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EFFECT OF *CONVOLVULUS PLURICAULIS* CHOIS. ON BLOOD GLUCOSE AND LIPID PROFILE IN STREPTOZOCIN INDUCED DIABETIC RATS

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ABSTRACT: Objective: To evaluate the hypoglycemic activity of ethanolic extracts, of *Convolvulus pluricaulis* in normal and streptozocin-induced diabetic rats.

Materials and Methods: Ethanolic extract of leaves of *C. pluricaulis* were orally tested at the dose of 400 mg/kg, 600 mg/kg & 800 mg/kg for hypoglycemic effect in normal and streptozocin-induced diabetic rats. In addition, changes in body weight, serum cholesterol, triglyceride and total protein levels, assessed in the ethanol extract-treated diabetic rats, were compared with diabetic control and normal animals.

Results: 800 mg/kg of *C. pluricaulis* produced a significant reduction in fasting blood glucose levels in the normal and streptozocin-induced diabetic rats. Apart from 400 mg/kg, 600 mg/kg showed activity from day 14. Significant differences were observed in serum lipid profiles (cholesterol and triglyceride), serum protein, and changes in body weight by 800 mg/kg treated-diabetic animals, when compared with the diabetic control and normal animals.

Conclusion: 800 mg/kg of *C. pluricaulis* exhibited significant antihyperglycemic activity in normal and streptozocin-induced diabetic rats. They also showed improvement in parameters like body weight and serum lipid profiles and so might be of value in diabetes treatment.

INTRODUCTION: Diabetes mellitus is one of the common metabolic disorders, and 2.8% of the population suffer from this disease throughout the world and it may cross 5.4% by the year 2025. Diabetes mellitus, often simply referred as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced¹. The oral therapy of non-insulin-dependent diabetes mellitus presently relies upon compounds from two chemical classes, sulfonylurea's and biguanidies².

C. pluricaulis (family Convolvulaceae) is one of four plants that is referred to as Shankhapushpi, and appears to be the 'true' form of Shankhapushpi according to the Ayurvedic Pharmacopoeia with the other three herbs (*Clitorea ternatea*, *Evolvulus alsinoides* and *Canscora decussata*) being used as replacements for *Convolvulus*³. *C. pluricaulis* is medicinally used for a brain tonic, nervine tonic, alternative and laxative as well as to reduce anxiety, neurosis, cognitive decline, and has some reported usage for fertility and seminal issues⁴. *C. pluricaulis* contain convolamine, Scopoletine, Ceryl alcohol, β -sitosterol, palmitic, myristic, and linoleic acid⁵.

Earlier studies have shown that the extract of the plant possesses Antioxidant activity⁶, Anti-convulsant activity⁷, Antidepressant activity⁸, Anxiolytic activity^{9, 10}, Learning behavior &

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memory enhancement activity^{11,12}, Anti Thyroid activity¹³, Antiulcer activity¹⁴, Anti-obsessive activity¹⁵, Neuroprotective activity^{16, 17}, Hepato-protective activity¹⁸, Anti-bacterial activity¹⁹, Antiviral activity²⁰, Nootropic activity²¹. So review of literature revealed that the antidiabetic activity of this plant has not been subjected to scientific evaluation. Since the leaves are one of the important parts of the plant *Convolvulus pluricaulis*, it was used for the pharmacological investigation.

METHOD AND MATERIAL:

Collection and authentication of plant material:

The leaves of *C. pluricaulis* was collected from village raksa near Jhansi, (U.P) India and authenticated by Dr. Neelema Sharma, (Research Officer Incharge) in National Vrکشayurveda Research Institute (N.V.R.I.) Department of AYUSH, Ministry of Health & Family Welfare. Jhansi, with accession no. 21706.

Extraction of plant material: Leaves of *C. pluricaulis* was air dried in the shade and coarsely powder by using grinder. The powder plant material (300 gm) was packed in the Soxhlet apparatus and continuously extracted by ethanol at the temperature 70°C. The percentage yield was calculated against 300 g of powder drug. It was 11.89%.

