Research Article

# BIOCHEMICAL EVALUATION OF HYPOGLYCEMIC ACTIVITY OF INORGANIC CONSTITUENTS OF *ACHYRANTHES ASPERA* SEEDS IN STZ INDUCED EXPERIMENTAL DIABETES IN RATS

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**ABSTRACT**

The present study has been made to analyze the inorganic elements present in the *Achyranthes aspera* (*A. aspera*), a very common weed of wastelands, and their role on diabetes related biochemical alterations in experimental diabetes. Experimental diabetes was induced using streptozotocin. STZ induced experimental diabetic rats were treated with *A. aspera* seed ash at a concentration of 90mg/Kg b.w orally for 30 days. Trace element analysis was performed. The biochemical parameters such as blood glucose, plasma insulin, total protein, serum creatinine, urea, uric acid and urine sugar were determined. The levels of hemoglobin and glycosylated hemoglobin were also determined. The levels of serum aminotransferases and alkaline phosphatase were also assayed. Diabetic rats treated with *A.aspera* seed ash at a concentration of 90mg/Kg b.w orally for 30 days showed significant hypoglycemic activity. The altered biochemical parameters were reverted to near normalcy upon treatment with ash. The trace element analyses indicate the presence of several hypoglycemic activity possessing elements in the seeds. The results of the study indicates that the plant *A. aspera* is rich in minerals like copper, magnesium, manganese, vanadium, chromium, calcium, zinc, sodium, and potassium. The presence of these inorganic elements in appreciable amounts in the seeds may play a direct or indirect role on insulin secretion or action in a synergetic manner. The hypoglycemic activity of the seed ash was comparable with gliclazide, a standard hypoglycemic drug.

**Keywords:** *Achyranthes aspera* seeds, inorganic elements, hypoglycemic activity, streptozotocin diabetes.

## INTRODUCTION

The abnormalities in the metabolism of zinc, chromium, magnesium and manganese have been associated with diabetes mellitus (DM)[1]. Clinical results also suggest that the homeostasis of essential trace elements can be disrupted in diabetes mellitus[2]. DM is a chronic metabolic disorder that arises due to absolute or relative insulin deficiency. It is recognized by elevated levels of fasting and postprandial blood sugar levels accompanied by symptoms of profuse urination, polyphagia and weight loss[3]. Herbs are considered to be the richest source of micro and macronutrients and they are prescribed because of their abundant availability, less cost and they elicit no side effects[4]. Experimental studies reported so far on medicinal herbs were mostly with their organic active principles. Very fewer reports were cited on inorganic constituents. It is to be noted that potassium, zinc, calcium, manganese, magnesium, vanadium and traces of chromium play a major role in the impaired glucose tolerance and thereby acting as essential nutrients in the treatment of DM[5-7]. Calcium and potassium aids in insulin release from β cells of pancreas [8]. Magnesium improves glucose disposal by stimulating glycolysis [9]. Vanadium mimics insulin activity in intact cell systems [10]. Hence, the determination of trace elements in the medicinal plants continues to identify their possible role in controlling the disease conditions.

*Achyranthes aspera*, Linne (*A.aspera*) belongs to the family *Amaranthaceae* and it has been used for centuries in ethno medicine for several medicinal purposes including diabetes mellitus. It is commonly found as weed on the way side and at waste places throughout India. The leaves have both wound healing and antioxidant activities[11]. The aqueous and methanolic extracts of whole plant is said to possess hypoglycemic activity[12]. The plant is said to possess various other activities like hepatoprotective[13], cancer preventive[14], anti-inflammatory, antiarthritic[15], thyroid stimulating and antiperoxidative and seeds contain high proteins [16, 17]. The seeds are reported to yield a saponin which possesses diuretic activity[18]. Since there was no report on the systematic study, the present study was designed to evaluate the antidiabetic nature of inorganic constituents in seed ash.

