucose and Lipid Profile Determination

The blood samples were centrifuged in a bench-top centrifuge at 3000 rpm for 5 min and serum collected. Serum glucose level was estimated by the glucose oxidase method (Trinder, 1969; Barham and Trinder, 1977) using Randox glucose oxidase kits.

Liver and Muscle Glycogen Determination

Two groups of three albino rats each labelled test and control were fasted for 36 h. The animals in the test group were administered 100 mg kg^{-1} body weight of the pure hypoglycaemic compound orally per day. Twenty four hours after the last administration the animals were anaestisized with

chloroform vapour in a gas jar. The animals were dissected and the liver and femur skeletal muscle excised and assayed for glycogen content based on a coloured reaction reported by Kemp and Van Heijninger (1954).

Intestinal Carbohydrate Digestion

The effect of the active principle on the intestinal digestion was studied by monitoring the feed intake and faeces output of the animals used for liver and muscle glycogen determination. The feed and faeces were subjected to proximate composition determination and the percentage carbohydrate absorbed was calculated from the difference in carbohydrate consumed and excreted (AOAC, 1975).

Intestinal Glucose Absorption

A modified oral glucose tolerance test was used for estimation of intestinal glucose absorption. Three rats each per group were fasted for 36 h before the administration of glucose. The blood glucose was measured using one touch glucometer. The tip of the tail of each of the animal was sterilized using methylated spirit and cut slightly using a sterilized sharp scissors. The administered solution was made up of the following (in g L^{-1}): 7.37 NaCl, 0.20 KCl, 0.065 $NaH_2PO_4.6H_2O$, 1.02 $CaCl_2$, 0.6 $NaHCO_3$ and 54.0 glucose at pH 7.5. The extract was added at 100 mg kg^{-1} body weight of the animal to the administered solution. In the control group the administered solution contained no extract. The blood glucose was monitored at 30 min interval for 120 min hours, with baseline at time zero. The blood glucose was recorded as percentage of the baseline glucose level.

Peripheral Glucose Consumption

This was studied using rat diaphragm preparations from animals fasted for 36 h before sacrifice. The diaphragms were incubated at 37°C with constant oxygenation for 90 min and shaking at 90 cycles min^{-1}. The nutrient solution was made up of 125 mL of 13 % $NaHCO_3$ (aerated for 3 min) and 750 mL of saline solution. The resultant solution was aerated for 10 min and used immediately. The saline solution was made up of the following (in g L^{-1}): 9.5 NaCl, 0.40 KCl, 0.30 $CaCl_2$, 0.35 $NaHCO_3$, 0.35 $MgSO_4.7H_2O$ and 0.20 KH_2PO_4. Glucose was added to a final concentration of 300 mg dL^{-1}. The amount of glucose consumed was calculated as mg glucose consumed/min/g fresh tissue. The glucose concentrations of the incubating media before and after incubation were used for the calculation of glucose consumed. The influence of the hypoglycaemic agent(s) was studied by incubating the diaphragm in the presence (test) and absence (control) of 1% (w/v) of the agent(s) in the incubating solution (Medina *et al*., 1994). Glycogen concentration of the tissues was also determined.

Qualitative Phytochemical Analysis

The aqueous extracts of the medicinal plants used in the current study and the second elution fraction (H_2) of the hexane fraction of the aqueous leaf extract of *A. chevalieri* were screened for the presence of various phytochemicals using the methods described in Harborne (1973) and El-Olemy *et al.* (1994).

Ultraviolet/visible Spectra Studies

The absorption spectrum of the apparently pure fraction (H_2) was determined using HEλOSα UV-Visible spectrophotometer V 2.5. The spectrum was plotted between 200-700 nm at bandwidth of 2.0 nm changing the lamp at 325 nm.

Statistical Analysis

The results are expressed as mean±standard error of the mean of five animals. The results for the hypoglycaemic effects were analysed using ANOVA. Post Hoc Tests Multiple Comparisons using

LSD was utilized to identify differences in means. The results of the mechanism of action were also analysed using student t-test. In all cases SPSS windows version 10 was employed for the analysis.

