Research Article

Bee Venom for the Treatment of Rabbit Arthritis Caused by *Staphylococcus aureus*

Heba S.S. Salem[1], Hend M. Megahed[2], Marwa M. Sarhan[2], Maha M. El Alem[3], Gehan N. Alagmy[3*]

[1]Department of Bacteriology (Zagazig branch), Agriculture Research center (ARC), Animal Health Research Institute (AHRI), Egypt; [2]Department of Biochemistry (Zagazig branch), Agriculture Research center (ARC), Animal Health Research Institute (AHRI), Egypt; [3]Department of Pathology, (Zagazig branch), Agriculture Research center (ARC), Animal Health Research Institute (AHRI), Egypt.

Abstract | This study was taken to assess the effect of administration of bee venom alone or added to systemic antibiotics for curing septic arthritis caused by *Staphylococcus aureus (S. aureus)*. Thirsty-five naturally infected cases of rabbits with arthritis were screened for the prevalence of *S. aureus* as a causative agent. *S. aureus* was diagnosed in 40% of the examined cases via traditional culture methods and molecular confirmation using PCR. All tested *S. aureus* isolates were positive for virulence-associated genes (*nuc*, *icaA*, and *Hlg*). In an experimental trial, a total of 75 healthy rabbits were divided equally into five groups (n =15/each group). The first group served as a negative control, the second group was injected intra-articulately with *S. aureus*, did not receive any treatment, and served as a positive control group, the third group received a subcutaneous injection of bee venom (BV), the fourth group was injected intra-articulately with *S. aureus* and treated with a subcutaneous injection of BV, the fifth group was injected intra-articulately with *S. aureus* and treated with a subcutaneous injection of BV and gentamicin. On the 7th and 14th days, post-infection animals were examined for hematological, biochemical, and histopathological assessments. The obtained results revealed that *S.aureus* infection caused marked alterations in hematological and biochemical parameters compared to the negative control, while treatment with BV alone or combined with gentamicin revealed a significant improvement of these parameters. Likely, treatment with BV caused an improvement in the histopathology compared with the untreated groups. In conclusion, BV treatment is of value in the treatment of *S. aureus*-induced arthritis in rabbits.

Keywords | Antibacterial Activity; Bee Venom; *Staphylococcus aureus*; Septic Arthritis

Received | July 05, 2022; **Accepted** | August 08, 2022; **Published** | August 20, 2022
***Correspondence** | Gehan N Alagmy, Pathology Department (Zagazig branch), Agriculture Research center (ARC), Animal Health Research Institute (AHRI), Egypt; **Email:** d.gehanelagmy@gmail.com
Citation | Salem HSS, Megahed HM, Sarhan MM, El Alem MM, Alagmy GN (2022). Bee venom for the treatment of rabbit arthritis caused by *Staphylococcus aureus*. Adv. Anim. Vet. Sci. 10(9): 2004-2012.
DOI | http://dx.doi.org/10.17582/journal.aavs/2022/10.9.2004.2012
ISSN (Online) | 2307-8316

INTRODUCTION

Staphylococcus aureus (S. aureus) is an opportunistic pathogen that causes a wide spectrum of pathologies inducing losses in commercial rabbits. Remarkably, septic arthritis in the joints of the rabbits showed histological evidence of damage affecting the knees (Marcheix et al., 2018). Hypertrophy of the synovial membrane, infiltration with neutrophils, lymphocytes, histiocytes, giant cells, and plasmatic cells, arthrocentesis, and finally cartilage and bone damage were the typical pathological changes caused by *S. aureus* in rabbits (Ainara et al., 2021). Rapid diagnosis and treatment are required for better management of joint degenerations and to control the high mortality rates (Ghosh

and Bishayi, 2015).

The abuse of antimicrobials in rabbit farms under intensive rearing programs led to the development of antimicrobial resistance among pathogens including *S. aureus*. Thus, new treatment strategies need to be continuously developed (Wenhua et al., 2006). A combination of the use of traditional antibiotic therapy and immune stimulation for treating rabbit arthritis caused by *S. aureus* was developed (Ghosh and Bishayi, 2015).

Bee venom (BV) has drawn attention as a complementary and alternative treatment for a variety of clinical conditions, including rheumatism, arthritis, discomfort, and cancer. BV includes various pharmacologically and biochemically active compounds (Permual et al., 2006; Wehbe et al., 2019). There have been numerous effects associated with bee venom, including antibacterial, antiviral, and anti-inflammatory actions. It has biological activity against osteoarthritis, rheumatoid arthritis, pain, and arthritis-related rheumatism (El-Bahnasy et al., 2022). Peptides, melittin, apamin, mast cell, degranulation (MCD), adolapin, enzymes (phospholipase A2 (PLA2) and hyaluronidase), amino acids, and volatile chemicals are only a few of the active substances found in bee venom (Hegazi et al., 2015). About 50 percent of the dry weight of the venom is made up of a substance called melittin, which has been shown in some studies to have anticancer, antiviral, and antibacterial activities, particularly against Gram-positive organisms than Gram-negative ones (Akkaya et al., 2012; Leandro et al., 2015).

Thus, the objectives of the present study were first to isolate and identify *S. aureus* from field cases of arthritis in rabbits in Sharkia governorate, Egypt. Second, to investigate the efficacy of the treatment with BV alone or in combination with gentamicin against *S. aureus*-induced arthritis in rabbits. Third, the hematological, immune, biochemical, and pathological changes associated with *S. aureus*-induced arthritis were evaluated.

MATERIAL AND METHODS

Field study

Sampling: Specimens were collected from joints of 35 rabbits suffering from swelling of the joints and clinical manifestations of septic arthritis. Specimens were collected from different localities of Sharkia governorate, Egypt during the period from July 2021 to October 2021.

Isolation and identification of *S. aureus*: Under the aseptic condition, isolation and identification of *S. aureus* were done by using Baird parker media (Himedia, India) according to Cruickshank et al. (1979).

