

Original Article

STUDIES ON THE EFFECT OF ANTIDIABETIC ACTIVITY OF *ACHYRANTHES ASPERA* L. ON ALLOXAN INDUCED WISTAR RATS

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ABSTRACT

Objective: The present study was designed to investigate the effect of anti-diabetic activity of *Achyranthes aspera* on alloxan induced wistar rats.

Methods: Diabetes was induced by administration of alloxan mono hydrate (150 mg/kg body weight i. p) to albino wistar rats. Diabetic rats were stabilized for four days and from fifth-day aqueous extract of *A. aspera* were administered at the dose of 250 mg/kg and 500 mg/kg for 45 days. Metformin 1 mg/kg was used as a standard. The effects of *A. aspera* and standard drug on following parameters was recorded-fasting blood glucose, glycogen, plasma insulin, glycosylated hemoglobin and protein were analyzed in blood samples. Glucose-6-phosphatase, Glucose-6 phosphate dehydrogenase, tissue protein, reduced glutathione, and lipid peroxide were estimated in liver tissues.

Results: Our results collectively suggested that administration of aqueous extracts of *A. aspera* considerably lower the blood glucose level which was comparable to standard anti-diabetic drug metformin (1 mg). Also, the extract shows that considerable increase ($p < 0.05$) in glucokinase activity when compared to untreated diabetic rats. Lipid peroxidation and reduced glutathione (GSH) level were also studied and the *A. aspera* aqueous extract and metformin-treated groups shows that the significant ($p < 0.05$) reduction in lipid peroxide and marked elevation in reduced glutathione levels.

Conclusion: Aqueous extract of *A. aspera* possess anti-diabetic action in alloxan induced diabetic rats.

Keywords: *Achyranthes aspera*, Anti-diabetic, Blood glucose, Glucokinase, Glycosylated hemoglobin.

INTRODUCTION

Diabetes is a syndrome characterized by disordered metabolism of carbohydrate, protein and lipid with abnormally high blood sugar (hyperglycemia) resulting from low levels of the hormone insulin with or without abnormal resistance to insulin's effect [1]. Diabetes mellitus is considered as one of the five leading causes of death in the world. About 150 million people are suffering from diabetes worldwide, which is almost five times more than the estimates ten years ago and this may double by the year 2030. India leads the way with its largest number of diabetic subjects in any given country. It has been estimated that the number of diabetes in India is employed to increase 57.2 million by the year 2025 [2, 3].

The prevalence of diabetes mellitus is expected to rise more rapidly in the future because of increasing obesity and reduced activity levels [4]. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as anti-diabetic and antihyperlipidemic remedies [5]. More than 400 plant species with hypoglycemic activity are available in the literature [6].

Achyranthes aspera is a common plant found in wastelands and agricultural fields. The plant is highly esteemed by traditional healers. *A. aspera* L. known by different names in India viz. Latjira in Hindi, Apamargah in Sanskrit, Apang in Bengali, Katalati in Malayalam, Nayurivi in Tamil, Uttaren in Telugu and Prickly-chaff flower plant in English [7]. Different parts of the plant have been used as an expectorant, stomach tonic, laxative, anthelmintic, diuretic, lithontriptic, sudorific, demulcent, anti-inflammatory and haematinic in indigenous medicine [8]. Nowadays many studies have been reported diverse actions of *A. aspera* e. g. antiviral [9], antibacterial activity [10, 11], antifertility [12], antidiabetic [13], positive inotropic effect, spasmolytic to smooth muscle [14], diuretic and purgative [15]. The major aim of the study is to prove the hypoglycemic activity of *A. aspera* leaf extract. Which is found to be futile with evidence base literature. The rationale of the present study is to find out the level of efficacy and potency of the leaf extract in accordance with hypoglycemic activity. The present study differ from other literature evidence in means of increased dose

level of signifying the expected pharmacological activity along with the control population. The *A. aspera* leaves were investigated for its antidiabetic *in vivo* in Alloxan induced diabetic rat model and to compare the same with metformin, a standard hypoglycemic drug.

