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Evaluation of Anti-diabetic Activity of Methanol and Aqueous Extracts of *Asteracantha longifolia* (Linn.) Nees

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Abstract: To evaluate the anti-diabetic activities of alcoholic and aqueous extracts of *Asteracantha longifolia* in Alloxan induced diabetic rats. Methanol and aqueous extracts (250 mg kg^{-1} b.w.) of *A. longifolia* was administered to Alloxan induced diabetic mice for 21 days and blood glucose levels of the diabetic rats were monitored at intervals of hours and days throughout the duration of the experiments. Oral administration of alcoholic extract of *A. longifolia* leaves to diabetic rats for 21 days significantly reduced the levels of blood glucose levels in both acute and sub acute study.

Key words: Glycosylated haemoglobin, insulin, creatine, Alloxan monohydrate, glibenclamide

INTRODUCTION

Diabetes mellitus is a non-communicable disease considered to be one of the three leading causes of death worldwide (Chauhan *et al*., 2010). Diabetes is a deadly disease that affects an estimated 135 million people worldwide and the numbers are increasing in rural and poor populations throughout the world (Chakraborty and Das, 2010). India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 (Sharma *et al*., 2011). The oral anti-diabetic agents suffer from various adverse effects, thus managing diabetes without any side effects is still a challenge to the workers and hence the search for more effective and safer therapeutic agents in eradiating diabetic syndromes has continued to be an important area of investigation. Several hypoglycemic agents have been used for the treatment of diabetes mellitus but are reported to produce serious adverse side effect such as liver problems, lactic acidosis and diarrhea. In addition, they are not suitable for use during pregnancy. Therefore, the search for more effective agents with low cost and without side effect from plant source has continued to be an important area of research because of their ready availability, affordability and low adverse side effect. According to world ethnobotanical information reports, almost 800 plants may possess anti-diabetic potential. India is well known for its herbal wealth. Medicinal plants like *Trigonella foenum graecum*, *Allium sativum*, *Gymnema slyvestre* and *Syzigium cumini* have been studied for treatment of diabetes mellitus (Grover *et al*., 2002). However, detailed studies on the efficacy, mechanism of action and safety of plant extract are needed (Prasad *et al*., 2009).

The genus *Asterocantha*, perennial angiospermic plant of family Acanthaceae is a commonly found herb in India being used as vegetable in some states like Odisha, Chhattisgarh and West Bengal. Boiled aerial parts of succulent plant of pre-flowering and flowering stages are used extensively to increase the haemoglobin status by the rural people of these states. This herbal remedy is devoid of any side effects with proven effectiveness. *Asteracantha longifolia* Nees [Synonym (s) *Hygrophila spinosa* T. Anders] contains various groups of phyto-constituents viz. phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, carbohydrates, hydrocarbons, flavonoids, terpenoids, vitamins, glycosides, etc., and is useful in the treatment of anasaraca, diseases of urinogenital tract, dropsy of chronic bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhoea, gonorrhoea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagea, etc. (Rastogi and Mehrotra, 1993; Annonymous, 2002; Sharma *et al*., 2002; Asolkar *et al*., 2005). Therefore, the present study was aimed to investigate the antidiabetic activity of methanolic and aqueous extracts of *Asteracantha longifolia*.

MATERIALS AND METHODS

Plant materials: Fresh plant parts (leaves) were collected randomly from the gardens and villages of Trichy District, Tamil Nadu from the natural stands. The botanical identity of these plants was confirmed by Dr. V. Sampath Kumar, Scientist-C, Botanical Survey of India (Southern Circle), Coimbatore, Tamil Nadu. A voucher specimen has been deposited at the Department of Botany, National College (Autonomous), 620 001 Tiruchirapalli, Tamil Nadu, India.

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Preparation of extracts

Aqueous extraction: About 100 g of dried powder were extracted in distilled water for 24 h. After 24 h, the extract was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected. This procedure was b repeated twice and after 6 h the supernatant was concentrated to make the final volume one-fifth of the original volume.

Solvent extraction: About 100 g of dried plant powdered samples were extracted with 200 mL of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fifth of the original volume. It was stored at 4°C in airtight bottles for further studies.

