Consumption of *Citrullus colocynthis* Fruit Extract Causes Histological and Immunological Alterations in Mice

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Accepted December 08, 2020 Published online December 30, 2020 Issue online December 31, 2020

Original article


Traditionally, the usage of *Citrullus colocynthis* (CCT) causes severe side effects. The side effects of CCT fruit extract administered orally at different doses related to gastric tissues and circulating cytokines profiles were reported. Thirty-five adult male albino mice were divided into 4 groups, a control group (G1) and three experimental groups. They orally received aqueous fruit extract over 20 days at different doses; 100, 200, and 300 mg/kg of body weight/day. Total body weight, stomach tissue, peripheral blood, anti-inflammatory, and pro-inflammatory cytokines were evaluated. Body weight significantly decreased in groups 2 and 3 over four weeks. Neutrophils, lymphocytes, monocytes, and basophils were significantly (p<0.05) elevated in group 4; while hemoglobin and mean corpuscular hemoglobin concentration (MCHC) were significantly decreased (p<0.05). Interleukin-8 (IL-8) level was significantly elevated in groups 3 and 4 versus the control group (p<0.05). Interleukin-6 (IL-6) levels showed a significant (p<0.05) increase in group 4 only. Anti-inflammatory cytokines showed a significant (p<0.05) decrease in all treated groups. The stomach tissues revealed that the extract induced a superficial focal loss of the surface mucous protective epithelium, the atrophy of peptic cells, and a thickening of mucosal connective tissue. The submucosa showed vascular congestion and inflammatory cell infiltration. Severe histological changes were reported in group 4. Using the extract for 20 days led to the elevation of the differential white blood cell (WBC) count as well as the destruction of the gastric mucosal lining at high doses. This could be due to an increase in pro-inflammatory and declining anti-inflammatory cytokines. It is not recommended to use CCT in high doses or for long periods.

Key words: *Citrullus colocynthis*, extract, mice, oral route, stomach, cytokines.

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*Citrullus colocynthis* (L.) Schrad (CCT) is a profitable cucurbit plant belonging to the Cucurbitaceae family. Some common individuals of the family are melon, cucumber, pumpkin, and bitter apple. CCT is a little scarbid perennial inching plant that has either a harsh or precise stem, having smooth circular fruits that are colored green when unripe, turning yellow once ripe (PRAVIN et al. 2013). CCT fruit is well known in Saudi Arabia as Handal or Sherry (AL-YAHYA et al. 2000). It’s also called Colocynth/Bitter Apple, bitter-cucumber, colocynth, wild gourd, vine-of-sodom. The plant grows in Saudi Arabia, Kuwait, Iraq, Jordan, Turkey, India, China, Pakistan, and other Asian, African, and European countries (AL-SNAFI 2016). Different CCT plant parts such as the seeds, leaves, fruit, stem, and root, are utilized as oil or aqueous extracts, dried or fresh, and are believed to have anti-diabetic (RAHIMI et al. 2012), anti-hyperlipidemic (RAHBAR and NABIPOUR 2010), laxative (MARZOUK et al. 2010), anti-inflammatory (MARZOUK et al. 2010), analgesic (MARZOUK et al. 2010), hair-growing (DHANOTIA et al. 2011), vermifuge (RAHMI et al. 2012), antifungal (MARZOUK et al. 2009), antibacterial (MARZOUK et al. 2009), and antioxidant properties (TANNIN-SPITZ et al. 2007).

