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Therapeutic effect of some honeybee products on alleviating symptoms of osteoarthritis in male albino rats

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ABSTRACT

Background and Aim: Honeybee products, besides their nutritional benefits, have a role in relieving the symptoms of many diseases. Thus, this work aimed to evaluate the therapeutic effect of honey, royal jelly, and venom on alleviating osteoarthritis symptoms in male albino rats.

Materials and Methods: This work was carried out from March to June 2017; 50 male albino rats were used and divided into five groups: first 10 rats as controlled group, second 10 rats as infected of arthritis by injected monosodium iodoacetate, 10 arthritic rats treated with honey as third group, 10 arthritic rats treated with royal jelly as fourth group, and 10 arthritic rats treated with venom as fifth group. From all groups, blood samples were collected, the hematological parameters—RBC, WBC, Hg, HCT, RF and CRP and biochemical parameters—serum level of sodium, potassium, and calcium were measured.

Results: Data obtained showed that significant differences with all hematological and biochemical parameters in arthritic rats cured by royal jelly, honey, and venom in RBC, Hg, and RF, respectively, whereas WBC, HCT, and CRP showed more effect in arthritic rats treated with honey, venom, and royal jelly, respectively. In the serum level of sodium and potassium, the superior effects on arthritics rats were caused by honey, followed by venom and royal jelly, respectively. Serum level of calcium was recorded high level in arthritic rats cured by venom, honey, and royal jelly, respectively.

Conclusion: The results of this work indicated that the symptoms of arthritis were alleviating in rats treated with honey, venom, and royal jelly, respectively.

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Introduction

Apitherapy or bee therapy is the medicinal use of products collected from honeybee colonies [1,2]. Products are honey, pollen grains, beeswax, propolis, royal jelly, and bee venom. The ancient Egyptians used honey in very many different medicines [3].

Bee honey is the sweet substance, which is produced by honeybee from the secretion parts of many plants [4]. Honey is produced worldwide by over 500 bee species and naturally presents some amounts of antioxidants (including flavonoids, phenolics, and carotenoids), organic acids, Maillard reaction products and amino acids in its composition, and specific sugar profile and acidity that bestow unique sensory characteristics [5].

Honeybee defends their colonies against enemies by a bee sting. Bee venom that is collected from bee workers (*Apis mellifera* L.) contains several pharmacologically active [6] substances such as melittin and histamine; Dopamine and other peptides were used as medicine for many years to cure the inflammatory diseases such as arthritis and rheumatoid conditions by direct sting or injections [7].

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Royal jelly is a natural substance, which is considered as one of the most important products of bee colonies as high nutritional, functional, and biological properties [8]. It is secreted from two glands in the bee workers head: hypopharingeal and mandibular glands, which serve as food for bee queen and young larvae [9]. Royal jelly was composed of proteins, lipids, sugars, vitamins and amino acids, and complex vitamins such as B1, B2, B6, and biotin. Moreover, it contains different minerals, trace elements with biological functions, and fatty acid (10-HDA), which play an important role in boosting the immune system and has anticancer activity [10].

Arthritis is a chronic inflammatory disorder characterized by cellular infiltration and proliferation of synovium, leading to progressive destruction of the joints [11]. Effectively treating arthritis pain is one of the most difficult challenges that clinicians can face. The pain can be debilitating and can severely restrict a patient's activities of daily living [12]. There are many remedies that purport to ease this pain; prescription pharmaceuticals and a wide range of alternative therapies such as bee products as apitherapy [13].

Thus, the aim of this work was evaluating the therapeutic effect of some bee products on male albino rats that are infected with arthritis by injection of monosodium iodoacetate.

Materials and Methods

Preparing materials of bee products

The present study was conducted from March to June months of 2017 in the apiary at Giza governorate which planting the marjoram crop and Faculty of Agriculture apiary, Cairo University. Twenty honeybee colonies of Carniolian first hybrid *Apis mellifera carnica*, which are equal in strength and exposed to the routine work during the experimental period, were used for this study to produce the honey, royal jelly, and venom.

