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THERAPEUTIC PHARMACOLOGICAL PROPERTIES OF *CITRULLUS COLOCYNTHIS* FRUIT PULPS METHANOLIC CRUDE EXTRACT AGAINST POTASSIUM OXONATE-INDUCED HYPERURICEMIC GOUT RAT MODEL

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This study was carried out to study the anti-hyperuricemic and antioxidant properties of C. colocynthis fruit pulps methanolic crude extract. The intra-peritoneal injection with 250 mg/kg/day potassium oxonate (PO) was used to develop hyperuricemic gout rat model. Our results reported that C. colocynthis pulps oral administration introduced potent hypouricemic and antioxidant properties against hyperuricemic gout rat model. The oral administration with C. colocynthis extract greatly alleviated the levels of ankle joint swelling rates (%), serum uric acid, xanthine oxidase (XOD), and thiobarbituric acid reactive substances (TBARS/MDA) as well as reduced the fold of differences of the renal mRNA URAT1 and mRNA GLUT9 urate re-absorption transports. Furthermore, C. colocynthis extract highly increased the levels and activities of serum total proteins content, glutathione peroxidase (GSHPx), and superoxide dismutase (SOD) enzymes. Moreover, Cc pulps extract greatly improved and reduced damage, inflammation and cytotoxicity in the liver and ankle joint specimens. Finally, C. colocynthis fruit pulps greatly introduced powerful therapeutic properties to improve the harmful hyperuricemia features compared to the allopurinol (Allo) administration.

Keywords: *Citrullus colocynthis fruit pulps; xanthine oxidase (XOD); urate anion transporter 1 (URAT1); glucose transporter 9 (GLUT9); antioxidant phytochemicals.*

INTRODUCTION

The liver is a critical organ for the *de novo* biogenesis of purines and uric acid (UA). UA is considered as a final enzymatic endproduct of the purine nucleosides and free bases degradation in humans and Great Apes[1&2]. Furthermore, UA is a weak diprotic acid that predominantly presents as a monosodium urate (MSU) ion at the physiological pH of 7.4. The serum uric acid (SUA) homeostasis depends on the balance between the hepatic UA production, secretion, its catabolic metabolism, renal re-absorption, and excretion through the kidney tubules[3]. Under normal physiological conditions, 90% of the renal glomerular-filtered urate is re-absorbed back into the blood stream, and 10% of it is excreted in the urine[4&5]. The renal urate re-absorption and excretion transporters play a critical role in the maintenance of the levels of the human SUA under physiological concentrations[6]. The renal urate re-absorption transporters are introduced as UA uptake transporters including urate anion transporter 1 (URAT1/SLC22A12), organic anion transporter 4 (OAT4/SLC22A11) and glucose transporter 9(GLUT9/SLC2A9)[3&6]. The renal urate excretion transporters includes OAT1 (SLC22A6), OAT3 (SLC22A8)[7], urate transporter (UAT), multidrug resistance protein

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4 (MRP4/ABCC4), ABCG2/BCRP (ATP-binding cassette sub-family G member 2/breast cancer resistance protein)[8] and sodium-dependent phosphate transport protein[3&4]. Under normal conditions, renal UA transporters regulate urate re-absorption and excretion to maintain SUA homeostasis [7]. The disturbance in the work of these renal UA transporters causes hyperuricemia (HUA)[6&9].

Hyperuricemia (HUA) upregulates activities of xanthine oxidoreductase (XOR) enzymes and induces oxidants generation[3]. The purines and/or fructose rich diets, prolonged alcohol intake, excessive exercises, and some malignant tumors (after chemotherapy) can cause HUA features[10&11]. HUA is also characterized by the high levels of SUA and the deposits of the urate crystals in the kidneys and body joints[3&5]. Furthermore, XOR enzymes catalyze the degradation process of purines, which regulate the oxidation process of hypoxanthine and xanthine to form UA and oxidants[3&12]. In the *de novo* UA biogenesis, xanthine oxidase (XOD), a XOR enzyme oxidized form, uses oxygen molecules to produce free radical byproducts and UA[3].

The management of HUA and gout treatment is presented to regulate the hepatic UA production and its renal re-absorption and excretion, alleviate the high levels of SUA and its deposits in the body joints and tissues, reduce the oxidants generation, attenuate the acute gouty inflammatory attacks, and upregulate the antioxidants production[13&14]. Allopurinol, an uricostatic synthetic drug, is a potent XOR inhibitor that regulates hepatic UA production to maintain SUA homeostasis[1]. Previous studies indicated that the long-term treatment with allopurinol (chronic therapy) can cause multiple harmful side effects, including skin rashes, allergic and hypersensitivity reactions, fever, gastrointestinal toxicity, hepatotoxicity, neurological diseases, and kidney stones[3&5]. Furthermore, allopurinol cannot eliminate the free radicals byproducts that are produced from the action of XOD enzyme[1]. Allopurinol is also used to treat HUA but is ineffective to attenuate the acute gouty inflammatory arthritis features[1&15].