Behind most of these therapeutic potentials are cucurbitacin, flavonoids, alkaloids, and phenolic acids (HUSSEIN et al. 2017; ZHENG et al. 2020). A phytochemical analysis of CCT fruit revealed the presence of amino acids, carbohydrates, tannins, proteins,
saponins, flavonoids, terpenoids, phenolics, anthranol, alkaloids, steroids, J, L, caffeic acid, Cucurbitacin A, B, C, D, and E (α-elaterin), and cardiac glycoloids (HUSSAIN et al. 2014; AL-SNAFI 2016; BASHA et al. 2019). Despite the medicinal properties and long therapeutic history of CCT (ZHENG et al. 2020), it has serious side effects that seem related to either over-consumption, patient allergy, or an extended period of usage. It can cause gastrointestinal upset as intestinal damage and rectorrhagia. Studies regarding CCT are quite different, mainly in respect to fruit parts being examined for toxic and physiological properties, the utilized solvent for the extraction from plant parts, doses given with lyophilized extract, route of administration, and acute or chronic extract effects. Despite being motivated by delineating plant treatment actions, these differences restrict the possibilities for comparing different results (SANADGOL et al. 2011). The toxicity complication effects of CCT ethanolic extract on the kidneys, heart, liver, spleen, stomach, and intestines, with varied severity, was definitive evidence and dose-dependent (MARZOUK et al. 2010; HUSSAIN et al. 2014; SHOKRZADEH et al. 2013; AL-SNAFI 2016). The acute median lethal dose (LD50) of the extract was found to be 1311.45 mg/kg (at a 95% confidence limit of 1037.80 to 1657.27 mg/kg) for male rats. The LD16 and LD84 were 825.61 mg/Kg and 2083.19 mg/Kg, respectively (SOUFANE et al. 2013). We used an aqueous extract according to (MUHSEN & ALALI 2010) which seems more close to reality than ethanol extract, and alternative medicine usually advises the patient to take CCT with water. Because of this, the current study follows these extraction methods and selected doses to avoid mortality, 00-300 mg/ kg/ day for 20 consecutive days. The current study aimed to evaluate the possible side effects of ingesting various CCT fruit extract doses on mouse stomachs and possible mechanisms.

Material and Methods

Aqueous extract preparation

The matured fruit of CCT was collected from local open farmland between Jeddah and M ecca. The entire fruit was thoroughly washed, dried with tissue paper, and ground to a powder. The powder (18 g) was then mixed in 600 ml of hot distilled water on a magnetic stirrer for 1 hour at room temperature and then left overnight. Next the solution was filtered through surgical gauze and then Whitman No.1 filter paper to obtain the aqueous extract which was used in the experiment. Extract preparation and the given doses (100, 200 and 300 mg) were determined according to previously published protocols (MUHSEN & ALALI 2010).

Experimental animals

All experimental procedures are approved by the Unit of Biomedical Ethics Research committee at King Abdulaziz University (KAU) and proceed with its guidelines (Reference No. 585-18). Thirty-five albino male mice of SWR strain aged eight weeks and weighing 35-40 grams were enrolled in this study. Mice were obtained from the house of an animal unit at King Fahad Medical Research Centre (KFMRC), KAU, Jeddah, Saudi Arabia. The animals were acclimatized to the laboratory environment for one week before experiment initiation. The mice were put in plastic cages (5 mice/ cage) and kept in controlled laboratory conditions, light: dark cycle (12:12 h), temperature (20±1ºC), and humidity (65%) and fed ad libitum with astandard diet containing 11.7% fat calories (20% soybean, 8% concentrated proteins, 50% wheat, 21% corn, and 1% vitamins & salts) and had free access to tap water.

Experimental design

Mice were randomly divided into 4 groups as follows: a control group (n =5) and the treated groups; group 2, group 3, and group 4 (10/each). The treated groups 2, 3, and 4, were administrated aqueous CCT fruit extract in doses of 100, 200 & 300 mg/kg of body weight/day via oral gavage for 20 consecutive days. The animals were kept under observation after dosing to check for any morphological symptoms or behavioral changes. The animals appeared normal in behavior and no interesting symptoms were observed. The weight of all animals was measured weekly till the end of the experiment, without any animals dying. All blood samples were collected over a period using the retro-orbital vein method on EDTA - or without. At the end of the experiment, the animals were anesthetized using Isoflurane inhalation. Their blood was collected from the jugular vein, and the stomachs were collected and weighed using the standard protocol (ANTAL et al. 2007; VALENTIM et al. 2008).

Hematological data

Complete blood count (CBC) was measured by an automated analyzer for the determined number of each cell type. Neutrophil, lymphocyte, and monocyte indexes were calculated (NFAMBI et al. 2015).

Determination of pro-inflammatory and anti-inflammatory cytokines

Serum samples were obtained by centrifuging (2000 rpm for 5 min) the blood samples, aliquoting, and then storing them at -20ºC till used. The anti-inflammatory cytokines (IL-10) & pro-inflammatory (IL-8 & IL-6) concentration profile was estimated in all serum samples using commercially available ELISA kits, according to the kit instructions.

