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Preparation, Identification and Antioxidant Evaluation of *Citrullus Colocynthis* Root and Fruit Extracts Against Doxorubicin in Male Rats

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Abstract: Millions of people are affected by diseases in developing or developed countries. *Citrullus colocynthis* is a high-antioxidant wildflower, it is healthy to eat and may be used as a source of energy or medication. The goal of this research was to conduct a phytochemical analysis of *Citrullus colocynthis* fruit extract, which is abundant in Egypt. Different extracts from that plant were used, including root and fruit extracts. Biochemical analysis and various hematological parameters were among the parameters estimated. These aqueous extracts were also evaluated for their protective action on various antioxidants, renal and histological parameters, and molecular changes of both extracts. According to the present findings, this plant can be used as an effective natural source of antioxidants and it was used to lessen oxidative stress's consequences in male rats produced by doxorubicin, a powerful chemotherapeutic drug used to treat a variety of cancers and known to generate oxidative stress. The results reveal that by increasing total antioxidant capacity, decreasing TBARS, increasing catalase, GPx, and GST, and improving molecular and histopathological changes, these extracts were able to lower the oxidative damage caused by this chemotherapeutic agent on the majority of measured parameters. Fruit extract was more efficient than root extract, highlighting the benefits of using such a powerful antioxidant source as a nutritional supplement in patients suffering from a variety of diseases caused by various sources of oxidative stress, including chemotherapeutic agents, to help them to overcome their complications.

Keywords: *Citrullus Colocynthis*, Doxorubicin, Antioxidants, Oxidative Stress, Histopathological, TNF

Introduction

Doxorubicin (Adriamycin) is a chemotherapeutic medicine used to treat diseases such as breast cancer, bladder cancer, kaposi's sarcoma, lymphoma, and acute lymphocytic leukemia. It is present on the essential list of medicines (WHO, 2019) and it is administered intravenously. It has the potential to cause tissue damage at the injection site, bone marrow suppression, and allergic reactions such as anaphylaxis. It belongs to antitumor, antibiotic classes, and an anthracycline (Greene, 2016). Doxorubicin functions partly by interfering with DNA's role through the induction of oxidative stress (Mai *et al*., 2016). World Health World Organization is emphasizing the fact that about 70-80% of the world's population relies on non-mainstream medicine, mostly natural products, for their primary healthcare. In developing countries, this is particularly true, where the price of seeing a doctor and the price of medicine is out of reach for many people (Dyson, 1998). Herbal medicines have become increasingly common in recent years because their active ingredients are related to

other molecules, they are still in a state of biological equilibrium and therefore do not accumulate in the body and, as a result, do not cause side effects, giving them a major advantage over chemical drugs. From conventional medicinal plants, hundreds of biologically active compounds have been produced (Marles and Farnsworth, 1995). *Citrullus colocynthis* is a source of tannins, saponins, anthranol, alkaloids, phenolics, isolated amino acids, flavone glucosides, protein, flavanoids, terpenoids, hormones, cucurbitacins, saponarin, acidic glycoloids carbohydrate, trace elements and many other chemical classes that are antioxidants, anti-diabetic, antimicrobial, anticancer and anti-inflammatory agents (Al-Snafi, 2016).

The plant is thought to be native to Africa and the Middle East and it is most likely a genetic form of watermelon. It has the potential to be a long-lived perennial that grows wild in deserts under extreme xerophytic conditions. When primed, youthful natural products are crisply dappled with dull green and often turn yellow (Agarwal *et al*., 2012). Furthermore, the effects of this plant have all confirmed its traditional uses (Al-Snafi, 2016). The root was used to treat breast inflammation and joint pain; it was also used in ophthalmia and uterine torments. Water was rubbed into the natural product and root, which was then linked to bubbles and pimples. The broadened guts of children are connected to a root glue (Kirtikar and Basu, 1988). Root was overused in cases of hacking, asthma, and breast enlargement, in addition to ulcers, urinary infections, and stiffness. Seed oil is used to treat noxious chomps, bowel complaints, and seizures and also to darken hair. Ascites, jaundice, worms, biliousness, colic, cerebral congestion, fever, constipation dropsy, and sciatica were all treated with the fruit (Dey, 1980). *Citrullus colocynthis* was a popular item that is commonly used as an abortifacient as well as to treat clogging, oedema, bacterial infections, cancer, and diabetes (Rajangam and Christina, 2013). TNF (Tumor Necrosis Factor) is a cytokine that causes tissue inflammation, which is mediated by the transcription factor NF-B, it activates latent cytoplasmic NF-B, which then reaches the nucleus and causes inflammatory and antiapoptotic gene expression programs. NF-B has recently been discovered to have two distinct modes of activation, monophasic and oscillatory, depending on the stimulus size. The characterization of temporal patterns of expression for the NF-B network, as well as the determination of these genes under monophasic or oscillatory regulation, has not been studied. TNF can be a prototypical pro-inflammatory and immunomodulatory cytokine that is transmitted indelibly by activated macrophages, monocytes, neutrophils, T-cells, and NK-cells (Beutler, 1995).

This research aimed to use fruits and roots extracts as natural antioxidants, a source to diminish oxidative stress induced by chemotherapeutic agents (doxorubicin).

Materials and Methods

Preparation of Aqueous Extract of Fruit Citrullus Colocynthis

Roots and Fruits of the plant were purchased from a spices shop in Alexandria, Egypt, cleaned, and grinded to a fine powder using an electric grinder. The extraction was gotten by blending five grams of natural product powder of *C. colocynthis* with 100 mL of bubbling refined water with mixing at 100 rpm a few times overnight. Extracts were centrifuged at 2147 × g for 30 min and filtered using filter papers then lyophilized at -50°C (Telstar Model 50, Spain), and the powder was collected and kept in glass vials at 20°C until used (Hamad *et al*., 2015).