Animals: The animals of both sexes were used for these experiments. They were obtained from Animal House, RVS Pharmaceutical Sciences, Coimbatore, Tamil Nadu. The animals were housed in standard cages and were maintained on a standard pelleted feed and water was given *ad libitum*. All the experiments were carried out according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments of Animals (CPCSEA), Government of India.

Induction of diabetes: The animals were fasted for 24 h and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of Alloxan monohydrate (150 mg kg^{-1} b.w.) in sterile normal saline. About 72 h later, mice with Blood Glucose (BGL) levels above 200 mg dL^{-1} were considered diabetic and selected for the experiment.

Experimental design: The animals were randomly divided into 7 groups with 6 rats in each group and treated as follows:

- Group 1: Normal control (Saline) (by using an intragastric catheter tube (IGC)
- Group 2: Diabetic control
- Group 3: Diabetic rats received *A. longifolia* Methanol extract (250 mg kg^{-1} b.w.) for 21 days by IGC
- Group 4: Diabetic rats received *A. longifolia* Aqueous extract (250 mg kg^{-1} b.w.) for 21 days by IGC
- Group 5: Diabetic rats received Glibenclamide (10 mg kg^{-1} b.w.) daily orally for for 21 days by IGC

The change in body weight and Fasting Plasma Glucose (FPG) levels of all the rats were recorded at regular intervals during the experimental period. For acute toxicity study, FPG were monitored after 30, 60, 120 and 180 min of administration of single dose of the extracts and at the end of 0, 7th, 14th and 21st days for sub acute study. Blood was drawn from the ventricles and centrifuged.

Biochemical analysis: Blood samples were taken into centrifuged at 3000 rpm for 15 min. Serum biochemical parameter such as blood glucose (Sasaki *et al*., 1972), Plasma insulin (Anderson *et al*., 1993), glycosylated haemoglobin (Karunanayake and Chandrasekharan, 1985), creatine (Owen *et al*., 1954) and urea (Varley, 1976).

Statistical analysis: All the data were subjected to Duncan's Multiple Range Test (DMRT) was done by using the SPSS version 2007 WINSAT Software.

RESULTS

The methanol and aqueous extracts of *A. longifolia* was administrated orally to the rats at the doses of 100, 200, 400, 800, 1200 and 1600 mg kg^{-1} b.w. did not produce any significant changes in the autonomic, behavioural or neurological alteration. Acute toxicity studies revealed the non-toxic nature of both extracts of *A. longifolia*. The signs and symptoms in all groups were found to be normal. Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight on day 7, 14 and 21. There were observable changes in the body weight of treated diabetic rats. Alloxan caused body weight reduction which is reversed by alcoholic and aqueous extracts of *A. longifolia* after 7, 14 and 21 days of treatment. The same trend was noted in glibenclamide treated groups (Table 1).

A dose-dependent reduction in blood glucose levels was observed in Alloxan induced diabetic rats treated with aqueous and methanol extracts of *A. longifolia*. After a single dose-of the extract give to the Aoxan induced

Table 1: Effect of methanol and aqueous extracts of *A. longifolia* on body weight of Alloxan induced diabetic rats

Treatments	Day 0 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)
Normal control	172.20±2.07	165.25±0.450	169.68±2.15	170.44±3.55
Diabetic control	164.66±1.28	159.37±2.230	158.65±0.05**	158.18±1.28**
ALME (250 (250 mg kg^{-1} b.w.)	161.15±1.07	164.88±2.250	167.54±4.01^{a}	170.16±0.36^{aa}
ALAE (250 mg kg^{-1} b.w.)	164.32±1.75	164.93±1.350	165.22±1.28^{a}	166.94±2.36^{a}
Glibenclamide (10 mg kg^{-1} b.w.)	164.63±1.02	165.48±0.032	165.95±0.45^{a}	171.47±1.25^{aa}

Each value is SEM±5 individual observations; *, **, ***p<0.05, 0.01, 0.001 compared normal control vs. diabetic rats; $^{a, aa}$p<0.05, 0.01 compared diabetic rats vs. drug treated

