

**IN VIVO LETHAL AND CYTOTOXICITY ASSESSMENT OF *TRICHOSANTHES CUCUMERINA***Singh Sandeep Kumar^{*1}, Prakash Veeru¹, Kumar Arun², Kumar Ranjit², Ali Mohammad²¹Department of Biochemistry & Biochemical Engineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India²Research Department, Mahavir Cancer Sansthan & Research Centre, Phulwari Sharif, Patna, Bihar, India

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ABSTRACT

Trichosanthes Cucumerina is being used as food as well as traditional medicine for long days. The aim of the present study was to evaluate the lethal dose and cytotoxicity of ethanolic extract of *Trichosanthes cucumerina* fruits (TCFEE) and leaves (TCLEE). For lethal dose estimation graded doses ranging from 200-2000mg/ kg body weight (BW) of both extracts were administered orally and observed mortality up to 72 h. In lethal toxicity study the LD₅₀ value of TCFEE and TCLEE was found as 1400 mg/kg BW and 1300 mg/kg BW. The cytotoxicity study of both extracts was done by evaluation of comparative difference of hepatorenal biochemical parameters (SGOT, SGPT, ALP, Urea, Uric acid and Creatinine) and histopathological slides of liver and kidney of both normal control and treated rats. Results showed no significant difference ($p > 0.05$) between biochemical parameters and no changes in anatomy of liver and kidney. Hence the results suggest that ethanolic extracts of *T. cucumerina* fruit and leave are quite safe and can be used in the treatment of the chronic diseases like diabetes without any toxicity.

Keywords: *Trichosanthes Cucumerina*, LD₅₀, Ethanolic extract, Cytotoxicity, Histopathological study, Charles foster strain rats.

INTRODUCTION

Herbal plants and their processed products are big concern of today's modern pharmacological approach for remedial of several disease due to their medicinal properties and less side effects. Generally people of developing countries go to use of traditional medicine in primary medical problems.¹ But without the proper information about efficacy and safety of these traditional medicines sometimes it would be harmful.

Cucurbitaceae family having a large no of vegetables plants used in daily diet of people. *Trichosanthes cucumerina* (ver. Snake tomato) is one such plant that is frequently being used as food as well as traditional medicine for long days. The plant is richly constituted with a series of chemical constituents like flavonoids, carotenoids, phenolic acids which makes the plant pharmacologically and therapeutically active. It has a prominent place in alternative systems of medicines due to its various pharmacological activities like antidiabetic, hepatoprotective, cytotoxic, anti inflammatory, larvicidal effects.² Fruit is regarded as anthelmintic, purgative, vomitive.³ Diabetic patients are advised to consume young fruits as it is having low sugar and excellent sources of fibres, vitamins, minerals and proteins.⁴ Leaf is cardiogenic, antipyretic, antiperiodic, useful for intestinal worms and its juice rubbed over the liver in remittent fever⁵, skin disease, biliousness, emetic, externally applied over bald patches of alopecia⁶. Its ethanolic extract showed antiovaratory activity in female albino rats⁷ while methanolic extracts showed antibacterial activity.⁸

Although its traditional use and extensive phytopharmacological studies are known but the toxicity profile, especially of its ethanolic extracts, has not been yet explored. The present investigation was therefore carried out to study the lethal dose and cytotoxicity of ethanolic extract of *Trichosantes cucumerina* fruits (TCFEE) and leaves (TCLEE).

MATERIALS AND METHODS**Plant Material**

Fresh fruits and leave of *Trichosanthes cucumerina* were collected from Allahabad district and authenticated by an Agronomist, Department of Agronomy, SHIATS, Allahabad, India.

Preparation of ethanolic extract

The Fresh fruits and leave of *T. cucumerina* were collected, washed, dried under shade and powdered into fine particles. The 50g powder was macerated in 600 ml of 95% ethanol at room temperature for 48 hours with occasional shaking at 8 hours. It was then filtered by Whatmann filter paper (size no.1) and the filtrate evaporated on rotary evaporator to concentrate in crude extract form at 40°C. Reddish orange and dark green crude residues (8.33% w/v) were obtained. The liquid suspensions were made by using 4% (v/v) ethanolic solvent and kept in air tight bottle in a refrigerator until used.

