Original Article

The Protective Role of Starch on Modulating Toxic Effects of *Citrullus colocynthis* on Rat Liver and Intestine

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ABSTRACT

Background: Despite using *Citrullus colocynthis* on treatment of various diseases, serious gastrointestinal disorders like bleeding are reported. In Traditional Iranian Medicine (TIM), administering equal weights of starch with this plant is suggested to produce more tolerable preparations from it. Hence, we assessed histopathological changes in rat liver and intestine after using starch as corrective agent.

Methods: We designed three experiments in Veterinary Medicine School of Shahid Bahonar University in Kerman, Iran in 2016. The procedure was applied in 2016 for 15 days. In the first experiment, group No. 2 and 3 received single daily dose of alcoholic pulp extract of *C. colocynthis* at 300 and 600 mg/kg extract consecutively. In the second experiment, group No. 4 and 5 received 300 and 600 mg/kg extract plus the same amount of starch consecutively. In the third experiment, group No. 6 and 7 received extract at 300 and 600 mg/kg plus the three times weight of starch consecutively. The live rats were euthanized and their liver and intestine were removed for histopathology examination. The samples were stained with hematoxyline-eosin (H&E).

Results: Rats in all of the groups died from bleeding and diarrhea except for group No.6 that showed no symptoms seen in other rats. Microscopic examination of their intestine showed no histopathological lesions or other degenerative changes of the epithelium.

Conclusion: Clearly further works in modern phytotherapy will be required to delineate the role of starch in reducing *C. colocynthis* toxicity. Consumption of adequate weight of starch with the toxic dose of *C. colocynthis* make it safe for digestive system but could not prevent necrotic changes in the liver.

Keywords: *Citrullus Colocynthis*, Histopathology, Phytotherapy, Starch.

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INTRODUCTION

Citrullus colocynthis, is an ancient medicinal plant, widely used in Traditional Iranian Medicine (TIM) and distributed in the desert areas of the world, including the southwest (Khuzestan, Fars), southeast (Kerman, Bandar Abbas, Baluchistan), central (Yazd) and eastern parts (Lout desert) of Iran [1]. It is known by different names such as Kabast and Sharang [bitter thing], Khiar Talkh (bitter cucumber), Kharboze talkhak (bitter melon) [2,3].

The principle medicinal part of the plant is the fruit pulp used medicinally in treatment of various diseases because of its wide range of biological activities, such as antioxidant [4-6], antidiabetic [7], antilipidemic [8], insecticide [9] and antimicrobial [10] properties. Curcurbitacins A, B, C, D, E, I, J, K, and L, colocynthosides A, and B, flavonoids, alkaloids, fatty acids and essential oils were recorded as bioactive chemical constituents of the fruit [11-13].

Among mentioned applications, many investigations had been done on antidiabetic effect of pulp. For example Oryan and his coworkers in (2014) revealed that hydro-ethanol extract of Citrullus colocynthis reduced blood glucose in alloxan induced diabetic rats [14]. In another study treatment of diabetic rats with *C. colocynthis* pulp extract (300 mg kg-1 body weight) resulted in a significant decrease in plasma glucose, glycosylated Hb, and increased insulin levels. This significant increase in insulin may explain the increase in the activity of liver hexokinase, with concomitant decrease of glucose 6-phosphatase and fructose-1, 6-bisphosphatase [15]. Citrullus colocynthis pulp extract could also modulate the levels of factors

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related to lipid metabolism in serum and liver of diabetic rats with hyperlipidemia [4]. Regardless of its significant efficacy in treatment of some diseases, *C. colocynthis* was classified as a top 10 toxic herb because of adverse effects following use of the pulp [16].

There are some tips about using this plant in TIM that make it possible to prevent adverse events, for example, it is suggested to apply equal weight of starch with the fruit to make it more tolerable [2, 17]. However, this point in TIM has not been considered in modern phytotherapy yet. To demonstrate the role of starch in reducing toxic effects of *C. colocynthis*, we evaluated liver and intestine histopathological changes in rat after using starch as corrective agent with the fruit pulp for the first time.

MATERIALS AND METHODS

Preparation of the C.Colocynthis Pulp Extract

C. colocynthis fruits were obtained in 2016 from the local market in Kerman, Iran. The black seeds of *C. colocynthis* were separated manually from pulp of dried fruit and the pulp was grounded into powder completely. The powder was extracted by 1 L of hydro-ethanol mixture (20/80, v/v) for 8 h. This step was repeated for four times. The filtrate was pooled and concentrated under vacuum at a temperature not exceeding 50 °C. The alcoholic extract was stored at -70 °C until being used.