Histological data analysis

Immediately after blood sampling, general anatomy was performed and the stomach was isolated from all animal groups. The collected stomachs were dissected along the greater curvature, washed with nor-
mal saline to eradicate stomach remnants and clots of blood and then inspected via a 10x magnifier lens for ulcers. Midline strips along the stomachs’ lesser curvature were fixed in 10% formalin neutral, buffered, processed, & embedded in paraffin. 4 µm sections were dissected and stained with hematoxylin and eosin (H&E) according to the standard protocol (CARDIFF et al. 2014). The normal histological procedure was applied on the lining mucosa to study the possible histopathological alterations of the gastric mucosa cells.

Statistical analysis

Data were analyzed by IBM SPSS Statistical program for Windows, version 23 (IBM SPSS, IBM Corp., Armonk, N.Y., USA). Data expressed as mean ± standard deviation (x±SD). Percentage changes of total body weight were calculated as group weight minus control weight divided by control weight then multiplied by 100 (%weight - C.weight/C.weight x100). Differences between the groups were made using the One Way ANOVA test followed by the least significant test (LSD) for normally distributed parameters. * p-value <0.05, **p-value<0.01 was significant and *** p-value<0.001 was highly significant.

Results

Animal weights

The animals total body weights were recorded weekly. Although there were fluctuations in weight in some animals, average weights were increasing in all the experimental groups as shown below due to an increase in age. Total body weight was significantly decreased in groups 2 and 3 versus the control group on the 1st day (p<0.05), 8th day (p<0.05), 15th day (p<0.05) and 20th day (p<0.05). Meanwhile there were insignificant changes regarding body weight between group 4 and the control group on the 1st day (p<0.05), 8th day (p<0.05), 15th day (p<0.05), and 20th day (p<0.05). While the percentage of the change in the body weight was detectably decreased in groups 2, 3, and 4 versus the control group at different durations (Figs 1 and 2).

Hematological analysis

Blood samples were being collected from all animals for CBC. In group 4, total WBC counts were significantly higher than in any other group. However, the WBC differential counts were highly significant (p<0.001), elevated for neutrophils, lymphocytes, and monocytes, and significantly increased for basophils (p<0.05). The animals in both groups 2 and 3 showed a total and differential WBC count comparable to the control group. There were insignificant changes to any CBC measured parameters between groups 2 and 3 compared to the control group. Neutrophil, lymphocytes, and monocytes indexes as calculated (under material and methods) were significantly (p<0.001) high in group 4. The leucocytes index was presented as the average of each cell type. The lymphocyte index value was the highest followed by monocytes and the neutrophil index, especially in the animals of group 4 (Fig. 3).

![Graph showing body weight changes](image)

Fig. 1. Aqueous CCT extract effects. The diagram shows the effect of the aqueous extract of CCT fruit pulp on the bodyweight of albino mice at different durations (strucks indicate if the values were significant *, more significant **, or highly significant***). The bodyweight of each group is presented as average ± standard deviation (x±SD).
MCHC were significantly (p<0.05) decreased in the animals of group 4, lower than the control group (Figs 4, 5 and 6).

Determination of pro-inflammatory/anti-inflammatory cytokines profile

To study CCT extract effects on the cytokine’s serum levels, both pro-inflammatory (IL-8 & IL-6) and anti-inflammatory (IL-10) cytokines were measured (Fig. 7). At the experimental end, the serum levels of IL-8 were significantly and exponentially elevated from group 3 (p<0.05) to group 4 (p<0.001) in comparison to the control group, while the second pro-inflammatory cytokine (IL-6) level was significantly increased in only group 4 in comparison to the control (p<0.05) group. Meanwhile, the anti-inflammatory cytokine (IL-10) level was exponentially decreased in groups 2, 3, and group 4 serum levels. This threefold decline between the control and group 4 was highly significant (p<0.001).
Fig. 4. Aqueous CCT extract effects. The diagram shows the effect of aqueous CCT fruit pulp extract on RBC and WBC counts in albino mice. The presented values are the average ± standard deviation (x±SD) of the calculated numbers of the WBC and RBC /each animal group, (***) indicate that the values were highly significant.