Antimicrobial susceptibility testing: Antimicrobial sensitivity of the recovered *S. aureus* isolates to eight of the commonly used antimicrobial agents in Egypt was performed using the disc diffusion method (Bauer et al., 1966). The interpretation of the obtained results was done according to the criteria recommended (CLSI, 2017) for antimicrobial susceptibility testing. The tested antibiotics were doxycycline, tetracycline, ciprofloxacin, penicillin, amoxicillin / clavulanic acid, ceftriaxone, vancomycin, and gentamicin.

Molecular detection of virulence-associated genes of *S. aureus* strains: DNA extraction for the detection of the virulence-associated genes of *S. aureus* strains (*nuc*, *icaA,* and *Hlg)*. DNA extraction was done using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer's recommendations. The used primers in the present study were purchased from Metabion, Germany, and presented in Table 1. The PCR master mix (25 µl) contained 12.5 µl of Dream Taq Green PCR Master Mix (2X) (Thermo Scientific), 1 µl of each primer (20 pmol), 5.5 µl of DEPC-treated water, and 5 µl of DNA template. An Applied biosystem 2720 thermal cycler was used for such amplifications. The amplification reaction started with an initial denaturation at 94˚C for 5 min, followed by 35 cycles including a denaturation step at 94˚C for 1 min, annealing at 50˚C (*nuc)*, 49˚C (*icaA),* and 55˚C (*Hlg)* for 1 min, and an extension step at 72˚C for 2 min. A final extension was employed at 72˚C for 10 min. PCR products were separated on 1% agarose gel (Applichem, Germany) in 1x TBE buffer at room temperature using a 100 bp DNA ladder (Fermentas, Thermo Scientific, Germany), and visualized on a gel documentation system (Alpha Innotech, Biometra).

Determination of the minimum inhibitory concentration (MIC) of BV against *S. aureus:* Bee venom (Sigma Aldrich, Egypt) was diluted in sterile nutrient broth into five concentrations starting with 1000, 500, 250, 125, and 62.5 µg/ml. A loopful of *S. aureus* culture and 0.5 McFarland standards (EUCAST, 2003) was inoculated into test tubes containing 2 ml of the tested concentrations of BV. These tubes were incubated at 37˚C for 24 h and observed for bacterial growth. A loopful of broth from each test tube that did not exhibit growth was then inoculated into a plate of nutritional agar (CLSI, 2017).

Experimental study

Ethics of the animal study: The use of animals in this study complied with the formal approval of the Animal Health Research Institute's policy on animal use and ethics. This study received approval on the protocol number ARC-AH-22-09.

Table 1: Primers used in the present study

Target gene	Primers sequences (5'-3')	Product size (bp)	Reference
Nuc	F- GCGATTGATGGTGATACGGTT R- -AGCCAAGCCTTGACGAACTAAAGC	270	(Brakstad et al., 1992)
icaA	F- CCTAACTAACGAAAGGTAG R- AAGATATAGCGATAAGTGC	1315	(Ciftci et al., 2009)
Hlg	F- GCCAATCCGTTATTAGAAAATGC R- CCATAGACGTAGCAACGGAT	937	(Kumar et al., 2009)

Animals: A-75 New Zealand clinically healthy male rabbits aged six months old and weighted from 1200 to1600 grams were obtained from the Faculty of Agriculture (Laboratory and Research Agricultural Center) in Sharkia governorate. All rabbits were inspected to ensure that they are free from bacteriological or parasitological infection. Animals were caged separately and had free access to water and commercial feed *ad libitum*.

Experimental design: Rabbits were divided into five equal groups each of 15 rabbits: Group (Gp1) was assigned as a control negative, not infected, and not treated. Group (Gp2) was assigned as a control positive group infected with 0.1 ml of inoculum having 8×10^8 CFU/ml of *S. aureus* (Georgieva et al., 2016) into the hip joint and not treated. Group (Gp3) was treated with S/C injection of BV 1mg/kg/day for 5 successive days (Ozlem Nisbet et al., 2011). Group (Gp4) was infected with 0.1 ml of inoculum having 8×10^8 CFU/ml of *S. aureus* into the hip joint and treated with S/C injection of BV 1mg/kg/day for 5 successive days. Group (Gp5) was infected with 0.1 ml of inoculum having 8×10^8 CFU/ml of *S. aureus* into the hip joint and treated with S/C injection of BV 1mg/kg/day and gentamicin (Gentacure-10®, Pharma Swede, Egypt) at a dose of 5 mg/kg body weight for 5 successive days (Fig. 1). Samples including synovial fluids and blood samples (collected from the ear veins) were obtained on the 7th and14th days post-inoculation.

Bacteriological examination of the infected joints and re-isolation of *S. aureus*: Bacterial swaps from the synovial fluids collected from the hip joints and periarticular abscess were done for all infected groups (2, 4, and 5) for re-isolation and identification of *S. aureus* (Boerhout et al., 2016).

Hematological and biochemical assays: A hematological picture was done according to Feldman et al. (2000). The biochemical assays including malondialdehyde (MDA) (Satoh, 1987), and glutathione peroxidase (GPx) (Miller and Slebodzinska, 1993) were assayed. Serum nitric oxide (NO) was also estimated using a nitric oxide assay kit (ab65328). Serum IL-1ß was analyzed by ELISA Kit (CSB-E08055r). Serum IL-6 was analyzed by ELISA Kit (CSB- E04640r). Serum TNF- α was analyzed by ELISA Kit (CSB-E11987r).

Histopathological examination: After the sacrifice of the experimental animals, the infected joints were fixed, then decalcified in 10% EDTA for 14 days. Specimens were imbedded in molten paraffin wax, after being dehydrated in progressively stronger alcohols. Using a microtome (Leica®), paraffin slices (5 µm) were cut, and they were stained with hematoxylin and eosin for microscopic analysis (Suvarna et al., 2018).

Statistical analysis: Statistical analyses among the experimental groups were evaluated using the analysis of variance (ANOVA) followed by Duncan's test, where a *p*-value less than 0.05 was considered to be significant. All Statistical analyses were done using the SPSS program.

