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The Role Of Therapeutic Bee Venom Compared To Prednisolone In Male Albino Rats Induced With Arthritis

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ABSTRACT

The current study was conducted with the aim of knowing the effects of bee venom in reducing the physiological and immune effects in experimentally induced arthritis rats and comparing it with prednisolone.

24 male Albino rats were used, their ages ranged between 10-12 weeks and their weights 150-135 g. The study included conducting an experiment in laboratory animals where the animals were randomly divided into six groups (4 animals for each group), the first treatment group was injected with 0. 1 ml of formaldehyde at a concentration of 2% in the peritoneum of the right sole of the foot, twice on the first and third days of the experiment (returned as a positive control group with induced arthritis). As for the second treatment group, it included healthy animals injected intraperitoneally with bee venom at a rate of 1 mg/kg body weight in When the third treatment group (the healthy group) was injected orally with prednisolone at a rate of 5 mg/kg of body weight, and the fourth treatment group included experimentally induced arthritic animals injected intraperitoneally with bee venom at a rate of 1 mg/kg of body weight, while the fifth treatment group (the group of experimentally induced arthritis) orally with prednisolone at a rate of 5 mg/kg of body weight and finally the sixth treatment group (a group with experimentally induced arthritis) injected intraperitoneally with bee venom and b It was 1 mg/kg of body weight as it was orally dosed with prednisolone and at 5 mg/kg of body weight.

After the end of the study period, the animals in each group were sacrificed and blood samples were taken for the purpose of conducting biochemical tests

The results of the current study and for both experiments showed a significant increase ($p \leq 0.05$) in the average right foot thickness in the first treatment when compared with the control group and the rest of the study groups. The results also showed a significant decrease ($p \leq 0.05$) in body weight in the first treatment when compared with the first treatment The rest of the totals and during a period of 14

It is concluded from the current study that the experimental infection with rheumatoid arthritis caused clear negative effects, by affecting the biochemical parameters. The use of prednisolone, because prednisolone has many negative effects with long-term use.

KEYWORDS: bee venom; prednisolone; male albino rats; arthritis**.**

INTRODUCTION

The term Arthritis consists of two parts: Arth, meaning joint, and ritis, meaning inflammation. This term is used to describe many pains that affect joints and bones. The development in civil life played a major role

in the spread of many diseases, including diabetes, high cholesterol, cardiovascular diseases, and rheumatoid arthritis. And osteoporosis and tooth decay and this was the result of following incorrect lifestyles and lack of movement and lack of exercise, and arthritis is one of the most prevalent diseases at the present time and affects both sexes and at different ages. and jaw and spine **(Hafstorm et al., 2001).**

Inflammatory factors such as TNF-α and interleukins such as IL-1β, IL-6, IL-17, IL-18, IL-23 and inflammatory enzymes such as Inducible Nitric Oxide Synthase (INOS) and Cycloxygenase-2 (COX2) An important role in the pathogenesis of rheumatoid arthritis **(Ganesan et al., 2016)**.

Among the complex mechanisms that lead to inflammatory reactions in arthritis is that inflammatory mediators such as nitric oxide (NO) and prostaglandins, which act on special receptors located on the cell surface, may participate in inflammatory reactions **(Cuzocera et al., 2002),** where Cytokines such as IL-1β and TNF stimulate the Nitric Oxide Synthase pathway in bone cells, since NO derived from this pathway potentiates cytokines and induces inflammation in bone loss. An elevated level of prostaglandins involved in these complex interactions leads to the deterioration of articular cartilage and articular bone **(Trebinoet al., 2003;Qasim and Al-Mayali,2019).**

Bee venom

Bee venom is used to treat various diseases. It is used to treat many pathological conditions such as rheumatoid arthritis in humans and animals, tumors and other diseases **(Mirshafiey, 2007; Son et al., 2007; Cho et al., 2012).** Bee venom contains at least 18 active ingredients, which include enzymes, peptides and amines, which have broad therapeutic qualities. In stimulating the production of cortisol by the adrenal glands, which in turn acts as an anti-inflammatory **(Son et al., 2007).**

Bee venom is one of the most important products for honey bees, as it comes from the venom bag, which is located at the level of the last piece of the abdomen of honey bee workers, as the amount of bee venom depends on the age and sex of the bees, and its components depend on the pollen that they consume. Apismellifera honey bees can give 0.1-0.012 mg) of venom during their stinging. Melittin is the main component of bee venom. Apamin is a moderate neurotoxin. Dried bee venom contains 3-2% of Apamin**(Son et al., 2007).** Bee venom is used as a treatment in many countries of the world, about 22 countries, especially in East Asia **(Kwon et al., 2002).**