## MATERIALS AND METHODS

### Plant Material

The plant *A.aspera* was collected in local grazing areas of Chengalpattu, Tamilnadu, India. Seeds were carefully removed using forceps. The plant was identified and authenticated by Dr. V. Kaviyarasan, Centre for Advanced studies in Botany, University of Madras. A voucher specimen has been deposited in the department herbarium.

### Preparation of ash

The seeds were shadow dried, finely powdered using electrical grinder. One hundred gram of properly powdered seeds were taken in a viterosil crucible and placed in an electrical muffle furnace overnight

maintaining its temperature between 430-450˚ C because the loss of zinc may occur at >450˚ C and loss of potassium occurs if the temperature is too high (>480˚ C). The ash was then removed and dried in vacuum desiccator. The yield of ash in the powdered seeds was found to be 6.03g/100g.

**Trace element analysis**

2g of ash was digested with a triple acid mixture comprising of nitric acid, sulphuric acid and perchloric acid in the ratio of 11:6:3 respectively for the complete removal of organic content. The digested sample was made up to 100 ml using deionized water and this sample is used for the assay of trace elements through atomic absorption spectroscopy using hollow cathode lamps.

**Instrumentation and analytical procedures**

The determination of the trace element content of *A.aspera* seeds was carried out using an atomic absorption spectrometer (GBC-Avanta, Australia). The operating conditions for the AAS and the detection limits of the investigated elements are listed in Table 1.

**Table 1:** Operating conditions of the GBC- Avantha AAS detection limits of the investigated elements

| Elements | Detection wave length (nm) | Drying Temperature (°C) | Melting Point (°C) | Detecting limits (ppm) |
|---|---|---|---|---|
| Fe | 248.3 | 120 | 1535 | 0.1-9.0 |
| Mn | 279.5 | 120 | 1244 | 0.5-5.0 |
| K | 766.5 | 120 | 63.65 | 0.2-5.0 |
| Zn | 213.9 | 120 | 419.60 | 0.0-1.50 |
| V | 318.4 | 120 | 1890 | 0.1-90.0 |
| Na | 589.0 | 120 | 97.81 | 0.0-1.0 |
| Mg | 285.2 | 120 | 648.8 | 0.1-0.5 |
| Cr | 357.9 | 120 | 1857 | 0.1-15.0 |
| Ca | 422.7 | 120 | 839 | 0.1- 2.0 |
| Cu | 324.8 | 120 | 1083 | 0.0-5.0 |

**Experimental animals**

Healthy Wistar rats weighing 160-180g were purchased from Tamilnadu Veterinary and Animal Sciences University (TANUVAS) Chennai. The rats were fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), composition of 5% fat, 21% protein, 55% nitrogen-free extract, and 4% fiber (*w/w*) with adequate vitamin levels for the animals and had free access to water. They were acclimatized to standard husbandry conditions. The experiments were conducted according to ethical norms approved by Ministry of Social justices & Environment, Government of India and Institutional Animal Ethical Committee guidelines [IAEC NO, 01/079/09].

**Induction of experimental diabetes**

A freshly prepared solution of Streptozotocin (50mg/kg b.w) in 0.1 M cold citrate buffer of pH 4.5 was intraperitonially injected to the overnight fasted rats[19]. Rats were supplied with 5% glucose solution for 48h after STZ injection in order to prevent severe hypoglycemia. After 1 week time for the development and aggravation of diabetes, the rats with moderate diabetes having persistent glycosuria and hyperglycemia (blood glucose range around 250mg/dl) were considered as diabetic rats and used for further experiment. The treatment was started on the 8$^{th}$ day after STZ injection and this was considered as 1$^{st}$ day of treatment. To select a dose for the inorganic part of the plant sample, adequate precautions were taken to avoid metal toxicity. For this recommended dietary allowances by the food and nutrition board, USA (1980) were followed. The dose calculated and selected was 90mg pure ash/kg body weight suspended in water as a vehicle solution [20].