Phytochemical Screening

Preliminary phytochemicals screening of the extracts were carried out using standard methods.⁹

Experimental Animals

Normal Charles foster strain rats of either sex weighing 200 ± 20 gm were used for whole study and maintained on standard pellet diet and water ad libitum. The temperature of housing environment was maintained at 25 ± 2 °C with 12 hours repeated light/dark cycle. The study was approved by the Institutional Animal Ethics Committee by Ethic No. IAEC/MCS/2011/12/03.

Experimental design and Selection of doses

Experimental rats were divided into 6 groups (4 rats/ group). In order to select optimum dose of TCFEE and TCLEE, different doses were designed as 200, 400, 800, 1200, 1600 and 2000 mg/kg BW respectively. Lethal toxicity study¹⁰ was

carried out after 72 hours by oral feeding of these doses to respective group rats.

In another experiment 8 rats are divided in 2 groups for each extract treatment. Group I having normal control healthy rats receiving normal saline for 7 days while Group II having normal healthy rats receiving ethanolic extracts at a doses of 200 and 400 mg/kg BW for 7 days. The animals were observed for toxicity signs and analyzed for post treatment cytotoxicity by assay of liver and kidney function markers.

Biochemical Analysis

The effects of TCFEE and TCLEE on liver and kidney function markers were evaluated by estimation of SGOT¹¹, SGPT¹¹, ALP¹², Urea¹³, Uric acid¹⁴ and Creatinine¹⁵ in plasma samples of normal control and extract treated rats. All analyses were performed by standard enzymatic methods using commercially available kit from CREST BIOSYSTEM LTD.

Histopathological Study

All treated rats with selected dose of TCFEE and TCLEE were subjected to death after blood samples collection under guidelines of IAEC. The tissue samples of liver and kidney were obtained, washed and fixed in 10% neutral formalin and used for histopathological slide preparation as described by Lillie, R. D. (1965).¹⁶ Slides were observed using X200, X400 objectives and results recorded.

Statistical Analysis

All results of liver and kidney function are expressed as mean $\pm$ SD. Data were analysed by one way ANOVA and followed by Dunnett's multiple comparison test using graph pad prism software (version 5.03) for windows (Graph Pad Software, San Diego, USA).

Table-1. Percentage mortalities in rats at different oral doses of *Trichosanthes cucumerina* fruit's ethanolic extract for one week

Group	No. of Animals	Dose (mg/kg)	No. of death	% Mortality
1	4	200	0	0
2	4	400	0	0
3	4	800	1	25
4	4	1200	1	25
5	4	1600	2	50
6	4	2000	4	100

Table-2. Toxicity signs observed in rats at single oral doses of 200 and 400 mg/kg body weight of *Trichosanthes cucumerina* fruit's ethanolic extract for one week

Dose (mg/kg body weight)	Sign of toxicity					
	Inappetence	Depression	Aggressiveness	Respiratory distress	Body weight loss	Death
200	-	-	-	-	-	-
400	-	-	-	-	-	-

+ = present, - = absent

Table-3. Percentage mortalities in rats at different oral doses of *Trichosanthes cucumerina* leave's ethanolic extract for one week

Group	No. of Animals	Dose (mg/kg)	No. of death	% Mortality
1	4	200	0	0
2	4	400	0	0
3	4	800	1	25
4	4	1200	2	50
5	4	1600	2	50
6	4	2000	4	100

Table-4. Toxicity signs observed in rats at single oral doses of 200 and 400 mg/kg body weight of *Trichosanthes cucumerina* leave's ethanolic extract for one week

Dose (mg/kg body weight)	Sign of toxicity					
	Inappetence	Depression	Aggressiveness	Respiratory distress	Body weight loss	Death
200	-	-	-	-	-	-
400	-	-	-	-	-	-