Animals

Twenty-eight female rat ~ (250) g body weight were selected for this study and randomly divided into seven groups (6 experimental and 1 control). Each group contained 4 rats. All animals were housed under constant temperature (20 °C) with a 12 h/12 h light/dark (L/D) schedule (lights on 7:00 am). Animals were allowed to adapt for 7 d before the first day of experiment. Food and drinking water were available *ad libitum*. In all of the experiments, *C. colocynthis* extract was given to rats orally via gavage.

Test Procedure

The experimental procedure was applied to 15 d and the first day of gavage was considered as day one. We designed three experiments, including 2 groups in each one (No.2-7). Group No.1 was considered as control group. Control rats were treated with normal saline via gavage. In the first experiment, group No.2 was treated with single daily dose (300 mg/kg) of alcoholic extract of *C. colocynthis* whilst group No.3 received 600 mg/kg extract. In the second experiment, group No.4 was treated with 300 mg/kg of extract plus the equal weight of starch (300 mg/kg) whilst group No.5 received 600 mg/kg extract plus the same weight of starch (600 mg/kg). In the third experiment, group No. 6 received extract 300 mg/kg plus the three times weight of starch (900 mg/kg) whilst group No.7 received 600 mg/kg extract plus the three times weight of starch (1800 mg/kg).

Sampling

The rats that were live until the last day of the experiment were euthanized on day 16 by ether and their liver and intestine were immediately removed for histopathology examination.

Histopathological Evaluation

The samples were fixed in 10% buffered formalin. Specimens were processed routinely and sections in 5-µm thickness were prepared and stained with hematoxyline-eosine (H&E). Prepared tissues were evaluated qualitatively (morphologic) using a light microscope.

Animal Ethics

This experiment was accomplished under the approval of the State Committee on Animal Ethics, Shahid Bahonar University of Kerman, Iran. The animals were treated and handled humanly in accordance with the standard principle of CCAC, 1984 [18].

RESULTS

Clinical Signs and Mortality

The Clinical signs and number of days that rats had been alive in each group are shown in Table 1. In the first experiment, all of the rat's in-group No.2 (300 mg/kg extract) showed decrease in heart rate as well as ulcer in hand and foot palms eventually died after 4 d of gavage. All of the rats in group No.3 (600 mg/kg extract) died after the first gavage and showed bleeding in their hand palms. In the second experiment, all of the rat's in-group No.4 (300 mg/kg extract plus the same amount starch) showed decrease in heart rate as well as ulcer in hand and foot palms eventually died after 4 d of gavage. In group No.5 (600 mg/kg extract plus the equal weight starch), rats lived longer but died after the 7 d of gavage and showed no bleeding in their palms and no changes in heart rate. In the third experiment, group No.6 which received extract 300 mg/kg plus the three times weight of starch (900 mg/kg) showed no symptoms seen in other rats such as bleeding, diarrhea and even they gained more weight and we euthanized them in day 16. All of the rats in the other group (No.7) died after day 4. Control rats in-group (No.1) showed no symptoms seen in other rats such as bleeding and diarrhea and we euthanized them in day 16.

Table 1. Number of days that rats had been alived and clinical signs, seen in each group after, consume different doses of *C. colocynthis* extract and starch via gavage.

Groups	*Alive days*	*Clinical signs*
Control	16	No symptoms such as bleeding and diarrhea
Group No.2 (300 mg/kg extract)	4	Decreasing in heart rate as well as ulcer in hand and foot palms
Group No.3 (600 mg/kg extract)	1	Bleeding in their hand palms
Group No.4 (300 mg/kg extract plus the same amount starch)	4	Decreasing in heart rate as well as ulcer in hand and foot palms
Group No.5 (600 mg/kg extract plus the equal weight starch)	7	Showed no bleeding in their palms and no changes in heart rate
Group No.6 (300 mg/kg plus the three times weight of starch (900 mg/kg)	16	No symptoms such as bleeding and diarrhea
Group No.7 (600 mg/kg extract plus the three times weight of starch (1800 mg/kg)	4	No symptoms such as bleeding and diarrhea

Histopathological Findings

Liver

Control rats had normal structure of liver (Fig. 1). The hepatocytes were arranged in single cell cords. The portal tracts contained bile duct, hepatic artery, and portal vein. Hepatocytes were polygonal with central nucleus and eosinophilic cytoplasm. In rats treated with *C. colocynthis*, 300 mg/kg BW as well as 900 mg/kg BW starch (group No.6), the hepatic cords were disorganized. Hepatocytes showed swelling and cytoplasmic vacuolar degeneration. Central veins were congested. Necrotic changes including pyknotic nuclei and increasing eosinophilic staining of cytoplasm were observed in some hepatocytes (Fig. 2).