The complementary and/or alternative therapeutic strategies of gout are aimed to use the natural products and their isolated compounds for controlling the gout features, SUA levels and the gouty inflammatory arthritis[16]. *Citrullus colocynthis* (L.) Schrad. belongs to family Cucurbitaceae, which is considered as a perennial herbaceous creeping plant. The seeds, fruits, cortexes, roots, stems, and leaves as either aqueous or oil extracts, dried or fresh forms of *C.colocynthis* are targeted to use for several pharmacological, phytochemical and nutritional investigations[17&18]. Previous studies demonstrated that *Cc* seeds extract includes 17-19% fixed essential oils with high proportion of unsaturated fatty acids [19]. Furthermore, *Cc* fruits extract includes high amounts of powerful antioxidant and anti-inflammatory phytochemicals such as alkaloids, tocopherols, polyphenolics, sterol, flavonoid glycosides, cucurbitacin glucosides, tannins, flavonoids, terpenoids, carbohydrates, and cucurbitacin derivatives as well as volatile compounds, vitamins, and minerals[17&20&21]. Polyphenols and flavonoids are bioactive secondary metabolities that have antioxidant, anti-inflammatory and free radicals scavenging properties[17]. *Cc* fruits also have many clinical pharmacological properties including analgesic, anti-epileptic, anti-hyperlipidemic, antioxidant and anti-inflammatory[18] as well as anti-hyperuricemic, anti-gouty arthritic pain, anti-fungal, anti-bacterial and anti-mycotoxigenic[17&22&23]. Previous study explained that *C.colocynthis* methanolic crude extract, a powerful antioxidant extract, has a potent scavenging efficacy toward the action of XOD enzyme under *in vitro* inhibitory studies[21]. In the present study, we aimed to examine the antioxidant, uricostatic, and uricosuric properties of *Cc* fruit pulps methanolic crude extract against the potassium oxonate-induced HUA gout rat model.

MATERIALS AND METHODS

Chemicals, Kits, and Primers

Allopurinol (xanthine oxidase inhibitor, $C_5H_4N_4O$, Mw 136.11 g/mole, A8003) was purchased from Sigma-Aldrich (Merck, St. Louis, MO, USA). Other materials, chemicals, and reagents were an analytical grade and used as received. Ultrapure water (deionized water) produced by the Milli-Q synthesis system (Millipore Corp., Billerica, MA, USA). In this

study, all solutions and buffer were prepared in ultrapure deionized water. The levels of serum TBARS/MDA (nmol/mg protein), total proteins content (g/dL), and uric acid (µmol/L) were determined using Biodiagnositic kits, Egypt according to the kit instructions. Serum xanthine oxidase (XOD, EC 1.17.3.2) concentration (ng/mg protein) was measured by quantitative sandwich enzyme immunoassay technique using a rat XOD quantitative immunoassay ELISA kit according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanghai korain Biotech Co., LTD, China. Cat. No: E1263Ra). Furthermore, the activities of serum GSHPx (EC 1.11.1.9) and SOD (EC 1.15.1.1,) enzymes in mU and IU/mg protein, respectively were evaluated using commercially available assay kits (Biodiagnositic Co., Ltd., Egypt) according to the manufacturer's recommendations. GLUT9, URAT1 and β-actin primers (F/R) were purchased from Thermo Fisher Scientific Inc. (Invitrogen, USA).

Preparation of *Citrullus colocynthis* Fruit Pulps Methanolic Crude Extract

The mature fruits of *C. colocynthis* were collected between September and November 2020 from a local grocery store of Assiut city, Southern Arab Republic of Egypt. These fruits were oven dried for 5 days at 40-45℃ to manually separate the fruit pulps from their seeds. The pulps with peel were pulverized and powdered using an electrical grinding knife mill. Each 500 g from the *Cc* fruit pulps powder was soaked and extracted in 1000 mL of a water/methanol mixture (30/70, v/v) for 72 h using Soxhlet extraction method with continuous stirring at room temperature [24]**.** The fruit pulps methanolic crude extract was filtered using sterile gauzes and Wattman filter papers no. 1. This hydro-methanolic extract solution was concentrated using a rotary evaporator under vacuum at a temperature of 40-60℃. The remained concentrated *C. colocynthis* fruit pulps water extract was dried at oven temperature of 50℃. Finally, 3-5 g from the hydro-alcoholic dried extract powder was obtained, weighed, labeled and kept in a refrigerator at 4 or -20°C until usage[21&22].

Animals and Experimental Design

Fourty eight adult Wistar Albino male rats (130-150 g) were purchased from the experimental animal house, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt. The experimental animals, use, and handling procedures were conducted following the Institutional Animal Care and Use Committee (IACUC) Guidelines, which was approved by Assiut University, Institutional Animal Ethics Committee (IAEC) using ethics IRB local approval code (No. 17101772). The animals were kept in polypropylene cages covered with metallic grids and maintained at proper standard laboratory environmental conditions of temperature (22 ± 3^{0}C), light (12 h light/dark cycles) and relative humidity (60 ± 5%). All rats were supplied *ad libitum* with pure drinking water and standard normal diet (SND) during the experimental period [25]. All animals were acclimatized for one week before starting of the experimental study. The rats were randomly divided into 4 groups (**12 rats per each**) as the following **(Fig.** 1**):**

1. **Control Group:** the rats were orally administrated with 2 ml/kg/day of 0.9% saline for consecutive 14 day as a vehicle **(negative control group)**[20].
2. **PO Group:** the rats were orally administrated with 2 ml/kg/day of 0.9% saline for consecutive 14 day[20], as well as intra-peritoneal injected with 250 mg/kg/day potassium oxonate dissolved in 0.9% saline for the first continuous 7 days (**positive control group**)[25-27].
3. ***Cc*-PO Group:** the rats were orally administrated with 100 mg/kg/day methanolic crude extract of *C. colocynthis* fruits that dissolved in 0.9% saline for continuous 14 day [28, 29]**,** as well as intra-peritoneally injected with 250 mg/kg/day potassium oxonate dissolved in 0.9% saline for the first consecutive 7 days **(co-therapy group)**[25&26].
4. **Allo-PO Group:** the rats were orally administrated with 10 mg/kg/day allopurinol (reference drug) that dissolved in 0.9% saline for continuous 14 day[27&30], as well as intra-peritoneally injected with 250 mg/kg/day potassium oxonate dissolved in 0.9% saline for the first consecutive 7 days **(co-therapy group)**[25& 26].

