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Research article

Phytochemical Analysis and Evaluation of Antidiabetic Effects in Alloxan-Induced Diabetic Rats Treated with Aqueous Leaf Extract of *Acanthospermum hispidum*

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ABSTRACT

The current study is aimed at investigating the antidiabetic activity of the leaves of Acanthospermum hispidum, a medicinal plant traditionally used to treat diabetes mellitus in NorthWestern Nigeria. Material from the plant was extracted using water (guided by the traditional mode of extraction) and the phytochemicals contained in the extract were analyzed qualitatively and quantitatively. Acute toxicity study of the extract was conducted, and diabetic rats induced using alloxan (80 mg/kg administered intraperitoneally once daily for 3 days) were treated with the extract for 28 days at 3 incremental doses (70, 210 and 700 mg/kg). At the end of the experiment, fasting blood glucose and glycogen content of the liver and skeletal muscle were determined. The study findings indicate that the extract was relatively safe with LD_{50} above 5000 mg/kg. Treatment with Acanthospermum hispidum extract resulted in a significant ($P<0.05$) reduction in FBG as well as a significant improvement in oral glucose tolerance at all doses. A significant elevation in the hepatic content of glycogen was also observed at the highest dose (700 mg/kg). Results from this study have demonstrated the potent antidiabetic activity of the aqueous extract of the leaves of Acanthospermum hispidum, thus justifying the traditional claim.

Keywords: *Acanthospermum, phytochemicals, antidiabetic, LD_{50}, aqueous extract*

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INTRODUCTION

Type 2 diabetes is one of the leading causes of mortality and morbidity worldwide (Danaei *et al*., 2014) . The utility of the various available treatment options for the condition is limited by high cost, particularly in low-income countries (Seuring, 2015). Accordingly, the use of herbal products is very common among diabetic patients in resource-limited countries like Nigeria (Yusuff *et al*., 2008). Phytochemicals are major sources of drugs for treating various diseases afflicting Man. For example, the most frequently prescribed antidiabetic agent metformin was isolated from a plant species Galega officinalis (Rates, 2001).

Acanthospermum hispidum (Asteraceae), popularly called "kashin yawo" in Hausa, is used traditionally for managing diabetes in North-Western Nigeria. To our knowledge, no proper evaluation of the antidiabetic activity of the leaf extract of the plant has been published. The current study is therefore aimed at investigating the folkloric claim of the antidiabetic properties of the extract..

MATERIALS AND METHODS

Animals: Male albino Wistar rats (weighing 150–200 g), as well as non-pregnant female counterparts (weighing 110–120 g), were purchased from a research institute in Ibadan. Before the commencement of the experiments, the animals were acclimatized for 10 days and allowed free access to a pellet diet with water *ad libitum.* The study was approved by the Research Ethics Committee, Usmanu Danfodiyo University, Sokoto. The maintenance and handling of the rats were in line with the international guidelines for handling animals.

Drugs and Chemicals : Glibenclamide (Glanil®, Nigerian-German Company Plc., Otta, Nigeria) was obtained from a reputable Pharmaceutical store, and alloxan monohydrate was purchased from Burgoyne Burbidges & Co., Mumbai, India.

Plant Material: The plant was collected from a local herbalist in Illela and was identified at Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. A direct specimen (V/N/PCG/UDUS/AST/0001) of the plant was deposited at the herbarium. Fresh leaves of Acanthospermum hispidum were washed with distilled water, dried to constant weights and then crushed to a powder using an electric blender. The dried powder of the plant (100 g) was separately subjected to Soxhlet extraction with 1000 ml of distilled water at 60^0C over 12 hours. The resulting extract was filtered with Whatman No. 1 filter paper and the filtrate oven-dried at 50^0 - 55 ^{0}C, giving a yield of 3.8%.

Phytochemical Analysis : The qualitative analysis of the following phytoconstituents: alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, flavonoids and tannins was performed on the extract using standard protocols as described by Tiwari *et al*. (2011). The quantitative phytochemical analysis was conducted on the extract of *A. hispidum*. The quantity of alkaloids and tannins were determined according to the methods of Trease and Evans (1978). Saponins and cardiac glycosides were determined according to the methods of El-Olemy *et al*. (1994), while carbohydrates, flavonoids, steroids and phenols were quantified as described by Hedge and Hofreiter (1962), Bohm and Kocipai-Abyazan (1994), Okeke and Elekwa (2003) and Edeoga *et al*. (2005) respectively.