Physiochemical properties of bee product samples

The quality of Honey was determined based on the presence of water content by the measurement of its refractive index value using ABBE WAY-IS refractometer at 20°C. The quantity of sugars (glucose and fructose) was determined by HPLC according to the method of Bogdanov et al. [14]. The electrical conductivity was determined by the method of Nombre et al. [15], using EC meter model EN50081-1 at room temperature (2 g of honey sample was dissolved in 10 ml of distilled water and the results were expressed as ppm). The optical density and color of the honey samples were measured by using the relation between optical density and USDA standard as indicated by the Association of Official Analytical Chemists [16].

The royal jelly was produced from bee colonies by a grafted technique based on the method of Grout [17] and harvested after 3 days from grafting, and samples were kept in dark bottle and stored in the deep freezer for chemical analysis. The percentage of amino acids was determined by the method of Vinas et al. [18] as gm/100 gm using amino acid analyzer apparatus (AAA400, INGOS Ltd) and the percentage of fatty acids was determined by the method reported from AOAC [16] using liquid chromatography as gm/100 gm.

Bee venom was collected according to the method of Pence [19] and the venom samples were immobilized by quick freezing at −20°c until analyzed. The samples were determined the amino acids and peptides composition using HPLC-Pico-Tag method according to Cohen and Bianchine [20]. Physiochemical and biochemical analyses of bee products were conducted in "Elements laboratory, Campus of research laboratories, FARP," Faculty of Agriculture, Cairo University Research Park.

Experimental Albino male rats

Fifty male Swiss albino rats (*Rattus norvegicus*) ranging in weight from 150–200 gm, acquired from Schistosoma Biological Supply Program (SBSP) Theodor Bilharz Research Institute, were housed in clear plastic cages (4 animals/cage) with wood chips as bedding and given pellet rodent diet, in addition of water *ad-libitum*. They were kept under controlled environmental conditions, including a temperature of 25°C, 70% humidity, and 12:12 H light/dark cycle according to AOAC [16].

Rats were allocated into two main groups. The first main group was control rats (10 rats), served as negative control and kept under normal laboratory conditions during the whole period of experimentation and were fed on a standard diet, food and water were available ad *libitum* for 4 weeks, and the second main group was induced with osteoarthritis by the monosodium iodoacetate (MIA) model of arthritis, which was induced as previously described by Janusz et al.; Sagar et al. [21,22]. Knee joint inflammation was induced by intra-articular injection of MIA (1 mg per cavity in 50 µl sterile saline) by microsyringe through the suprapatellar

ligament and into the bilateral knee joint cavity (1 mg/50 ml; Sigma-Aldrich) [23,24]. The control group rats received an intra-articular injection of 50 µl sterile saline 0.9% or underwent transaction of the medial meniscus [24,25].

The second main group (infected rats induced by monosodium iodoacetate) was classified into four subgroups (10 rats per each): the first one of arthritic rats served as positive control. The second was arthritic rats treated daily dose of 10 ml marjoram honey/kg/5 ml of distilled water [26] through oral canola for 4 weeks; the third one was arthritic rats treated daily with royal jelly (1 g/kg b/wt, orally) for 4 weeks. The last one was arthritic rats treated daily with a direct sting in rats knee (three worker bees) used daily for 4 weeks.

Determination of hematological and biochemical parameters in albino male rats

Blood samples were collected from the two main groups of male rats by the orbital plexus by means of fine capillary glass tubes, with EDTA according to Lewis et al. [27] for hematological and biochemical parameters and samples were centrifuged at 1,200 rpm for 5 minutes to obtain the serum for analyses.

