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ANTI-DIABETIC ACTIVITY OF THE PLANT *ARISTOLOCHIA INDICA* (LINN.) ROOT

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ABSTRACT

The present study aimed that evaluation anti diabetic activity of root of the *Aristolochia Indica* L. roots by streptozotocin Induced method. The herbal extraction of roots screened for phytochemical study, acute oral toxicity studies, histopathological studies, determination of lipids, triglycerides, Estimation of serum, Estimation of HDL cholesterol, Estimation of LDL, Phytochemical investigation reveals the presence of alkaloids, flavonoids, carbohydrates and glycosides in ethanolic extraction and individual plant extraction. In acute toxicity studies no mortality was observed with the ethanolic extracts even at the dose level of 2000mg/kg body weight. In the present study, the ethanolic extracts reduced the cholesterol and triglycerides in a manner similar to the reduction facilitated by glibenclamide. The antidiabetic activities of glibenclamide and the ethanol extract of *Aristolochia Indica* L. root were evident in streptozotocin-induced diabetic rats.

Key words: *Aristolochia Indica* L. roots.

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INTRODUCTION

Diabetes is defined as a state in which homeostasis of carbohydrates and lipid metabolism is improperly regulated by insulin. Diabetes mellitus or simply diabetes is a disease condition characterized by high blood sugar level, i.e. hyperglycemia. The hormone 'insulin', which is secreted by pancreas, controls the sugar metabolism of the body (Anonymous 1). High blood sugar level is either due to less production of insulin or body's resistance to insulin and sometimes both. Symptoms of diabetes are increased hunger, thirst, loss in weight, blurred vision, etc., Resistance to the metabolic actions of insulin is one of the salient features of impaired glucose tolerance and NIDDM (Anonymous 2). Large, population-based studies in China, Finland and USA have recently demonstrated the feasibility of preventing or delaying, the onset of diabetes in overweight subjects with mild glucose intolerance. [7]Oral hypoglycemic drugs are of two kinds' sulphonamide derivative (sulphonylurea) and Guanidine derivative (biguanides). They are used by 30% of all diabetics. Medicinal plant contains so many chemical compounds which are the major source of therapeutic agents to cure human disease. *Aristolochia Indica* L. is distributed throughout India at low elevations. The young roots are light brown and fairly smooth, whereas the older ones are comparatively rough due to the development of cork, lenticles and the presence of scars of rootlets (Bhattacharya PK & Sarkar, 1986). The major chemical constituients are phenanthrene derivaties, terpenes, alkaloids, flavonoids, quinones and lactones. The roots are bitter, acrid, astringent, thermogenic, purgative, anthelmintic, stomachic, cardiotonic, anti-inflammatory.

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MATERIALS AND METHODS:

Collection of plant and authentification

The species for the proposed study that is *Aristolochia Indica* (Linn.) root parts were collected in the month of July 2014 from the town of Uthangarai, Krishnagiri (DT). The species for the proposed study was identified and authenticated as *Aristolochia Indica* (Linn.) By Joint Director, Government of India, Ministry of Environment & Forest, Botanical Survey of India, Southern Circle, T.N.A.U. Campus, Lawley Road, Coimbatore.

Extraction:

Air-dried & coarsely powdered (500 gm) of *Aristolochia indica* root was extraction in a soxhlet extractor using ethanol. The extract was carried out until the solvent found to be colourless. Then the solvent was filtered and distilled off. Final traces of ethanol were removed under pressure by using rotary vacuum flask evaporator and they were preserved in desiccators.

Preliminary Phytochemical Screening:

The crude ethanolic extract of root *Aristolochia Indica* L were tested for its different chemical groups such as alkaloids, flavonoids, tannins, steroids, saponins, fixed oils, tri-terpenoids, carbohydrayes and glycosides (Satyesh Chandra Prakashi *et al*., 1977).

Experimental animals:

Wistar albino rats (both sex), weighing 150 - 200g were used in this study. Protocols were in accordance with and approved by the institutional animal ethical committee. These animals were kept in an environment with temperature (22°C ± 2°C), humidity (45–57%) and photoperiod (12: 12-h light-dark cycle). All the animals were fed free access to the rat chow and also had free access to water. All animals were performed as per the guidelines of CPCSEA and IAEC. For the determination of LD_{50} value of *Aristolochia Indica* Linn., the procedure was followed by using OECD guidelines 423 (Acute toxic class method) (Roll R *et al*., 1986; Lipnnik RL *et al., 2003*).

ANTI DIABETIC ACTIVITY:

STREPTOZOTOCIN INDUCED DIABETIC MODEL[4]:

The animals were acclimatized for one week before initiation of the experiment. After overnight fasting, diabetes was induced by intraperitoneal injection of streptozotocin (STZ) was freshly dissolved in 0.1 m sodium citrate buffer, pH 4.5, at a dose of 60 mg/kg body weight. After 48 hours of STZ administration rats with moderate diabetes having hyperglycemia (200-290 mg/dL) were used in this study. The treatment was started on the third day after the STZ injection and this was considered the first day of treatment. The treatment was continued for 21 days.

