

Antidiabetic activity of *Achyranthes aspera* L. with alloxanised mice for the estimation of level of glucose and cholesterol

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ABSTRACT

Achyranthes aspera Linn. (*A.aspera*) is an annual, stiff erect herb, about 0.3 to 0.9m high and found commonly as a weed throughout India. Diabetes mellitus is a chronic metabolic disease with life-threatening complications. Despite the enormous progress in conventional medicine and pharmaceutical industry, herbal-based medicines are still a common practice for the treatment of diabetes. This paper explains evidence based-information regarding the antidiabetic activity of ethanol extract of *Achyranthes aspera* with alloxanised mice for the estimation of level of glucose and cholesterol. In case of alloxanized mice due to administration of ethanol extract of *A.aspera* the level of blood glucose was found to be increased by 123% and 128% on the fifteenth and thirtieth day of exposure respectively. It was obvious from the result that, the ethanol extract of *A.aspera* played a beneficial role in the maintenance of glucose level in alloxanised mice. Due to administration of lone effect of ethanol extract of *A.aspera* the level of cholesterol was found to be decreased in mice by 3.6% on fifteenth day of exposure. However, after completion of exposure period the tendency to decrease in the state of hypocholesterolemia was pronounced and was found to be 5.5%. From the results of the present study it is obvious that the *A. aspera* has potent anti diabetic effect in alloxan induced diabetic mice and could therefore be used as a remedy for the treatment of diabetes mellitus.

Key words: *Achyranthes aspera*, anti-diabetic, alloxan, glucose and cholesterol.

INTRODUCTION

Diabetes mellitus is one of the most common disease affecting millions of people. At least 30 million people throughout the world suffer from diabetes mellitus. Life expectancy may be halved by this disease, especially in developing countries where its prevalence is increasing and adequate treatment is often unavailable. Diabetes not only kills, but it is a major cause of adult blindness, kidney failure, neuropathy, heart attack and strokes. It is a chronic disease characterized by deranged secretions of effects of insulin and /or glucagons, extensive disturbances of carbohydrates, proteins and lipid metabolism, thickening of capillary basement membrane throughout the body leading to microangiopathy and macroangiopathy and long term complications involving eye (cataract), kidney (renal failure), peripheral nervous system (autonomic neuropathy) and circulation (cardiomyopathy and atherosclerosis).

Insulin is essential for maintaining glucose homeostasis and regulating carbohydrate, lipid and protein metabolism (Rosen, 1989). Diabetes is a condition where insulin is absent, deficient or defective, so that sugar- glucose cannot enter into the cells and remains in the blood in excessive amounts. Diabetes basically can be categorized into two types (WHO, 1985). It is characterized by the virtual absence of β (beta) -cells from the islets of Langerhans in the pancreas. It is an autoimmune disease in which the body's own immune system attacks the pancreas rendering it unable to produce insulin that regulates blood glucose (Ali *et al.*, 1993 and Guhabakshi *et al.*, 1999). Common signs and symptoms of type I diabetes include high levels of sugar in the blood and urine, weight loss, extreme thirst, weakness and exhaustion, irritability and mood swings, nausea and vomiting. NIDDM represents a variety of diabetic states in which β -cells are usually low in number relative to a (α) -cells and insulin secretion is usually

sufficient to oppose the ketogenic actions of glucagons but not to prevent hyperglycemia. It is often associated with obesity. It is a heterogeneous metabolic disorder characterized by the impaired insulin secretion from the pancreatic beta cells and the insulin resistance in the peripheral tissues such as liver, adipose tissue, and skeletal muscle (Ward *et al.*, 1984).

Several drugs such as biguanids and sulfonyl urea are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for a new class compounds is essential to overcome these problems (Kamaeswara Rao *et al.*, 2001). *Achyranthes aspera* L. (Amaranthaceae) is an indigenous medicinal plant of Asia, South America, and Africa. It is commonly used by traditional healers for the treatment of fever, especially malarial fever, dysentery, asthma, hypertension and diabetes (Girach and Khan, 1992). *A. aspera* leaves have been assessed for cancer chemo preventive activity (Chakraborty *et al.*, 2002). Hence in the present investigation attempt to find out the antidiabetic effect of *A.aspera*.

