

# Royal jelly protects against experimentally-induced ulcerative colitis in adult male albino rats : A histological study

Original  
Article

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## ABSTRACT

**Introduction:** Ulcerative colitis (UC) is a chronic inflammatory condition characterized by acute episodes of colonic inflammation. Its pathogenesis is associated with decreased antioxidant capability. Royal Jelly (RJ) is usually used as a complementary therapy in various diseases because of its antioxidant, anti-inflammatory and immunomodulatory effects.

**Aim of the work:** To evaluate the protective effect of RJ against acetic acid-induced UC in adult male albino rats.

**Materials and Methods:** Thirty rats were divided equally into 3 groups. Group I was the control group. Group II included rats subjected to intracolonic acetic acid (AA) for induction of UC. Group III 10 rats treated with RJ (250 mg/kg/day orally) for 7 days, thereafter subjected to AA. RJ was administered for another 14 days. Rats were sacrificed after 21 days. Serum glutathione (GSH) and malondialdehyde (MDA) were assessed. Colonic sections were subjected to H&E, Periodic Acid Schiff (PAS), toluidine blue, Mallory's trichrome stains and cyclooxygenase-2 (COX-2) immunohistochemical stain.

**Results:** Group II showed significant decrease in GSH, significant increase in MDA and marked histological alterations in colon. There was a significant decrease in the number of goblet cells, but significant increases in the number of mast cells, area% of collagen fibers and COX-2 immunoreactivity compared to the control. In group III, GSH was significantly increased and MDA was significantly decreased compared to group II. The colon showed minimal changes, significant increase in the number of goblet cells, significant reduction in the number of mast cells, collagen deposition and COX-2 immunoreactivity compared to group II. When compared to the control, there was no significant difference regards the serological, histological and morphometric results, except for the number of goblet cells that revealed significant decrease.

**Conclusion:** RJ proven to protect against acetic acid-induced UC in albino rats.

**Received:** 15 November 2017, **Accepted:** 12 February 2018

**Key Words:** Acetic acid, inflammation, oxidative stress, royal jelly, ulcerative colitis.

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**ISSN:** 1110-0559, Vol. 41, No.2

## INTRODUCTION

Ulcerative colitis (UC) is an idiopathic, chronic inflammatory disorder of the intestine where patients suffer from frequent acute relapses. It is characterized by recurrent abdominal pain, prolonged diarrhea and stool with pus, blood and mucus<sup>[1]</sup>. It is featured by inflammatory remitting and relapsing lesions affecting the large intestine, involving the rectum, the sigmoid colon, descending colon, and sometimes the entire colon<sup>[2]</sup>. Several etiological factors such as genetic, immunological, and environmental have been linked with the pathophysiology of the disease<sup>[3]</sup>. Although the etiology is not completely understood, it has been usually associated with increased free radical production such as reactive oxygen species (ROS) together with reduced antioxidant capacity. Over production of ROS leads to lipid peroxidation (LPO) that inhibits the cellular antioxidant capability and finally results in prominent colonic inflammation<sup>[4]</sup>. UC is associated with migration of macrophages and lymphocytes to the intestinal mucosal tissue. Activated white blood cells produce free radicals

in the intestinal mucosal tissue, causing LPO, increased the permeability of blood vessels, thus, increasing the entrance of neutrophils and expanding of inflammation in the intestinal mucosa<sup>[5]</sup>.

Nowadays, the clinical management of UC is based on using anti-inflammatory agents such as corticosteroids, aminosalicylates, and immunosuppressants. These drugs are known to have serious adverse effects, and are not always able to keep the patients alleviated for a long time. Therefore, for disease control and prevention, numerous studies have been conducted on natural remedies particularly those with antioxidant properties<sup>[1, 5]</sup>.

Several natural products, including honey, have been suggested to have antioxidant, anti-inflammatory, and anti-ulcerative properties. Royal jelly (RJ) is a secretion of the mandibular and hypopharyngeal glands of worker honeybees to feed the adult queen bee and young larvae. Laboratory studies have reported that it exhibits anti-hyperglycemic, antioxidant, anti-inflammatory and

immunomodulatory properties. It is commonly used to supplement the medical treatment of various diseases. RJ contains numerous important compounds with biological activity, such as proteins, minerals (e.g. iron and calcium), sugars, free amino acids, fatty acids, and vitamins (mainly thiamine, niacin, riboflavin)<sup>[6,7]</sup>.

Cyclooxygenase-2 (COX-2) is an inflammatory mediator where its inducible isoform expressed in response to different pro-inflammatory molecules. It catalyzes the synthesis of prostaglandins from arachidonic acid, and the most important of which found throughout the gastrointestinal tract is Prostaglandin E2. COX-2 regulates numerous physiological functions in the digestive tract, including gastrointestinal secretion and motility, mucosal protection, and is implicated in the pathophysiology of colitis. It is up-regulated by pro-inflammatory cytokines, growth factors, bacterial toxins, tumor promoters, and in cases of colitis. There is sufficient data that inhibition of this enzyme is beneficial during treating colitis and also that RJ is capable of reducing the colonic mast cells (MCs) infiltration, that stimulate the production of COX-2<sup>[8]</sup>.

The present work aimed to study the possible protective role of royal jelly on acetic acid-induced ulcerative colitis in adult male albino rats; this was done using serological and histological methods.

## MATERIALS AND METHODS

### Chemicals

Royal Jelly (RJ) was purchased from Pharco Pharmaceutical, Alexandria, Egypt. It was in the form of gelatinous capsules, each containing 200 mg of natural royal jelly. It was prepared in distilled water.

Acetic acid (AA) solution was purchased from Merck Chemicals GmbH, Darmstadt, Germany. It was prepared in 0.9% NaCl to get a concentration of AA 4% v/v.

### Animals

Thirty adult male albino rats (200-220 gm) were housed in the laboratory animal house unit of Kasr Al-Ainy Faculty of Medicine, Cairo University. They were provided with ordinary rat chow, bred and housed in standard stainless-steel cages at a 12 h cycle of light and dark, room temperature was kept at 24±2°C and humidity maintained at 50%. All animals were kept under the same environmental conditions and had free access to water and food. They were treated in accordance with guidelines approved by the Animal Use Committee of Cairo University.

### Induction of ulcerative colitis

Rats were anesthetized with ether after fasting for 24 hours, and a 2.7 mm-diameter soft pediatric catheter was lubricated with gel and inserted 6-8 cm proximal to the anus. Two mL of 4% acetic acid v/v (prepared at the Biochemistry

Department, Faculty of Medicine, Cairo University) was instilled into the colon and rats were maintained in a supine Trendelenburg position for 30 seconds to prevent leakage. Thereafter, 1 mL of 0.9% NaCl was instilled in order to withdraw the previous solution from the colon. Exactly similar procedure was performed to the control animals but using equal volume of 0.9% NaCl instead of AA solution<sup>[3]</sup>.

### Experimental design

A total of 30 albino rats were divided equally into three groups as follows:

Group I (Control group): rats received standard rat chow diet and water and subdivided equally into:

-Subgroup IA: received 2 ml intracolonic 0.9% NaCl for 30 seconds.