The hematological parameters are count of white blood cells (WBC) x 10^3 mm^3, count of red blood cells (RBC) million/mm^3, hemoglobin concentration (Hg) % and hematocrit value (HCT) %, rheumatoid factor (RF) (IU/ml), and C-reactive protein (CRP) (mg/l). Besides that, the serum levels of sodium (mmol/l), potassium (mmol/l), and calcium (mg/100 ml) were analyzed according to the standard techniques described by Baker et al. [28].

Statistical analysis

The two-way statistical analysis of variance (ANOVA), mean separation, and correlation required subprogram of MSTAT [29] microcomputer statistical program. Simple and multiple linear regression analysis were applied and the Student "*t*"-test was used to express as the mean ± SE. Significance was considered at a level of $p < 0.01$.

Results and Discussion

Physiochemical properties of bee product samples

Bee honey

As shown in Table 1, there were clear significant differences in all tested parameters. The moisture was 18.10% ± 0.17% in marjoram honey where the electrical conductivity (EC) was 0.01% ± 0.00%, whereas the pH value was 3.84 ± 0.11 in the tested samples of marjoram honey. On the other hand, the obtained data showed that glucose content was 28.00% ± 0.00% and the fructose content was recorded 37.50 ± 0.00, while the optical density (OD.) was 0.25 ± 0.00 OD in tested samples of marjoram bee honey. The moisture values which obtained in this study are similar to those found in South Asia honey: from 15.3 to 21.7 g/100 g [30] and in North African honey (from 14.6 to 21.8 g/100 g) [31–33].

Table 1. Physiochemical parameters of the marjoram honey samples.

Physiochemical parameters (mean ± S.E)	
Moisture %	18.10 ± 0.17
Electrical conductivity %	0.01 ± 0.00
pH	3.84 ± 0.11
Glucose %	28.00 ± 0.00
Fructose %	37.50 ± 0.00
Optical density	0.25 ± 0.00

EC values were in the same range as those reported by other authors in Burkina Faso honey [15,34,35]. The sugar contents (glucose and fructose sugars) of bee honey samples are similar to those found with Escriche et al. [36], which detected the levels of glucose (from 27.8 to 31.9 g/100 g) and fructose (38.3 and 42.7 g/100 g), while pH value agrees with that obtained by Rateb [37].

Royal jelly

The analysis was done to determine the amino acids (essential and nonessential) and fatty acids percentage. As shown in Table 2, the essential amino acids means recorded 0.977% ± 0.02%, 0.965% ± 0.03%, 0.744% ± 0.02%, and 0.532% ± 0.02% in lysine, leucine, valine, and threnine, respectively, while the non-essential amino acids: aspartic, glutamic, serine, and glysine were scored 2.411% ± 0.23%, 1.101% ± 0.16%, 0.982% ± 0.03%, and 0.789% ± 0.04%, respectively.

On the other hand, the percentage of fatty acids in the royal jelly samples were 10-hydroxy-2-decenoic acid, tetracosanoic acid, and eicosanoic acid reached to 3.177% ± 0.16%, 0.312% ± 0.02%, and 0.201% ± 0.03%, respectively.

The data obtained were similar with Nabas et al. [38] who mentioned that the amino acids: valine,

Table 2. The essential, non-essential amino acids, and fatty acids of royal jelly samples.

Essential amino acids %	
Lysine	0.977±0.02
Leucine	0.965±0.03
Valine	0.744±0.02
Threnine	0.532±0.02
Non-essential amino acids %	
Aspartic	2.411±0.23
Glutamic	1.101±0.16
Serine	0.982±0.03
Glysine	0.789±0.04
Fatty acids %	
10-hydroxy-2-decenoic acid	3.177±0.16
Tetracosanoic acid	0.312±0.02
Eicosanoic acid	0.201±0.03

Table 3. Amino acids and peptides of the collected bee venom samples.