MATERIALS AND METHODS

24 male laboratory rats of the type Albino rats were used in this study, their ages ranged between 12-10 weeks and their weights ranged between 150-135 g. They were placed in plastic cages with 4 rats in each cage. periodically to maintain the cleanliness of the rats, and the animals were subjected to similar laboratory conditions in terms of ventilation, temperature 2±22 °C and lighting (12 hours light to 12 hours dark), and food and water were provided to them openly ad libitium, as the ration was made in the form of fingers **(Ward,1970).**

The bee venom obtained by the American company Sigma was used in the form of packages containing 1 g of the compound and a dose of 1 mg / kg of body weight was prepared from it, according to the method described in **(Lee et al.,2014).** As for prednisolone, it was obtained from local pharmacies and a dose of 1 mg/kg of body weight was prepared. 5 mg/kg body weight, according to the method described in **(Barua et al.,2017).**

- The first treatment group (T1): Injected with formaldehyde 0.1 ml at a concentration of 2% in the peritoneum of the right posterior sole of the foot on the first and third days of the experiment, and it was considered a positive control group (in which arthritis developed).
- The second treatment group (T2): a healthy group was injected intraperitoneally with bee venom at a rate of 1 mg/kg of body weight for the duration of the experiment.
- The third treatment group (T3): a healthy group dosed orally with prednisolone at a concentration of 5 mg/kg of body weight for the duration of the experiment.
- Fourth treatment group (T4): A group in which arthritis developed and injected intraperitoneally (I.P) with bee venom at a rate of 1 mg/kg of body weight for the duration of the experiment.
- Fifth treatment group (T5): A group in which arthritis developed and was administered orally with prednisolone at a concentration of 5 mg/kg of body weight for the duration of the experiment.
- Sixth treatment group (T6): A group in which arthritis developed and was injected intraperitoneally (I.p) with bee venom at a rate of 1 mg / kg of body weight, and it was orally dosed with prednisolone at a concentration of 5 mg / kg of body weight for the duration of the experiment.

Arthritis in rats was induced with formaldehyde, where 0.1 ml of formaldehyde at a concentration of 2% was injected into the right hind paw on the first and third days of the experiment **(Kore et al., 2011).** The arthritis induced by formaldehyde is a chronic inflammation, and the changes it causes in the joints are similar to the changes found in rheumatoid arthritis in humans **(Okoli et al., 2008).**

Arthritis index

The thickness of the right foot of the animals that were treated with formaldehyde was measured by the Caliper Verneir machine and with a unit of measure mm, measured before the start of the treatment (day zero) and on the tenth day of the experiment, as the evidence of arthritis was determined according to the method described by Coelho et al. (2004).

Collection of blood samples

After the end of each experiment, the animals were anesthetized by injecting a mixture of 0.3 ml ketamine and 0.1 ml xylazine per kg body weight in the peritoneum (IP), and blood was drawn from the heart of each animal directly by pricking the heart using a sterile 5-capacity syringe. Ml. Save 2 ml of blood in tubes containing an anticoagulant substance (EDTA) for the purpose of making antioxidants.

RESULTS

Paw thickness

The results of the current study showed that injecting rats with formaldehyde (0.1) ml at a concentration of 2% in the peritoneum of the sole of the right foot, it led to the occurrence of arthritis, as many changes were seen in the sole of the feet represented by redness, swelling and the appearance of lesions, as well as a significant increase in ($p \leq 0.05$) in the thickness of the right foot in treatment ((T1 and T4) and (T5) and (T6) during the study period (14), as in Table (1).

The results of the study also showed that there were no significant differences in the thickness of the right foot ($p \geq 0.05$) in treatment ((T2 and treatment T3)) when compared during the 14-day study period, as shown in Table (1), and these two groups showed a significant decrease ($p \leq 0.05$) when compared with treatment (T1) and the rest of the study groups, and there was a significant decrease ($p \leq 0.05$) in the mean of right foot thickness in treatment (T4), treatment (T5) and treatment (T6) when compared with treatment T1)) during the two study periods. Also, treatment (T6) showed a significant increase ($p \leq 0.05$) in the mean

of right foot thickness when compared with treatment (T4) and an insignificant decrease (p≥ 0.05) when compared with treatment (T5) during a period of 14.