-Subgroup IB: received 1 ml distilled water orally for 7 days, then were injected with 2 ml intracolonic 0.9% NaCl at day 8, and the distilled water was continued for another 14 days.

Group II (Ulcerative Colitis, UC group): rats were subjected to 2 ml intracolonic acetic acid (AA) 4% for induction of UC. And were sacrificed after 14 days<sup>[3]</sup>.

Group III (RJ group): rats received 250 mg/Kg/day of RJ orally for 7 days, followed by injection of 2 ml intracolonic AA 4% at day 8 for induction of UC, and treatment with RJ was continued for another 14 days. One capsule of RJ was dissolved in 4ml distilled water and 1 ml was received for each rat<sup>[7]</sup>.

### Serological investigations

At the end of the experiment, blood samples were taken from tail vein in collecting heparinized tubes, and serum levels of oxidative enzymes; glutathione (GSH) and malondialdehyde (MDA) were measured at the Biochemistry Department, Faculty of Medicine, Cairo University.

### Light microscopic studies

Animals from all groups were sacrificed by intraperitoneal injection of thiopental sodium (50 mg/kg)<sup>[9]</sup>. Specimens from the distal colon were fixed in 10% buffered formalin. Paraffin-embedded serial sections were cut at 5-7 µm thickness and were subjected to:

- Hematoxylin and Eosin stain<sup>[10]</sup>.
- Periodic Acid Schiff reaction<sup>[11]</sup>.
- Toluidine blue stain<sup>[11]</sup>.
- Mallory trichrome stain<sup>[11]</sup>.

• Immunohistochemical stain<sup>[10]</sup> for cyclooxygenase-2 (COX-2) antibody, which is a rabbit polyclonal antibody (Lab Vision Corporation Laboratories, catalogue number PA1-37505). Cox-2 immunopositive reaction appears as brown cytoplasmic deposits. A photomicrograph of

positive control from lung immunostained with COX-2 (catalogue number PA1- 21054) was obtained from Lab Vision Corporation Laboratories. Its detection system is Ultravision Detection System (Lab Vision Corporation Laboratories, catalogue number TP - 015- HD). Reagents Supplied in this kit were; Hydrogen Peroxide Block, Ultra V Block, Biotinylated Goat Anti-Polyvalent secondary antibody, Streptavidin Peroxidase, DAB Plus Substrate and DAB Plus Chromogen.

### **Steps of Immunohistochemical staining**

Ultrathin sections were incubated in hydrogen peroxide for 15 minutes then rinsed in phosphate buffered saline (PBS, Sigma Chemical CO.P.3813 USA). Antigen retrieval was done by boiling the sections in 10Mm citrate buffer pH 6 (Lab Vision Corporation Laboratories, cat no AP 9003) for 10 minutes and left to cool in room temperature, then rinsed in PBS. To eliminate non-specific background, sections were incubated immediately with 2 drops (=100 µl) of Ultra V Block for 5 minutes at room temperature. This was followed by incubation for 60 minutes with 2 drops of the primary antibody (this step was omitted in the negative control). Afterwards, slides were incubated for 10 minutes with 2 drops of Biotinylated Goat Anti-Polyvalent secondary antibody at room temperature then rinsed well with PBS. Sections were incubated with 2 drops of streptavidin peroxidase for 10 minutes at room temperature then washed in PBS. One drop of DAB Plus Chromogen was mixed with 2 ml of DAB Plus Substrate then applied to the slides and incubated for 10 minutes at room temperature then rinsed well with distilled water. Slides were then counterstained with Mayer's hematoxylin (Lab Vision Corporation Laboratories, cat no TA-060-MH).

### **Morphometric study**

We used Leica Qwin 500" software image analyzer computer system (Leica image system Ltd; Cambridge, England), with an objective lens of x40 magnification, whereas 10 non-overlapping randomly chosen fields were studied for each section. The following parameters were measured:

- a) The number of PAS positive goblet cells.
- b) The number of mast cells stained with toluidine blue.
- c) The area percent of collagen deposition using Mallory trichrome stain
- d) The area percent of COX-2 immunoexpression.

### **Statistical analysis**

Serum levels of GSH, MDA enzymes and the morphometric results were recorded and statistically analyzed. Data were expressed as mean and standard deviation (SD) for the quantitative variable. Data were statistically analyzed using statistical package SPSS version 16 (SPSS Inc., Chicago, USA). Comparisons between

groups were done using ANOVA (analysis of variance) followed by post hoc test for multiple comparisons between each 2 groups. The results were considered significant when  $p < 0.05$ <sup>[12]</sup>.

## **RESULTS**

### **Serum enzymes level (GSH and MDA)**

Serum GSH level decreased significantly in group II (UC group) compared to the control and group III, but it increased significantly in group III (RJ group) when compared to group II, and showed no significant difference as compared to the control. Considering MDA, there was significant increase in group II as compared to the control and group III, alongside with significant decrease in group III when compared to group II, and showed no significant difference as compared to the control (Histogram 1).

### **Histological results**

Macroscopic examination of the distal colons from rats of group II (UC group) exhibited gross lesions in the form of hyperemia, edema, inflammation and ulcerations. Whereas colons from group III (RJ group) had gross picture comparable to the control.

### **H&E-stained sections**

Colon sections from both control subgroups (IA and IB) revealed no histological difference. They showed normal mucosa, submucosa, musculosa and serosa. The mucosa was folded, intact and continuous, lined with simple columnar epithelial cells and had close regularly arranged crypts that appeared tubular in structure (Fig. 1). Group II (UC group) displayed distorted mucosa; absence of folding, loss of columnar surface cells and goblet cells, and malformed or even absent crypts (Fig. 2). There was heavy inflammatory infiltration in the mucosa and in the widened submucosa together with the presence of congested dilated blood vessels (Fig. 2). Numerous mast cells and eosinophils could be seen (Fig. 3). Group III (RJ group) showed mostly normal folded mucosa lined with surface columnar epithelial cells and close regular crypts. However, few inflammatory cells and slightly dilated blood vessels were seen in the mucosa and the minimally wide submucosa (Fig. 4). Mast cells were not detected in the control or RJ groups.

### **PAS-stained sections**

Section from the control group exhibited numerous PAS-positive goblet cells in the crypts of colonic mucosa (Fig. 5). Group II showed very few PAS-positive goblet cells (Fig. 6). Whilst group III displayed many PAS-positive goblet cells (Fig. 7).

### **Toluidine blue-stained sections**

Mast cells were not apparent in the mucosa and submucosa of the colons from the control rats (Fig. 8).

Group II showed numerous mast cells with toluidine blue positive reaction in the submucosa, with the presence of degranulated ones (Fig. 9). As regards group III, there were very few mast cells in the colonic mucosa (Fig. 10).

