ORIGINAL RESEARCH PAPER
VOLUME-6 | ISSUE-6 | JUNE-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

Effect of ethyl acetate - aqueous extract of *Acanthospermum hispidum* DC. on hyperglycemic condition in STZ induced diabetic rats

Botany

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ABSTRACT

This study aimed to testify the anti hyperglycemic effect of ethyl acetate aqueous extract of *Acanthospermum hispidum*, DC. .a member of the family Asteraceae. In this study it is investigated in both normal and streptozotocin induced diabetic rats and compared this effect with the effect of glibenclamide, a standard hypoglycemic agent. Blood glucose and urine sugars are estimated. Oral glucose tolerance test is conducted. Insulin level is estimated in Plasma of normal and STZ induced diabetic rats by ELISA method. Insulin is assayed. It is found that the extract of *A.hispidum* normalized the hyperglycemic condition in STZ induced diabetic rats, because of its potent anti hyperglycemic activity. This study confirmed that *A. hispidum* plant can mitigate and assist in combating diabetic complications.

KEYWORDS:

Hyperglycemic, diabetes I&II, blood sugar, induced diabetes, glibenclamide, etc.

Introduction:

Diabetes mellitus is one of the most devastating diseases in the world. It is the third leading cause of death in many developed countries. The worldwide survey reported that the diabetes is affecting nearly 10% of the population (Jong, et al., 2006). The vast majority of cases of diabetes fall into two broad etio pathogentic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and inadequate compensatory insulin secretary response. Diabetes mellitus is an international public health problem since ancient days. The condition is predominantly more severe in developing countries like India where, life is more sedentary due to the even changing lifestyles in this fast-paced global scenario. The disease is now highly visible across all sections of society within India. There is now the demand for urgent research and intervention at regional and national levels to try to mitigate the potentially catastrophic increase in diabetes that is predicated for the
upcoming years.

The management of diabetes is considered as a global problem. The synthetic anti diabetic agents like sulfonylurea's, biguanides, thiazolidinedione and α-glucosidase inhibitors reduce the blood glucose level but have different adverse effects, thus limiting their use and made diabetes and the related complications continued to be a major medical problem. The limitation of the pharmaceutically available anti diabetic drugs paved the way to search for the alternative medicine. Use of medicinal plants to cure various ailments including diabetes is the old tradition in Indian systems of traditional medicine, till it is practiced successfully by the Ayurvedic medicinal practitioners. Plants are the major source of pharmaceutical drugs, because of their diverse Phyto constituents. The searching for new anti diabetic drugs from natural plants is still attractive because their usage was safe and with minimal side effects. However only a few of these medicinal plants have received scientific scrutiny till to date despite, the fact that the World Health Organization has recommended the medicinal and scientific examinations of such plants should be undertaken. Ethno Botanical information identifies about 800 Indian plants which may have anti-diabetic potential. Research findings, include plant parts used, methods of extraction and preparation and specific compounds isolated and tested for their anti-diabetic effects (Grover et al., 2002). The exhaustive presentation of research into 45 plants used in traditional Indian medicine and found to have potential in trading diabetes mellitus is existed.

Acanthopermum hispidum is a weed growing along the roads and in moist habitats throughout India, belongs to the family Asteraceae. In literature it is stated that this plant is useful in Urinary tract disorders and has anti diabetic and anti hyper lipedemic effects. It is reported to possess antioxidant, antibacterial and anti inflammatory activities. The present work thus attempts to analyze the wide potential traditional plant *A.hispidum* to identify or testify the hypoglycemic and anti hyperglycemic effect to ethyl acetate extract of the plant. *A.hispidum* is investigated in both normal and streptozotocin induced diabetes in the existing literature and offers immense scope for researchers engaged in validation of the traditional claims and development of safe and effective herbal drug having anti diabetic activity. The present study is focused on evaluating the anti hyperglycemic effect of *A.hispidum,* isolation and identification of active constituent, preparation of standardized dose and dosage regimen which plays a significant role in improving the hypoglycemic action. In the current study it is investigated the anti-hyperglycemic effect of ethyl acetate extract of *A.hispidum* in STZ induced diabetic rats and compared this effect with the effects of glibenclamide, a standard hypoglycemic agent.

Methodology:

Collection of Plant material:

Leaves of *A. hispidum* (Fig. 1) are collected from Rangampet (vi.), Sri Venkateswara University campus and surrounding areas of Chittoor district, in August 2012. The plant is identified with the help of herbarium and floras. The voucher specimen is deposited in the herbarium of Department of Botany, Sri Venkateswara University, Tirupati.