The rats were divided into four groups comprising six animals in each group as follows:

Group 1: Control rats given only CMC (2% w/v), p.o.

Group 2: Diabetic controls (STZ 65 mg/kg body weight of rats).

Group 3:

Diabetic rats treated with Ethanolic Extract of *Aristolochia indicia* (Linn.) root (250 mg/kg body weight of rats/day) dissolved in carboxy methyl cellulose (CMC) by orally for 21 days.

Group 4:

Diabetic rats treated with Ethanolic Extract of *Aristolochia indicia* (Linn.) root (500 mg/kg body weight of rats/day) dissolved in carboxy methyl cellulose (CMC) by orally for 21 days.

Group 5:Diabetic rats treated with Glibenclamide (6mg/kg body weightof rats/day) p.o, for 21 days[11].

After 21 days of treatment period the overnight fasted rats were sacrificed by cervical decapitation and the blood was collected by retro orbital puncture using mild anaesthesia. Serum was separated by centrifugation. After centrifugation at 2,000 rpm for 10 min, the clear supernatant was used for the analysis of various biochemical parameters. The liver and pancreas were excised and rinsed in ice-cold saline and kept in formalin solution.

1. Estimation of body weight

Body weights are estimated for starting day of the experiment and after 21 days of the experiment are estimated per week of all groups of the rats.

Estimation of plasma glucose

Glucose level in plasma was estimated by glucose oxidase/peroxidase method using a commercial kit of SD Check Ozone Biochemical Pvt., Ltd.

The level of glucose is expressed as **mg/dl.**

3. Estimation of Serum insulin

Insulin was assayed in plasma using a commercial kit by enzyme linked immunosorbant assay (ELISA) technique.25 µl of the plasma was dispensed in micro wells coated with anti-insulin antibody. To this, 100 µl of the enzyme conjugate was dispensed into each well, mixed for 5 sec and incubated at 25°C for 30 min. The wells were rinsed five times with washing buffer. Then, 100 µl of solution A and then 100 µl of solution B were dispensed into each well. This was incubated for 15 min at room temperature. The reaction was stopped by adding 50 µl of 2 N HCl to each well and read at 450 nm. The values are expressed as **µU/ml** plasma.

4. Estimation of glycosylated haemoglobin (Hb A_1C)

Glycosylated haemoglobin was estimated by the method using a commercial kit of Asritha Quality Processes. Quality Diagnostics Hyderabad followed by

Trivelli, L.A., Ranney, H.M. and Lai, H.T., New Eng.J.Med.284, 353(1971). [5]It includes hemolysate preparation, glyco hemoglobin (GHb) separation, total hemoglobin (THb) estimation 5 ml of distilled water was taken in a glass tube. To this 0.02 ml of hemoglysate from Step A was added, mixed well and the absorbance was measured at 420 nm in a colorimeter.

The GHb in % was calculated by the following formula

GHb = Abs. of GHb /Abs. of THb X 4.61,

Where, 4.61= Assay factor

From this GHb % (A1), HbA1c (A1C) and Mean blood glucose (MBG) was calculated by the standard chart provided with kit.

The values were expressed as **mg/g haemoglobin.**

5. Estimation of total cholesterol

0.1 ml of extract was evaporated to dryness and 5.0 ml ferric chloride- acetic acid reagent was added, mixed and centrifuged. To the supernatant 3.0 ml of concentrated sulfuric acid was added and the absorbance was read after 20 min at 560 nm against a reagent blank. A Standard in the concentration range of 40-200 µg was treated similarly.

Values were expressed as **mg/dL** serum.

6. Determination of triglycerides

An aliquot of serum/lipid extract was evaporated to dryness. 0.1 ml of methanol was added followed by 4 ml of isopropanol. 0.4 g of alumina was added to all the tubes and shaken well for 15 min. Centrifuged and then 2 ml of the supernatant was transferred to labeled tubes. The tubes were placed in a water bath at 65°C for 15 min for saponification after adding 0.6 ml of the saponification reagent followed by 0.5 ml of acetyl acetone reagent. After mixing, the tubes were kept in a water bath at 65°C for 1 h, the contents were cooled and absorbance was read at 420nm. A series of standards of concentrations 8-40 µg triolein were treated similarly along with a blank containing only the reagents. All the tubes were cooled and read at 420nm.

The triglyceride content was expressed as **mg/dl**.

7. Cholesterol content in lipoprotein fractions

Cholesterol in the lipoprotein fractions was also determined by the method as described earlier. HDL cholesterol was analyzed in the supernatant obtained after precipitation of plasma with phosphotungstic acid/Mg^{2+}. LDL cholesterol and VLDL cholesterol were calculated as follows:

VLDL-C = Triglycerides/5,

LDL-C = Total cholesterol - (HDL-C + VLDL-C).

The levels of HDL, LDL and VLDL-cholesterol are expressed as **mg/dl**.