Fig. 5. Aqueous CCT extract effects. The diagram shows the effect of aqueous CCT fruit pulp extract on platelet count in albino mice. The presented values are the average ± standard deviation (x±SD) of the platelet count /each animal group.

Fig. 6. Aqueous CCT extract effects. The diagram shows the effect of aqueous CCT fruit pulp extract on the concentration of Hemoglobin, Hematocrit, MCH, MCV, MCHC, RDW, and MPV in mice. The presented values are the average ± standard deviation (x±SD) of the hemoglobin (g/dl), hematocrit (%), MHC (pg), MCV (fl), MCHC (pg), RDW (%), and MPV (fl) count /each animal group, (***) indicate that the values were significant.
Macroscopic examination of the stomachs did not show any noticeable morphological changes in the treated animals in comparison to those of the control group. The histological examination of the control group tissues showed normal stomach wall layers, mucosa, submucosa muscle layers, and outer connective tissue layers. Higher magnification revealed the intact surface epithelium which secretes a protective mucous. Both HCL-secreting oxyntic cells and Chief peptic cells were in normal density population and integrity with no features of hypertrophy or degeneration (Fig. 8).

Histological changes were observed in the animals that received 100 mg/kg of fruit extract. A slight superficial ulceration was observed, with a marked enlargement of HCl-secreting cells, while the lower mucosa showed prominent peptic cells secreting gastric enzyme pepsin (Fig. 9). The animals in group 2 who

Fig. 7. Aqueous CCT extract effects. The diagram shows the effect of aqueous CCT fruit pulp extract on serum levels of interleukin (IL-8), (IL-6), and (IL-10) in albino mice. The presented values are the average ± standard deviation (X±SD) of the circulating concentration of interleukin-8 (IL-8), interleukin-6 (IL-6), and interleukin-10 (IL-10) in each animal group, the asterisks indicate that a value was significant.

Fig. 8. Sections from the control group’s stomachs (fundic region) stained by H&E. Low power ×40 (A): all stomach wall layers’ mucosa (MU) with upper and lower layers (double head arrows), submucosa (SM), muscle layer (ML). Upper mucosa ×400 (B) showing intact normal surface epithelium (black arrows) normal population of HCL-secreting cells (oxyntic cells) with a acidophilic pink cytoplasm and rounded active nuclei (black arrow). Lower mucosa ×400 (C) showing peptic cells (dark blue stained basophilic cells (dotted arrow)).

Fig. 9. Sections from group 2’s stomachs (100 mg/kg) (fundic region) stained by H&E to show the effects of aqueous CCT extract on stomach mucosa. Low power ×40 (A) showing surface ulceration (black arrows) and deep mucosa degeneration and increase of HCl-secreting cells. Upper mucosa ×400 (B), showing superficial slight surface epithelium ulceration (black arrows) and atrophy of mucous neck cells. An increased population of HCL-secreting cells (oxyntic cells) with dark stained or degenerated cytoplasms and rounded active nuclei (black arrow). Lower mucosa ×400 (C), showing atrophy of peptic cells (dark blue stained basophilic cells (dotted arrow) and enlarged oxyntic cells (black arrows)). Submucosa shows congested vessels (star).
received 200 mg/kg showed an increase in the histological features of superficial ulceration with slight atrophy of the glandular cells (Fig. 10). Most histopathological changes were observed in group 4 which received 300 mg/kg of fruit extract. Frequent patches of ulceration were commonly observed, glandular epithelium naps were staved and were shrunk and degenerative in appearance (Fig. 11).

Discussion

The goal of this research was to shed light on the side effects of various concentrations of aqueous CCT fruit extract on hematological parameters, the stomach tissue structure, and the immunological changes accompanied. The current results revealed that the total body weights of the mice in the different experimental groups were increased over different weeks compared to the 1st week which could be contributed to an increase in animal age. Meanwhile, the percentage changes of the total body weight of the different experimental groups decreased compared to the control. The weights of the 2nd and 3rd groups were significantly lower than the control group which might be due to the toxic effects of CCT on the gastrointestinal tract as revealed by microscopic examination of the stomach tissues. The first sign was the change of the experimental animal’s body masses. Body mass was decreased in groups 2, 3, and 4 due to the unhealthy status of animals which was directly affected by the extract. We believe that severe damage may have occurred at the physiological level which induces a kidney or liver function disorder. While the body mass of group 4 increased with the highest dose, this may be due to the subjects retaining too much water.