Fig.1. *Acanthospermum hispidum* twig:

Preparation of plant extracts:

The plant is shade dried in the laboratory and then grinded it into powder. The powder is placed in a Soxhlet's apparatus and extracted with different solvents i.e. water and ethyl acetate following continuous hot extraction for 48 hours. The extracts are used for the necessary tests.

Experimental animals:

Healthy male albino wistar rats aged three and half months (Body weight~180-250) procured from Sanzyme P. Ltd. , Hyderabad, Telangana are used in this study. The animals are fed on pellet diet (Hindustan Lever, India).

Chemicals:

Streptozotocin (STZ), Adenosine tri phosphate, glucose-6-phosphate, Sodium dodecyl sulphate, Glutathione reduced are purchased from sigma chemical company, USA. Organic solvents are obtained from commercial sources in higher purity available and insulin kit purchased from BARC, Mumbai, India.

Experimental induction of diabetes:
Diabetes is induced in male wistar rats aged 4 months (Body weight ~180-250mg) by intra peritoneal administration of STZ. All the animals are allowed to free access to tap water and pellet diet and maintained at room temperature.

Experimental design:
Every batch rats are divided into 6 groups and each group consisted of 6 rats. Group 1: Untreated normal rats; Group 2: Untreated diabetic rats; Group 3: Diabetic rats treated with 150 mg/ kg plant extract; Group 4: Diabetic rats treated with 300 mg/ kg. plant extract;

Group 5: Diabetic rats treated with 450 mg./kg. plant extract.

Ethyl acetate extract of *A.hispidum* is dissolved in distilled water and administered orally for 15 days. After an overnight fast the plant extract suspended in distilled water is fed by gastric intubation using a force feeding needle. Group 1 and Group 2 rats are fed distilled water alone. Blood samples are collected for the measurement of blood glucose from the tail vein before plant extract treatment and after plant extract treatment. Blood glucose is measured and the results are compared with those of normal rats.

Determination of blood glucose and urine sugar:
Blood glucose is determined by the O-toluidine method 10. 0.1 ml of blood is precipitated with 1.9 ml of 10% TCA and the precipitate is removed after centrifugation. 1 ml of supernatant was mixed with 4 ml of O-toluidine reagent and kept in a boiling water bath for 15 minutes and cooled. The absorbance was read at 620 nm. Glucose is expressed as mg/dl of blood. Urine glucose is assessed in fresh urine using glucose indicator stick (Boerhinger Mannheim, Germany).

Oral glucose tolerance test:
OGTT is performed at the end of the experimental period. Prior to OGTT rats are fasted over night (at least 12 hours). 30 minutes following the various treatment schedules, each rat is given an oral glucose load, 2 g/kg body weight. Blood is withdrawn from the retro orbital sinus at 30 minutes (Just before the administration of the extract), time 0 (prior to the glucose load), 30, 60 and 120 minutes after the glucose load. Blood glucose concentration is estimated using a glucose oxidase –peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, USA).

Quantitative determination of plasma insulin:
Insulin level is estimated in Plasma of normal and STZ induced diabetic rats by ELISA method. Streptozotocin (STZ) induces diabetes in almost all the species. Diabetes does vary with the species and the optimal dose required to produce diabetes in rat is found to be 50-60mg/kg ip or iv. Due to its low stability the rapid iv injection appears to be the best root of administration. STZ diabetes can be induced by two ways either by single injection of STZ or by multiple low dose injections of STZ. Initial hyperglycemia is observed by 1hour after the injection and again a hyperglycemia state at 48 hours, the elevated blood glucose level is observed by 48-72 hours (peak effect) and is maintained thereafter.

Estimation of blood glucose:
Estimation of blood glucose is carried out by using dextrostics with Ames Glucometer.

Insulin assay:
The insulin is assayed by modified method of Herbert, et al., (1965) using insulin radio immuno assay kit obtained from BARK, Mumbai, India. Blood samples are collected in EDTA and plasma is kept frozen 20°C till the assay. The flow chart given is as table source for the procedure of the assay.

Results & Discussion:
It is observed that there are 3 – 4 times higher fasting blood glucose levels in diabetic rats than in normal control rats. After the oral administration, at5th and 7th hour, a significant attenuation in the blood glucose (P < 0.0001) is produced on rats at the dose of 200mg/kg. ethyl acetate aqueous plant extract. But less significant attenuation is observed in the rats administered with 200mg/kg of plant extract even at 9th hour (Table – 1).