RESULTS

Effect of Aqueous Leaf Extract of *A. chevalieri* on Body Weight Changes of Diabetic and Normal Rats

The changes in body weights of normal and alloxan-induced diabetic rats treated with aqueous extract of *A. chivalieri* are presented in Fig. 1. The result indicated that alloxan injection significantly ($p<0.05$) decreased body weight of the animals. Treatment with the extract however resulted in significant ($p<0.05$) improvement in the body weights.

Effect of Aqueous Leaf Extract on Serum Glucose Level of Diabetic and Normal Rats

Table 1 shows the serum glucose of rats treated with the aqueous extract of *A. chevalieri*. Intraperitoneal injection of alloxan induced significant ($p<0.05$) hyperglycaemia in the experimental animals. Treatment with the water extract of the leaf caused significant ($p<0.05$) decrease in the blood glucose levels of both diabetic and normal rats.

Fractionation of the Aqueous Leaf Extract of *A. chevalieri*

The aqueous extract was partitioned between different organic solvents and the results of the effects of these extracts on serum glucose of both alloxan-induced diabetic and normal rats showed that the hypoglycaemic agent(s) is partitioned in the hexane fraction of the extract (Table 2).

The hexane fraction was fractionated into three (3) fractions on column chromatography and the second fraction (H_2) had highest hypoglycaemic effect (Table 3).

Effect of the Aqueous Leaf Extract on Muscle and Liver Glycogen and *in vivo* in Carbohydrate Digestion

The results indicated that liver glycogen g^{-1} fresh tissue was significantly ($p<0.05$) higher than the muscle glycogen (Table 4). The liver glycogen of the animals treated with the leaf extract of *A. chevalieri* was about twice as high as the value obtained for the untreated group. There was no significant difference ($p>0.05$) in the muscle glycogen of the treated and untreated groups. The results of the % *in vivo* carbohydrate digestion indicated no significant difference ($p>0.05$) between the animals that were treated with the extract and those that were not.

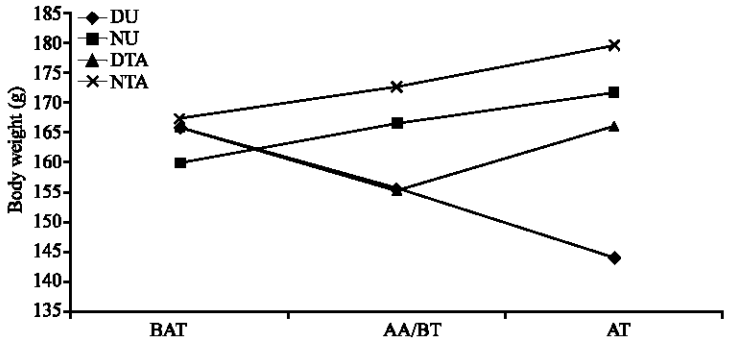

Fig. 1: Changes in growth of normal and diabetic rats treated with *A. sieberana*, DU: Diabetic untreated rats, NU: Normal untreated rats, DTR: Diabetic treated with *A. chevalieri*, NTR: Normal rats treated with *A. chevalieri*

Table 1: Serum glucose of rats treated with crude water extracts of *A. chevalieri*

Group	Serum glucose (mmol L^{-1})
NU	6.46±0.60[a]
DU	11.43±0.4[0b]
NTA	4.68±0.47[c]
DTA	7.84±0.67[d]

NU Normal untreated rats, DU diabetic untreated, NTA normal rats treated with *A. chevalieri*; DTA diabetic rats treated with *A. chevalieri* , a,b,c,d: values bearing different superscript horizontally differ significantly ($p<0.05$)

Table 2: Serum glucose (mmol L^{-1}) of albino rats treated with organic solvents fractions of the aqueous extract of *A. chevalieri*

Group	Normal	Diabetic
Untreated (Water)	6.46±0.60	11.43±0.4
Hexane	3.85±0.91*	5.16±1.28*
Petroleum ether	5.13±1.24	8.58±1.51*
Chloroform	5.13±0.47	10.35±6.54
Last water extract	6.23±0.53	10.19±1.29

