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Syeda Shahana
Y.B. Chavan College of Pharmacy, Aurangabad, Maharashtra, India

Anna Pratima G Nikalje
Y.B. Chavan College of Pharmacy, Aurangabad, Maharashtra, India

Correspondence
Syeda Shahana
Y.B. Chavan College of Pharmacy, Aurangabad, Maharashtra, India

Development and evaluation of antidiabetic formulation of *Trichosanthes dioica* fruit extract

Syeda Shahana and Anna Pratima G Nikalje

Abstract
Many plants and vegetables have therapeutic activity due to the presence of various phytoconstituents. *Trichosanthes dioica* commonly called Parwal is an annual or perennial herb commonly consumed in India as a vegetable. The present research work was undertaken to formulate and evaluate anti-diabetic activity of tablets prepared from aqueous extract of the plant. The formulation was stable and exhibited statistically significant anti-diabetic activity. Standard parameters of tablets were also evaluated (weight variation, friability, hardness and disintegration time and the formulated tablets complies with the standard range.

Keywords: Antidiabetic, herbal tablets, *Trichosanthes dioica*, Streptozotocin, flavonoids

Introduction
Trichosanthes dioica known by the common name *parwal*, is one of the important vegetables in India [1]. The fruits and leaves are edible parts of the plant which are cooked in many ways either alone or in combination with other vegetables or meats [2]. The fruit is rich in vitamins and contains 9.0 mg Mg, 2.6 mg Na, 83.0 mg K, 1.1 mg Cu, and 17.0 mg S per 100 g edible part [3]. Two main phytosterols present in *T. dioica* are, namely, 24α-ethylcholest-7-enol and 24β-ethylcholest-7-enol [4]. Diabetes is a common metabolic disorder affecting millions of people worldwide. With ever increasing diabetic population and shortcomings of conventional hypoglycemic drugs have necessitated the need to explore alternative therapies. Natural products are also assumed to be safe with less adverse effects. Though oral hypoglycemic act by different mechanisms still the plant extracts can be explored for added benefits or synergistic action with conventional drugs. Phytoconstituents present in plants are usually carotenoids, flavonoids, terpenoids, alkaloids, glycosides which usually have anti-diabetic effects [5]. As diabetes is a multifactorial disease which develops several complications later, for holistic treatment it requires multiple therapeutic approach. Major limitation in inclusion of herbal medicine in modern medical practices is absence of scientific and clinical data proving their efficacy and safety. Many formulations are available in market which are used regularly by diabetic patients on the advice of physicians. Many different plants are being used individually or in formulations for treatment of diabetes and its complications. One of the major problems with this herbal formulation is that the active ingredients are not well defined. It is important to know the active component and their molecular interaction, which will help to analyze therapeutic efficacy of the product and also standardize the product. Efforts are now being made to investigate mechanism of action of some of these plants using model systems [6]. Phytochemicals have tremendous potential to greatly affect the onset and progression of diabetes, oxidative stress and ageing. They act as bio enhancers of several physical and biochemical processes [7]. Hence the present study was undertaken to explore the potential of *Trichosanthes dioica* fruit extract tablets in diabetes and associated complications.

Material and Methods
Plant Material: Collection of fresh unripe fruits of *Trichosanthes dioica* was done from local market of Aurangabad region and authenticated by taxonomist, Maulana Azad College, Dept. of Botony, Aurangabad with specimen number MACH-12453. The fruits were shade dried and size reduced in mixer. Extract was obtained by continuous hot boiling up to 40 hours and filtered and concentrated in rotary evaporator and reduced under pressure.

Experimental Animals: Male wistar rats 8-10 weeks old in the range of 200-225 gms body weight were obtained from Wockhartd Ltd, Aurangabad, India. Animals were housed under standard lab conditions 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-chem26/2015-16/116.

Induction of Diabetes: Diabetes was induced by a single intra-peritonial injection of freshly prepared streptozotocin at a dose of 65 mg/kg b.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 after STZ injection was achieved. Animals exhibiting 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) [8] and Group 4 with tablets of *Trichosanthes dioica* extract in dose of 500 mg / kg b.w for 21 days. Control rats (group 1 and 2) received vehicle (distilled water only) orally regularly once a day up to 21 days.

LD_{50}: Acute oral toxicity test for the aqueous extract of fruits of *Trichosanthes dioica* was carried out as per Organization for Economic Co-operation and Development Guidelines 425. Extract was found to be safe up to 2000 mg/kg.
Phytochemical Screening of *Trichosanthes 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 [9].

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

Formulation of Aqueous extract
The aqueous extract of *Trichosanthes dioica* were formulated into tablets with following specifications.

Table 1: The Ingredients batch function

Ingredients		G/batch	Function
	Mg/tablet	**4000**	
Extract	50 mg		
Avicel PH101	70	280	Compression binder
Lactose 200M	25	100	Diluent/filler
Povidone k 30	3	12	Dispersing/ suspending agent
Mag Stearate	0.5	2	Lubricant
Sodium starch glycolate	5	20	Disintegrant/suspending agent
	156	414	

Tablets were prepared as

Granulation → drying → blending → lubrication → compression

PVK-30 15% solution used in granulation and also extra water used for granulation.

