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Research Article

# EVALUATION OF ANTIDIABETIC POTENTIAL OF A*CHYRANTHES ASPERA* LINN. ON ALLOXAN INDUCED DIABETIC ANIMALS

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**ABSTRACT**

The present study, aims to evaluate the antidiabetic potential of aqueous extract of *Achyranthes aspera* Linn. against alloxan induced diabetic rats. Wistar strain of albino rats of either sex were divided into five groups comprising of six rats each. Group I served as normal control, group II served as disease control (alloxan induced), group III & IV animals, received aqueous extract of *A.aspera* Linn at a dosage of 250mg/kg body weight and 500mg/kg body weight for 45 days, groupV served as standard drug control (glibenclamide1mg/Kg body weight). After the experimental period the blood and tissue samples were collected and subjected to various biochemical and enzymic parameters. There were profound alteration in fasting blood glucose, serum insulin, glycosylated hemoglobin ($HbA_1C$) and liver glycogen levels in alloxanized rats. Glucose-6-phosphatase, glucokinase, and glucose-6-phosphate dehydrogenase activity were also altered in diabetic rats. The alterations were observed to resume ($P<0.05$) back to normal on treatment with plant drug. The effect of plant extract was found to be dose dependent. The present investigation reveals the antidiabetic potential of aqueous extract of whole plant of *A.aspera* L

**Keywords:** Diabetes mellitus, *Achyranthes aspera* Linn., Alloxan, Glycosylated hemoglobin, Glucokinase.

## 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 a major public health problem worldwide. The prevalence of diabetes mellitus is expected to rise more rapidly in the future because of increasing obesity and reduced activity levels[2].

The currently available therapeutic options for diabetes like oral hypoglycemic agents and insulin are considered to have limitations of their own, hence herbal medicines have been recommended for the treatment of diabetes[3]. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as anti-diabetic and antihyperlipidemic remedies. Antihyperglycemic effect of these are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or by inhibiting the intestinal absorption of glucose or by facilitating the metabolites in insulin dependent processes. More than 400 plant species with hypoglycemic activity are available in literature[4].

*Achyranthes aspera* Linn. belonging to family Amarathaceae, is commonly found as a weed on way side throughout India. It is known as Apamarg in Sanskrit, Chirchitta in Hindi and Prickly chaff flower in English, Naayuruvi in Tamil. The plant is used for treating asthmatic, cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhea and abdominal pain[5,6,7, 8& 9] It also stimulates the immune system and enhances the antigen clearance[10]. It also possess anti inflammatory effect[11]and anticancer activity[12]. Decreases lipid peroxidation[13]and also claim to have hypoglycemic activity [14]. Owing to its hypoglycemic[14] and antioxidant[13] properties the present investigation aims in evaluating the commonly available plant source, *Achyranthes aspera* Linn. for its antidiabetic potential.

## MATERIAL AND METHODS

### Preparation of plant extract

*Achyranthes aspera* Linn. were obtained from places in and around Trichy identified and authenticated with the herbarium specimen of RAPINAT herbarium of St.Joseph's College, Trichy, Tamilnadu, India. The collected plant materials (Aerial parts) were shade dried and coarsely powdered with electrical blender

200g of Achyranthes aspera Linn. coarse powder was taken and extracted with water. To one part of the material six parts of water was added, boiled and reduced to one third and the filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to pre-clinical screening. The percentage yield of extract was 52.

### Experimental Animals

Healthy adult wistar strain of albino rats of either sex, weighing 150-200g was used as experimental models. Animals were kept in well-ventilated cages and fed with standard rat chow pellet obtained form Sai Durga Food and Feeds, Bangalore, India and water *ad libitum*. Animals were maintained under standard laboratory conditions (Temperature 24-28ºC, relative humidity 60-70%). All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (approval No: 790/03/ac/CPCSEA).

### Alloxan induction

Diabetes mellitus was induced in a batch of normoglycemic albino rats, starved for 16 hours. 150mg/kg body weight of alloxan monohydrate was dissolved in physiological saline and injected intraperitoneally (IP). This dose of alloxan produced persistent hyperglycemia after 4 days as revealed by determination of sugar levels by the analysis of blood and urine samples[15].