*Values bearing asterisk differ significantly ($p<0.05$) from the respective untreated groups

Table 3: Serum glucose (mmol L^{-1}) of albino rats treated with column chromatographic fractionated hexane and petroleum ether fractions of the aqueous extract of *A. chivalieri*

Group	Normal	Diabetic
Untreated (Water)	6.46±0.60	11.43±0.40
H_1	5.59±0.66	8.32±5.80
H_2	1.97±0.23*	3.39±0.63*
H_3	4.05±0.63	8.16±5.15

*Values bearing asterisk differ significantly ($p<0.05$) from the respective untreated group

Table 4: Liver and muscle glycogen (glucose equivalent) and % intestinal carbohydrate digestion of albino rats treated with *A. chevalieri*

Group	Test	Control
Liver (mg g^{-1} fresh tissue)	49.68±12.2[a]	28.44±1.02[b]
Muscle (mg g^{-1} fresh tissue)	15.65±1.03[c]	13.05±1.01[c]
Carbohydrate digested (%)	70.48±4.53	68.47±8.40

Values are mean±standard error of mean of 5 animals, a,b, c: values bearing different superscripts differ significantly

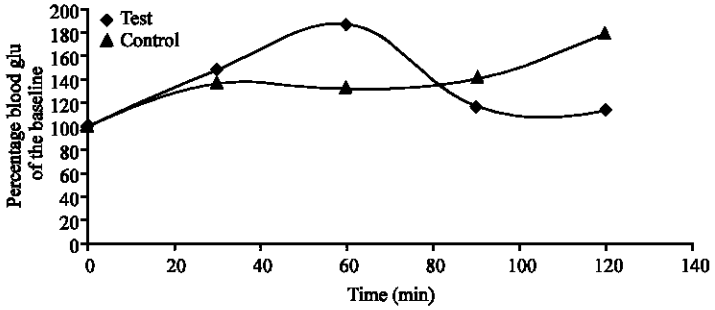

Fig. 2: *In vivo* intestinal glucose absorption of rats treated with *A. chevalieri*

The Effect of the Extract on *in vivo* Glucose Absorption and Peripheral Glucose Utilization

The *in vivo* effect of the extract on glucose absorption is presented in Fig. 2. The results indicated that the extract increased the rate of absorption of glucose from the intestine and glucose clearance from the blood.

The results indicated that although there was no significant difference ($p>0.05$) in glucose consumed, the amount of glycogen formed by the tissue in the presence and absence of the extract differs significantly ($p<0.05$) (Table 5).

Table 5: *In vitro* peripheral (diaphragm) glucose utilization in the presence and absence of *A. chevalieri*

Group	Glucose consumed (mg/min/g fresh tissue)	Glycogen formed (mg glucose equivalent g^{-1} fresh tissue) in 90 min
Test	0.13±0.023[a]	13.14±0.61[a]
Control	0.14±0.021[a]	5.38±0.80[b]

Values are mean±standard error of mean of 5 animals, a,b: values bearing different superscript vertically differ significantly ($p<0.05$)

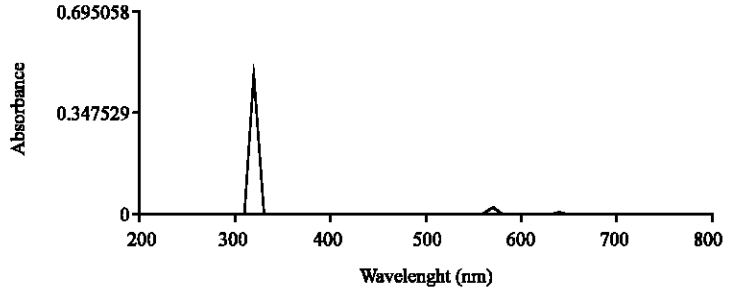

Fig. 3: UV visible is ible absorption spectrum of apparently pure hypoglycaemic agent of *A. chevalieri*

Phytochemical Screening of the Aqueous Extract

The results of phytochemical screening of the crude extract indicated the presence of saponins (++), tannins (+), terpenes (+), steroids (+), balsams (+), alkaloids (+), flavonoids (++) and glycosides (+).

