Republic of Iraq
Ministry of Higher Education
& Scientific Research
University of Al-Qadisiyah
College of Veterinary Medicine

Effect of bee venom on some immunological parameters in male albino rates induced by arthritis in comparison with prednisolone drug.

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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2021 A.D. 1442 A.H.

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ ۗ وَلَا تَعْجَلْ بِٱلْقُرْءَانِ مِن قَبْلِ أَن يُقْضَىٰٓ
إِلَيْكَ وَحْيُهُۥ ۖ وَقُل رَّبِّ زِدْنِى عِلْمًا ﴿١١٤﴾

صَدَقَ ٱللَّهُ ٱلْعَظِيمُ

من سورة طه

Certificate of Supervisor

I certify that the project entitled (**Effect of bee venom on some immunological parameters in male albino rates induced by arthritis in comparison with prednisolone drug)** was prepared by Karrar Hussain Hashem under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah.

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We certify that Karrar Hussain Hashem has finished his/her Graduation Project entitled (**Effect of bee venom on some immunological parameters in male albino rates induced by arthritis in comparison with prednisolone drug**) and candidate it for debating

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Abstract

The aim of the present study was to investigate the effects of bee venom on immunological parameters in male albino rates with arthritis and compare it with prednisolone drug (30) mature male albino rates (135-150) gm were divided into (6) groups, (5) male rates for each group, the experiment continued for 28 days. The animals were divided as follow:

the first group (G1) was injected by (0.1ml/animal) from physiological normal saline (0.9% Nacl),the second group(G2)was injected by (0.1ml/animal) formaldehyde, the third group (G3) was injected by bee venom(I.P) (1mg / kg of B.W), the fourth group (G4) was treated orally with prednisolone (5mg / kg of B.W), the fifth group (G5) was injected by bee venom(I.P) (1mg /kg of B.W), the sixth group (G6) was treated orally by prednisolone (5 mg / kg of B.W

Our results showed that G2 appeared a significant increase ($p \leq 0.05$) in the level of CRP, TNF -α, IL-B, HSP-70 and RF compared with control group and others groups the G3 and G4 groups showed a significant decrease ($p \leq 0.05$) in the level of CRP, TNF-α, IL-1B, HSP-70 and RF compared with G2. Also the results showed decrease ($p \leq 0.05$) in the G5 and G6 groups, in the levels of CRP TNF-α, TL-1B, HSP-70 and RF compared with G2.

We conclude from the present study that bee venom attenuates Rheumatoid arthritis to be developed by reducing the levels of immunological parameters such as CRP, TNF-α, IL-1B, HSP-70 and RF and bee venom was being more safety from prednisolone because the adverse effects of prednisolone drug.

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Chapter one

Introduction

Rheumatoid arthritis is one of the most autoimmune disease prevalence in the present time and it is the most common in the female at ratio 3:1 (Tripath., 2008), it causes many adverse effects in the body systems include lymphatic, hematology, respiratory and muscular system as well as kidneys (Nicola *et al*., 2006).

There are several factors involved in the pathogenesis of Rheumatoid arthritis, including environmental factors, genetic factors, chronic infections, sex hormones, smoking and stress (Lipsky, 2005). There are several proinflammatory cytokines such as Tumor neurosis Facbor α (TNF-α), IL1B, IL-6, IL-17, IL-18 and inflammatory enzyme like inducible nitric oxide (iNos) and cyclooxygenase -2 (COX-2) play a central role in the pathogenesis of RA(Ganeson *et al*.,2016). Many cytokines are known as mediators of cartilage metabolism (Han *et al*.,2016), one of these, inter leukin-1B (IL 1B) which plays a central role in the pathogenesis of Rheumatoid arthritis (Kirwan, 1995).