**Experimental design**

The animals were divided into four groups, comprising a minimum of six animals in each group as follows,

Group 1 - Control rats

Group 2 - STZ induced diabetic rats

Group 3 - Diabetic rats treated with *A. aspera* seed ash (90mg/kg b.w /day) in aqueous solution orally for 30 days.

Group 4 -Diabetic rats treated with gliclazide (5 mg /Kg. b.w/day) in aqueous solution orally for 30 days.

At the end of experimental period, the animals were fasted overnight, anesthetized and then killed by cervical decapitation. Blood was collected in tubes as whole blood for serum and with EDTA for plasma. The estimation of glucose by o-toluidine method[21], protein by Lowry's method[22], hemoglobin by the Drabkin and Austin method[23] glycosylated hemoglobin by Nayak and Pattabiraman method[24] were performed. Blood urea was determined by Natelson method [25]. Serum was used for the determination of creatinine[26] and uric acid[27]. Plasma was used for insulin assay using radioimmunoassay kit for rats (Linco Research, Inc., USA) and serum for the assay of Aspartate transaminase (AST), Alan ine transaminase(ALT)[28] and Alkaline phosphatase (ALP)[29].

**Statistical analysis**

The results were expressed as mean ± SD of six rats per group and the statistical significance was evaluated by one way analysis of variance (ANOVA) using the SPSS (version 15.0) program followed by LSD. Values were considered statistically significant when $p < 0.05$.

## RESULTS

Table 1 shows the operating conditions of AAS and Table 2 shows the levels of inorganic elements present in the *A. aspera* seed ash. In total, ten elements have been analyzed. The concentration of various elements in the seed ash decreases in the following order, K > Mg > Ca > Na > Zn > Fe > Mn > Cu > V > Cr.

**Table 2:** Concentration of inorganic elements present in seed ash

| Element | Concentration (µg/g) |
|---|---|
| Potassium (K) | 2598 |
| Magnesium (Mg) | 1085 |
| Calcium (Ca) | 950 |
| Sodium (Na) | 120 |
| Zinc (Zn) | 88 |
| Iron (Fe) | 60 |
| Manganese (Mn) | 30 |
| Copper (Cu) | 8.5 |
| Vanadium (V) | 0.55 |
| Chromium (Cr) | 0.22 |

Table 3 shows the levels of blood glucose, plasma insulin, urea, uric acid, total proteins, serum creatinine and urine sugar in control and experimental groups of rats. The levels of blood glucose, urea, serum creatinine, and uric acid were found to be elevated in STZ-induced diabetic group of rats whereas the levels of plasma insulin and total proteins were significantly decreased. The altered biochemical parameters were reverted back to near-normal values after treatment with seed ash as well as gliclazide. Urine sugar present in diabetic rats was absent in rats treated with seed ash as well as gliclazide.

Table 4 shows the levels of total hemoglobin and glycosylated hemoglobin in control and experimental groups of rats. A significant increase in the level of glycosylated hemoglobin along with a concomitant decrease in the level of hemoglobin were noticed in diabetic rats, which were reverted back to near- normal level after treatment with seed ash for a period of 30 days.

The activities of AST, ALT and ALP in the serum of control and experimental groups are given in Table 5. A significant ($p<0.05$) elevation in the levels of AST and ALT were noted in serum of STZ induced diabetic rats. Oral administration of *A.aspera* seed ash brought down the activity of AST, ALT and ALP to near normal in serum of diabetic rats.

**Table 3:** Effect of treatment of *Achyranthes aspera* seed ash on biochemical markers in control and experimental groups of rats