MATERIALS AND METHODS

Induction of diabetes

Diabetes was induced in mice by the intraperitoneal (i.p.) injection of alloxan at a dose of 100 mg/kg. Animals were kept for a period of 15 days to stabilize the diabetic condition. Animals showing fasting blood glucose levels above 200 mg/dl were considered as diabetic.

Experimental protocol

A total number of 30 mice were randomized into 5 groups of control and experimental animals. Lethal dose of plant extract and alloxan was analyzed using Sprague (1963) method. Based on the 96 hrs LD₅₀ value a sub lethal concentration of 0.2g/kg of alloxan and a sublethal concentration of 12.5g/kg of plant extract were chosen for the treatment. The analysis was carried out 15th and 30th days of experimental period.

Group I: Served as untreated control (normal) and did not receive any treatment.

Group II: Animals were treated daily with single intraperitoneal (i.p) injection of alloxan monohydrate (0.2g / kg) after overnight fast for 12 hours.

Group III: Animals were received ethanol extract of *A. aspera* leaves (12.5g / kg) for 30 days after the diabetic state was assessed in alloxan induced diabetic mice.

Group IV: Animals were received ethanol extract of *A. aspera* leaves (12.5g / kg) for 30 days.

Group V: Animals were received tolbutamide (standard antidiabetic drug) (100 mg/ kg) for 30 days after inducing diabetes.

During treatment pellet feed and water were given in *ad libitum*. Food consumption, general condition and other symptoms were observed daily and body weight was recorded fort nightly.

Biochemical Estimations

Biochemical estimation was carried out in blood homogenate sample of control and experimental animals in each group. It includes the estimation of glucose and cholesterol in blood.

Preparation of blood and liver homogenate samples

Blood samples were collected from control and treated animals into heparinized tubes using cardiac puncture bleeding techniques. Plasma was separated from the blood sample by centrifugation at 1000rpm for 15minutes.

Estimation of Glucose

The glucose level was qualitatively tested in urine samples using Benedict's method. Five ml of Benedict's solution was boiled, and then added to 0.5 ml (8 drops) of urine samples collected from control and treated animals. The color change indicated the presence of glucose in urine sample and the intensity of glucose level is denoted with +signs, green color (+), yellowish green color (++) and reddish brown color (+++). If glucose obtained in samples, then the blood and liver tissue homogenate samples were taken and estimated the level of glucose in the samples.

Glucose level was estimated by the method of O-toluidine using the modified reagent of Sasaki *et al.* [1972]. Separately 0.1 ml of freshly drawn blood and 0.1 mg of liver tissue homogenate samples were immediately mixed with 1.9 ml of 10% TCA to precipitate the proteins and then centrifuged. One ml of the supernatant was mixed with 4.0 ml of O-toluidine reagent and was kept in boiling water bath for 15 minutes. When green color developed, it was read calorimetrically at 620 nm. The level of glucose was calculated using standard graph of known concentration of glucose level (20-100mg). The concentration of glucose was expressed as mg/dl of blood.

Cholesterol estimation: (Zak *et al.*, 1954).

0.1ml of the serum was added to 9.9ml of ferric chloride acetic acid reagent and centrifuged. It was then stoppene, condensed and mixed well then allowed to stand for 10 minutes to precipitate proteins. 5ml of supernatant was used as test.

To a set of other test tubes named standards 0.5, 1.0, 1.5, 2.0 and 2.5ml were taken. All the tubes were made up to 2.5ml using ferric chloride acetic acid reagent and another tube containing 2.5ml of ferric chloride- acetic acid reagent was marked as blank. To all the tubes 3ml conc.H₂SO₄ was added and mixed by complete inversion. The tubes were allowed to stand for 30 minutes. The color developed was read at 550nm. Total cholesterol values were expressed as mg/dl for plasma and mg/ml protein for erythrocyte membrane and mg/g for tissues.