Percentage of amino acids	
Histadin	12.69±0.18
Alanine	8.01±0.27
Cysteine	7.12±0.16
Glutamic	4.77±0.33
Tyrosine	3.87±0.16
Percentage of peptides in dry weight venom	
Melittin	49.7±0.80
Apamine	2.7±0.40
Adolapin	1.2±0.51

leucine, and lysine were recorded 0.734%, 0.965%, and 0.986%, respectively, while the fatty acids were recorded 10-hydroxy-2-decenoic acid and tetracosanoic acid reached 3.158% and 0.298%, respectively. The main fatty acid present in RJ is 10-hydroxy-trans-2-decenoic acid (10-HDA); it plays an important role in boosting the immune system, anticancer activity [39].

Bee venom

The chemical composition of bee venom was analyzed to determine the amino acids and protein fraction. Table 3 illustrates that the major amino acids were histadin 12.69% ± 0.18%, alanine 8.01% ± 0.27%, and cysteine 7.12% ± 0.16%, followed by glutamic and tyrosine, which recorded 4.77% ± 0.33% and 3.87% ± 0.16%, respectively. Furthermore, the protein and peptides components were scored in dry weight of venom 49.7% ± 0.80%, 2.7% ± 0.40%, and 1.2% ± 0.51% of melittin, apamine, and adolapin, respectively.

Obtained data were similar with Rady et al. [40] who stated that the melittin, a major peptide component of bee venom, which accounts for 40%–50% of dried bee venom, is an attractive candidate for therapy of many diseases. The broad bioactive potential of BV includes antioxidant, anti-inflammatory, and cytotoxic activities. Despite the identification of the most abundant molecules in BV, some other minor compounds, together with synergistic/antagonistic effects at specific concentrations, could be involved in the reported bioactivities [41].

Determination of hematological and biochemical parameters in treated albino male rats with bee products

After injection of MIA, all of the arthritic treated rats were shown significant arthritis and hyperalgesia as compared with normal control group manifested as a significant increase in all arthritic hematological and biochemical parameters. As shown in Figure 1, the red blood cells count was affected with arthritis treatment, as well as curing by bee products; the RBC was recorded 8.567 ± 0.577 million/mm^3 in the control rats, while it reached to 7.933 ± 0.115 million/mm^3 in infected rats. On the other hand, the count of RBC in the curing rats with honey was recorded 8.008 ± 0.190 million/mm^3, whereas in curing with royal jelly was scored 8.733 ± 0.144 million/mm^3 and with bee venom was recorded 8.207 ± 0.315 million/mm^3.

The WBC count indicated clear significant differences; it was recorded 8.163 ± 0.234 × 10^3 mm^3 in control rats, while in infected rats reached to 13.53 ± 0.635 × 10^3 mm^3, and furthermore, the count of WBC in the curing rats with bee products scored 7.567 ± 0.533, 7.110 ± 0.650, and 6.407 ± 0.087 × 10^3 mm^3 with honey, royal jelly, and bee venom, respectively.

On the other hand, Figure 1 shows that the concentration of hemoglobin was recorded 13.88 ± 0.621 g/dl in control rats, while in arthritis rats, the Hg concentration was reached to 11.80 ± 0.264 g/dl, whereas in the treated albino rats with bee products, the Hg scored 11.68 ± 0.106 g/dl with honey, 12.05 ± 0.320 g/dl with royal jelly, and 11.59 ± 0.193 g/dl with curing by venom. Besides that, the HCT appears clearly, which signifies the differences between the control, arthritis, and curing albino rats. HCT in control rats was recorded 42.47% ± 0.665%, whereas in arthritic rats scored 37.17% ± 0.237%. Furthermore, HCT was reached

41.43% ± 1.433%, 42.07% ± 2.286%, and 42.97% ± 1.070% in curing rats with honey, royal jelly, and venom, respectively.