Table 1. shows the effect of bee venom and prednisolone on the right foot thickness (mm) of healthy and experimentally infected male laboratory rats with arthritis

Groups	**right foot thickness inDay 0 of the experiment (mm)**	**right foot thickness inDay 10 of the experiment (mm)**	**right foot thickness inDay 14 of the experiment (mm)**
T1	9.45±0.24	12.25±0.09 A	12.00 ±0.04 A
T2	9.42±0.23	9.62±0.03 B	9.62±0.037 D
T3	9.74±0.04	9.4 2±0.23 B	9.4 2±0.23 D
T4	9.72±0.01	12.15±0.33 A	10.72±0.08 C
T5	9.38±0.29	12.06 ±0.25 A	11.57 ±0.06 B
T6	9.62±0.03	12.32±0.15 A	11.40±0.03 B
LSD	N.S	0.512	0.334

The results of the current study showed a significant increase (p≤0.05) in the level of each of malondialdehyde, ceruloplasmin and uric acid in the treatment (T1) when compared with the control group and during the study period, table (2), while the treatment (T2 and (T3) has It showed a significant decrease (p≤0.05) in the level of each of malondialdehyde, ceruloplasmin and uric acid when compared with treatment (T1) and during the study period (14), except for treatment (T3), while treatment (T2) did not differ significantly (p≤ 0.05) in the level of malondialdehyde, ceruloplasmin, uric acid, and albumin from the control group, and treatment (T3) showed a significant (p≤ 0.05) increase in the level of ceruloplasmin, and insignificant (p≥0.05) in the level of malondialdehyde, uric acid, albumin and bilirubin than the control group. During a period of 14 days, as in Table (2)

Table 2. shows the effect of bee venom and prednisolone on the oxidative stress indices of healthy and experimentally infected male laboratory rats with arthritis for 14 days.

Groups	**MDA mg/dl)**	**Ceruloplasmin(mg/dl)**	**Uric acid (mg/l)**
T1	3.00± 0.043 A	19.48± 0.27 A	2.77± 0.10 A
T2	1.56± 0.017 D	16.79± 0.09 E	1.66± 0.02 D
T3	1.71± 0.035 D	17.18± 0.02 D	1.77± 0.03 CD
T4	1.99± 0.006	17.58± 0.02	1.84± 0.01

	C	C	C
T5	2.41± 0.010 B	17.96± 0.02 B	2.11± 0.04 B
T6	2.43± 0.041 B	18.33± 0.46 F	2.19± 0.05 B
LSD	0.21	0.35	0.12

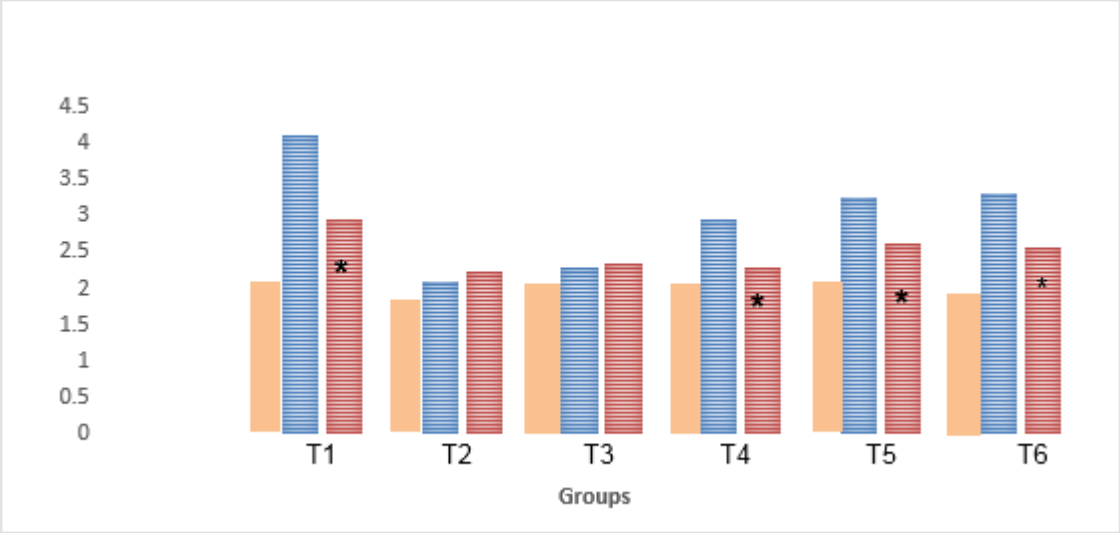

Figure 1. Show Effect of bee venom and prednisolone on albumin concentration (g/dl) of healthy and experimentally infected male laboratory rats with arthritis for 14.

