

Hematological, Biochemical and Cytotoxic Effect of Ethanolic Raw Extract of Egyptian *Citrullus colocynthis* in Sprague Dawley Rats

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Abstract

Background: The extract of *Citrullus colocynthis* is considered as a source of drug or adjuvant to other therapeutics agents as the pharmacological activities of *Citrullus colocynthis* studied previously for this plant as antidiarrheal, antioxidant, antimicrobial, anti-diabetic and antihyperlipidemic properties.

Aims: To better understand the biochemical and cytotoxic effect of ethanolic raw extract of Egyptian *Citrullus colocynthis* in Sprague Dawley rat short term of toxicity bioassay.

Methodology: Ethanolic extract of fruits of *Citrullus colocynthis* was investigated for its sub chronic toxicity on sprague Dawley rats, in order to develop safe doses, we use 30 male rats gavage with 0, 12.5 and 25 mg/kg of *Citrullus colocynthis* extract twice per week for 8 weeks.

Results: The raw extract of fruits of *Citrullus* at dose of 12.5 mg and 25 mg/kg induced significant increase in level of creatinine and total protein and increased non-significantly levels of glucose and blood urea while reduced level of triglycerides, total cholesterol and high-density lipoprotein-cholesterol significantly when compared with control group. Histopathological examination revealed that kidney of rat treated with *Citrullus colocynthis* at dose of 12.5 mg/kg show interstitial chronic inflammatory cell infiltrate while kidney of SD-rat treated with *Citrullus colocynthis* at dose of 25 mg/kg show interstitial fibrosis stained with mallory trichrome stain. While liver show lytic necrosis replaced by leukocyte infiltration at dose of 12.5 mg/kg of raw extract and rat treated with *Citrullus colocynthis* at dose of 25 mg/kg show interstitial fibrosis stained with mallory trichrome stain.

Conclusion: Raw extract of fruits of *Citrullus* had adverse effect on liver and kidney while in improving lipid profile and hematology in a novel manner in Sprague Dawley rats at selected doses.

Keywords: *Citrullus colocynthis*; Liver; Kidney; Toxicity; Sprague Dawley rat

Introduction

Citrullus colocynthis is commonly used as diuretic, purgative and abortifacient. Its fruit is pungent, bitter and used as anthelmintic, purgative, antipyretic, carminative, cured leukoderma, tumors, ulcers and asthma. Its root is useful in ascites, urinary disease, rheumatism and jaundice [1]. *Citrullus colocynthis* fruit extract had a protective effect against hepato-renal toxicity of cisplatin anticancer drug [2].

Citrullus colocynthis extract made pathological alteration in hepatic cells. Notably, collagen and reticular fibers deposition were increased in liver parenchyma only at 400 g/kg. *Citrullus colocynthis* raw extract may enhance fibrosis and necrosis on liver of treated rat [3].

Pulp extracts of *Citrullus colocynthis* at doses of 100 mg/kg/day of caused severe lesions in the small intestine, kidney, and liver of rabbits. In contrast to pulp extract *Citrullus colocynthis*, animals treated with 100 or 200 mg/kg/day of seed extract showed only little intestinal alteration [4]. The treatment of extract of *Citrullus colocynthis* ripe-fruit enhanced adverse effects on kidneys and liver of treated rats. The hepato-nephrotoxic of the extract *Citrullus colocynthis* was evidenced by increase significantly of ALT, AST, urea and creatinine. The effect of the methanol-extract of *Citrullus colocynthis* on hematology at dose of 131 mg/kg had qualitatively but not quantitative changes [5].

The rationale of this study to evaluate the toxicity of *Citrullus colocynthis* raw extract in male SD-rat as we plan to study the effect of same doses of extract on hepatorenal injury induced by carbon

tetrachloride.

Materials and Methods

Experimental animals

30 male SD-rat weight 100 ± 5 gm obtained from medical experimental center, faculty of pharmacy, Mansoura University, Egypt. Animals were apparently clinical healthy and were housed in stainless steel cages with wood shavings as bedding. Animal were accommodating to laboratory condition for 2 weeks before being experimented. SD-rat was maintained on balanced ration prepared in lab of forensic medicine and toxicology department, faculty of veterinary medicine, mansoura university. Water and feed were added *ad-libitum* along the experiment.