Table 2: Antidiabetic effect of methanol and aqueous extracts of *A. longifolia* on blood glucose level of Alloxan-induced mice during acute study

Treatments	0 (min)	30 (min)	60 (min)	120 (min)	180 (min)
Normal control	102.06±0.40	144.90±0.48	157.86±0.41	173.16±0.47	195.10±0.26
Diabetic control	182.03±0.75	215.80±0.43	225.03±0.25	240.63±0.70	256.86±0.61*
ALME (250 mg kg^{-1} b.w.)	194.90±0.26	172.10±1.65	160.33±0.70	123.03±0.95^{aa}	115.20±2.10^{*aa}
ALAE (250 mg kg^{-1} b.w.)	192.43±0.45	170.26±0.35	159.13±0.90	142.00±0.80	120.76±0.76
Glibenclamide (0 mg kg^{-1} b.w.)	193.90±0.36	168.23±0.20	142.13±0.32	130.23±0.25*	105.36±0.90*

Table 3: Effect of methanol and aqueous extracts of *A. longifolia* on blood glucose level of Alloxan-induced rats during sub-acute study

Treatments	0 day	7 day	14 day	21 day
Normal control	94.16±2.35	99.46±2.40	90.16±1.40	95.26±2.55
Diabetic control	241.33±3.98	262.23±1.51	294.16±2.30*	310.13±4.50*
ALME (250 mg kg^{-1} b.w.)	248.30±1.45	190.63±3.32	143.10±1.36^{*aa}	115.26±6.30^{*aa}
ALAE (250 mg kg^{-1} b.w.)	260.46±1.30	194.50±1.45	158.06±2.35*	129.16±2.28*
Gli. (10 mg kg^{-1} b.w.)	278.23±3.32	183.50±6.20	141.40±3.55*	111.16±1.15*

Each value is SEM±5 individual observations; $^{*, aa}$p<0.05, 0.01 compared diabetic rats vs. drug treated

Table 4: Effects of *A.longifolia* extracts on non-protein compounds and glycosylated haemoglobin levels in normal and Alloxan induced diabetic rats

Treatments	Insulin (Iu L^{-1})	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})	Hb A1c (%)
Normal control	0.656±0.36	12.20±2.25	0.61±0.03	3.71±0.27
Diabetic control	0.155±0.24**	30.45±1.35*	1.27±0.75*	9.49±0.20**
ALME (250 mg kg^{-1} b.w.)	0.562±0.06^{*aa}	15.89±2.02	0.75±0.35	3.86±0.98^{aa}
ALAE (250 mg kg^{-1} b.w.)	0.497±0.23^{a}	17.21±4.17^{a}	0.83±0.25	4.13 0.51^{aa}
Gli. (10 mg kg^{-1} b.w.)	0.578±0.32^{aa}	14.55±3.01^{a}	0.62±0.02	3.76±0.20^{aa}

Each value is SEM±5 individual observations; $^{*, **, ***}$p<0.05, 0.01, 0.001 compared normal control vs. diabetic rats $^{a, aa}$p<0.05, 0.01 compared diabetic rats vs. drug treated

diabetic rats, there was a significant p<0.05 reduction in blood glucose levels of the diabetic rats within the period of acute study compared to control.

The maximum effect was observed at 180 min with the methanol extract exerting comparable to effect of aqueous extract that exerted a more pronounced effect (Table 2). The increased blood glucose level in Alloxan induced diabetic rats was significantly p<0.05 by crude extracts (methanol and aqueous) treatment and it was found to be lowered up to 129.16 and 115.26 at the dose of 250 mg kg^{-1} of body weight, respectively (Table 3).

The insulin and glycosylated haemoglobin levels of diabetic rats treated with methanol and aqueousextracts of *A. longifolia* and glibenclamide, a known hypoglycemic drug, resulted from a significant decrease in glycosylated haemoglobin where as increase insulin levels when compared with Alloxan alone treated rats. The maximum reverse the trend of insulin and glycosylated haemoglobin levels against Alloxan induced diabetic aberrations was achieved with the optimum dose 250 mg kg^{-1} body weight both (aqueous and methanol) extracts of *A. longifolia*. Among the 2 extracts treated, the methanol extract showed significant changes in insulin and glycosylated haemoglobin.

