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Therapeutic Potentials of Honey, Royal Jelly and Bee Venom on Testosterone Deficiency in Male Albino Rats Infected by $AlCl_3$

Erkek Albino Sıçanlarda $AlCl_3$ Maruziyetiyle İndüklenen Testosteron Eksikliği Üzerine Bal, Arı Sütü ve Arı Zehirinin Terapötik Potansiyeli

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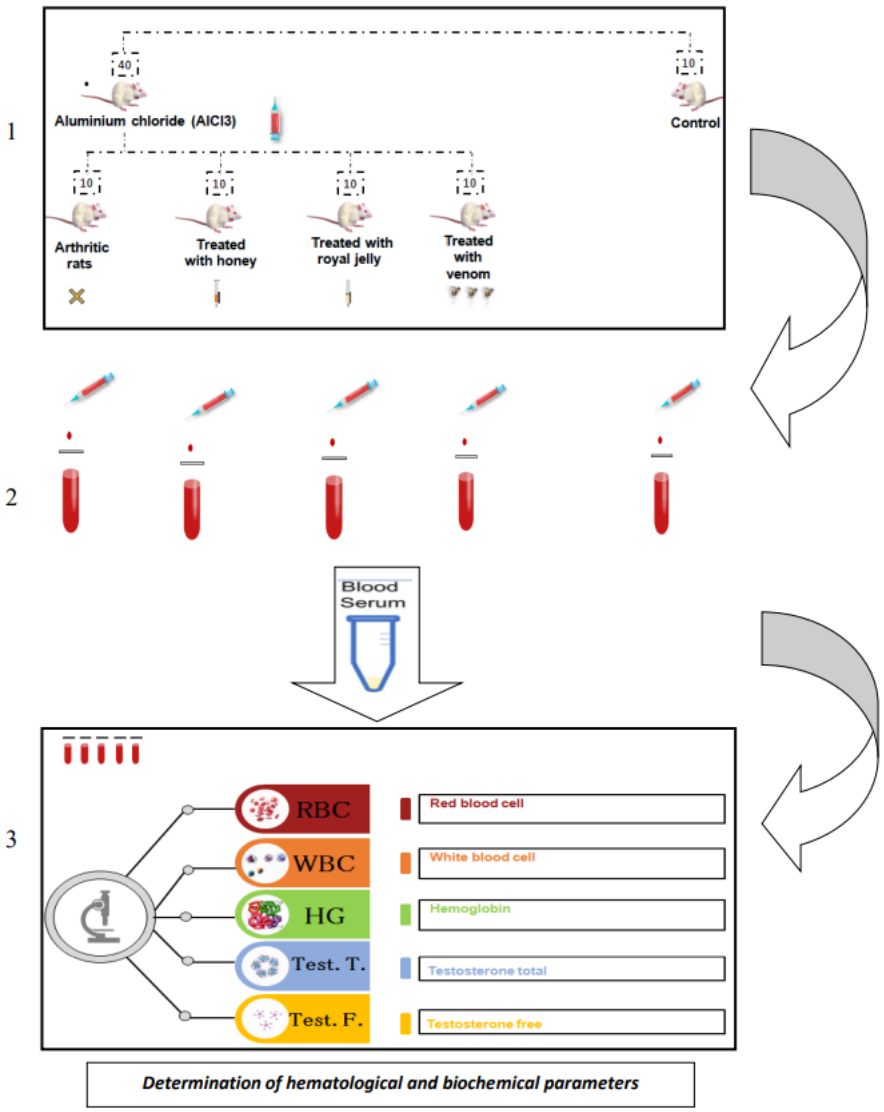
Graphical Abstract



