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IN VIVO ANTIHYPERLIPIDEMIC EFFECTS OF LEAF EXTRACT OF TRICHOSANTHES CUCUMERINA

[1]Mohammad Waseem, [2]Balweer Singh Kirar, [3]Dr. Alok Pal Jain

[1]RKDF College of Pharmacy, SRK University, Bhopal M.P.

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Abstract:

The objective of the present research work was to investigate the antioxidant and antihyperlipidemic activity of extract of leaves of Trichosanthescucumerina in vivo effects on High Fat diet induced hyperlipidemia respectively. Animals were divided into different groups. Briefly, the normal group received a standard chow diet and all other groups received a high-cholesterol diet consisting of normal chow diet 92%, cholesterol 2%, cholic acid 1%, and coconut oil 5% for 7 days. The reference drug (Atorvastatin 50 mg/kg) and extracts were administered once daily between 8:00 and 9:00 a.m. for 7 days. The daily food intakes were determined before treatments.There was a significant increase in the serum levels of TC, TG, LDL-C, VLDL-C, and HDL-C in the hyperlipidimic control group as compared with the normal control group. All the treatment groups produced a significant decrease in serum TC, TG, HDL-C, and VLDL-C levels. In addition to the above, the serum LDL-C levels were significantly decreased by the Trichosanthescucumerina leaves extracts. In the present study, we have selected the Ethanolic extracts Trichosanthescucumerina leaves have shown significant antioxidant activity. By the virtue of its antioxidant activity, Trichosanthescucumerina leaves show antihyperlipidemic activity.

Key words:*Trichosanthes cucumerina, Antihyperlipidemic, antioxidant, High Fat diet induced.*

Corresponding author:

Balweer Singh Kirar,

rkdfcp95@gmail.com

QR code

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INTRODUCTION:

Recent studies have demonstrated that increased formation of free radicals/reactive oxygen species (ROS) contribute to cardiovascular disease (CVD) progression [1,2]. Reactive oxygen species induce cardiac dysfunction and cardiac apoptosis and/or necrosis in heart failure [3]. Reactive oxygen species are formed intracellular and are controlled by antioxidant defense. The generation of large amounts of reactive oxygen species can overwhelm the intracellular antioxidant defense, causing activation of lipid peroxidation, protein modification, and DNA breaks [4]. Reactive oxygen species induced depletion of antioxidants is a key factor for the initiation of atherosclerosis and the development of CVD[1] .

Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for susceptibility to cardiovascular diseases [5]. World Health Organization reports that high blood cholesterol contributes to approximately 56% cases of cardiovascular diseases worldwide and causes about 4.4 million deaths each year[5]. Hyperlipidemia is a metabolic disorder, specifically characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), Very Low Density Lipoprotein Cholesterol (VLDL-C), and Triglycerides (TAG) with a concominant decrease in the concentrations of High Density Lipoprotein Cholesterol (HDL-C) in the blood circulation [6].

Currently, the use of complimentary/alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long-term use[1].

Trichosanthes cucumerina is a tropical or subtropical vine. Its variety *T. cucumerina* var. anguina raised for its strikingly long fruit. In Asia, it is eaten immature as a vegetable much like the summer squash and in Africa, the reddish pulp of mature snake gourd is used as an economical substitute for tomato[7]. Common names for the cultivated variety include snake gourd serpent gourd, chichinda and padwal. *Trichosanthes cucumerina* is found in the wild across much of South and Southeast Asia, including India, Bangladesh, Nepal, Pakistan, Sri Lanka, Indonesia, Malaysia, Myanmar(Burma) and southern China (Guangxi and Yunnan).Tricosanthescucumerina is used in the treatment of head ache, alopecia, fever, abdominal tumors, bilious, boils, acute colic, diarrohea, haematuria and skin allergy[8].

The objective of the present research work was to investigate the antioxidant and antihyperlipidemic activity of extract of leaves of *Trichosanthescucumerina*in vivo effects on High Fat diet induced hyperlipidemia respectively.