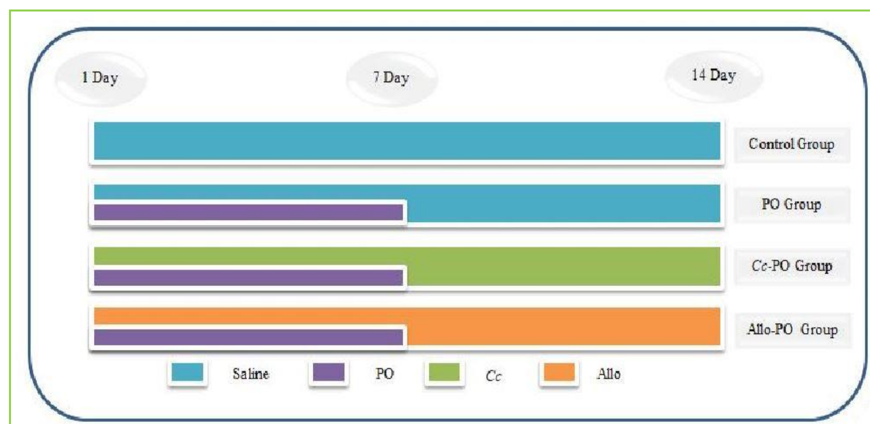

Fig. 1: Schematic diagram explains our experimental hyperuricemic gout rat model. PO, potassium oxonate; *Cc*, *C. colocynthis* fruit pulps methanolic crude extract; Allo, allopurinol as a synthetic reference drug.

Preparation of Blood, Liver, Kidney and Ankle Joint tissue Samples

The rats were sacrificed on the 14th day of the experiment under anesthesia after 12 hrs from the last drug administration. The collected blood samples were allowed to clot for 1 h at room temperature, and centrifuged at 3000-5000 rpm for 15 min at 4°C to obtain serum. The serum samples were stored at -20°C until the biochemical assays were performed. The serum samples were used to determine the levels of SUA, total proteins content, and TBARS/MDA as well as the activities of XOD, GSHPx and SOD enzymes. Furthermore, the liver and ankle joint samples were excised and rinsed in ice-cold 0.9% saline then fixed in 10% neutral buffered formalin for the pathological investigations. For molecular examinations, kidneys were quickly removed, washed in ice-cold 0.9% saline, sliced to pieces of renal tissues and stored at -80°C refrigerator until these renal pieces were used to perform the quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) analysis[25].

qRT-PCR analysis

Total RNA was extracted from the kidney tissues using an ABT total RNA mini extraction kit (spin column, ABT002, Applied Biotechnology group, USA). Total RNA was measured by an UV/Vis-Spectrophotometer (111292, Gene Quant 1300, USA). A reverse transcriptase ABT cDNA synthesis kit (Applied Biotechnology group, USA) was used to produce complementary DNA (cDNA) strands using 1-5 µg from the total isolated RNA. Furthermore, the cDNA was magnified using specific designed PCR primers for the specific target genes and 2X Willowfort *HERA^{PLUS}* SYBR® Green qPCR Master Mix Kit (WF1030800X, Willowfort, Birmingham Research and Development Park, Vincent Drive, Birmingham, B15 2SQ) by Veriti qRT-PCR thermocycler (2990218112, Applied Biosystems, USA). The used primer sequences were **URAT1** F, 5′-GACCTGCAAGCCCTAGGAAG-3′ and R, 5′-CGAAGGATCCCCATCTCACG-3′; **GLUT9** F, 5′-ATGGACAGCCCATAGATCCG-3′ and R, 5′-GTTGTTGACCAGCAGTGTGT-3′; **β-actin** F, 5′-ACGTCAGGTCATCACTATGG-3′ and R, 5′-GGCATAGAGGTCTTTACGGATG -3′[31]. The thermocycling conditions of the routine qRT-PCR analysis were 10 min at 95°C for 40 cycles, including denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s, with final elongation at 72°C for 5 min. The threshold copy numbers (C_T) of the target genes were normalized with the values (C_T) of the housekeeping gene (β-actin) (ΔC_T) that represented as a fold of control group ($\Delta\Delta C_T$). The relative mRNA expression levels of target genes were determined using the $2^{-\Delta\Delta C_T}$ method. Our qRT-PCR work was performed in the Molecular Biology Researches and Studies Institute, Faculty of Medicine, Assiut University, Assiut, Egypt.

Pathological Examinations

To evaluate ankle swelling rates (%), the perimeter (circumference) of the ankle was determined with vernier caliper at 0, 1, 2, 3, 4,

5, 6 and 7 days after intra-peritoneal injection with 250 mg/kg/day PO compared with the perimeter of the ankle control rats at the same duration [25]**.** The circumference (perimeter) was measured based on the mean of the long and short diameters of the ankle joint multiplied by 3.14. They were introduced as 0, 1, 2, 3, 4, 5, 6 and 7 perimeters. The swelling rate (%) was calculated as the following equation:

$$\text{Swelling rate (\%)} = (\text{perimeter}_n - \text{perimeter}_0)/\text{perimeter}_0 * 100$$

Where; perimeter_n represents the circumference at different days, and perimeter_0 represents the circumference at 0 day after intra-peritoneal injection with PO.