### Mallory's trichrome-stained sections

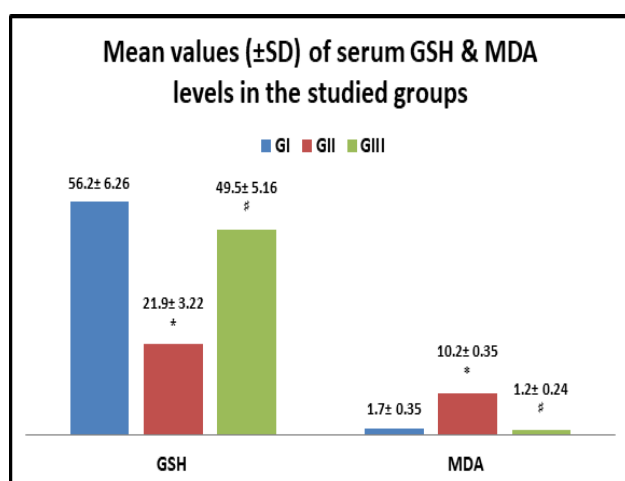
Both the control and RJ groups revealed mild collagen fibers deposition between the crypts and in the submucosa (Figs. 11 and 13). In group II, there was marked deposition of interrupted collagen fibers in the same locations (Fig. 12).

### Immunohistochemical stain results

The control and RJ groups revealed scarce brown cytoplasmic immunoreaction for COX-2 in the epithelial cells and connective tissue cells of the mucosa and submucosa (Figs. 14 and 16). Group II displayed wide-spread brown cytoplasmic immunoreaction in the same locations (Fig. 15).

### Morphometric and statistical results

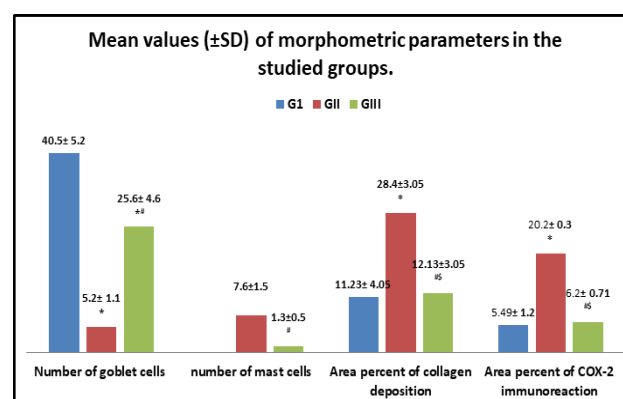
Group II (UC group) showed significant decrease in the mean number of goblet cells, with significant increase in the mean area% of collagen deposition and COX-2 immunoreaction when compared to the control. Group III (RJ group) displayed no significant difference regarding the mean area% of collagen deposition and COX-2 immunoreaction, but there was significant decrease in the mean number of goblet cells as compared to the control. However, it showed significant increase in the mean number of goblet cells, and significant decrease in the mean number of mast cells, the mean area% of collagen deposition and COX-2 immunoreaction compared to group II (Histogram 2).



**Histogram 1:** Mean values (±SD) of serum GSH and MDA levels in the studied groups

\* Significant difference versus both groups I and III.

# No significant difference versus group I.

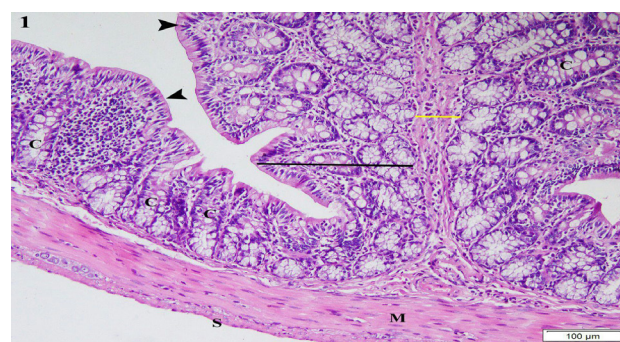


**Histogram 2:** Mean values (±SD) of morphometric parameters in the studied groups.

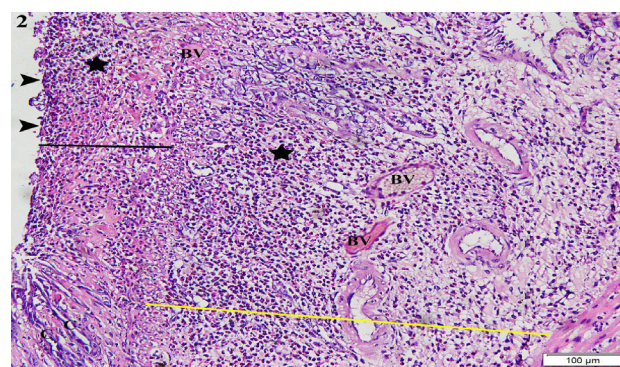
\* Significant difference versus groups I.

# Significant difference versus group II.

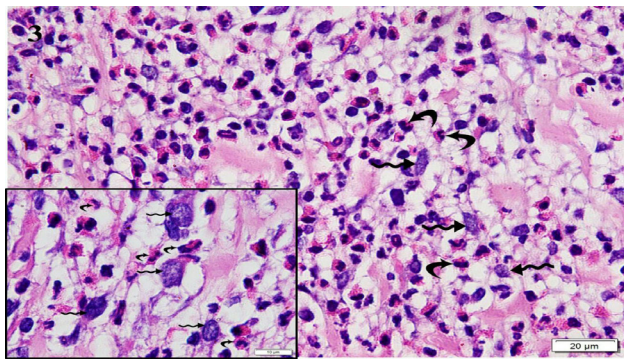
\$ No significant difference versus group I.



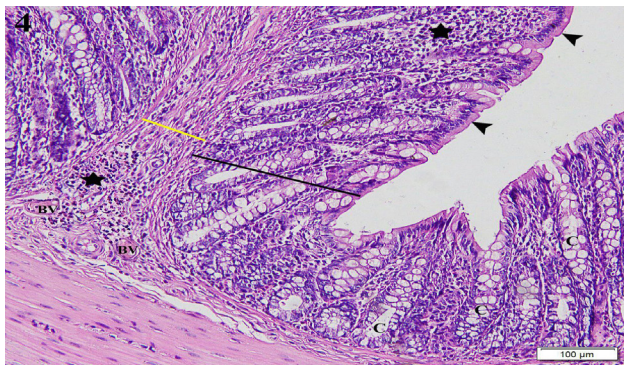
**Fig. 1:** Photomicrograph of a section in the colon from a control rat (group I) shows normal mucosa (black line), submucosa (yellow line), musculosa (M) and serosa (S). The mucosa is folded, intact and continuous, lined with simple columnar epithelial cells (arrowheads) and has close regularly arranged tubular crypts (C). (H&E, x100)



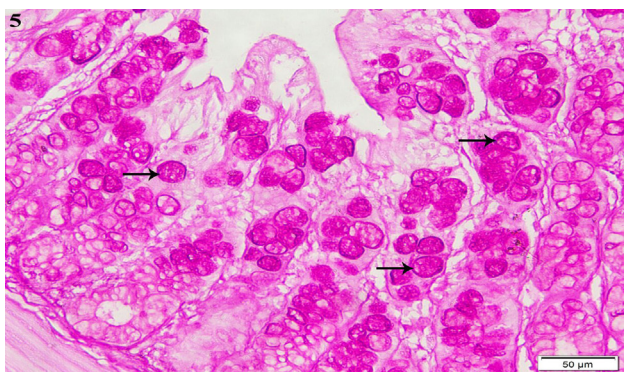
**Fig. 2:** Photomicrograph of a section in the colon from a rat with UC (group II) shows distorted mucosa (black line) with absence of folding, loss of surface columnar epithelial cells (arrowheads) and malformed or even absent crypts (C). There was heavy inflammatory infiltration (stars) in the mucosa (black line) and in the widened submucosa (yellow line), together with the presence of congested dilated blood vessels (BV). (H&E, x100)