Abstract

Honeybee products have several health benefits and claim toward various types of diseases in addition to being food. Thus, the therapeutic potentials of some bee products on testosterone deficiency in male albino rats were studied. This work was carried out from February to June 2019; fifty male rats were used and divided into five groups: 10 rats as controlled group, 10 rats were infected with Aluminium chloride ($AlCl_3$) intraperitoneally, 10 infected rats treated with honey as third group, 10 infected rats treated with royal jelly as fourth group, and 10 infected rats treated with venom as fifth group. From all groups, blood samples were collected, hematological parameters; red blood cells, white blood Cells, mean corpuscular volume, packed cell volume and hemoglobin concentration. Besides that, serum level of aldosterone hormone and blood glucose, liver functions and testosterone levels were measured. Data obtained showed significant differences with all parameters in defected rats cured by royal jelly, venom, and honey in RBCs, MCV, PCV, and Hb respectively, whereas WBCs showed more effect in infected rats treated with honey, venom, and royal jelly, respectively. Serum level of testosterone hormone, aldosterone hormone and blood glucose, superior effects on infected rats were caused by royal jelly, followed by venom and honey, respectively. On the other hand, ALP, AST and ALT were recorded high level in infected rats cured by venom, royal jelly, and honey, respectively. The results indicated that the symptoms of testosterone deficiency were alleviating in rats treated with royal jelly, venom, and marjoram honey, respectively.

Keywords: Apitherapy, Bee products, Testosterone hormone, Haematological

Özet

Bal arısı ürünlerinin sağlık üzerine birçok yararları vardır ve gıda olmanın yanında çeşitli hastalık türlerine yönelik iddiaları bulunmaktadır. Bundan dolayı erkek albino sıçanlarda bazı arı ürünlerinin testosteron eksikliği üzerindeki terapötik potansiyelleri çalışılmıştır. Bu çalışma Şubat-Haziran 2019 tarihleri arasında gerçekleştirilmiş olup elli erkek sıçan kullanıldı ve beş gruba ayrıldı: Kontrol grubu olarak 10 sıçan, intraperitoneal olarak alüminyum klorür ($AlCl_3$) ile enfekte edilen 10 sıçan, üçüncü grup olarak bal ile tedavi edilen 10 enfekte sıçan, dördüncü grup olarak arı sütü ile tedavi edilen 10 enfekte sıçan, ve beşinci grup olarak arı zehri ile tedavi edilen 10 enfekte sıçan şeklindedir. Tüm gruplardan kan örnekleri alınarak hematolojik parametreler; kırmızı kan hücreleri, beyaz kan hücreleri, ortalama korpüsküler hacim, paketlenmiş hücre hacmi ve hemoglobin konsantrasyonu, bunların yanında serum aldosteron hormonu, kan şekeri seviyeleri, karaciğer fonksiyonları ve testosteron seviyeleri ölçülmüştür. Elde edilen veriler, sırasıyla RBC'ler, MCV, PCV ve Hb değerlerinde arı sütü, arı zehri ve bal ile tedavi edilen kusurlu sıçanlarda tüm parametrelerde önemli farklılıklar gösterirken, WBC'ler sırasıyla bal, arı zehri ve arı sütü ile tedavi edilen enfekte olmuş sıçanlarda daha fazla etki göstermiştir. Testosteron hormonu, aldosteron hormonu ve kan şekerinin serum düzeylerinin, enfekte sıçanlar üzerindeki üstün etkileri sırasıyla arı sütü, ardından arı zehri ve bal tarafından sağlanmıştır. Öte yandan ALP, AST ve ALT sırasıyla arı zehri, arı sütü ve bal ile tedavi edilen enfekte sıçanlarda yüksek düzeyde bulunmuştur. Sonuçlar, sırasıyla arı sütü, arı zehri ve mercanköşk balı ile tedavi edilen sıçanlarda testosteron eksikliği semptomlarının hafiflediğini göstermiştir.

Anahtar kelimeler: Apiterapi, Arı ürünleri, Testosteron hormonu, Hematolojik

parameters, Biochemical parameters, Testosterone deficiency.
parametreler, Biyokimyasal parametreler, Testosteron eksikliği

Abbreviations: RBC, Red blood cells; WBC, White blood cells; MCV, Mean corpuscular volume; PCV, Packed cell volume; Hb, Hemoglobin Concentration; ALP, Alkaline Phosphatase; AST, Aspartate aminotransferase; ALT, Alanine Aminotransferase

1. INTRODUCTION

Apitherapy is the medicinal use of products collected from colonies of honeybee (Habryka et al., 2016; Stawiarz & Dyduch, 2014). Bee products are honey, beeswax, pollen grains, royal jelly, propolis and venom. The Egyptians ancient used bee products such as honey in very many different medicines (Hellner et al., 2008).

Bee honey is a sweet viscous substance, which is produced by worker honeybees from the sugary secretion parts of many plants (Osho & Bello, 2010). Honey is produced worldwide by more than 500 bee species and naturally presents some amounts of antioxidants (including flavonoids and phenolics), organic acids and amino acids in its composition, and specific sugar profile and acidity that bestow unique sensory characteristics (Singh et al., 2012).