Spectral Studies of the Active Agent of *A. chevalieri*

The UV/Visible absorption spectrum of the H_2 fraction was taken and presented in Fig. 3. The spectrum indicated the highest peak at 320 nm and the lowest at 620 nm with an intermediate peak at 570 nm.

DISCUSSION

Reduction in body weight as a result of alloxan treatment may not be unconnected to the destruction of the β cells in the diabetic animals. Insulin, which is produced by these cells, is known to stimulate lipogenesis and protein synthesis. As a result of the destruction of the β cells insulin: glucagon ratio decreases. This effect has lipolytic activity and cause diminished entry of amino acid into muscle cell, which may explain the observed decrease in weight in the diabetic animals. One of the symptoms of diabetic mellitus is weight loss in spite of adequate caloric intake (Murray *et al*., 1996). The improvement in weight of the rats as a result of treatment may be due to the reversal of these processes. Chemical compounds that selectively damage the pancreatic β cells are used as diabetogenic drugs. Alloxan, a cyclic derivative of urea is one of such compounds (Rho *et al*., 2000). Its ability to damage β cells is related to the generation of cytotoxic reactive oxygen species (Takasu *et al*., 1991). The beta cells are sensitive to oxidative stress because of their weak intracellular antioxidant defence mechanisms (Rasilainens *et al*., 2002). Oxidative stress is thus, suggested to be a potential contributor to the development of diabetes mellitus and the associated complications (Mohamad *et al*., 2004). The observed hyperglycaemia as result of alloxan administration in the current study may not be unconnected to the destruction of the β cell by this agent resulting in non/decreased production of insulin. There are special transport channels in cell membranes through which glucose from the blood can enter a cell. The most important of these transporters as far as insulin stimulated glucose homeostasis is concerned is the GLUT-4, otherwise called insulin sensitive glucose transporter,

uniquely expressed in skeletal muscle, cardiac muscle and adipose tissue (Olefsky, 1999). These channels are, indirectly, under insulin control. Lack of circulating insulin or insulin insensitivity prevent glucose from entering those cells (as in the case of diabetes mellitus). In either of these cases, there is cell starvation ironically associated with hyperglycaemia.

Treatment of the normal and alloxan-induced diabetic rats with aqueous extract of *A. chevalieri* caused a significant ($p<0.05$) reduction in serum glucose level when compared with the normal and diabetic untreated rats respectively (Table 1). This suggests that the extract contains a hypoglycaemic principle(s).

In the current study, activity guided fractionation of the active agent on the basis of its biological activity, rather than fractionation based on a particular class of compound (Hostettamnn *et al*., 1995; Evans, 1999) was used with outstanding results. During the fractionation the efficacy of the *hypoglycaemic agent* increased from about 30% to above 70%. The fraction 2 of the hexane fraction of the aqueous extract, which is the fraction with the highest hypoglycaemic activity, had only one component on TLC using silica gel as the stationary phase and water as the mobile phase. This may be an indication that the active hypoglycaemic agent might have been successfully fractionated.Anumber of plant extracts have been reported to possess significant hypoglycaemic effect. Bijan *et al*. (2003) reported that leaf extract of *Urtica dioica* lower serum glucose level of both normal and streptozotocin induced diabetic rats. Other plants reported with hypoglycaemic effect comparable to the leaf extract of *Albizzia chevalieri* include *Momordica cymbalaria (*Kameswara *et al*., 1999), *Arachis hypogaea* (Bilbis *et al*., 2002) and *Stevia rebaudiana* (Jeppensen *et al*., 2003). Teixeira *et al*. (2000) however reported that a tea prepared from the leaves of *Syzygium cumini* and *S. jambos* could not induced antihyperglycaemia in streptozotocin and normal rat models.