The experimental liver and ankle joint specimens were fixed for 24 hrs at room temperature in 10% neutral buffered formalin for the pathological analysis. The ankle joints were decalcified using 10% ethylenediaminetetraacetic (EDTA) acid solution[25]. After fixation, these tissues were gradually dehydrated using different concentrations of ethanol and then embedded in paraffin. The solid paraffin sections were cut with a thickness of 5-6 µm, which were stained with haematoxylin and eosin (H&E) stain for the histological investigations using a light microscope, and photomicrographs were taken[26&32].

Statistical Analysis

Data were statistically expressed as means ± standard errors of means (SEM) for 10 rats in each group. Statistical significance differences between the experimental groups ($p < 0.05$) were evaluated using analysis of one-way ANOVA with the LSD *post hoc* test of SPSS Windows Version 16.0 (SPSS, Inc., Chicago, IL, USA)[22&26].

RESULTS AND DISCUSSION

Results

C. colocynthis Crude Extract Attenuated the Levels of Serum Uric Acid, TBARS/MDA, and XOD Enzyme as well as Elevated the Levels and Activities of Serum Total Proteins Content, GSHPx, and SOD Enzymes

As described **Table 1**, after 250 mg/kg/day intra-peritoneal injection with potassium oxonate (PO) for consecutive 7 days, the hyperuricemic gout rat model demonstrated a significant ($p < 0.05$) increase in the levels of serum SUA, TBARS/MDA, and XOD enzyme and decrease in the levels of serum total proteins content and activities of serum GSHPx and SOD enzymes compared to the control group. Upon, the *Cc*- and Allo-oral administration introduced a significant ($p < 0.05$) reduction in the levels of serum SUA, TBARS/MDA, and XOD enzyme and elevation in the levels of serum total proteins content and activities of serum GSHPx and SOD enzymes compared to the PO group. The oral administration of *Cc* fruit pulps methanolic crude extract introduced a powerful anti-hyperuricemic, anti-gout, and antioxidant properties that significantly ($p < 0.05$) regulated the levels and activities of these serum biochemical parameters compared to the Allo-treated rats.

Citrullus colocynthis Crude Extract Downregulated the Relative Renal mRNA URAT1 and mRNA GLUT9 Expression Levels

As shown in Error! Reference source not found., the intra-peritoneal injected with 250 mg/kg/day PO developed a hyperuricemic gout rat model, which introduced a significant ($p < 0.05$) upregulation in the relative expression levels (fold of changes) of the renal mRNA GLUT9 urate re-absorption transporters and non-significant ($p > 0.05$) induction in the relative expression levels (fold of changes) of the renal mRNA URAT1 re-absorption transporters compared with the control group. The *Cc* fruit pulps extract- and Allo-treated hyperuricemic rats demonstrated a significant ($p < 0.05$) attenuation in the relative expression levels of the renal mRNA URATl and mRNA GLUT9 urate re-absorption transporters compared with the PO group. Furthermore, the oral administration with *Cc* fruit pulps methanolic crude extract demonstrated a significant ($p < 0.05$) improvement in the HUA features and alleviation in the relative expression levels of these renal urate transporters compared with the Allo-PO group (**Figure 2**).

Table 1: Changes in the levels and activities of some serum biochemical parameters within the different experimental rat groups.Experimental groups

Experimental groups	SUA (µmol/L)	Total proteins (g/dL)	TBARS/MDA (nmol/mg protein)	XOD (ng/mg protein)	GSHPx (mU/mg protein)	SOD (IU/mg protein)
Control	179.82 ± 3.77	6.78 ± 0.095	0.94 ± 0.026	0.157 ± 0.022	11.71 ± 0.64	3.41 ± 0.14
PO	353.87 ± 3.19[a]	5.31 ± 0.082[a]	2.23 ± 0.028[a]	0.881 ± 0.036[a]	4.46 ± 0.39[a]	1.28 ± 0.11[a]
Cc-PO	237.23 ± 3.49[ab]	6.61 ± 0.072[ab]	1.31 ± 0.014[ab]	0.411± 0.0311[ab]	8.67 ± 0.42[ab]	2.79 ± 0.13[ab]
Allo-PO	263.51 ±3.28[abc]	6.16±0.066[abc]	1.73 ±0.023[abc]	0.579 ± 0.012[abc]	5.84 ±0.19[abc]	2.18 ± 0.12[abc]

Data values are expressed as means ± SEM (n = 10/group). Statistical significance differences ($p < 0.05$) were evaluated using analysis of one-way ANOVA with LSD *post hoc* test. [a]$p < 0.05$ vs. Control group; [b]$p < 0.05$ vs. PO group; [c]$p < 0.05$ vs. *Cc*-PO group. PO, Potassium Oxonate; *Cc*, *Citrullus colocynthis* fruit pulps; Allo, Allopurinol as a reference synthetic drug; SUA, serum uric acid level; TBARS/MDA, thiobarbituric acid reactive substances (malondialdehyde) level; XOD, xanthine oxidase level; GSHPx, glutathione peroxidase activity; SOD, superoxide dismutase activity.