RESULTS AND DISCUSSION

Level of Blood glucose in Serum

The level of blood glucose in mice maintained as control, alloxanized mice, mice treated with lone effect of *A.aspera* and in combination with alloxan and also individual effect of tolbutamide, was estimated and the results were reported in Table 1.1. In mice maintained as control there was no distinct variation in blood glucose level after exposure period.

Table 1. Effect of administration of ethanol extract of *A. aspera* leaves on blood glucose level in alloxan induced diabetic mice

Groups	Treatment	Blood sugar (mg / 100ml)		
		Initial	Day 15	Day 30
I	Control	85.1 ± 1.8	86.0 ± 0.2	86.0 ± 1.8
II	Alloxan	86.0 ± 2.0	370 ± 20	383 ± 21
III	Alloxan + <i>A. aspera</i> extract	85.0 ± 2.0	190 ± 16	180 ± 15
IV	<i>A. aspera</i> extract only	82.0 ± 1.5	81.0 ± 0.2	81.0 ± 2.1
V	Alloxan + Tolbutamide	83.0 ± 2.0	22.0 ± 0.8	18.0 ± 9.5

All values are mean ± SD of six animals in each group

In the present study, due to administration of alloxan the level of blood glucose was found to be increased by 330% on the fifteenth day of exposure period. However, after the completion of exposure period (30 days) the tendency to increase in the level of blood sugar was not much and was found to be only 340%. The percentage difference of the level of blood glucose on 15th and 30th day of exposure was 10. Increase in blood glucose in Swiss albino mice due to alloxan was reported by various researchers (Moram, 2001, Hanefi, 2004 and Zulfiker *et al.*, 2010).

Further, due to tolbutamide, the level of blood glucose was reduced in mice by 73% on the fifteenth day of exposure and the same was 90% on thirtieth day. The percentage difference in the level of blood glucose on 15th and 30th day of exposure was 17. In case of alloxanized mice due to administration of ethanol extract of *A.aspera* the level of blood glucose was found to be increased by 123% and 128% on the fifteenth and thirtieth day of exposure respectively. The percentage difference in the level of blood glucose on 15th and 30th day of exposure was 4. When compared the result of ethanol extract of *A.aspera* in combination with alloxan with that of the lone effect of alloxan, the combined effect exhibited a favourable result. The result was in accordance with the reports of Moram 2001 and Zulfiker, 2010 in alloxanized mice due to the extracts of *Panax quinquefolius* and *Scoparia dulcis* respectively.

