

Full Length Research Paper

***Citrullus colocynthis* as a medicinal or poisonous plant: A revised fact**

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***Citrullus colocynthis* is a conventional herbal medicine with wide therapeutic applications, but also with some toxic side effects. The aim of the present study was to investigate the toxic effects of *C. colocynthis*. Thirty male rabbits were randomly divided into 5 groups. Test animals were treated with 100 or 200 mg/kg/day of either pulp or seed extract. One month later, surviving animals were sacrificed and specimens of small intestine, kidney, and liver were prepared for morphological evaluation. No animals treated with 200 mg/kg/day of pulp extract survived. Animals treated with 100 mg/kg/day of pulp extract displayed sever lesions in the small intestine, kidney, and liver. Interestingly, animals treated with either 100 or 200 mg/kg/day of seed extract displayed only minor intestinal insult. In contrast to seed extract, pulp extract of *C. colocynthis* can be fatal to rabbits. Therefore, seed extract may be the preferred route for therapeutic application.**

Key words: *Citrullus colocynthis*, fatal, rabbits, toxicity.

INTRODUCTION

Citrullus colocynthis, a member of the Cucurbitaceae family (Ziyyat et al., 1997), is known as Bitter Apple in the English language (Patrick et al., 1960), Hindal in Arabic (Al-Ghaithi et al., 2004), and Abujahl watermelon in Persian. The extract of the fruit is called colocynthine due to its extreme bitter favor. Known compounds found in *C. colocynthis* include glycosides (Estrols classified to groups A, B, K, L, and E curcurbitacins), alkaloids, and flavonoids (Abdel-Hassan et al., 2000; Adam et al., 2001; Chen et al., 2005; Delazar et al., 2006; Yoshikawa et al., 2007). However, unknown compounds may also contribute to its therapeutic reputation. The curcurbitacins

(highly oxygenated tetracyclic glycosides) have a broad range of applications due to their wide spectrum of biological activities (Tannin-Spitz et al., 2007). They are found mainly in plants belonging to the Cucurbitaceae family, but have also been found in several other families of the plant kingdom (Tannin-Spitz et al., 2007). In folk medicine, *C. colocynthis* is widely used by rural inhabitants as a purgative, anti-diabetic, anti-neoplastic, anti-rheumatic, and anti-allergic agent (Tannin-Spitz et al., 2007). They are found mainly in plants belonging to the Cucurbitaceae family, but have also been found in several other families of the plant kingdom (Tannin-Spitz et al., 2007). Although, the whole fruit is often used for the treatment of the aforementioned diseases, but some particular parts of the fruit are also used for specific purposes. One of such example is the traditional application of the dried pulp and seed extract of *C. colocynthis* for the treatment of constipation and diabetes

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(Kumar et al., 2008; Nmila et al., 2000). Despite the wide therapeutic potentials attributed to this fruit, the development of complications during treatment is not uncommon. Some adverse effects include bloody diarrhea and toxic colitis, and are responsible for *C. colocynthis* classification as a toxic plant (Grover et al., 2002; Nmila et al., 2000), where it is considered among the top 10 (Jouad et al., 2001). Interestingly, most of the studies on the toxic effects of this medicinal plant are performed on the whole fruit extract (colocynthine). Since different parts of the fruit, such as the pulp or the seed, are claimed to exert different therapeutic effects, it is reasonable to suggest that a particular therapeutic benefit, or toxic side effect could be attributed to one part of the fruit, and not another. Therefore, the aim of this study was to investigate the toxic effects of either pulp or seed extract of *C. colocynthis* in rabbits.

MATERIALS AND METHODS

Extraction

C. colocynthis fruit was prepared by separating the seeds from the pulp, and then both were rind and milled mechanically into fine particles. The seed powder was defatted by n-hexan. Then, defatted seeds and pulp were extracted using methanol 70%. This extraction process was repeated three times and dried at low temperature (< 40°C) under 100 mmHg pressure in a rotary evaporator. The dried extracts were stored in zero temperature until application. For administration, 100 or 200 mg of dried extract was reconstituted in 1 ml of distilled water in order to make a 10 or 20% solutions (w/v). In all the experiments, the same amounts were applied (Harborne, 1984).

