The therapeutic efficacy of *Aloe vera* gel ointment on staphylococcal pyoderma in dogs

Ahmed Kamr, Ali Arbaga, Amanallah El-Bahrawy, Ahmed Elsify and Hany Hassan

1. Department of Animal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt; 2. Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Egypt.

**Corresponding author:** Hany Hassan, e-mail: hany.youssef@vet.usc.edu.eg

**Co-authors:** AK: ahmed.basha@vet.usc.edu.eg, AA: ali_a.azem@vet.usc.edu.eg, AE: amanallah.elbahrawy@vet.usc.edu.eg, AhE: ahmed.elsify@vet.usc.edu.eg, HK: hadeer.khaled@vet.usc.edu.eg

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**Abstract**

**Background and Aim:** Staphylococcus pyoderma is a common problem in dogs that need a novel treatment rather than antibiotic therapy. The aim of this study was to investigate the therapeutic efficacy, anti-inflammatory, and antioxidative properties of *Aloe vera* (*Aloe barbadensis*) gel ointment on dogs’ Staphylococcus pyoderma compared to gentamicin ointment.

**Materials and Methods:** The inhibition zone of *A. vera* extract 20% and 40% and gentamicin 1% against *Staphylococcus aureus* was determined on well diffusion agar. Twenty Baladi local breed dogs were used as control negative group before intradermal inoculation with 10⁵ CFU *S. aureus*. The animals were classified into four equal groups, control positive group without treatment (n=5), treated groups by 20% *A. vera* gel ointment (n=5), 40% *A. vera* gel ointment (n=5), and gentamicin ointment 1% (n=5). Topical application of *A. vera* and gentamicin ointments was carried out twice daily for 2 weeks until complete healing of dogs’ pyoderma. Clinical evaluation was recorded. Inflammatory, oxidant, and antioxidant parameters were measured in serum.

**Results:** The inhibition zone of *A. vera* extracts 20% and 40% was 19 mm and 23 mm, respectively, while gentamicin 1% was 18 mm. The half maximal inhibitory concentration (of *A. vera* 20% and 40% were 13.70 with R²=0.98. Dogs’ pyoderma treated with *A. vera* gel ointment 20% and 40% were more likely to have low haptoglobin and tumor necrosis factor-α concentrations than gentamicin 1% ([odds ratio [OR]=4.6; 95% confidence interval [CI]=1.31-17.40; p<0.05]; [OR=5.2; 95% CI=1.04-22.30; p<0.05]), respectively.

**Conclusion:** It seems evident that *A. vera* has therapeutic effect, antibacterial, and anti-inflammatory effects against dogs’ staphylococcal pyoderma than gentamicin that would support its further use rather than antibiotics in one health arena.

**Keywords:** catalase, dogs, interleukins, malondialdehyde, pyoderma, serum amyloid A.

**Introduction**

*Aloe vera* (*Aloe barbadensis*) is a medicinal plant that has many functions include wound healing, immunomodulation, and hepatoprotection in addition to its antiviral, skin protective, antioxidant, antidiabetic, anti-inflammatory, antimicrobial, and anticancer properties [1]. *A. vera* is characterized by the presence of many active compounds, inclusive of amino acids, minerals, anthraquiones, chromones, nutrients, lipids, anthrones, carbohydrates, and flavonoids [2]. Canine superficial pyoderma is described as superficial bacterial contamination of the dermis and hair follicles that caused by *Staphylococcus* spp. [3]. Pyoderma can be caused by infectious, neoplastic, and/or inflammatory etiologies with consequences of the accumulation of neutrophilic exudates and associated with increased free radical production, oxidative stress [4,5]. Of interest, *Staphylococcus aureus*, which is considered methicillin-resistant, was isolated from dogs with recurrent pyoderma [6].

Acute-phase response is a distinguished systemic reaction of the body within 24-48 h after local or systemic diseases due to infection, tissues damage, surgery, trauma, and immunological impairment [7-9]. Serum amyloid A (SAA), haptoglobin (HP), and ceruloplasmin (CP) were produced by liver and considered prognostic biomarkers for acute inflammation in dogs as a result of tissue infection or inflammation [10,11]. Cytokines are small glycoproteins (~5–20 kDa) that have autocrine, paracrine, and endocrine signaling. Cytokines include chemokines, interferons, interleukins (IL), lymphokines, and tumor necrosis factors (TNF) are produced by many types of cells that include macrophages and lymphocytes that are essential in host immune responses to infection, inflammation, trauma, sepsis, cancer, and reproduction [12].
However, the inflammatory and immunomodulatory roles of A. vera in dogs’ pyoderma need further investigation.