### Experimental design

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

**Group I**- Normal control

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

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

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

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

### Collection of blood, liver from the rat:

After the experimental period, animals were sacrificed by cervical decapitation. Blood was collected and serum was separated by

centrifugation (for 15min at 2000rpm). The liver were dissected out and washed in ice-cold saline. Tissues were cut into small pieces and homogenized, in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for various biochemical and enzymatic analysis.

**The parameters studied:**

Fasting blood glucose by folin-Wu's method[16], glycogen[17], plasma Insulin[18], Glycosylated hemoglobin ($HbA_1C$) by Nayak *et al*[19] and protein were analyzed in blood sample. Glucokinase[20], Glucose-6-phosphatase[21&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 ± S.E.M. 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 the levels of blood sugar, glycosylated hemoglobin ($HbA_1C$) and plasma insulin in diabetes induced untreated as well as plant drug treated animals. Effect of aqueous extract of *A. aspera* L. in alloxan induced diabetic rats was evident from the results of group III & IV (250mg/Kg bw & 500mg/Kg bw respectively). *A. aspera* L. at a dose of 500mg/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 standard drug also showed a significant reduction in blood glucose and $HbA_1C$ 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 treatment *A.aspe*ra extract on blood glucose, glycosylated hemoglobin ($HbA_1C$) serum insulin and Glycogen levels of experimental animals**

| S. No. | Group | Blood glucose(mg /dl) | $HbA_1C$(%) | Serum Insulin(µU/ml) | Glycogen(mg /g) |
|---|---|---|---|---|---|
| 1. | Normal | 84.3±9.21 | 2.4±0.34 | 23.4±6.72 | 42.1± 9.92 |
| 2. | Diabetic control | 292.3±36.97* | 6.6±0.42* | 9.8±2.52* | 10.4±5.12* |
| 3. | Diabetic+*A.aspera* (250mg/kg) | 180.4±29.53** | 4.2±1.12** | 17.2±5.81** | 24.3±5.74** |
| 4. | Diabetic+*A.aspera* (500mg/kg) | 90.5±11.52** | 3.1±0.75** | 22.4±7.06** | 38.5±8.52** |
| 5. | Diabetic+glibenclamide (1mg/kg) | 83.3±13.15 | 3.0±0.72 | 20.6±6.33 | 40.7±7.66 |

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

**Table 2: Effect of treatment *A.aspe*ra extract on Glucokinase, Glucose-6-phosphate, Glucose-6-phosphodehydrogenase activity in experimental animals**

| S. No. | Group | Glucokinase (µmoles/ml/min) | Glucose-6-phosphatase (U/ml/min) | Glucose-6-phosphate dehydrogenase (U/ml/min) |
|---|---|---|---|---|
| 1. | Normal | 118 ± 32.34 | 6.92 ± 1.41 | 11.3 ± 2.22 |
| 2. | Diabetic control | 94.3± 10.45* | 15.02 ± 2.53 * | 7.2 ± 1.16* |
| 3. | Diabetic+*A.aspera* (250mg/kg) | 100.4 ± 23.02** | 10.32± 2.63** | 9.4 ± 1.74** |
| 4. | Diabetic+*A.aspera* (500mg/kg) | 115.7± 26.15** | 7.05 ± 1.92** | 11.5 ± 2.64** |
| 5. | Diabetic+glibenclamide (1mg/kg) | 107.5 ± 22.58 | 11.18 ± 3.56 | 8.1 ± 1.03 |

Values are Mean ± SEM (n=6)
*P < 0.05.statistically significant when compared Group II with Group I,
**P < 0.05.statistically significant when compared Group III, & IV 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* L aqueous extract (250mg &500mg/kgb.w) for 45 days significantly increases (P<0.05) glucokinase activity when compared to untreated diabetic rats. Group II Diabetic rats showed a profound increase in the activity of glucose -6-phosphatase. Administration of *A. aspera* L plant extract were found to be effective in resuming (P<0.05) the activity of glucose -6-phosphatase. Glucose-6-phosphate dehydrogenase is one of important glucose oxidizing enzyme, and was found to be altered significantly (P<0.05) in diabetic untreated rats (group II). Administration of aqueous plant extract elevated the activity of glucose -6-phosphate dehydrogenase in a dose dependent manner. The effect was also compared with standard drug (group V). Total tissue and serum protein showed significant variation between diabetic and plant treated animals. The levels of protein decreased in untreated diabetic animals and resumed back to normal after oral administration of plant drug **(Table 3).**

Lipid peroxide (LPO) and reduced glutathione (GSH) levels were also studied to evaluate the antioxidant status in alloxan-induced untreated and treated animals. *A.aspera* L and glibenclamide treated groups showed significant (P<0.05) reduction in the lipid peroxide level and marked elevation in reduced glutathione levels **(Table 3)**.