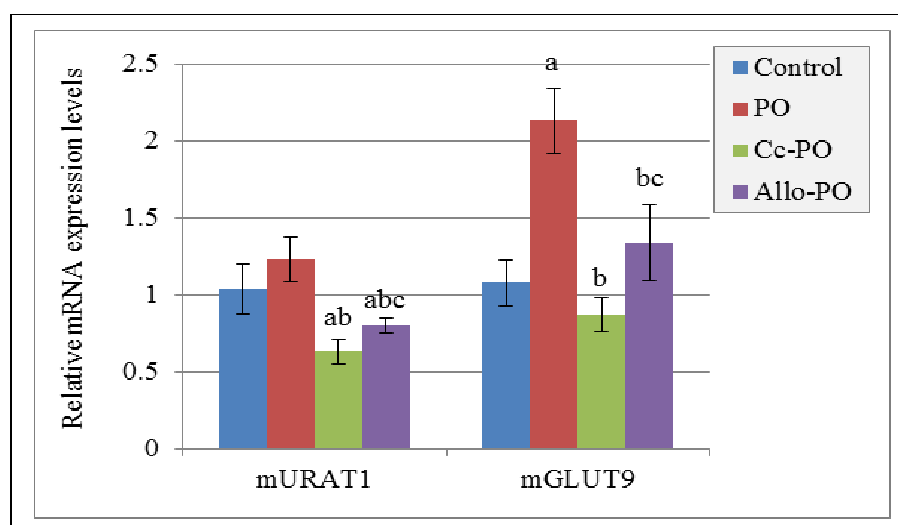

Fig. 2: Effects of *C.colocynthis* fruit pulps methanolic crude extract and allopurinol on the relative mRNA expression levels (fold of differences) of the renal mURAT1 and mGLUT9 urate re-absorption transporters of the hyperuricemic gout rat model. The relative mRNA expression levels of the renal URAT1 and GLUT9 genes were analyzed by real time RT-PCR technique, which normalized to the values of β-actin (housekeeping gene) and presented as fold of differences (fold of changes) to the control group. Data values are expressed as means ± SEM (n = 10/group). Statistical significance differences ($p < 0.05$) were evaluated using analysis of one-way ANOVA with LSD *post hoc* test. [a]$p < 0.05$ vs. Control group; [b]$p < 0.05$ vs. PO group; [c]$p < 0.05$ vs. *Cc*-PO group. PO, Potassium Oxonate; *Cc*, *Citrullus colocynthis* fruit pulps; Allo, Allopurinol as a reference synthetic drug.

Hepatoprotective and Anti-gouty Effects of *C. colocynthis* Crude Extract

As demonstrated in **Figure 3**, the intra-peritoneal injection with 250 mg/kg/day PO showed harmful effects on the rat ankle swelling rates (%). After the day 5 from consecutive intra-peritoneal injection with PO, the hyperuricemic gout rat model demonstrated a significant ($p < 0.05$) increase in the ankle swelling rates (%) compared to the control rats.

The *Cc*- and Allo oral administration introduced a significant ($p < 0.05$) decrease in the ankle swelling rates (%) compared to the PO group. Furthermore, the oral administration with 100 mg/kg/day *C. colocynthis* fruit pulps methanolic crude extract induced powerful antioxidant and anti-inflammatory properties that significantly ($p < 0.05$) reduced the rat ankle swelling, inflammation, and edema features compared to the Allo-treated rats.

To evaluate the pathological features of the liver sections, the negative control group (C) specimens showed a normal hepatic architecture with normal central vein and radiating cords of hepatocytes. These cords of hepatocytes were also separated by blood sinusoids that were lined with Kupffer cells **(Figure 4, upper panel).** The liver sections of the hyperuricemic gout rat model (PO) demonstrated a diffuse vascular degeneration in hepatocytes and showed presence of inflammatory cell reactions in the portal area (PO-I). The hepatocytes of the PO-treated rats greatly included focal areas of the necrobiotic infiltration by mononuclear cells (PO-II). The *C. colocynthis* fruit pulps oral administration (*Cc*-PO) demonstrated normal hepatocytes with slight congestion in the blood vasculates (blood vessels) **(Figure 4, upper panel)**. Furthermore, the oral administration with allopurinol (Allo-PO) as a reference synthetic drug introduced congestion in the blood sinusoids with a mild degeneration in hepatocytes **(Figure 4, upper panel).** Besides, the histopathological features of the ankle joint were also investigated. The negative control group (C) showed a normal articular surface and dermal tissue as well as a normal skin (epidermis and dermal tissues) and subcutaneous tissues above an ankle joint. The pathological ankle joint specimens of the hyperuricemic rat gout model (PO) included an extensive edema congestion in the blood vessels as well as inflammatory cell reaction and degeneration in an articular surface (PO-I and PO-II) **(Figure 4, lower panel).** The *Cc*-treated (*Cc*-PO) group showed normal subcutaneous tissues above the ankle joints with widening in an articular area. Moreover, the oral administration with allopurinol (Allo-PO) as a reference synthetic drug introduced a normal articular surface and normal skin (epidermis and dermal tissues) with a slight edema in the subcutaneous tissues **(Figure 4, lower panel).**