The level of urea and creatine in normal, diabetic and treated animals were shown in Table 4. The normal function of the kidney was assessed as blood urea level. The urea level in diabetic was found to be 30.45 mg dL^{-1}, it was altered from treated animals of 15.85 mg dL^{-1} against 12.20 mg dL^{-1} (control). The level of creatine in group 2 and 5 showed significant variation when compared to control. Among the two extracts treated, the methanol extract showed significant changes in urea and creatine.

DISCUSSION

Diabetes mellitus is a major health problem is developed and developing countries. Management of diabetes without any side effects is still a challenge to the modern medicine. Traditionally, a number of plants have been used in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically. The present investigation reports the anti-diabetic effect of aqueous and methanol extracts of *A. longifolia*. The observation and preliminary idea of the mechanism of its action reported here offer scientific explanation for the potential use of this plant for the treatment of diabetes mellitus. Diabetes inducing agents like Alloxan and streptozotocin are reported to induce diabetes with generation of free radicals, a significant reduction in antioxidant enzyme level is indicated as the potential reason for the susceptibility of organs to atrophy in diabetes states. Alloxan, a beta-cytotoxin causes a massive destruction of the islets of langerhans resulting in reduced synthesis and releases of insulin (Nammi *et al*., 2003). However, the animals survived without insulin treatment and showed improvement by glibenclamide which act by stimulating residual beta cells of the pancreas indicate incomplete destruction of pancreatic beta cells of the diabetic rats in

the present study. Chronic treatment with aqueous and methanol extracts of *A. longifolia* reduced blood glucose level throughout the experimental period in duration dependent manner indicating its antihyperglycemic activity. However, blood glucose levels were not altered in normoglycemic mice further strengthening the antidiabetogenic potential of the extract.

In diabetes mellitus, body cells are unable to utilize glucose as a source of energy due to which proteins are spared as energy source. This leads to decrease in protein storage which in turn reduces body weight (Guyton and Hall, 1996). In Alloxan induced diabetic rats, increased food consumption and decreased body weight were observed. This indicates polyphagic condition and loss of weight due to excessive breakdown of tissue protein. In the present study, Alloxan diabetic mice show decrease in body weight throughout the experimental period. Oral treatment with aqueous and methanol extracts of *A. longifolia* significantly improved the body weight loss in diabetic rats as compared to diabetic control indicating possible role of the extract in restoration of protein metabolism.

In diabetic mellitus, due to persistent hyperglycemia, the excess blood glucose reacts with haemoglobin in a nonenzymatic process to form glycosylated haemoglobin. Since, the glycation rate is directly proportional to blood glucose concentration, level of glycosylated haemoglobin indicates glycemic control in the diabetic state (Monnier, 1982). Estimation of haemoglobin is a well established parameter useful in the 22 management and prognosis of the disorder (Chang and Nobel, 1979). In the present study, administration of methanol and aqueous extracts of *A. longifolia* leaves significantly reduced the elevated glycosylated haemoglobin levels in Alloxan-diabetic rats further substantiating its potential in long term glycemic control of diabetes mellitus.

Urea and uric acid are organic waste products produced during the breakdown of amino acids. Creatinine is generated in the skeletal muscle tissue by the breakdown of creatinine phosphate. Their increased level in serum is an indication kidney disorder. It was found that urea and creatinine levels were normal in all the experimental groups. Many oral hypoglycemic agents are normally metabolized or cleared by the kidneys and so accumulate in uraemic patients thus increasing the risk of hypoglycemia and toxicity (Cynthian and Rajeshkumar, 2012). The results on the creatinine and urea are very close to normal range and are significant. The remarkable hypoglycaemic potential of *A. longifolia* was quite competent with standard drug. Further studies, are necessary to elucidate details of active phytochemicals and their mechanism of hypoglycaemic action. Isolation and study of active principles are under process.

CONCLUSION

These results suggested that the methanol extract of *A. longifolia* possess anti-diabetic effect on Alloxan induced diabetic rats and it can be recommended for the prevention of diabetes mellitus.

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