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Original article

Evaluation of Acute and Subacute Toxicity of Coriander Triphala Tablet in Wistar Rats

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Abstract

Background and objectives: This study evaluated the acute and subacute toxicity of the Coriander Triphala tablet (CTT) in Wistar rats. **Methods**: The CTT was prepared according to the method described in our previous work. In the acute toxicity study, five female Wistar rats received 2000 mg/kg CTT and five female rats were administered distilled water as control. In the subacute test, sixty male and female rats were randomly divided into six groups. Three groups received 200, 500, and 1000 mg/kg of the tablet; the satellite group was treated with 1000 mg/kg of CTT, and controls received distilled water for 28 days. Body weights and food and water intake of rats were recorded. Toxicity signs were recorded and hematological, biochemical, and histopathological analyses were performed. **Results:** No remarkable toxic effect of the tablet was observed in the rats after receiving a single dose of 2000 mg/kg. This indicated that the median lethal dose (LD_{50}) was more than 2000 mg/kg. In the subacute toxicity study, different doses of CTT didn't change hematological parameters. However, the tablet increased the levels of cholesterol, creatinine, and aspartate aminotransferase (AST) in males and alanine aminotransferase (ALT) and AST in females at high doses. Histopathological evaluation of liver samples from both sexes showed congestion and hydropic degeneration of hepatocytes. Renal histopathology revealed varying degrees of tubular cell necrosis. **Conclusion:** Our data indicated the toxic effects of CTT on the liver and kidney, suggesting the need for special precautions in administration of this medication to the patients.

Keywords: acute toxicity; Coriander Triphala; Iranian traditional medicine; rat; subacute toxicity

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Introduction

Natural herbal medicines have been shown to cure human diseases for years and are the sources of more than 25% of modern medicines [1, 2]. The use of herbal medications and plant-derived natural compounds is increasing globally, and about 80% of the world's population use these herbal compounds as part of their primary health care [3, 4]. The increasing desire for herbal medicines may arise from the fact that herbal medicines may effectively treat some diseases where classical therapies or chemical drugs are not good enough or impose high costs and side effects on the patients [5]. Thus, it seems that future health care will shift towards the use of

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combined herbal products with multiple pharmacological effects on different medicinal targets [6]. Iranian traditional medicine has suggested many natural recommendations for a variety of health conditions [7-10]. Coriander Triphala is an Iranian traditional formulation (oral semisolid form) made from the fruits of *Terminalia chebula*, *Terminalia bellirica*, *Phyllanthus emblica*, and *Coriandrum sativum* along with almond oil and honey. It has been widely used to treat digestive disorders and as a cleanser [11, 12]. *Terminalia chebula* is a plant species of the Combretaceae family, which has three types of fruit in different stages of maturity; immature, mature, and fully matured fruits [13]. *Terminalia chebula* has been numerously used in traditional medicine for various conditions such as bleeding, dysentery, liver diseases, digestive disorders, diarrhea, infections, asthma, cough, and skin disorders [14, 15]. It mainly contains different tannins such as gallic acid, ellagic acid, methyl gallate, ethyl gallate, chebulagic acid, chebulagic acid, corilagin and hexahydroxydiphenic acid ester [16-18]. *Phyllanthus emblica* is a medicinal plant used in traditional medicine for the treatment of various diseases such as diabetes, hyperlipidemia, central nervous system disorders, and eye diseases. It is a main source of tannins, vital amino acids, and vitamins [19]. As a well-known spice, *Coriandrum sativum* has been traditionally used to treat flatulence, diarrhea, dysentery, fever, vomiting, indigestion, and memory loss [20]. In a previous investigation, a film-coated tablet from the traditional form of Coriander Triphala has been made and quality control of the tablet has been performed [21]. As mentioned above, Coriander Triphala contains many compounds especially high levels of tannins and these ingredients may cause toxic effects on a variety of body organs such as the liver, kidney, heart, and others. Therefore, this present study aimed to explore the acute and subacute toxicity of Coriander Triphala tablets (CTT) in Wistar rats according to the Organization for Economic Co-operation and Development (OECD) guidelines [22, 23].

Material and Methods

Ethical consideration

The experimental design was approved by Ethical Committee of Shahid Beheshti University of Medical Sciences with the code of IR.SBMU.RETECH.REC.1400.1214. All experiments were performed according to the National Institute of Health's (NIH) guidelines for the care and use of laboratory animals.