MATERIALS AND METHODS:

Plant materials:

Leaves of Selected plant *Trichosanthescucumerina*from the family (Cucurbitaceae) were collected from the Bhojpur M.P, India. The plants were identified and authenticated by a Botanist.

Preparation of crude extracts:

The plant Material (Leaves) was defatted with petroleum ether (40°-60°C) for about 12 hrs separately & complete defatting was ensured by placing a drop from the thimble on a filter paper which did not exhibited any oily spot. The defatted material was removed from the Soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted plant drug was subjected to extraction by ethanol as solvent. The process was carried out for about different timings for different solvents.

Preliminary phytochemical screening:

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents, present in the different extracts of *Trichosanthes cucumerina,* was subjected to the phytochemical tests as per standard methods. Qualitative analysis was done to identify the presence of the following phytoconstituents; alkaloids, flavonoids, tannins and phenols, steroids and terpenoids, saponins, carbohydrates, glycosides, proteins and amino acids using standard procedures. 19, 20

Quantitative Analysis of chemical constituents:

Total Phenolic Content [9]:

The total phenolic content was estimated by FolinCiocalteu method. The estimation is based on the principle that, in the alkaline medium, electrons from phenolic compounds are transferred to phosphomolybdic /phosphotungstic acid which is determined spectrophotometrically at 765 nm. Gallic acid was used as standard. The total phenol values were expressed as gallic acid equivalents in milligram per gram (GAE mg/g) of crude extract.

Total Flavonoid [10]:
The total flavonoid content was determined by aluminium chloride colorimetric technique. Acid stable complexes are formed by AlCl3 with C-4 keto group and either C- 3 or C- 5 hydroxyl groups of flavones and flavonols. They also form acid labile complexes in the A or B ring of flavonoids with orthodihydroxyl groups which can be measured spectrophotometrically at 415 nm. Quercetin was used as the standard and the concentration of flavonoids was expressed as quercetin equivalents in milligram per gram (QE mg/g) of crude extract.

Qualitative antioxidant activities:
Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods and natural products. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods are based on the direct interaction with reactive molecules or on their reactivity with metal ions and the effects are monitored by chemical measurements. Examples are determination of peroxyl radical scavenging, the ORAC assay, total antioxidant scavenging activity, the DPPH test or the FRAP method. In addition to these methods, chemical approaches have been developed which allow the detection of radical specific DNA- modifications in vitro.

DPPH radical scavenging:
DPPH scavenging activity was measured by the spectrophotometer with slightly modification [11] Stock solution (6 mg in 100ml methanol) was prepared Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517.

$$\% \text{ of Inhibition} = \frac{(A0 - A1)}{A0} \text{x}100$$

Where, A0 = absorbance of the control (without test samples)
A1 = absorbance of test samples.

Experimental work:
Animals:
Animal's Albino rats (SD strain) weighing 150–200g of either sex were used in the present study. The animals were procured from College of Veterinary Science and Animal Husbandry Mhow, Indore (M.P), India. They were provided normal diet and tap water ad labium and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee (RKDF College of pharmacy, Bhopal.

Drugs and extracts:
All the extracts were suspended in distilled water using 1% w/v gum acacia. The reference drugs Atorvastatin suspended in distilled water using 1% carboxymethyl cellulose (CMC). The control group received 1% w/v gum acacia in distilled water and 1% CMC solution as vehicles.

Acute oral toxicity study:
Adult Albino rats (SD strain) weighing 150–200g of either sex, fasted overnight and were used for acute toxicity study, as per the Organization for Economic Co-Operation and Development (OECD 423) guideline. Four groups of mice of both sexes were fasted overnight. The first control group mice received 0.5% carboxymethyl cellulose (CMC) suspension in distilled water while the other groups received ethanolic extracts of *Trichosanthes cucumerina* in 0.5% CMC at doses of 200, 600, and 2000 mg/kg. Animals were observed closely for first 4 hours, for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 14 days and no mortality was reported within the study period.