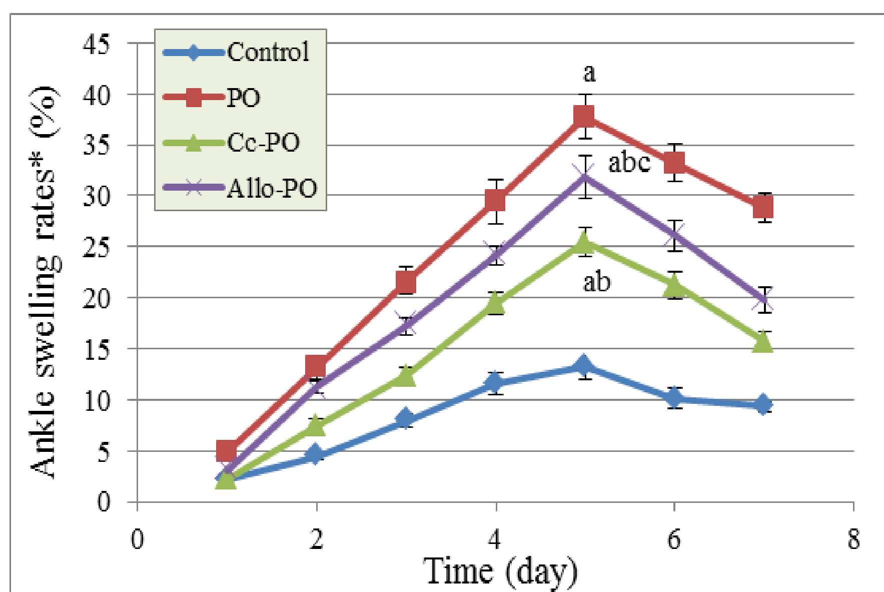

Fig.3: Changes in the swelling rates (%) of ankle joints of the different experimental rat groups.
Data values are expressed as means ± SEM (n = 10/group). Statistical significance differences (*$p < 0.05$) were evaluated by using analysis of one-way ANOVA with LSD *post hoc* test. $^a p < 0.05$ vs. Control group; $^b p < 0.05$ vs. PO group; $^c p < 0.05$ vs. *Cc*-PO group. PO, potassium oxonate; *Cc*, *Citrullus colocynthis* fruit pulps; Allo, Allopurinol as a reference synthetic drug; *, statistical significance difference ($p < 0.05$) within groups after intra-peritoneal injection with 250 mg/kg/day PO at the day 5.

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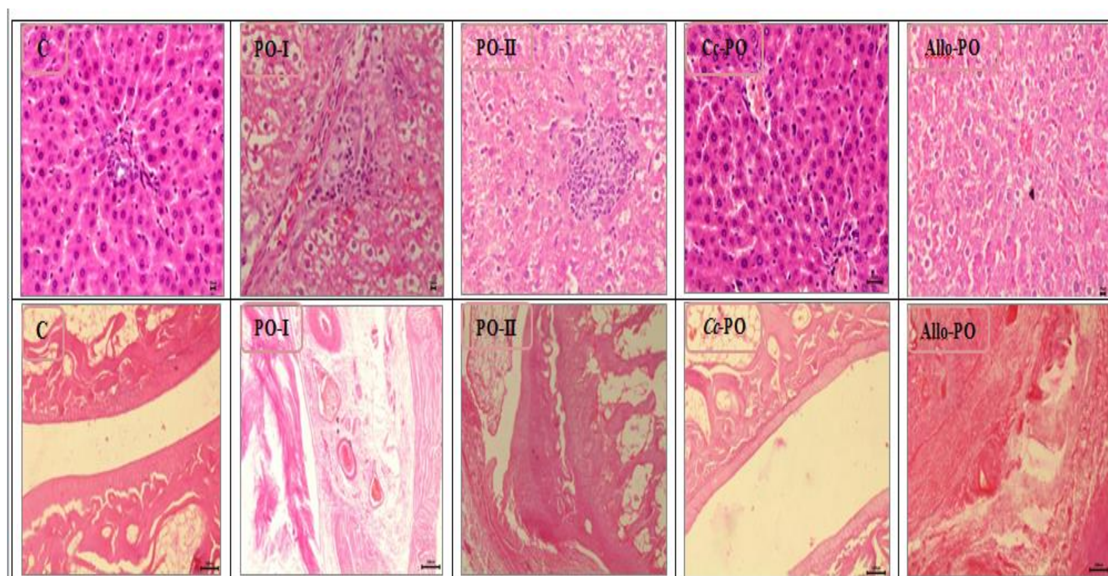

Fig. 4: The pathological evaluations of the liver and ankle joint sections via H&E staining. **C**, negative control group; **PO**, potassium oxonate-induced hyperuricemic gout rat model; ***Cc*-PO**, *C. colocynthis* fruit pulps oral administration-treated hyperuricemic rats (co-therapy group); **Allo-PO**, allopurinol oral administration-treated hyperuricemic rats (co-therapy group). The upper panel explains the different hepatic light photomicrographs with 200x magnification (n = 4). Scale bar = 20 µm. The lower panel shows the different ankle joint light photomicrographs with 200x magnification (n = 4). Scale bar = 100 µm.

Discussion

Uric acid (UA) can be handled by the physiological work of the renal tubules that regulate the renal urate glomerular filtration, re-absorption and its tubular excretion. The renal urate re-absorption and excretion transporters play a critical role to maintain the levels of the human SUA under physiological concentrations[6]. Furthermore, URAT1 is considered as a renal UA re-absorption transporter in the kidney proximal tubules, which uptakes UA from the renal lumen into the epithelial cells. GLUT9 is also considered as a renal urate re-absorption transporter that facilitates the transportation of UA from the epithelial cells into the blood stream. The disturbance between the expression levels and the activity work of these renal urate re-absorption and excretion transporters greatly introduces an increase in the levels of the renal tubular re-absorption of urate and/or decrease in the levels of its renal excretion[25]. This disturbance also elevates the blood UA level, causes HUA and increases the risk for the gout flares[9].

Gout, a MSU crystals metabolic disorder, is characterized by the high levels of SUA, the deposits of the MSU crystals into the synovial joints, tendons and the soft surrounding tissues, and acute inflammatory arthritis[32&33]. Moreover, edema, redness, acute inflammation, severe pain and stiffness of the affected joints and connective tissues are considered as the most common signs and symptoms of gout [34]. In this study, the principle strategy of gout and HUA treatment aimed to use traditional synthetic agent (Allo) and natural product (*Cc*) to examine their urate-lowering, anti-gout, and antioxidant properties against the hyperuricemic gout rat model. As reported by Yao *et al*., allopurinol, a uricostatic traditional synthetic drug, alleviated acute gouty inflammatory arthritis features and reduced the high levels of SUA through blocking the action of XOD enzyme as a XOD inhibitor[32].