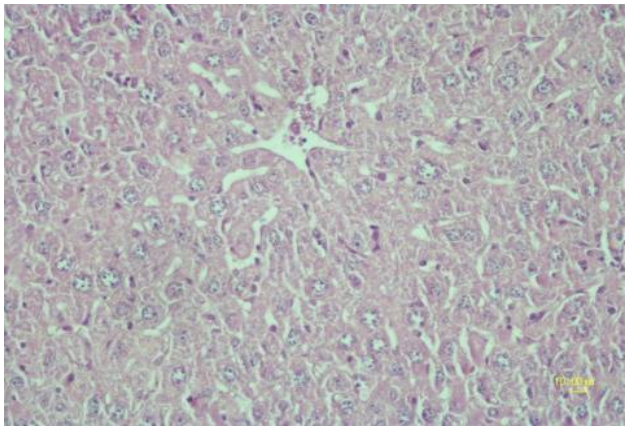

Figure 1. Normal structure of liver in control rats group [No. 1]. The hepatocytes are arranged as single cell cords around the central vein [HE, Bar=10 μm].

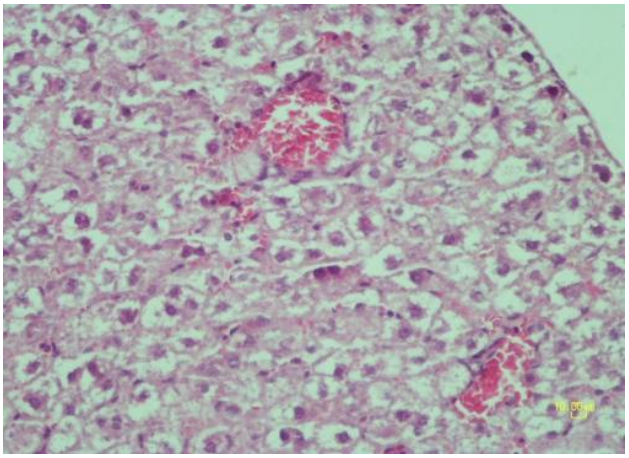

Figure 2. Disorganized hepatic architecture, congestion of central vein and vacuolar degeneration of hepatocytes in rats received *Citrullus colocynthis* 300 mg/kg BW as well as 900 mg/kg BW starch [group No. 6] [HE, Bar=10 μm].

Intestine

Control rats had normal structure of intestine. In rats, in-group (No.6) received *C. colocynthis* 300 mg/kg BW as well as 900 mg/kg BW starch, no histopathological lesions were observed in the intestine tracts. The mucosa and submucosa layers were normal, and there was no denudation or other deterioration of the epithelium in the examined specimens (Fig. 3).

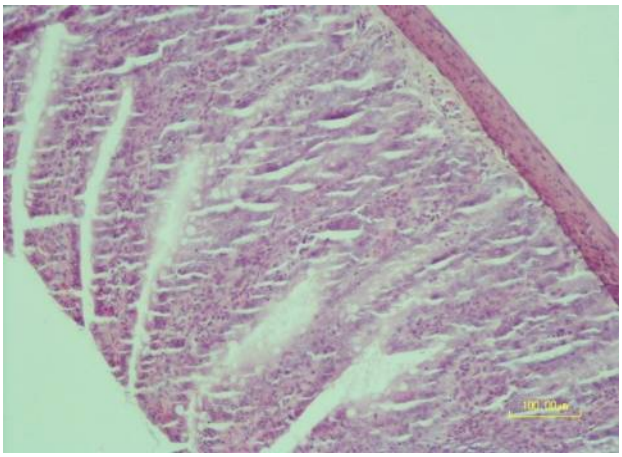

Figure 3. The photograph shows normal histology of intestine in control rats group [No. 1] and in rats received *Citrullus colocynthis* 300 mg/kg BW as well as 900 mg/kg BW starch group [No. 6] [HE, Bar=100 µm].

DISCUSSION

In our experiment, starch provided the wall around toxic materials in the extract and changed the release profile of the toxic agents. Therefore, no clinical signs and histopathological lesions in the rats that consumed extract with suitable dose of starch.