Acute Toxicity Study: Based on the recommendation of OECD guidelines (OECD, 2008)**,** female non-pregnant Wistar rats (weighing 110 – 120 g) were used to investigate the acute toxicity of the extract. Median lethal dose (LD50) of the extract was determined using a limit test dose of 5000 mg/kg (OECD, 2008).

Experimental Protocols

Assessment of Hypoglycemic Effect of the Extract in Normal Rats : Twenty-five normal male rats were randomly distributed to five groups of five rats each. After 8 hours of fasting, the rats were administered orally with the following drugs and extracts:
Animals in Group 1 (normal control) were administered with 10 ml/kg of distilled water.
Rats in group 2 (standard control) were treated with 0.60 mg/kg aqueous suspension of glibenclamide (Prince and Menon, 1999).
Groups 3, 4 and 5 consist of animals administered with escalating doses of aqueous leaf extract of Acanthospermum hispidum (at 70, 210 and 700 mg/kg) suspended in distilled water.

Assessment of Anti-hyperglycemic Effect of the Extract in Alloxan-Induced Diabetic Rats: Numbers were assigned to sixty male rats and then randomised. Rats corresponding to the first 6 random numbers were selected as non-diabetic control. Animals corresponding to the remaining 54 random numbers were subjected to diabetes induction through intraperitoneal injection of a freshly prepared aqueous solution of alloxan monohydrate at 80 mg/kg administered once daily for 3 days (Kato and Miura, 1994). Following each dose, glucose was added to drinking water of the animals as a preventive measure against the development of hypoglycaemia (Sebai *et al.*, 2013). One week after the last dose, hyperglycaemia was confirmed using a standardised glucometer, "On call plus glucometer" (ACON Laboratories, Inc. San Diego, CA 92121, USA) based on glucose oxidase. Rats with moderate diabetes, taken as fasting blood glucose of 198 – 252 mg/dl (11 – 14mmol/l) (Jaouhari *et al*., 2000), were used for the experiments. The successfully-induced rats were randomly divided into five groups (six animals each). The normal rats (group 1) were administered with distilled water (10 ml/kg) to serve as a non-diabetic control. Two groups of successfully induced diabetic rats were dosed with either distilled water (10 ml/kg) or an aqueous suspension of 0.60 mg/kg of glibenclamide (Prince and Menon, 1999) at 10 ml/kg. The remaining 3 groups were administered with 10 ml/kg of Acanthospermum hispidum extract (at incremental doses of 70, 210 or 700 mg/kg for the extract) respectively.

Collection of Samples and Determination of Biochemical Parameters: Oral glucose tolerance was assessed in each rat from all the six groups at days 0, 14 and 28. Blood samples were collected from tail veins (by excision) of the rats at 0, 1, 2, 3 and 4 hours following their treatment with the standard drug or the various doses of the extract. Plasma glucose was determined from the samples using a standardised glucometer. At day 29, after eight-hour fasting, the rats were anaesthetized with chloroform vapour in a gas jar. The animals were then dissected and the liver and skeletal muscle of the femur excised and their glycogen content determined colorimetrically according to the methods of Kemp and Van Heijningen (1954).

Statistical Analysis: The data was analysed using GraphPad Prism 7.0 and the results were expressed as mean ± standard error of the mean (SEM). The level of significance of differences between groups was determined using one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparison tests. P value < 0.05 was considered significant.

RESULTS

Phytochemical Analysis: Findings from qualitative and quantitative phytochemical analysis showed the presence of tannins, flavonoids, saponins, cardiac glycosides, carbohydrates, phenols, phytosterols and alkaloids (Table 1).

Acute Toxicity Study: *A. hispidum* extract was found to be relatively safe with a median lethal dose above 5000 mg/kg. No sign of toxicity was noted throughout the 14-day period of the observation.

