Artículo Original | Original Article

Evaluation of the toxic potential of standardized extracts (hot water extract and cold ethanolic extract) of *Trichosanthes cucumerina* Linn. aerial parts

[Evaluación de la toxicidad de extractos estandarizados (obtenidos con agua caliente y etanol frio) de partes aéreas de Trichosanthes cucumerina Linn.]

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Abstract

Trichosanthes cucumerina Linn. is one of the medicinal plants that is often used in Sri Lankan traditional systems of medicine for the preparation of formulations to treat a variety of disease conditions. However, the toxic effects of T. cucumerina are not known. The aims of the present study were to (a) standardize hot water (HWE) and cold ethanolic (CEE) extracts of T. cucumerina aerial parts, and (b) evaluate toxic potential of the plant extracts. Both extracts were standardized by developing their densitograms and HPLC fingerprints and determination of physico – chemical parameters such as total ash, water soluble ash and acid insoluble ash. Administration of the HWE or CEE to mice did not result in acute or chronic toxic effects as evident from their effects on (a) liver and kidney functions and (c) hematological parameters and (d) fertility of male or female mice.In conclusion, the results of this study have revealed that standardized extracts of T. cucumerina at the doses tested do not produce any serious toxic side effects.

Keywords: Trichosanthes cucumerina, extract standardization, renotoxicity, hepatotoxicity, hematology parameters, fertility.

Resumen

Trichosanthes cucumerina Linn. Es una de las plantas comunmente utilizadas en el sistema de medicina tradicional de Sri Lanka, en la preparación de formulaciones para el tratamiento de diversas enfermedades. Debido a que los efectos tóxicos de T. cucumerina no se conocen, los objetivos de este estudio fueron: (a) estandarizar los extractos obtenidos con agua caliente (EAC) y con etanol frío (EEF), y (b) evaluar la toxicidad de ambos extractos. Ambos extractos fueron estandarizados por obtención de sus densitogramas y huella digital con HPLC. Adicionalmente se determinaron parámetros fisicoquímicos, tales como: cenizas totales, cenizas solubles en agua y cenizas solubles en ácido. La administración de EAC y EEF a ratones no mostró efectos tóxicos agudos ni crónicos. Las funciones renales, hepáticas, estudios hematológicos y de fertilidad en machos y hembras fueron normales. Se concluye que los extractos estandarizados de T. cucumerina, a las dosis ensayadas no producen ningún efecto tóxico.

Palabras Clave: Trichosanthes cucumerina, estandarización, nefrotoxicidad, hepatotoxicidad, parámetros hematologicos, fertilidad

INTRODUCTION

Traditional medicine is widely spread globally and it is the almost exclusive source of primary health care for 80% of the world's population (Farnsworth, 1998). Herbal medicines are regarded by the public and some health care providers to be gentle and safe, despite a paucity of scientific evidence to support such beliefs. The active ingredients of plant extracts are chemicals that are similar to those in purified medications, and they have the same potential to cause serious adverse effects (De Smet, 2004).

The usefulness of any drug depends not only on its therapeutic efficacy but also on its lack of toxicity or adverse side effects. According to published reports, there are several herbal preparations that can produce adverse effects. For example, popular Chinese herbal preparation ''jin bu huan'' which is used as a pain and insomnia remedy, has been linked with several cases of acute hepatitis. Germanium, a non essential mineral commonly found in many herbal products, has been associated with chronic kidney failure. Kombucha tea, commonly used in the hopes of preventing cancer, relieving arthritis, curing insomnia and stimulating hair regrowth can cause several metabolic disorders (Whitney and Rolfes, 1999).

Trichosanthes cucumerina Linn (Family Cucurbitaceae) is an annual, dioecious climber belonging to the family Cucurbitaceae. It is widely distributed in Asian countries including Sri Lanka, India, Malay Penisula and Philippine (Jayaweera, 1980). The whole plant including roots, leaves, fruits, seeds have medicinal properties. The root is used as a cure for bronchitis, headache and boils. Externally, the leaf juice is rubbed over the liver to relieve liver congestion. Both the root and fruit are considered to be cathartic. The fruit is used as an anthelmintic in French Guiana. The seeds are used for stomach disorders in Malabar Coast and is also considered antifebrile and anthelmintic. The aerial parts of *T. cucumerina* are used along with other plant materials for indigestion, bilious fevers, boils, sores, skin eruptions such as eczema, dermatitis, psoriasis, ulcers and diabetes (Anonymous, 1976; Jayaweera, 1980; Anonymous, 2002).