Generally, the hematological pictures reflect the immune response of the host to external agents. Differential leukocytes such as lymphocytes and neutrophils are produced by erythrocyte tissue and are responsible for the induction of the immune response with monocytes (JANEWAY et al. 1999). The results of the complete blood count showed that the animals in group 4, which received a higher the dose of aqueous CCT fruit extract, had significantly higher total WBC and differential leukocyte counts; while hemoglobin and M CHC were significantly lower than in the control group. The observed increase in WBC and differential leukocytes could have been due to increased inflammation as proven by our histological results of the stomach and a significant increase of pro-inflammatory circulating signals (IL-8 and IL-6). The decline in hemoglobin and M CHC could be explained by decreased iron absorption due to the damage to the stomach mucosa as the stomach is responsible for the ionization of iron. In this respect, (SHAH et al. 1989) mentioned that CCT had significant hematological dysfunction effects. Meanwhile, (ELGERWI et al. 2013) reported a significant increase
in RBC count, hemoglobin concentration, and hematoctrit after CCT administration in albino rats. The authors explained these effects as a CCT dehydrating effect as the animals used in this experiment had diarrhea. Also, there was a significant elevation in the total WBC and differential leukocyte counts. These observed results were similar to previous studies performed elsewhere (ELAWAD et al. 1984 and ATOLE et al. 2009).

The phytochemical analysis of CCT fruit demonstrates that, in addition to alkaloids, tannins, saponins, flavonoids, terpenoids, phenolic, anthranol, steroids, J, L, caffeic acid, Cucurbitacin A, B, C, D, E (α-elaterin), and cardiac glycoloids, it contains amino acids, proteins, lipids, and carbohydrate nutrients (RAHIM et al. 2012; MIAO et al. 2012 and AL-SNAFI 2016). A dose-dependent observed increase in WBC and differential leukocyte counts could be also attributed to these micronutrients in the CCT fruit extract. Extracted Cucurbitacin E glucoside escalating inhibits the testosterone-induced experimental BPH (Benign prostatic hyperplasia) in mice via, at least partly, its antiproliferative, antioxidant, anti-inflammatory, and antifibrotic effects (BASHA et al. 2019). The Cucurbitacin E glucoside seems to exhibit anti-inflammatory activity as it decreased cyclooxygenase-2 and interleukin-1β protein expression in prostatic tissues (BASHA et al. 2019). While, synthesized Cucurbitacin E glucoside (1) and its derivatives (2 and 3) inhibited the elevation of proinflammatory cytokines (TNF-α, IL-6, and IL-23) in the livers of t-BHP-treated rat models (HUSSEIN et al. 2017).

These compounds are fundamental for the development and maturation of the immune system, specifically the cellular components of hemopoiesis (MAGGINI et al. 2007). Neutrophils are not only one of the main components of innate immunity but are considered a Cinderella of the innate immune system (KUMAR & SHARMA 2010). They have always been considered tissue-destructive cells responsible for inflammatory tissue damage occurring during acute responses (KUMAR & SHARMA 2010). They also contribute to the clearance of foreign bodies by recognition and migration to the foreign body, phagocytosis, and destroying these foreign agents (JANEWAY et al. 1999). Our depicted results showed a dose-dependent increment in the neutrophils index which may indicate an acute activation and migration. It is important to note that this is the first reported link of the neutrophil activation index with CCT fruit extract consumption. The significant elevation of both the lymphocytes and monocytes indexes also supports the above finding. The vigorous inflammatory signals elicited by CCT fruit extract could therefore be used when the immune system is immunocompromised to recovery and improve the cell-mediated immune response as this extract potentiates immune responses (SHOKRIADEH et al. 2013). They do not exclude the possibility that some CCT fruit extract metabolites were absorbed and transported from the treated mice’s gastrointestinal region into their livers, where Kupffer cells (the liver is the main source of monocytes) are nourished (JANEWAY et al. 1999). This may explain and substantiate the higher monocyte index in the treated groups especially group 4, which could be reconsidered a defense mechanism exerted by the animals.