Diet-induced hyperlipidemia in rats:
The method of Blank et al. (1963), with modification, was used to produce diet-induced hyperlipidemia. Animals were divided into different groups (Table 2). Briefly, the normal group received a standard chow diet and all other groups received a high-cholesterol diet consisting of normal chow diet 92%, cholesterol 2%, cholic acid 1%, and coconut oil 5% for 7 days.

The reference drug (Atorvastatin 50 mg/kg) and extracts were administered once daily between 8:00 and 9:00 a.m. for 7 days. The daily food intakes were determined before treatments. On the last day, animals were deprived of food but not water. Blood samples were collected by retro orbital puncture technique under light anesthesia. The animals were sacrificed and liver tissues were collected and preserved at −40°C for further analysis. The fecal matters of the last 24h before fasting were collected, immediately dried in an oven at 80°C for 1h, and stored at −40°C for further analysis.

Table 1: Diet-induced hyperlipidemia model: summary of animal groups and treatments.

S. No	Groups	Treatments
1.	Normal	Vehicles (1 mL of 1% gum acacia and 1% CMC)
2.	Hyperlipidimic control	High cholesterol diet
3.	Treated with Standard (Atorvastatin)	High cholesterol diet + Atorvastatin (50mg/kg, p.o.)
4.	Treated with EtTC 200mg/kg	High cholesterol diet + EtTC (200mg/kg, p.o.)
5.	Treated with EtTC 400mg/kg	High cholesterol diet + EtTC (400mg/kg, p.o.)

EtTC: Ethanolic extract of *Trichosanthe scucumerina*

Estimation of biochemical parameters:

Lipid profile:

The serum lipid profile was determined on day 8 in the case of diet-induced hyperlipidemia. The total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) levels were estimated using commercially available kits (Erba; Transasia Bio-Medicals Ltd., Daman, India). Very low-density lipoprotein cholesterol (VLDL-C) was calculated as TG/5. LDL-cholesterol (LDL-C) levels were calculated using Friedewald's formula [12] The atherogenic index was calculated using the formula: atherogenic index.

$$(\mathrm{AI}) = \frac{(\mathrm{VLDL-C} + \mathrm{LDL-C})}{\mathrm{HDL-C}}$$

Estimation of serum lipid profiles:

Estimation of lipid profiles were placed major role in obesity condition. Usually, in obese condition the levels of lipids were higher than normal. So that, to know the activity of plant extract lipid profiles was studied.

Estimation of Cholesterol:

Estimation of cholesterol was carried out by the method of [13]

Procedure:

Ferric chloride-acetic acid (9.9 ml) reagent was added to 0.1 ml of serum for deproteinization. The contents were centrifuged at 3000 rpm for 15 min. 5 ml of the supernatant was taken and to this added 3 ml of concentrated Sulphuric acid and kept for 20 min at room temperature. The pink colour formed was read at 540 nm against a blank containing 5 ml of ferric chloride-acetic acid reagent. A set of standards were also performed in the similar manner.

Estimation of Triglycerides:

Plasma triglycerides were measured by the method[14]

Procedure:

Plasma (0.1 ml) was taken in a glass stoppered centrifuge tube and to this added 4 ml of isopropanol and 400 mg of alumina. The tubes were tightly capped and shaken vigorously for 10 min.The tubes were centrifuged at 3000 rpm for 15 min and 2 ml of the supernatant was pipette into clean, dry test tubes. To these added 0.6 ml of alcoholic KOH and kept at 70°C for 15 min. The tubes were cooled to room temperature. To this added 0.5 ml of acetyl acetone reagent, 1.0 ml of meta periodate reagent and incubated at 50ºC for min. Standard was also run in the same fashion with triolein instead of plasma. The colour developed was read at 405 nm against the reagent blank.