RESULTS AND DISCUSSION

Bacteriological examination of rabbits with arthritis from field samples revealed isolation of *S. aureus* from 14 out of 35 cases at a prevalence rate of 40%. Likely, Mowafy et al. (2018) isolated *S. aureus* from septic arthritis cases in rabbits at 36.7%.

Antimicrobial susceptibility testing of the recovered *S. aureus* isolates revealed 93% susceptibility to ciprofloxacin and, gentamycin followed by 85.71% susceptibility to ceftriaxone, and vancomycin, 71.43% to tetracycline, and doxycycline, 64.29 % to amoxicillin / clavulanic acid, then 35.71% to penicillin. In agreement with the recorded results of the present study Mowafy et al. (2018) recorded that isolates of *Staphylococcus spp.* recovered from rabbits were highly sensitive to gentamicin, and 37.5% of the isolates were resistant to penicillin.

PCR confirmation of the recovered *S. aureus* isolates via amplification of the species-specific *nuc* gene which is encoding the thermostable nuclease of *S. aureus* revealed detection of *nuc* gene in all recovered isolates. Similarly, all recovered *S. aureus* isolates harbored both *icaA* and *Hlg* genes (Data are not shown). *S. aureus* typically combines several virulence genes, which were assumed to contribute to the pathogenicity. Two virulence determinants were tested in the current study, the *Hlg* gene (β-hemolysin-

Table 2: The effect of experimental infection with *S. aureus* and treatment with bee venom and gentamicin on hematological parameters of rabbits at 7 days post inoculation (n=5)

Group	RBCs (10^6×mm^3)	Hb (g/dL)	PCV (%)	WBCs (10^3×mm^3)	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)
Group 1	5.1 ± 0.14^a	14.1 ± 0.18^a	38.7 ± 0.25^a	7.3 ± 0.12^c	84.7 ± 0.15^a	3.5 ± 0.13^a	8.0 ± 0.57^c
Group 2	3.9 ± 0.10^b	11.5 ± 0.34^c	36.6 ± 0.60^b	8.9 ± 0.22^a	82.52±0.57^a	3.9 ± 0.10^a	12.1 ± 0.28^a
Group 3	5.0 ± 0.07^a	13.7 ± 0.17^{ab}	38.5 ± 0.18^a	7.5 ± 0.17^c	84.6 ± 0.45^a	3.7 ± 0.12^a	8.3 ± 0.88^c
Group 4	4.6 ± 0.11^a	12.8 ± 0.23^b	37.5 ± 0.38^{ab}	8.4 ± 0.19^b	83.3 ± 0.37^a	3.8 ± 0.33^a	10.8 ± 0.44^{ab}
Group 5	4.9 ± 0.22^a	13.2 ± 0.17^b	38.2 ± 0.31^a	8.1 ± 0.15^b	83.9 ± 0.44^a	3.7 ± 0.15^a	9.2 ± 0.72^{bc}

RBCs: Red blood cells; Hb: Hemoglobin; PCV%: Packed cell volume; WBCs: White blood cells

Table 3: The effect of experimental infection with *S. aureus* and treatment with bee venom and gentamicin on hematological parameters of rabbits at 14 days post inoculation (n=5)

Group	RBCs (10^6×mm^3)	Hb (g/dL)	PCV (%)	WBCs (10^3×mm^3)	Lymphocyte (%)	Monocyte (%)	Neutrophil (%)
Group 1	5.0 ± 0.14^a	14.6 ± 0.22^a	39.1 ± 0.28^a	7.9 ± 0.20^b	83.9 ± 0.36^a	3.7 ± 0.08^b	7.6 ± 0.33^b
Group 2	4.0 ± 0.04^b	12.2 ± 0.18^c	36.3 ± 0.20^b	9.2 ± 0.13^a	82.6 ± 0.63^a	3.9 ± 0.02^a	9.5 ± 0.28^a
Group 3	5.1 ± 0.11^a	14.3 ± 0.39^{ab}	38.9 ± 0.20^a	8.4 ± 0.14^b	83.6 ± 0.51^a	3.7 ± 0.08^b	7.8 ± 0.16^b
Group 4	4.7 ± 0.13^a	13.5 ± 0.13^b	38.4 ± 0.29^a	8.4 ± 0.11^b	82.7 ± 0.43^a	3.8 ± 0.05^{ab}	8.8 ± 0.24^{ab}
Group 5	4.8 ± 0.97^a	14.2 ± 0.43^{ab}	39.0 ± 0.19^a	8.2 ± 0.11^b	82.9 ± 0.63^a	3.8 ± 0.11^{ab}	8.2 ± 0.44^{ab}

RBCs: Red blood cells; Hb: Hemoglobin; PCV%: Packed cell volume; WBCs: White blood cells
Means with different letters at the same column are significantly different (P <0.05)

converting bacteriophages) and *icaA*, a gene connected to host adherence and invasion, (Edwards et al., 2010; Penadés et al., 2020). The hemolysins and leukocidins produced by the *Hlg* gene have hemolytic functions that target erythrocytes and leukocytes and have been demonstrated to cause inflammation, facilitate osteoblast invasion, and kill osteoblasts, exacerbating *S. aureus*-induced osteomyelitis (Jin et al., 2021).

the minimum inhibitory concentration (MICs) were taken as the lowest concentration of tested venoms that inhibited visible bacterial growth (Burt, 2004). Testing of MIC of BV against *S. aureus* showed that using BV at a-178.3 µg/ml, a complete inhibition for the growth of *S. aureus* was achieved. This value was similar to previous reports (Kim et al., 2006; Mahmoudi et al., 2020).