| Groups | Blood glucose (mg/dl) | Plasma Insulin (µU/ml) | Total Protein (g/dl) | Serum creatinine (mg/dl) | Blood urea (mg/dl) | Serum uric acid (mg/dl) | Urine sugar |
|---|---|---|---|---|---|---|---|
| **Control** | 95.24± 6.25 | 17.10 ± 0.54 | 8.48 ± 0.18 | 0.87 ± 0.04 | 22.34 ± 2.11 | 2.87 ± 0.06 | Nil |
| **Diabetic control** | 284.14± 21.08$^{a*}$ | 8.18 ± 0.19$^{a*}$ | 4.20 ± 0.19$^{a*}$ | 2.09 ± 0.32$^{a*}$ | 44.73 ± 5.92$^{a*}$ | 6.72 ± 0.17$^{a*}$ | +++ |
| **Diabetic + *A. aspera*** | 102.98 ± 4.69$^{b*}$ | 13.04 ± 0.47$^{b*}$ | 6.61 ± 0.12$^{b*}$ | 0.93 ± 0.06$^{bc*}$ | 25.81 ± 1.84$^{b*}$ | 2.99 ± 0.03$^{b*}$ | Nil |
| **Diabetic + Glyclazide** | 99.18 ± 3.47$^{b*}$ | 12.09 ± 0.69$^{b*}$ | 7.25 ± 0.14$^{b*}$ | 0.89 ± 0.04$^{b*}$ | 26.17 ± 1.23$^{b*}$ | 2.97 ± 0.05$^{b*}$ | Nil |

Results are expressed as mean ± S.D. [n=6]. One-way ANOVA followed by post hoc test LSD.*$p<0.05$. The results were compared with $^{a}$control, $^{b}$Diabetic control. $^{c}$Diabetic + Glyclazide.

**Table 4:** Levels of total hemoglobin and glycosylated hemoglobin in control and experimental group of rats.

| Groups | Hemoglobin (g/dl) | Glycosylated hemoglobin (% Hb) |
|---|---|---|
| **Control** | 14.71 ± 4.32 | 7.05 ± 1.82 |
| **Diabetic control** | 6.53 ± 2.47$^{a*}$ | 17.22 ± 4.64$^{a*}$ |
| **Diabetic + *A. aspera*** | 11.97 ± 2.14$^{b*}$ | 8.02 ± 1.63$^{b*}$ |
| **Diabetic + Glyclazide** | 12.09 ± 2.54$^{b*}$ | 7.95 ± 0.99$^{b*}$ |

Results are expressed as mean ± S.D. [n=6]. One-way ANOVA followed by post hoc test LSD.*$p<0.05$. The results were compared with $^{a}$control, $^{b}$Diabetic control.

**Table 5:** Levels of serum AST, ALT and ALP in control and experimental group of rats.

| Enzymes | Control | Diabetic control | Diabetic + *A. aspera* | Diabetic + Glyclazide |
|---|---|---|---|---|
| AST | 81.16 ± 2.04 | 135.10 ± 5.00$^{a*}$ | 99.00 ± 2.36$^{b*}$ | 91.16 ± 1.60$^{b*}$ |
| ALT | 23.00 ± 2.09 | 53.83 ± 2.56$^{a*}$ | 33.66 ± 1.36$^{b*}$ | 29.50 ± 1.51$^{b*}$ |
| ALP | 80.50 ± 1.87 | 157.33 ± 1.75$^{a*}$ | 92.00 ± 2.09$^{b*}$ | 87.00 ± 1.54$^{b*}$ |

Enzyme activities are expressed as, AST and ALT - µmoles of pyruvate/h/mg of protein, ALP - µmoles of phenol liberated/min/mg of protein. Results are expressed as mean ± S.D. [n=6]. One-way ANOVA followed by post hoc test LSD.*$p<0.05$. The results were compared with $^{a}$control, $^{b}$Diabetic control.

## DISCUSSION AND CONCLUSION

Despite heavy competition from other drug discovery methods, medicinal plants are still providing their fair share of new clinical candidates and proven drugs. The use of metals in therapeutic drugs has become gradually more important over the last couple of decades resulting in a variety of exciting and valuable drugs such as *cis*-platin and auranofin. Some metals are essential for biological functions and are found as cofactors required for various biological processes. Safety pharmacology is a discipline where safety assessment on vital body systems/functions is carried out. The dosing may be single dose or dosing escalating over a few days until a maximum tolerated dose is reached. Before, during and after dosing, vital toxicological and biochemical parameters are monitored by optional methods, after developed for use in human beings. The regulatory authorities validate the side effects of a drug candidate observed in non-clinical safety testing. Based on the results obtained in the physiological observations and biochemical parameters analyzed (data not shown) the optimal dosage for treatment was fixed as 90mg of seed ash/kg body weight/day.