Chemicals

Ketamine-xylazine solution was purchased from Sigma Company (Sigma, USA); formaldehyde solution for the histological study was provided from Merck Company (Germany); absolute ethanol was purchased from Razi Yeast and Alcohol Company, Iran; hematoxylin-eosin was from Sigma, Germany; biochemical parameters were measured using pars Azmoon laboratory kits (Pars Azmoon Company, Iran). Hematology whole blood control was purchased from Man Company, Iran.

Coriander Triphala tablet formulation

The Coriander Triphala tablet (CTT) was prepared according to the method described by Choopani et al. [21]. The tablet consisted of 98 mg of each species (*T. chebula* (fruits), *T. bellirica*, *Ph. emblica*, *C. sativum*), 14 mg almond oil, and 148 mg honey. The tablet was made by the wet granulation method. Quality control tests including weight variation, friability, hardness, disintegration time, assay, and dissolution were performed. It was standardized according to total tannins with the use of Folin-Ciocalteu reagent. During this method, first total phenolics were determined, and then tannins were removed from the sample by hide powder [17]. The difference between total phenolics before and after adding hide powder was considered as total tannins

Experimental animals

Fifty Wistar rats of both sexes were obtained from Animal House, Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. All animals were kept under standard laboratory conditions in a room with a 12 h light/dark cycle, a temperature of 23-25 °C, 50% humidity, and appropriate ventilation. All animals had free access to food and water during the experiments. All experiments were performed according to the international guidelines for animal laboratory use and care.

Acute oral toxicity study

The acute toxicity study was achieved according to the OECD guideline 425 [22]. Ten female

Wistar rats weighing from 100 to 150 g with the age of 8-12 weeks were used and acclimatized in the laboratory conditions for five days before dosing. The rats were fasted overnight prior to the prescription of doses. Subsequently, five animals were treated with a single dose of 2000 mg/kg CTTs dissolved in distilled water via oral gavage. Five control rats received only distilled water at a volume of 1 mL/100g body weight. All CTT-treated and control rats were observed periodically during the first 24 h after dosing and daily thereafter for 14 days. Observations included the changes in skin and fur, eyes and mucus membranes, behavioral patterns, mortality, salivation, respiratory disorders, lethargy, diarrhea, and tremors. Food and water intake as well as the body weights of all treated and control rats were also recorded daily. All animals were subjected to gross necropsy to find possible macroscopic pathological changes in the animal body.

Subacute oral toxicity study

The subacute oral toxicity of CTT was performed in accordance with the OECD guideline 407 with minimal modifications [23]. Forty Wistar rats weighing from 100–120 g were randomly divided into five equal groups (n = 10, 5 females and 5 males). Three groups were treated with aqueous solution of CTT at doses of 200, 500 and 1000 mg/kg by oral gavage for 28 continuous days. The control group received distilled water at volume of 1 mL/100 g body weight of the rats for the same time. A satellite group of 20 animals (10 males and 10 females) also received the highest dose (1000 mg/kg) and distilled water (1 mL/100 g body weight) for 28 days. After 28 days, dosing was stopped for 14 days and then blood samples were collected. An observer recorded the changes in body weight, water and food intake, physical signs or symptoms of toxicity, and behavioral parameters of all animals daily up to the end of experiments. Finally, the rats were anesthetized by using ketamine-xylazine solution (10:1) and then the blood samples were collected by cardiac puncture. Biochemical and hematological parameters were analyzed in serum specimens. The rats were then sacrificed and liver and kidney organs were removed.

Histopathological examinations

As mentioned above, the liver and kidneys were excised and removed from all of the animals. Subsequently, the dissected organs were placed in a 10% formalin solution to fix the tissues. Formalin-fixed tissues were then dehydrated by absolute ethanol and embedded in paraffin blocks. Each tissue block was cut by microtome into sections with a thickness of 5–7 μm and stained using hematoxylin-eosin. All tissue sections were analyzed by an expert pathologist.

Statistical analysis

The obtained data were statistically analyzed by GraphPad PRISM software version 8. The numerical results obtained from each group were analyzed using one-way ANOVA followed by Tukey's multiple comparison test. The data were expressed as mean ± standard deviation of five replicates. P-values less than 0.05 were considered statistically significant.

Results and Discussion

The total tannins content of pyrogallol was 64.19 mg/tab. The results of the limit test of the acute toxicity study showed that the treatment of all five rats with an aqueous suspension of CTT at a dose of 2000 mg/kg did not lead to death in these animals. None of the animals displayed drug-related acute toxicity effects such as increased salivation, lethargy, diarrhea, tremors, convulsions, and death during the first 24 hours after the administration of CTT aqueous suspension. No signs of adverse changes in skin, behavior, breathing, water and food intake, body weight, and temperature were observed. According to the OECD guideline 425, the tablets appeared to be safe at the doses of 2000 mg/kg and the median lethal dose (LD_{50}) value was considered to be more than 2000 mg/kg. Therefore, there was no need to perform the main test. The body weight and water and food consumption during the limit test are shown in Tables 1 and 2.