Honeybee defends their colonies against enemies by a sting. The bee venom which collected from bee workers (*Apis mellifera* L.) contains several pharmacologically active substances (Bae et al., 2016) as follows; melittin, histamine, Dopamine and other peptides were used as medicine for a long time to cure the inflammatory diseases by direct sting or injections (Ali, 2014).

Royal jelly is a honeybee secretion that is used in the nutrition of worker and drone larvae, as well as larvae and adult queens (Vucevic et al., 2007), which is considered as one of the most important products of bee colonies as high nutritional, functional, and biological properties (Qu et al., 2007). It is secreted from two glands in the head of nurse bee workers: mandibular and hypopharingeal glands. Royal jelly was consisting of lipids, proteins, sugars, vitamins, amino acids, and complex vitamins; 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 (Ishmuratov et al., 2008). Recently, it can use the royal jelly to alleviating symptoms of many diseases such as testosterone deficiency (Suemaru et al., 2008).

Testosterone is the male sex hormone that is made in the testicles, testosterone hormone levels are most important to normal male sexual development and functions (Kok-Yang & Soelaiman,

2017). Testosterone levels generally decrease with age, so older men tend to have low blood testosterone levels which called testosterone deficiency, and it can be used the hematological parameters such as Red Blood Cells, White Blood Cells, mean corpuscular volume, packed cell volume and hemoglobin concentration to determine the testosterone hormone deficiency (Kok-Yang & Soelaiman, 2017). Whereas there are some conditions in which the level of the hormone testosterone decreases despite the young age, the reason may be due to men who have diabetes or overweight (Yakubu et al., 2017). There are many remedies that purport to ease this symptom; prescription pharmaceuticals and a wide range of alternative therapies such as bee products as Apitherapy (Abdel-Rahman et al., 2013).

Thus, the aim of this work was evaluating the therapeutic potentials of some bee products on male albino rats that are infected with testosterone deficiency by injection of Aluminium chloride ($AlCl_3$).

2. MATERIALS and METHODS

2.1. Preparing Sample of Bee Products

The present study was conducted from February to June months of 2019 at Giza governorate which planting the marjoram crop and Faculty of Agriculture apiary, Cairo University. Fifteen 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 marjoram honey, royal jelly and bee venom.

2.2. Physiochemical Properties of Bee Product Samples

The honey quality 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. Quantity of sugars (glucose and fructose) was determined by HPLC according to the method of Bogdanov et al. (2007). The electrical conductivity was determined by the method of Nombre et al. (2010), by using EC meter model EN50081-1 at room temperature (2g of honey sample was dissolved in 10 mL of distilled water and the results were expressed as ppm). Optical density as well as color of the honey samples was measured by using the relation between optical density and USDA standard as indicated by the Association of Official Analytical Chemists (AOAC, 2016).

The royal jelly was produced from bee colonies by a grafted technique based on the method of Grout (Grout, 1992) 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 according to Vinas et al. (1997) 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 (2016) using liquid chromatography as gm/100 gm.

Bee venom was collected according to the method of Pence (1981) 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 (1995). Physiochemical and biochemical analyses of bee products were conducted in "Elements laboratory, Campus of research laboratories, FARP," Faculty of Agriculture, Cairo University Research Park.

2.3. 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 (2016).

Two main groups of treated male rats were divided. 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 testosterone deficiency by the Aluminium chloride ($AlCl_3$) (5 mg/ kg body weight) intraperitoneally for four weeks which was induced as described by Moselhy et al. (2012).

The second main group (infected rats induced by $AlCl_3$) was classified into four subgroups (10 rats per each): the first one of infected rats served as positive control. The second was infected rats treated daily dose of 10 ml marjoram honey/kg/5 ml of distilled water (Busserolles et al., 2012) through oral canola for 4 weeks; the third one was infected rats treated daily with royal jelly (1 g/kg b/wt, orally) for 4 weeks (Busserolles et al., 2012). The last one was infected rats treated daily with a direct sting in rats knee (three worker bees) used daily for 4 weeks (Choe et al., 1986).