Bacterial re-isolation of *S. aureus* from the infected joints after 7 days post-inoculation was successful in 100% of group 2, 60% of group 4, and 40% of group 5. While after 14 days post-inoculation, 100% of group 2, and 10% of group 4 showed positive re-isolation of *S. aureus.*

The hematological pictures of the treatment group were recorded in Tables 2 and 3. The obtained results showed a significant decrease in the RBCs count, Hb concentration, and packed cell volume in the infected and non-treated groups. Leukopenia and neutrophilia were also detected in group 2 compared with group 1. Such results were in agreement with Dimitrova et al. (2000) and Petrov and Mircheva (2013) who reported a reduction of the hematological parameters in rabbits with *S. aureus* infection and this decline might be due to the suppression in hematopoietic function caused by the *S. aureus* infection. Treatment with either BV alone or in a combination with gentamicin evoked a significant increase in the tested hematological parameters compared with group 1. Similarly, Son et al. (2007) and Mohammed and Hassan (2019) assumed an improvement in these parameters due to the action of BV in increasing coronary and peripheral circulation which improves circulation of blood in the micro blood vessels and stimulates erythropoiesis. Besides, the reduction in the leucocytic count might be due to the immunosuppressive effect of bee venom (Mohammed and Hassan, 2019).

S. aureus is considered the primary cause of bacterial arthritis which is characterized by massive inflammation and the release of cytokines from macrophages such as TNF-α, IL-1β, and IL-6 (Kwan et al., 2004). The recorded results of the biochemical parameters in Tables 4, and 5 revealed a significant increase (P <0.05) in IL 1-β, IL 6, TNF-α, nitric oxide, and malondialdehyde in group 2 compared with group 1. This was explained by Bitschar et al. (2017) who reported that inflammatory response due to *S. aureus* infection begins with the reorganization of lipopeptides and peptidoglycan components of the *S. aureus* cell wall by toll-like receptors (TLR-2) in the immune system and resulted in the stimulation of pro-inflammatory cytokine production such as TNF-α, IL-1β, and IL-6. Meanwhile,

Table 4: The effect of experimental infection with *S. aureus* and treatment with bee venom and gentamicin on some biochemical parameters of rabbits at 7 days post inoculation (n=5)

Group	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)	Nitric oxide (µmol/L)	Malondialdehyde (mg %)	Glutathione peroxidase (mg %)
Group 1	491.3 ± 26.3^{d}	406.3 ± 20.9^{c}	404.3 ± 28.4^{c}	13.9 ± 0.74^{c}	6.5 ± 0.33^{c}	7.4 ± 0.37a
Group 2	1016.0 ± 29.2^{a}	814.3 ± 30.4^{a}	983.3 ± 35.8^{a}	50.7 ± 1.3^{a}	29.7 ± 1.14^{a}	3.1 ± 0.34^{c}
Group 3	534.3 ± 30.4^{d}	412.3 ± 14.3^{c}	425.3 ± 29.5^{c}	14.7 ± 0.64^{c}	7.3 ± 0.46^{c}	7.6 ± 0.47^{a}
Group 4	778.6 ± 15.3^{b}	546.1 ± 29^{b}	565.6 ± 35.4^{b}	35.2 ± 1.7^{b}	15.3 ± 0.47^{b}	5.4 ± 0.15^{b}
Group 5	665.6 ± 25.7^{c}	499.6 ± 25.6^{bc}	499.0 ± 25.6^{bc}	30.3 ± 0.87^{b}	14.3 ± 0.51^{b}	6.3 ± 0.40^{b}

(TNF): tumor necrosis factor; IL-1β: interleukin1β; IL-6: interleukin 6

Means with different letters at the same column are significantly different (P <0.05)

Table 5: The effect of experimental infection with *S. aureus* and treatment with bee venom and gentamicin on some biochemical parameters of rabbits at 14 days post inoculation (n=5)

Group	IL-1β (pg/ml)	IL-6 (pg/ml)	TNF-α (pg/ml)	Nitric oxide (µmol/L)	Malondialdehyde (mg %)	Glutathione peroxidase (mg %)
Group 1	505.3 ± 10.8^{c}	398.6 ± 32.0^{c}	411.2 ± 22.0^{c}	12.9 ± 0.92^{b}	7.6 ± 0.23^{c}	7.5 ± 0.20^{a}
Group 2	940.0 ± 27.2^{a}	829.3 ± 22.0^{a}	703.6 ± 25.0^{a}	40.3 ± 1.15^{a}	30.2 ± 1.20^{a}	3.6 ± 0.43^{b}
Group 3	512.6 ± 22.2^{c}	404.1 ± 20.0^{c}	400.4 ± 20.0^{c}	14.4 ± 0.40^{b}	7.7 ± 0.27^{c}	7.7 ± 0.18^{a}
Group 4	587.3 ± 12.4^{b}	506.2 ± 17.0^{b}	545.2 ± 23.0^{b}	15.1 ± 1.25^{b}	11.8 ± 0.22^{b}	7.0 ± 0.22^{a}
Group 5	567.6 ± 10.0^{bc}	409.0 ± 32.0^{c}	474.0 ± 22.0^{bc}	14.2 ± 0.78^{b}	10.2 ± 0.57^{b}	7.2 ± 0.15^{a}

(TNF): tumor necrosis factor; IL-1β: interleukin1β; IL-6: interleukin 6

Means with different letters at the same column are significantly different (P <0.05)

Table 6: Clinical and histopathological finding in joints of rabbits after intra-articular *S. aureus* infection (n=5)

Time of observation/ groups	Day of examination	No. of animals with clinical signs	Periarticular abscess formation	Predominant inflammatory cells in synovitis		Pannus formation	Clustering of chondrocytes
				PMNCs	MCs		
Group 2	7th	4	4	++++	+	4	5
	14th	5	5	++	++++	5	4
Group 4	7th	3	2	+++	+	3	3
	14th	0	0	+	+++	1	1
Group 5	7th	3	0	+++	+	2	1
	14th	0	0	+	+	1	0

+ minimal infiltration; ++ mild infiltration; +++ moderate infiltration; ++++, massive infiltration