+ = present, - = absent

Table-5. Liver function markers in plasma of TCFEE and TCLEE treated rats at the dose of 400 mg/ kg bw. (values are mean $\pm$ sd)

Liver function markers	Group I	Group II	
		TCFEE treated	TCLEE treated
SGPT (U/ml)	72.87 $\pm$ 1.44	73.25 $\pm$ 0.87*	71.75 $\pm$ 0.87*
SGOT (U/ml)	86.25 $\pm$ 0.72	83.50 $\pm$ 1.22*	85.00 $\pm$ 1.77*
ALP (KA units/ml)	9.25 $\pm$ 0.69	8.83 $\pm$ 0.58*	8.74 $\pm$ 0.69*

Table-6. Kidney function markers in plasma of TCFEE and TCLEE treated rats at the dose of 400 mg/ kg bw. (values are mean $\pm$ sd)

Kidney function markers	Group I	Group II	
		TCFEE treated	TCLEE treated
Urea (mg/dl)	33.86 $\pm$ 0.67	30.20 $\pm$ 1.73**	32.86 $\pm$ 1.56*
Uric acid (mg/dl)	6.43 $\pm$ 0.38	6.02 $\pm$ 0.20*	6.23 $\pm$ 0.37*
Creatinine (mg/dl)	1.19 $\pm$ 0.24	1.25 $\pm$ 0.29*	1.25 $\pm$ 0.24*

*means are significantly not different as $p > 0.05$ for treated group II vs. control group I; **means are significantly different as $p < 0.05$ for treated group II vs. control group I

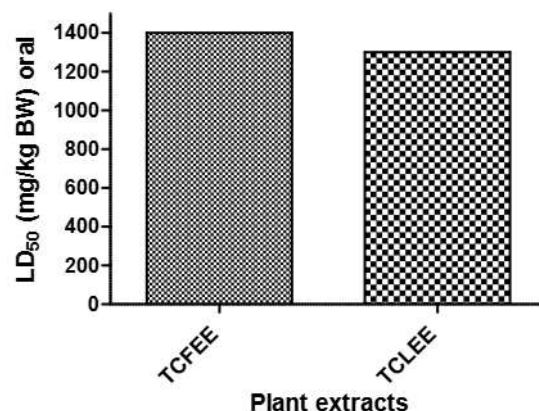


Figure 1: LD₅₀ value of ethanolic extract of *T.cucumerina* fruit (TCFEE) and leave (TCLEE)

Histopathological results

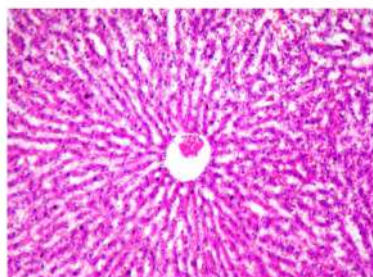


Figure 2A: Liver of normal control rat at (10 × 20 X)

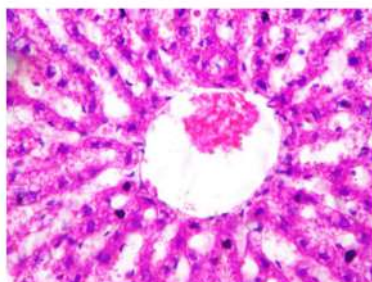


Figure 2B: Liver of TCFEE treated (400mg/kg BW) rat. (10 × 40 X)

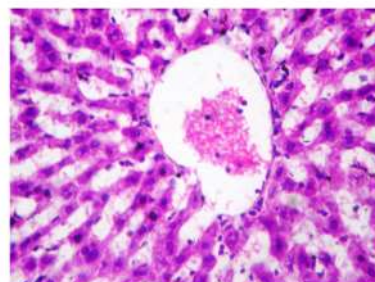


Figure 2C: Liver of TCLEE treated (400mg/kg BW) rat. (10 × 40 X)

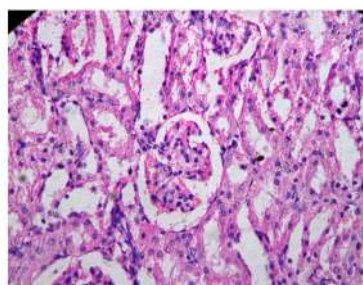


Figure 3A: Kidney of normal control rat at (10 × 40 X)