**Table 3: Effect of treatment *A.aspe*ra 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 | 42.3 ± 9.44 | 14.1±2.11 | 46.4 ± 8.54 | 0.9 ± 0.51 |
| 2. | Diabetic control | 30.4 ± 8.92* | 10.2 ± 2.24* | 23.3 ± 7.54* | 2.5 ± 0.24* |
| 3. | Diabetic+*A.aspera* (250mg/kg) | 36.5 ± 8.41** | 12.1 ±1.73** | 30.8 ± 5.77** | 1.9 ± 0.76** |
| 4. | Diabetic+*A.aspera* (500mg/kg) | 44.8 ± 7.06** | 13.8 ± 1.34** | 38.6 ± 5.43** | 1.4 ± 0.32** |
| 5. | Diabetic+glibenclamide (1mg/kg) | 40.2 ± 6.78 | 15.7 ± 2.17 | 40.4 ± 7.28 | 1.5 ± 0.17 |

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

**DISCUSSION**

Currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs[27-29].

Induction of diabetes with alloxan is associated with the characteristic alterations in metabolic profile. Liver functions as a "glucosat" and play a vital role in the maintenance of blood glucose level and hence it is of interest to examine the possible role of *A.aspera* on key enzymes of carbohydrate metabolism in liver. Liver is the main site for glycolysis, a process where glucose is oxidized and gluconeogensis , a process where glucose is synthesized from non carbohydrate sources [30].

The glucokinase activity was decreased in diabetic group II rat **(Table 2).** This may be due to insulin deficiency. Treatment with aqueous extract of *A.aspera* L. elevated the activity of glucokinase in the liver. *A.aspera* L. may stimulate insulin secretion, which activates glucokinase, thereby enhances the utilization of glucose leading to profound decrease in blood glucose levels (Group III & IV) **(Table 1).**

Insulin decreases gluconeogenesis by decreasing the activity of key enzyme, glucose-6-phosphatase[31]. In *A.aspera* L treated rats, the activity of enzyme glucose-6-phosphatase was seen significantly reduced in 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 that play a major role in the glycogen metabolism[31]. The reduced glycogen store in diabetic rats has been attributed to reduced activity of glycogen synthase[32] and increased activity of glycogen phosphorylase[33]. Treatment with *A.aspera* elevated the activity of glycogen synthesing enzymes in liver.

The observed increase in the level of glycosylated hemoglobin ($HbA_1C$) in diabetic control group of rats is due to the presence of excessive amount of blood glucose. Mechanism by which increased oxidative stress is involved in the diabetic complications are partially known, including activation of transcription factors advanced glycated end product(AGEs) and protein kinase C. Glycosylated hemoglobion has been found to be increased over a long period of time in the diabetic mellitus[34]. There is an evidence that glycation may itself induce the generation of oxygen-derived free radicals in diabetic condition[35].

Treatment with *A.aspera* extract showed a decrease in the glycosylated hemoglobin with a concomitant increase in the level of total hemoglobin in diabetic rats. Animals, which received standard drug glibenclamide, also showed 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. This can cause increased GSH consumption and can be correlated to the increase in the level of oxidized glutathione (GSSH). 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[36].

The study suggested that 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 beneficial for correcting the hyperglycemia and preventing diabetic complications due to lipid peroxidation and free radicals. The *A.aspera* plant is having a hypoglycemic effect and it also controls the antioxidant level.

**CONCLUSION**

Many indigenous Indian medicinal plants are used as remedies against various diseases. In the present scenario herbal medicines and herbal research is coming to lime light. The present investigation evaluates the antidiabetic efficacy of *A.aspera*, further investigation have to be extended by analyzing the mechanism of action of *A.aspera* is combating altered glycemic and lipid profile in diabetic condition.

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