Chemicals and drugs: All the chemicals and solvents were of analytical grade and were procured from Loba Chemie Pvt. Ltd. 107, Wodehouse Road, Mumbai, India. STZ was procured from Himedia laboratories Pvt. Ltd., Mumbai-400086, India. Metformin sample is taken from Indian drug pharmaceutical Ltd. Rishikesh (Utrakhnad), India.

Experimental Animals: Experiments were performed on adult male Wistar rats (body weight range 150–200 g), 10 to 11 weeks of age. Animals were housed and maintained at 22°C under a 12-h light/12-h dark cycle, with free access to food and water. Experiments were carried out during the normal light/dark cycle, always starting at the same hour (10:00 AM). Efforts were made to minimize animal suffering and to reduce the number of animals used. After randomization into various groups and before initiation of experiment, the rats

were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity. All experiments complied with the guidelines on ethical standards for the investigation in animals. The experimental protocol was approved by the Institutional Animal Ethics Committee of the Bundelkhand University. (Reference number BU/Pharm/IAEC/12/025).

Acute oral toxicity studies: Acute toxicity studies were performed on albino wistar rats according to Organization for Economic Co-operation and Development (OECD) – 425 guidelines the animals were kept fasted for 2 hours with free access to water. The *C. Pluricaulis* extract were administered orally at a dose of 50 mg/kg. The dose at which mortality was observed in two out of three albino Wistar rats, it was considered as toxic dose²². However, if no mortality was observed, the procedure was repeated with higher dose such as 100, 300, 500, 2000, 5000 mg/kg body weight. Toxic manifestations like abnormal motor activity, alteration in water or food intake, respiration, sedation and moribund stages were observed for 6 h and mortality for 24 h. There was no mortality amongst the graded dose groups of albino Wistar rats up to a dose of 5000 mg/kg for duration of 72 h. This finding probably suggests that *C. puricaulis* extract are relatively safe or non-toxic in albino Wistar rats at the doses used for this study.

Assessment of Extracts of *C. pluricaulis* on

Normal Fasted Rats: For the normoglycemic study, rats were divided into five groups (n=6) and were administered 2% gum acacia solution, Metformin at the dose of (500 mg/kg)²³ and ethanolic extract of *C. pluricaulis* at the dose of (400, 600 and 800mg/kg). The blood glucose levels were measured just prior to and 2, 4, and 6 h after drug administration²⁴.

Anti-diabetic activity (STZ induced model): The animals fasted overnight and diabetes was induced experimentally by a single intraperitoneal injection of freshly prepared solution of STZ (Himedia, Mumbai) at a dose of (50 mg/Kg body weight of rats) in sterile normal saline solution²⁵. After 72 h. blood was collected with all aseptic precautions from the tail vein of the rat under ether anaesthesia and blood glucose level were determined using the Glucometer, accucheck-COMFORT (Roche-Diagnostics)²⁶.

Control rats were injected with normal saline solution alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on third day after the STZ injection and this was considered the first day of treatment. Fasting blood glucose estimation was done at 0, 2, 4, and 6 h after treatment. Treatment was continued for 21 consecutive days. The fasting blood glucose levels were estimated on days 0, 1, 7, 14, and 21²⁷.

The rats were divided into five groups with six rats in each group as follows;

1. Group I: Normal control rats given only 2% gum acacia solution.
2. Group II: Diabetic control rats (STZ 50 mg/kg body weight of rats).
3. Group III: Diabetic rats that received *C. pluricaulis* extract (400 mg/kg body weight);
4. Group IV: Diabetic rats that received *C. pluricaulis* extract (600 mg/kg body weight);
5. Group V: Diabetic rats that received *C. pluricaulis* extract (800 mg/kg body weight);
6. Group VI: Diabetic rats that received Metformin (500 mg/kg body weight)

These doses were given to albino wistar rats for a period of 21 days orally.