The pharmacological management of RA relies on treatment regimens include the use of non-steroidal anti-inflammatory drugs (NSAIDs), disease modifying anti rheumatic drugs (DMARDs) and corticosteroids, where corticosteroids are potent suppressors of the inflammatory response in many diseases (Kumar *et al*.,2015)

The researchers have been directed towards use of anti-inflammatory from natural sources due to the adverse effects of drugs that used to treat arthritis. Bee venom has both anti-inflammatory and anti-microbial effects involving no side effects in animal model (Han *et al*., 2006).

Bee venom composed of melittin, phospholipase A2, apamin and adolapin (Sciani *et al*.,2010 ;Ferreira-Junior *et al*., 2016) and has pro inflammatory, and anticancer effects (orsolic *et al*., 2003) and it is effective in the treatment of many diseases such as rheumatoid arthritis, tendinitis, multi sclerosis and wounds (Kirwan, 1995; Costanbader *et al*., 2008)

The aim of the present study was to investigate the effect of bee venom on some immunological parameters in male albino rates with arthritis and compare it with prednisolone drug.

Chapter Two

2.1 History of Rheumatoid Arthritis

The history of rheumatoid arthritis goes back to 3000 BC. When skeletal remains in North America supported the existence of the disease which was discovered 2400 years ago when Hippocrates suggested ailments of the joint (Rothschild and Woods, 1990). Sydenham (1676) and Landry-Beauvais (1800) were the first group who described this disease in their writings, but Alfred Baring Garrod in 1859 was the first who used the term rheumatoid arthritis (Fraser, 1982).

Since the early years of the 20th century, while the possibility that RA might result from an infection has been evolved, and still in the modern medicine. This disease like many other diseases of unknown causes was strongly thought to result from infection (Lipsky, 2005) .

The discovery of IgM rheumatoid factor in the serum of patients with RA was reported by Waaler (1940), In 1942 Klemperer and his workers considered that this disease might result from diffused primary degeneration of collagen. This leads to the inclusion of RA in the group of collagen diseases (Klemperer *et al.*, 1942).

In 1970, Stastny says that the first findings of an increase in HLA-Dw4 and DR4 ,associations of the different DRB1 alleles in various ethnic groups with rheumatoid arthritis susceptibility has increased gradually (Weyand and Goronzy, 1990).

The cytokines expression in rheumatoid joints began to appear in pathogenesis and treatment of disease in 1985, while Tumor Necrosis Factor-alpha (TNF-α) has early reckoned in 1988 as a potent cytokine that exerts diverse effects by stimulating a variety of cells. in RA treatment (Paramalingam *et al.*, 2007).

2.2 Epidemiology :-

Rheumatoid Arthritis is an ancient autoimmune disease. The prevalence of RA is consistent worldwide, affecting 0.5-1 % of the general population. Although the disease effects people all over the world, certain populations demonstrate particularly low or high prevalence (Wolfe, 1968; Markenson, 1992). As many as 2%–3% of those over age 60 (Rasch *et al*., 2003;Kvien, 2004).

Although no present reports about clustering in special place or time, the prevalence estimate has varied from 0.5% to 2% for Caucasian European and 0.9% to 1.1% for North America (Cope, 2008). Despite similar prevalence estimates for these geographically diverse populations, greater diversity has been documented for rural African populations. The prevalence is as low as 0.1% in Native America including the Pima, Yakima and Chippewa tribes. The prevalence may be reached 5.3% to 6% (Sliman and Hochberg, 1993). However, there are some interesting exceptions for incidence in China, Indonesia and Japan, somewhat rare or lower than (0.2% - 0.3%), while there is no a single find of RA case in Nigeria (Klippel *et al.*, 2001; Cope, 2008)Other studies have showed the prevalence rates of RA in developing countries approximately 0.5% - 1% of adult populations (Gabriel and Michaued , 2009). Overall, the prevalence rates of rheumatoid arthritis in Egypt is clearly lower (0.2%), while in Oman is (0.4%), and (0.5%) in Turkey (Gabriel and Michaued, 2009).(0.22%) in Saudi Arabia (Al-Dalaan *et al.,* 1998).