MATERIALS AND METHODS

Collection and authentication of the plant material

The leaves of *Achyranthes aspera* were collected in the month of March 2010 from Tropical area of Western Ghats region of Coimbatore district. Coimbatore lies between 11° 00' N latitude and 77° 00' E longitude. It covers an area of 246.8 km². The plant was authenticated at the Botanical Survey of India (BSI), Southern circle Coimbatore, Tamil Nadu, India. Where the voucher specimens were deposited (Herbarium vouchers no BSI/SC/5/23/09-10/Tech-266). The samples were shade dried at room temperature experimental purpose.

Preparation of plant extract

The collected plant leaf materials were dried and finely powdered with an electrical blender. 200 g of *A. aspera* coarse powder was taken and extracted with water. To one part of the material and four parts of water was added, boiled and reduced to one-third and the filtrate was evaporated to dryness. Paste form of the extract was obtained and its subjected to pre-clinical screening. The percentage yield of extract was 49 % w/v.

Experimental animals

Healthy adult wistar strain of albino rats of either sex, weighing 150-200 g was used as experimental models. Animals were kept in well-ventilated cages and fed with standard rat chow pellet obtained from Sai Durga Food and Feeds, Bangalore, India and water *ad libitum*. Animals were maintained under standard laboratory conditions (Temperature 24-28 °C, relative humidity 60-70%). All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining the necessary clearance from the Committee (Approval No: NCP/IAEC/Ph. D/2010-001).

Alloxan induction

Diabetes mellitus was induced in a batch of normoglycemic albino rats, starved for 12 h. 150 mg/kg body weight of alloxan monohydrate was dissolved in physiological saline and injected intraperitoneally (IP). This dose of alloxan produced persistent hyperglycemia after four days as revealed by the determination of glucose levels by the analysis of blood and urine samples [16].

Experimental design

The rats were divided into five groups and each group comprising of six rats.

Group I-Normal control

Group II-Animals treated with alloxan monohydrate in normal saline at a dosage of 150 mg/kg body weight IP.

Group III-Animals were treated as in Group II. After 4 days of diabetes-induction, treated with *A. aspera* aqueous extract 250 mg/kg body weight, orally for 45 days.

Group IV-Animals were treated as in Group II. After 4 days of diabetes-induction, treated with *A. aspera* aqueous extract 500 mg/kg body weight orally for 45 days.

Group V-Animals were treated as in Group II. After 4 days of diabetes-induction, treated with standard drug metformin 1 mg/kg body weight orally for 45 days.

The parameters studied

Fasting blood glucose by folin-Wu's method [17], glycogen [18], plasma insulin [19], glycosylated hemoglobin (HbA1C) [20] and protein were analyzed in the blood sample.

Glucokinase [21], Glucose-6-phosphatase [22], Glucose-6-phosphate dehydrogenase [23], tissue protein by Lowry's method [24], Reduced glutathione (GSH) [25] and Lipid peroxide (LPO) [26] were estimated in liver tissues.

Statistical analysis

All the results were expressed as mean±SEM. The data were statistically analyzed by one-way analysis of variance (ANOVA) between plant extract treated groups and disease control group. P values<0.05 were considered as significant.

RESULTS

Diabetes mellitus was induced by intra peritoneal injection of alloxan monohydrate to all the group of animals except Group I (Normal control) that caused severe diabetes in rats. Table 1 shows that the levels of blood sugar, glycosylated hemoglobin (HbA1C) and plasma insulin in diabetes-induced untreated as well as plant drug treated animals. Effect of aqueous extract of *A. aspera* in alloxan induced diabetic rats was evident from the results of Group III & IV (250 mg/kg bw & 500 mg/kg bw respectively). *A. aspera* at a dose of 500 mg/kg produced a significant (p<0.05) fall in blood sugar and glycosylated hemoglobin level in diabetic rats.

Plasma insulin level that was decreased in disease control was found to be increased in plant extract treated group of rats (Group III & IV). Animals treated with the standard drug also shows that a significant reduction in the blood glucose level and HbA1C level compared to Group II (p<0.05). The level of liver glycogen was reduced in alloxanized diabetic Group II rats. Administration of test drug for 45 days caused significant (p<0.05) elevation in liver glycogen levels.