Phytochemical Qualitative Analysis

The qualitative phytochemical analysis was performed to test for tannins, flavonoids, alkaloids, reducing sugars, volatile oils, saponins (Foam Test), glycosides, terpenoids (Salkowki's Test), amino acids and proteins, and steroids according to (Mbatchou and Kosoono, 2012; Hamad *et al*., 2020).

Determination of Total Phenolic Content in Citrullus Colocynthis Extract

The complete phenolic content of the *Citrullus colocynthis* extract has been calculated with the spectrophotometric method of Folin-Ciocalteu conferring (Chavan and Doshi, 2013). Folin-Ciocalteu was included in a 2 mL of *Citrullusc colocynthis* with 0.1 mL of reagent. The mix was left to sit for 15 min. After that, 3 mL of saturated 2% sodium carbonate (Na_2CO_3) was added. The total phenolic content was determined using a spectrophotometric technique at 760 nm after 30 min room temperature (LaboAmerica, USA). It made use of regular gallic acid. Using a standard basic linear regression analysis, the total phenol value can be estimated in milligrams of gallic acid per gram of sausage. Gallic acid calibration curve; $y = 28.291x + 0.4643$. All samples were examined in triplicates (Hamad *et al*., 2018).

Antioxidant Activity Evaluation

Citrullus colocynthis extracts' free radical scavenging measures DPPH (antioxidant) was calculated using the normal procedure of Hamad *et al*. (2015) and Brand-Williams *et al*. (1995). Adopted with suitable modifications (Hamad *et al*., 2018). At an absolute concentration of 1 mg/mL, a stock solution of methanol-growing extract was made. *Citrullus colocynthis* extracts serial dilutions were made; about 1 mL of each dilution

was mixed with 1 mL of methanolic solution of DPPH in a concentration of 1 mg/mL. Following 30 min of dark incubation, the absorbance was consistent at 517 nm. IC_{50} values were calculated using a non-linear regression algorithm from the percentage inhibition versus concentration map, after estimation of the inhibition percentage by the previous calculation; Inhibition % = [(A of control - A of the sample)/A of control] x100.

Phenolic Compounds in Citrullus Colocynthis Extract by HPLC

HPLC analysis was carried out using an Agilent 1260 series (Agilent, USA). The separation was carried out using the Eclipse C18 column (4.6 x 250 mm i.d., 5 μm). The column temperature was maintained at 40°C. The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1 mL/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0-5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 μL for each of the sample solutions (Hamad *et al*., 2021).

Experimental Animals

Fifty-four male Wistar rats weighing 120-150 g (10 weeks old) were being utilized within the entire investigation experiment. Rats were maintained on experimental diets and drinking water which were presented ad libitum and then were maintained under normal operating conditions which agreed to the guidelines of the NIH. After two weeks of acclimation, creatures were partitioned into six bunches, 9 rats each: Control, the root extricate (50 mg/kg BW orally), the fruit extract (50 mg/kg BW orally), doxorubicin (15 mg/kg BW, IP), the root extract plus doxorubicin and the fruit extract plus doxorubicin respectively. The research was carried out for 14 days; doses of both extracts were given daily however doxorubicin was given on the 14th day (Othman *et al*., 2020).

Blood Collection and Tissue Preparation

Blood samples were obtained from the sacrificed animals and quickly put into the snow. Plasma samples were obtained by centrifuging at 3000 rpm for 20 min and stored at -80°C. Tissues in the ice-cold sodium and potassium phosphate buffer (0.01 m, pH 7.4) were minced and homogenized separately. At 4°C the homogeneous was centrifuged at 3,000 rpm for 20 min and the resulting supernatant was used (Othman *et al*., 2019).

Biochemical Parameters

Stored plasma samples were analyzed for total protein, albumin, urea, creatinine, LDH (Lactate Dehydrogenase), γ-GT (γ- Glutamyl Transferase), AST (Aspartate Transaminase), and ALT (Alanine Transaminase), total antioxidant capacity, GSH (Glutathione reduced), GPx (Glutathione Peroxidase), catalase and GST (Glutathione S-Transferase) were calculated using kits from BioSystems S.A. Costa Brava, 30. 08030 Barcelona (Spain). Whereas thiobarbituric acid was purchased from Sigma Chemical Company, St Louis, MO, USA.

Hematological Parameters

24 h after the last treatment, blood samples were taken from the slaughtered animals and placed on ice. Heparin was used as an anticoagulant and non-coagulated blood was tested, shortly after collection, for Red Blood Cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Platelets (PLT) by HA-VET CLINDIAG.

TNF-α Gene Expression

At the end of the *in vivo* investigation, total kidney RNAs were extracted using an RNA extraction kit purchased from Thermo scientific. cDNA was synthesized using Strand cDNA synthesis Thermo scientific purchased kit. Rt-PCR was conducted utilizing B-actine (F: GCACCACACCTTCTACAATG; R: TGCTTGCTGATCCACATCTG) title as an inside control reference by Qiagen Syber Green ace blend. Molecular changes were detected using changes in the TNF gene (F: CACAGAAAGCATGATCCGCGACGT; R: CGGCAGAGAGGAGGTTGACTTTCT), the Tumor Necrosis Factor (TNF) mRNA expression level polymorphism (the genetic exposure component of IBD development) was calculated.

Thiobarbituric Acid Reactive Substances and Antioxidants

Esterbauer and Cheeseman's (EC, 1990) method was used to measure the reactive substances of kidney Supernatant Thiobarbituric Acid (TBARS), total antioxidant efficiency was measured according to Koracevic *et al*. (2001) and the method of Beutler (1963) was used to calculate reduced glutathione content.

Histological Section Preparation

Kidney samples were obtained from rats and treated directly with 10% formalin, acceptable for treatment with standard grade xylol and paraffin-embedded alcohol, and sequestered at a thickness of 4-6 cm. Sections had been maintaining records with Haematoxyl and Eosin (H&E) recolor for consideration of histopathological examination.

Statistical Analysis

Data were analyzed using SPSS software package version 18.0 (SPSS Chicago, IL, USA) (Hagen, 2002).

