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EFFECT OF *TRICHOSANTHES DIOICA* AQUEOUS FRUIT EXTRACT IN DIABETES AND DIABETIC COMPLICATIONS

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ABSTRACT: Anti-diabetic and protective activity of *T. dioica* aqueous extract in diabetic complications in streptozotocin (STZ)-induced diabetic rats was evaluated. Albino rats (n = 24) were divided into four groups, of six animals each. Group 1 (normal control) and Group 2 (diabetic control) received normal saline (10 ml/kg/day p.o.) whereas Group 3 (Standard, insulin 6 U/kg/day s.c) and Group 4 received 1000 mg/kg/day p.o. of *T. dioica* extract for 28 days. Extract treated animals showed significant decrease in various biochemical parameters like blood glucose, SGOT, SGPT, creatinine, urea, LDL, Cholesterol and glycated hemoglobin. Extract was also effective in diabetic complications like neuropathy and learning and memory dysfunction evaluated by behavioral models like elevated plus maze, object recognition, open field test, rotarod, hot plate, tail flick, immersion in hot and cold water *etc*. It can be concluded that *T. dioica* possess significant anti-diabetic and protective activity in diabetic complications in STZ-induced diabetic rats.

INTRODUCTION: Diabetes mellitus is the most common metabolic disorder widely prevalent worldwide thus creating urgent need to reflect about life style alteration, diet, stress management, alternative medication *etc*. The secondary metabolites of plants, termed phytochemicals, are naturally occurring, non-nutritional constituents having biological and pharmacological activities, such as antioxidant, anti-inflammatory, antimicrobial, anti-allergic, antibiotic, hypoglycaemic *etc* [1]. Prolonged hyperglycemia and oxidative stress leads to development of diabetic complications. Some of the active principles originate from edible plants and their inclusion in the diet would undoubtedly be of some value because of their hypoglycemic potential [2]. *Trichosanthes dioica*is a vine plant of Cucurbitaceae family, perennial and dioecious.

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The fruits are green with white or no stripes. Present study of the aqueous extract of the fruits of *T. dioica* was undertaken to evaluate its effect in diabetes and its associated complications in streptozotocin induced diabetic rats.

Botanical name: *Trichosanthes dioica*

Common name: Pointed Gourd, Parwal

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Cucurbitales

Family: Cucurbitaceae

Genus: *Trichosanthes*

Species: *dioica*

MATERIALS AND METHODS:

Plant Material: Fresh unripe fruits (6 kg) of *T. dioica* were purchased from the local market and authenticated by Taxonomist, Department of Botany, Maulana Azad College Aurangabad, India with herbarium number MACH - 012453.

The fruits were cut into small pieces, shade dried, mechanically crushed and extracted with distilled water using boiling temperature (100 °C) up to 42 hrs. The extract was filtered and concentrated in rotatory evaporator and reduced under pressure (yield: 15.9 %w/w).

Experimental Animals: Male wistar rats were obtained from Wockhartd Ltd., Aurangabad, India. Animals were housed under standard environmental conditions (25 ± 2 °C temperature, 50 ± 5 % humidity with a 12 h each of dark and light cycle) and maintained with free access to water and standard laboratory diet *ad libitum*. The study was approved by the Institutional Ethics Committee with Ref. number CPCSEA /IAEC/pharm-chem-26/2015-16/116. Experiment was performed on 8-10 weeks old, healthy, male wistar albino rats of body weight from 150-200 grams.

Induction of Diabetes: Diabetes was induced by a single intraperitonial injection of freshly prepared streptozotocin (purchased from Sigma Aldrich Chem. Co. USA.) at a dose of 65 mg/kgb.w in 0.1 M citrate buffer (pH 4.5) to a group of overnight fasted rats. After 3 days of STZ administration, fasting blood glucose level was estimated and postprandial glucose (PPG) was checked regularly till stable hyperglycemia, generally 1 week after STZ injection was achieved. Animals having marked hyperglycemia (> 250 mg/dl) were selected for the study.

Experimental Design: The experiment was carried out on four groups (1, 2, 3 and 4) of six rats each. Group 1 normal (control) treated with *vehicle* Group 2 severely diabetic (control) treated with vehicle, Group 3 severely diabetic treated with Insulin (6 U/kg/day/s.c)[3] and Group 4 with 1000 mg/kg of *T. dioica* extract for 28 days. Control rats (group 1 and 2) received vehicle (distilled water only) orally regularly once a day up to 28 days.

LD_{50}: Acute oral toxicity test for the aqueous extract of fruits of *T dioica* was carried out as per Organisation for Economic Co-operation and Development Guidelines 425. Extract was found to be safe up to 2000 mg/kg. Dose of 1000 mg/kg was selected for the study. The dose of 1000 mg/kg of aqueous extract was found to be more effective [4].

Phytochemical Screening of *T dioica* Aqueous Fruit Extract: The aqueous fruit extract was subjected to qualitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, diterpenes, triterpenes and phenols as per the standard methods [5].

Statistical Analysis: Data were statistically evaluated using one-way ANOVA, followed by Dunnet's test.