The hypoglycaemic effect of *A. chevalieri* may be attributable to one or more of the numerous secondary plant metabolites found in the plant extract. The qualitative analysis of the extracts of the current study indicated the presence of compounds such as saponins, flavonoids, steroids, volatile oils and resins, alkaloids balsams, glycosides, cyanogenic glycosides and saponin glycosides. The UV/Visible spectral studies of F_2 fraction indicated that the active agent absorbs maximally at 320 nm with minor absorptions at 570 and 620 nm. This may suggests that the hypoglycaemic agent may contain benzene/phenol ring(s) with conjugated double bonds (Harborne, 1973). Hypoglycaemic effect of *Parmentiera edulis* has been attributed to one of the guaianolide, lactucin-8-0-methyl acrylate from the chloroform extract of the dry fruit (Perez *et al*., 2000). The blood glucose lowering component of the herbal prototype for biguanides, *Galega oficinalis* is a guanidine, structurally similar to metformin (Setter *et al*., 2003).

The preliminary results of the mechanism of action in the current study indicated that the extract did not affect the intestinal digestion of carbohydrate or intestinal absorption of glucose. It however exerts significant effect on the hepatic store of glycogen. Additionally, the extract caused significant ($p<0.05$) increase in glucose stored as glycogen by the extrahepatic tissue, diaphragm. This was reflected in the amount of glycogen stored by this tissue when incubated for 90 min in glucose solution containing the extract. There was however no significant ($p>0.05$) difference in glucose consumed by diaphragm over the 90 min incubation period, but the amount of glycogen stored in the tissues differed significantly ($p<0.05$) as a result of treatment. How the extract stimulates glycogen synthesis is unclear, but may be through the stimulation of glycogen synthase activity or UDP-glucose pyrophosphorylase. It may also be due to the stimulation of the phosphorylation of glucose by hexokinase. The fruit extract of *M. cymbalaria* has also been reported to induce hypoglycaemia by stimulating glycogenesis (Kameswara *et al*., 1999). It may also be through insulin mimetic activity of the extract. The mechanism of hypoglycaemic action of the extract may be similar to that of metformin, a biguanide. *Galega oficinalis* is the herbal prototype of the biguanides and was used to synthesize several antidiabetic compounds (Bailey and Day, 1989). In the peripheral tissues, metformin enhances

glucose transport and utilisation by skeletal muscle. It does this by improving the non-oxidative glucose disposal (including conversion of glucose to lactate and incorporation of glucose into triacylglycerols) and glycogen synthesis. An interesting aspect of the current result is the fact that the hypoglycaemic effect may not necessarily be due to the presence of circulating insulin. This assertion is due to the fact that the agent caused hypoglycaemia even in the alloxan-induced diabetic animals. Though there are reports suggesting that there is regeneration of β-cells following alloxan injection (Lazarow, 1952), in the current study, the treatment period was not long enough to warrant the regeneration. This is attested to by the fact that the diabetic untreated animals still had substantial hyperglycaemia at the termination of the experiment. Acarbose, an alpha glucosidase inhibitor, has been reported to improve glycaemic control of elderly overweight type 2 diabetic patients whose glycaemic control has been poor on other oral hypoglycaemic agents and insulin regimens (Sangiorgio *et al*., 2000). The current study ruled out the possibility of inhibition of the digestive enzymes and delayed absorption of glucose in rats as a result of treatment with *A. chevalieri*. The effect of the extract on insulin secretion was not studied in the current study. Some plant extracts are known to induced hypoglycaemia by induction of insulin secretion (Bijan *et al*., 2003).

It is apparent therefore to assert that *A. chevalieri* used by traditional medical practitioners for the treatment of diabetes mellitus possesses significant hypoglycaemia. The hypoglycaemic agent may be a phenolic compound that stimulates hypoglycaemia by inducing glycogen synthesis. These may confirm the claim of the practitioners that *A. chevalieri* has anti-diabetic property.