Figure 2 illustrates the differences between the controlled, arthritic, and curing rats on the serum level of the rheumatoid factor (RF) and C-reactive protein (CRP). The RF was recorded 16.233 ± 0.152 IU/ml in controlled rats, while in arthritis albino male rats reached 22.867 ± 0.461 IU/ml. On the other hand, RF was recorded 15.883 ± 0.503 IU/ml in cured rats with marjoram honey and in rats which were cured by royal jelly, the RF has scored 16.567 ± 0.534 IU/ml, while with cured rats by venom, it was reached to 15.367 ± 0.199 IU/ml.

The CRP showed significant differences between controlled rats with all treated rats; in control male rats, the CRP was recorded 6.310 ± 0.144 mg/l while in arthritis rats, it was reached to 8.040 ± 0.970 mg/l, whereas in curing rats, it was scored 6.320 ± 0.100, 5.820 ± 0.440, and 5.850 ± 0.600 mg/l with honey, royal jelly, and venom, respectively.

Data obtained in Figure 3a illustrates that the serum level of sodium in control male rats was recorded 137.1 ± 0.907 mmol/l, while it was reached to 178.1 ± 1.900 mmol/l in arthritic rats. With therapeutic by honey, royal jelly, and bee venom, the serum level of sodium was scored 140.0 ± 0.412, 139.2 ± 0.383, and 139.8 ± 0.560 mmol/l, respectively. On the other hand, Figure 3b shows that the level of potassium in the serum of control rats was 5.897 ± 0.310 mmol/l, whereas, in arthritic male rats, it was reached to 4.020 ± 0.069 mmol/l. In cured rats with honey, the level of potassium scored 5.577 ± 0.096 mmol/l, whereas with royal jelly and venom were recorded 5.260 ± 0.520 and 5.407 ± 0.907 mmol/l, respectively.

Figure 3c illustrates that the calcium level in control albino male rats was scored 10.6 ± 0.953 mg/100 ml;

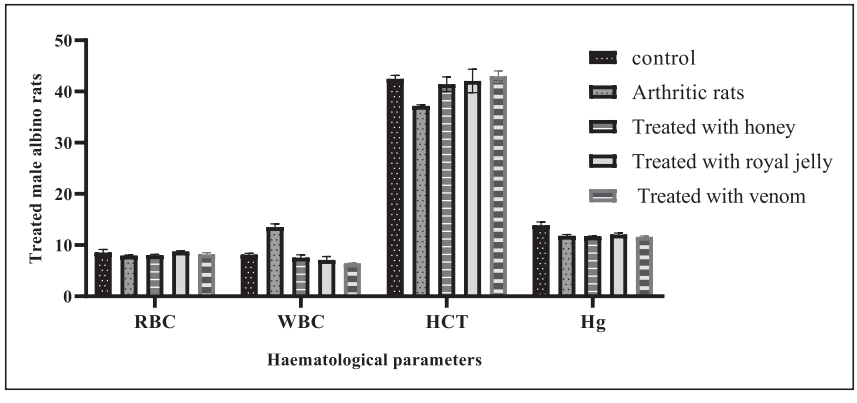

Figure 1. Effects of honey, royal jelly, and venom on the hematological parameters of RBC (million/mm^3), WBC (×10^3 mm^3), HCT (%), and Hg (g/dl) in arthritic male albino rats induced by monosodium iodoacetate.