This study investigated the beneficial healing effect, antibacterial, anti-inflammatory, and antioxidative roles of A. vera in dogs’ staphylococcal pyoderma. We hypothesized that acute-phase proteins, cytokines, and oxidants will be elevated, while antioxidant biomarkers will be reduced in dog’s staphylococcal pyoderma; furthermore, A. vera gel ointment will modulate the clinical image inflammatory response in a better manner than gentamicin against staphylococcal pyoderma in dogs.

Materials and Methods

Ethical approval

All dogs were used and treated according to Animal Ethics Committee at College of Veterinary Medicine, University of Sadat City (Approval code VUSC-007-1-19).

Study period and location

This study was conducted between September 2018 and May 2019. The experiment was conducted at Animal Medicine and Infectious Diseases Department, teaching hospital and laboratory, University of Sadat City.

Animals’ criteria

A total of 20 Baladi local male adult dogs 2-3 years old were housed in individual cages for acclimatization for 3 weeks, with free access to food and water. According to the production company Pharma Swede, Egypt, dogs were dewormed by subcutaneous injection of ivermectin (Paramectin®) at a dosage of 10 mg/50 kg as a routine protocol against external parasites that would interfere with experimental induction of canine pyoderma.

Experimental design

Twenty male adult dogs of Baladi local breed were clinically healthy based on their physical examination. The animals were considered as a control negative group before induction of pyoderma (n=20). The dogs were inoculated intradermally in chest region with 1 mL broth containing 10⁴ CFU staphylococcus [6,13]. The injected dogs were further classified into further subgroups after appearance of pyoderma lesions into control positive group without treatment (n=5), treated groups by 20% A. vera gel ointment (n=5), 40% A. vera gel ointment (n=5), and gentamicin ointment 0.1% (n=5). Topical application of A. vera and gentamicin ointments (Garamycin® 0.1%; Schering-Plough Company, USA) was carried twice daily for 2 weeks until complete healing of dogs’ pyoderma.

In vitro evaluation of antibacterial effect of A. vera extract

Antibacterial activity of A. vera extract was evaluated using agar diffusion test in which 15-20 mL of nutrient agar (Oxoid®) was poured on glass Petri plates of same size and allowed to solidify. Agar surface of each plate was streaked by a sterile cotton swab with S. aureus equivalent to 0.5 McFarland standards. Agar plate was punched with a sterile cork borer of 4 mm size; then, A. vera fresh extract 20% and 40% concentration, gentamycin 0.1%, pure A. vera, and pure ethanol were filled into its wells. The plate was allowed to stand by for 30 min. The plate was incubated at 37°C for 48 h and examined. The diameter of inhibition zones was measured [14].

A. vera gel ointment 20% and 40% preparation

Preparation of A. vera gel ointments 20% and 40% formula was carried out by adding 20 g and 40 g of A. vera gel, respectively, 3 g of wax and 5 mL of paraffin oil with 72 g and 52 g of Vaseline™, respectively. All components were mixed well into mortar until obtaining homogenous ointments. Gentamycin ointment was purchased as a commercial product from the pharmacy (Garamycin® 0.1%; Schering-Plough Company, USA). A. vera concentrations of 5%, 10%, and 15% did not give the optimal results against dogs’ pyoderma, so we used 20% and 40% A. vera concentrations in this study.

Sampling

Blood samples were collected from admitted dogs in EDTA and serum clot tubes for hematobiochemical analysis before induction and on 3rd, 7th, 10th, and 14th day after induction and treatment of Staphylococcus pyoderma. Samples were centrifuged at 2000×g for 10 min at 4°C. Serum and plasma were aliquoted into smaller volumes and stored at −80°C until analyzed.

Clinical examination and complete blood count

Physical and clinical examinations were determined for all dogs according to methods described by Englar [15]. The hemogram and leukogram examinations were measured and calculated for each dog according to methods described by Turgeon [16]. Dog’s pyoderma lesions diameter (Ø) was measured and expressed as mm.

Oxidant, antioxidant, and inflammatory biomarkers measurements

Serum malondialdehyde (MDA), total antioxidant capacity (TAC), and catalase concentrations were measured by commercial kits (Bio Diagnostics, Ltd., Egypt) according to methods described by Okhawa et al. [17], Koracevic et al. [18], Aebi [19], respectively. Serum CP, SAA, HP, and ILs; IL-1, IL-6, and IL-10 and TNF-α were determined by commercial ELISA kits (Shanghai Coon Koon Biotech., Ltd.; China) with inter- and intra-coefficient of variations <10 and good linearity (R²=0.95). All procedures were performed as described in the instructions of the manufactures.