Phytochemical analysis

Sterols investigation

Determination of sterols was based on the Liebermann-Burchard reaction (Harborne, 1984). The reaction was conducted at 37°C for 30 min. Sterols give a specific blue-greenish colour, evaluated at 430 nm. Observation of a blue-green color after the addition of anhydride acetic to the concentrated sulfuric acid indicates the presence of sterol (Cook, 1961).

Flavonoids investigation

Cyanidine test was applied in this regard. Extract (2 ml) was heated and then hydrochloric acid (HCl) and magnesium (Mg) were added. The red or orange color change after addition of HCl and Mg ribbon in alcoholic milieu indicates the presence of flavonoids (Karumi and Ogugbuaja, 2004).

Saponin investigation

Saponin was investigated as previously described (Karumi and Ogugbuaja, 2004). Briefly, 5 ml of ether, methanol or water extract was vigorously shaken with 10 ml distilled water for 2 min. Shaking aqueous extract leads to the formation of permanent foam (minimum time: 15 min). Formation of emulsion in the case of olive

oil addition in the main solution indicates the presence of saponin.

Alkaloid investigation

Extract (0.2 ml) was stirred and placed in 1% aqueous HCl on a steam bath. Then 1 ml of the filtrate was treated with Mayer's reagent, while another portion was similarly treated with Dragendorff reagent (Karumi and Ogugbuaja, 2004). Turbidity and deposition of alkaloids in acidic solution (1% HCl) facilitated by the application of Mayer or Dragendorff detergent helped in the identification of alkaloid compounds (Kumar et al., 2008).

Animals

This study was conducted using 3 months old New Zealand white male rabbits, weighing approximately 3 kg. Thirty rabbits were randomly assigned into 5 groups: a control group receiving regular rabbit chow for 4 weeks, and four test groups receiving either seed or pulp extract of *C. colocynthis* at either 100 or 200 mg/kg/day, in addition to the normal rabbit diet. At the end of the first month, surviving animals were sacrificed after anesthesia with intravenous injection of pentobarbital sodium. Specimens from liver, intestine, and kidney were fixed in 10% buffered formalin for at least 2 days. Samples were stained with Hematoxylin and Eosin and periodic acid-Schiff (PAS) staining after thin sectioning, and were evaluated for histopathologic and morphological changes using light microscopy. All animal experiments were approved by the local animal ethics committee of Tabriz University of Medical Sciences, Tabriz, Iran.

Image analysis

Morphometric analysis of intestinal cells was performed by measuring changes in length and height of microvilli between control and test groups. Image analysis was performed using image analysis software (ImageJ).

Statistical analysis

Statistical evaluation was performed using either Microsoft Excel or Prism GraphPad software packages. Results were expressed as mean \pm standard error (SE) and were analyzed by one way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. All tests were done at the 5% level of significance and statistical significance was defined as $P < 0.05$.

RESULTS

Animals in test groups receiving pulp extract of *C. colocynthis* were afflicted with watery diarrhea and anorexia. All test animals treated with pulp extract at 200 mg/kg/day died after the first dose. Additionally, 50% of animals treated with pulp extract at 100 mg/kg/day did not survive to the end of the study. Intestinal sections of surviving animals treated with pulp extract at 100 mg/kg/day showed disappearance of the absorptive microvilli, and a significant reduction in height and length of absorptive cells ($P < 0.0001$) (Figure 1d). In these samples, nuclei of absorptive intestinal cells were more