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Plant materials

One and half kilogram of dried fruits of *Citrullus colocynthis* Shard was brought from Hassasien Market. *Citrullus colocynthis* Shard was authenticated by Dr. Zein Elaabdeen Metwaly and the extraction methods were done inside the research laboratory of pharmacognosy department, faculty of pharmacy, Mansoura University.

Plant identification and extraction

Preparation of *Citrullus colocynthis* ethanolic extract: The dried fruits were minced and sieved. The powder was add in 10 liters of ethanol (5 times), 2 liters each respectively by slow percolation at room temperature. The filtrate was separated and evaporated with a rotatory evaporator then allowed to concentrate under reduced pressure till a brownish yellow viscous paste was obtained which weighted 68.0 g and stored in air tight dark glass bottle in the refrigerator at 4o C till using. A fresh solution of the extract was dissolved in ethanol/water for SD-rat treatment at different concentration according selected doses.

Pharmacological evaluation (determination of maximum ultraviolet absorbance (λ_{max}) and construction of the calibration curves of raw plant extracts): An evaluation inside the pharmaceutics laboratory, faculty of pharmacy, Mansoura University was done to confirm that each 1 gm of the obtained extracts contained an exact 1 g of the plants extract substance to avoid the miss calculation of the administered doses of each plant.

For the calibration curves, the standard solutions were prepared by dissolving 10 mg of the raw extract in 100 ml of phosphate buffer (Ph 6.6) to give a solution contains 100 μ g of extract/ml. Different volumes of the prepared solutions transferred to volumetric flasks, each completed with 0.1 NaOH to give concentration of 0.2, 0.6, 1, 1.4, 2 and 4 μ g/ml. these solutions were scanned spectrophotometrically at different wavelengths (200 - 400 nm) to determine the maximum wavelength (λ_{max}) (Figure 1). The standard calibration curves of the extract were constructed by plotting the absorbance of different

concentrations of the extract at 230 nm in the range of 0.2 to 4 μ g/ml versus the concentration.

Animals and treatments

The animals were kept along the experiment with accordance to the ethical guidelines prescribed by the Faculty of veterinary science, University of Mansoura, Egypt. The male Sprague Dawley rats were divided into three groups.

Group 1: Male SD rats were received 0.5 ml DW orally, keep on the standard diet and were served as control group.

Group 2: Male SD rats gavage with 12.5 mg/kg (1/8 of LD50 of extract of fruits*) of *Citrullus colocynthis* extract

Group 3: Male SD rats gavage with 25 mg/kg (1/4 of LD50 of extract of fruits*) of *Citrullus colocynthis* extract

* LD50 of ethanolic extract was calculated as 100 mg/kg by up and down methods before start of main experiment (data not shown)

Pathological evaluation

The treated animals and their controls were sacrificed by overdose of CO₂ after 8 weeks of treatment. Their kidneys were removed and fixed in 10% neutral formalin. Fixed tissues were embedded in liquid paraffin wax and after solidified cut into sections of 5 mm thickness. Slides were stained with E&H for histological examination.

Biochemical assays

For biochemical study, sera of all rats were separated by centrifugation of the blood samples and put at 20°C until assayed for the biochemical parameters by colorimetric method for estimation of creatinine [6], urea [7], serum glucose [8], Total cholesterol [9], serum triglyceride [10], total protein [11], albumin [12] and globulin levels [13].