Pro-inflammatory cytokines contain Th1-type cytokines (interferon (IFN) -γ and tumor necrosis factor (TNF) -α) and others [interleukin (IL)-1β and IL-6], whereas anti-inflammatory cytokines are Th2-type cytokines [IL-4 and IL-10] and others [transforming growth factor (TGF)-β1] (WILCZYŃSKI 2005). The results of this study revealed that oral administration of aqueous CCT fruit extract induced a significant elevation in the serum levels of IL-6 in groups 3 and 4 which received 200 and 300 mg/kg of body weight/day and in IL-6 in group 4 only. On the other hand, IL-10 was significantly decreased in all the treated groups. In a similar line, it was reported that the gastric mucosal levels of IL-6 increased in H. pylori-associated gastritis (SUGIMOTO et al. 2010) and H. pylori-positive individuals with gastric carcinoma (MEJIAS-LUQUE et al. 2008). Also, a high expression of IL-8 was shown in gastric mucosa infected with H. pylori (KUSUGAMI et al. 1997). IL-8 leads to chemotaxis and inflammatory cell activation in gastric mucosa infected with H. pylori (NAITO et al. 2010). Meanwhile, IL-10 downregulates cell-mediated immune actions and cytotoxic inflammatory actions (KIM et al. 2012). Interleukin-10 suppresses pro-inflammatory cytokines (TNF-α & IL-6) released via human macrophage/monocyte in action for polymethyl methacrylate (PMMA, spherical 1-10 μm), the challenge of a particle in vitro (TRINDADE et al. 2001). However, others reported that aqueous CCT fruit extract (4 mg/kg) resulted in an anti-inflammatory action upon using a carrageenan-made edema of paw assay in rats (MARZOUK et al., 2010). In vivo, the anti-inflammatory actions of various components of CCT extract were found utilizing a carrageenan-made edema of paw assay in albino rats (ALY & NADDAF 2006). A iso using a carrageenan-made edema of paw assay in rats, research about CCT crude extract from immature fruits from South of Tunisia revealed an anti-inflammatory action (MARZOUK et al. 2011). A previous study reported that an oral intake of 50 mg/kg of CCT extract for 6 weeks resulted in anti-inflammatory action by decreasing both IL-6 and TNF-α levels, while not affecting the IL-10 steady-state in obese mice (SANADGOL et al. 2011).

The results of this study revealed that the macroscopic examination of the stomachs in situ did not show any morphological changes in the treated animals in different treated groups compared with control mice. Microscopic examination however revealed that CCT resulted in the superficial focal loss of the
surface mucous protective epithelium, the atrophy of peptic cells secreting enzymes, and a thickening of sum mucosal connective tissues. The submucosa showed vascular congestion and inflammatory cells in the treated animals which received 300 mg/kg of body weight/day. Surface degeneration was more frequent and deep in the high dose group (group 4), with marked atrophy of both oxyntic and peptic cells. The severity of damage to the stomach mucosa reported in this study was positively proportional with the increasing CCT doses, this may indicate that the toxicity was due to toxic components such as saponin and alkaloids that lead to toxicity according to the dose used. The detected lesions of the stomach presented in this study point out the direct local irritant action of CCT extract on the mucosa. CCT toxicity at high doses was reported in human cases and in experimental animals (BAKHiet & ADam 1995; AL-YAHAYA et al. 2000; BARTH et al. 2002), but the fruit extract also has significant anti-proliferative potential against MCF-7 and AGS cell lines of gastric adenocarcinoma and breast cancer. These cytotoxicity effects were dose-dependent and probably induced by apoptosis (REZAI et al. 2018). Systemic CCT use causes adverse side effects such as acute rectorrhagia, abortifacient, liver intoxication, cardiac-suppression, and vomiting (KUMAR et al. 2008; AKHZARI et al. 2015; ALHAWITI 2018). In 1989, 3 cases of acute toxic colitis after admission of CCT were recorded by (GOLDFAIN et al. 1989). The main symptom was dysenteric diarrhea. Colonoscopies revealed hyperemia and congestion with abundant exudates of the mucosa that faded within 14 days after the ceasing CCT administration (GOLDFAIN et al. 1989). CONTRERAS et al. (1996) recorded that poisoning by colocynth is a rare cause of acute diarrhea. KHAN et al. (2003) observed that five cases of toxicity caused by colocynth intake in Saudi Arabia for a period of more than two years resulted in acute severe bloody diarrhea. Four cases of colocynth intoxication were symptomatized with acute rectorrhagia after mucosal diarrhea associated with tenesmus, that progressed to rectorrhagia and bloody diarrhea in 3 to 4 hours. Colonoscopies showed ulcers on the mucosa that had completely disappeared by the next colonoscopy 14 days later (JAVADZADEH et al. 2013).