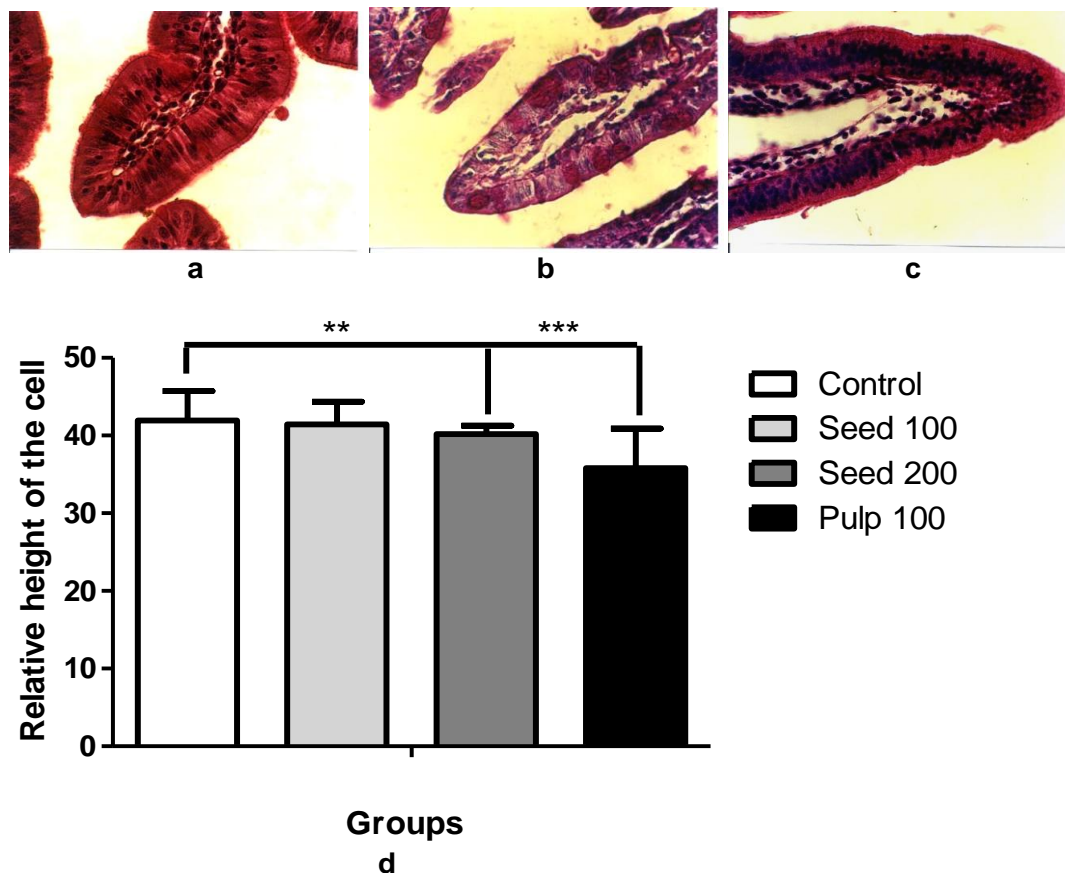


Figure 1. Photomicrographs of intestinal sections and comparison of the relative height of intestinal cells. (a) Photomicrograph of intestinal section of control rabbits (H&E $\times 660$). (b) Photomicrograph of intestinal section of rabbits after administration of pulp extract of *C. colocynthis* (100 mg/kg/day; PAS staining $\times 660$). (c) Photomicrograph of intestinal section of rabbits after administration of seed extract of *C. colocynthis* (100 mg/kg/day; H&E staining $\times 660$). (d) Relative height of absorptive cells of intestine.

condense than in the control group (Figure 1). Furthermore, the basement membrane of intestinal cells was discontinuous (demonstrated by PAS staining), and hemorrhagic segments were also observed in the gut lamina propria. Liver sections from these animals demonstrated nuclear pyknosis, dilatation of sinusoids and bile canaliculi (Figure 2a to f), while renal damage included loss of microvilli from the epithelial cells lining the proximal tubules, glomerular bleeding, and congestion (Figure 2a to f).

In general, the toxic side effects of pulp extract at 100 mg/kg/day dramatically exceeded the toxic side effects of seed extract, and all animals treated with seed extract survived the duration of the study. In the animals treated with seed extract at either dose, intestinal damage was concomitant with loss of microvilli and pyknosis of nuclei in a limited number of cells. With respect to the height of absorptive cells, a significant decrease was only observed in animals treated with seed extract at 200 mg/kg/day (Figure 1d; $P < 0.001$). No hepatic or renal injury was observed in seed-treated animals.

DISCUSSION

Our data demonstrates that, within *C. colocynthis* extract, pulp extract constitutes the main toxic component. *C. colocynthis* has been demonstrated to induce diarrhea and intestinal damage in treated animals (Al-Faraj, 1995), and colitis in humans (Jouad et al., 2001). Moreover, previous studies in mice have revealed that saponins, a component of colocynthine, can induce similar enterohepatorenal effects as have been observed in our study. Furthermore, the basement membrane of intestinal cells was discontinuous (demonstrated by PAS staining), and the main extract is likely to be saponin.