Table 1. Effect of Coriander Triphala tablet on body weights after limit test

Groups	Body weights (g)		
	Week 0	Week 1	Week 2
Control	156.60±8.50	160 ± 7.34	164.40±7.63
Test (2000 mg/kg CTT)	161.44 ± 8.00	166.60±6.94	173.60±7.50

CTT: Coriander Triphala tablet

Table 2. Effect of Coriander Triphala tablet on food and water intake after limit test

Groups	Food (g/day)	Water (ml/day)
Control	15.96 ± 3.39	43.50 ± 8.50
Test (2000 mg/kg CTT)	18.85 ± 2.80	38.91 ± 7.60

CTT: Coriander Triphala tablet

The subacute toxicity-inducing effect of CTT was evaluated by treating the rats with daily oral doses of 200, 500, and 1000 mg/kg body weight for 28 days. The control group also received 1 mL/100g of distilled water. At the end of the experiments, hematological, biochemical, and histopathological analyses were performed. Besides, general clinical symptoms and behavioral signs were recorded. It should be noted that no toxic side effects such as increased salivation, lethargy, diarrhea, tremors, convulsions, and mortality were observed in the rats during the 28 days of CTT treatment.

As shown in Table 3, daily oral gavage of the aqueous suspension of CTT revealed no significant change in the body weight of rats before, during, and after the treatments compared with the control rats ($p<0.05$). The daily oral administration of the aqueous suspension of CTT was conducted at doses of 200, 500, and 1000 mg/kg for 28 days.

Table 4 demonstrates the effect of daily oral gavage of the aqueous suspension of CTT on food and water intakes both in male and female rats. The results indicated no significant change in the food and water intakes of animals before, during and after the CTT administration in comparison to the controls ($p<0.05$). The daily oral administration of the aqueous suspension of CTT was conducted at doses of 200, 500, and 1000 mg/kg for 28 days.

The effects of the subacute doses of CTT on hematological parameters are shown in Table 5. According to data, all the measured hematological indices, including white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet, red cell distribution width (RDW%), reticulocyte count (Retic%), prothrombin time (PT), partial thromboplastin time (PTT), and the percentage of neutrophil, lymphocyte, monocyte, eosinophils, and basophile remained unchanged in comparison to the control rats ($p<0.05$).

According to the results shown in Table 6, the treatment of male rats with doses of 200, 500, and 1000 mg/kg of the aqueous suspension of CTT had no significant effect on the serum levels of glucose, urea, alanine aminotransferase (ALT), total protein, albumin, sodium, potassium, and total bile acids compared with the control rats.

However, serum levels of cholesterol and creatinine significantly increased after oral consumption of CTT aqueous suspension at the doses of 500, and 1000 mg/kg ($p<0.01$) and $p<0.05$). In addition, CTT increased the level of liver enzyme aspartate aminotransferase (AST) at the dose of 1000 mg/kg ($p<0.05$). On the other hand, the treatment of female rats with doses of 200, 500, and 1000 mg/kg had no significant effect on the serum levels of glucose, urea, cholesterol, creatinine, total protein, albumin, sodium, potassium, and total bile acids.

Table 3. Effect of Coriander Triphala tablet on body weights after subacute toxicity study

Groups	Body weights (g)				
	Week 0	Week 1	Week 2	Week 3	Week 4
Male					
Control	149.20 ± 5.50	155.20 ± 6.22	168.40 ± 5.60	179.60 ± 5.62	194.40 ± 7.50
CTT (200 mg/kg)	156.20 ± 6.50	168.50 ± 4.34	174.40 ± 7.63	184.40 ± 7.63	196.20 ± 6.62
CTT (500 mg/kg)	146.60 ± 8.30	157.35 ± 6.30	167.40 ± 3.40	177.20 ± 6.50	192.30± 7.20
CTT (1000 mg/kg)	152.60 ± 5.50	168.65 ± 5.34	17.40 ± 5.60	184.40 ± 7.62	190.60 ± 8.60
Female					
Control	151.42 ± 60	165.60 ± 5.94	178.60 ± 7.20	183.20 ± 6.50	193.60 ± 5.50
CTT (200 mg/kg)	161.30 ± 50	167.60 ± 5.94	175.60 ± 7.50	189.60 ± 8.50	199.60 ± 5.50
CTT (500 mg/kg)	161.40 ± 80	168.60 ± 5.94	179.60 ± 7.50	189.60 ± 8.50	198.60 ± 5.50
CTT (1000 mg/kg)	160.35 ± 50	168.60 ± 6.90	178.60 ± 7.50	182.50 ± 4.50	193.50 ± 5.50

CTT: Coriander Triphala tablet; No significant differences were found ($p>0.05$).