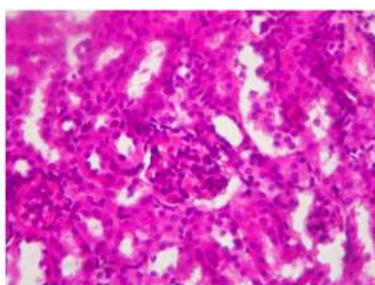


Figure 3B: Kidney of TCFEE treated (400mg/kg BW) rat. (10 × 40 X)

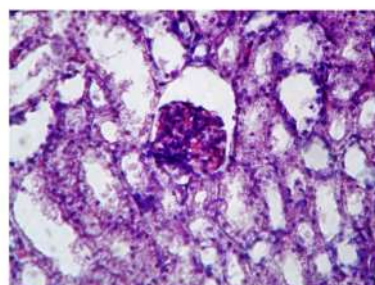


Figure 3C: Kidney of TCLEE treated (400mg/kg BW) rat. (10 × 40 X)

RESULTS

Preliminary phytochemical screening of TCFEE and TCLEE revealed the presence of polyphenolics, flavonoids, carotenoids, non protein thiols, vitamin - C and carbohydrates. In lethal toxicity study the LD₅₀ value of TCFEE and TCLEE was found as 1400 mg/kg BW and 1300 mg/kg BW oral respectively (Figure 1). The dose dependent mortality rates of TCFEE and TCLEE are showed in Table 1 & 3. However it did not show any positive signs of toxicity (Inappetence, Depression, Aggressiveness, Respiratory distress, Body weight loss and Death) on selected dose (Table 2 & 4). Commonly there was no significant difference between biochemical parameters (SGOT, SGPT, ALP, Urea, Uric acid and Creatinine) level of treated rats to control rats as $p > 0.05$ (Table 5 & 6). Histological study of the liver and kidney tissue of both normal control rat as well as extract

treated rat exhibited not any major or specific anatomical changes (Figure 2A, 2B, 2C, 3A, 3B, and 3C).

DISCUSSION

The use of herbal medicines has received a great attention as alternatives to synthetic pharmaceutical products in recent times, leading to the increase in their demand. Experimental screening method including a thorough toxicity study is therefore important to ascertain the safety and efficacy of these herbal drugs.¹ According to the toxicity scale of Hodge and Serner, any compound with an oral LD₅₀ of between 500 – 1000 mg/kg should be considered practically non toxic.¹⁷ At present the following chemical labelling and classification of acute systemic toxicity based on oral LD₅₀ values are recommended by the Organisation for Economic Co-operation and Development (OECD): very toxic, < 5 mg/kg

body weight; toxic, $> 5 < 50$ mg/kg; harmful, $> 50 < 500$ mg/kg; and no label, $> 500 < 2000$ mg/kg. The concept of basal cytotoxicity states that the mechanisms of action of most toxic chemicals are related to biochemical processes expressed in all cells.^{18,19} In present study the LD₅₀ value of ethanolic extract of *T. cucumerina* fruits (TCFEE) and leave (TCLEE) was found as 1400 mg/kg BW and 1300 mg/kg BW oral respectively which exhibits its safe oral dose for therapeutical uses. Increase in the level of SGPT, SGOT, ALP, Urea, Uric acid and Creatinine reflects the structural and functional dysfunction of liver cell and kidney cell membrane or cell rupture or inflammation.¹ Biochemical analysis of present study revealed not any significant difference in level of liver and kidney function markers of normal control versus extracts treated rats as $p > 0.05$. It

shows safety profile of extracts on liver and kidney function. Histological observations correlate our results showing the normal cellular anatomy of liver and kidney in the treated group of animals.

CONCLUSION

It is therefore concluded that the dose below LD₅₀ value of ethanolic extracts of fruits and leave of *T. cucumerina* may safely use for therapeutically benefits.

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