Estimation of Biochemical Parameters: After the completion of the treatment on day 21, the animals were sacrificed by cervical dislocation under mild anesthesia. Blood was collected on decapitation in two different tubes, one with anticoagulant for plasma and another without anticoagulant for serum separation. Serum and plasma was separated by centrifugation at 2,000 rpm for 15 min, and utilized for biochemical studies. The serum was analyzed for total protein (Biuret method)²⁸, TC, TG and HDL were analyzed by kits (Roche Diagnostics GmbH, Mannheim, Germany) on

Hitachi auto analyzer. LDL, VLDL were calculated using the formula of Friedewald *et al*²⁹.

Statistical analysis: All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean \pm standard error of mean (S.E.M.). The results are analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered significant.

RESULTS:

Preliminary Phytochemical Screening: Preliminary phytochemical screening of the extract of *C. pluricaulis* revealed the presence of alkaloids, glycosides, proteins, sterols, carbohydrates, flavonoids, and tannins.

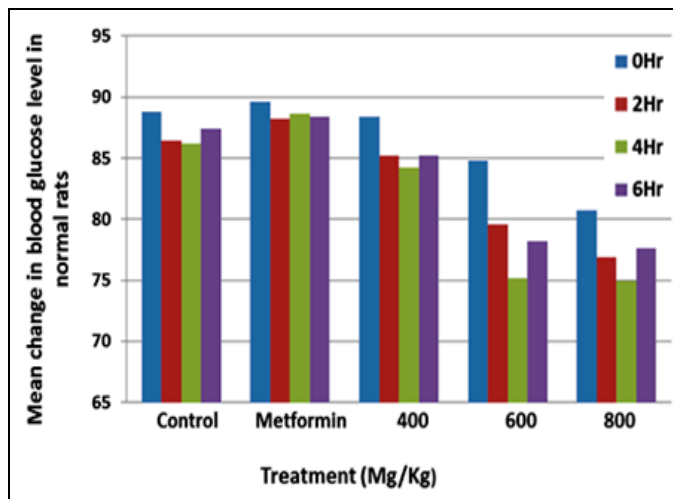
Acute toxicity study: There was no mortality amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 5000 mg/kg. This finding suggests that the ethanolic extract is safe or non-toxic to rats and hence dose of 400 mg/kg, 600 mg/kg & 800 mg/kg, p.o. were selected for the study.

Antihyperglycaemic activity screening in normal and STZ induced diabetic rats: The antidiabetic effects of ethanolic extracts of *C. pluricaulis* on the fasting blood sugar level of normal and diabetic rats are shown in **Tables 1 and 2**. In normal animals, significant ($P < 0.05$, $P < 0.01$) reduction in the blood glucose level was observed by the ethanolic extract as compared to the control [**Table 1**]. 800 mg/kg of *C. pluricaulis* exhibited significant antihyperglycemic activity in normal and streptozocin-induced diabetic rats. However, treatment with 400 mg/kg and 600 mg/kg of *C. pluricaulis* could not bring back the sugar to normal levels.

TABLE 1: THE DOSE RESPONSE EFFECT OF *C. PLURICAULIS* LEAVES ON BLOOD GLUCOSE LEVEL IN NORMAL FASTED RATS

Group	Treatment	0 hour	2 hour	4 hour	6hour
I	Normal control	88.8 \pm 2.3607	86.4 \pm 1.81659	86.2 \pm 1.64317	87.4 \pm 1.14018
II	Standard	89.6 \pm 1.14018	88.2 \pm 1.48324**	88.6 \pm 2.19089**	88.4 \pm 1.81659**
III	400 mg/kg	88.4 \pm 1.14018	85.2 \pm 2.48998	84.2 \pm 1.30384	85.2 \pm 2.16795
VI	600 mg/kg	84.8 \pm 0.83666	79.6 \pm 1.14018	75.2 \pm 3.49285	78.2 \pm 2.38747
V	800mg/kg	80.7 \pm 0.7876	76.9 \pm 1.237*	74.9 \pm 2.987**	77.6 \pm 1.3879**