Hypoglycaemic Activity of the Extract in Normal Rats: In normal rats, no significant hypoglycaemic effect was observed in any of the treated groups compared with the controls, even after four hours of administration of the respective treatments (data not shown).

Table 1:
Composition of aqueous leaf extract of *Acanthospermum hispidum*

Constituent	Qualitative analysis	Quantitative analysis (g per 100 g of sample)
Carbohydrates	Positive	20.75
Alkaloids	Positive	3.50
Glycosides	Positive	0.25
Saponins	Positive	2.00
Phytosterols	Positive	0.01 (steroids)
Phenols	Positive	0.06
Flavonoids	Positive	2.50
Tannins	Positive	8.25

Antihyperglycaemic Effect of the Extract in Alloxan-Induced Diabetic Rats

As indicated in Fig 1A, rats injected with alloxan monohydrate showed significantly higher levels of blood glucose compared with normal controls. There was no significant difference in fasting blood glucose at baseline between different groups of alloxan-induced diabetic animals (Fig. 1A).

After 2 weeks of treatment, a significant reduction in fasting blood glucose concentration was demonstrated in the diabetic rats treated with either glibenclamide (the standard drug) or a high dose (700 mg/kg) of the extract of *A. hispidum* (Fig. 1C). Also, the extract (at all doses), as well as the standard drug, produced improvement in oral glucose tolerance (Fig. 1B).

At day 28, as seen in Fig 1C, treatment with *A. hispidum*, at all doses, produced a significant dose-related reduction in fasting blood glucose (the high dose almost restored the glucose level to normal). The effect of the high dose of the extract was comparable to that produced by the standard drug.

When compared with the normal control group, a clear, though nonsignificant reduction in the glycogen contents of the liver and muscle was observed in the rats from the diabetic control group (Table 2). Rats treated with high dose (700 mg/kg) of *A. hispidum* extract exhibited a significant elevation in glycogen content of the liver (Table 2). However, the increase in the content of skeletal muscle glycogen has not attained statistical significance (Table 2).