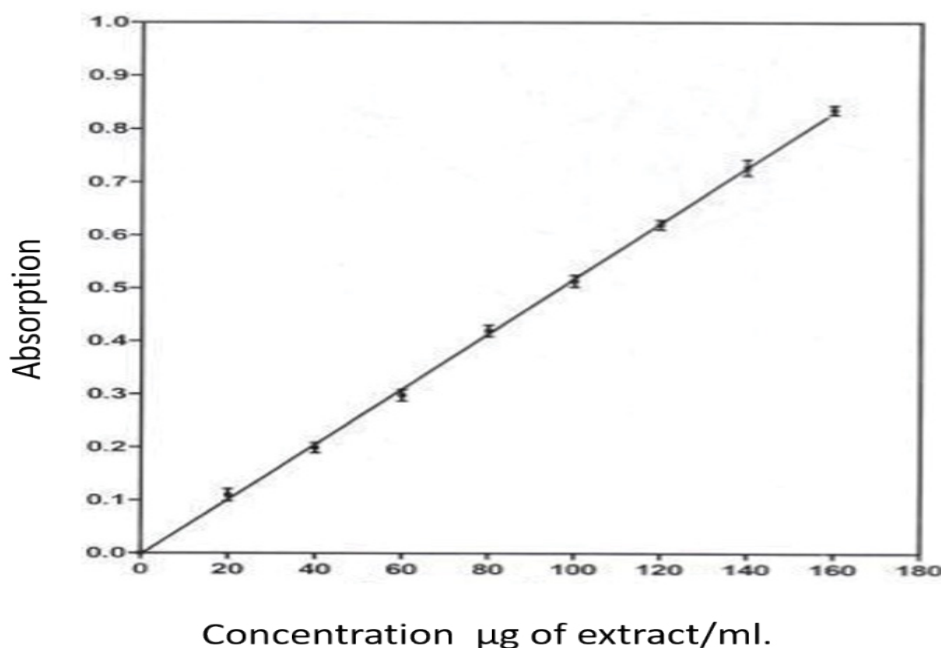


Figure 1: Show standard calibration curve of concentration of raw extract of *Citrullus colocynthis* scanned spectrophotometrically at different wavelengths (200–400 nm).

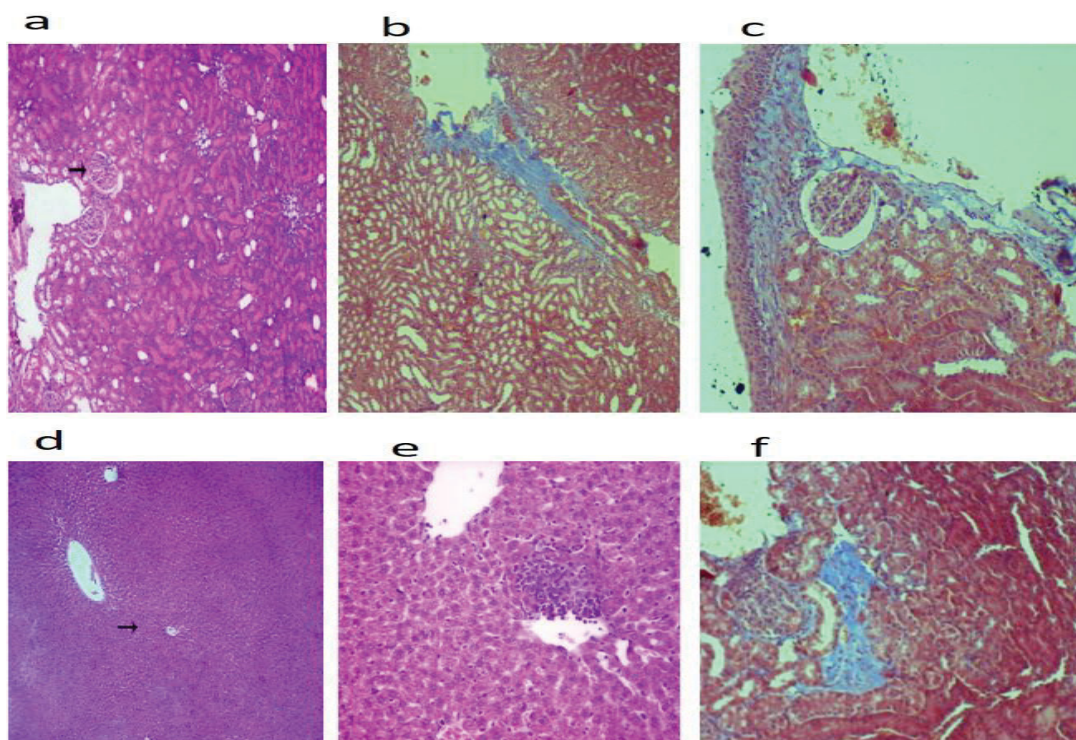


Figure 2: a. Normal renal architecture b. Kidney of rat treated with *Citrullus colocynthis* at dose of 12.5 mg/kg interstitial chronic inflammatory cell infiltrate. c. Kidney of rat treated with *Citrullus colocynthis* at dose of 25 mg/kg show interstitial fibrosis stained with mallory trichrome stain. d. Normal liver architecture e. Liver shows lytic necrosis replaced by leukocyte infiltration rat treated with *Citrullus colocynthis* at dose of 12.5 mg/kg f. Rat treated with *Citrullus colocynthis* at dose of 25 mg/kg show interstitial fibrosis stained with mallory trichrome stain.