2.4. 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. (2006) 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 (Hb) (g/dl), mean corpuscular volume (MCV) (fl), packed cell volume (PCV) (%). Besides that, the serum levels of Aldosterone hormone (ng/l), testosterone total (pg/ml), testosterone free (pg/ml), blood glucose (mg/dl), as well as liver functions; Aspartate aminotransferase (AST) (IU/L), Alanine aminotransferase (ALT) (IU/L) and Alkaline phosphatase (ALP) (IU/L) were analyzed according to the standard techniques described by Baker et al. (1998).

2.5. Statistical Analysis

The two-way statistical analysis of variance (ANOVA), mean separation, and correlation required subprogram of MSTAT (1989) 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 $p < 0.01$.

3. RESULTS and DISCUSSION

3.1. Physiochemical Properties of Bee Product Samples

3.1.1. Marjoram Honey

As shown in Table 1, there were clear significant differences in all tested parameters. The moisture was 19.30% ± 0.18% in marjoram honey where the electrical conductivity (EC) was 0.02% ± 0.00%, whereas the pH value was 4.04 ± 0.13 in the tested samples of marjoram honey. On the other hand, the obtained data showed that glucose content was 28.04% ± 0.01% and the fructose content was recorded 36.90 ± 0.02, while the optical density (OD.) was 0.29 ± 0.01 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 (Chuttong et al., 2016) and in North African honey (from 14.6 to 21.8 g/100 g) (28–29). EC values were in the same range as those reported by other authors in Burkina Faso honey (Escriche et al., 2016; Nombre et al., 2010; Schweitzer et al., 2013).

Table 1. Physiochemical parameters of the marjoram honey samples.

Physiochemical parameters (mean ± S.E)	
Moisture %	19.30 ± 0.18
pH	4.04 ± 0.13
Fructose %	36.90 ± 0.02
Glucose %	28.04 ± 0.01
Optical density	0.29 ± 0.01
Electrical conductivity %	0.02 ± 0.00

The sugar contents (glucose and fructose sugars) of bee honey samples are similar to those found with Escriche et al. (2017), 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 (2005).

3.1.2. Collected 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.

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

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

The data obtained were similar with Nabas et al. (2013) who mentioned that the amino acids: valine, 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 (Yang et al., 2010).

3.1.3. Collected 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.

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

Percentage of amino acids	
Alanine	8.01±0.27
Glutamic	4.77±0.33
Tyrosine	3.87±0.16
Histadin	12.69±0.18
Cysteine	7.12±0.16
Percentage of peptides in dry weight venom	
Apamine	2.7±0.40
Adolapin	1.20±0.51
Melittin	49.70±0.80

Obtained data were similar with Rady et al. (2017) 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 bee venom includes antioxidant, anti-inflammatory, and cytotoxic activities. Despite the identification of the most abundant molecules in bee venom, some other minor compounds, together with synergistic/antagonistic effects at specific concentrations, could be involved in the reported bioactivities (Sobral et al., 2016).

3.2. Determination of Hematological and Biochemical Parameters in Treated Albino Male Rats with Bee Products

All of the infected treated rats were shown significant testosterone deficiency as compared with normal control group manifested as a significant increase in all infected hematological and biochemical parameters. As shown in Figure 1, the red blood cells count was affected with

$AlCl_3$ treatment, as well as curing by bee products; the RBC was recorded 8.57 ± 0.33 million/ mm^3 in the control rats, while it reached to 6.28 ± 0.16 million/mm^3 in infected rats. On the other hand, the count of RBC in the curing rats with honey was recorded 7.55 ± 0.24 million/mm^3, whereas in curing with royal jelly was scored 8.28 ± 0.20 million/mm^3 and with bee venom was recorded 8.11 ± 0.23 million/mm^3.