Studies on the pharmacological activities have shown the presence of anti-inflammatory activity in root tubers (Kolte et al., 1996 – 1997) and aerial parts (Arawwawala et al., 2010a), antidiabetic activity in seeds (Kar et al., 2003) and aerial parts (Kirana and Srinivasan, 2008; Arawwawala et al., 2009), antioxidant activity in fruit pulp (Adebooye, 2007), hepatoprotective activity (Kumar et al., 2009) and gastroprotective activity (Arawwawala et al., 2010b) in aerial parts. The root and fruit also contain components that are cytotoxic to some cancer cell lines (Kongtun et al., 2009). However, no extensive safety studies have been conducted on extracts of *T. cucumerina* to date. Investigations have therefore, been carried out to determine whether acute or chronic administration of *T. cucumerina* standardized extracts would produce any unacceptable toxic side effects using mice as an experimental model.

MATERIALS AND METHODS
Plant material

Trichosanthes cucumerina Linn. (Family Cucurbitaceae) plants were collected from Western province of Sri Lanka between the period of August – September. The plant was identified and authenticated by the curator of National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. A voucher specimen (TS 01) was deposited in the Industrial Technology Institute, Colombo 7, Sri Lanka.

Animals

Healthy adult ICR mice (weigh: 25 - 30 g; age: 6 weeks old) were purchased from Medical Research Institute, Borella, Sri Lanka for the experimentation. They were housed under standardized (temperature: 28 - 31 ºC, photoperiod: approximately 12 hours natural light per day, relative humidity: 50 - 55%) animal house conditions and fed with standard rat feed prepared according to W.H.O. standards and water ad libitum. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use and care and guide lines stated in the rules of the ethical committee, University of Kelaniya, for animal experimentations.

Preparation of hot water extract (HWE)

T. cucumerina aerial parts were cut into small pieces and air dried. Plant material (60 g) was boiled in 1.9 L of distilled water (DW) and the final volume was reduced to 240 mL by gentle boiling over 4 h. The hot water extract was freeze dried and stored at 4 ºC until use (yield 12.5% dry weight basis). Qualitative testing revealed that polyphenols, flavonoids, tannins,

alkaloids, steroids and saponins were present in the HWE (Arawwawala et al., 2009).

Preparation of cold ethanolic extract (CEE)

T. cucumerina aerial parts were air dried in the shade, cut into small pieces and macerated with ethanol (500 mL) and kept for 48 h at room temperature (28 - 30 °C). The extract was filtered and evaporated to dryness under reduced pressure at 50 °C (yield 7.5% w/w dry weight basis) and stored at 4 °C until use. Qualitative testing revealed that polyphenols, flavonoids, tannins, alkaloids, steroids and saponins were present in the CEE (Arawwawala et al., 2009).

Standardization of *T. cucumerina* HWE and CEE

T. cucumerina extracts were standardized using physico-chemical parameters and phytochemical fingerprints as follows:

Determination of physico-chemical parameters of *T. cucumerina* extracts

The following physico-chemical parameters were determined forT. cucumerina *extracts according to methods recommended by the WHO (1998).*

Determination of total ash

Accurately weighted extract (2.5 g) was placed in a crucible. The extract was spread in an even layer and ignited to a constant weight by gradually increasing the heat to 500-600 °C using a muffle furnace (L5/C6H, Nabertherm®, Germany).

Determination of acid insoluble ash

2M HCl (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the acid insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight.

Determination of water soluble ash

Water (25 mL) was added to the crucible containing the total ash and boiled for 5 min. The water insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The weight of this residue was subtracted from the weight of total ash and the content of water soluble ash calculated.

Development of phytochemical fingerprints of *T. cucumerina* extracts

Both extracts (HWE and CEE) of *T. cucumerina* were standardized using densitometric and HPLC fingerprints.

Developments of densitometric fingerprints

The chromatographs were performed by spotting 5 µL of each extract (5 mg/mL) on pre-coated Silica gel-GF$_{254}$ plates. Plates were developed using mobile phases consist of methanol: dichloromethane: diethyl amine (0.5: 4: 0.5 v /v) and dichloromethane: ethyl acetate (1:1 v/v) for HWE and CEE respectively. Subsequent to the development, thin layer chromatography plates were air - dried and densitometric scanning was performed on a densitometer (CS – 9301PC, Shimadzu, Japan) at 254 nm.