The main pathophysiological mechanism underlying these toxic effects is likely to be the membranolytic activity of saponin present in the *C. colocynthis* pulp extract. Saponin acts on the plasma membrane via interaction with cholesterol and phospholipids (George et al., 2002; Kalinin, 2000). Micelle formation disrupts the structure of the cell membrane and affects the function of plasma membrane proteins including the Na^+/K^+ ATPase

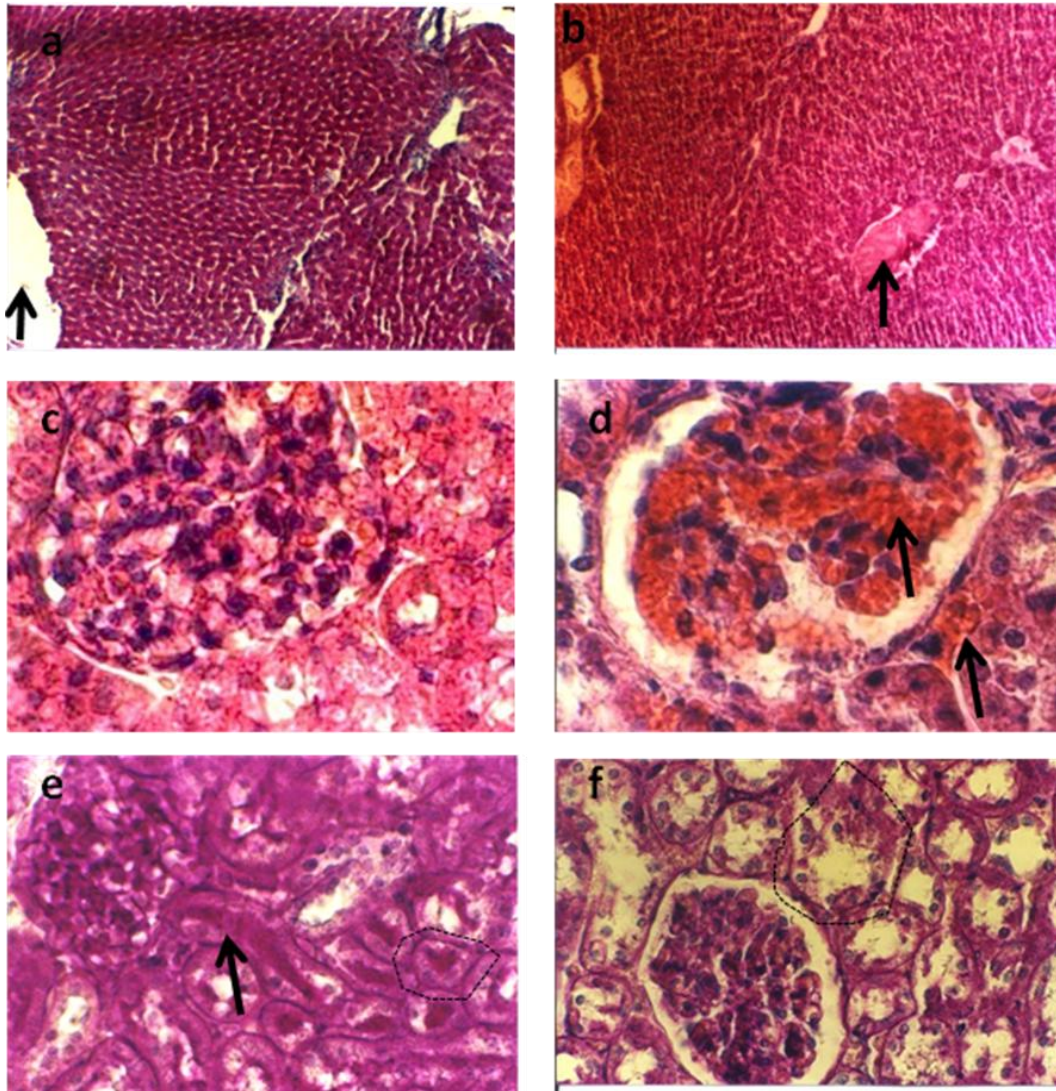


Figure 2. Photomicrographs of the liver and kidney. (a) Photomicrograph of the liver of control rabbits (Arrow shows central vein; H&E staining, original size $\times 165$). (b) Photomicrograph of the liver after administration of pulp extract of *C. colocynthis* (100 mg/kg/day) (arrow shows central vein with in experimental group congestion; H&E staining, original size $\times 165$). (c) Photomicrograph of renal cortex of control rabbits (H&E staining $\times 660$). (d) Photomicrograph of renal cortex of rabbit after reception of pulp extract of *C. colocynthis* (100 mg/kg/day) (arrows indicate red blood cells in renal corpuscle and adjacent structures, H&E staining $\times 660$). (e) Photomicrograph of renal cortex of control rabbits (PAS positive microvilli is demonstrated by arrow and intact is outlined with dashed line $\times 660$). (f) Photomicrograph of renal cortex of rabbit after reception of pulp extract of *C. colocynthis* (100 mg/kg/day) disappeared microvilli and discontinued basement membrane (PAS staining $\times 660$).