PMNCs: polymorph-nuclear cells; MNCs; mononuclear cells

treatment with either BV alone or in a combination with gentamicin evoked a significant decrease in these parameters in groups 4, and 5 when compared with group 2. This might be attributed to the anti-inflammatory activity of melittin which is a more abundant component in BV as it blocks the TLR-2 and finally causes inhibition of the production of TNF-α, IL-1β, and IL-6 that proving the anti-inflammatory effect of BV (Lee and Bae, 2016; Wilson et al., 2022). Moreover, apamin in the bee venom consider an anti-inflammatory agent due to its ability to cause the inhibition of cyclooxygenase-2 and lower the levels of TNF-, IL-1, IL-6, and NO (Shin et al., 2018; Lee et al., 2020). Infection with *S. aureus* caused a significant decrease (P <0.05) in glutathione peroxidase enzyme in group 2 compared with group 1 during the experimental period. While groups (4, and 5) treated with either BV alone or BV with gentamicin revealed a significant increase in such parameters compared with group 2. The increase in the inflammatory cytokines, particularly IL6 is a major factor for the onset of oxidative stress, BV inhibits the activity of IL-6 and therefore decreases oxidative stress. In agreement with this assumption, Sobral et al. (2016) stated that BV has significant antioxidant effects. The results in the current work showed that the MDA level was significantly increased in group 2 compared with group 1 and significantly decreased after treatment with bee venom, our results agreed with El-Hanoun et al. (2020), this antioxidant activity might be due to the ability of melittin and

apamin (the main components of BV) to inhibit the lipid peroxidation (Park et al., 2018), moreover, Suh et al. (2006) reported that BV inhibited oxidative damage through the inhibition of ROS production.

Clinical examination of the infected groups with septic arthritis revealed discomfort and difficulty in moving the joint. Symptoms included fever, lameness, hotness, redness, and swelling of the infected limbs with no recorded mortalities. These signs decreased gradually in the treated groups (4, and 5) as shown in Table 6. Likely, Brooks and Jefferson (2012) and Nasser et al. (2020) revealed that *S. aureus* is a significant bacterial disease of rabbits associated with synovitis and osteomyelitis symptoms. As shown, the total scores of the treated animal groups were lower in the clinical signs in comparison with the untreated group 2. This lower lesion score might be due to the lower damage as evidenced by pannus formation, proteoglycan depletion, and synovitis (Wysenbeek et al., 1998; Al-Ani et al., 2015). Histopathological examination on the 7th day of the *S. aureus*-infected group without treatment (Group 2) showed degenerative changes with fibrillation within the cartilage of articular surfaces besides the presence of pannus formation. Synovial membrane within the joint cavity revealed septic synovitis which was formed from intense aggregates of the inflammatory cells mainly neutrophils inter-mixed with fibrin exudates and dilated capillaries (Fig. 2, lanes 1-3). Group 4 which received treatment with BV alone showed restoration of all zones of chondrocytes at articular surfaces with hypertrophied chondrocytes. But synovial membrane showed moderate inflammation with cellular proliferation and exudation in the synovium within the joint cavity (Fig. 2, lanes 4, 5). Group 5 which received treatment with BV and gentamicin showed a reduction of synovitis area within the joint cavity. Hypercellularity of the articular surfaces with reactive chondrocytes. (Fig. 2, lanes 6, 7). While histopathological examination on the 14th day of the *S. aureus*-infected group without treatment (Group 2) showed destructive and septic arthritis which was represented by replacement of the superficial and transitional zones of articular cartilage by eosinophilic and basophilic materials besides the presence of fissures and clefts in the transitional and radial zones with fibrillation and pannus formation. Moreover, the synovial membrane which lined the joint cavity showed cellular proliferation with large areas of inflammatory exudates mainly monocytes, and periarticular abscess formation with neutrophils and fibrin threads (Fig. 3, lanes 8-10). Group 4 which received treatment with BV alone showed articular surfaces with smooth flattened chondrocytes in the superficial zone and normal chondrocytes in moderate and deep zones. However, the synovial membrane showed mild synovitis which was formed from a minute number of inflammatory cells and fibroblasts (Fig. 3, lanes 11, 12). Group 5

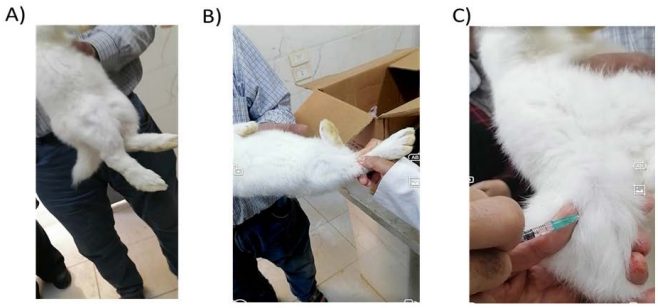

Figure 1: Representative images for A) group 1 normal rabbit, B) group 2 control positive with enlarged joint, and C) group treated with BV

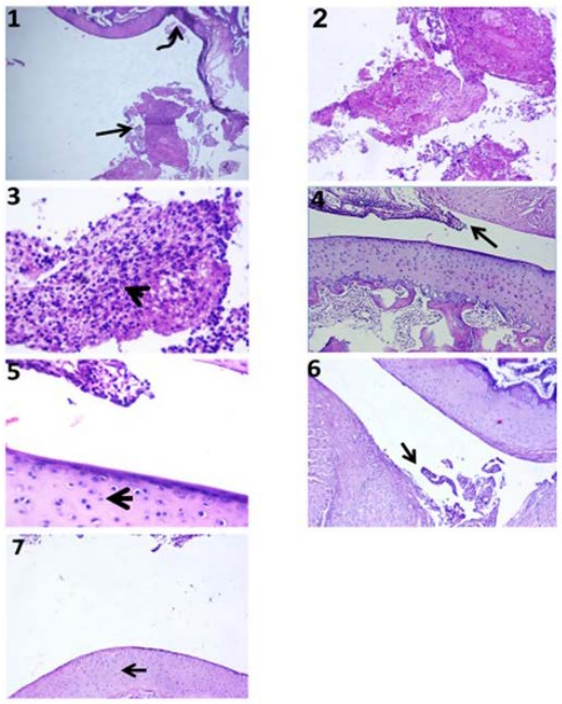

Figure 2: Histopathological findings of *S. aureus*-inoculated rabbit jointson the7 th day post inoculation
Lane 1 showing group 2 with degenerative changes with fibrillation within the cartilage of articular surfaces (curved arrow) with pannus formation (arrow) (5X), lane 2 showing septic synovitis formed from aggregates of inflammatory exudates (10X), lane 3 showing aggregation of neutrophils (arrowhead) intermixed with fibrin exudates within synovium (40X). Lanes 4 and 5 showing group 4 with restoration of all zones of chondrocytes at articular surfaces with hypertrophied chondrocytes (arrowhead) beside presence of moderate synovitis with cellular proliferation and exudation in the synovium within the joint cavity (arrow) (5, 10X). Lanes 6 and 7 showing group 5 with reduction of synovitis area within the joint cavity (arrow) and hypercellularity of the articular surfaces with reactive chondrocytes (arrowhead) (H&E staining) (10, 40X).