DISCUSSION

The results of the current study showed an increase in the right paw thickness on the tenth day of injecting rats with formaldehyde (0.1 ml) at a concentration of 2%, and the emergence of some symptoms that indicate inflammation such as redness, swelling and the appearance of lesions, as there was a significant increase In the mean of the thickness of the right foot ($p \leq 0.05$) in the treatment T1, T4, T5 and T6 when compared with each other, the reason for this is that the injection of formaldehyde (0.1 ml) at a concentration of 2% led to the release of some substances such as histamine, serotonin and prostaglandin at the injection site. Histamine and prostaglandins are the main mediators of inflammatory hyperalgesia through the activation of local pain receptors and nerve endings that cause hypersensitivity in the injury area **(Praveen &Janarthan, 2013),** where the inflammatory process leads to the activation of inflammatory causes such as free radicals derived from active oxygen species. Reactive oxygen species, cytokines, NO, and prostaglandins, causing Free radicals cause many inflammatory diseases **(Valko et al., 2006;Tahmasebi et al.,2021),** and inflammatory cytokines, especially IL-8, IL-1, TNF-α, play an important role in attracting inflammatory cells to the synovial membrane, which leads to the destruction of joints and bones**(Karray et al., 2011).**

The results of the study showed a significant decrease in the average right foot thickness ($p \leq 0.05$) on day 14 of treatment in treatment T4 (a group infected with arthritis and injected with bee venom at a concentration of 1 mg/kg of body weight) when compared with treatment T1 (a group infected with inflammation). The reason for this is that the use of bee venom in effective concentrations causes inhibition

of some inflammatory factors and thus stimulates anti-edema responses **(Yamasaki et al., 2015)**, as Melittin, the main component of bee venom, possesses therapeutic power through complex formation. Melittin-PLA2 complex after injection of bee venom, as this compound is able to inhibit the development of inflammation **(Saini et al., 1997),** and Melittin and PLA2 enzyme have the ability to inhibit the production of Neutrophil superoxide **(Somerfeild et al., 1986;Mohammed and Qasim,2021).**

The results of the study showed a significant decrease in the rate of foot thickness ($p \leq 0.05$) in treatment T5 (a group suffering from arthritis and dosed with prednisolone at a concentration of 5 mg/kg) during the two study periods compared with T1, and the reason for this is due to the inhibition of prednisolone for inflammatory reactions, and the use of Prednisolone is effective in controlling foot size in rats induced with paw edema, as foot size is considered an indicator of inflammation **(Yamazaki et al., 2013).** As for the significant decrease in the thickness of the right foot ($p \leq 0.05$) in treatment T6 (a group suffering from arthritis and injected with bee venom at a concentration of 1 mg/kg and also dosed with prednisolone at a concentration of 5 mg/kg) and during the two study periods, it is due to the use of both bee venom and prednisolone in the treatment of inflammation joints, which agreed with **(Wood, 2009;Shabgah et al.,2021)**, as this is due to the role of bee venom in reducing the level of tumor necrosis factor-alpha, as its decrease has a close relationship with the improvement of foot edema, and bee venom has a characteristic As a pain reliever when used at concentrations higher than 1 mg/kg of body weight **(Kwon et al., 2001),** prednisolone has anti-inflammatory activity by binding to glucose cortical receptors and thus stimulating signal transmission pathways, as well as inhibiting inflammatory cytokines. It stimulates neutrophils **(Taieb et al., 2000),** but it has poor pharmacological efficacy, which makes it ineffective in treatment. This requires the use of high doses and repeated treatment, and thus causes many harmful systemic effects such as Diabetes Mellitus, Osteoporosis and Hypertension **(Czock et al., 2005).**

CONCLUSION

The experimental infection with rheumatoid arthritis caused clear negative effects, by affecting the biochemical parameters, and the study showed that bee venom and prednisolone have clear effects because they are antioxidant and anti-inflammatory substances, and the use of bee venom is safer than the use of prednisolone, because Prednisolone has many negative effects with long-term use.

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