In Iraq, however, lower incidence has been reported by (Al- Rawi *et al.*,1997) that they have showed that prevalence of around 1% of definite cases has been observed in adult population. Women are more likely to be affected than men, for unknown reasons (Woolf& Pfleger, 2003). The course of the disease varies widely, but is generally associated with progressive disability and early mortality (Kvien, 2004).

As with other autoimmune conditions women are affected more than male at a ratio 4 : 1 (Koopman , 2001; Buch and Emery, 2002), whereas Iraqi patients according to Al-Rawi *et al*. (1997) showed that female to male ratio is 3.4:1. Although its prevalence increases with age and its peak is between fourth and sixth decades, with 80% of patients

developing the disease between the age 35 – 50 years (Kraag, 1989; Lipsky, 2005). While (Abdulla , 2010) results of studied group according to gender showed the majority of RA patients are females (84%) with a ratio of (5.2: 1) while the frequency of females among SLE group –for comparsion- is (70%) with a ratio of 2.3: 1. (Doran *et al*., 2002 ; Kaipiainen-Seppanen& Kautiainen, 2006).

2.3 Heat Shock Protein 70 :-

Heat shock protein or HSPs are being synthesized under different kind of stress conditions and act as molecular chaperones for protein molecules. Because theses protein were first found in cell that were exposed to high temperature (Lindquist and Craig ,1988; Lai *et al*., 1995). HSP70 , a 70 Kd a protein which is strongly induced in all organisms (Ritossa,1962).

Hsp70 is usually in an ATP bound state. Hsp70 by itself is characterized by a very weak ATPase activity, such that spontaneous hydrolysis will not occur for many minutes (Prakken *et al* ., 2004) the presence of a peptide in the binding domain stimulates the ATPase activity of Hsp70(Kampinga *et al*., 2009). These co chaperones dramatically increase the ATPase activity of Hsp70 in the presence of interacting peptides (Gaston , 2002).

Table2-1 : Heat-Shock Proteins Involved in Autoimmune Arthritis (Hattori *et al* ., 2000; Alcaraz,2003; Johnson *et al* ., 2005; Chang *et al* .,2007; Huang 2009)

Protein Family	HSP (alternative name; origina)	Major Functions
GroES (chaperonin)	HSP10 (chaperonin 10); HSP10 (rat); HSP10 (GroES; Mtb, *E coli*)	Function intracellularly in protein folding in coordination with HSP60
Small HSPs	HSP22 (HSPB8); _-crystallin; HSP27; HSP20 (rat)	A highly diverse group; may be important in hermotolerance
Heme Oxygenase	HSP32 (HO-1); HSP32 (HO-1; rat)	Transform heme into iron, biliverdin, and CO to reduce oxidative stress and subsequent damage
DnaJ homolog subfamily B	HSP40 (HDJ-1)	Interacts with HSP70 to facilitate peptide loading and stimulate its ATPase activity
HSP40 (dnaJ)	HSP40 (dnaJ; *E coli*, *A actinomycetemcomitans*)	Binds to HSP70 to stimulate its ATPase activity and facilitate the disassembly of protein complexes toprevent aggregation under stressful conditions
Serpin	HSP47 (colligin)	Collagen-specific chaperone; may be involved in the biosynthesis of collagen.
HSP60 (chaperonin)	HSP60; HSP60 (HSP65; rat); HSP60 (GroEL; *E coli*); HSP65 (GroEL; Mtb, *M bovis*, *M vaccae*); HSP65 (*M leprae*)	Function with HSP10 in the ATP-dependent protein folding and refolding process with high efficienc
HSP70	HSP70 (HSP72); HSP70 (rat); HSP70 (dnaK; Mtb, *M bovis*, *E coli*); HSP70-Hom; GRP78	High affinity for ATP; disrupt protein-protein interaction to help with various cellular processes, including DNA replication, transport of proteins across