Table 4. Effect of Coriander Triphala tablet on food and water intake after subacute toxicity study

Groups	Food (g/day) Male	Water (ml/day) Male	Food (g/day) Female	Water (ml/day) Female
Control	15.50 ± 2.45	44.20 ± 2.30	14.60 ± 2.40	42.50 ± 7.50
CTT (200 mg/kg)	16.80 ± 2.50	40.90 ± 6.40	16.85 ± 2.80	39.91 ± 5.60
CTT (500 mg/kg)	17.70 ± 2.40	43.50 ± 6.50	16.96 ± 2.30	40.50 ± 8.50
CTT (1000 mg/kg)	18.25 ± 2.80	39.90 ± 5.60	17.20 ± 2.35	42.90 ± 6.60

CTT: Coriander Triphala tablet; No significant differences were found ($p>0.05$).

Table 5. Effect of Coriander Triphala tablet on hematological parameters after subacute toxicity study

Parameters	Male				Female			
	Control	CTT (200 mg/kg)	CTT (500 mg/kg)	CTT (1000 mg/kg)	Control	CTT (200 mg/kg)	CTT (500 mg/kg)	CTT (1000 mg/kg)
WBC ($\times10^3$/µL)	10.96±3.76	8.08±0.93	7.44±2.33	8.18±1.13	8.57±2.47	6.32±1.07	8.36±0.63	6.38±1.66
RBC ($\times10^6$/µL)	7.36±0.64	7.88±0.30	7.79±0.93	8.21±0.36	7.48±0.57	7.13±0.44	7.42±0.51	7.35±0.36
Hb (g/dL)	15.78±0.74	15.78±0.46	15.52±1.67	16.32±0.64	14.87±1.62	14.84±0.32	15.86±0.81	15.82±0.82
Hct (%)	40.90±1.95	42.34±1.87	40.46±4.78	42.50±2.16	40.60±1.30	38.52±1.14	40.80±2.30	40.70±2.47
MCV (fL)	53.94±1.03	52.90±1.21	53.00±0.90	53.18±1.25	55.87±0.82	53.90±2.31	54.70±1.80	55.10±1.76
MCH (pg)	20.80±0.48	20.22±0.69	20.32±0.34	20.42±0.63	22.07±0.63	20.80±0.91	21.26±0.68	21.42±0.62
MCHC (g/dL)	38.58±0.37	37.50±0.76	38.34±0.64	38.40±0.64	39.52±1.23	38.54±0.89	38.86±0.25	38.88±0.21
Platelet ($\times10^3$/µL)	536.40±67.18	553.60±58.28	586.00±66.66	575.00±74.43	569.33±46.19	624.20±83.86	594.00±36.05	549.75±30.34
RDW (%)	12.82±1.03	12.06±0.60	12.66±1.96	12.34±0.37	12.15±0.26	12.28±0.38	12.63±0.15	12.28±0.28
Retic (%)	2.70±0.28	2.00±0.67	1.75±0.77	2.36±1.08	2.53±1.72	2.04±0.92	2.70±0.45	2.72±0.86
PT (s)	16.72±0.99	15.22±1.63	14.56±1.36	15.82±1.45	15.25±0.85	16.12±0.25	14.72±1.21	15.65±1.23
PTT (s)	14.42±1.03	13.25±0.86	13.56±1.14	15.72±0.75	14.85±0.55	14.5 ±0.23	13.8 ±0.75	14.25±0.50
Neutrophils (%)	69	68	70	74	70	69	75	70
Lymphocytes (%)	31	32	30	26	30	31	25	30
Monocytes (%)	0	0	0	0	0	0	0	0
Eosinophils (%)	0	0	0	0	0	0	0	0
Basophils (%)	0	0	0	0	0	0	0	0

CTT: Coriander Triphala tablet; WBC: white blood cells; RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; Retic: reticulocyte; PT: prothrombin time; PTT: partial thromboplastin time; No significant differences were found ($p>0.05$).