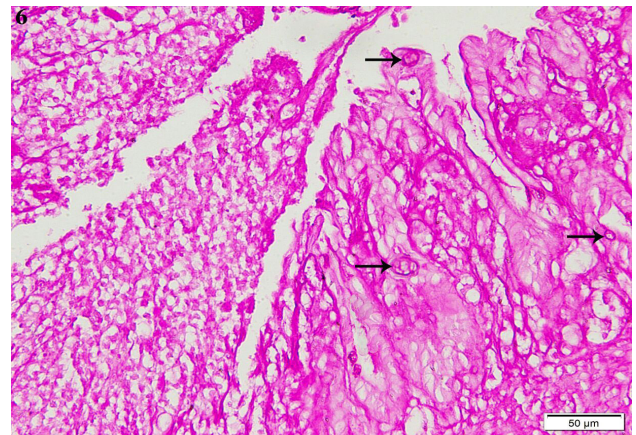
**Fig. 3:** Photomicrograph of a section in the submucosa of colon from a rat with UC (group II) shows heavy inflammatory infiltration with numerous mast cells (wavy arrows) and eosinophils (curved arrows). The inset in the lower left quadrant is for mast cells (wavy arrows) and eosinophils (curved arrows). (H&E x200, x1000)



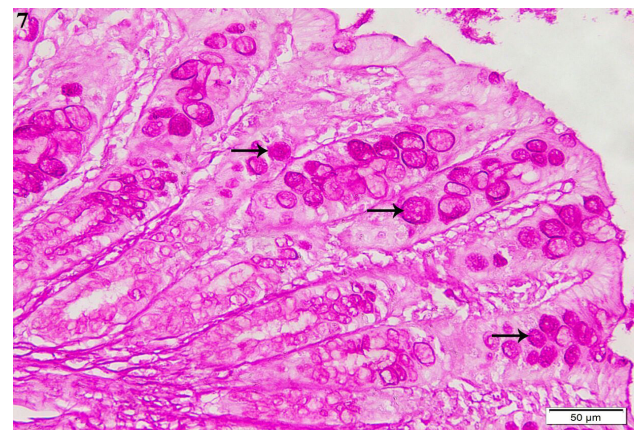
**Fig. 4:** Photomicrograph of a section in the colon from a treated rat (group III) shows mostly normal folded mucosa (black line) lined with simple columnar cells (arrowheads) and regular crypts (C). Few inflammatory cells (stars) are seen in both the mucosa (black line) and the minimally wide submucosa (yellow line). Also slightly dilated blood vessels (BV) are present in the submucosa. (H&E, x100)



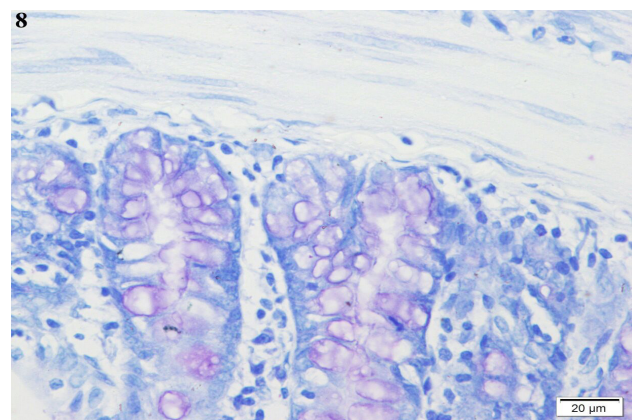
**Fig. 5:** Photomicrograph of a section in the colon from a control rat (group I) shows numerous PAS-positive goblet cells (arrows) in the crypts of colonic mucosa. (PAS, x200)



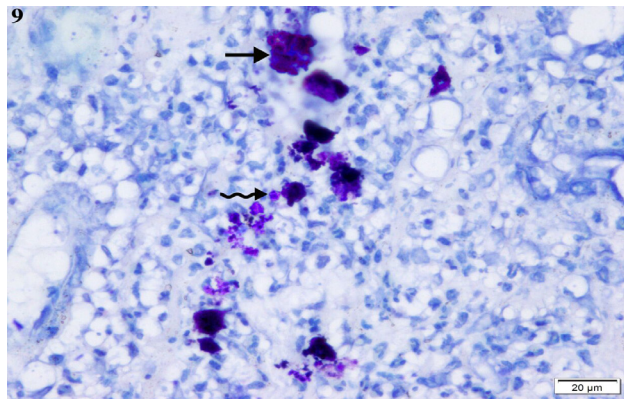
**Fig. 6:** Photomicrograph of a section in the colon from a rat with UC (group II) shows very few PAS-positive goblet cells (arrows) in the mucosal crypts. (PAS, x200)



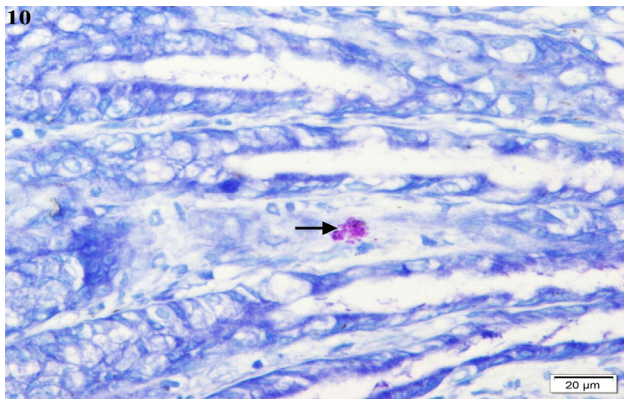
**Fig. 7:** Photomicrograph of a section in the colon from a RJ treated rat (group III) shows many PAS-positive goblet cells (arrows) in the crypts of the mucosa. (PAS, x200)



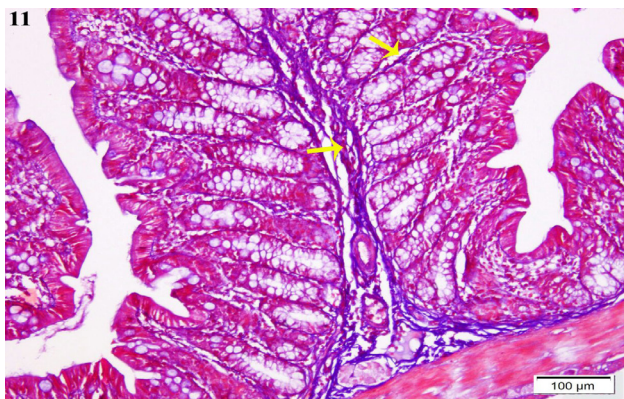
**Fig. 8:** Photomicrograph of a section in the colon from a control rat (group I) shows no apparent mast cells in the mucosa or the submucosa. (Toluidine blue, x400)