Table 1: Effect of *A. aspera* extract on blood glucose, glycosylated hemoglobin (HbA1C) serum insulin and Glycogen levels of experimental animals

S. No.	Group	Blood glucose (mg/dl)	HbA1C (%)	Serum insulin (µU/ml)	Glycogen (mg/g)
1	Normal	82.31±10.07	2.82±0.64	22.75±6.02	41.17±10.92
2	Diabetic control	289.51±39.77*	6.96±0.38*	10.01±3.12*	10.82±4.88*
3.	Diabetic+ <i>A. aspera</i> (250 mg/kg)	172.44±31.53**	3.95±1.72**	16.72±6.01**	23.08±5.14**
4.	Diabetic+ <i>A. aspera</i> (500 mg/kg)	91.53±12.32**	2.94±0.95**	23.62±6.96**	39.25±7.32**
5	Diabetic+Metformin (1 mg/kg)	84.05±12.78**	3.25±0.98**	19.68±7.83**	41.01±8.48**

Values are mean±SEM (n=6), *P<0.05. statistically significant when compared to Group II with Group I, **P<0.05 statistically significant when compared to Group III, IV & V with Group II.

Table 2 shows the activity of glucokinase in the liver of both control and experimental groups of rats. Oral administration of *A. aspera* aqueous extract (250 mg/kg & 500 mg/kg bw) for 45 days significantly increases (p<0.05) glucokinase activity when compared to untreated diabetic rats. In group II, diabetic rats showed a profound increase in the activity of glucose-6-phosphatase. Administration of *A. aspera* plant extract were found to be effective in resuming (p<0.05) the activity of glucose-6-phosphatase. Glucose-6-phosphatase dehydrogenase is one of the important glucose

oxidizing enzyme and it was found to be altered significantly (p<0.05) in diabetic untreated rats (Group II). Administration of aqueous plant extract was elevated in the activity of glucose-6-phosphatase dehydrogenase in a dose-dependent manner. The effect was also compared with the standard drug (Group V). Total tissue and serum protein shows that the significant variation between diabetic and *A. aspera* extract treated animals. The levels of protein decreased in untreated diabetic animals and resumed back to normal after oral administration of *A. aspera* plant extracts.

Table 2: Effect of *A. aspera* extract on Glucokinase, Glucose-6-phosphatase, Glucose-6-phosphodehydrogenase activity in experimental animals

S. No.	Group	Glucokinase (µmol/ml/min)	Glucose-6-phosphatase (U/ml/min)	Glucose-6-phosphatase dehydrogenase (U/ml/min)
1	Normal	114.02±29.54	7.02±1.76	12.04±1.92
2	Diabetic control	92.32±12.45*	14.92±2.13 *	7.91±1.08*
3	Diabetic+ <i>A. aspera</i> (250 mg/kg)	99.45±22.02**	9.82±3.13**	8.93±1.94**
4	Diabetic+ <i>A. aspera</i> (500 mg/kg)	113.92±29.75**	8.95±2.02**	11.73±2.44**
5	Diabetic+Metformin (1 mg/kg)	108.15±21.58**	10.18±4.76**	7.93±1.13**

Values are mean±SEM (n=6), *P<0.05. Statistically significant when compared to Group II with Group I, **P<0.05 statistically significant when compared to Group III, IV& V with Group II.

Table 3 shows that Lipid peroxide/lipid peroxidase (LPO) and reduced glutathione (GSH) levels were also studied to evaluate the antioxidant status in alloxan-induced untreated and treated animals.

A. aspera and metformin-treated groups shows that significant ($p < 0.05$) reduction in the lipid peroxide level and marked elevation in reduced glutathione levels.

Table 3: Effect of *A. aspera* extract for 45 days on serum and tissue protein, reduced glutathione and lipid peroxide levels in experimental animals

S. No.	Group	Serum protein (mg/dl)	Tissue protein (mg/100g)	Liver GSH (mM/100g)	Liver LPO (mM/100g)
1	Normal	38.13±9.44	16.13±2.44	42.42±9.54	0.92±0.67
2	Diabetic control	28.24±9.92*	11.23±2.78*	24.31±6.54*	1.95±0.14*
3	Diabetic+ <i>A. aspera</i> (250 mg/kg)	33.52±10.41**	11.33±1.54**	29.68±6.24**	1.51±0.56**
4	Diabetic+ <i>A. aspera</i> (500 mg/kg)	43.38±8.16**	14.81±1.84**	37.60±5.03**	1.82±0.12**
5	Diabetic+Metformin (1 mg/kg)	39.82±5.26**	14.93±2.07**	39.41±7.98**	1.32±0.19**

Values are mean±SEM (n=6), * $P < 0.05$ statistically significant when compared to Group II with Group I, ** $P < 0.05$ statistically significant when compared to Group III, IV & V with Group II.