There is accumulating evidence that the metabolism of several trace element is altered in diabetes mellitus and that these nutrients might have specific role in the pathogenesis and progression of disease. In the present study, the hypoglycemic potential of inorganic constituents in *A. aspera* seed ash was evaluated. Trace elemental analysis showed the presence of essential trace elements in the seeds. According to Akhtar and Iqbal (1991), the plant *A. aspera* is rich in minerals like copper, magnesium, manganese and zinc and also the whole plant was said to be hypoglycemic in nature. Focusing on the inorganic constituents present in the seeds, the present study was made to assess the hypoglycemic potential of the seeds. It is well known that trace elements play a pivotal role in various biochemical and physiological process in humans. Trace elements alone or as a component may play a significant role in the development and control of DM.

STZ induced diabetic rats are considered as one of the widely accepted animal models of diabetes mellitus as it emulates the pathophysiological conditions of diabetes in human beings[30]. Streptozotocin is a nitrosourea analogue, preferentially uptaken by pancreatic beta cells via the low-affinity GLUT2 glucose transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD+ and ATP. Enhanced ATP dephosporylation after STZ treatment supplies substrate for xanthine oxidase resulting in the formation of superoxide radicals NO moiety is liberated from STZ leading to the destruction of β cells by necrosis[31]. This condition contributes a number of features similar with Type 2 DM, and is exemplified by stable hyperglycemia, glucose intolerance, and significantly altered glucose-stimulated insulin secretion. Hence, in the present study, streptozotocin induced diabetes in experimental rats was chosen as the animal model to evaluate the hypoglycemic nature of seed ash.

Blood glucose is a biochemical index for the diagnosis of diabetes and is primarily maintained by insulin that regulates the uptake, storage and utilization of glucose. streptozotocin administration causes irreversible damage to the β-cells of pancreas, which resulted in increased levels of blood glucose. From the results obtained, it is evident that STZ induced diabetic rats had much higher blood glucose levels than control rats. Oral administration of *A. aspera* seed ash significantly decreased the blood glucose level in diabetic rats. This suggests the hypoglycemic effect of *A. aspera* seed ash in diabetic rats.

During diabetes there is increased catabolism of proteins because of deranged glucagon mediated regulation of cyclic AMP formation in insulin deficiency[32]. Urea is the end product of protein catabolism. Due to increased catabolism of proteins, blood urea level gets elevated in diabetic rats. Diabetic animals manifest a negative nitrogen balance. Impaired nitrogen balance coupled with lowered protein synthesis leads to increased concentration of urea in blood[33]. Oral administration of *A. aspera* seed ash significantly decreased the blood urea level and increased the level of plasma proteins.

In diabetic rats, it is also observed that there is a decrease in total hemoglobin and an increase in glycosylated hemoglobin. Glycosylated Hemoglobin level was found to increase in patients with DM to approximately 16% and the amount is directly proportional to the fasting blood glucose level[34]. During diabetes excess glucose present in the blood reacts with hemoglobin irreversibly to form glycosylated hemoglobin[35]. Treatment of *A. aspera* seed ash for 30 days decreased the glycosylated hemoglobin level and normalized blood hemoglobin level.

Aminotransferases, such as alanine aminotransferase and aspartate amino transferase measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatases act as markers of biliary function and cholestasis. It is hypothesized that elevation in ALT, AST and ALP are considered as predictors of diabetes[36]. The increased activities of ALT, AST and ALP in the serum of diabetic rats may be primarily due to the leakage of these enzymes from liver cytosol into blood stream as a consequence of the hepatotoxic effect of STZ[37]. Further, the elevation in the levels of these gluconeogenic enzymes whose gene transcription is suppressed by insulin could indicate impairment in insulin signaling rather than purely hepatocyte injury[38]. As a result, their activities in serum increase. Restoration of normal levels of these parameters is achieved by oral administration of *A. aspera* seed ash which indicates the normal functioning of liver.