Estimation of HDL-Cholesterol (HDL-C):

Determination of plasma HDL-Cholesterol was carried out by the method [15].

Procedure:

Plasma (0.5 ml) was taken in a centrifuge tube and to this added 0.25 ml of Phosphotungstic acid reagent and 0.25 ml of $MgCl_2$ and was centrifuged at 1500 x g for 30 min in a refrigerated centrifuge and the amount of cholesterol was determined in the supernatant.

Estimation of vldl and ldl cholesterol (vldl-c and ldl-c):

By using Freidwald formula the concentration of VLDL and LDL cholesterol in serum were calculated.

LDL-C = (TC) - (HDL-C) - (TG/5)

RESULTS AND DISCUSSION:

Table 2: Preliminary phytochemical screening *Trichosanthes cucumerina* leaves.

S.N.	Phytoconstituents	Test Name	Hydroalcoholic Extract
1	Alkaloids	Mayer's Test	Present
2	Glycosides	Raymond's Test	Present
3	Carbohydrates	Molisch's Test	**Absent**
5	Flavonoids	Lead acetate	Present
6	Resins	Color detection with ferric chloride	**Absent**
7	Steroids	Libermann- Bur chard Test	Present
8	Proteins & Amino acids	Biuret Test	Present
		Ninhydrin Test	Present
9.	Phenols	Ellagic Acid Test	Present

Estimation of total phenolic content:

Gallic acid is used as a standard compound and the total phenols were expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.005x + 0.0344$, $R^2 = 0.981$, Where y is absorbance at 760 nm and x is total phenolic content in the Hydroalcoholic extract of *Trichosanthes cucumerina* (Leaves). The results were expressed as the number of equivalents of Gallic acid (µg/mg of extract). The results were presented in Fig.

Table 3: Total Phenolic Content of Hydroalcoholic extract of leaves of *Trichosanthes cucumerina*

Sample	Total phenolic content GAE mcg/ml
Hydroalcoholic extract of *Trichosanthes cucumerina* 100µg/ml	29.27± 0.154

n=3, values are given in SEM

Estimation of total flavonoids content:

Flavonoid content was calculated from the regression equation of the standard plot ($y=0.006x+0.090$, $R^2 =0.988$) and is expressed as quercetin equivalents (QE) (fig.). Total Flavonoid content was 10.612 ± 0.005 mg/g quercetin equivalent in MELP. Flavonoids are the most common and widely distributed group of plant's phenolic compounds,.

Table 4: Total Flavonoid content of Hydroalcoholic extract of leaves of *Trichosanthescucumerina*

Sl. No.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mcg/ml
1	Hydroalcoholic extract (100µg/ml)	10.612 ± 0.005

n=3, values are given in SEM

Table No. 5: % Inhibition of ascorbic acid and extracts of *Trichosanthes cucumerina* using DPPH method (Leaves)

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	44.65	25.45
2	20	48.62	35.56
3	40	65.34	45.58
4	60	69.65	52.23
5	80	77.41	61.45
6	100	84.13	69.98
IC 50		17.68	53.37

Acute oral toxicity study:

The acute toxicity study did not result in any mortality of treatment rats and no toxic effect was observed throughout the 14 days study period. Physical observation of the test article-treated rats throughout the study indicated that none of the them showed signs of toxic effect such as changes on skin and fur, eyes and mucus membrane, behavior pattern, tremors, salivation, diarrhea, sleep and coma. No mortality was observed in any of the rats.

Food intake:

No significant difference in food intake among the different groups was observed (Table).