	(BiP); HSP73 (HSC70)	membranes, binding of proteins in the endoplasmic reticulum, and uncoating of the coated vesicles
HSP90	HSP90; HSP90B1 (gp96); HSP90 (rat)	Abundantly expressed cytosolic protein; functions to keep various proteins, such as steroid receptors and tyrosine kinases, inactive until the proper signal for activation is received
Hsp110	Hsp110 (human) Apg-1 (mouse) Hsp105	Thermal tolerance Protein refolding

2.1 Table Heat-Shock Proteins Involved in Autoimmune Arthritis

he Hsp70/Hsp90 Organizing Protein can bind to both Hsp70 and Hsp90 table (2-1) at the same time, and mediates the transfer of peptides from Hsp70 to Hsp90. Hsp70 also aids in trans membrane transport of proteins, by stabilizing them in a partially folded state **(** Wegele *et al*., 2004).

Interaction with CHIP (Carboxyl-terminus of Hsp70 Interacting Protein) anE3 ubiquitin ligase–allows Hsp70 to pass proteins to the internal cell' sub-iquitination and proteolysis pathways (Lüders *et al*., 2000). In addition to improving overall protein integrity, Hsp 70 directly inhibits apoptosis. (Beere *et al*., 2000) .

Hsp70 proteins can act to protect cells from thermal or oxidative stress. These stresses normally act to damage proteins, causing partial unfolding and possible aggregation. By temporarily binding to hydrophobic residues exposed by stress, Hsp70 prevents these partially denatured proteins from aggregating, and allows them to refold (Albani *et al*.,

1995). Low ATP is characteristic of heat shock and sustained binding is seen as aggregation suppression, suggesting a second mode of binding regulation based on oxidative stress (Mac *et al*., 2000)

Hsp70 seems to be able to participate in disposal of damaged or defective proteins. A potential hypothesis is that some autoantibodies and T-cells exist that recognize epitopes shared by the HSP of both the infectious agent and host cells. This would facilitate cross-reactivity of lymphocytes with host cells, triggering an immunological reaction. This is referred to as molecular mimicry (Kaufmann, 1990; Burmester *et al*., 1991; Li *et al*., 1992).

New hypotheses, extracellular heat shock proteins (Hsp70) may represent an ancestral danger signal of cellular death or lysis-activating innate immunity. Recent studies demonstrating a dual role for Hsp70 as both a chaperone and cytokine provided support for the hypothesis that extracellular Hsp70 is a messenger of stress (Zolta´*et al*., 2002).

2.4 Rheumatoid Factor RF :-

Since the discovery of RF in 1940, much research has linked this autoantibody to the pathophysiology of severe rheumatoid arthritis from this time a vast amount of work has been performed on the incidence, nature and specificity of RA (Mageed, 1996).

It have confirmed previous reports that RF production is associate with smoking in RA patients. An association was also found between RF positivity and carriage of the SE, although significance was reduced or lost after correction for previous or current smoking. In agreement with a number of previous studies, found that the association between the SE and RF positivity was primarily due to HLA–DRB1*0401, and that other

common SE alleles were not associated. also found that the DRB1*040 association remained significant after correction for previous or current smoking. This suggests that DRB1*0401 and smoking are independently associated with the production of RF. However, there also appears to be an additive effect because smoking further increases the likelihood of RF production in DRB1*0401 patients. A major difference between DRB1*0401 and other SE alleles is the amino acid encoded at position 71 of dependent on the amino acid substitution at 71 (Olerup and Zetterquist, 1992). In this regard, it is interesting that heavy cigarette smoking is markedly more prevalent in RA patients without a family history of the disease (Eklund and Blaschke, 1986).