Results and Discussion

Phytochemical Analysis

Tannins, alkaloids, flavonoids, volatile oils, reducing sugars, glycosides, amino acids, saponins, proteins, terpenoids, and steroids for *Citrullus colocynthis* were determined as detailed in Table 1. HPLC analysis of phenolic compounds in *Citrullus colocynthis* root and fruit extracts is presented in Fig. 1 and 2 and illustrated also in Tables 2 and 3.

Total Phenolic Content in Citrullus Colocynthis Extract

The Total Phenolic content of *Citrullus colocynthhis* fruit extract was found to be 3.218 g gallic acid equivalent per 100 g aqueous extract by using the equation of standard curve (y = 28.291 + 0.4643). Concentrations of Gallic acid (grade stander, Sigma-Aldrich) used for the standard curve were 0.01- 0.18 mg/mL.

Antioxidant Activity Evaluation

The ability of *Citrullus colocynthis* extracts to scavenge DPPH free radicals (antioxidant) was calculated using the standard method and data as detailed in Table 4. *C. colocynthis* fruit, through its antioxidant and hypoglycemic activities, has a positive effect on the treatment of diabetic neuropathy. *C. Colocynthis* treatment has also appeared effective in improving the weight of diabetic animals. The fruits of *A. leucotrichus* and *C. colocynthis* were very rich in polyphenols including flavonoids, tannins, and coumarins (Halla *et al*., 2019).

Phenolic Compounds Analysis

HPLC was used to detect chlorogenic acid, gallic acid, catechin, methyl gallate, coffeic acid, syringic acid, pyro catechol, rutin, ellagic acid, coumaric acid, vanillin, ferulic acid, naringenin, querectin, cinnamic acid, kaempferol and hesperetin in *Citrullus colocynthis* extract (Table 5).

Biochemical Parameters

Body weight did not change significantly. These results are consistence with Ostovar *et al*. (2020) who discovered that *C. colocynthis* treatment enhanced metabolic results (blood glucose level and body weight) when in comparison to a placebo. Fruit extract was more able to overcome the significantly decreased total protein and albumin and increased urea, creatinine, LDH, and GT. ALT and AST upon doxorubicin injection alone than the root extract (Table 6). Results confirmed the effect of *C. colocynthis* on blood glucose reduction, not only in oral form (reported in several studies) but also in topical administration. Results however showed that oral consumption could be more efficient (Ostovar *et al.,* 2020).

Oxidative Stress and Antioxidants

Oxidative stress was induced by doxorubicin administration of a single i.p. dose (15 mg/kg BW, i.p.) on the 14th day which was confirmed by the obtained results in which plasma TBARS were increased and decreased their TAC, GSH, GST, and GPx. Both extracts' administration deteriorated this oxidative stress via decreasing plasma TBARS and increasing TAC and various antioxidants in rats as shown in Table 7. Both root and fruit extracts significantly decreased kidney TBARS and increased TAC, GSH, catalase, and GPx. Fruit extract showed a better effect on most of the evaluated parameters (Table 7). *C. colocynthis* antioxidant ability and its influence on therapy were also mentioned by Ostovar *et al*. (2020). Our results proved the capacity of bitter apple to alleviate the complications of doxorubicin which is collinear with Hussain *et al*. (2014) who stressed the therapeutic effects of several edible and medicinal plants that are used in traditional medicine that are usually attributed to their polyphenolic compounds. *Citrullus colocynthis* extract represents a plentiful supply of antioxidants (plantsterol and polyphenol) (Sebbagh *et al*., 2009). Some *in vitro* studies conducted on the fruit extracts revealed their excellent antioxidant potential (Kumar *et al*., 2008). Our data confirmed the capacity of both extracts to improve various biochemical parameters and increase various antioxidants; alternatively, decreasing TBARS (fruit extract was more effective). *Citrullus colocynthis* antioxidant effect was demonstrated by measuring the markers of oxidative stress (malondialdehyde, superoxide dismutase, and catalase). The results supported powerfully the use of *Citrullus colocynthis* as a natural source of antioxidant substances (Hussain *et al*., 2014). Species of Reactive Oxygen (ROS) can harm proteins, biomembrane, and nucleic acid. If cellular defense mechanisms fail, severe dysfunction or cell death may result, events that are part of the pathogenic process in a statement. There is accumulating evidence that plant-derived antioxidants may reduce or prevent oxidative stress and have a beneficial influence on animal and human health. Improving data on various antioxidants is consistent with Abd El-Baky and Amin (2011) who demonstrated that *Citrullus colocynthis* fruit extract was beneficial in enhancing Glutathione levels as well as GPx and SOD activities.

Hematological Parameters

Fruit and root extract significantly increased HGB and HCT whereas RBC, HGB, HCT, and PLT were decreased in rats treated with doxorubicin while MCH increased. Treatment with *Citrullus colocynthis* fruit and root extracts with doxorubicin minimized its toxic effect on various estimated hematological parameters (Table 8). Ostovar *et al*. (2020) performed metabolic assessments by measuring the body weight and blood glucose levels of the C-treated animals. *C. colocynthis* (especially an oral dose of 100 mg/kg), which occurred to be effective for

improving diabetic animal weight. The results of their studies have shown considerable prevention of oxidative damage with *C. colocynthis* treatment. Involved in the development of neuropathy by including a therapeutic agent, which is a commonly performed, traditional use for neuropathy, their work investigated the therapeutic, not preventative, effect of *C. colocynthis*. The antioxidant capacity of *C. colocynthis* was illustrated in their study. Oral *C. colocynthis* treatment with a 100 mg/kg dose was recommended which gave the best results among all *C. colocynthis*-treated rats and further, metabolic consequences (body weight and blood glucose level) were value-added in the *C. colocynthis* treated groups as paralleled to control. Their results confidently confirmed that hot-plate and tail-flick tests correspondingly had lower invisibility in the *C. colocynthis*- treated groups and a predictable quantity of oxidative stress markers (catalase, malondialdehyde, and superoxide dismutase) displayed the antioxidant consequence of *C. colocynthis*.