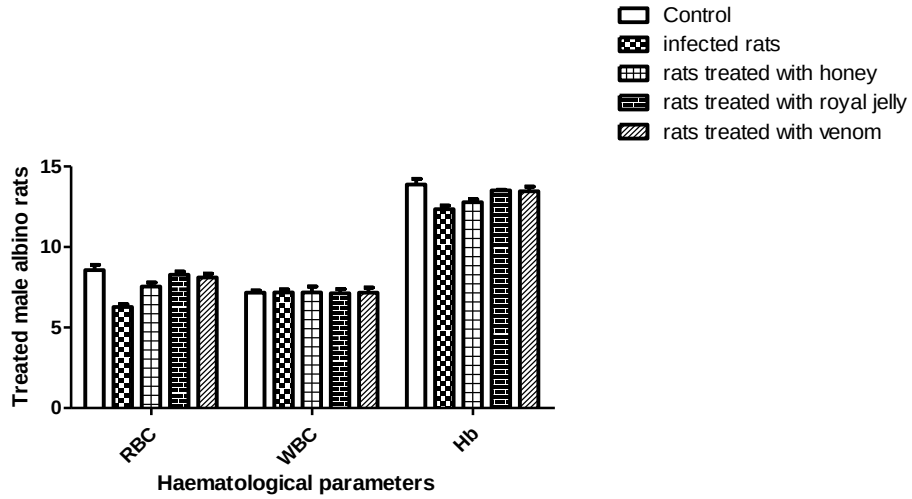


Figure 1. Effects of marjoram honey, royal jelly and venom on the haematological parameters of RBC (million/mm^3), WBC (×103 mm^3) and Hb (g/dl) in infected male albino rats induced by Aluminium chloride ($AlCl_3$).

The WBC count indicated clear significant differences; it was recorded 7.16 ± 0.14 × 10^3 mm^3 in control rats, while in infected rats reached to 7.18 ± 0.18 × 10^3 mm^3, and furthermore, the count of WBC in the curing rats with bee products scored 7.18 ± 0.36, 7.13 ± 0.26, and 7.16 ± 0.32 × 10^3 mm^3 with honey, royal jelly, and bee venom, respectively.

On the other hand, the concentration of hemoglobin was recorded 13.88 ± 0.35 g/dl in control rats, while in infected rats, the Hb concentration was reached to 12.35 ± 0.22 g/dl, whereas in the treated albino rats with bee products, the Hb scored 12.78 ± 0.19 g/dl with honey, 13.51 ± 0.05 g/dl with royal jelly, and 13.46 ± 0.29 g/dl with curing by venom.

Figure 2 illustrates the differences between the controlled, infected, and curing albino rats on the serum level of the Mean Corpuscular Volume (MCV) and Packed Cell Volume (PCV). The MCV was recorded 49.91 ± 1.99 % in controlled rats, while in infected albino male rats reached 69.17 ± 0.55 %. On the other hand, MCV was recorded 56.31 ± 1.99 % in cured rats with marjoram honey and in rats which were cured by royal jelly, the MCV has scored 51.31 ± 0.55 %, while with cured rats by venom, it was reached to 52.41 ± 1.30 %.

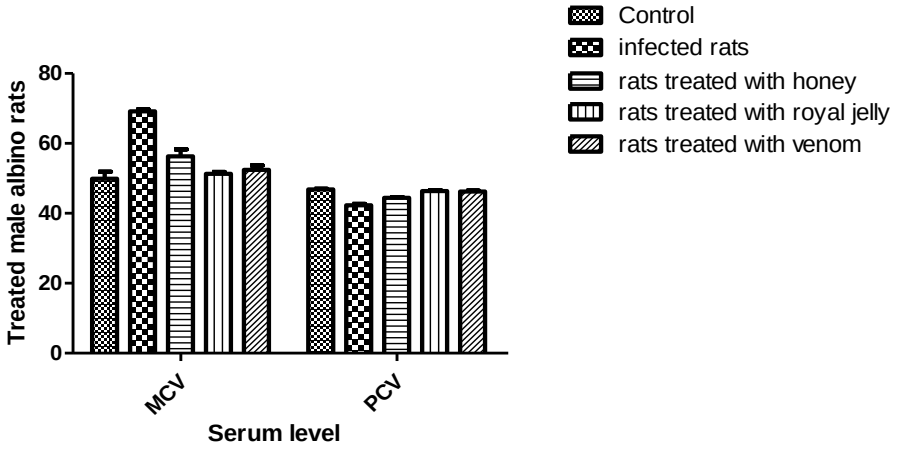


Figure 2. The effects of marjoram honey, royal jelly and venom on the serum levels of MCV (fl) and PCV (%) in testosterone deficiency male rats.

The PCV showed significant differences between controlled rats with all treated rats; in control male rats, the PCV was recorded 46.83 ± 0.22 fl while in infected rats, it was reached to 42.27 ± 0.41 fl, whereas in curing rats, it was scored 44.42 ± 0.24, 46.39 ± 0.26, and 46.22 ± 0.29 fl with honey, royal jelly, and venom, respectively.