Table 6. Effect of Coriander Triphala tablet on biochemical parameters

Parameter	Male				Female			
	Control	CTT (200 mg/kg)	CTT (500 mg/kg)	CTT (1000 mg/kg)	Control	CTT (200 mg/kg)	CTT (500 mg/kg)	CTT (1000 mg/kg)
Glucose (mg/dL)	175.00±22.38	186.00±25.45	168.60±18.33	174.20±24.23	172.00±18.35	168.80±17.85	172.24±15.41	182.20±28.59
Urea (mg/dL)	48.80±6.21	54.40±12.50	49.9±9.46	42.50±1.54	52.50±5.01	49.80±5.98	44.80±4.00	42.30±4.67
Cholesterol (mg/dL)	33.25±4.48	44.30±11.57	**57.80±17.19****	**57.62±9.39****	42.87±5.97	52.12±6.88	50.37±7.63	44.00±5.96
Creatinine (mg/dl)	0.49±0.06	0.61±0.28	**0.74±0.11***	**0.74±0.05***	0.67±0.02	0.72±0.16	0.74±0.17	0.66±0.10
AST (U/L)	185.25±17.72	189.50±11.50	232.00±27.92	**232.25±20.90***	202.00±13.07	202.25±9.67	243.33±27.02	**253.00±23.66***
ALT (U/L)	32.33±3.37	32.26±5.85	31.50±6.53	45.66±11.25	52.00±4.58	53.75±6.92	55.00±5.56	**84.66±10.06***
Total protein (g/L)	6.66±0.59	6.58±0.69	6.44±0.98	6.54±0.56	6.40±0.46	6.68±0.56	6.97±0.80	6.78±0.56
Albumin (g/L)	3.13±0.49	3.40±0.46	3.29±0.85	3.45±0.77	3.26±0.58	3.43±0.55	2.78±0.62	3.01±0.23
Sodium (mEq/L)	141.60±2.70	141.00±1.58	142.80±4.65	145.60±3.13	141.50±4.50	140.40±1.14	142.20±3.83	142.00±3.67
Potassium (mEq/L)	4.81±0.56	5.15±0.67	5.04±0.66	4.86±0.61	4.68±0.29	4.62±0.64	4.88±0.49	4.68±0.53
Total bile (µmol/L)	14.80 ± 1.61	17.20 ± 1.16	16.60 ± 1.68	17.82±1.65	18.25±1.85	19.12±1.25	19.72±1.21	20.65±1.23

CTT: Coriander Triphala tablet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; *$p<0.05$,**$p<0.01$

However, the serum amount of AST and ALT enzymes significantly increased following oral administration of CTT suspension at a dose of 1000 mg/kg in comparison to the control group ($p<0.05$). Histopathological evaluation of liver samples from both male and female rats is illustrated in Figure 1. Normal histology of liver tissues at 200 mg/kg CTT-treated and control groups are evident. As depicted in the figure, treating the rats with 500 and 1000 mg/kg of CTT resulted in congestion and hydropic degeneration (mild to moderate) of hepatocytes. Figure 2 shows the histological micrographs of kidney sections from different groups. Control and CTT-treated animals at the dose of 200 mg/kg showed normal histology of the kidney tissue of male and female rats.

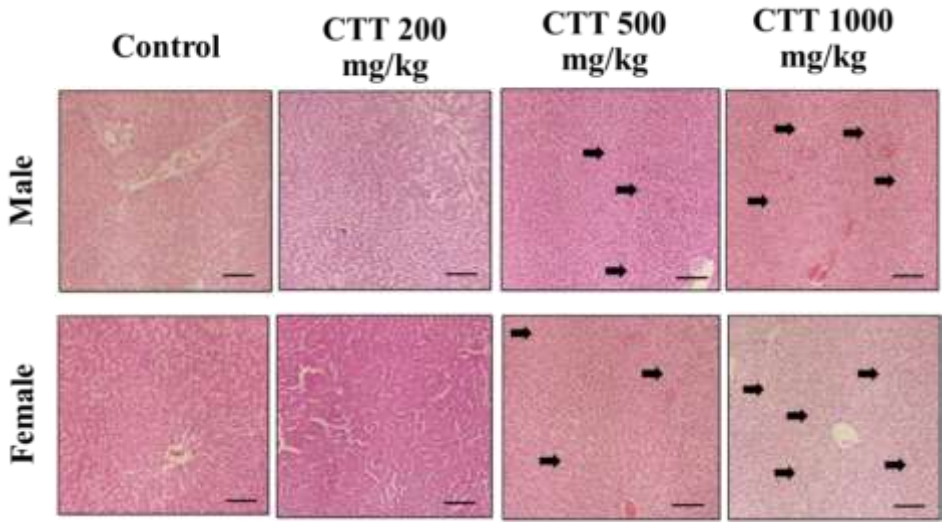

Figure 1. Histologic images of liver specimens of the different experimental groups; black arrows: hydropic degeneration of hepatocytes; The images were stained using the H&E staining technique. CTT; Coriander Triphala tablet; Scale bar = 100 µm