Developments of HPLC fingerprints

For determination of the HPLC profiles, the HWE and CEE redissolved in distilled water and ethanol respectively (20 mg/mL concentration from each extract). After filtration through 0.45 µm, 13 nm millipore filter, 20 µL of each sample were injected into an Inertsil 5U ODS-2, C-18 reverse phase column (250 mm x 2.6 mm) of a High Performance Liquid Chromatography System (Shimadzu, Japan) connected to a SPD – M 10 Avp uv/vis photodiode array detector. The HPLC analysis was performed using acetonitrile – water mobile phase (40 : 60 v/v), with gradient elution at a flow rate of 1.0 mL/min. The detection wavelength was 254 nm.

Evaluation of toxicity of *T. cucumerina* extracts
Determination of LD$_{50}$

To determine the dose of the HWE or CEE that would cause the death of 50% of the test mice (LD$_{50}$), ICR mice were randomly divided into 12 groups (n = 10/group; 5 male mice & 5 female mice). Mice in groups 1 - 6 were orally treated with a single dose of the HWE at 1.5, 3, 6, 12, 15, 30 g/kg respectively while the mice in groups 7 – 12 were orally treated with a single dose of the CEE at 1.5, 3,

6, 12, 15, 30 g/kg respectively. After single dosing with different concentrations of the HWE or CEE, mice were observed for 7 days for any mortality, overt signs of toxicity (salivation, diarrhoea, lacrymation, tremors, ataxia, yellowing of hair, loss of hair, postural abnormalities or behavioral changes), stress (fur erection or exophthalmia) and aversive behaviors (biting paw and penis, intense grooming behavior, scratching behavior, licking at tail or vocalization).

Short term and long term toxicity studies
For both short term and long term toxicity studies, a dose of 1.5 g/kg/day, of each extract was administered each day since this dose corresponds to the normal therapeutic dose of *T. cucumerina* administered to adult humans as calculated on the basis of relative surface areas of humans and mice (Paget and Barnes, 1996).

Short term toxicity: ICR mice were randomly divided into 4 groups (n = 12/group; 6 male mice & 6 female mice). Mice in groups 1 and 2 were orally treated with 1 mL of DW/day and 1 mL of Tween 80 (1% in DW) solution/day respectively for 14 consecutive days while the mice in groups 3 and 4 were orally treated with the HWE and CEE at a dose of 1.5 g/kg/day respectively for 14 consecutive days.

Long term toxicity: ICR mice were randomly divided into 4 groups (n = 12/group; 6 male mice & 6 female mice). Mice in groups 1 and 2 were orally treated with 1 mL of DW/day and 1 mL of Tween 80 (1% in DW) solution/day respectively for 42 consecutive days while the mice in groups 3 and 4 were orally treated with the HWE and CEE at a dose of 1.5 g/kg/day respectively for 42 consecutive days.

Mice were observed twice daily (9.00 h and 16.00 h) for general toxic effects such as overt signs of toxicity (salivation, diarrhoea, lacrymation, tremors, ataxia, yellowing of hair, loss of hair, postural abnormalities or behavioral changes), stress (fur erection or exophthalmia), aversive behaviors (biting paw and penis, intense grooming behavior, scratching behavior, licking at tail or vocalization) and mortality. Percentage weight gain and, food and water intake were determined weekly during the period of treatment for each group. The consistency of faeces and color of urine were noted daily. At the end of short term (14 days) or long term (42 days) toxicity studies, effects on hematological parameters and

serum enzyme levels, were determined. In addition, effects on external morphology and histopathology of selected organs were also determined at the end of the long term toxicity study.

Determination of effects on hematological parameters, serum enzyme levels and bio – chemical profiles
On day 1 post treatment (in both short term and long term toxicity study), approximately 2 mL blood was collected by cardiac puncture of the treated mice (either with the HWE or CEE or respective controls) under mild ether anesthesia and divided into two equal parts. To one part ethylenediamine tetra-acetic acid (EDTA) was added and red blood cell (RBC) counts, white blood cell (WBC) counts, differential WBC, % packed cell volume (% PCV) and hemoglobin (Hb) concentration were determined according to the methods described by Manual of Procedures in Hematology (1994). Using the above data, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) were calculated.

The second part was allowed to clot (25 - 30 min.) at room temperature (28 - 30 °C) and subjected to 15 min. centrifugation at 3200 rpm for the collection of serum for assessment of effects on liver and kidney functions and lipid profiles.

Determination of effects on liver and renal function
To evaluate the hepatic functions, the concentrations of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined using enzyme kits purchased from Randox Laboratories Ltd., Antrium, UK.

The effects on kidney function were evaluated by determination of the serum creatinine and urea concentrations using enzyme kits purchased from Randox Laboratories Ltd., Antrium, UK.