Oxidative tissue injury results from the imbalance between the oxidants generation and the antioxidant defense signaling, which attenuates the activities of the antioxidant modulators and accelerates the oxidative damage of the intracellular components[35].

Under pathological conditions, XOD enzyme greatly increases the levels of SUA and oxidants[26]. Hyperuricemia (HUA) triggers accumulation of intracellular free radicals and lipid peroxidation, reduces synthesis of antioxidants and enhances oxidative stress and inflammatory responses [26]. *C. colocynthis* (L.) Schrad (bitter apple fruits) is an important medicinal plant that belongs to the family Cucurbitaceae[36]. Previous studies demonstrated that *C. colocynthis* different extracts had potent antioxidant capacities and powerful scavenging activities toward several oxidant and free radical inducers and lipid peroxidation, which included several active secondary metabolites[21&37].

In the current study, the oral administration with 100 mg/kg/day *C.colocynthis* fruit pulps methanolic crude extract for 14 consecutive days significantly ($p < 0.05$) decreased the levels of serum SUA, XOD and TBARS/MDA and increased the levels and activities of serum total proteins content, GSHPx and SOD enzymes compared to the PO and Allo-PO groups. This demonstrates that *C.colocynthis* fruit pulps had hypouricemic and antioxidant potentials, which markedly reduced the harmful effects and the cytotoxicity of the elevated levels of oxidants (SUA, XOD and TBARS/MDA) and upregulated the antioxidant mechanisms and hepatic homeostasis (total proteins content, GSHPx and SOD) compared to the PO and Allo-PO groups. In accordance with our results, the study of Ostovan *et al.* demonstrated that the extract of *C. colocynthis* pulps had a potent clinical therapeutic efficacy and oxidants scavenging capacity toward lipid peroxidation, which reduced the levels of TBARS/MDA in diabetic rats[20]. Ostovan *et al.* study also reported that *C. colocynthis* pulps had several polyphenolic compounds with antioxidant properties[20]. Park *et al.* also found that the ethanol extract of *Aster glehni* leaves inhibited the activity of XOD enzyme and decreased the levels of SUA in the PO-induced HUA rat model, which confirmed our results toward using the traditional medicinal plants as natural antioxidant sources in the treatment of HUA and gout features[38]. Moreover, previous studies reported that *Citrullus colocynthis* introduced powerful *in vitro* oxidants scavenging values toward the activity of XOD enzyme as 14.40% at 200 µg/mL *Cc* crude extract[39&40]. Recent studies reported that the alcoholic fruits extract of *C. colocynthis* had good antioxidant and anti-inflammatory properties and free radicals scavenging activities due to presence of several potent phytochemicals including gallic acid and phenolics[36]. In addition, the ethanolic fruits extract of *C. colocynthis* introduced hepatoprotective and nephroprotective actions by stimulating the antioxidant defense systems, which markedly induced hepatic and renal homeostasis, reduced the levels of MDA and nitrites and increased the levels and activities of reduced glutathione, catalase and SOD enzymes[41].

As estimated by Zhu *et al.* work, the treatment with 250 mg/kg PO developed a hyperuricemic mice model, which activated the XOD enzyme cascades, elevated the levels of SUA and upregulated the relative expression levels of mRNA URAT1 and mRNA GLUT9 urate re-absorption transporters compared to the normal mice group, which confirmed our constructed HUA gout rat model[26]. Zhu *et al.* results also indicated that the PO administration stimulated renal urate re-absorption, inhibited renal urate excretion, accumulated UA in the bloodstream and developed HUA features in the HUA mice model[26]. Our results demonstrated that the oral administration with *C. colocynthis* extract introduced a potent curative degree toward the HUA gout rat model, which markedly attenuated action of renal urate re-absorption and decreased relative mRNA expression levels of URAT1 and GLUT9 urate re-absorption transporters compared with the PO and Allo-PO groups. Previous studies introduced that several traditional medicinal plants, phytochemicals, natural products and their purifications were used to treat and manage HUA and gout, which confirmed our findings[26&31&40]. These natural products also had powerful antioxidant potentials, which alleviated HUA and gout complications, reduced the elevated levels of SUA, lipid peroxidation, oxidants generation and XOD enzyme and down-regulated the relative renal mRNA expression levels of GLUT9 and URAT1 urate transporters of the HUA animal models[26&31&40].

Our pathological evaluations for the different experimental liver and ankle joint

specimens indicated that the oral administration with *C. colocynthis* fruit pulps extract greatly improved cytotoxicity, necrotic and inflammatory features in its specimens compared to the PO and Allo-PO groups. As reported by Li et al., the intra-peritoneal injection with 300 mg/kg PO significantly accumulated UA in the animal body and induced precipitation of the MSU crystals in the joint cavity, which greatly developed ankle joint swelling, severe painful arthritis, inflammation, edema and harmful ankle joint pathological alternations[42]**.** Celery seed extracts, a natural product rich with antioxidant medicinal phytochemicals, could significantly ameliorate inflammatory responses and ankle swelling rates (%) and improve edema features of the HUA animal model[42], which confirmed our results that *Cc* fruit pulps extract as a natural antioxidant extract could greatly restore the harmful gout features of our hyperuricemic gout rat model. In addition, *Helianthus annuus*, sunflower head powder extract, strongly suppressed the high levels of SUA, XOD and ankle swelling rates (%) of the HUA and acute gouty inflammatory arthritis animal models[15]. Under pathological examinations, sunflower head powder extract also reduced the inflammatory cells and increased the ankle joint space compared with the MSU-induced acute gout model[15], which confirmed our results that introduced and administrated *Cc* fruit pulps extract to improve and restore the pathological features in the liver and ankle joint specimens of the HUA gout rat model compared with the PO and Allo-PO groups.