Various biochemical pathways for the induction of RF synthesis may be possible. Recently it has been shown in a large population study in Finland there is an association between coffee drinking and RF production (Stolt *et al*., 2003) and that excess coffee consumption (4 or more cups per day) may be a risk factor for sera positive RA, independent of smoking. Again, the mechanism for this is not known, but it was suggested that RF production could be triggered by the presence of coffee diterpenes, which are also known to raise low density lipoprotein (LDL) cholesterol levels (Symmons *et al* .,1997). A positive association between serum LDL cholesterol levels and RF was found in the Finnish study (Stolt *et al*., 2003). Recent observations from our laboratory suggest that in women with RA, there is a higher frequency of RF production in smokers who lack expression of the glutathione S-transferase (GST) M1 enzyme (Klareskog *et al*., 2004). GSTM1 is believed to play a role in the detoxification of chemicals in cigarette smoke, and we have speculated that lack of this enzyme in smokers may promote increased RF production through a failure to detoxify smoke-derived chemicals. These include a variety of free radicals that have the potential to damage IgG

and stimulate the formation of immune complexes(Monach *et al*., 2004) . In conclusion, the production of auto antibodies against the Fc portion of immunoglobulin G. These auto antibodies are generally known as rheumatoid factors (RF) and may be of the IgA, IgG, or IgM isotype. IgM RF has been measured most commonly in RA, with many studies showing that the majority of RA patients (60–80%) are positive for this RF. RF is not specific to this disease and is found in some other chronic inflammatory conditions. IgA RF in particular has been found in diseases of the gut such as Crohn's and coeliac disease (Mac *et al*., 1993; Sokjer *et al* ., 1995).

2.5 Tumor Necrosis Factor – Alpha (TNF-α) :-

This cytokines is a potent one that exerts diverse effects by stimulating a variety of cells, it is a soluble 17-kd protein composed of three identical subunits. It is produced mainly by monocytes-macrophages series, but also by B cells and fibroblasts. Newly synthesized TNF- *a* is inserted into the cell membrane and subsequently released through the cleavage of its membrane-anchoring domain by a serine metalloproteinase.

Thus, TNF- *a* secretion might be suppressed by inhibitors of this enzyme. Perhaps the best studied aspect of TNF-*a* is its ability to promote inflammation. It is an autocrine stimulator as well as a potent paracrine inducer of other inflammatory cytokines, including interleukin-1, interleukin-6, interleukin-8, and granulocyte-monocyte colony-stimulating factor (Nawroth *et al.*, 1986; Hawroth *et al.*, 1991; Butter *et al*., 1995).

Chapter Three
Materials and Methods

3.1 Experimental animals :-

30 male albino rats (135-150) gm. were obtained from animal house of vet. med. college /University of AL-Qadisiyah. The animals were placed in plastic cages in an air conditioned room with temperature maintained at 25 ± 2 Cº. The experiment was performed under controlled conditions (temperature, humidity and 12 h light –dark cycle. Rats were given sterile food pellets and tap water**.** The experiment continued for 28 days. All rats were divided in to 6 groups,5 rats for each one and treated as below:

1.First group(G1)

Which treated as negative control group, it was injected with (0.1 ml/ animal) from physiological normal saline (0.9% Nacl) in the first and third day of the experiment.

2.Second group(G2)

which treated as positive control it was injected with (0.1 ml/ animal) of formaldehyde (2%) in the first and third day of the experiment(Arthritis group).

3.Third group(G3)

it was normal group, injected by Bee venom (I.P) (1 mg/ kg of B.W once daily.

4.Four group(G4)

it was normal group, treated orally with prednisolone (5 mg/ kg of B.W once daily.

5.Fifth group(G5)

it was arthritis group, injected by BV (I.P) (1 mg/ kg of B.W once daily.

6.Sixth group(G6)

it was arthritis group, treated orally with prednisolone (5mg/ kg of B.W once daily.

3.2 Chemicals :-

Bee venom (BV) was obtained from (Sigma-Aldrich, USA) in packages contain (1gm) of BV powder, prepare at a dose (1mg/ kg of B.W) according to(Lee *etal.*, 2004) .Prednisolone was obtained from market and prepare at a dose (5mg/ kg of B.W) according to (Barua *etal*., 2017).