Determination of effects on lipid profile
To evaluate the effects on lipid profile, concentrations of triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol were determined using a Randox enzyme kits purchased from Randox Laboratories Ltd., Antrium, UK.

Effects on external morphology and wet weights of selected organs

At the end of 42 days post treatment with the *T. cucumerina* extracts, mice were sacrificed by exposure to an over dose of ether. The liver, kidneys, heart, spleen and lungs were excised, blotted free of blood and their wet weights recorded. Weights of the organs were expressed as a percentage of the body weight. These organs were then examined for any external pathological abnormalities.

Effects on histopathology of selected organs

Small pieces of organs (liver, kidney, heart, spleen and intestine) were fixed in 10% formalin and routinely processed for histopathological examinations. These pieces were processed for sectioning at 5 mm thickness and stained with hematoxylin and eosin. The sections were viewed under a light microscope (Olympus CH30) at magnifications of 40 and 100.

Examination of gastric ulceration

Stomachs of rats treated with *T. cucumerina* for 42 days were removed, opened along the greater curvature and observed for any gastric lesions.

Effects of reproductive ability

Experiments were carried out to determine the effects of *T. cucumerina* extracts on reproductive ability of mice. The effects of the HWE and CEE on ovulatory, early abortifacient and implantation activities were determined *in vivo* while the spermicidal activity was determined *in vitro* by the methods described below.

Effects on ovulatory activity

ICR female mice (12 to 14 weeks of age) with normal oestrus cycle (average 4 – 6 days of pro-oestrus, oestrus, metoestrus and dioestrus) were dived into 4 groups (n = 7/group). Mice in groups 1 and 2 received 1 mL of DW/day and 1 mL of Tween 80 (1% in DW) solution/day respectively while the mice in groups 3 and 4 received 1.5 g/kg of HWE/day and 1.5 g/kg of CEE/day respectively for 12 consecutive days. Vaginal smears were examined daily to check the stage of oestrus they were in. Persistent dioestrus, non – cyclic and lengthy oestrus cycles were considered as

toxic signs. And different stages of the oestrus cycle were determined.

Effects on early abortifacient activity and implantation

Regularly cyclic female virgin mice (12 to 14 weeks of age) were mated. Presence of copulation plugs or sperms in the vaginal smear on the following morning were regarded as conformation of mating. The day copulation plugs or sperms appeared was assumed the day one of pregnancy. Pregnant female mice were randomly divided into 4 groups (n = 7/group) and treated in following manner: mice in groups 1 and 2 received 1 mL of DW/day and 1 mL of Tween 80 (1% in DW) solution/day respectively while the mice in groups 3 and 4 received 1.5 g/kg of HWE/day and 1.5 g/kg of CEE/day respectively from days 1 to 7 of pregnancy.

During pregnancy, the mice were evaluated for survival, altered appearance and any clinical signs of toxicity such as changes in food and water intake, piloerection, diarrhoea, changes of locomotor activity and vaginal bleeding. Autopsies were performed on the 10th day and the number of implantation sites, the number of live/dead fetuses and the number of corpora lutea of pregnancy were recorded.

Determination of the spermicidal activity

Masturbated human semen was taken from proven males and allowed to liquefy at 37 ^{o}C for 30 min. Three slides were taken and one drop of the semen placed on each slide. One drop of HWE (reconstitute in saline) or CEE [reconstitute in Tween 80 (1% in saline)] or saline was added onto each semen drop and mixed well. Finally, the mortality of the human sperms was observed under the microscope.

RESULTS

Table 1 summarizes the physico-chemical parameters of *T. cucumerina* extracts. There was no significant ($P \geq 0.05$) difference between HWE and CEE in terms of total ash content, water soluble ash content and acid insoluble ash content. Further, as evident from densitometric and HPLC fingerprints (Figure 1 & 2) of the HWE and the CEE, a greater number of constituents were found in the CEE when compared to the HWE.

Table1. Physico-chemical parameters of *Trichosanthes cucumerina* extracts

PHYSICO-CHEMICAL PARAMETERS	% IN DRY WEIGHT BASIS	
	Hot water extract (HWE)	Cold ethanolic extract (CEE)
Total ash	6.7 ± 0.19	5.9 ± 0.11
Acid insoluble ash	0.17 ± 0.01	0.11 ± 0.01
Water soluble ash	4.5 ± 0.14	3.6 ± 0.18