All values are expressed as mean \pm SEM (n=6); * $P < 0.05$, ** $P < 0.01$ vs Group I



GRAPH 1: THE DOSE RESPONSE EFFECT OF *C. PLURICAULIS* LEAVES ON BLOOD GLUCOSE LEVEL IN NORMAL FASTED RATS

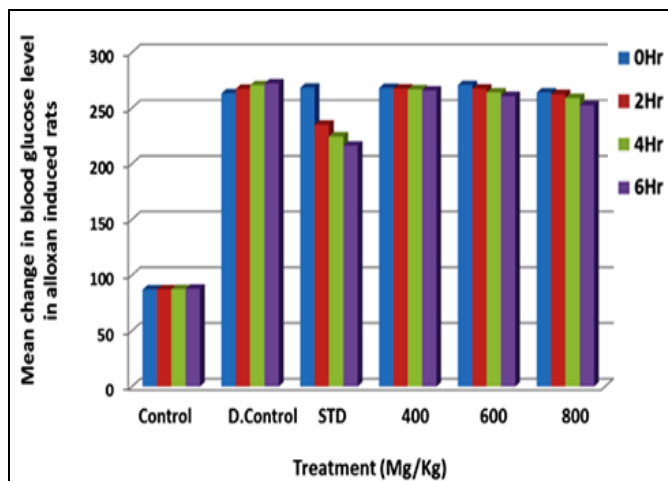
Effect of extracts of *C. pluricaulis* on blood glucose level in STZ (50 mg/kg i.p.)-induced diabetic rats: Administration of STZ (50 mg/Kg body weight of rats) lead to elevation of fasting blood glucose levels, Acute and chronic treatment of the extract of *C. pluricaulis* (400 mg/kg) in STZ-induced diabetic rats resulted in a significant ($P<0.01$) decrease in the elevated blood glucose levels as compared to the control. Acute treatment with 400 mg/kg and 600 mg/kg of *C. pluricaulis* could not bring back the sugar to normal level. However in repeated dose treatment, 600 mg/kg extract showed significant anti-hyperglycemic activity from day 14 and 800 mg/kg extract showed significant anti-hyperglycemic activity from day 7 [Tables 2.1 and 2.2].

TABLE 2.1: THE DOSE RESPONSE EFFECT OF *C. PLURICAULIS* LEAVES ON BLOOD GLUCOSE LEVEL IN NORMAL AND ALLOXAN INDUCED RATS

Group	Treatment	0 Hour	2 Hour	4 Hour	6 Hour
I	Control	87.16±1.47196	87.33±1.36626	87.5±1.0488	87.83±0.752
II	D + Control	263.33±10.801	267.33±8.755**	270.6±9.72**	272.1±11.0**
III	Metformin	268.33±6.8313	235.16±18.66*	224.6±14.7**	216.3±11.6**
IV	400 mg/kg	268.16±11.356	267.5±11.6232	266.8±12.56	265.8±10.628
V	600 mg/kg	270.83±2.041	267.5±4.1833	264.16±4.91	260.83±4.915
VI	800 mg/kg	264.16±8.6120	262.5±6.8920**	259.16±4.9**	252.5±7.58**

TABLE 2.2: THE DOSE RESPONSE EFFECT OF *C. PLURICAULIS* LEAVES ON BLOOD GLUCOSE LEVEL IN NORMAL AND ALLOXAN INDUCED RATS.