8. Alkaline Phosphatase

20 µl of plasma was added to 1 ml of working reagent mixed well. The absorbance was measured at 0, 30, 60 and 90 seconds in a auto analyzer and the mean absorbance was taken (ΔA). The ALP was calculated by the following formula

ALP in U/L = ΔA / min X 2713

Where, 2713 = Standard factor

The activity of the enzyme is expressed as U/litre of plasma.

RESULTS AND DISCUSSION:

Table: 1. Effect of Ethanol extract of *Aristolochia indica* and Glibenclamide on glucose tolerance test.

Group	Treatment	Blood glucose level (mg/dl)				
		Fasting	After 30 min	After 60 min	After 90 mins	After 120 mins
I.	Glucose,2 gm/kg	71.16± 2.21	162.5± 2.66	151.41± 2.91	140.15± 2.91	132.41± 2.76
II.	EEAI 250 mg/kg	71.02± 2.11	148.4± 1.91[b]	135.4± 2.35[b]	126.32± 2.87[a]	120.13 ± 2.39[a]
III.	EEAI 500 mg/kg	70.66± 2.85	126.9± 2.28[C]	112.4± 2.76[C]	101.16± 3.1[C]	95± 2.85[C]
IV.	Glibenclamide 6 mg/kg	70± 2.85	111.5± 2.32[C]	100.7± 3.11[C]	89.17± 2.96[C]	80.4± 2.64[C]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at c-p<0.001, b= p<0.01, a = p<0.05.

Table: 2. Body weight changes in EEAI and Glibenclamide of control and experimental groups of rats.

Treatment	Body weight (g) Initial	Body weight (g) Final
Control	179.16 ± 3.52	196.83 ± 4.33
Diabetic Control	166.66 ± 4.42[b]	147.54 ± 3.83[c]
Diabetic + EEAI (250 mg/kg) p.o	175.82 ± 6.26	196.23 ± 6.14[c]
Diabetic + EEAI (500 mg/kg) p.o	175 ± 5.34[a]	184.55 ± 3.83[c]
Diabetic + Glibenclamide (6 mg/kg)	175 ± 4.86[a]	155.16 ± 4.41[b]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at c - P<0.001, b – P<0.01, a – P<0.05.

Table: 3. Effect of EEAI and Glibenclamide on Blood Glucose Level

Groups	Treatment dose/kg, b.w	Blood glucose level (mg/dl)			
		0 Day	7th Day	14th day	21st day
I	Normal control	93.16 ± 3.25	93.13 ± 2.73	93 ± 4.65	92 ± 2.59
II	Diabetic control	277.83 ± 6.10^{C}	288.66 ± 5.86^{C}	289.5 ± 5.15^{C}	296.1 ± 4.26^{C}
III	Diabetic + EEAI 250mg/kg	283.6 ± 4.02^{C}	256.6 ± 6.21^{C}	230.8 ± 6.59^{b}	188.5 ± 3.63^{C}
IV	Diabetic + EEAI 500mg/kg	249 ± 4.59^{C}	203.5 ± 4.26^{C}	179.3 ± 3.64 ^{C}	150.5 ± 2.56^{C}
V	Diabetic + Glibenclamide 6 mg/kg	280.83 ± 3.58^{C}	227.6 ± 3.61^{C}	227.6 ± 3.24^{C}	129.26 ± 3.29^{C}

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at C - p<0.001.

Table 4. Effect of EEAI and Glibenclamide on Total cholesterol, Triglycerides, HDL cholestrol, LDL cholestrol, VLDL cholestrol of control and experimental groups of rats

Groups	Total cholestrol (mg/dl)	Triglycerides (mg/dl)	HDL cholestrol (mg/dl)	LDL cholestrol (mg/dl)	VLDL cholestrol (mg/dl)
Control	144 ± 5.01	83.5 ± 2.10	38.83 ± 1.58	92.33 ± 1.76	17.5 ± 1.50
Diabetic control	260.83 ± 4.73^{c}	193 ± 2.94^{c}	32.83 ± 2.18	188.5 ± 2.78^{c}	31.0 ± 1.18^{c}
Diabetic + EEAI (250 mg/kg)	174 ± 2.31^{c}	98.8 ± 2.55^{c}	42 ± 3.11	96.66 ± 2.85^{c}	25 ± 2.05
Diabetic + EEAI (500 mg/kg)	168.66 ± 2.34^{c}	92.5 ± 2.22^{c}	47.16 ± 3.61^{c}	88.16 ± 4.24^{c}	25.83 ± 1.74
Diabetic + Glibenclamide (6 mg/kg)	155.16 ± 2.27	88.16 ± 3.56^{c}	54.16 ± 2.39^{c}	77.66 ± 2.64	19 ± 1.06^{c}

c – P<0.001

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at ^{c}P<0.001**.**

CONCLUSION:

Plants have played a significant role in human health care since the ancient time. Thus, it is concluded that the ethanol extract of root *Aristolochia Indica* L. Produced a significant anti-diabetic activity against streptozotocin induced diabetic model[12]. However the exact mechanism responsible for activities is currently unclear. Therefore, further investigations need to be carried out to isolate the specific compound.

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