Values are expressed as mean ± S.E.M., n = 6
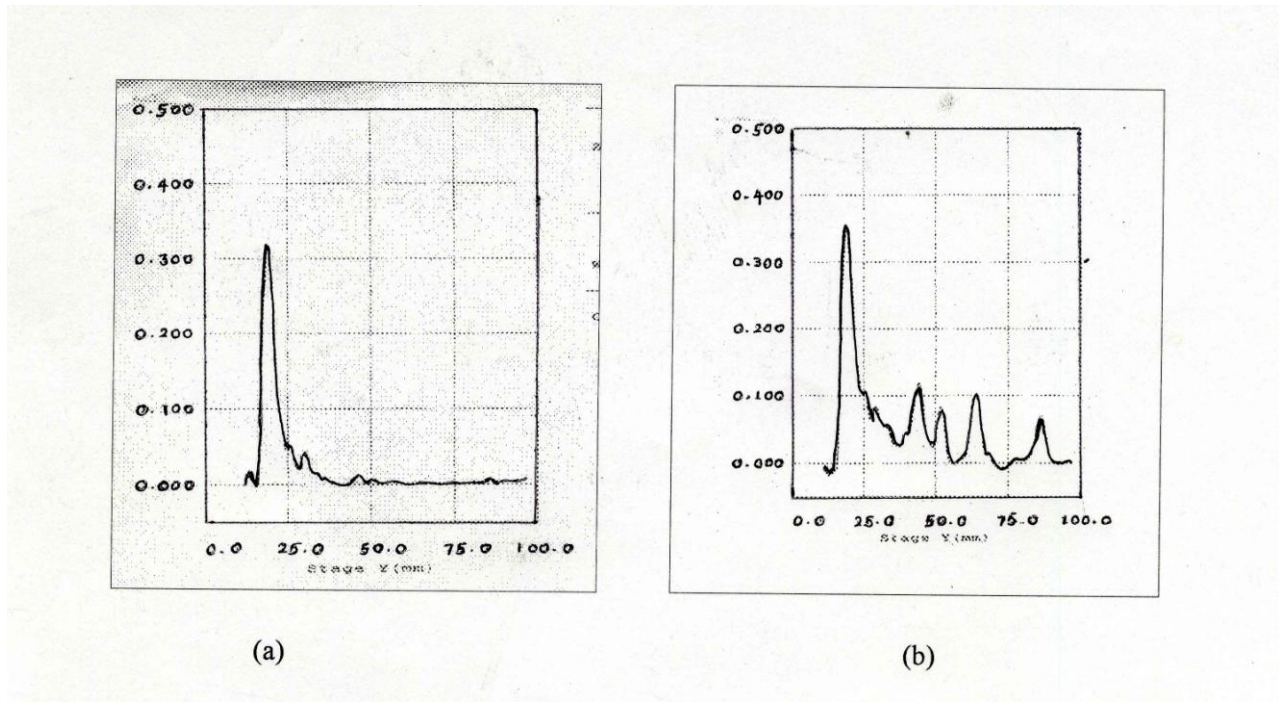No significant different between the values of the physico-chemical parameters between HWE and CEE

Figure 1. The densitograms of TLC fingerprint profiles of (a) hot water extract (HWE) and (b) cold ethanolic extract (CEE)
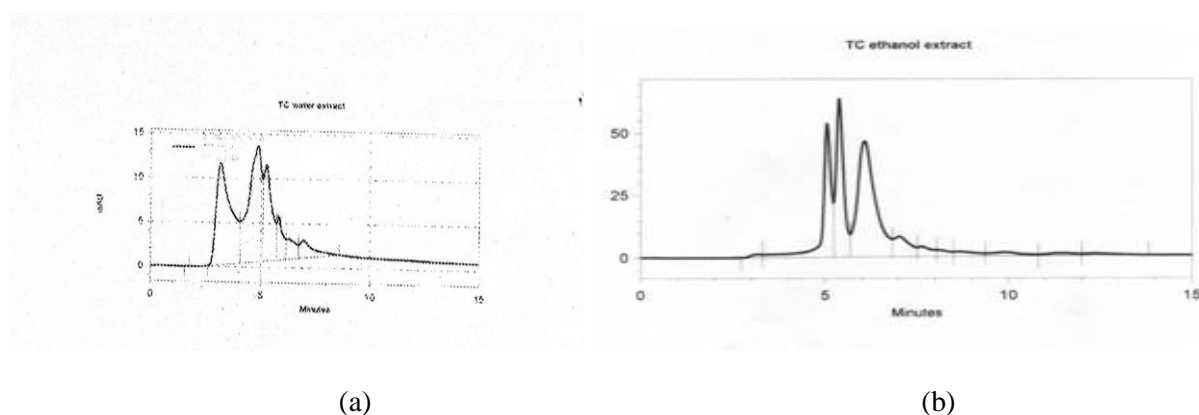
(a)   (b)