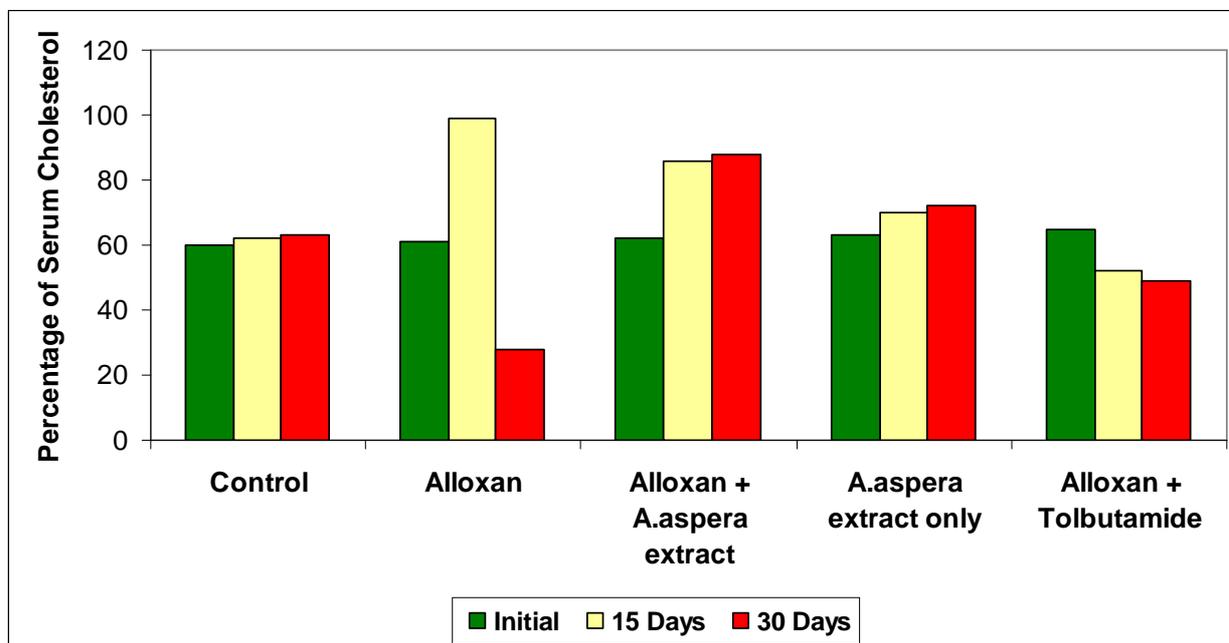
In the present study, due to administration of ethanol extract of *A.aspera* the level of blood glucose was found to be increased by 20% on the fifteenth day of exposure. However, after the completion of exposure period the tendency to increase in blood glucose level was not much and was found to be only 24.8%. The percentage difference in the level of blood glucose on 15th and 30th day of exposure was 4.8. Increased level of blood glucose observed in mice of the present study coincided the result observed in mice due to petroleum, ether, methanol and aqueous extracts of *Terminalia catappa* (Nagappa *et al.*, 2003).

When compared the blood glucose level in alloxanised mice with that of mice treated with alloxan in combination in ethanol extract of *A.aspera* the sugar level was found to decrease 370 – 190 and 383 – 180 mg/ ml on fifteenth and thirtieth day of exposure. However, when compare the hypoglycemic effect of ethanol extract of *A.aspera* with that of standard antidiabetic drug, a reduced, hypoglycemic activity was observed in mice due to administering plant extract.

Level of Cholesterol in Serum

The level of cholesterol in mice maintained as control, alloxanized mice, mice treated with lone effect of *A.aspera* and in combination with alloxan and individual effect of tolbutamide was estimated and the results were given in Fig 1.2. In mice maintained as control there was no distinct variation in cholesterol level, during the exposure period.

Fig 1. Percentage variation of Serum Cholesterol activity in alloxanised mice due to ethanol extract of *A.aspera*



The alloxanised mice showed hypercholesterolemic by 50.3% on the fifteenth day of exposure. However, after completion of exposure period of 30 days the tendency to increase in cholesterol level was not much and was found to be only 53.6%. The percentage difference in the level of cholesterol on 15th and 30th day of exposure was 3.3. Increase in cholesterol in alloxanised mice was due to reported by various researchers (Asokan *et al.*, 2010, Edem, 2010 and Gupta, 2011).

Further, in mice treated with tolbutamide with the increase of exposure period, the condition of hypocholesterolemic was also found to increase and was 4.8 and 11.5% on fifteenth and thirtieth days of exposure. The percentage difference in the level of cholesterol on 15th and 30th day of exposure was 6.7. In alloxanized mice due to administration of ethanol extract of *A.aspera* the cholesterol level was found to be decreased by 34.2% and 56.5% on the fifteenth and thirtieth day of exposure respectively. The percentage difference in the level of cholesterol on 15th and 30th day of exposure was 22.3.

When compared the level of cholesterol in alloxanised mice with that of mice treated with alloxan in combination with ethanol extract of *A.aspera*, the cholesterol level was found to decrease by 212 - 161% and 219 - 170% on fifteenth and thirtieth day of exposure (Table 3.11). However on comparing the level of cholesterol in mice due to ethanol extract of *A.aspera* and tolbutamide, a reduced level of cholesterol was observed in mice due to the administer of plant extract. A favorable influence was observed in alloxanised mice regarding the total cholesterol level due to extract of *A.aspera*. The result coincided with the report of Edem, 2010 in alloxanized mice due to the extract of *Persea Americana mill*.