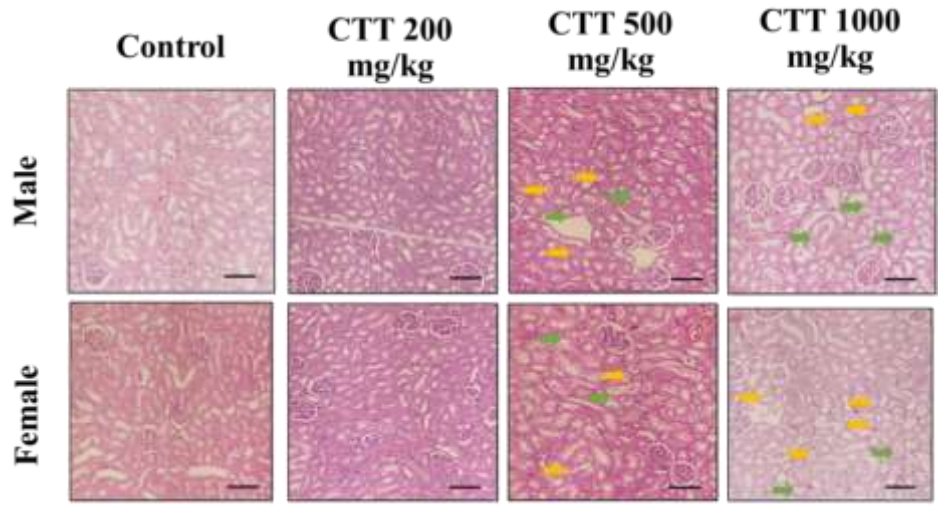

Figure 2. Histologic micrographs of the kidney tissue sections from various experimental groups; The yellow arrows show renal tubules that contain necrotic cells and debris in their lumen and are only partially covered by epithelium. The green arrows point to single-cell necrosis foci, where the necrotic cells have dense eosinophilic cytoplasm (karyorrhexis). The tissue sections were stained using H&E method. CTT; Coriander Triphala tablet; Scale bar = 100 µm

The kidney sections from the female and male rats treated with CTT at the doses of 500 and 1000 mg/kg showed different degrees of tubular cell necrosis. Extensive tubular epithelial necrosis (sloughed necrotic epithelial cells) with degeneration was evident in both treatment groups. According to our observations, no marked toxic symptoms or behavioral changes were indicated in the satellite group. As demonstrated in Table 7, none of the hematological indexes was changed in the satellite group due to the treatment of the rats with 1000 mg/kg CTT. However, the levels of some biochemical parameters significantly altered in the satellite group compared with the controls. Based on the data shown in Table 8, the treatment of both male and female rats of the satellite group with CTT (1000 mg/kg) significantly increased serum levels of cholesterol and creatinine ($p<0.01$ and $p<0.05$). The satellite group also considerably elevated the amounts of liver enzyme ALT in comparison to the control group ($p<0.05$). Other biochemical parameters showed no difference between the

CTT-treated and control animals. The results of biochemical and hematological parameters in satellite and CTT 1000 mg/kg were relatively similar.

In Iranian traditional medicine, the fruits of *T. chebula*, *T. bellirica*, *Ph. emblica*, and *C. sativum* are combined to produce an oral semisolid form of Coriander Triphala, which is used to cure digestive diseases and other ailments [11, 12]. Choopani, et al. formulated a film-coated form of CTT and tested the quality control of the tablet [21]. The major plant materials of CTT contain different phytochemical compounds such as the tannins, which may pose toxic effects on a variety of body organs [24]. The general assumption that herbal medications and their formulations are safer than allopathic medicine leads to indiscriminate use of them without considering their potential adverse side effects [25, 26]. Therefore, this study evaluated the acute and subacute toxic effects of CTT on Wistar rats according to the OECD guidelines.

To assess the acute toxicity of CTT, an oral dose of 2000 mg/kg of the aqueous suspension of the tablet was administered to the rats. The results uncovered that the animals receiving CTT at a single dose of 2000 mg/kg showed no acute toxicity signs such as increased salivation, lethargy, diarrhea, tremors, convulsions, and death during the first 24 hours after CTT administration. Moreover, no considerable adverse change was observed in their skin, behavior, breathing, water and food intake, body weight, and temperature. Sholikhah, et al conducted a study to investigate acute and subchronic toxicity of a polyherbal formulation in rats.