RESULTS:

TABLE 1: EFFECT OF *T. DIOICA* AQUEOUS EXTRACT ON BLOOD GLUCOSE LEVELS AT TIME INTERVALS

Group	Treatment	Initial (mg/dl)	1st week (mg/dl)	2nd week (mg/dl)	3rd week (mg/dl)	4th week (mg/dl)
1	Normal Control	103.3 ± 2.2**	100.1 ± 2.4*	100.8 ± 2.3*	97.6 ± 2*	98 ± 2.1*
2	Diabetic Control	101.8 ± 1.57**	377.5 ± 8.7**	370.3 ± 4.8*	359.1 ± 7.4*	327 ± 6.4*
3	Standard Control	101 ± 1.98**	360 ± 5.3**	151 ± 4.2*	127 ±3.3*	116 ± 2.1*
4	*T. dioica* Extract	99 ± 1.8**	354 ± 7.3**	182 ± 2.8*	135.8 ± 4.7*	122 ± 3.1*

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.*P < 0.01, **P < 0.05 *vs.* Control.

TABLE 2: EFFECT OF *T. DIOICA* AQUEOUS EXTRACT ON DIABETIC NEUROPATHY MODELS

Group	Treatment	Hot Plate Paw Withdrawal Latency (in Sec)	Tail Flick Withdrawal Latency (in Sec)	Hot Water Immersion Tail withdrawal latency (in sec)	Cold Water Tail Immersion withdrawal latency (in sec)	Rota rod Fall off Latency (in sec)
1	Normal Control	12.8±0.6*	4.1±0.16*	5.3± 0.21*	12.3± 0.21*	258±4.2*
2	Diabetic Control	6.6±0.33*	1.66±0.33*	2.1±0.30**	4.1±0.3*	35.8±5.5*
3	Standard Control	10.5±0.42*	3.33±0.33*	3.1±0.30**	9.8±0.47*	224±3.5*
4	*T.dioica* Extract	11.5±0.42*	3.5±0.42*	3.5±0.22*	11.1±0.30*	204±.3.9*

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.*P < 0.01, **P < 0.05 *vs.* Control.

TABLE 3: EFFECT OF *T. DIOICA* AQUEOUS EXTRACT ON LEARNING AND MEMORY DYSFUNCTION MODELS

Group	Treatment	Elevated Plus Maze Transfer latency in sec		Object recognition Test Exploration in sec		Open Field Test Number of squares/rearing	
		Central Platform (sec)	Closed Arm (sec)	Familiar Object (sec)	Novel Object (sec)	Locomotor Activity (Num of squares)	Rearing (Num)
1	Normal Control	5.6 ± 0.33*	19.5 ± 0.42*	5.6 ± 0.33*	7.1 ± 0.3*	47.6 ± 1.8*	33.8 ± 0.87*
2	Diabetic Control	15.8 ± 0.6*	53.5 ± 1*	3 ± 0.25*	2.8 ± 0.3*	27.8 ± 0.87*	17.3 ± 0.91*
3	Standard Control	7 ± 0.36*	21.8 ± 1.1*	5 ± 0.25*	6.1 ± 0.3*	39 ± 0.51*	31.3 ± 0.49*
4	*T. dioica* Extract	6.6 ± 0.66*	24.5 ± 0.42*	4.1 ± 0.3**	5.8 ± 0.3*	36.1 ± 0.83*	28.6 ± 0.80*

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.*P < 0.01, **P < 0.05 *vs.* Control.

TABLE 4: EFFECT OF *T. DIOICA* AQUEOUS EXTRACT ON VARIOUS BIOCHEMICAL PARAMETERS

Group	Treatment	CRP (mg/dl)	LDL (mg/dl)	Liver Glycogen (mg/2gm liver wt)	SGOT (mg/dl)	SGPT (mg/dl)	Triglycerides (mg/dl)	Urea (mg/dl)	Creatinine mg/L
1	Normal Control	0.29±0.00*	36±0.81*	2.4±0.03*	59.8±0.54*	50±0.57*	91.3±1.1*	37.3±0.61*	1.21±0.14*
2	Diabetic Control	1.48±0.00*	78.5±0.84*	1.6±0.28*	171.5±0.92*	158.6±1.7*	192±3*	77.6±0.91*	2.4±0.04*
3	Standard Control	0.59±0.01*	40.8±0.83*	2.23±0.04**	75.1±1.1*	66.1±1*	107.5±2.1*	41.6±0.76*	1.26±0.04*
4	*T. dioica* Extract	0.77±0.00*	42.3±1.2*	2.15±0.22**	95±1.2*	79.3±0.49*	115.8±2.88*	47.1±0.47*	1.33±0.01*

Results are expressed as Mean ± SEM (n=6). The data was analysed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test.*P < 0.01, **P < 0.05 *vs.* Control.