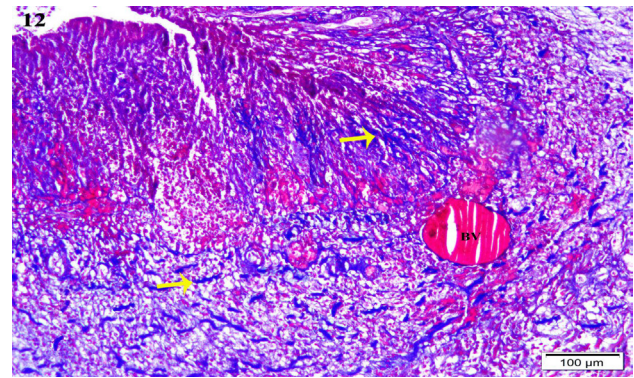
**Fig. 9:** Photomicrograph of a colonic section of a rat from UC group (group II) showing multiple mast cells with their coarse granules in the submucosa (arrow), with the presence of degranulated ones (wavy arrow). (Toluidine blue, x400)



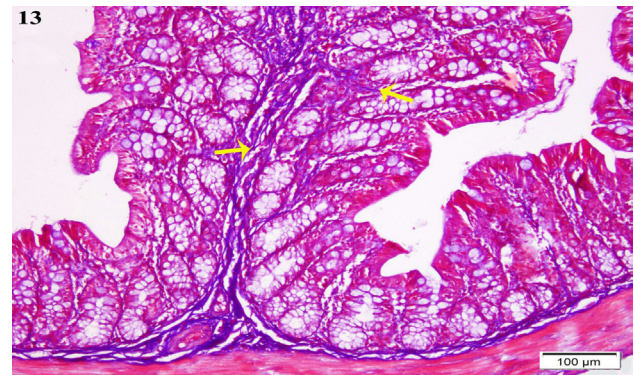
**Fig. 10:** Photomicrograph of a section in the colon of a rat from RJ group (group III), it shows a mast cell with its characteristic purple granules in the mucosa (arrow). (Toluidine blue, x400)



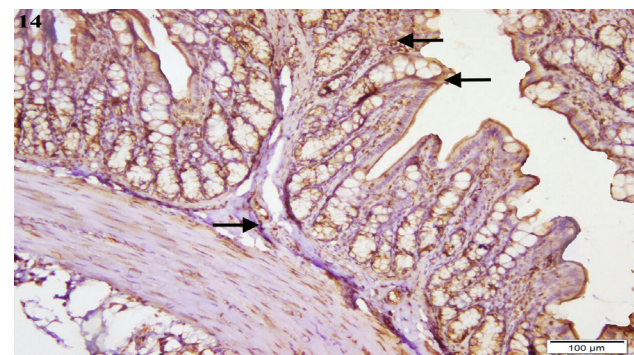
**Fig. 11:** Photomicrograph of a section in the colon from a control rat (group I) shows mild collagen fibers deposition between the crypts and in the submucosa (yellow arrows). (Mallory's trichrome, x100)



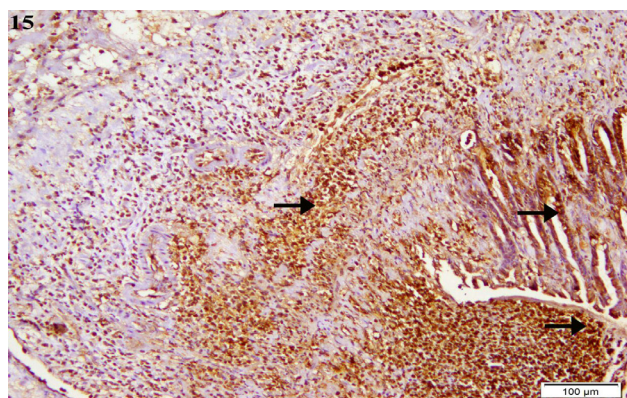
**Fig. 12:** Photomicrograph of a section in the colon from a rat with UC (group II) shows marked deposition of interrupted collagen fibers between the crypts and in the submucosa (yellow arrows). Note the presence of dilated congested blood vessel (BV) in the submucosa. (Mallory's trichrome, x100)



**Fig. 13:** Photomicrograph of a section in the colon from a treated rat (group III) shows mild collagen fibers deposition between the crypts and in the submucosa (yellow arrows). (Mallory's trichrome, x100)

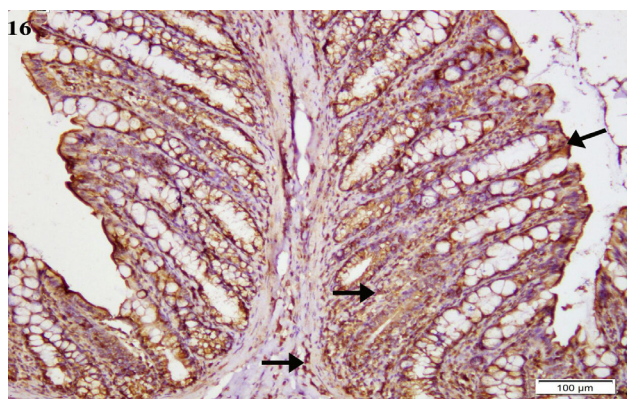


**Fig. 14:** Photomicrograph of a section in the colon from a control rat (group I) shows scarce brown cytoplasmic immunoreaction in the epithelial cells and connective tissue cells of the mucosa and submucosa (arrows). (Anti COX-2 immunohistochemical stain, x100)



**Fig. 15:** Photomicrograph of a section in the colon from a rat with UC (group II) shows wide-spread brown cytoplasmic immunopositivity in the epithelial cells and connective tissue cells of the mucosa and submucosa (arrows).

(Anti COX-2 immunohistochemical stain, x100)



**Fig. 16:** Photomicrograph of a section in the colon from a treated rat (group III) shows scarce brown cytoplasmic immunoreaction in the epithelial cells and connective tissue cells of the mucosa and submucosa (arrows).

Anti COX-2 immunohistochemical stain, x100)

## DISCUSSION

In the present study, intracolonic application of acetic acid (AA) was used in albino rats to induce acute colitis that resembles the acute episodes of ulcerative colitis (UC)<sup>[3]</sup>. Rectal administration of 4% AA to experimental rodents is a well-established animal model to induce UC, which phenotypically resembles the colon inflammation in humans<sup>[3, - 6, 13, 14]</sup>. Also, AA-induced colitis carries close resemblance to human UC in terms of pathogenesis, histopathological features and inflammatory mediator profile. The mechanism by which AA induces colitis includes the entry of protonated form of acid into the epithelial cells, where it dissociates to liberate protons resulting in intracellular acidification that may account for the epithelial injury. Moreover, it is quite possible that oxidative stress plays an essential role in the initiation and progression of colitis and appears to be the crucial

pathogenic factor where ROS production is increased in UC patients. Similarly, the characteristic feature of AA-induced colitis in animals is the imbalance between oxidant and antioxidant substances. It has been documented that inflammatory cells infiltration leads to superoxide anion production and initiation of a cascade for the production of different reactive species. This may lead to the generation of hydroxyl radicals and peroxides, which significantly result in tissue necrosis and mucosal dysfunction. Furthermore, inflammatory cells release proteases and lipid mediators that additionally contribute to intestinal injury<sup>[2]</sup>.