However, ethyl acetate aqueous extract of *A.hispidum* at a dose of 400 mg/kg caused a significant attenuation (P<0.0001) in the blood glucose at 7th and 9th hour when compared to the normal treated group (Table - 3).

Slight body weight loss is observed in STZ-induced diabetic rats and almost normalized by treatment with extract of *A. hispidum* extract (Table – 2). In diabetic rats, this slight loss of weight may be due to tissue protein break down and muscle wasting via unavailability of carbohydrate as an energy source and catabolism of fats (Gouggean et al., 2008). However, the normal treated rats gained weight with intake of food and water in comparison with the diabetic group after 21 days treatment.

Ethyl acetate aqueous extract are able to reduce blood glucose level significantly compare to the normal diabetic control rats. Of these two extracts of *A.hispidum* tested, the highest dose at 450 mg/kg of extract is effective and improving of glucose tolerance test. Hence this dose is selected for the long term study for 2 weeks (15 days). The findings in this study showed that, the ethyl acetate aqueous extract of *A.hispidum* normalized the hyperglycemic condition in STZ induced diabetic rats, because of its potent anti hyperglycemic activity. This study reports the preliminary anti-diabetic potential of *A.hispidum*. They have significant anti-hyperglycemic effect in diabetic rats and when its effect is comparable to that of insulin. Thus this study confirms that the plant *A.hispidum* can mitigate postprandial hyperglycemia and therefore assist in combating diabetic complications. This medicinal plant is considered to be effective and alternative treatment for diabetes with cardiovascular disease

Table 1: Effect of different doses of Acqueous extract of *Acanthospermum hispidum* plant extract on fasting blood glucose levels (mg/dl) of diabetic treated rats (Mean±S.D):

Groups	Mean ±SD %change	3 h	5 h	7 h	9h
Group1	Mean ±SD	81.52 ±6.11[a]	78.14 ±6.65[a]	75.24 ±5.62[a]	76.24 ±6.07[a]
Group2	Mean ±SD	285.96 ±6.32[d]	281.72 ±4.62[d]	282.42 ±6.09[d]	289.64 ±5.66[c]
Group3	Mean ±SD	255.54 ±11.40[c]	248.2 ±7.25[c]	244.59 ±10.22[c]	252.45 ±8.15[d]
Group4	Mean ±SD	265.44 ±7.25[b]	255.63 ±6.12[b]	225.12 ±5.16[b]	204.26 ±5.52[c]
Group5	Mean ±SD	217.17 ±8.43[b]	153.68 ±5.46[b]	132.76 ±6.42[b]	125.82 ±5.46[b]
F-Value		459.45	882.56	642.882	952.285
significance		0	0	0	0

T-test: Values are expressed as mean ± SEM (n=6). ***P<0.0001 compared with diabetic control (one way ANOVA followed by Duncan post-hoc tests)

2. **Effect of *A. hispidum* plant extract on Changes in2body weight**

Groups	Mean ± SD % change	Changes in body weight(g)
Group1	Mean ±SD	23.16[a] ±2.12
Group2	Mean ±SD	43.58[c] ±3.10
Group3	Mean ±SD	28.66[b] ±5.04
Group4	Mean ±SD	19.88[a] ±3.35
F-Value		51.821
Significance		0

Table 3: Changes in plasma glucose, plasma insulin normal, diabetic treated rats (Mean ±S.D):

Groups	Mean±SD %change	Plasma glucose		Plasma insulin	
		Before treatment	after treatment	Before treatment	after treatment
Group 1	Mean ±SD	79.62 ±2.86[a]	24.12 ±2.85[b]	76.68 ±4.13[a]	23.42 ±2.62[c]
Group2	Mean ±SD	282.34 ±5.26[b]	9.25 ±2.12[a]	276.45 ±3.46[d]	9.12 ±1.26[a]
Group3	Mean ±SD	276.62[b] ±6.12[b]	8.56 ±1.13[a]	145.26 ±5.26[c]	14.11 ±2.09[b]
Group4	Mean ±SD	276.23 ±5.62[b]	8.52 ±1.02[a]	126.12 ±3.48[b]	13.16 ±1.15[b]
F-Value		1142.70	152.31	1156.842	78.135
Significance		0	0	0	0

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