Group	Treatment	1 Day	7 Day	14 Day	21 Day
I	Control	88.16±0.752	88.83±0.752	89.66±2.6583	90.66±2.33809
II	D + Control	273.1±11.0**	283.5±8.38**	290.6±9.244**	302.83±12.20**
III	Metformin	205.16±6.4**	148.5±32.7**	118.5±6.892**	98.5±6.89202**
IV	400 mg/kg	264.8±8.95	263.1±8.495	262.5±7.5828*	260.83±6.645**
V	600 mg/kg	259.16±8.01	252.5±9.87**	245.8±10.6**	237.5±6.8920**
VI	800 mg/kg	250.83±6.6**	214.1±16.8**	180.8±25.38**	130.83±14.28**



GRAPH 2: THE DOSE RESPONSE EFFECT OF *C. PLURICAULIS* LEAVES ON BLOOD GLUCOSE LEVEL IN NORMAL AND ALLOXAN INDUCED RATS

Effect of extracts of *C. pluricaulis* on body weight: Vehicle control animals were found to be slightly increased in their body weight but diabetic rats showed significant reduction in body weight during 21 days. STZ caused body weight reduction, which is reversed by ethanolic extract at high dose (800 mg/kg). Ethanolic extract (800 mg/kg) is more effectively than the ethanolic extract at low dose (600 mg/kg) after 21 days of treatment [Table 3].

Biochemical Parameters: STZ treatment will increase the serum enzymes levels such as cholesterol, LDL, creatinine, and alkaline phosphatase and decrease the HDL level, but Metformin (500 mg/kg) and plant extracts of *C. pluricaulis* reversed the above STZ induce changes.

Significant differences were observed in serum lipid profiles (cholesterol and triglyceride) and serum protein at (800 mg/kg)-treated diabetic animals, when compared with the diabetic control and normal animals ($P < 0.01$) [Tables 4.1 and Table 4.2].



PHOTOGRAPH 1: BLOOD WITHDRAWAL FROM DIABETES TREATED WISTAR RAT

TABLE 3: EFFECT OF *C. PLURICAULIS* LEAVES ON CHANGE IN BODY WEIGHT IN NORMAL AND ALLOXAN INDUCED RATS

Group	0 day	7 day	14 day	21day
Control	152.4±1.1	154.6±1.6	157.4±1.1	156.8±1.9
D + Control	163.0±2.2	158.2±1.6	152.4±2.3	147.2±2.5
Metformin	160.4±2.0	162.6±1.1	164.8±2.3	166.2±2.1
400 mg/kg	153.6±2.0	154.6±2.3	155.8±2.2	156.4±2.3
600 mg/kg	164.4±2.1	166.8±1.9	168.0±2.3	168.2±2.4
800 mg/kg	172.3±2.3	174.6±2.0	176.1±1.7	178.5±1.9

TABLE 4.1: EFFECTS OF AQUEOUS EXTRACT OF *C. PLURICAULIS* ON SOME BIOCHEMICAL PARAMETERS IN ALLOXAN-INDUCED DIABETIC RATS.

Group	Control	Cholesterol	TG	HDL-Cholesterol	LDL-Cholesterol
I	Control	123.8±4.02	92.4±5.5497	57.6±4.03733	79.6±4.5055
II	D + Control	252.2±2.5**	290.8±2.7**	44.2±4.54973**	175.6±4.15**
III	D + Metformin	89.6±3.50**	103.6±5.0**	59.4±3.36155**	77.4±5.272**
IV	400 mg/kg	201.6±3.8**	235.2±3.8**	66.6±2.70185**	164.2±4.54**
V	600 mg/kg	170.8±3.0**	161.8±4.3**	111.2±5.7619**	124.2±4.54**
VI	800 mg/kg	120.2±4.1**	107.6±3.6**	122.6±3.974**	72.8±3.834**

All values are expressed as mean ± SEM (n=6); Group II is compared with Group I, Group III and Group VI is compared with Group I; (** $P < 0.01$).