Table 6. Effects of different treatments on food intake of diet-induced hyperlipidimic rats

Group (n = 6)	Daily food intake (g)						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Normal	20.63±0.44	21.57±0.23	20.75±0.21	21.92±0.44	21.15±0.40	20.78±0.45	20.92±0.55
Hyperlipidimic control	20.79±0.21	20.44±0.41	20.52±0.39	20.68±0.78	21.75±0.31	21.13±0.50	20.48±0.54
Atorvastatin	19.36±0.52	20.48±0.42	21.12±0.34	21.03±0.32	22.77±0.75	20.41±0.41	22.57±0.48
Treated with EtTC 200mg/kg	20.33±0.31	21.03±0.52	21.66±0.32	20.17±0.42	22.65±0.58	22.15±0.29	22.21±0.54
Treated with EtTC 300mg/kg	21.04±0.35	22.07±0.35	20.44±0.42	22.53±0.47	20.20±0.45	20.19±0.62	20.30±0.52

Note. All values represent mean ± SEM from six animals. Statistical analysis was carried out using one-way ANOVA followed by Tukey's test. $p<0.05$ was considered statistically significant.

Diet-induced hyperlipidemia:

There was a significant increase in the serum levels of TC, TG, LDL-C, VLDL-C, and HDL-C in the hyperlipidimic control group as compared with the normal control group. All the treatment groups produced a significant decrease in serum TC, TG, HDL-C, and VLDL-C levels. In addition to the above, the serum LDL-C levels were significantly decreased by the *Trichosanthes cucumerina* leaves extracts.

Table 7. Effect of *Trichosanthes cucumerina* leaves extracts on serum lipid profile of diet-induced hyperlipidemia in rats.

Group (n = 6)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Normal	132.64±2.97	101.46±4.16	62.12±1.32	53.43±2.45	21.09±0.83
Hyperlipidemic control	329.23±6.95*	304.80±12.59*	105.56±3.54*	163.91±9.58	61.76±2.52*
Atorvastatin	163.00±6.97**	163.99±1.95**	67.87±2.47†	63.53±6.48**	33.60±0.39**
Treated with EtTC 200mg/kg	243.26±3.45**	109.42±2.12**	67.50±2.41†	155.08±3.61	22.68±0.42**
Treated with EtTC 300mg/kg	208.56±8.91**	131.51±5.43**	65.99±2.31†	117.46±7.73**	27.10±1.09**

Note. All values represent mean ± SEM from six animals. *Compared with normal group (p<0.05), **compared with hyperlipidemic control group (p<0.05), † significant reduction compared with hyperlipidemic control group (p<0.05). TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol.

Fecal bile acid and cholesterol excretion:

The *Trichosanthescucumerina* leaves extracts significantly increased fecal cholesterol excretion (Table). The fecal bile acid excretion was significantly increased by all the treatment groups except atorvastatin.

Table 8: Effect of *Trichosanthescucumerina* leaves extract on fecal cholesterol and bile acid excretion in diet-induced hyperlipidimic rats.

Group	Fecal cholesterol (mg/g of fecal matter)	Fecal bile acid‡ (mg/g of fecal matter)
Normal	2.05±0.09	1.29±0.08
Hyperlipidimic control	3.18±0.07*	1.12±0.05
Atorvastatin	2.27±0.07	2.56±0.06
Treated with EtTC 200mg/kg	4.83±0.13**	3.69±0.04**
Treated with EtTC 300mg/kg	8.50±0.13**	3.97±0.10**

Note. All values represent mean ± SEM from six animals. *Compared with normal group (p<0.05), **compared with hyperlipidimic control group (p<0.05), a compared with atorvastatin , ‡ as cholic acid equivalent.

CONCLUSION:

In the present study, we have selected the *Trichosanthescucumerina* leaves for evaluating antihyperlipidemic activity since the phytochemical constituents Are found to be in excellent quantity. In our study high fat diet induced hyperlipidemia in rats model was used. From the results, it is evident that Ethanolic extracts *Trichosanthescucumerina* leaves can effectively decrease plasma cholesterol, triglyceride, LDL, and VLDL and increase plasma HDL levels. In addition, the Ethanolic extracts *Trichosanthescucumerina* leaves have shown significant antioxidant activity. By the virtue of its antioxidant activity, *Trichosanthescucumerina* leaves show antihyperlipidemic activity.

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