pump, the major enzymatic ion transporter of the cell membrane (Abe et al., 1978; de Souza et al., 2004; George et al., 2002). This disruption of membrane protein function has been suggested as the main cause of the secondary biochemical responses induced by saponin (George et al., 2002). Alkaloids, glycosides, and other components of *C. colocynthis* pulp may have some possible synergistic or antagonistic effects in combination with saponin, which will need to be investigated in further studies. Intestinal epithelium has previously been

demonstrated to undergo some histological changes in response to colocynthine administration or its saponin component (Al-Faraj, 1995; Al-Yahya et al., 2000; Barth et al., 2002; Diwan et al., 2000). Intestinal epithelium exposed to saponin compounds display damage to the absorptive cells, especially to the microvilli cell surface (Gee et al., 2004). Moreover, saponin causes rapid membrane depolarization of the small intestinal mucosal cells, even at low concentrations (less than 50 $\mu\text{g/ml}$) (Gee et al., 1993). Hemorrhagic events in the renal cortex

and intestinal mucosa of the pulp extract-treated animals are consistent with previous studies on either colocythine or saponin compounds of pulp extract or rind form of *C. colocynthis* (Abdel-Hassan et al., 2000; Al-Faraj, 1995; Al-Yahya et al., 2000; Diwan et al., 2000). Furthermore, the membranolytic capacity of saponin could explain the disintegration of the endothelial layer of the capillaries (George et al., 2002), leading to the hemorrhagic events observed in this setting. In addition, cucurbitacins interact with the cytoskeletal protein Vimentin, found in microvilli, which may cause reduction in water absorption and nutrients by impaired transportation mechanisms (Duncan et al., 1996). Disrupted transport in intestinal cells can result in inappropriate water migration from the blood to the intestinal lumen, contributing to the occurrence of watery diarrhea. Such effects have not yet been observed by any known components of pulp extract.

For definitive attribution of the toxic effects to saponin, further investigations ruling out the involvement of other components found in pulp extract of *C. colocynthis* is needed. Such evaluations will also be needed to reveal beneficial therapeutic components, those that are safe and without significant side effects, as a remedy for the aforementioned diseases. In this regard, purgative effects of the pulp extract appear to be a pathologic sign, indicating toxicity rather than a true treatment. However, this event should not be considered as a negligible side effect of treatment, as some of the beneficial effects of the pulp extract may be related to its effects on the absorptive intestinal surface. As a possible mechanism, the observed hypoglycemic effects of pulp extract may be due to wounded intestine and injured renal proximal tubules and their subsequent reduced ability to regulate glucose transportation. In addition, impaired hepatic function and glucose metabolism may be a contributing mechanism for induced hypoglycemia. Hepatic toxicity induced by carbon tetrachloride has been shown not to be alleviated by *C. colocynthis*, but instead, it is enhanced by applying higher doses of pulp extract, in some instances (Barth et al., 2002).

Animal death after administration of evidently high doses of pulp extract of *C. colocynthis* is an interesting finding, consistent with its toxic reputation. Some possible explanations may be dehydration due to severe diarrhea, heart failure due to a cardio-stimulant effect, hepatorenal insults or hypoglycemia.

Our data demonstrates that the seed extract of *C. colocynthis* has no major toxic effects on the intestine, liver or kidney. While some minor intestinal changes were observed in our study, the overall estimation is that the seed extract of *C. colocynthis* is a safe candidate for the treatment of the earlier mentioned diseases. The seed extract of *C. colocynthis* may exert the same therapeutic effects, with less untoward complications, as the application of the whole fruit.

C. colocynthis has great promise as a therapeutic agent. However, it is a fatal agent for rabbits when

administered in the form of pulp extract. In contrast, its seed extract is safe, with only minor intestinal side effects, which may be negligible. Therefore, seed extract may be the preferred route for *C. colocynthis* application as a therapeutic agent; however, its efficacy needs to be investigated in future studies.

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