In the current study, the balance between oxidative and anti oxidative systems was assessed by measuring the serum levels of GSH and MDA. Whereas, GSH is a vital antioxidant that belongs to the non-enzymatic antioxidant defense system and scavenges free radicals directly, while MDA is an indicator of lipid peroxidation that is formed by free radicals during tissue damage, so it is used to measure oxidative stress (15). There was significant decrease in GSH and significant increase in MDA in UC group as compared to the control, and the reverse revealed in RJ group consistent with previous studies<sup>[1, 3, 16]</sup>.

Under normal physiological conditions, there is a balance between oxidants and antioxidants that keeps the normal function of the intestinal mucosa. Once the quantity of oxidant production exceeds the capability of endogenous antioxidant defense, the gut would be potentially subject to injury<sup>[16]</sup>. Studies have shown that GSH protects normal cells against oxidative damage by reducing the sulfhydryl groups of proteins and guarding them from reacting with free radicals. Oxidative stress and its consequent LPO are able to exacerbate free radical chain reactions and disrupt the integrity of intestinal mucosa barrier, besides, trigger inflammatory mediators, resulting in increased MDA levels<sup>[1]</sup>.

Examination of colonic sections from UC group revealed disrupted mucosa, absence of folding, distorted or even absent crypts, loss of the surface epithelium and depletion of goblet cells. There was heavy inflammatory infiltration with numerous mast cells and eosinophils in the mucosa and in the wide submucosa, with the presence of congested dilated blood vessels. These findings are consistent with earlier studies<sup>[6, 14, 17]</sup>. In addition, intra-colonic administration of AA revealed increased inflammatory cells infiltration in the intestinal tissue, massive necrosis of mucosa, vascular dilation and congestion, and submucosal edema<sup>[2]</sup>, subsequently resulted in absent crypts, congested vessels together with the widened submucosa found in the present work.

Intestinal goblet cells, secretory highly polarized cells present throughout the intestinal tract, are most abundant in the distal colon and rectum. These cells play an important defensive role in the intestine by synthesizing several mediators, including the mucin and trefoil factor-3. Under

basal conditions and following host or bacterial stimuli, goblet cells release the mucin into the lumen forming the mucus gel layer overlying the epithelium<sup>[18]</sup>. The colonic mucus plays a chief protective role against chemically-induced ulceration, and may also assist the repair of the damaged epithelium<sup>[3]</sup>. In addition, this layer lubricates the intestinal surface, limits passage of molecules from the lumen into the mucosa, and acts as a substrate and niche where the commensal-flora can colonize and derive their nutrients. Trefoil factor-3 is a potent inducer of cell migration and an inhibitor of apoptosis, so it plays a critical role in wound healing by promoting epithelial restoration following mucosal injury. Also, it synergizes with colonic mucins to augment the protective barrier properties of the mucus layer<sup>[18]</sup>. In the current work, there was significant reduction in the number of goblet cells in the mucosa of rats with UC when compared to the control group, which was in agreement with other researchers<sup>[18,19]</sup>. A unique feature of UC pathology is the major depletion of mucin producing-goblet cells and the mucus layer, which correlates with increased microbiota-induced inflammation, as UC is characterized by dysfunction of the mucosal barrier, outstandingly in epithelial goblet cells and mucus production<sup>[19]</sup>.

The colons of rats from UC group were heavily infiltrated with mast cells (MCs) and eosinophils, besides the other inflammatory cells, confirmed morphometrically by the significant increase in the number of mast cells as compared to both groups I and III. These findings are in accordance with previous authors who identified changes in MCs in cases of UC<sup>[3, 6, 20, 21]</sup>. The pathogenesis of UC is characterized by passage of granulocytes and other leucocytes to the inflamed mucosa and the superficial ulcers, elevating the levels of pro-inflammatory cytokines e.g. tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ ). These cytokines play a crucial role in modulating the mucosal immune system, as macrophages and neutrophils are responsible for disrupting the epithelium and causing colon injury<sup>[3]</sup>. The ability of MCs to release immunoregulatory and proinflammatory mediators has led to assumption that they are involved in gastrointestinal pathologies, as intestinal allergy, coeliac disease and UC. Whereas the number of intestinal MCs was increased in patients with UC, and accounted for 60% of all mucosal cells immunoreactive for TNF- $\alpha$ <sup>[6]</sup>. In addition, *in vitro* studies on MCs isolated from intestinal tissue showed increased release of histamine and eicosanoids; including eosinophil chemotactic factor and this could explain the heavy eosinophilic infiltration found in the present work. Such data support the hypothesis that MCs are prepared and pre-activated by the local inflammatory environment<sup>[22]</sup>.

The mean area % of both the collagen fibers and COX-2 immunoexpression in UC group was significantly increased when compared to the control group, coincides

with previous research<sup>[1, 6, 23, 24, 25]</sup>. Intestinal fibrosis is a common complication of UC and Chron's disease, directly following the distribution and location of inflammation. There is evidence that chronic exposure of fibroblasts to inflammatory mediators may drive their transition to activated myofibroblasts with consequent abnormal collagen production and tissue remodeling necessary for initiating the fibrotic process<sup>[26]</sup>. Noteworthy, the mechanisms regulating fibrosis appear to be distinct from those regulating inflammation. Fibrosis is a progressive and chronic process that acts through complex cell/matrix/cytokine and growth factors interactions. It results from an abnormal reaction to local injury, where there is abnormal production and deposition of extra cellular matrix by the activated myofibroblasts, these include collagen type I, II and IV, laminins and fibronectins<sup>[27]</sup>.

In our research, RJ proved its capability to protect against the AA-induced oxidative damage where it restored the balance between the oxidative enzymes to be comparable to the control; the GSH level increased significantly and the MDA level decreased significantly when compared to UC group. Additionally, it was able to protect against injury of colon tissues confirming its strong antioxidant and anti-inflammatory properties. Examined sections from RJ group demonstrated almost normal folded mucosa with regular crypts and lined with columnar epithelial cells. However, very few inflammatory cells and MCs were detected, together with slightly dilated blood vessels. Also the mean number of goblet cells was significantly increased, and the mean area % of both the collagen fibers and COX-2 immunoexpression in RJ group was significantly decreased compared to group II, consistent with previous studies carried out on RJ and its effects on UC<sup>[6,7]</sup>.

Apitherapy is the use of bee products for medical purposes. Recently, there is a growing interest, particularly in food industry, in the functional foods that are globally accepted to have benefits on human health. The widely known bee products, honey, propolis and RJ have a significant place among these functional foods (28). RJ has been proved to possess antioxidant effects on different tissues as the testis<sup>[29]</sup>, the heart<sup>[30]</sup>, the liver<sup>[31]</sup>, and in type 2 diabetes<sup>[32]</sup>. It contains water (60%-70%), proteins (12%-15%), sugar (10%-16%), fats (3%-6%), vitamins, salts, and amino acids (2%-3%). RJ exerts antibacterial and immunomodulatory effects attributed to some biologically active chemicals as 10-hydroxyl-2-decenoic acid and antibacterial proteins. Its composition differs depending on geography and climate. There is evidence that RJ increases energy, relieves anxiety, sleeplessness, and moodiness, and alleviates memory loss<sup>[30]</sup>. It is also proven to have a hypotensive effect, antitumor activity, insulin-like action<sup>[32]</sup>, anti-inflammatory, wound-healing, antibiotic and antifungal activity, and cholesterol-lowering effects. Moreover, RJ improves the fertility in men by increasing



the quality of their sperms, and in women by increasing the quality of their ova<sup>[33]</sup>.