Data obtained in Figure 3 illustrates that the serum level of Aldosterone hormone in control male rats was recorded 306.40 ± 0.67 ng/l, while it was reached to 272.33 ± 0.32 ng/l in infected rats. With therapeutic by honey, royal jelly, and bee venom, the serum level of Aldosterone hormone was scored 283.33 ± 3.33, 300.00 ± 0.00, and 295.23 ± 6.67 ng/l, respectively. Whereas the serum level of glucose showed significant differences between controlled rats with all treated rats; in control the glucose level reached to 166.30 ± 0.33 mg/dl, while in infected male rats recorded 157.29 ± 1.20 mg/dl. On the other hand, glucose was recorded 165.11 ± 0.24 mg/dl in cured rats with marjoram honey and in rats which were cured by royal jelly has scored 165.26 ± 0.33 mg/dl, while with cured rats by venom; it was reached to 164.89 ± 1.20 mg/dl.

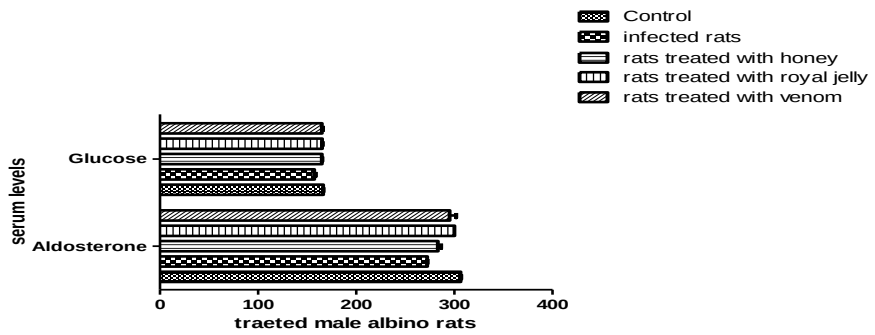


Figure 3. Effects of honeybee products on the serum level of aldosterone hormone (ng/l) and blood glucose (mg/dl) in infected male rats induced by $AlCl_3$.

Figure 4 shows that the level of liver functions; AST, ALT and ALP in the serum of control rats were 114.00 ± 0.57, 226.00 ± 0.58 and 222.31 ± 0.67 IU/L, respectively. Whereas, in infected male rats, there was reached to 116.12 ± 0.58, 252.00 ± 1.15 and 256.01 ± 1.10 IU/L, respectively. In cured rats with honey the level of liver functions scored 115.24 ± 0.33, 230.01 ± 0.33 and 228.23 ± 0.33 IU/L, respectively. Whereas with royal jelly were recorded 114.22 ± 0.33, 226.26 ± 0.25 and 225.34 ± 0.37 IU/L, respectively. While the AST, ALT and ALP in the serum of cured rats with venom scored 114.20 ± 0.38, 226.22 ± 0.32 and 225.12 ± 0.42 IU/L, respectively.

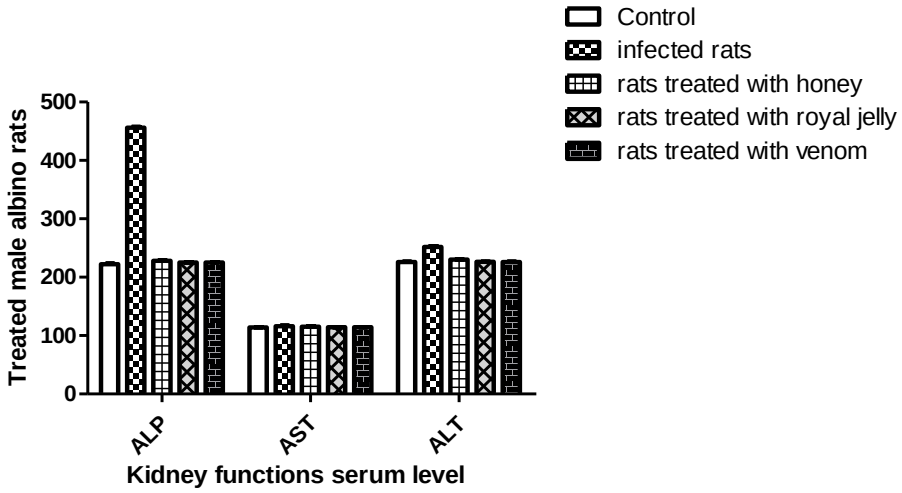


Figure 4. The serum levels of kidney functions; ALP (IU/L), AST (IU/L), and ALT (IU/L) in testosterone deficiency male rats cured by bee products.