TNF Gene Expression

TNF gene expression patterns were associated to detect molecular changes between the different groups under investigation. The expression of the TNF mRNA gene was measured using the related quantitative reverse transcription PCR. The extent of TNF-α gene expression in the five cases of the study (group 2: Root extract, group 3: Fruit extract, group 4: Doxorubicin, group 5: Root extract + Doxorubicin, and group 6: Fruit extract + Doxorubicin) compared with control (group 1). Figure 3 illustrates the expression level of TNF mRNA levels between the different groups. On the molecular level, Sanadgol *et al*. (2011) result indicated that the extract of *C. colocyn* affectedly reduced the expression of TNF in overweight mice. They explained the potential advantage of phytochemicals is that they may act through multiple pathways and reduce the development of resistance by cells. They concluded that herbal therapy will provide an added advantage over the currently available conventional therapies.

Table 1: Phytochemical screening of *C. colocynthis* extract

Chemical constituents	Water extract
Alkaloid	+
Tannins	+++
Saponins	+
Flavanoid	+
Terpenoid	++
Glycosides	+
Steroid	+
Flavonoids	+
Reducing sugars	-
Carbohydrate	+
Protein	-
Amino acid	+
Total phenolic compound	+

Table 2: Root extract HPLC analysis of antioxidants

Peak #	Ret time (min)	Type	Width (min)	Area (mAU*s)	Area %	Name
1	2.486		0.0000	0.00000	0.0000	
2	2.610		0.0000	0.00000	0.0000	
3	2.837		0.0000	0.00000	0.0000	
4	3.051	MM	0.0885	63.58703	10.3552	Gallic acid
5	3.636	MM	0.1407	22.73729	3.7028	Chlorogenic acid
6	4.092		0.0000	0.00000	0.0000	Catechin
7	4.361	MM	0.1442	42.61789	6.9403	
8	4.929	VB	0.1079	2.99409	0.4876	Methyl gallate
9	5.215	BV	0.1609	34.46970	5.6134	Coffeic acid
10	5.771	VB	0.1668	9.62992	1.5682	Syringic acid
11	6.216	BV	0.1420	2.50434	0.4078	Pyro catechol
12	6.874	BB	0.1483	2.11177	0.3439	Rutin
13	7.566	MM	0.2171	190.34041	30.9970	Ellagic acid
14	8.063		0.0000	0.00000	0.0000	Coumaric acid
15	8.655	BV	0.1932	62.32138	10.1490	Vanillin
16	9.484	MM	0.1409	5.38756	0.8774	Ferulic acid
17	10.084		0.0000	0.00000	0.0000	Naringenin
18	11.078	BV	0.1419	72.87646	11.8679	
19	11.904	BV	0.0998	78.89358	12.8478	
20	12.812	BB	0.0958	1.70786	0.2781	Querectin
21	14.149		0.0000	0.00000	0.0000	Cinnamic acid
22	15.090	MM	0.1138	13.65920	2.2244	Kaempferol
23	15.554	VB	0.1733	8.22284	1.3391	Hesperetin
Totals:				614.06131		

Table 3: Fruit extract HPLC analysis of antioxidants

Peak #	Ret time (min)	Type	Width (min)	Area (mAU*s)	Area %	Name
1	2.486		0.0000	0.00000	0.0000	
2	2.610		0.0000	0.00000	0.0000	
3	2.837		0.0000	0.00000	0.0000	
4	3.045	MM	0.0834	78.70303	2.1287	Gallic acid
5	3.767	BV	0.1291	177.66365	4.8053	Chlorogenic acid
6	4.092	VV	0.1444	296.02286	8.0067	Catechin
7	4.966	BV	0.1446	398.12656	10.7683	Methyl gallate
8	5.344	VB	0.1579	231.11531	6.2511	Coffeic acid
9	5.821	BV	0.1339	183.10789	4.9526	Syringic acid
10	6.011	VB	0.1673	200.53180	5.4239	Pyro catechol
11	7.032	BB	0.1803	242.32448	6.5543	Rutin
12	7.804	BV	0.1659	158.93285	4.2987	Ellagic acid
13	8.063	VV	0.2066	387.50558	10.4810	Coumaric acid
14	8.684	VB	0.2150	289.68179	7.8351	Vanillin
15	9.399	MM	0.1950	156.88800	4.2434	Ferulic acid
16	10.086	VB	0.1259	154.67921	4.1837	Naringenin
17	12.934	MM	0.1736	102.64160	2.7762	Querectin
18	14.150	BB	0.1742	302.47455	8.1812	Cinnamic acid
19	15.317	BV	0.1801	109.85464	2.9713	Kaempferol
20	15.883	VB	0.2206	226.95558	6.1386	Hesperetin
Totals:				3697.20974		

Table 4: DPPH inhibition percentage of ascorbic acid and *C. colocynthis* fruit extract

Extract	Concentration (µg/ml)	DPPH inhibition	
Percentage (%) (µg/ml)	IC50		
Ascorbic acid	1.0	39.86	1.98
	2.0	50 34	
	4.0	67.79	
	6.0	89.50	
	8.0	98.63	
Citrullus colocynthis extract			
	5.0	42.13	14.80
	10.0	47.79	
	15.0	51.67	
	20.0	69.24	
	25.0	90.13	

Table 5: Phenolic compounds analysis of *Citrullus colocynthis* extract via HPLC

Phenolic compound	Concentration (µg/ml)
Gallic acid	13.57
Chlorogenic acid	3.58
Catechin	ND.00
Methyl gallate	0.08
Coffeic acid	2.68
Syringic acid	0.90
Pyro catechol	0.36
Rutin	0.53
Ellagic acid	41.08
Coumaric acid	ND.00
Vanillin	2.78
Ferulic acid	0.43
Naringenin	ND.00
Querectin	0.21
Cinnamic acid	ND.00
Kaempferol	1.49
Hesperetin	0.48