There are two fundamental mechanisms for the action of antioxidants: First, mechanism of removal of ROS initiators that is mainly based on the inhibition of enzymes involved in the generation of ROS as xanthine oxidase and lipoxygenase. Second, a chain breaking mechanism; where the antioxidants scavenge free radicals by donating an electron to neutralize them<sup>[30]</sup>. Authors have disclosed that the protecting role of RJ against oxidative status may be via the chain breaking mechanism and extra electron donation, so scavenging free radicals<sup>[30, 32]</sup>. In addition, there is evidence indicating that vitamin E and vitamin C (which are strong antioxidants) are the main vitamins in the composition of RJ and they work mainly through the chain breaking mechanism, thus preventing the peroxidation of membrane phospholipids<sup>[30, 31]</sup>.

Several studies have discussed the anti-inflammatory effects of RJ; such as in the kidneys<sup>[28]</sup> or in soft tissues<sup>[33]</sup>. Scientists have attributed the immunomodulatory properties of RJ to the presence of neopterin that acts as a chain breaking antioxidant. This neopterin is produced by stimulated monocytes or macrophages and it depresses both the antibody production and the proliferation of immunocompetent cells.

## CONCLUSION

Oral pretreatment and co-treatment with RJ were proven to be effective in protecting the colon of rats against oxidation and inflammation caused by AA. Further studies to evaluate more parameters involved in experimental colitis are recommended.

## CONFLICT OF INTEREST

There are no conflicts of interest.

## REFERENCES

1. Yang J, Liu XX, Fan H, Tang Q, Shou ZX, Zuo DM, Zou Z, Xu M, Chen QY, Peng Y, Deng SJ, Liu YJ. Extracellular vesicles derived from bone marrow mesenchymal stem cells protect against experimental colitis via attenuating colon inflammation, oxidative stress and apoptosis. *PLoS One*. 2015; 10: e0140551.
2. Randhawa PK, Singh K, Singh N, Jaggi AS. A Review on Chemical-Induced Inflammatory Bowel Disease Models in Rodents. *The Korean Journal of Physiology and Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology*. 2014; 18(4): 279-288.
3. Al-Rejaie SS, Abuhashish HM, Al-Enazi MM, Al-Assaf AH, Parmar MY, Ahmed MM. Protective effect of naringenin on acetic acid-induced ulcerative colitis in rats. *World J Gastroenterol* 2013; 19: 5633–5644.
4. Tahan G, Aytac E, Aytakin H, Gunduz F, Dogusoy G, Aydin S, Tahan V, Uzun H. Vitamin E has a dual effect of anti-inflammatory and antioxidant activities in acetic acid-induced ulcerative colitis in rats. *Can J Surg*. 2011; 54: 333-338.
5. Dugani A, Dakhil B, Treesh S. Protective effect of the methanolic extract of malva parviflora l. leaves on acetic acid-induced ulcerative colitis in rats. *Saudi J Gastroenterol*. 2016; 22(3): 226-233.
6. Karaca T, Bayiroglu F, Yoruk M, Kaya MS, Uslu S, Comba B, Mis L. Effect of royal jelly on experimental colitis Induced by acetic acid and alteration of mast cell distribution in the colon of rats. *Eur J Histochem*. 2010; 54(4): e35.
7. Karaca T, Uz YH, Demirtas S, Karaboga I, Can G. Protective effect of royal jelly in 2,4,6 trinitrobenzene sulfonic acid-induced colitis in rats. *Iran J Basic Med Sci*. 2015; 18: 370-379.
8. Manzo LP, de-Faria FM, Dunder RJ, Rabelo-Socca EA, Consonni SR, de Almeida AC, Souza-Brito AR, Luiz-Ferreira A. Royal Jelly and its dual role in TNBS colitis in mice. *ScientificWorldJournal*. 2015; 2015: 956235.
9. Rafsanjani FN, Adeli S, Ardakani ZV, Ardakani JV, Ghotbi P. Effects of diabetes mellitus on gastric motility in rats. *Pak J Physiol*. 2009; 5: 20-24.
10. Bancroft J and Gamble M: *Theory and Practice of Histological Techniques*. 7th ed., staining methods, Churchill Livingstone, Edinburgh, London, Madrid, Melbourne, New York and Tokyo. 2008; pp: 121135- and 263 -325.
11. Suvarna SK, Layton C, Bancroft JD: *Bancroft's theory and practice of histological techniques*, 7th edition .Elsevier Health sciences, Churchill Livingstone. 2012; pp. 215- 239.
12. Emsley R, Dunn G, White IR. Mediation and moderation of treatment effects in randomised controlled trials of complex interventions. *Stat. Methods Med. Res*. 2010; 19: 237-270.
13. Hartmann RM, Morgan Martins MI, Tieppo J, Fillmann HS, Marroni NP. Effect of Boswellia