2.2.1 Induction of arthritis :-

Arthritis was induced in rats by administration of (0.1 ml) formaldehyde (2%) in the right hind paw in the first and third day of the experiment (Kore *etal*., 2011).

2.2.2 Blood collection :-

Blood collection was done at 28 days of the experiment via abdominal vein. Blood samples were collected after 28 days placed (3ml) of blood placed in test tubes without anti- coagulated that allowed coagulating for 15 min. Serum was separated from coagulated blood samples by centrifugation at 3000 rpm for 15 min and then kept in the frozen at -20Cº until using to estimate Immunological parameters.

3.3 Estimation of Immunological parameters :-

1- Determination of serum C-Reactive protein

Creative protein was measured by using Latex kit according to (Maron & Romer, 1990).

2- Determination of serum TNF-α, HSP-70 and IL1B

TNF-α and HSP-70 were measured by Elisa using technique sandwich enzyme.

3- Determination of serum Rheumatoid factor:

Rheumatoid factor by using Latex kit.

3.4 Statistical analysis :-

The data was statistically read by spss program, methods of testing include one way ANOVA for comparisons among groups followed by least significant differences (LSD) test to compare among groups, P value of $P \leq 0.05$ were considered to record significant differences, all data were expressed as mean ± standard error (SE) (Leech *etal*., 2011).

Chapter four

Results:-

our results showed a significant increase ($p \leq 0.05$) in CRP, TNF-α IL-1B, HSP-70 and RF at G2 compared with control and other groups, except the G6 which shown no significant difference from G2 in HSP-70 and RF, as in Table (1).

G3 and G4 groups showed a significant decrease ($p \leq 0.05$) in CRP, TNF-α, IL-1B, HSP-70 and RF compared with G2. Also, G3 showed a significant decrease ($p \leq 0.05$) in CRP, IL-1B and RF and a significant increase ($p \leq 0.05$) in TNF-α and HSP-70 compared with G1 (control group), while G4 showed a significant increase ($p \leq 0.05$) in CRP, TNF-α, IL-1B, HSP-70 and RF compared with G1 , as in table (1).

The results showed also a significant decrease ($p \leq 0.05$) in the levels of CRP, TNF-α, IL-1B, HSP-70 and RF in G5, G6, G7 groups compared with G2 except G5 showed non-significant decrease ($p \geq 0.05$) in the level of CRP and RF compared with G2.

Table (4-1): Effect of Bee venom and prednisolone drug on immunological parameters in studied groups after 28 days.

R-factor(%)	HSP-70(ng/ml)	IL-1β(pg/ml)	TNF-α(pg/ml)	CR-p(mg/L)	parameters Groups
9.06± 0.02 D	75.00± 0.40 G	80.79± 0.23 E	43.07± 0.39 F	6.60 ±0.05 D	**G1**
12.11± 0.03 A	125.7± 0.40 A	100.1± 0.13 A	58.8± 0.25 A	8.44±0.07 A	**G2**
8.87± 0.13 E	80.2± 0.25 F	76.72± 0.31 F	45.03± 0.31 E	6.41±0.02 E	**G3**
9.58± 0.02 C	90.60± 0.28 E	81.28± 0.27 G	48.32± 0.28 D	7.42±0.07 C	**G4**
11.05± 0.01 B	95.0± 0.40 D	92.52± 0.19 D	52.70± 0.57 C	7.30±0.04 C	**G5**
12.05± 0.002 A	109.0± 0.40 B	97.3± 0.23 B	56.28± 0.18 B	8.32±0.04 A	**G6**
0.13	0.85	0.58	0.82	0.12	**LSD**

4.1 Effect of Bee venom and prednisolone drug on immunological parameters in studied groups after 28 days.

Results expressed as mean± S.E

-Different letters refer to significant differentiations between groups at ($P \leq 0.05$).