REFERENCES

Alarcon-Aguilar, F.J., M. Jimenez-Estrada, R. Reyes-Chilpa, B. Gonzalez-Paredes, C.C. Contreras-Weber and R. Roman-Ramos, 2000. Hypoglycemic activity of root water decoction, sesquiterpenoids and one polysaccharide fraction from *Psacalium decompostum* in Mice. J. Ethnopharmacol., 69: 207-215.

AOAC., 1975. Official Methods of Analysis. 12th Edn., In: Association of Official Analytical Chemists. Horowitz, W. (Ed.), Washington DC., pp: 546-579.

Bailey, C.J. and C. Day, 1989. Traditional plant medicines as treatments for diabetes. Diabetes Care, 12: 553-564.

Barham, D. and P. Trinder, 1977. Quoted in Cheesbrough, M. (1992). Medical Laboratory Manual for Tropical Countries, Vol. 1. 2nd Edn., ELBS, Cambridge, pp: 527-545.

Bijan, F., D. Ahmadvand, S. Vardasbi, F.J. Majin and Sh. Khaghani, 2003. Induction of insulin secretion by a component of *Urica dioica* leave extract in perfused islets of langerhans and its *in vivo* effects in normal and streptozotocin diabetic rats. Ethnopharmacology, 89: 47-53.

Bilbis, L.S., R.A. Shehu and M.G. Abubakar, 2002. Hypoglycaemic and hypolipidaemic effects of aqueous extract of *Arachis hypogaea* in normal and alloxan-induced diabetic rats. Phytomedicine, 9: 553-555.

El-Olemy, M.M., F.J. Al-Muhtadi and A.A. Afifi, 1994. Experimental Phytochemistry: A Laboratory Manual. King Saud University Press. Saudi Arabia, pp: 3-19.

Evans, W.C., 1999. Trease and Evans Pharmacognosy. 14th Edn., Saunders, London, pp: 5-7, 119-130, 438-440, 488-491.

Harborne, J.B., 1973. Phytochemical Methods, Chapman and Hall, London, pp: 1-32.

Hostettamnn, K., A. Marston and J.L. Wolfender, 1995. Strategy in the Search for New Biologically Active Plant Constituent. In: Phytochemsitry of Plants Used in Traditional Medicine. Hostettmann, K., A. Marston, M. Maillard and M. Hamburger (Eds.), Clarendon Press, Oxford, pp: 17-45.

Jafri, M.A., M. Aslam, K. Javed and S. Singh, 2000. Effect of *Punica granatum* lin (flowers) on blood glucose level in normal and alloxan-induced hyperglycemic diabetic rats. J. Ethnopharmacol., 70: 309-314.

Jaouhari, J.T., H.B. Lazrek and M. Jana, 2000. The hypoglycaemic activity of *Zypoghyllum gaetulum* extracts in alloxan-induced hyperglycemic rats. J. Ethnopharmacol., 69: 17-20.

Jeppensen, P.B., S. Gregersen, S.E.D. Rolfsen, M. Jepsen, M. Colombo, A. Agger, J. Xiao, M. Kruhoffer, T. Orntoft and K. Hermansen, 2003. Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism, 52: 372-378.

Joy, K.L. and R. Kuttan, 1999. Anti-diabetic activity of *Picrorrhiza kurroa* extract. J. Ethnopharmacol., 67: 143-148.

Kameswara, B.R., M.M. Kesavulu, R. Giri and Ch.A. Rao, 1999. Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook fruit powder in alloxan diabetic rats. J. Ethnopharmacol., 67: 103-109.

Kato, A. and T. Miura, 1994. Hypoglycemic action of the Rhizomes of *Polygonatum officinale* in normal and diabetic mice. Planta Med., 60: 201-203.

Kemp, A. and A.J.M.K.V. Heijningen, 1954. A colorimetric micro-method for the determination of glycogen in tissues. Biochem. J., 56: 64-648.

Lazarow, A., 1952. Spontaneous recovery from alloxan diabetes in the rat. Diabetes, 1: 363-372.

Medina, E.S.de., M.J. Gamez, L. Jiminez, J. Jimenez, J.I. Osuna and A. Zarzuelo, 1994. Hypoglycemic activity of Juniper Berries. Planta Med., 60: 197-200.