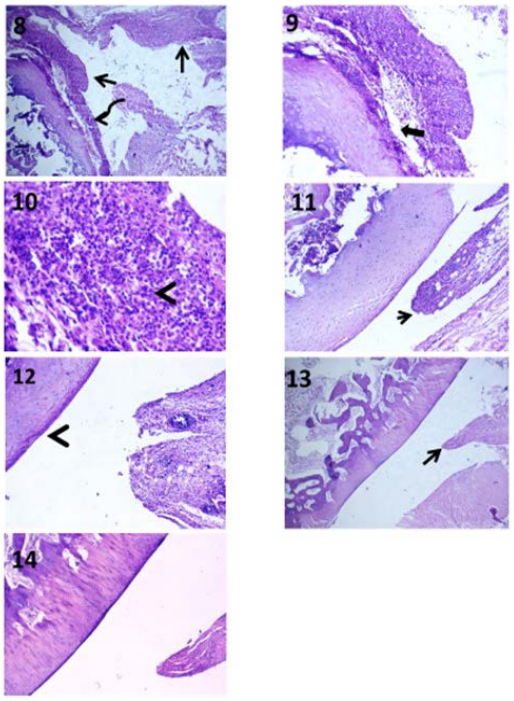

Figure 3: Histopathological findings of *S. aureus*-inoculated rabbit jointson the7 th day post inoculation Lane 8 showing group 2 with destructive and septic arthritis beside presence of fibrillation (curved arrow), ostiomalacia and pannus formation (arrows) (5X). Lane 9 showing group 2 with replacement of the superficial and transitional zones of articular cartilage by eosinophilic and basophilic materials (thick arrow) (10X). Lane 10 showing group 2 with cellular proliferation with large areas of inflammatory exudates mainly neutrophils (arrowhead) and fibrin threads in synovial membrane (40X). Lanes 11 and 12 showing group 2 with articular surfaces showing smooth flattened chondrocytes in superficial zone and apparently normal chondrocytes in mid (arrowhead) and deep zones beside mild synovitis (arrow) (10, 40X). Lanes 13 and 14 showing group 5 with reduced area of pannus formation (arrow) and apparently normal histomorphological structures of superficial, mid, and deep zones of chondrocytes at the articular joints (5, 10X) (H&E staining).

which received treatment with BV and gentamicin showed a reduced area of pannus formation which formed mainly from macrophages, other inflammatory cells, and fibroblasts. Articular surfaces of the knee joint revealed normal histomorphology structures of chondrocytes which are organized into superficial, mid, and deep zones (Fig. 3, lanes 13, 14). In agreement with the reported histopathological alterations in the infected group, Linhart et al. (1990) supported these points as acellular cartilage, erosions, clustering, pannus formation, proteoglycan depletion, and severe synovitis. Such pathological effects might be attributed to the bacterial virulence factors and the reduction of the host immune status (Priscila and Alexandrina, 2014). The beneficial effects of BV agreed with AL-Ani et al. (2015) who confirmed the antimicrobial activity of BV against several bacterial species. Therefore, this study shows a promising tool for the treatment of bacterial septic arthritis using a combination of BV and antibiotics, particularly gentamicin.

CONCLUSION

BV could have therapeutic activities on *S. aureus*-induced arthritis in rabbits and could improve the influenced hematological, biochemical parameters, immune response, and pathological alterations. This improvement became more prominent by using a combination of BV and gentamicin. However, respect of the withdrawal times and observing the adverse effects of gentamicin are highly recommended before its use in rabbit medicine.

ACKNOWLEDGMENT

We acknowledge the kind support of professor Dr. Nahed A. Kamoura, professor of Clinical pathology.

CONFLICT OF INTEREST

None.

NOVELTY STATEMENT

This study investigated the hematological, biochemical, and histopathological alterations of S. aureus-induced septic arthritis in rabbits. In addition, the therabeutic use of bee venom alone or in a combination with gentamicin against such bacterial disease was suggested.

AUTHORS' CONTRIBUTION

All authors contributed equally to this study.

REFERENCES

Ainara A, Sango MG, Escudero-Jimenez A, Losa-Palacios S, Berenguer-Romero MD, Geronimo-Pardo M (2021). Development of an experimental model of septic knee arthritis in rats through intra-articular inoculation of *Staphylococcus aureus*.Lab. Animals, 55(3): 270–280. https://doi.org/10.1177/0023677220978678

Akkaya S, Nazali M, Frda AK (2012). Cefazolin-sodium has no adverse effect on fracture healing in an experimental rabbit model. Eklem Hastalik Cerrahisi Turkey, 23(1): 44-8.