- **Step 1:** PVK-30 added to purified water (108.0 g), clear solution found (solid content 10.0 %).
- **Step 2:** Take half quantity of extract and to it added 50g purified water.
- **Step 3:** Avicel PH-101 and lactose 200m sifted through #20 American Standard Test Sieve and loaded in 2 liter RMG (Rapid Mixer Granulator) and dry mix for 5 minutes.
- **Step 4:** Step 2 solution sprayed on Step 3 material using spraying gun.
- **Step 5:** Step 1 solution sprayed on Step 4 material and kneading for 30 seconds.
- **Step 6:** Unloaded the material from RMG and dried in FBD at 60^{o} C with 40 air flow for 1 hour. LOD at 105^{0} C for 5 minutes = 1.3-1.4%.
- **Step 7:** Sifted the dry granules through #20 ASTM sieve and loaded in 2 liter double cone blender with Presifted sodium starch glycolate for 5 minutes.
- **Step 8:** Step 7 material lubricated with mag stearate for 5 minutes.
- **Step 9:** Step 8 material used in compression.

Fig 1: Tablets formulated of *Trichosanthes dioica* Aq extract

Table 2: Quality control tests of formulated tablets of *Trichosanthes dioica*

S. No	Test	Value	Conclusion
1	Tablet Hardness	70-80N	Complies
2	Friability Test	< 1%	Complies
3	Tablet Diameter	7mm	Complies
4	Tablet Weight Variation	152-155mg	Complies
6	Disintegration test	3-4 minutes	Complies

Table 3: Blood glucose of STZ induced diabetic rats

S. No	Treatment	Blood glucose of rats in mg/dl		
		Initial	4th Day	21st Day
1	Normal control	92.3±1.4**	91±1.4*	91±1*
2	Diabetic control	93.3±1.6	355±8.5	325.3±6.2
3	Standard(Insulin)	94.8±1**	350.8±3.5*	115.3±3.5*
4	*Trichosanthes dioica* (Tablets)	92.5±1.6**	352±1.9*	125.1±11*

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< 0.1 ** p< 0.5 vs control.

Table 4: Biochemical parameters of Kidney and Liver profile

Groups	Treatment	Biochemical parameters of Kidney and Liver profile				
		Creatinine mg/dl	Urea mg/dl	Liver Glycogen mg/gm tissue	SGOT mg/dl	SGPT mg/dl
1	Normal control	0.90±0.02*	35.5±1.1*	42.1±0.3*	47.1±2.2*	42.3±2.3*
2	Diabetic control	2.55±0.06	70.3±2.1	15.3±0.2	153.3±6	145±7
3	Standard(Insulin)	1.12±0.04*	44.8±1.4*	34±0.21*	55.5±2.2*	56.8±2.6*
4	*Trichosanthes dioica*	1.25±0.04*	49±2.5*	31±0.2*	59±4*	60.8±2.7*

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< 0.1 ** p< 0.5 vs control.

Table 5: Biochemical parameters of lipid profile

Groups	Treatment	Biochemical parameters of lipid profile				
		Total Cholesterol	Triglycerides	LDL	HDL	VLDL
1	Normal control	114±1.4*	86.6±2.4*	34±1.1*	31±1.2*	27.1±0.6*
2	Diabetic control	175.1±3.3	179.1±4.3	84.8±2.3	20.6±0.5	37.8±0.8
3	Standard(Insulin)	119.6±2.7*	100.1±2.7*	40.6±1.5*	29.5±0.34*	30.5±0.3*
4	*Trichosanthes dioica*	125.5±2.1*	118.3±2.4*	44±2.4*	26.1±0.45*	31.8±0.3*

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< 0.1 ** p< 0.5 vs control.

Table 6: *In vitro* antioxidant analysis

S. No	Concentration	% Reduction					
		NBT Method		Metal Chelating Activity		DPPH Method	
		Std.(Ascorbic Acid)	*Trichosanthes dioica*	Std. (EDTA)	*Trichosanthes dioica*	Std. (Ascorbic acid)	*Trichosanthes dioica*
1	50	35.5	18.2	35.3	25.2	30.5	15.3
2	100	66.1	39.8	59.5	44.5	56.1	37.8
3	150	78.5	50.3	78.2	55.3	75.2	46.3
4	200	93.3	57.3	94.1	60.5	92.3	55.7
IC_{50} µ/ml		10.5	47.3	13.5	45.5	14.5	55.3