ND; not detected

Table 6: Effect of *Citrullus colocynthis* Extracts administration on various biochemical parameters

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Body weight (gm)	130 ±3.73^{ab}	145±6.24^{a}	136±5.0^{ab}	114±2.10^{b}	126±6.21^{ab}	132±3.55^{ab}
Biochemical						
Total protein (g/L)	70.6±1.17^{a}	69.0±1.57^{a}	68.4±0.80^{a}	53.6±0.64^{c}	61.2±1.43^{b}	58.5±0.31^{bc}
Albumin (g/L)	42.2±0.82^{b}	43.6±0.40^{b}	54.8±0.42^{a}	22.9±0.20^{d}	30.5±0.53^{c}	27.6±0.74^{c}
Urea (mg/dl)	35.5±0.91^{cd}	22.2±0.90^{c}	36.9±1.92^{d}	56.3±1.94^{a}	45.5±1.23^{b}	39.8±0.25^{bc}
Creatinine (mg/dl)	0.90±0.02^{d}	0.87±0.03^{d}	0.93±0.02^{d}	2.62±0.11^{a}	1.34±0.03^{c}	2.03±0.11^{b}
LDH (U/L)	190±4.75^{c}	183±4.97^{c}	187±4.06^{c}	456±19.82^{a}	294±12.14^{b}	328±10.92^{b}
γ-GT (U/L)	20.9±0.35^{c}	20.4±0.15^{c}	18.9±0.18^{c}	30.1±0.78^{a}	25.1±1.07^{b}	26.7±1.19^{b}
ALT (U/L)	32.3±2.06^{c}	31.8±1.80^{c}	29.3±0.98^{c}	64.0±4.26^{a}	58.3±2.82^{b}	50.1±1.22^{b}
AST (U/L)	28.3±0.57^{c}	27.7±0.58^{cd}	24.3±0.82^{d}	35.3±1.06^{a}	32.8±1.35^{b}	34.6±0.87^{a}

Data are presented as Mean ± S.E, S.E: Standard Error.
Mean values within a row not sharing common superscript letters (a, b, c, d) were significantly different, $p<0.05$.
Group 1: Control group, Group 2: Root extract group, Group 3: Flower extract group, Group 4: Doxorubicin group, Group 5: Root extract + doxorubicin group, Group 6: Flower extract + doxorubicin group.

Table 7: Effect of *Citrullus colocynthis* Extracts on plasma and kidney antioxidants

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Antioxidants plasma						
TAC (mm/L)	1.75±0.18^{c}	2.33±0.05^{b}	2.70±0.10^{a}	1.09±0.13^{e}	1.41±0.13^{d}	1.52±0.14^{d}
TBARS (nmol/ml)	0.91±0.02^{d}	0.64±0.03^{e}	0.53±0.03^{f}	2.28±0.15^{a}	2.02±0.13^{c}	2.26±0.03^{b}
GSH (µmole/ml)	4.76±0.07^{b}	5.60±0.11^{a}	5.27±0.16^{a}	3.70±0.10^{c}	4.70±0.16^{b}	4.33±0.17^{b}
Catalase(U/ml)	40.3±1.25^{a}	41.0±1.62^{a}	43.3±0.60^{a}	37.7±1.09^{c}	38.9±1.26^{b}	39.1±1.01^{b}
GPx (U/ml)	9.07±0.21^{ab}	9.09±0.23^{ab}	9.27±0.17^{a}	8.31±0.17^{c}	8.35±0.18^{c}	8.66±0.09^{bc}
GST (µmol/hr/ml)	1.41±0.14^{ab}	1.46±0.11^{a}	1.76±0.14^{a}	1.03±0.09^{d}	1.15±0.14^{c}	1.25±0.13^{bc}
Kidney						
TAC (mm/L)	0.82±0.02^{b}	0.95±0.01^{a}	0.96±0.01^{a}	0.53±0.02^{d}	0.57±0.02^{c}	0.60±0.02^{c}
TBARS (nmol/gm wet tissue)	20.1±0.30^{c}	18.4±0.17^{d}	18.8±0.46^{d}	30.1±1.56^{a}	29.1±0.46^{b}	27.3±0.73^{b}
GSH (µmole/gm wet tissue)	4.26±0.04^{b}			3.40±0.06^{d}	3.64±0.09^{c}	3.69±0.11^{c}
Catalase (U/mg protein)	50.4±0.80^{b}	51.6±1.07^{a}	52.0±1.67^{a}	38.9±0.25^{e}	41.3±0.50^{d}	43.4±0.76^{c}
GPx (U/mg protein)	28.4±0.29^{b}	29.1±1.09^{ab}	29.6±1.34^{a}	24.4±0.44^{d}	25.5±0.70^{cd}	25.9±0.91^{c}
GST (µmol /hr/mg protein)	0.80±**0**.02^{b}	0.82±0.02^{a}	0.84±0.02^{a}	0.58±0.01^{d}	0.65±0.02^{c}	0.70±0.02^{c}

Data are presented as Mean ± S.E, S.E: Standard Error
Mean values within a row not sharing a common superscript letters (a, b, c, d, e) were significantly different, $p<0.05$
Group 1: Control group, Group 2: Root extract group, Group 3: Flower extract group, Group 4: Doxorubicin group, Group 5: Root extract + doxorubicin group, Group 6: Flower extract +doxorubicin group

Table 8: Effect of *Citrullus colocynthis* extracts administration on various hematological parameters