As shown in Figure 5, the serum level of total testosterone hormone in the control male rats was scored 2.56 ± 0.08 pg/ml. While it was reached in infected rats 0.81± 0.08 pg/ml, whereas in cured rats with honey, royal jelly and venom the level of total testosterone hormone recorded 2.25 ± 0.22, 2.56 ± 0.06 and 2.46 ± 0.28 pg/ml, respectively. On the other hand, the serum level of free testosterone hormone in the control rats was scored 1.06 ± 0.08 pg/ml. While it was reached in infected rats 0.42± 0.00 pg/ml, whereas in cured rats with honey, royal jelly and venom the level of free testosterone hormone recorded 0.53 ± 0.04, 1.04 ± 0.03 and 1.01 ± 0.03 pg/ml, respectively.

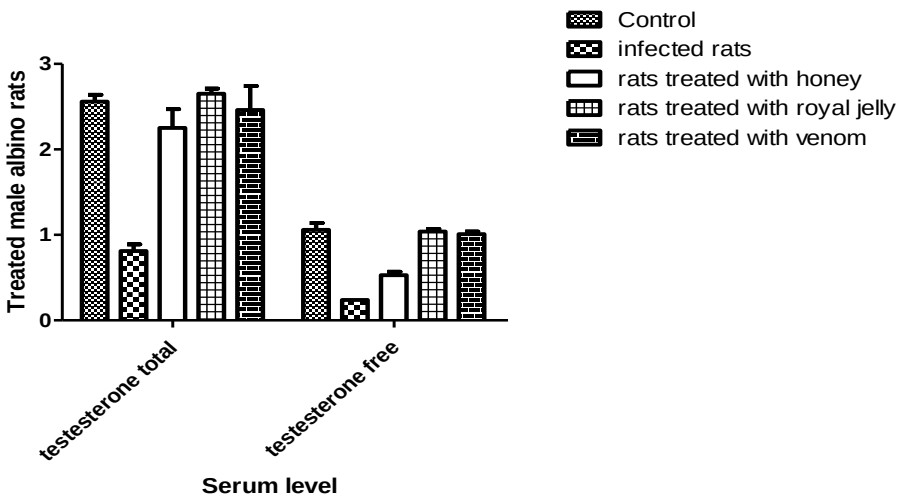


Figure 5. The serum levels of total and free testosterone hormone in testosterone deficiency male albino rats treated with marjoram honey, royal jelly and bee venom.

The present results were strengthened by those of (Nuhair, 2015; Yakubu et al., 2017) who reported that oral administration of Aluminium chloride resulted in a significant decrease in the serum testosterone in adult rats. $AlCl_3$ caused an increase in oxidative damage to sperm members, proteins and DNA (Aziz et al., 2007). This was associated with alterations in signal transduction mechanisms that affected male fertility (EbischI et al., 2006).

The organization of World Health Organization (WHO) stated that, levels of Reactive Oxygen Species (ROS) production in semen were negatively correlated with the percentage of normal sperm forms, these supported the results of the present study which indicated that there was a relationship between oxidative stress induced by Aluminium chloride ($AlCl_3$) and decrease in testosterone level (Geeta & Jain, 2017). Aluminium chloride was able to induced oxidative stress by produced a remarkable significant decrease in the ALP, AST and ALT by worsening liver functions (Huang, 2006).

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 testosterone deficiency, 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.

4. CONCLUSION

The apitherapy is a branch of alternative medicine that deals with the use of honeybee products for the therapeutic and prevention of various diseases. Obtained results illustrated that the royal

jelly acts as a superior effect on curing of testosterone deficiency rats in several parameters: RBC, MCV, PCV, Hb, Aldosterone hormone, blood glucose, free and total testosterone hormone than other products under this study followed by bee venom and honey, respectively. On the other hand, other parameters: ALP, AST, ALT and WBC showed active curing of the infected rats with bee venom, royal jelly and honey, respectively.

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