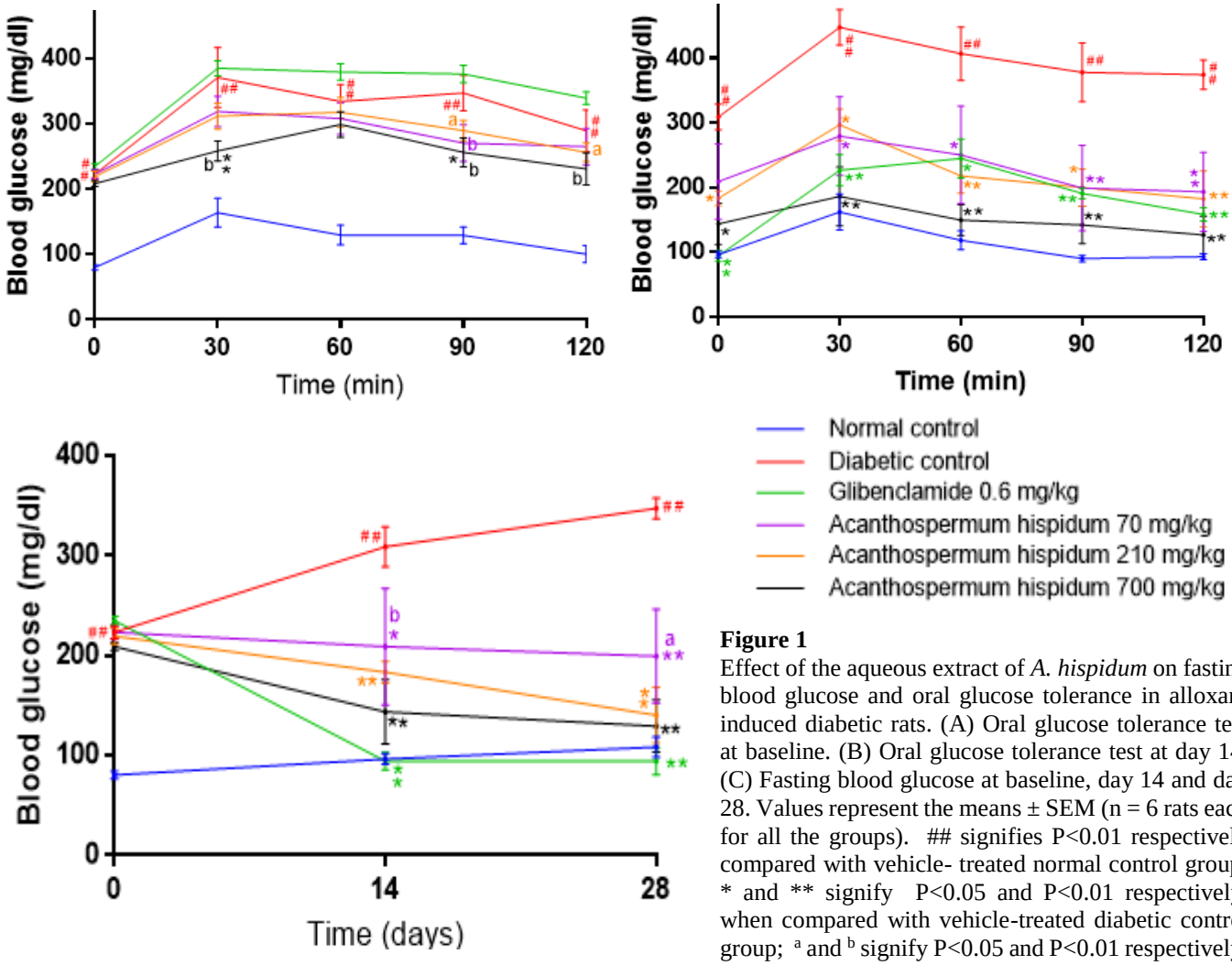

Figure 1
Effect of the aqueous extract of *A. hispidum* on fasting blood glucose and oral glucose tolerance in alloxan-induced diabetic rats. (A) Oral glucose tolerance test at baseline. (B) Oral glucose tolerance test at day 14. (C) Fasting blood glucose at baseline, day 14 and day 28. Values represent the means ± SEM (n = 6 rats each for all the groups). ## signifies P<0.01 respectively compared with vehicle- treated normal control group; * and ** signify P<0.05 and P<0.01 respectively, when compared with vehicle-treated diabetic control group; [a] and [b] signify P<0.05 and P<0.01 respectively, when compared with positive (glibenclamide-treated) diabetic control group.

Table 2
Effect of the aqueous leaf extract of *A. hispidum* on glycogen content in the liver and skeletal muscle alloxan-induced diabetic rats at day 29 of the study

Treatment groups	Mean glycogen concentration ± SEM (mg/g of tissue)	
	Liver	Muscle
Normal control (n = 6)	18.77 ± 4.21	4.75 ± 1.51
Diabetic control (n =6)	6.62 ± 0.64	2.43 ± 0.68
Glibenclamide (0.6 mg/kg; n = 6)	44.91 ± 11.05**	14.36 ± 2.67
AH (70 mg/kg; n = 6)	17.69 ± 2.41	5.81 ± 0.96
AH (210 mg/kg; n = 6)	17.73 ± 7.32	7.07 ± 0.69
AH (700 mg/kg; n = 6)	33.84 ± 5.16**	10.98 ± 0.93

Key: AH, aqueous leaf extract of *Acanthospermum hispidum*; SEM, standard error of mean.
*** P value <0.01 compared with vehicle-treated diabetic control group.*

DISCUSSION

Alloxan-induced hyperglycaemic rat, a widely employed animal model of diabetes, was used to investigate the folkloric claim of antidiabetic property of the plant under study. In the current study, the animals injected with alloxan exhibited a marked rise in fasting blood glucose (>198 mg/dl), suggestive of successful induction of diabetes mellitus (Jaouhari *et al*., 2000).