Parameters Hematological	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Plasma RBC's(10^6/µL)	7.00±0.51^{ab}	7.88±0.18^{a}	7.63±0.12^{b}	6.85±0.31^{c}	8.68±0.33^{ab}	7.79±0.08^{ab}
HGB (g/dL)	9.04±0.55^{c}	11.4±0.34^{b}	11.3±0.30^{b}	10.1±0.42^{d}	13.5±0.64^{a}	12.0±0.28^{a}
HCT (%)	32.1±1.92^{c}	39.2±0.87^{ab}	37.7±1.08^{b}	34.9±1.06^{d}	43.8±1.38^{ab}	41.3±0.76^{a}
MCV (fL)	47.0±1.24^{c}	50.2±0.99^{b}	51.2±1.13^{ab}	51.6±1.14^{c}	53.4±0.69^{a}	55.2±0.87^{a}
MCH (Pg)	13.9±0.22^{d}	14.4±0.21^{cd}	15.0±0.39^{ab}	15.6±0.19^{ab}	14.9±0.95^{bc}	15.9±0.45^{a}
PLT (10^3/µL)	339±11.3^{a}	95.0±5.6^{a}	85.2±4.74^{a}	188±16.7^{c}	275±13.0^{b}	292±5.54^{b}

Data are presented as Mean ± S.E, S.E: Standard Error.
Mean values within a row not sharing a common superscript letter (a, b, c, d) were significantly different, $p<0.05$.
Group 1: Control group, Group 2: Root extract group, Group 3: Flower extract group, Group 4: Doxorubicin group, Group 5: Root extract + doxorubicin group, Group 6: Flower extract + doxorubicin group.

Histopathological Results

Kidney specimens were collected following scarification, fixed in 10% neutral buffered Formalin solution. After 24 h, following steps of dehydration in ascending grades of ethanol, cleared in xylene and then embedded in paraffin wax. Tissue sections (3-5 microns thick) were sliced and stained with hematoxyl and eosin (H&E), thus according to Banchroft and Stevens (1996) and the light microscopy was confined to histological changes.

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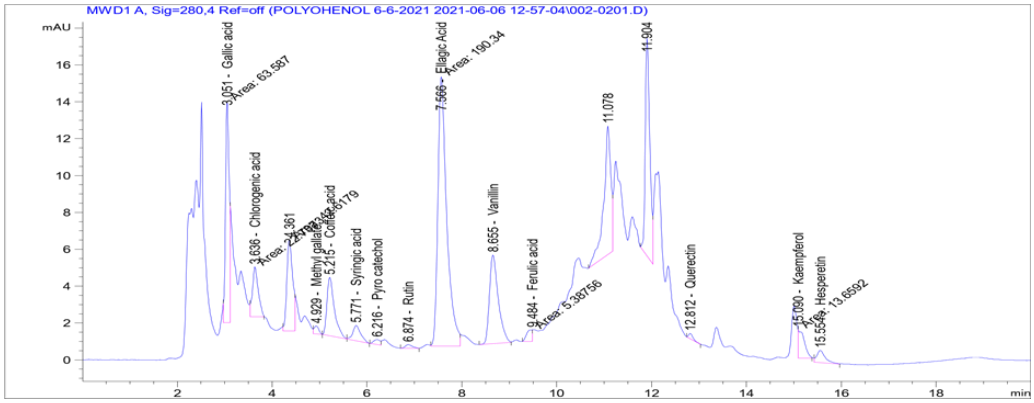

Fig. 1: HPLC analysis of phenolic compounds in *Citrullus colocynthis* root extract

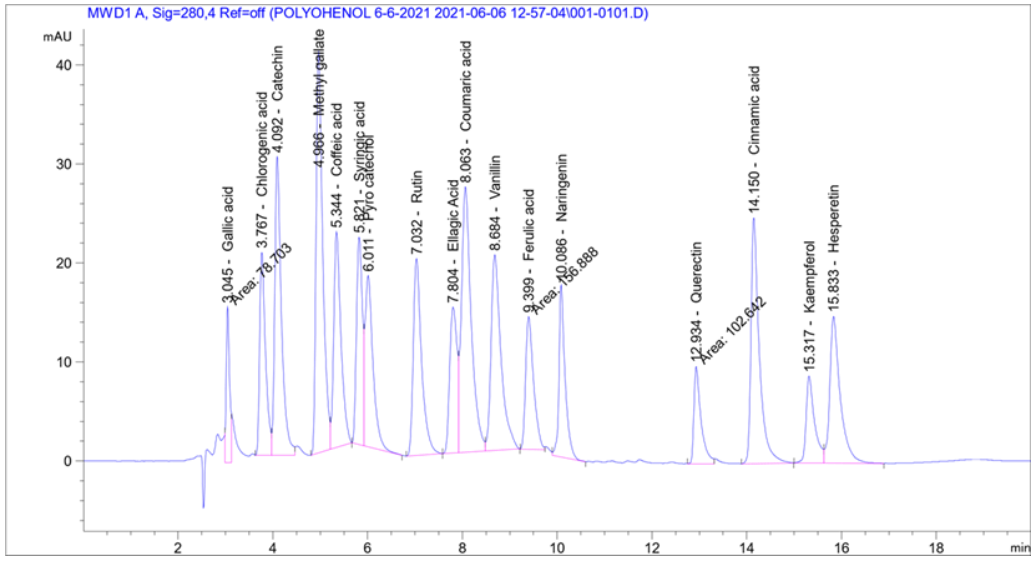

Fig. 2: HPLC analysis of phenolic compounds in *Citrullus colocynthis* fruit extract