Figure 2. The HPLC fingerprints of hot water extract (HWE) and cold ethanolic extract (CEE)

No deaths or any sign of toxicity were observed in the 7 days following a single oral administration of several different doses of the HWE or CEE (1.5 g/kg – 30 g/kg). These extracts appear to be well tolerated at the doses ranging from 1.5 g/kg - 30 g/kg. Even the highest dose (30 g/kg) did not produce any apparent adverse effects. Therefore, the LD_{50} could not be estimated. Oral treatment of the HWE or CEE for 14 days or 42 days failed to bring about any overt signs of toxicity, stress, aversive behaviors and mortality. Further, HWE and CEE treated mice showed normal food and water intake. The consistency of faeces and color of urine of the HWE and CEE treated mice were similar to that of respective control groups.

Table 2. Effects of the hot water extract (HWE) and the cold ethanolic extract (CEE) of *Trichosanthes cucumerina* on hepatic enzyme levels in mice treated for 14 and 42 consecutive days.

Groups	Short term toxicity study (14 consecutive days)			Long term toxicity study (42 consecutive days)		
	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control 1 (1 mL of DW)	28.2 ± 1.4	38.2 ± 2.7	121.0 ± 5.7	28.6 ± 1.2	42.4 ± 2.0	138.0 ± 4.3
HWE (1.5 g/kg/day)	29.8 ± 2.2	37.5 ± 3.3	128.6 ± 4.4	30.8 ± 2.0	40.0 ± 1.9	139.5 ± 3.4
Control 2 [1 mL of Tween 80 (1 % in DW)]	30.2 ± 2.5	41.1 ± 2.6	130.6 ± 6.0	28.9 ± 1.9	39.5 ± 2.2	136.2 ± 4.0
CEE (1.5 g/kg/day)	29.8 ± 2.5	39.8 ± 3.7	139.5 ± 5.0	31.0 ± 2.3	40.3 ± 1.0	133.5 ± 5.1

Values are expressed as mean ± S.E.M., n = 12
Values of the test groups were not significant when compared to the respective control groups; $P \geq 0.05$
ALT: alanine aminotransferase; **AST:** aspartate aminotransferase; **ALP:** alkaline phosphatase

Table 3. Effects of the hot water extract (HWE) and the cold ethanolic extract (CEE) of *Trichosanthes cucumerina* on renal functions in mice treated for 14 and 42 consecutive days.

Groups	Short term toxicity study (14 consecutive days)		Long term toxicity study (42 consecutive days)	
	Creatinine (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	Urea (mg/dL)
Control 1 (1 mL of DW)	0.73 ± 0.02	36.0 ± 2.5	0.76 ± 0.02	33.0 ± 1.8
HWE (1.5 g/kg/day)	0.74 ± 0.02	33.7 ± 2.9	0.75 ± 0.02	33.1 ± 1.5
Control 2 [1 mL of Tween 80 (1 % in DW)]	0.76 ± 0.02	34.5 ± 2.1	0.75 ± 0.01	32.9 ± 1.0
CEE (1.5 g/kg/day)	0.74 ± 0.02	35.7 ± 1.9	0.73 ± 0.01	31.4 ± 1.7

Values are expressed as mean ± S.E.M., n = 12
Values of the test groups were not significant when compared to the respective control groups; $P \geq 0.05$

Table 4. Effects of the hot water extract (HWE) and the cold ethanolic extract (CEE) of *Trichosanthes cucumerina* on lipid profile in mice treated for 14 and 42 consecutive days.

Groups	Short term toxicity study (14 consecutive days)				Long term toxicity study (42 consecutive days)			
	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	TG (mg/dL)	Total cholesterol (mg/dL)	HDL cholesterol (mg/dL)	LDL cholesterol (mg/dL)	TG (mg/dL)
Control 1 (1 mL of DW)	102.5 ± 3.5	57.4 ± 3.4	18.4 ± 1.3	25.9 ± 2.0	108.2 ± 2.8	59.2 ± 3.3	22.3 ± 1.5	23.3 ± 1.2
HWE (1.5 g/kg/day)	100.7 ± 3.0	59.4 ± 2.4	16.6 ± 1.1	23.3 ± 1.2	104.6 ± 2.6	71.1 ± 3.6 *	20.6 ± 1.1	22.9 ± 1.3
Control 2 [1 mL of Tween 80 (1% in DW)]	98.4 ± 4.9	60.4 ± 3.0	19.8 ± 1.4	22.1 ± 1.0	105.1 ± 3.5	57.3 ± 2.6	20.4 ± 1.4	24.1 ± 1.2
CEE (1.5 g/kg/day)	100.2 ± 3.6	61.1 ± 3.9	18.7 ± 1.2	23.2 ± 1.3	102.2 ± 4.4	73.9 ± 2.8 *	20.5 ± 1.2	25.3 ± 1.3