The inorganic elements have been investigated as a potential preventive and treatment agents for both type1 and type2 DM[39]. The differences in the concentration of these elements are attributed to the soil composition and climate in which a plant grows. It has been found that the plant *A.aspera* is rich in minerals like copper, magnesium, manganese, vanadium, chromium, calcium, zinc, sodium, and potassium which do play a pivotal role in insulin metabolism.

Chromium is an important trace element found to be mainly associated with glucose homeostasis, especially in the metabolism of glucose and lipid molecules. Cr facilitates binding of insulin to its receptors. In other words, Cr increases the number of insulin receptors thereby increasing the affinity of insulin to its receptors[40].

Vanadium facilitates the glucose uptake thereby enhances the insulin sensitivity. Vanadium mimics most of the actions of insulin. Vanadium up regulates the insulin receptors[41]. Recently we have also reported the hypoglycemic, hypolipidemic and antioxidant properties of macro cyclic oxovanadium complexes in STZ induced diabetic rats [42–44].

The other important element found to be essential for humans is magnesium, an important constituent of plasma is involved as a cofactor for more than 300 enzymes. It is essential for all energy-dependant transport systems, glycolysis, oxidative energy metabolism and cell membrane stabilization. Magnesium supplementation has a mild positive effect on insulin sensitivity[45]. Potassium plays a key role in insulin release from β cells of pancreas[7]. Sodium and potassium ions play an important role in the diseases related to renal disorder[46].

Zinc is involved in the synthesis, storage, secretion, conformational integrity of insulin monomers and that zinc assembles to a dimeric form for storage and secretion as crystalline insulin[47]. Zn has been regarded as a possible candidate molecule for the prevention if diabetes due to its beneficial effects on glycemic control in type 1 and type 2 diabetic animals. Moreover, Zn is an important stimulator of insulin mediated signal transduction mechanisms leading to increased glucose uptake in insulin dependent cells[48].

Manganese is a cofactor for a number of enzymes. Mn is essential for hemoglobin formation. Mn is required for normal insulin synthesis and secretion[49, 50]. Cu is an important component of enzymes like cytochrome oxidase, lysyl oxidase. Cu is involved in insulin binding and copper deficiency may be reflected in increased glycosylated hemoglobin, indicative of raised blood sugar level.

Calcium constitutes a large proportion of the bone, human blood and extracellular fluid. Calcium is essential for normal functioning of cardiac muscles, blood coagulation, milk clotting, and the regulation of cell permeability. Oral calcium loads could stimulate insulin secretion in pancreatic islets. Calcium and potassium aids in insulin release from β cells of pancreas[8].

The results obtained from the present study shows that the trace elements that plays a main role in the insulin metabolism are present in appreciable levels. It is to be noted that administration of ash that contained purely of these inorganic elements could normalize the levels of biochemical parameters that were altered in the diabetic group of rats. Earlier, we have reported the hypoglycemic activity of inorganic constituents in *Nelumbo nucifera* seeds and *Eugenia jambolana* seeds on STZ- induced diabetes in rats[51, 52]. We have also reported the mineral contents of *Aloe vera* leaf gel and some medicinal plants used in the treatment of diabetes mellitus [53, 54].

In conclusion, present investigation show the positive speculation that the presence of biologically important inorganic constituents in the seeds of the plant *A. aspera* are responsible for its potent hypoglycemic activity. The seeds might have provided essential minerals thereby compensating the mineral deficiency that occurs during diabetes mellitus. Thus it is concluded that trace elements present in seeds of the plant play a direct or indirect role in ameliorating STZ- induced experimental diabetes in rats. The observed hypoglycemic nature of *A. aspera* may be due to the synergetic effects of trace elements with known antidiabetic activity present in the seeds.

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