Due to administration of lone effect of ethanol extract of *A.aspera* the level of cholesterol was found to be decreased in mice by 3.6% on fifteenth day of exposure. However, after completion of exposure period the tendency to decrease in the state of hypocholesterolemia was pronounced and was found to be 5.5%. The percentage difference in the level of cholesterol on 15th and 30th day of exposure was 1.9. Similar kind of improvement in the cholesterol level in mice was noticed due to methanol and aqueous extracts of *Glinus Oppositifolius* (Gobinda Mohan *et al.*, 2010).

Table 2. Effect of ethanol extract of *A. aspera* leaves on serum Cholesterol level in alloxan induced diabetic mice

Groups	Treatment	Cholesterol ($\mu\text{g} / \text{ml}$)		
		Initial	15 days	30 days
I	Control	140.0 \pm 1.5	142.5 \pm 2.6	143.0 \pm 2.1
II	Alloxan	141.0 \pm 2.4	212.3 \pm 6.2	219.0 \pm 2.1
III	Alloxan + <i>A. aspera</i> extract	142.0 \pm 1.9	161.0 \pm 3.9	170.0 \pm 4.2
IV	<i>A. aspera</i> extract only	143.0 \pm 1.4	146.2 \pm 3.1	151.0 \pm 3.9
V	Alloxan + Tolbutamide	145.0 \pm 1.2	138.0 \pm 3.4	127.0 \pm 4.6

The data obtained in the present study due to the treatment of alloxan in mice aptly demonstrated the extent of effect exerted by alloxan on the carbohydrate metabolism particularly the hyperglycemic and hypoinsulinemic. Hyperglycemia can be considered as a direct reflex to the marked hypoinsulinemia caused by the selective destructive cytotoxic effect of alloxan on the cells of pancreas (Bolaffi *et al.*, 1986). From the biochemical point of view, increased hepatic glucose output in diabetes mellitus may be due to gluconeogenesis. The high rate of gluconeogenesis observed in the present work may be the reason for hyperglycemia of the diabetic mice as explained by Shibib *et al.*, 1993, Ravi *et al.*, 1998.

Amelioration of serum glucose concentration besides elevating insulin concentration in alloxanised mice due to the ethanol extract of *A.aspera* of the present study was similar to the result of Moram, 2001 in rats due to aqueous extracts of green tea, sage and ginseng. Data of the present study were in good agreement with other investigators (Abdel- Moneium, 1998 and Broadhurst *et al*, 2000) who explained the positive effects of specific plant extracts and insulin activity and also suggested a possible role of plants in improving glucose and insulin metabolism.

The results of the present study the possible reason for these hypoglycemic states observed in diabetic mice are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Holt, 2008). This may be the reason for the increased level of serum enzyme in the alloxanised mice of the present study, since alloxan is toxic to liver.

From the results of the present study it is obvious that the *A. aspera* has potent anti diabetic effect in alloxan induced diabetic mice and could therefore be used as a remedy for the treatment of diabetes mellitus.

CONCLUSION

Diabetes is a metabolic disorder basically a disease of glucose metabolism resulting from dysfunction of pancreatic beta cells and insulin resistance. It is specifically characterized by hyperglycemia. Using of synthetic antidiabetic agents to treat diabetic can lead to undesirable side effects. Further, the high cost and low availability of synthetic drugs and the recommendation insisted by WHO on diabetes mellitus have led to force the researchers to find hyperglycemic drugs of plant origin. Considering the above facts, the plant *A.aspera* was chosen to find its antidiabetic efficacy against alloxan induced diabetic Swiss albino mice. In diabetic mice administration of ethanol extract exhibited significant hypoglycemic activity. It is obvious from the present study that *A. aspera* has beneficial effect on blood glucose level as well as liver function due to diabetes. These results could be used in the medical treatment in case of deficiency of insulin hormone by using medicinal plant.

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