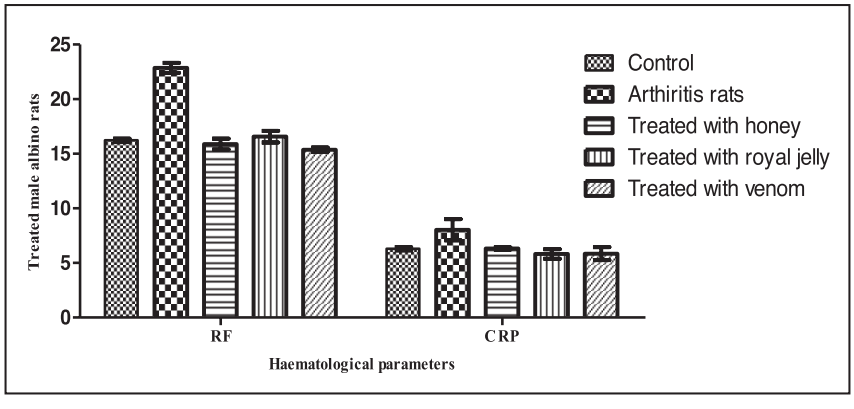

Figure 2. Effects of honey, royal jelly, and venom on the hematological parameters of RF (IU/ml) and CRP Quantities (mg/l) in arthritic male rats induced by monosodium iodoacetate.