Discussion

Blood glucose is the most significant parameter in evaluating antidiabetic activity. Blood glucose was checked initially and then every week till three weeks. The diabetic control group had initial level around 93.3 mg/dl blood glucose which increased to 325.3 mg/dl by the end of third week. Insulin obviously prevented increase in blood glucose levels. *Trichosanthes dioica* tablets had significant effect in lowering blood glucose (Table 3). Kidney and Liver are the most affected organs in prolonged hyperglycemia. To evaluate the effect of a particular drug creatinine and urea are considered as renal parameters and Liver glycogen, SGOT and SGPT are considered as hepatic parameters (Table 4). Lipid profile is also disturbed in diabetes with elevated levels of cholesterol, triglycerides, LDL, VLDL and reduced levels of HDL. Diabetic control group exhibited massive increase in all these lipid levels and reduced HDL levels. Insulin had maximum effect in maintaining the lipid profile in normal range and extract was also effective in normalizing lipid profile (Table 5). The in-vitro antioxidant activity was evaluated using DPPH, NBT and Metal chelating activity. The extract exhibited significant in vitro antioxidant activity, increasing with increasing concentration. In DPPH method the standard used was ascorbic acid which had IC_{50} value of 14.5 µ/ ml. Extract had IC_{50} values of 55.3 µ/ml. Superoxide scavenging activity was evaluated using NBT method. The IC_{50} value of standard used i.e ascorbic acid was 10.5µ/ml and *Trichosanthes dioica* was 47.3 µ/ml. Metal chelating activity was also evaluated using EDTA as standard. The IC_{50} value of EDTA was 13.5µ/ml whereas *Trichosanthes dioica* was 45.5 µ/ml (Table 6). Controlling hyperglycemia can decrease the onset and development of diabetic complications. Antioxidant defence and cellular redox mechanisms may be main therapy in diabetes and its complications [10]. Antioxidant therapy combined with other treatments for diabetic complications can lead to holistic effect in ameliorating diabetic complications [11]. *Trichosanthes Dioica* possess hypoglycemic and antioxidant activity due to presence of flavonoids [12]. Besides *Trichosanthes dioica* also corrects metabolic disturbances [13]. Its fruit has a steroidal saponin 24-α-ethyl-20-ene-7-hydro-stigmast-8-β:14 β-di-3-O-β-D-xylofuranoside. It also has beta sitosterol and luteolin-7-glycoside. Its effect may also be credited to presence of phenols and flavonoids [14]. It was also found that *Trichosanthes dioica* has 2′,4′5,-trihydroxy-5′,7-dimethoxyflavone, with name 5-hydroxy-2-(2,4-dihydroxy-5-methoxyphenyl)-7-methoxy-4H-chromen-4-one, second compound 2′,5,7-trihydroxy-5′-methoxyflavone with name 5,7-dihydroxy2-(2-hydroxy-5-methoxyphenyl)-4H-chromen-4-one and third compound 7-Hydroxy-4H-chromen-4-one. Calcium, potassium, magnesium, aluminium and iron improve glucose tolerance. Calcium and other trace elements are involved in release of insulin from β cells of islets of Langerhans. Potassium plays a role in carbohydrate metabolism, glycogen and glucose metabolism by regulating glucose to glycogen conversion stored in the liver for energy. Magnesium is a cofactor in various enzyme pathways in glucose oxidation besides modulating glucose transport across cell membrane. Due to urinary loss of magnesium its deficiency causes insulin resistance. Thus its supplementation may improve insulin resistance. All these elements are present in *Trichosanthes dioica* [15]. Diabetes causes increased expression of drug metabolizing gene CYP 450 which was drastically inhibited after treatment with *Trichosanthes dioica* extract. The inhibition of gene expression level can be linked with the exogenous metabolism. Also the level of antioxidant enzymes was increased. Polar phytosterols especially cucurbitacins from fruits and seeds may be responsible for

these pharmacological effects [16]. As *Trichosanthes diocia* has various types of phytoconstituents belonging to different chemical classes like alkaloids, flavonoids, glycosides, terpenes etc which inhibits alpha glucosidase and decrease glucose transport through the intestinal epithelium. Imidazoline compounds increase insulin secretion in a glucose dependent manner. Polysaccharides elevate the level of serum insulin and decrease the blood glucose level and they also raise tolerance to glucose. Flavonoids inhibit the glucose level, saponin stimulates the release of insulin and blocks the formation of glucose in blood hence it is effective in diabetes [17]. Flavonoids are also insulin-mimetics, regenerate beta cells of pancreas, protect from environmental stress and has free radical scavenging and anti-inflammatory activity. This may be beneficial in neurodegenerative disorders like diabetes. Saponins reduce glucose and cholesterol uptake from gut and decrease metabolic load of liver. Saponins are found in *Trichosanthes dioica* [18]. *Trichosanthes dioica* fruit is effective in treating diabetes and increased activity of enzymatic antioxidants in diabetics, it also has protective action against lipid peroxidation [19]. It can also be used as an adjuvant to achieve a better glycemic control, to prolong the action of metformin, to avert the loss of body weight and also to elevate the glucose tolerance in diabetic patients [17].

Conflict of Interest

The authors declare that there is no conflict of interest.

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