Mohamad, S., A. Taha, R.N.K. Bamezai, S.F. Basir and N.Z. Baquer, 2004. Lower doses of vanadate in combination with *Trigonella* restore altered carbohydrate metabolism and antioxidant status in alloxan-diabetic rats. Clin. Chim. Acta, 342: 105-114.

Moussa, Y., 2004. Traditional medicinal uses of *Albizzia chevaliri*. Personal Communication.

Murray, R.K., D.K. Granner, P.A. Mayes and V.W. Rodwell, 1996. Harper's Biochemistry. 24th Edn., Prentice-Hall International, USA, pp: 581-598.

Olefsky, J.M., 1999. Insulin stimulated glucose transport. J. Biol. Chem., 274: 1863.

Onoagbe, T.O., A.O. Ebhota, H.C. Udegbe, M. Omondia, D. Edeni and S.O. Ebengho, 1999. Assessment of some medicinal plants for hypoglycaemic activities in rats and rabbits. Biosci. Res. Commun., 11: 159-163.

Pari, L. and J.U. Maheswari, 1999. Hypoglycaemic effect of *Musa sapientum* L. in alloxan induced diabetic rats. J. Ethnopharmacol., 68: 321-325.

Perez, R.M., C. Perez, M.A. Zavala, S. Perez, H. Hernandez and F. Lagunes, 2000. Hypoglycaemic effects of lactucin 8-0-methylacrylate of *Parmentiera edulis* fruit. J. Ethnopharmacol., 71: 391-394.

Rasilainens, S., J.M. Nieminen, A.L. Levonen, T. Otonkoski and R. Lapatto, 2002. Dose dependent cysteine-mediated protection of insulin-producing cells damage by hydrogen peroxide. Biochem. Pharmacol., 63: 1297-1304.

Rho, H.W, J.N. Lee, H.R. Kim, B.H. Park and J.W. Park, 2000. Protective mechanism of glucose against alloxan-induced b-cell damage: Pivotal role of ATP. Exp. Mol. Med., 32: 12-17.

Sangiorgio, L., T. Attardo, L. Condorelli and M. Lunetta, 2000. Effects of the treatment with acarbose in elderly overweight type 2 diabetic patients in poor glycaemic control with oral hypoglycaemic agents or insulin. Arch. Gerontol. Geriatrics, 31: 27-34.

Sarah, W., R. Gojka, G. Anders, S. Richard and K. Hilary, 2004. Global prevalence of diabetes. Diabetes Care, 27: 1047-1053.

Setter, S.M., J.L. Iltz, J. Thams and R.K. Campbell, 2003. Metformin hydrochloride in the treatment of type 2 diabetes mellitus: A clinical review with a focus on dual therapy. Clin. Therapeutics, 25: 2991-3025.

Sobngwi, E., F. Mauvais-Jarvis, P. Vexiau, J.C. Mbanya and J.F. Gautier, 2001. Diabetes in Africans. Diabetes Metab., 27: 628-634.

Stanely, P., M. Prince and V.P. Menon, 2000. Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. J. Ethnopharmacol., 70: 9-15.

Takasu, N., T. Asawa, I. Komiya, Y. Nagasawa and T. Yamada, 1991. Alloxan-induced DNA strand breaks in pancreatic islets: Evidence for H_2O_2 as an intermediate. J. Biol. Chem., 266: 2112-2114.

Trinder, P., 1969. Annals of Clinical Biochemistry; 6, 24. In: Medical Laboratory Manual For Tropical Countries. Cheesbrough, M. (1992); Vol. 1. 2nd Edn., ELBS, Cambridge, pp: 527-545.

WHO, 1994. WHO Study Group Report on Prevention of Diabetes Mellitus (WHO Technical report Series No. 844).WHO Geneva, pp: 1-92.

Wild, S., R. Gojka, G. Anders, S. Richard and K. Hilary, 2004. Global prevalence of diabetes. Diabetes Care, 27: 1047-1053.