DISCUSSION

Since there are some clinical limitations and demerits with the currently available treatment regimens for diabetes mellitus, there is a requirement for safer and more potent Anti-diabetic drugs [27, 28]. Alloxan-induced Diabetes is associated with the specific dysfunction in the metabolic index. The liver plays a vital role in the maintenance of blood glucose level and hence it is of interest to evaluate the possible role of *A. aspera* on key enzymes of carbohydrate metabolism in the liver. The liver is the main site for glycolysis, a process where glucose is oxidized and gluconeogenesis, a process where glucose is synthesized from noncarbohydrate sources [29].

The glucokinase activity was decreased in diabetic Group II rat. This may be due to insulin deficiency. Treatment with aqueous extract of *A. aspera* elevated the activity of glucokinase in the liver. *A. aspera* may stimulate insulin secretion, which activates glucokinase, thereby enhances the utilization of glucose leading to the profound decrease in blood glucose levels (Group III & IV) (table 1).

Insulin decreases gluconeogenesis by decreasing the activity of the key enzyme, glucose-6-phosphatase [30]. In *A. aspera* treated rats, the activity of enzyme glucose-6-phosphatase was seen significantly reduced in the liver. This may be due to increased insulin secretion, which is responsible for the repression of one of the gluconeogenic key enzyme.

The regulation of glycogen metabolism *in vivo* occurs by the multifunctional enzyme glycogen synthase and glycogen phosphorylase plays a major role in the glycogen metabolism. The reduced glycogen stored in diabetic rats has been attributed to reduced activity of glycogen synthase [31] and increased activity of glycogen phosphorylase [32]. Treatment with *A. aspera* elevated the activity of glycogen synthesis enzymes in the liver.

The observed increase in the level of glycosylated hemoglobin (HbA1C) in the diabetic control group of rats is due to the presence of an excessive amount of blood glucose. Mechanisms by which increased oxidative stress is involved in the diabetic complications are partially known, including activation of transcription factors advanced glycation end product (AGEs) and protein kinase C. Glycosylated hemoglobin has been found to be increased over a long period of time in the diabetic mellitus [33]. There is an evidence that glycation may itself induces the generation of oxygen-derived free radicals in diabetic conditions. Treatment with *A. aspera* extract showed a significant decrease in the glycosylated hemoglobin with a concomitant increase in the level of total hemoglobin in diabetic rats. Animals, which is received standard drug metformin, also shows that the similar result. Reduced glutathione is a potent-free radical scavenger GSH within the islet of β -cell and is an important factor against the progressive destruction. Depletion of GSH results in enhanced lipid peroxidation (table 3).

This can cause increased GSH consumption and can be correlated to the increase in the level of oxidized glutathione (GSH). Treatment of *A. aspera* resulted in the elevation of the GSH levels, which protect the cell membrane against oxidative damage by regulating the redox status of protein in the membrane [34].

In the present study reveals that the diabetic animals are exposed to oxidative stress and *A. aspera* can partially reduce the imbalances between the generation of reactive oxygen species (ROS) and the scavenging enzyme activity. According to these results, *A. aspera* could be a supplement, as an antioxidant therapy and may be useful for treating the hyperglycemia and preventing further diabetic complications. The *A. aspera* plants possess a significant hypoglycemic effect and it also controls the antioxidant level.

CONCLUSION

A lot of traditional Indian medicinal plants are used for treating various diseases and ailments which greatly focuses herbal medicines and herbal research into a spotlight. The present study estimated the Anti-diabetic effect of *A. aspera*. Further investigations are needed to quantify the mechanism of action of *A. aspera* in the treatment of hyperglycemic individuals with respect to its liver metabolic index and parameters.

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CONFLICT OF INTERESTS

Declared None

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