The membranolytic actions of different CCT constituents were blamed for the destruction of the intestine due to CCT’s cucurbitacin glycoside content (JAVADZADEH et al. 2013). The fruit extract of CCT had teratogenic effects if taken in early pregnancy in rats (ELGERWI et al. 2013). In a study where rabbits administered with 100 or 200 mg/kg of body weight/day of CCT seed or extract for 30 days, all rabbits treated with 200 mg/kg of body weight/day of extract died. The rabbits that were administrated 100 mg/kg of body weight/day of extract showed massive damage to their kidneys, liver, and small intestine (SHAFAEI et al. 2012). A single daily dose of CCT ethanol extract (50, 100, 200, and 400 mg/kg of body weight) given intraperitoneally, for 14 days, induced hepatocyte necrosis and liver fibrosis, in a dose-dependent pattern (DEHGHAN & PANJEH 2006). Meanwhile, ICKERT (1980) observed that extract from dried CCT fruits had no toxicological action in Wister rats when administered subcutaneously for four weeks and advised the administration of the extract as a cathartic purgative.

The proper therapeutic dose of CCT fruit varied from 0.6 to 1.75 grams/day and 0.1 to 0.4 grams/day according to Traditional Iranian Medicine (TIM) and modern phytotherapy. CCT seed must be given at 120 to 300 mg/day (maximum: 600 mg), and root powder at 0.2 to 0.4 grams/day. If the fruit is given with its correctives as a gum of Arabic, side effects are decreased and larger doses are allowed. The toxic dosage of CCT reported was 600–1000 mg and may lead to hematochezia, abdominal colic, diarrhea, nephrosis of the kidneys, and frequent vomiting. Fatal doses (≥2 g) led to paralysis, fits, and death due to the collapse of the circulation system (RAHIMI, et al. 2012). However, SACHIN & ARCHANA (2009) examined (aqueous and alcoholic extracts) of CCT for their anti-ulcer actions in a pylorus ligation induced ulcer model in male Wistar rats. They reported that aqueous and alcoholic extracts had significant anti-ulcer actions due to flavonoids, tannins, alkaloids, saponins glycosides, and phenolic substances. These active compounds enhance bicarbonate, mucus, and prostaglandin secretions and antagonized the deteriorating actions of reactive oxidants in the lumen of the gastrointestinal tract.

**Conclusion**

Traditional, natural herbal medicine would offer an advantage over conventional treatments but with the appropriate dose and duration. Current results provide biological evidence for the histological and immunological activities of aqueous CCT extract in experimental mice and may suggest and support the limitation of the traditional use of this plant. A point of interest is the side effects accompanied by a higher dose. At higher doses, CCT is suspected to induce injury to the mucosal layers of the stomach (300 mg/kg of body weight/day) and shouldn’t be taken for more than 20 consecutive days to avoid possible toxicity, through immunological inflammatory signals, which is congruent with TIM’s recommendation. Our study’s goal was to investigate the long-term actions and toxicity of CCT to prevent unexpected life-threatening complications. Our findings may suggest that the identification and characterization of different components of CCT fruit extract followed by their conversion to synthesized medicines will not only be helpful for determining an accurate estimation of the harmful effects of CCT but the permitted and toxic dosages as well.
Funding

This research received no external funding.

Author Contributions

Research concept and design: M.O.A.; Collection and/or assembly of data: R.S.B.; Data analysis and interpretation: R.S.B., E.M.R.; Writing the article: R.S.B.; Critical revision of the article: M.O.A., E.M.R.; Final approval of article: M.O.A., E.M.R.

Conflict of Interest

The authors declare no conflict of interest.

References