- serrata on antioxidant status in an experimental model of colitis rats induced by acetic acid. *Dig Dis Sci.* 2012; 57: 2038–2044.
14. Malago JJ, Sangu CL. Intraperitoneal administration of butyrate prevents the severity of acetic acid colitis in rats. *J Zhejiang Univ Sci B.* 2015; 16(3): 224-234.
  15. Rayaman P, Rayaman E, Cevikbaş A, Demirtunç R, Sehirli AO, Alagöz SG, Güner US. Effect of Antibiotics on Polymorphonuclear Leukocyte Functions and Myeloperoxidase Activity, Glutathione and Malondialdehyde Levels in Allergic Asthma. *Pol J Microbiol.* 2015; 64(1): 69-72.
  16. Achitei D, Ciobica A, Balan G, Gologan E, Stanciu C, Stefanescu G. Different profile of peripheral antioxidant enzymes and lipid peroxidation in active and non-active inflammatory bowel disease patients. *Digestive diseases and sciences.* 2013; 58(5): 1244–1249.
  17. Malago JJ, Nondoli H. Sodium arsenite reduces severity of dextran sulfate sodium-induced ulcerative colitis in rats. *J Zhejiang Univ Sci B.* 2008; 9(4): 341-350.
  18. Bergstrom KSB, Guttman JA, Rumi M, Ma C, Bouzari S, Khan MA, Gibson DL, Vogl AW, Vallance BA. Modulation of intestinal goblet cell function during infection by an attaching and effacing bacterial pathogen. *Infection and Immunity.* 2008; 76: 796–811.
  19. Nowarski R, Jackson R, Gagliani N, de Zoete MR, Palm NW, Bailis W, Low JS, Harman CC, Graham M, Elinav E, Flavell RA. Epithelial IL-18 equilibrium controls barrier function in colitis. *Cell.* 2015; 163(6): 1444-1456.
  20. Inamura H, Kashiwase Y, Morioka J, Suzuki K, Igarashi Y, Kurosawa M. Accumulation of mast cells in the interstitium of eosinophilic colitis. *Allergol Immunopathol (Madr)* 2006; 34: 228–230.
  21. Kashiwase PY, Inamura H, Morioka J, Igarashi Y, Kawai-Kowase K, Kurosawa M. Quantitative analysis of mast cells in benign and malignant colonic lesions: Immunohistochemical study on formalin-fixed, paraffin-embedded tissues. *Allergol Immunopathol (Madr)* 2008; 36: 271–276.
  22. Lorentz A, Sellge G, Bischoff S. Isolation and characterization of intestinal mast cells. From: *Methods in molecular biology*, Clifton, NJ. 2015; 1220: 163-177.
  23. Manetti M, Rosa I, Messerini L, Ibba-Manneschi L. Tercytes are reduced during fibrotic remodelling of the colonic wall in ulcerative colitis. *J Cell Mol Med.* 2015; 19(1): 62-73.
  24. Sklyarov AY, Panasyuk NB, Fomenko IS. Role of nitric oxide-synthase and cyclooxygenase/lipoxygenase systems in development of experimental ulcerative colitis. *J Physiol Pharmacol.* 2011; 62(1): 65-73.
  25. Sakthivel KM, Guruvayoorappan C. Amentoflavone inhibits iNOS, COX-2 expression and modulates cytokine profile, NF- $\kappa$ B signal transduction pathways in rats with ulcerative colitis. *Int Immunopharmacol.* 2013; 17(3): 907-916.
  26. Rieder F, Fiocchi C. Intestinal fibrosis in inflammatory bowel disease: progress in basic and clinical science. *Curr Opin Gastroenterol.* 2008; 24: 462–468.
  27. Specia S, Giusti I, Rieder F, Latella G. Cellular and molecular mechanisms of intestinal fibrosis. *World J Gastroenterol* 2012; 18: 3635-3661.
  28. Aslan Z, Aksoy L. Anti-inflammatory effects of royal jelly on ethylene glycol induced renal inflammation in rats. *Int Braz J Urol.* 2015; 41(5): 1008-1013.
  29. Delkhoshe-Kasmaie F, Malekinejad H, Khoramjouy M, Rezaei-Golmisheh A, Janbaze-Acyabar H. Royal jelly protects from taxol-induced testicular damages via improvement of antioxidant status and up-regulation of E2f1. *Syst Biol Reprod Med.* 2014; 60(2): 80-88.
  30. Malekinejad H, Ahsan S, Delkosh-Kasmaie F, Cheraghi H, Rezaei-Golmisheh A, Janbaze-Acyabar H. Cardioprotective effect of royal jelly on paclitaxel-induced cardio-toxicity in rats. *Iran J Basic Med Sci.* 2016; 19(2): 221-227.
  31. Nejati V, Zahmatkesh E, Babaei M. Protective Effects of Royal Jelly on Oxymetholone-Induced Liver Injury in Mice. *Iran Biomed J.* 2016; 20(4): 229-234.

32. Shidfar F, Jazayeri S, Mousavi SN, Malek M, Hosseini AF, Khoshpey B. Does Supplementation with Royal Jelly Improve Oxidative Stress and Insulin Resistance in Type 2 Diabetic Patients? *Iran J Public Health*. 2015; 44(6): 797-803.
33. Arzi A, Olapour S, Yaghooti H, Sištani Karampour N. Effect of royal jelly on formalin induced-inflammation in rat hind paw. *Jundishapur J Nat Pharm Prod*. 2015; 10(1): e22466.

## الملخص العربي

### غذاء ملكات النحل يحمي ضد التهاب القولون التقرحي المحدث تجريبياً في ذكور الجرذان البيضاء البالغة: دراسة نسيجية

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**المقدمة:** التهاب القولون التقرحي هو حالة التهابية مزمنة تتميز بنوبات حادة من التهاب القولون، وترتبط بطريقة تطور المرض مع انخفاض القدرة المضادة للأكسدة. عادة ما يستخدم غذاء ملكات النحل كمكمل علاجي في أمراض مختلفة، لما يحتوي من مواد مضادة للأكسدة ، مضادة للالتهابات ، ومعدلة للمناعة.

**الهدف من البحث:** تقييم التأثير الوقائي لغذاء ملكات النحل ضد التهاب القولون التقرحي المسبب بحمض الخليك في ذكور الجرذان البيضاء البالغة.

**مواد وطرق البحث:** قسمت ثلاثون من الجرذان الى ثلاث مجموعات متساوية، (المجموعة الأولى): المجموعة الضابطة، (المجموعة الثانية): تعرضت الجرذان لحمض الخليك داخل القولون لإحداث التهاب القولون التقرحي ، (المجموعة الثالثة) : تم إعطاء الجرذان غذاء ملكات النحل (250 ملجم/كجم/يوم) عن طريق الفم لمدة 7 أيام ، ثم تعرضت لحمض الخليك واستمر إعطاء غذاء ملكات النحل لمدة 14 يوماً أخرى. وتم التضحية بالجرذان بعد 21 يوماً. وقد تم تقييم إنزيمات الجلوتاثيون و المالوندايلدهايد. وتم صبغة شرائح القولون بصبغات الهيماتوكسيلين والإيوسين، حمض شيف الأيودي، التوليودين الأزرق، مالوري ثلاثي الألوان، والصبغة الهستوكيميائية المناعية ضد إنزيم الأكسدة الحلقية-2.

**النتائج:** أظهرت المجموعة الثانية نقص في إنزيم الجلوتاثيون وزيادة في إنزيم المالوندايلدهايد ذوا دلالة إحصائية، مصحوبا بتغيرات ملحوظة في نسيج القولون . كما وجد نقص ذو دلالة إحصائية في عدد الخلايا الكأسية، مع زيادة ذات دلالة إحصائية في عدد الخلايا البدينة، مساحة ألياف الكولاجين والظهور المناعي لإنزيم الأكسدة الحلقية-2 مقارنة بالمجموعة الضابطة. وقد أظهرت المجموعة الثالثة زيادة في إنزيم الجلوتاثيون ونقص في إنزيم المالوندايلدهايد ذوا دلالة إحصائية مقارنة بالمجموعة الثانية، كما أظهر القولون تغيرات قليلة، مع زيادة ذات دلالة إحصائية في عدد الخلايا الكأسية، وانخفاض ذو دلالة إحصائية في عدد الخلايا البدينة، مع ترسيب الكولاجين والتفاعل المناعي لإنزيم الأكسدة الحلقية-2 مقارنة بالمجموعة الثانية.

باستثناء الانخفاض ذو الدلالة الإحصائية في عدد الخلايا الكأس لم نسجل فرق ذو دلالة إحصائية في جرذان المجموعة الثالثة مقارنة بالمجموعة الضابطة وذلك فيما يتعلق بنتائج مصل الدم وكذلك النتائج والدراسات النسيجية والكمية القياسية.

**الاستنتاج:** يحمى غذاء ملكات النحل ضد التهاب القولون التقرحي المحدث تجريبياً في الجرذان البيضاء.