Values are expressed as mean ± S.E.M., n = 12
* Significant when compared to the respective control groups; $P \geq 0.05$

No significant ($P \geq 0.05$) differences were observed between controls and treated mice concerning the hematological parameters such as WBC, differential WBC, % PCV, Hb concentration,

MCV, MCH and MCHC. Further, there were no significant ($P \geq 0.05$) differences observed between controls and treated mice concerning the parameters checked for hepatic (Table 2) or renal (Table 3) functions. Further, apparent from Table 4, treatment with the extracts for 14 days did not result in any change in the serum levels of total cholesterol, HDL, LDL and TG. However, with prolonged treatment further 42 days either with HWE or CEE, there was a significant increase in HDL levels (HWE: by 20%; CEE: by 29%) without any significant ($P \geq 0.05$) differences observed in total cholesterol levels, LDL or TG levels between controls and treated mice.

Visual examination of liver, kidneys, heart, spleen and lungs appeared normal in treated mice.

Gastric lesions were not observed in any of the treated rats. There were no significant alterations *($P \geq 0.05$)* in the organ weights between the treated groups. In all groups under test, no apparent abnormalities were detected in sections of liver, kidneys, heart, spleen and intestine stained with hematoxylin and eosin.

The results of the effects of the HWE and CEE of *T. cucumerina* on ovulatory activity are shown in Table 5. Treatment of mice either with HWE or CEE did not significantly ($P \geq 0.05$) increase the duration of the dioestrus phase and the total length of oestrus cycle relative to the controls. Therefore, no anti ovulatory activity was observed with *T. cucumerina* extracts.

Table 5. Effects of the hot water extract (HWE) and the cold ethanolic extract (CEE) of *Trichosanthes cucumerina* on ovulatory activity

Groups	Persistent dioestrus stage (number of rats)	Appearance of regular cyclic stages (number of rats)	Duration of dioestrus cycle (number of days)	Duration of oestrus cycle (number of days)
Control 1 (1 mL of DW)	Nill	7.0 ± 0.0	1.2 ± 0.2	4.0 ± 0.0
HWE (1.5 g/kg/day)	Nill	7.0 ± 0.0	1.6 ± 0.4	4.0 ± 0.0
Control 2 [1 mL of Tween 80 (1 % in DW)]	Nill	7.0 ± 0.0	1.4 ± 0.2	4.0 ± 0.4
CEE (1.5 g/kg/day)	Nill	7.0 ± 0.0	1.5 ± 0.3	4.0 ± 0.2

Values are expressed as mean ± S.E.M., n = 7
Values of the test groups were not significant when compared to the respective control groups; $P \geq 0.05$

There were no signs of vaginal bleeding in the control groups and the HWE or CEE treated groups. Therefore, HWE or CEE showed no early abortifacient activity in mice. Table 6 shows that administration of 1.5 g/kg of HWE or CEE did not significantly ($P \geq$ 0.05) alter the number of implantation sites and the number of corpora lutea. Further, during the treatment period either with HWE or CEE , no deaths of pregnant rats were reported and no any clinical signs displayed of maternal toxicity such as changes in food and water intake, piloerection, diarrhoea and changes of locomotor activity. Both HWE and CEE showed no

spermicidal activity. These two extracts had hardly any effect on sperm mobility.

DISCUSSION

Standardization is an essential measurement for ensuring quality control of herbal drugs (Sanjay et al., 2009). Standardization of herbal products/drugs is more challenging than synthetic drugs. Herbal extracts contain a number of constituents of complex chemical nature and are inconsistent in composition (Jadhav et al., 2003). In most of the cases the biological activity is not exclusively dependent upon the so called active constituents, but is due to synergistic effect of all

chemical constituents of the plant. Even though biologically inert, many constituents affect the pharmacokinetics and stability of the active constituents (Handa, 1994). Densitograms and HPLC fingerprints have been utilized to standardize many medicinal plants extracts (Sanjay et al., 2009). The densitograms and HPLC fingerprints developed in the present study are also useful to ensure batch to batch consistency of *T. cucumerina* extracts in future investigations.