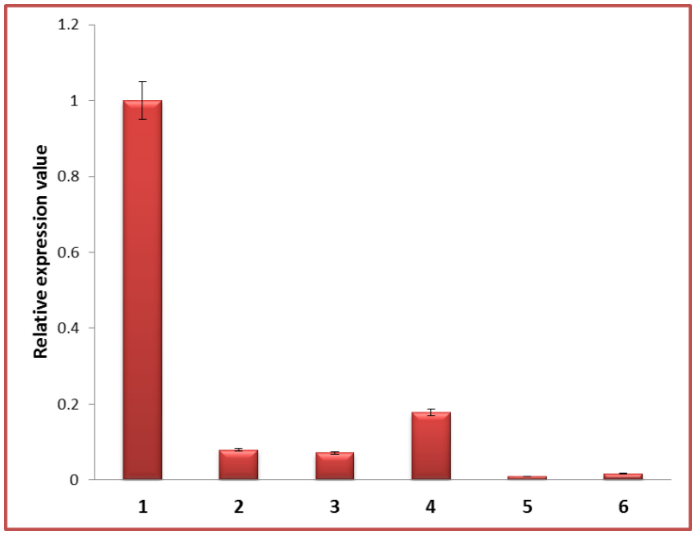

Fig. 3: Relative expression value of TNF gene of the different groups

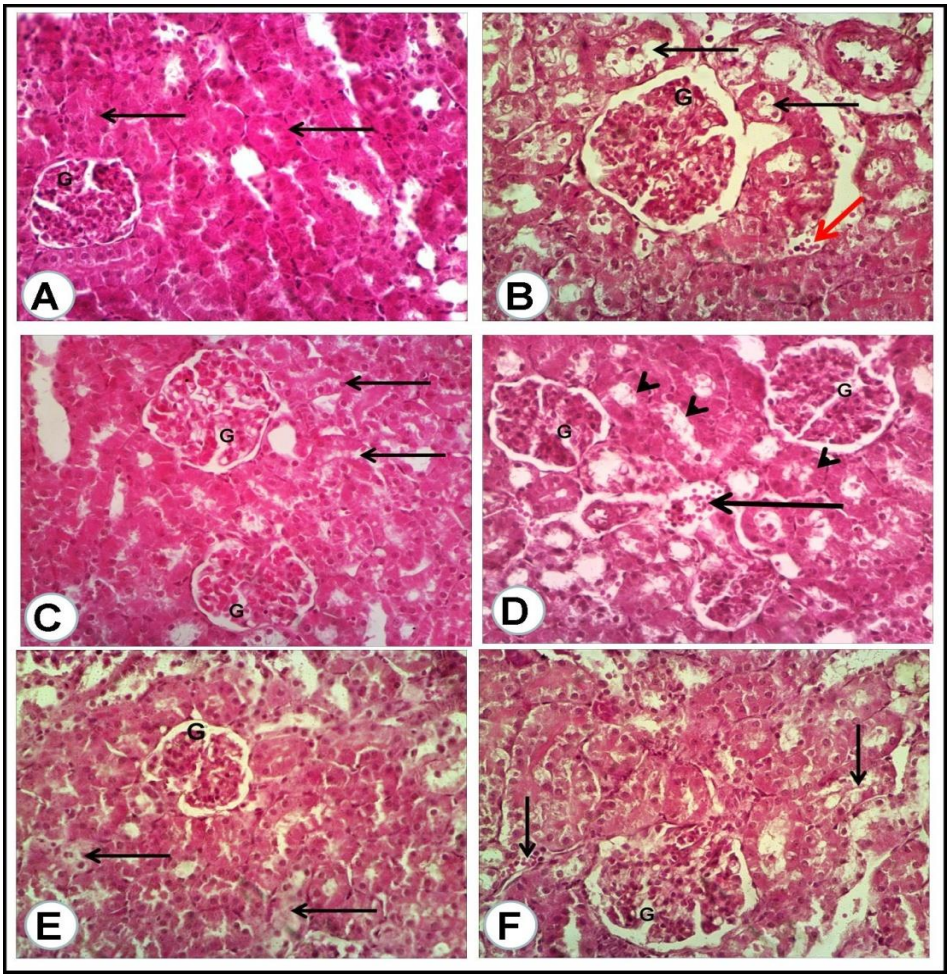

Fig. 4: Renal cortex of the rats of all groups (H&E, X400)

Changes in the renal cortex of the kidneys of all groups (Fig. 4): The light microscopy for the renal cortex of the control group showed no histopathologic changes (Fig. 4A), while the doxorubicin treated group showed obvious nephrotoxic changes in the cortex of the kidneys mostly in the convoluted tubules where their uroepithelium suffered degenerative and necrotic changes in addition to numerous interstitial lymphocytic infiltrations, while the glomeruli appeared swollen due to the hypercellular defensive mechanism (Fig. 4B). The cortex of the kidneys in the group administered with only root extract appeared with the normal histologic structure of the glomeruli (less swelling or hypercellularity) and uroepithelium of the convoluted tubules (Fig. 4C). The changes, after administration of root extract + doxorubicin, were only in form of mid degeneration of the tubular uroepithelium with the presence of some interstitial infiltration with lymphocytic cells, while the glomeruli appeared somewhat enlarged due to the hypercellularity (Fig. 4D). The cortex of the kidneys, in the group that received only fruit extract, showed nearly normal glomeruli (small and less cellular) and convoluted tubules, while only a few interstitial lymphocytic infiltrations (Fig. 4E) were seen. The last group treated with fruit extract + doxorubicin showed a less ameliorating effect on the renal cortex. The convoluted tubules are affected by degeneration and necrosis of its epithelium in addition to the presence of an excess and severe interstitial mononuclear cell (lymphocytes and macrophages) infiltrations. The glomeruli appeared also swollen and hypercellular (Fig. 4F).

Changes in the renal medulla of the kidneys of all groups (Fig. 5): The changes of toxicosis as a side effect of doxorubicin administration appeared more obvious in the kidney than in the liver. The detected hepatotoxic changes of doxorubicin were in the form of hepatocytic degeneration and necrosis, mononuclear cell infiltration, dilation, and vascular congestion and hemorrhages. The detected nephrotoxic changes of doxorubicin in the renal cortex of the kidneys were in form of degenerative and necrotic changes of the uroepithelium of the convoluted tubules in addition to the interstitial infiltrations with lymphocytes. The glomeruli became swollen due to the