Although the antidiabetic activity of ethyl acetate extract of aerial parts of Acanthospermum hispidum has been reported by Vasundharamma *et al*. (2016), the current research is the first to investigate the effect of the plant on the glycogen content of the liver and skeletal muscle of diabetic rats. There are other important differences between the previous study (Vasundharamma *et al*. (2016) and the current investigation. First, the geographical location where the plant material was obtained differed in the two studies (Nigeria versus India). Secondly, a different part of the plant (leaf in the current study versus aerial parts in the previous research) was investigated. Thirdly, unlike the earlier study in which ethyl acetate extract of the plant was used, the current study evaluated the extract which is traditionally employed in North-Western Nigeria (i.e., the aqueous extract). It has been well documented that the geographical location where a particular plant species is obtained (Eldridge and Kwolek, 1983), the part of the plant used (Eldridge and Kwolek, 1983) as well as the method of extraction (Shan *et al*., 2007) are factors that can affect the phytochemical composition, and hence the pharmacological activity, of a plant extract.

The aqueous leaf extract of *A. hispidum* was found to be effective in reducing blood glucose in the diabetic animals. The groups of diabetic rats treated with the extract (at all 3 doses) exhibited significant antihyperglycaemic activity by day 28 of the study. Also, just like glibenclamide, *A. hispidum* produced an elevation of the hepatic content of glycogen, suggesting either increased glycogenesis or decreased glycogenolysis or both as possible mechanism(s) by which the extract exerts its glucose lowering effect. The increase in hepatic glycogen content observed in the group of rats treated with *A. hispidum* may be due to stimulation of insulin secretion or increased tissue sensitivity to the hormone. It has been documented that insulin increases the content of liver glycogen through both inhibition of glycogenolysis and stimulation of glycogenesis (Syed and Khandelwal, 2000). The fact that the extract showed significant ability to improve glucose tolerance in the diseased animals even at baseline, unlike glibenclamide, which improved glucose tolerance in the diabetic rats only after repeated dosing for 2 weeks suggests that the extract may act via a mechanism other than stimulation of insulin secretion (e.g. by enhancing sensitivity of tissues to insulin). This suggestion is supported by the findings of Vasundharamma *et al*. (2016) which revealed that ethyl acetate aerial part extract of the plant reduces the increased level of serum insulin in streptozocin-induced diabetic rats. However, the possibility of enhancement of insulin secretion being an additional mechanism by which the aqueous leaf extract exerts it action could not be ruled out in this study.

The failure of the extract to exhibit hypoglycaemic effect in normal rats as observed in this study is in agreement with the report of Pandhare *et al*. (2011) in an ethnopharmacological study of a different plant extract using Streptozotocin-induced rodent model of diabetes.

The human equivalent dose of the extract for rats is within the range of the doses used in this study. According to the description of the herbalists, 4 tablespoons of the powdered leaf of the herb is extracted in 250 ml of boiled water (yielding 1.15 g of the extract from our observation) and then taken orally daily. This translates to 16.43 mg/kg of *A.hispidum* for an average 70 kg man. For rats, this dose is equivalent to 101.87 mg/kg—a figure obtained by multiplying the traditional human dose (16.43 mg/kg) by a standard conversion factor of 6.2 based on surface area (FDA CDER, 2005). The current study has vindicated the claim of the herbalists that the extract has the ability to reduce blood glucose at such dose.

These findings highlight the utility of treating experimental animals for a prolonged period of time as well as employing semilogarithmic incremental doses in ethnopharmacological diabetic studies.

Qualitative phytochemical analysis of the extract under study revealed the presence of certain phytochemicals (namely flavonoids, tannins and saponins) known to possess antidiabetic properties (Firdous, 2014). The observed antidiabetic activity of the extract may be attributed to the presence of some of the above-mentioned phytoconstituents either individually or in combination. The value of the LD_{50}

observed for the extract was greater than 5000 mg/kg, indicating that it is relatively safe.

In conclusion, this study has validated the traditional claim that aqueous extract of the leaves of *Acanthospermum hispidum* possesses antihyperglycaemic activities in experimental diabetic rats following repeated oral administration (at a dose equivalent to traditional human dosage) for 28 days. The study, however, revealed that administration of *A. hispidum* at a dose higher than the traditional dosage may be more effective.

Conflict of interest statement

The authors declared that they have no conflicts of interest.

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