TABLE 4.2: EFFECTS OF AQUEOUS EXTRACT OF *C. PLURICAULIS* ON SOME BIOCHEMICAL PARAMETERS IN ALLOXAN-INDUCED DIABETIC RATS

Group	Control	VLDL	Total Protein(g/dl)	Alkaline Phosphate	Creatinine
I	Control	29.4±3.6469	6.6±1.14018	115.666±4.03	0.7666±0.103
II	D + Control	165.2±3.8**	4.2±0.8366**	249.16±17.4**	1.483±0.14**
III	D + Metformin	39.4±3.64**	6.4±1.1401**	143.3±10.80**	1.15±0.1870**
IV	400 mg/kg	124.6±4.3**	4.4±1.1401**	151.66±9.30**	0.66±0.216**
V	600 mg/kg	44.6±2.96**	6.2±1.4832**	150.8±7.359**	0.83±0.0816**
VI	800 mg/kg	27.4±3.97**	6.8±1.3038**	142.5±9.34**	1.35±0.4505**

All values are expressed as mean ± SEM (n=6); Group II is compared with Group I, Group III and Group VI is compared with Group I; (** $P < 0.01$).

DISCUSSION: In light of the results, our study indicates that ethanolic extract of *C. pluricaulis* exhibited significant anti-hyperglycemic activity in normal and Streptozocin-induced hyperglycemic rats. In normal rats, administration of ethanolic extract shows increase in the blood glucose levels on 2, 4, and 6 h, in which at 400 mg/kg its 85.2, 84.2, 85.2 respectively, at 600 mg/kg its 79.6, 75.2, 78.2 respectively, at 800 mg/kg its 76.9, 74.9, 77.6 respectively so, that means at 400 mg/kg the increase in blood glucose is at highest.

Streptozocin-induced diabetic rats administered with ethanolic extract showed less decline in the blood glucose level on 2, 4, and 6 h, in which at 400 mg/kg its 268.1, 267.5, 266.8 respectively, at 600 mg/kg its 270.8, 267.5, 264.1 respectively, at 800 mg/kg its 264.1, 262.5, 259.1 respectively, whereas they showed higher decline in the blood glucose level on 1, 7, 14, and 21 day, in which at 400 mg/kg its 264.8, 263.1, 262.5, 260.8 respectively at 600 mg/kg its 259.1, 252.5, 245.8, 237.5 respectively, at 800 mg/kg its 250.8, 214.1,

180.8, 130.8 respectively so, that means at 800 mg/kg the decline in blood glucose level is at highest level. They can also improve the condition of diabetes as indicated by parameters like serum cholesterol, serum triglyceride, total protein, HDL-Cholesterol, LDL-Cholesterol, VLDL, Alkaline Phosphate, and Creatinine.

In normal rats, administration of ethanolic extract shows increase and decrease in the body weight on 0, 7, 14 and 21 days, in which at 400 mg/kg its 153.6, 154.6, 155.8, 156.4 respectively, at 600 mg/kg its 164.4, 166.8, 168.0, 168.2 respectively, at 800 mg/kg its 172.3, 174.6, 176.1, 178.5 respectively. Streptozocin-induced diabetic rats administered with ethanolic extract showed increase and decrease in the biochemical parameters of Cholesterol, TG, LDL, VLDL, Total protein, Alkaline phosphate, Creatinine at 400 mg/kg its 201.6, 235.2, 66.6, 164.2, 124.6, 4.4, 151.6, 0.6 respectively at 600 mg/kg its 170.8, 161.8, 111.2, 124.2, 44.6, 6.2, 150.8, 0.8 respectively at 800 mg/kg its 120.2, 107.6, 122.6, 72.8, 27.4, 6.8, 142.5, 1.35 respectively.

In conclusion, *C. pluricaulis* exhibited significant antihyperglycemic activities in normal and Streptozocin-induced diabetic rats. The ethanolic extract of *C. pluricaulis* also showed improvement in lipid profile as well as regeneration of β -cell of pancreas and so might be of value in treatment of diabetes.

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