Variables	Body weight	Kidney weight	Renal to body weight ratio
Group 1	260 ± 12.4	1.9 ± 0.07	0.74 ± 0.01
Group 2	240 ± 13.0	1.6 ^{b1,3} ± 0.09	0.68 ± 0.02
Group 3	258 ± 5.00	1.9 ± 0.09	0.67 ± 0.02

b1, b2, b3 are significantly between groups at p ≤ 0.05

Table 1: Effect of *Citrullus colocynthis* on body weight, kidney weight and renal to body weight ratio.

Variables	Hb g/dL	RBCs 10 ⁹ /μl	HCT %	MCV fL(μm ³)	MCH pg	MCHC g/dL	WBCs 10 ³ / μl	Platelets 10 ³ / μl
Group 1	12.9 ± 0.3	7.1 ± 0.1	39.2 ± 0.7	54.9 ± 1.1	17.9 ± 0.1	32.8 ± 0.4	6.9 ± 1.2	696 ± 50
Group 2	12.3 ± 10.8	9 ^{b1,2} ± 1.6	39.4 ± 3	54 ± 2	16.8 ± 0.53	31.23 ± 0.34	9 ^{b1} ± 1.2	645 ± 33
Group 3	13.8 ^{b1} ± 0.3	7.5 ± 0.2	44 ^{b1} ± 0.8	58.5 ± 1.1	18.3 ± 0.52	31.26 ± 0.35	10.9 ^{b1} ± 2.4	764 ^{b1} ± 10

b1, b2, b3 are significant between groups when p ≤ 0.05

Table 2: Effect of *Citrullus colocynthis* on blood hematology indices.

Statistical analysis

Results obtained in this study were statistically analyzed by one way analysis for variance (ANOVA) and followed by non-parametric student t-test as described by Snedecor and Cochran and we use computerized SPSS program (1996) version 32 trial [14].

Results

The prepared solutions of *Citrullus colocynthis* extract were scanned spectrophotometrically at different wavelengths (200 – 400 nm) to determine the maximum wavelength (λ_{max}). It was confirming that each 1 gm of the obtained extract contained an exact 1 gm of the plants extract substance.

The group treated with *Citrullus colocynthis* at dose of 12.5 which reduced non-significantly when compared with control group. Notably, group treated with *Citrullus colocynthis* at dose of 25 mg/kg showed a little similar body weight to control group.

In the current study, kidney and liver weight were reduced significantly in group *Citrullus colocynthis* treated at dose of 12.5 mg/kg. Notably, kidney and liver weight of group treated with *Citrullus colocynthis* at dose of 25 shown normal level as well as control group. Moreover, Renal to body weight ratio of both treated groups was reduced non-significantly state when compared with control group as well as liver to body weight ratio (Table 1).

Citrullus colocynthis at dose of 25 mg/kg increased significantly

Variables	Glucose mg/dL	Creatinine mg/dL	Urea mg/dL	T.P mg/dL	Albumin g/dL	globulin g/dL	Triglyceride mg/dL	T. chol. mg/dL	LDL-C mg/dL	HDL-C mg/dL
Gr. 1	220 ± 6.4	0.5 ± 0.03	50.6 ± 3.9	7 ± 0.29	3.9 ± 0.1	3.1 ± 0.2	35.4 ± 6.5	57 ± 7.6	29.9 ± 6.3	23.2 ± 3.1
Gr. 2	281 ± 4.2	0.94 ^{b1,3} ± 0.04	59 ± 3	8.7 ^{b1,3} ± 0.4	3.4 ± 0.34	5.3 ± 0.4	16.8 ^{b1} ± 1	37.2 ^{b1} ± 5	11.4 ^{b1} ± 3.2	22.4 ± 3.6
Gr. 3	223 ± 1.1	0.64 ^{b1} ± 0.11	56.4 ± 5.8	9.7 ^{b1,3} ± 1.1	3.7 ± 0.26	6 ± 0.5	13.6 ^{b1} ± 3a	32.2 ^{b1} ± 6.5	16.6 ± 4.9	13.2 ^{b1} ± 2.6

b1, b2 are significant between groups when $p \leq 0.05$

Table 3: Effect of *Citrullus colocynthis* on serum biochemical parameters.