-Similar letters refer to no significant differentiations between groups at ($P \geq 0.05$).

-G1 group: control group.

-G2 group: first treatment group (Arthritis group).

-G3 group: second treatment group(normal group treated with bee venom at a dose 1mg/kg of B.W).

-G4 group: third treatment group(normal group treated with prednisolone at a dose 5 mg/kg of B.W).

-G5group:fourth treatment group(arthritis group treated with BV at a dose 1mg /kg of B.W).

-G6 group: fifth treatment group(arthritis group treated with prednisolone at a dose 5 mg/kg of B.W).

Chapter five

Discussion:-

The objective of the present study was to identify the effects of bee venom on immunological parameters in rates induced by arthritis. The results showed a significant increase in CRP, TNF-α IL-1B, HSP-70 and RF in G2 compared with control group, the cause of these results due to production of CRP from the liver because of production of pro inflammatory cytokines from monocytes and macrophages, as well as the pro inflammatory response causes secretion of IL-1B and TNF-α (Punzi *et al*., 2002; Punzi *et al*., 2005). The results of this study were agreed with (Abed *et al*., 2016) because of HSP-70 works as companion, cytokine and a messenger of stress (Zolta *et al*., 2002). TNF-α acts a central role in the pathogenic of Rheumatoid arthritis. Also the present results was agreed with (Al-Salih, 2013), the significant increase in the level of CRP due to a systemic inflammatory characteristic of Rheumatoid arthritis (Lubbert *et al*., 2005).

The results of the present study showed a significant decrease in the levels of CRP, TNF-α, IL1B, HSP-70 and RF in G5, the cause of these results due to bee venom that caused the development of arthritis to be inhibited (Lee *et al*., 2004).

These results was agreed with (Abedd- Gawad *et al*., 2016; shaldoum *et al*., 2018), as the decrease in the CRP due to the anti-inflammatory effect of bee venom (Nam *et al*., 2003).

The anti-inflammatory effect of bee venom through the inhibition of Rheumatoid arthritis (Abdel- Rahman *et al*., 2013). Bee venom and melttin that considered the main component of bee venom inhibited the inflammatory stimuli such as TNF-α through prevent translocation of p50 through the interaction between melittin and sulfhydryl group of p50, also the anti-inflammatory effect of bee venom be through suppress the transcription of cyclooxygenase genes and pro-inflammatory cytokines such as IL-1B, IL-6 and TNF-α (Park *et al*., 2007).

Also these result was agreed with (EL-Gendy *et al*., 2017; kim *et al*., 2018), as that decreased in TNF-α, IL-1B, IL-6 and NF-kB due to the NF-kB considered one the important regulators for pro-inflammatory genes such as TNF-α and COX2 (Surh *et al*., 2001), Also bee venom has anti-inflammatory activity through inhibition the activity of DNA binding NF-

kB through inhibition the phosphorylation of IkB and this lead to decrease the translocation of p50 (Martins *et al*., 2011).

The results showed a significant decrease in the level of CRP, TNF-α, IL-1B, HSP-70 and RF in G6 compared with G2, this result was agreed with (Barua *et al*., 2017), due to the role of NF-kB in the pathogenic of Rheumatoid arthritis (Ganesan *et al*., 2016), Glucocorticoids such as prednisolone cause inhibition the production of pro-inflammatory cytokines such as TNF-α and IL-6 that reduce by macrophages and monocytes through their spreading inside the cell and binding with receptors in the cytoplasm them moves to inside the nucleus and induced transcription of IKBa which in turn causes inhibition of NF-kB and reduces the production of pro-inflammatory cytokines (Scheinman *et al*., 1995). Also the result was agreed with (Gerlag *et al*., 2004; Abou-Raya *et al*., 2014), where they indicated for decreasing in the level of IL-1B, IL6, TNF-α and high sensitivity CRP of her treatment with prednisolone.

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