AL-Ani I, Zimmermann S, Reichling J, Wink M (2015). Pharmacological synergism of bee venom and melittin with antibiotics and plant secondary metabolites against multi-drug resistant microbial pathogens. Phytomed., 22(2): 245-255. https://doi.org/10.1016/j.phymed.2014.11.019

Bauer AW, Kirdy WM, Sherries JC, Turck M (1966). Antibiotic susceptibility testing by standardized signal disk method. Am. J. Clin. Pathol., 45(4): 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bitschar KC, Wolz B, Krismer A, Schittek B (2017). Keratinocytes as sensors and central players in the immune defense against *Staphylococcus aureus* in the skin. J. Dermatol. Sci., 87: 215-220. https://doi.org/10.1016/j.jdermsci.2017.06.003

Boerhout EM, Koets AP, Vernooij JC, Mols-Vorstermans TG, Nuijten PJ, Rutten VP, Bijlsma JJ, Eisenberg SW (2016). Reisolation of *Staphylococcus aureus* from bovine milk following experimental inoculation is influenced by fat percentage and specific immunoglobulin G1 titer in milk. J. Dairy Sci., 99(6): 4259-69. https://doi.org/10.3168/jds.2015-10648

Brooks JL, Jefferson KK (2012). Staphylococcal biofilms: quest for the magic bullet. Adv. Appl. Microbiol., 81: 63-87. https://doi.org/10.1016/B978-0-12-394382-8.00002-2

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. Int. J. Food Microbiol., 94(3): 223-53. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

CLSI (2017). Performance standards for antimicrobial susceptibility testing, 27th ed. CLSI document M100-S27. CLSI, Wayne, PA.

Cruickshank R, Duguid JP, Masion BP, Swain RH (1979). Medical Microbiology. 12th ed., Churchill Livingstone, Edinburgh, London, New York.

Dimitrova DJ, Pashov DA, Kanelov IN (2000). Flucloxacillin pharmacokinetics following intravenous and oral administration in dogs. BJVM., 3: 13-18.

Edwards AM, Potts JR, Josefsson E, Massey RC (2010). *Staphylococcus aureus* host cell invasion and virulence in sepsis is facilitated by the multiple repeats within FnBPA. PLoS Pathogens, 6(6):e1000964. https://doi.org/10.1371/journal.ppat.1000964

El-Bahnasy SA, Mahfouz HM, ElBassiony M, El-Shibiny A, El-Bolok DM (2022). Antibacterial activities of bee venom produced by two honeybee, *Apis mellifera L*., hybrids. Sinai J. Appl. Sci., 11(2): 257-70. https://doi.org/10.21608/sinjas.2022.117292.1090

El-Hanoun A, El-Komy A, El-Sabrout K, Abdella M (2020). Effect of bee venom on reproductive performance and immune response of male rabbits. Physiol. Behav., 223: 112987. https://doi.org/10.1016/j.physbeh.2020.112987

European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2003). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. Clin. Microbiol. Infect., 9: 1-7. https://doi.org/10.1046/j.1469-0691.2003.00790.x

Feldmann BV, Zinki JG, Jain NC (2000). Shalm's Veterinary Hematology. 5th ed. Lea and Fibiger, Philadelphia, USA.

Georgieva T., Petrov V., Lyutskanov M., Georgiev I., Zaprianova D., Marutsov P., Rusenova N., Parvanov P. (2016). Effect of Staphylococcus Aureus infection on plasma creatine kinase activity in rabbits. Trakia J. Sci. 14. 52-59. https://doi.org/10.15547/tjs.2016.01.007.

Ghosh C, Bishayi B (2015). Toll-like receptor 2 and 6 interdependency in the erosive stage of *Staphylococcus aureus* induced septic arthritis mediated by IFN-γ and IL-6. A possible involvement of IL-17 in the progression of the disease. Immunobiol., 220: 910-923. https://doi.org/10.1016/j.imbio.2015.01.012

Hegazi AG, EL-Feel MA, Abdel-Rahman EA, Al-Fattah A (2015). Antibacterial activity of bee venom collected from *Apis mellifera* carniolan pure and hybrid races by two collection methods. Int. J. Curr. Microbiol. App. Sci., 4(4): 141-149.

Jin T, Lu Y, He QX, Wang H, Li BF, Zhu LY, Xu QY (2015). The role of microRNA, miR-24, and its target CHI3L1 in osteomyelitis caused by *Staphylococcus aureus*. J. Cellular Biochem., 116(12): 2804-13. https://doi.org/10.1002/jcb.25225

Kim ST, Hwang JY, Sung MS, Je SY, Bae DR, Han SM, Lee SH (2006). The Minimum Inhibitory Concentration (MIC) of bee venom against bacteria isolated from pigs and chickens. Korean J. Vet. Serv., 29(1): 19-26.

Kwan TS, Padrines M, Théoleyre S, Heymann D, Fortun Y (2004). IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev., 15(1): 49-60. https://doi.org/10.1016/j.cytogfr.2003.10.005

Leandro LF, Mendes CA, Casemiro LA, Vinholis AH, Cunha WR, Almeida R (2015). Antimicrobial activity of apitoxin, melittin and phospholipase A_2 of honey bee (*Apis mellifera*) venom against oral pathogens. An. Acad. Bras. Cienc., 87(1): 147-155. https://doi.org/10.1590/0001-3765201520130511

Lee G, Bae H (2016). Anti-inflammatory applications of melittin, a major component of bee venom: Detailed mechanism of action and adverse effects. Molecules, 21: 616. https://doi.org/10.3390/molecules21050616

Lee YM, Cho SN, Son E, Song CH, Kim DS (2020). Apamin from bee venom suppresses inflammation in amurine model of gouty arthritis. J. Ethnopharmacol., 257: 112860. https://doi.org/10.1016/j.jep.2020.112860

Linhart WE, Steinwender G, Weybora W, Zadravec S, Esterbauer H (1990) Influence of superoxide dismutase on staphylococcal arthritis- A histological and biochemical investigation using an experimental animal model. Agents Actions. Mar., 29(3-4): 259-65. https://doi.org/10.1007/BF01966456

Marcheix PS, Martin C, Fiorenza F, Leclercq X, Sturtz F (2018). Intra-articular Gentamicin-loaded PLA Microparticle Injection for the Treatment of Septic Arthritis in Rabbits. J. Am. Acad. Orthop. Surg., 26(16): e349-e356. https://doi.org/10.5435/JAAOS-D-16-00883