The residue remaining after incineration of plant material is the ash content or ash value, which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to

it, as a form of adulteration. The ash value was determined by 3 different methods, which measured total ash, acid insoluble ash and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both "physiological ash" which is derived from the plant tissue itself and "non – physiological ash" which is the residue of the extraneous matter adhering to the plant surface. Acid insoluble ash measures the amount of silica or acid insoluble matter present. Water soluble ash is the water soluble portion of the total ash. These ash values are important quantitative standards (Singh and Sharma, 2010).

Table 6. Effects of the hot water extract (HWE) and the cold ethanolic extract (CEE) of *T. cucumerina* extract on implantation

Groups	Number of implantation sites	Number of live fetuses	Number of corpora lutea of pregnancy
Control 1 (1 mL of DW)	8.5 ± 0.6	6.8 ± 0.6	10.5 ± 0.6
HWE (1.5 g/kg/day)	9.3 ± 0.6	7.6 ± 0.8	10.6 ± 0.7
Control 2 [1 mL of Tween 80 (1 % in DW)]	7.8 ± 0.5	7.4 ± 0.4	9.2 ± 0.8
CEE (1.5 g/kg/day)	8.6 ± 1.1	7.0 ± 0.7	9.8 ± 1.3

Values are expressed as mean ± S.E.M., n = 7
Not significant when compared to the respective control groups; $P \geq 0.05$

The results of the toxicity study indicate that *T. cucumerina* HWE or CEE administration by oral route with the doses up 30 g/kg did not produce any sign of toxicity or death in mice. According to Kennedy et al (1986), substances that present LD_{50} higher than 30 g/kg by oral route can be considered to be practically non – toxic. Therefore, it may be suggested that acute toxicity of *T. cucumerina* is practically null by oral route.

Some herbal medicines may cause liver damage leading to hepatocellular carcinoma (Harizal et al., 2010). The measurement of serum AST, ALT and ALP levels serve as an indirect assessment of liver function (Hilaly et al., 2004). Generally, any damage to the parenchymal liver cells results in elevations of both transaminases in the blood (Mukinda and Eagles, 2010). However, as indicated by effects of the *T.*

cucumerina extracts on serum levels of AST, ALT and ALP and liver histopathology, both HWE and CEE have no adverse effects on liver function even after 42 days of continuous treatment.

Creatinine is a protein produced by muscle and released into the blood. If kidney function falls, the creatinine level will rise. On the other hand, urea is the major end product of protein nitrogen metabolism. The determination of serum urea nitrogen is an important index of kidney function. Impaired renal function or increased tissue protein breakdown are associated with increased urea nitrogen levels whereas kidney damage or pregnancy are associated with decreased levels (Champe and Harvey, 1994; Lameire et al., 2005). In this study, the creatinine and urea concentrations were normal compared to that of the

respective controls. Therefore, both HWE and CEE of *T. cucumerina* appear to be devoid of renotoxicity.

The hematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (Mukinda and Syce, 2007). In this study, tested hematological parameters (such as WBC, differential WBC, % PCV, Hb concentration, MCV, MCH and MCHC) showed no significant differences between the control and the treated groups indicating that *T. cucumerina* extracts had no effects on the circulating blood cells nor on their production. Compared to rats in control groups, there was a significant increase in HDL levels in *T. cucumerina* extracts (HWE: by 20%; CEE: by 29%) treated rats. The improvement of HDL level in the serum is a beneficial effect because HDL is a cardio protective lipoprotein (Barter, 2005).

Extracts prepared from *T. cucumerina* aerial parts exert significant gastroprotective activity (Arawwawala et al., 2010b). This may be the reason why no gastric lesions were observed in mice treated either with HWE or CEE. *Mormodica charantia*, a close relative to *T. cucumerina* has been reported to exert abortifacient activity in early and mid term pregnancy and reduced the sperm production in male rats (Grover, 2004). However, *T. cucumerina* does not appear to mediate any unacceptable effects on the fertility of males or females as evident from the effects of the HWE and CEE on early aborfacient activity and implantation in female rats and spermicidal activity *in vitro*.

CONCLUSIONS

In conclusion, standardized extracts (HWE and CEE) of *T. cucumerina* at a dose level which corresponds to the normal therapeutic dose administered to adult humans as calculated on the basis of relative surface areas of humans and mice, appear to be safe in terms of (a) hepatotoxicity (as judged by AST, ALT, ALP concentration), (b) renotoxicity (as judged by serum urea and creatinine) or (c) hemototoxicity (as judged by WBC, RBC counts and Hb concentration, %PCV, MCV, MCH and MCHC), (d) gross morphology and weights of organs, (e) stress or aversive behaviors and (f) fertility.

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