Table 7. Effect of Coriander Triphala tablet on hematological parameters in satellite group

Parameters	Male		Female	
	Control	CTT (1000 mg/kg)	Control	CTT (1000 mg/kg)
WBC ($\times10^3$/µL)	9.56±6.52	8.02±3.83	8.02±1.12	7.88±4.01
RBC ($\times10^6$/µL)	8.45±0.63	7.95±0.39	7.85±0.67	7.65±0.66
Hb (g/dL)	16.88±0.72	15.35±0.43	15.07±0.28	15.80±0.36
Hct (%)	42.60±0.92	41.65±1.18	40.50±0.30	39.80±1.87
MCV (fL)	54.84±0.83	53.08±2.02	54.65±0.25	54.00±0.06
MCH (pg)	22.62±1.36	22.32±0.54	21.12±0.65	20.32±0.42
MCHC (g/dL)	39.65±0.27	38.43±0.60	38.35±0.26	38.36±0.13
Platelet ($\times10^3$/µL)	564.65±74.20	580.10±72.32	580.21±35.32	555.05±25.32
RDW (%)	12.20±0.25	12.22±0.32	12.25±0.36	12.63±0.41
Retic (%)	2.00±0.28	2.02±1.01	2.36±1.12	2.35±0.84
PT (s)	17.65±0.36	15.36±1.14	16.32±0.65	15.23±1.65
PTT (s)	15.66±0.22	15.25±0.65	14.64±0.55	14.65±0.33
Neutrophils (%)	70	72	73	68
Lymphocytes (%)	28	30	32	26
Monocytes (%)	0	0	0	0
Eosinophils (%)	0	0	0	0
Basophils (%)	0	0	0	0

CTT: Coriander Triphala tablet; WBC: white blood cells; RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; Retic: reticulocyte; PT: prothrombin time; PTT: partial thromboplastin time; No significant differences were found ($p>0.05$).

Table 8. Effect of Coriander Triphala tablet on biochemical parameters in satellite group

Parameter	Male		Female	
	Control	CTT (1000 mg/kg)	Control	CTT (1000 mg/kg)
Glucose (mg/dL)	169.00±20.33	173.35±19.31	174.12±22.45	178.44±33.00
Urea (mg/dL)	45.55±8.00	44.89±3.66	50.22±4.50	47.44±5.05
Cholesterol (mg/dL)	40.00±2.87	**62.11±3.21****	36.23±4.05	**56.80±7.00****
Creatinine (mg/dL)	0.45±0.12	**0.78±0.04***	0.60±0.05	**0.74±0.28***
AST (U/L)	180.22±12.00	210.31±18.00	208.23±10.00	230.00±12.02
ALT (U/L)	32.02±1.50	**58.65±9.80***	48.90±5.00	**80.56±10.25***
Total protein (g/L)	5.49±0.65	5.44±0.75	5.80±0.74	5.20±0.55
Albumin (g/L)	3.40±0.24	3.50±0.32	3.80±0.22	3.74±0.69
Sodium (mEq/L)	142.00±1.02	144.05±2.03	145.00±1.80	140.30±1.87
Potassium (mEq/L)	4.32±0.45	4.65±0.35	4.41±0.96	4.84±0.65
Total bile (µmol/L)	15.65 ± 1.02	16.65±1.43	16.66±1.31	17.85±1.55

CTT: Coriander Triphala tablet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; *$p<0.05$, **$p<0.01$

In the acute study, they observed no signs of toxicity due to the treatment of animals with 2000 mg/kg of formulation and thus reported that the LD_{50} of that the poly herbal drug was more than 2000 mg/kg [27]. Jayesh and colleagues also evaluated the acute toxicity of the aqueous acetone extract of the fruit of *T. bellirica* in Wistar rats. They reported that a single dose of this plant material (2000 mg/kg) caused no acute toxic effect on female rats and thus the LD_{50} of the extract was reported to be more than 2000 mg/kg [28]. In the present study, the LD_{50} of CTT was estimated to be greater than 2000 mg/kg according to OECD guideline 425 [22]. This may suggest that acute consumption of up to 2000 mg/kg of CTT results in no remarkable clinically important acute effects. However, these data were obtained from an acute toxicity study on female rats and are not directly attributed to human subjects. Therefore, the study needs to be performed in human cases. Based on the results of the acute study, we decided to assess the subacute toxicity of CTT to reveal the long-term effects of CTT consumption.

In the subacute toxicity study, both female and male rats were orally treated with CTT at doses of 200, 500, and 1000 mg/kg for 28 days. Our observations revealed no considerable signs of toxic effects such as increased salivation, lethargy, diarrhea, tremors, convulsions, and death during 28 days of the experiment.