Miller J, Slebodzinska BA (1993). Oxidative stress, antioxidants and animal func-tion J. Dairy Sci., 76: 2812-2823. https://doi.org/10.3168/jds.S0022-0302(93)77620-1

Mahmoudi H, Alikhani MY, Imani Fooladi AA (2020). Synergistic antimicrobial activity of melittin with clindamycin on the expression of encoding exfoliative toxin in *Staphylococcus aureus*. Toxicon, 183: 11-19. https://doi.org/10.1016/j.toxicon.2020.05.004

Mohammed ZI, Hassan AJ (2019). Effect of bee venom on some blood and and biochemical parameters in formaldehyde induced arthritis male rats in comparison with prednisolone drug. J. Physics Conf Ser., 1234: 012066. https://doi.org/10.1088/1742-6596/1234/1/012066

Mowafy RE, Megahed HM, El Oksh AS (2018). Pathological studies on infertility in bucks as a sign to some bacterial infection. Int. J. Curr. Res. Biosci. Plant Biol., 5(11): 7-33. https://doi.org/10.20546/ijcrbp.2018.511.002

Nasser A, Azimi T, Ostadmohammadi S, Ostadmohammadi S (2020). A comprehensive review of bacterial osteomyelitis with emphasis on *Staphylococcus aureus*. Microb.

Pathogen.,148: 104431. https://doi.org/10.1016/j.micpath.2020.104431

Ozlem Nisbet H, Ozak A, Yardimci C, Nisbet C, Yarim M (2011). Evaluation of bee venom and hyaluronic acid in the intra-articular treatment of osteoarthritis in an experimental rabbit model. Res. Vet. Sci., 7: 5.

Park HG, Lee KS, Kim BY, Yoon HJ, Choi YS, Lee KY, Wan H, Li J, Jin BR (2018). Honeybee (*Apis cerana*) vitellogenin acts as an antimicrobial and antioxidant agent in the body and venom. Dev. Comp. Immunol., 85: 51-60. https://doi.org/10.1016/j.dci.2018.04.001

Penadés M, Viana D, García-Quirós A, Muñoz-Silvestre A, Moreno-Grua E, Pérez-Fuentes S, Pascual J, Corpa JM, Selva L (2020). Differences in virulence between the two more prevalent *Staphylococcus aureus* clonal complexes in rabbitries (CC121 and CC96) using an experimental model of mammary gland infection. Vet. Res., 51: 11. https://doi.org/10.1186/s13567-020-0740-1

Permual SR, Pachiappan A, Gopalakrishnakone P, Thwin MM, Hian YE, Chow VT (2006). *In vitro* antibacterial activity of natural toxins and animal venoms tested against *Burkholderia Pseudomallei*. BMC Infect. Dis., 6: 100. https://doi.org/10.1186/1471-2334-6-100

Petrov VS, Mircheva TM (2013). A study on the hematological changes inobese and non- obese rabbits with experimental Staphylococcus aureus infection. Bulgarian J. Vet. Med., 16: 121: 133.

Priscila MC, Alexandrina S (2014) Septic arthritis: immunopathogenesis, experimental models and therapy. J. Venom. Animals Toxins Trop. Dis., 20:19 https://doi.org/10.1186/1678-9199-20-19

Sang MH, Joung MK, Hong IP, Woo SO, Kim SG, Jang HR, Pak SC (2016). Antibacterial activity and antibiotic-enhancing effects of honeybee venom against methicillin-resistant *Staphylococcus aureus*. Molecules, 21: 79. https://doi.org/10.3390/molecules21010079

Satoh K (1987). Lipid peroxide (Malondialdehyde) colorimetric Methods. Clin. Chem. Acta, 90: 37.

Shin SH, Ye MK, Choi SY, Park KK (2018). Anti-inflammatory effect of bee venom in an allergic chronic rhinosinusitis mouse model. Mol. Med. Rep., 17: 6632-6638. https://doi.org/10.3892/mmr.2018.8720

Sobral F, Sampaio A, Falcão S (2016). Chemical characterization, antioxidant, anti-inf lammatory and cytotoxic properties of bee venom collected in Northeast Portugal. Food Chem. Toxicol., 94: 172-7. https://doi.org/10.1016/j.fct.2016.06.008

Son DJ, Lee JW, Lee YH, Song HS, Lee CK, Hong JT (2007). Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacol. Ther., 115(2): 246-70. https://doi.org/10.1016/j.pharmthera.2007.04.004

Suh SJ, Kim KS, Kim MJ, Chang YC, Lee SD, Kim MS, Kim CH (2006). Effects of bee venom on protease activities and free radical damages in synovial fluid from type II collagen-induced rheumatoid arthritis rats. Toxicol. In Vitro, 20: 1465-1471. https://doi.org/10.1016/j.tiv.2006.06.016

Suvarana SK, Layton C, Bancroft JD (2018). Bancroft's theory and practice of Histological techniques.8thed., Churchill Livingstone. Elsevier, England.

Wehbe R, Frangieh J, Rima M, El Obeid D, Sabatier J, Fajloun Z (2019). Bee Venom: Overview of main compounds and bioactivities for therapeutic interests. Molecules. 24(16): 2997 https://doi.org/10.3390/molecules24162997.

Wenhua R, Shuangquan Z, Daxiang S, Kaiya Z, Guang Y (2006). Induction, purification and characterization of an antibacterial peptide scolopendrin I from the venom of centipede Scolopendra subspinipes multilans. Indian J. Biochem. Biophys., 43: 88-93.

Wilson AW, Ripari N, Fernanda LC, Honorio MS, Sartori AA, Matucci RH, Sforcin JM (2022). An overview about apitherapy and its clinical applications. Phytomed. Plus, 12: 100239. https://doi.org/10.1016/j.phyplu.2022.100239

Wysenbeek AJ, Volchek J, Amit M, Robinson D, Boldur I, Nevo Z (1998). Treatment of staphylococcal septic arthritis in rabbits by systemic antibiotics and intra-articular corticosteroids. Ann. Rheum. Dis., Dis., 57(11): 687-690. https://doi.org/10.1136/ard.57.11.687