hypercellular of a defensive mechanism. The changes in the kidneys' medulla were only restricted to a focal protein's depositions in between the medullary tubules, as well as some cast formation inside the lumina of some tubules, while the uroepithelium mostly appeared nearly normal. The light microscopy for the renal medulla of the control group showed no histopathologic changes (Fig. 5A), while the changes of toxicosis in the group injected with doxorubicin were restricted on the focal depositions of a protein's globules in between the medullary tubules, while the uroepithelium of these medullary tubules appeared nearly normal (Fig. 5B). The medullary tubules, in the group that received only root extract, appeared nearly with normal epithelium, but a few intratubular infiltrations of fibrous elements and small lymphocytes (Fig. 5C) were seen. The group was given root extract + Doxorubicin showed only mild degeneration of the uroepithelium of the medullary tubules, while the mononuclear cell reactions appeared excess and involved numerous foci of the medulla (Fig. 5D). In the case of treatment with only fruit extract, the renal medullary tubules appeared normal and associate with only some intratubular infiltrations with lymphocytic cells (Fig. 5E). The group was given fruit extract + Doxorubicin showed a very less ameliorating effect on the renal medulla. The medullary tubules appeared widely separated by the excess of interstitial fibrosis and mononuclear cell infiltration in addition to the degeneration of its uroepithelium (Fig. 5F). Histological findings were parallel with Abd El-Baky and Amin (2011) who explained that the oral intake of *Citrullus colocynthis* fruit extracts showed partial protection of glomeruli and appeared nearly normal with wide tubules and many vacuolated cells. They added that pale dilated renal tubule with acidophilic hyaline material and thickening of blood vessels were observed. They suggested the ability of this extract to enhance renal functions and they emphasized the healing ability of this plant extract on tissues of the kidney. Bagherizadeh *et al*. (2015) added that *Citrullus colocynthis* fruit may have protective effects on the kidney functions and tissues and the results of their research revealed that colocynth pulp has the potential to treat diabetes and prevent the relevant kidney damages.

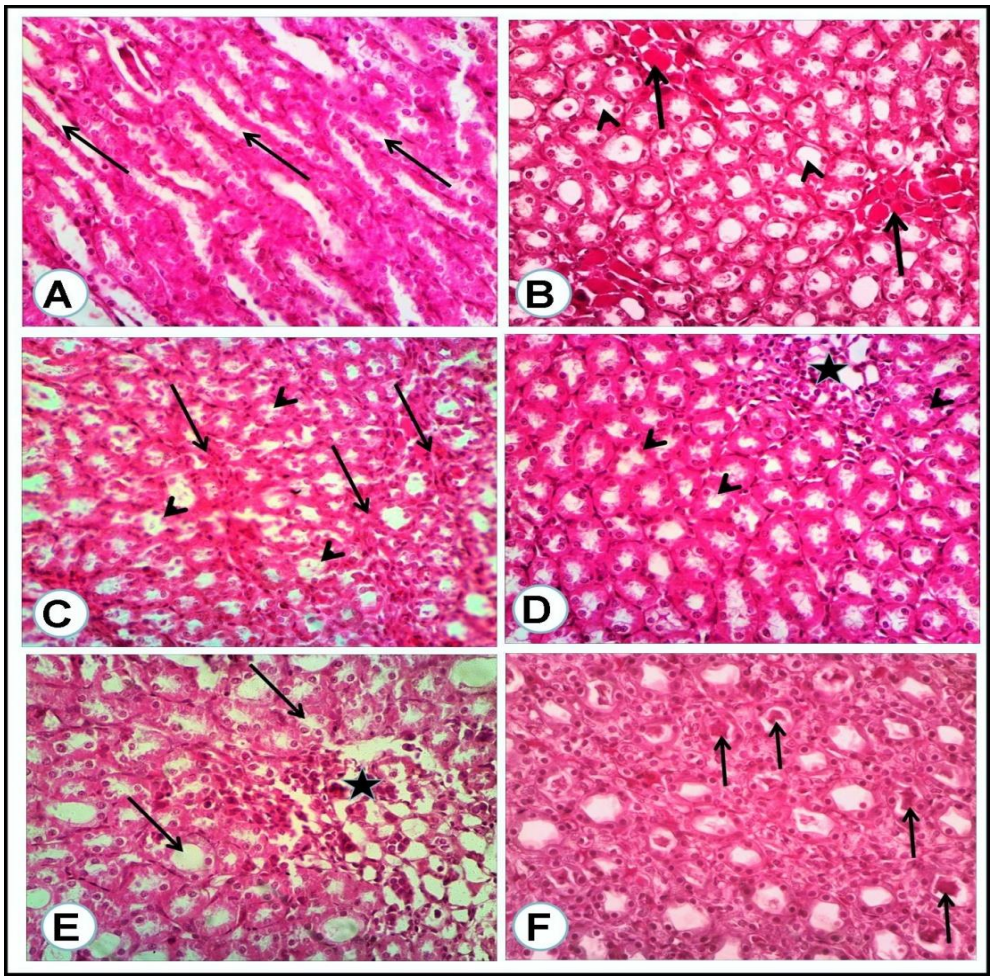

Fig. 5: Renal medulla of the rats of all groups (H&E, X400)

Conclusion

Fruit and root extracts of *Citrullus colocynthis* are natural mixtures of antioxidants and can be used as supplements for patients treated with drugs inducing oxidative stress including chemotherapeutic medications. Whereas, fruit extract showed better results on various estimated parameters as its chemical analysis showed higher contents of different antioxidant constituents. This extract is a promising natural source of various antioxidants that can be used in the pharmaceutical and food supplements industries.

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Author's Contributions

Sarah Samir Othman: Participated in planning, design, and coordination of the animal study, biochemistry study, and uptake assays and participated in the statistical analysis.

Gamal M. Hamad: Carried out of plant extract and determination of antioxidants, total phenolic and phytochemical analysis**.**

Sabria Abo Zaid Hassan: Carried out of plant extract and determination of total phenolic analysis.

Eman Fayad and Safaa M. Ali: Carried out the performed the DAT autoradiography. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

This experiment was carried out at the Pharmaceutical and Fermentation Industries Development Center in the City of Scientific Research and Technological Applications (SRTA-city) and approved by its ethics committee (IACUCs) l IACU # 11-1H-1019.

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