DISCUSSION: Effect of aqueous extract of *T. dioica* on blood glucose, various biochemical parameters, behavioral models of learning and memory dysfunction and neuropathy was done. In uncontrolled diabetes mellitus, there are functional changes in many tissues or organs like pancreas, liver, heart *etc* which are reflected as alterations in some of the metabolic pathways and many blood parameters [6]. *T dioica* extract affected themetabolic pathways such that liver, heart and other tissues show tendency to return to normal levels. Results in **Table 1** and **4** show that in the untreated diabetic rats various biochemical parameters were altered while in the extract treated animals blood glucose, urea, creatinine, SGOT, SGPT, LDL, CRP, Triglycerides and glycated hemoglobin were near normal levels at the end of 28 days treatment.

Extract was found to be safe from LD_{50} study as the behavior of the treated rats seemed normal and no mortality was observed. In behavioral models of neuropathy and learning and memory dysfunction the extract proved very effective. Paw withdrawal latency in hot plate, tail flick, immersion in hot and cold water and rotarod indicated that extract was very effective in preventing diabetic neuropathy as the readings are comparable with standard drug insulin in **Table 2**. *Trichosanthes* was very effective in a very important complication of diabetes *i.e*. learning and memory dysfunction as indicated by the results of object recognition, open field and elevated plus maze test in **Table 3**. Two main phytosterols present in *T. dioica* are namely, 24α-ethylcholest-7-enol and 24β-ethylcholest-7-enol 13 and 7-oxidihydrokarounidol-3-benzoate [7]. Plant sterols significantly reduce plasma cholesterol levels [8]. The main phytochemical groups present are alkaloids, glycosides, flavonoids, carbohydrates, fixed oils, steroids, tanninsand phenols [9].

T dioica aqueous fruit extract also has antioxidant activity [10]. From the fruits of Trichosanthes 14 cucurbitane glycosides (khekadaengosides A-J, M-N, cucurbitacin J 2-O-bglucopyranosideand cucurbitacin K 2-O-b-glucopyranoside), a hexanorcucurbitaneglucoside (khekadaengoside K) and octanorcucurbitane (khekadaengoside L) have been isolated along with two known cucurbitane glucosides (cucurbitacin 2-O-b-glucopyranosideand 25-O-acetyl-cucurbitacin 2-O-b-glucopyranoside) [11].

T. dioica is also rich in vitamins and contains 9.0 mgMg, 2.6 mg Na, 83.0 mg K, 1.1 mg Cu and 17.0 mg S per 100 g edible part [12] Vitamin A, Vitamin C, tannins, saponins [13] and flavonoids [14]. The probable mechanism of this benefit is due to its effect in controlling muscle wasting, *i.e*., by reversal of antagonism [15]. *T dioica* possess significant hypoglycemic as shown in **Table 1** and antioxidant activity in STZ rats due to inhibition of endogenous glucose production [16].

Flavonoids in *T. dioica* are responsible of the antioxidant activity in normal and STZ rats [17]. Oral administration of saponins from some medicinal plants, significantly reduce triglycerides and cholesterol levels in rat [18]. Hence it can be concluded that *T. dioica* possess significant anti-diabetic and protective activity in diabetic complications and the antioxidant and anti-hyperglycemic activity is likely due to presence of saponins and flavaonoids. Saponins regulate blood glucose level and prevent diabetic complications due to their antioxidant activity [19].

The chronic complications of diabetes are developed when cells or cellular components are chronically exposed to the high concentrations of glucose. The non-enzymatic glycosylation of proteins and the accumulation of polyol as the sorbitol, result in the formation of end products of the advanced glycosylation in the tissues, causing irreversible changes and this process culminates in cellular damage and complications such as neuropathy, retinopathy and nephropathy [20]. Hypomagnesemia in diabetic is usually observed in patients with deficient metabolic control, or associated with diabetes chronic complications [21, 22]. Trichosanthes is rich in magnesium so it can be assumed that it is effective in diabetic complication due to presence of magnesium also.

T. dioica possesses modulatory effects on blood lipid abnormalities associated with diabetes. A number of lectins are reported in *T. dioica*. Lactines are responsible for many erythrocyte surface alterations in diabetes causing impaired cell function so the improvement in diabetic condition on extract treatment might be due to the presence of lectins in *T. dioica* extract along with other active components for its antidiabetic and anti-lipidemic activity [23]. Many antioxidants like Vitamin C, beta carotene, carotene, saponins and tannins are present in *T dioica* [24] which reduces serum marker enzymes and has free radical scavenging activity may be making it effective in diabetic complications also [25].

CONCLUSION: *T. dioica* aqueous extract possess significant hypoglycemic, antioxidant and protective activity in diabetic complications. Though it can be concluded that its activity is due to various phyto-constituents present there is still scope to study the mechanism at molecular level and identify specific phytochemicals responsible for the activity [26].

Future Prospects of the Study: Isolation of active phytoconstituents using column chromatography and estimation of percentage value of phytochemicals using high-performance liquid chromatography would be more definite in predicting exact mechanism of action of the extract. Several ingredients discovered by compatibility studies, pharmacological effects of them, and compatibility with other herbs should be investigated, apart from studying a sole constituent or a solitary type of pharmacologically active substance [27].

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CONFLICT OF INTEREST: Nil

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