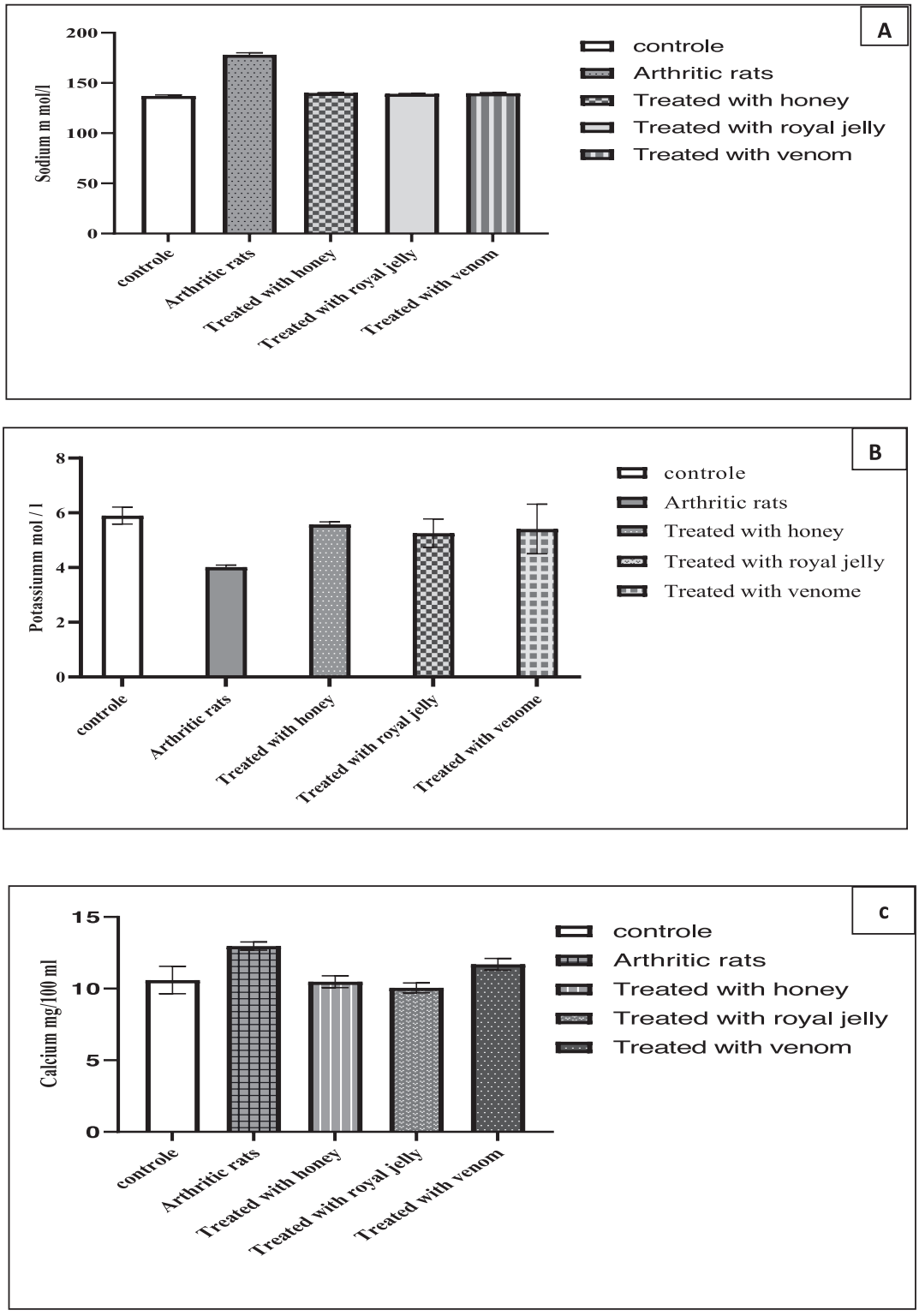

Figure 3. Effects of honey, royal jelly, and venom on the serum levels (a) sodium (mmol/l), (b) potassium (mmol/l), and (c) calcium (mg/100 ml) in arthritic male rats induced by monosodium iodoacetate.

furthermore, in serum arthritis rats, the calcium level was reached to 12.97 ± 0.288 mg/100 ml, whereas, the therapeutic of arthritic male rats with honey, royal jelly, and venom calcium level were recorded 10.48 ± 0.240, 10.05 ± 0.343, and 11.70 ± 0.408 mg/100 ml, respectively.

From the obtained data, we suggested that honey, royal jelly, and bee venom have active

pharmacological ingredients that alleviate the symptoms of many diseases; honey contains boron which avoids the hormonal unbalance that lead to osteoarthritis, and royal jelly was rich with amino acids. Venom contains at least 18 active components, including enzymes, peptides, and biogenic amines, which have a wide variety of pharmaceutical properties.

The hematological parameters: RBC, WBC, Hg, and HCT in arthritis rats were increased compared with the control group, while the therapeutic arthritis male albino rats with bee products were decreased in all hematological parameters near to the parameters in controlled rats. Furthermore, in the biochemical parameters, RF and CRP in arthritis rats were increased compared with the control group, whereas the therapeutic arthritis albino male rats with bee products were decreased in all biochemical parameters approach to the parameters in controlled rats.

In the serum level of sodium and potassium, the superior effects on arthritics rats were honey, followed by venom and royal jelly, respectively. Otherwise, the serum level of calcium was recorded high level in arthritic rats cured by venom, honey, and royal jelly, respectively.

The results we obtained were in agreement with Kohno et al. [42] who suggests that the royal jelly (RJ) contains factor(s) responsible for the suppression of pro-inflammatory cytokine secretion and it can alleviate the symptoms of arthritis. The conjugation of cell lytic peptide (melittin) with hormone receptors and gene therapy carrying melittin can be useful as a novel targeted therapy for arthritis and some types of cancer, such as prostate and breast cancer [43]. Hematologic and blood biochemistry analyses such as RBC, WBC, and Hg are valuable tools for evaluating wildlife health in diagnosing disease [44].

The venom has been found to be effective in the treatment of localized treatments of psoriasis, with minimal tolerable side effects [12]. The honey contains many components that lead to balance the hormonal factors in the body to avoid many problems such as osteoarthritis [5]. The treatment of an arthritic patient with bee sting only did not cause a significant effect on the markers of the rheumatoid patient [13]. Many studies confirm the effectiveness of Apitherapy and show mechanisms of bee products on the human body to curing some diseases such as rheumatoid arthritis [1].

Conclusion

The Apitherapy is a branch of alternative medicine that deals with the use of bee products for the therapeutic and prevention of various diseases. Obtained results illustrated that the royal jelly acts as a superior effect on curing of arthritic rats in several parameters: RBC, Hg, and RF than other products under this study followed by honey and bee venom, respectively. On the other hand, other parameters: WBC, HCT, and CRP showed active curing of the arthritic rats with honey, bee venom, and royal jelly, respectively.

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