In conclusion, the intra-peritoneal injection with 250 mg/kg/day PO for continuous 7 days developed a HUA gout rat model. HUA gout rat model was greatly characterized by elevation of the levels of serum SUA, TBARS/MDA, XOD enzyme, and ankle joints swelling rates (%), induction of the pathological changes in its liver and ankle joint specimens as well as upregulation of the relative mRNA expression levels of renal URAT1 and GLUT9 urate transporters. Moreover, HUA gout rat model was characterized by attenuation in the levels and activities of serum total proteins content, GSHPx and SOD enzymes. These features were alleviated upon treatment with *Cc* fruit pulp extract, a potent downstream XOD inhibitor that could greatly improve HUA features and gouty inflammatory arthritis, attenuate the levels of serum SUA, TBARS/MDA, XOD, ankle joint swelling rates (%), and renal URAT1 and GLUT9 urate transporters and increase the levels and activities of serum total proteins content, GSHPx and SOD enzymes of the HUA gout rat model compared to the Allo treatment.

Ethical Statement

The experimental animals, use, and handling procedures were conducted following the Institutional Animal Care and Use Committee (IACUC) Guidelines, which was approved by Faculty of Medicine, Assiut University, Institutional Animal Ethics Committee (IAEC) using an ethics IRB local approval code (No. 17101772).

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نشرة العلوم الصيدليـــة
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الخصائص الدوائية العلاجية للمستخلص الميثانولي الخام للب فاكهة نبات الحنظل ضد أكسونات البوتاسيوم المستخدمة في أستحداث النقرس تجريبيا في ذكور الجرذان

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وفي دراستنا الحالية، كان عملناعلى النحو التالي:–
إعداد المستخلص الميثانولي الخام من لب فاكهة نبات الحنظل.
أستحداث الأرتفاع الملحوظ لمستوي حمض اليوريك بالدم وبعض مشاكل النقرس وهي الألتهابات المفصلية الحاد والمزمنة في ذكور الجرذان من خلال حقنها ب ٢٥٠ مجم / كجم / يوم من أوكسونات البوتاسيوم لمدة سبعة أيام متواصلة من المدة التجريبية المحددة للتجربة.
أستخدام ١٠٠ مجم / كجم / يوم من المستخلص الكحولي للب فاكهة نبات الحنظل و ١٠ مجم / كجم / يوم من الألبيورينول كعلاج لمدة ١٤ يوم للتحقق من الخصائص العلاجية لكليهما نحو علاج العرض الأكثر أنتشارا لمسببات النقرس وهو الأرتفاع الحاد لمستويات حمض اليوريك بالدم.
وكان الهدف من هذه الدراسة تقييم فعالية وكفاءة مستخلص نبات الحنظل و الألبيورينول على نموذج من ذكور الجرذان تم أستحداث فيها أعراض وملامح مرض النقرس من خلال.
تحديد معدلات تورم مفصل الكاحل (٪) خلال سبعة أيام بعد الحقن ب ٢٥٠ مجم / كجم / يوم من أكسونات البوتاسيوم.
تحديد مستويات حمض اليوريك بالدم.
تحديد مستويات وكفائة بعض المتغيرات المسببة لتلف بالخلايا أو المضادة للأكسدة و تشمل TBARS/MDA، XOD، GSHPx و SOD.
تحديد مستويات التغيير في التعبير الجيني النسبي للناقلات الكلوية لل يوريك (mRNA URAT1 و mRNA GLUT9) والتي تعتبر المختصة بأعاده أمتصاص وأسترجاع اليوريك للدم مره أخري.
الفحص الميكرسكوبي لتقييم عينات أنسجة الكبد ومفصل الكاحل في مجموعات الجرذان التجريبية المختلفة.
في الختام ، أوضحت هذه الدراسة أن تناول ١٠٠ مجمم / كجمم / يوم من المستخلص الميثانولي الخام للب فاكهة نبات الحنظل لمدة ١٤ يومً مستمرة قد أظهرت نتائج ملحوظة لمعالجة الأرتفاع الملحوظ ل حمض اليوريك في الدم وبعض مضادات الأكسدة في نموذج الالتهاب المفصلي النقرسي الحاد في ذكور الجرذان. حيث أستطاع نبات الحنظل بشكل ملحوظ تقليل مستويات تورم مفصل الكاحل%، خفض

معدلات التعبير الجيني النسبي للناقلات والمختصة بأعاده الأمتصاص لحمض اليوريك و الزياده الملحوظه لنشاط بعض مضادات الأكسده.

علاوة علي ذلك، أدي تناول المستخلص الميثانولي الخام لنبات الحنظل الي تحسين وظائف الكبد من خلال تقييم التصوير الميكرسكوبي لقطاعات متعددة ومختلفة لأنسجة الكبد وأيضا من خلال تحسين المستوي الكلي للبروتين بالدم.

وأيضا أثبتت الجرعه المقررة بهذة الدراسة لمستخلص نبات الحنظل أنها لها قدره علاجية و أستشفائية قد ظهرت من خلال الفحص الميكرسكوبي لقطاعات متعددة ومختلفة لخلايا الكبد ومفصل الكاحل للجرذان المستحدثة بالنقرص مقارنتا بالألبيورينول.