A number of biochemical studies recorded *C. colocynthis* fruit as a therapeutic plant. Biological activities of this part of the plant were due to presence of highly oxygenated tetracyclic glycosides called cucurbitacins. They are found mainly in plants belonging to the Cucurbitaceae family [19]. Some of pharmacologic properties of *C. colocynthis* mentioned in TIM were well confirmed by modern research [20]. Despite the wide therapeutic potentials attributed to the fruit in folk medicine, ethnomedicinal preparations from *C. colocynthis* pulp extract may not be completely safe as an oral remedy; and long-term administration and at high concentration should be avoided. For example, alcoholic extract of *C. colocynthis* in concentration above 100 mg/kg could have toxic effects on hepatocytes, which might induce hepatocyte necrosis and liver fibrosis [21]. This research and the other investigations raised a question that why this plant had been used in folk medicine with such adverse effects? The answer is that in TIM, it is proposed to administer equal weights of starch with *C. colocynthis* [2, 17]. Unfortunately, point mentioned in TIM such as using *C. colocynthis* with correctives does not consider in modern phytotherapy.

As the common complications about consuming the fruit are colitis, diarrhea and bleeding, therefore, the aim of this project was to confirm this hypothesis that starch could attenuate toxic capacity of this plant on intestine. In addition, the liver is a sensitive organ and many substances including toxins accumulate in hepatocytes and induce liver toxicity, therefore we evaluated toxicopathology of the liver and intestine used two doses of starch intake in rats.

All of the rats consumed the starch with the same weight of extract (group No.4 and 5) died with diarrhea and bleeding whilst in the rats which consumed extract with suitable starch (group No.6). No clinical signs were seen and they also lived longer and microscopic examination of their intestine showed no histopathological lesions and no denudation or other degenerative changes of the epithelium (Fig. 2). We can see that application of starch with extract maybe encapsulate the toxic ingredients in *C. colocynthis,* prevented diarrhea, and bleeding. In these days, encapsulation evokes interest of food scientists because it provides an effective method to avoid flavors evaporation and unwanted reactions during storage by coating them with protective carrier materials. Fully biodegradable, widely available, inexpensive, easily modified into derivatives with various properties and other significant benefits make starch to be considered as an excellent wall material for encapsulation over the years [22]. From this point of view, it is possible that in-group (No.6) starch provided the wall around toxic materials in the extract and changes the release profile of the poisonous agents. For this reason, we could not see any histological variations including degenerative changes of the epithelium in microscopic examination of rat intestine (Fig. 3).

In a study, rabbits were treated with 200 or 100 mg/kg/day of pulp without starch [23]. Application of the fruit pulp without additives caused mortality of 100% and severe lesions in the small intestine consecutively. This is consistent with our first experiment in which all of the rats in-group No.2 (300 mg/kg extract), group No.3 (600 mg/kg extract) showed bleeding, and ulcer in their hand palms and died in a few days. However, in our investigation, livers of rat's in-group No.6 showed hepatocytes swelling and degeneration (Fig. 2). Necrotic changes seen in the liver of this group is attributed to the toxic dose of extract (300 mg/kg) consistence with a study on rat liver, which showed that alcoholic extract of *C. colocynthis* in concentration above 100 mg/kg could have toxic

effects on hepatocytes, may induce necrosis and liver fibrosis [21]. In another investigation, the influence of *C. colocynthis* pulp extract administered orally was studied in the intestine of rabbits. All of the animals given 200 mg/kg pulps extract orally and 46% given 100 mg/kg pulp extract, died just after receiving extract because of hepatorenal injury. In histopathological examination of latter group, 100 mg/kg pulp extract caused microvilli destruction and penetration of lymphocytes into intestinal mucose [24]. In our study, although we used toxic dose of pulp extract (300 mg/kg/day), they had been alive for 15 d like the control group.

One of our problems during the experiment was rat's mortality that caused postmortem changes so we could only evaluate histopathology changes of few groups. Another problem was dose of starch recommended in TIM as the same as the fruit pulp but since we used extract instead of pulp powder, we decided to apply two doses [the same and three times weight] of starch. In our first experiment, all of the rats showed intoxication clinical signs (decreasing in heart rate as well as ulcer in hand and foot palms) and died before day 4. All of the rats in the second experiment (groups No.4 and 5) which respectively received 300 and 600 mg/kg extract and the same weight of starch died in the first week of the experiment.

CONCLUSION

Clearly further investigations in this area will be needed to delineate the role of starch in reducing *C. colocynthis* toxicity, but this study was approval of traditional Iranian medicine that application of starch with the *C. colocynthis* fruit could prevent intestine from degeneration and changes of the epithelium. In order to prevent the liver from damage it is better not to use toxic doses of this plant.

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