level of hemoglobin (Hb) and total leukocyte count (WBCs) but non-significantly increase of red blood cells (RBCs) and platelets counts while *Citrullus colocynthis* at dose of 12.5 reduced significantly hemoglobin (Hb) level (Table 2).

Moreover, *Citrullus colocynthis* at dose of 12.5 mg and 25 mg/kg increased significantly levels of both creatinine and total protein and increased levels of both glucose and blood urea non-significantly while reduced levels of triglycerides, total cholesterol and HDL-cholesterol significantly when compared with control rats (Table 3).

Moreover, kidney of rat treated with *Citrullus colocynthis* at dose of 12.5 (Figures 2a and 2b) mg/kg show interstitial chronic inflammatory cell infiltrate while Kidney of rat treated with *Citrullus colocynthis* at dose of 25 mg/kg show interstitial fibrosis stained with mallory trichrome stain (Figures 2c and 2d) While liver show lytic necrosis replaced by leukocyte infiltration at dose of 12.5 mg/kg of raw extract (Figure 2e) and rat treated with *Citrullus colocynthis* at dose of 25 mg/kg show interstitial fibrosis stained with mallory trichrome stain (Figure 2f).

Discussion

Antioxidant effect of higher dose of raw extract of *Citrullus colocynthis* fruits maintained body weight and kidney weight [15-17], while low dose reduced body weight of treated male rats non-significantly [5]. *Citrullus colocynthis* at dose of 25 mg/kg increased significantly level of hemoglobin (Hb), total leukocyte count (WBCs) and non-significantly increase of red blood cells (RBCs) count and platelets count while *Citrullus colocynthis* at dose of 12.5 reduced significantly hemoglobin (Hb) level as similar notice of Soufane et al. and Elgerwi et al. [5,18]. In contrast to our finding, *Citrullus colocynthis* induced anaemia and leukopenia [19] or had no effects on hemoglobin concentration and packed cell volume [20]. The *Citrullus colocynthis* ethanolic extract increased significantly serum creatinine in the treated groups compared with control one [18] In contrast, the aqueous extract of *Citrullus colocynthis* at doses of 50 and 100 mg/kg of rats were safe [20].

Moreover, *Citrullus colocynthis* at both doses increased significantly levels of total protein; also they non-significantly increased levels of glucose and blood urea while reduced significantly levels of lipid profile when compared with control group [21]. *Citrullus colocynthis* fruits extract at dose of low dose induced interstitial chronic inflammatory cell infiltrate in renal tissue while kidney of rat treated with *Citrullus colocynthis* at higher dose shown interstitial fibrosis [22,23].

Conclusion

Ethanolic extract of *Citrullus colocynthis* has toxic effect in liver and kidney of rat and so separation, purification and isolation of beneficial active principal is essential to use in medicine instead of total raw extract due to potential antioxidant and improvement of hematology and immunity at high dose.

Author Conflict of Interest

No.

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Author's Contributions

1. Mahmoud M Elalfy: Planning and shared in the experiment sampling, records and write part of the manuscript. Gave final approval of the version to be submitted.

2. Ahmed Helmy: Shared in the experiment sampling, records and write part of the manuscript. Gave final approval of the version to be submitted.

3. Zain Matwaly: Shared in experiment sampling and records. Gave final approval of the version to be submitted.

4. Amany Farag: Shared in the experiment, sampling and records. Gave final approval of the version to be submitted.

5. Fathy Ali: Shared in Experimental design and Gave final approval of the version to be submitted. Limitation of this study is only that we studied two doses as we plan to evaluate its protective effect on hepatorenal induced injury.

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