The body weight of animals is used as an important indicator of the animal's physiological health [29]. Sufficient food and water intake is also essential for the health, production, and well-being of animals [30, 31]. The present study recorded both water and food intake and body weight changes in all experimental rats. Body weights as well as food and water intake were not changed in CTT-treated female and male rats in comparison to the control animals.

Both clinicians and researchers generally evaluate hematologic and biochemical parameters to estimate the health status of animals and humans [32]. Therefore, both hematologic and biochemical levels of female and male rats used in the subacute study were measured and evaluated. Results showed that the hematological parameters of CTT-administered animals showed no significant difference compared to the controls. However, treating male rats with CTT at the doses of 500 and 1000 mg/kg significantly elevated serum levels of cholesterol and creatinine and considerably increased the level of AST enzyme at a dose of 1000 mg/kg. Female rats receiving 1000 mg/kg CTT showed increased serum levels of AST and ALT enzymes in comparison to the control group. Histopathological analysis of liver tissues from both male and female rats demonstrated that CTT at the doses of 500 and 1000 mg/kg caused congestion and hydropic degeneration (mild to moderate) of hepatocytes. The female and male rats treated with CTT at the doses of 500 and 1000 mg/kg showed different degrees of tubular cell necrosis and degeneration in kidney tissues in comparison to the control animals. Both female and male rats treated with 200 mg/kg CTT demonstrated normal histology of the liver and kidney. The vital organs of the body include the liver, kidneys, heart, lungs, and spleen [33]. Xenobiotics are mainly metabolized and excreted by two major body organs including the liver and kidneys [34]. Therefore, the presence of adverse histological changes in liver and kidney tissues may aggravate the normal function of these organs posing many other risks to body health. According to the literature, liver injury is generally characterized by hepatocellular damage leading to elevated levels of serum ALT and AST enzymes [35]. In the present study, elevated levels of these enzymes were completely consistent with the congestion and degeneration of hepatocytes in CTT-administered groups at doses of more than 500 mg/kg, suggesting the development of liver parenchymal damage due to CTT consumption. Serum levels of creatinine reflect the normal function of the kidneys and indicate how well the kidneys remove the waste products from the bloodstream [36]. Thus, increased levels of creatinine in serum can be indicative of kidney dysfunction leading to the accumulation of toxic wastes in the body. This may either directly or indirectly affect the normal function of other body organs or pose many risks to body health. Increased levels of serum creatinine in the present study were also in line with tubular cell necrosis and degeneration in kidney tissues of rats treated with CTT. Kidney injury is generally characterized by increased serum levels of creatinine [37].

In the satellite group, the rats exhibited no change in the hematological parameters, behavioral indexes, and toxicity-related signs in comparison to the control group. However, the levels of some biochemical parameters including serum

cholesterol, creatinine, and liver enzyme ALT were significantly raised in comparison to the control group. Other biochemical parameters had no significant difference between the CTT-treated and control animals of the satellite group. These data suggest that the rats in the satellite group had no significant recovery from the subacute toxic effects of CTT and experienced no delayed occurrence of toxic effects.

Conclusion

The present data unraveled that CTT had no remarkable acute toxicity at a dose level of 2000 mg/kg in rats. In the subacute toxicity study, it was evidenced that CTT at a dose level of 200 mg/kg exerted no marked toxic effect on the biochemical, hematological, histological, wellness, and behavioral parameters of the animals. However, the drug increased biochemical parameters such as cholesterol, creatinine, AST, and ALT in the serum of the rats treated with 500 and 1000 mg/kg CTT. CTT also caused histopathological changes in the liver parenchyma and tubules of the kidneys. Consequently, these results may suggest that the long-term consumption of CTT can have detrimental effects on the normal functions of body organs, especially the liver and kidneys. Therefore, special medical precautions should be considered during the long-term use of CTT for therapeutic purposes.

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Author contributions

Homa Hajimehdipoor and Sadegh Rajabi designed the experiments and defined the methodology; Homa Hajimehdipoor and Maliheh Soodi edited the manuscript; Fatemeh Jafari and Fatemeh Ghorbannejad performed the experiments and collected the data; Sadegh Rajabi analyzed the data and wrote the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

CTT: Coriander Triphala tablet; LD50: median lethal dose; AST: aspartate aminotransferase; ALT: alanine aminotransferase; OECD: Organization for Economic Co-operation and Development; WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; Retic: reticulocyte count; PT: prothrombin time; PTT: partial thromboplastin time