Statistical analysis

Data were assessed for normality and were normally distributed based on Shapiro–Wilk test.
Data were expressed as mean with standard error. Comparisons between the groups were performed by one-way ANOVA using SPSS Statistics 24.0 version [20]. Receiver operating characteristic curve and the half maximal inhibitory concentration (IC$_{50}$) were calculated using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA). Univariate logistic regression includes odds ratio (OR) and 95% confidence interval (CI) was calculated. Significance was set at p<0.05.

**Results**

*In vitro* evaluation of antibacterial effect of *A. vera* extracts and drug efficacy on dogs' staphylococcal pyoderma

*A. vera* 20% and 40% extracts and gentamycin 0.1% inhibition zone diameter were 19 mm, 23 mm, and 18 mm, respectively, compared by raw *A. vera* gel and pure ethanol, as shown in Figure-1a. *A. vera* concentrations 20% and 40% had IC$_{50}$=13.70 with 95% CI=12.54-14.88 with R$^2$=0.98; p<0.05; (Figure-1b).

**Clinical findings between the studied groups of dogs**

There was a significant increase in diameter of pyoderma lesions in infected dogs on 3rd-10th day without treatment compared to control negative group (p<0.05; Table-1). In dogs treated with *A. vera* gel ointment 20% and 40% and gentamicin ointments 0.1%, the diameter of the pyoderma lesions was significantly reduced on the 3rd and 7th day post-treatment (DPT) compared to control positive group dogs (p<0.05; Figure-2a and b and Table-1), while there is no statistical variation between dogs treated with

![Figure-1: In vitro evaluation of antibacterial effect of Aloe vera extracts and drug efficacy on dogs’ staphylococcal pyoderma. (a) In vitro evaluation of antibacterial activity of different concentrations A. vera extracts 20% (1) and 40% (2), gentamicin (3), raw A. vera gel (4), and pure ethanol (5); (b) A. vera concentrations 20% and 40% had half maximal inhibitory concentration (IC$_{50}$) than A. vera 5%, 10%, and 15% concentrations (IC$_{50}$=13.70; 95% confidence interval=12.54-14.88 with R$^2$=0.98).](image)

| Variables | Ø of pyoderma lesion (mm) |
|-----------|--------------------------|
| Control negative dogs (before induction) (n=20) | No lesions |
| Infected dogs without treatment Group 1 (n=5) | No lesions |
| 3 DWT | 8.12±0.17* |
| 7 DWT | 6.1±0.2 |
| 10 DWT | 4.3±0.28# |
| 14 DWT | 2.2±0.18# |
| Dogs treated with *Aloe vera* gel ointment 20% Group 2 (n=5) | No lesions |
| 3 DPT | 6±0.2* |
| 7 DPT | 3.8±0.2# |
| 10 DPT | Cured |
| 14 DPT | Epidermal collarette (lesion with a circular scaly edge) |
| Dogs treated with *Aloe vera* gel ointment 40% Group 3 (n=5) | No lesions |
| 3 DPT | 6±0.3* |
| 7 DPT | 3.6±0.4# |
| 10 DPT | Epidermal collarette (lesion with a circular scaly edge) |
| 14 DPT | Complete healing |
| Dogs treated by gentamicin sulfate 0.1% ointment Group 4 (n=5) | No lesions |
| 3 DPT | 6.48±0.4* |
| 7 DPT | 3.85±0.11# |
| 10 DPT | Cured |
| 14 DPT | Epidermal collarette (lesion with a circular scaly edge) |

SE=Standard error, DWT=Day without treatment, DPT=Day post-treatment, n=Number, mm=Millimeter. Means with different letter superscripts in the same column are significantly different at p<0.05.
A. vera gel ointment 20% and 40% and gentamicin ointments 0.1% and control ones (p>0.05; Table-1). Control positive group with Staphylococcus pyoderma showed sloughing of epidermis, severe inflammatory cells infiltration in the dermis, necrosis, and collagen degeneration (Figure-3a). A. vera gel ointment treated group with concentration of 40% showed normal epidermis and few inflammatory cells infiltration in the dermis on the 14th day of treatment (Figure-3b). The mean values of body temperature, respiratory, and pulse rates were elevated in infected dogs on the 3rd-10th day without treatment compared to healthy control negative dogs (p<0.05; Table-2), but not with the 14th day without treatment (p>0.05). There was significant increase for the values of body temperature, respiratory, and pulse rates in dogs treated with A. vera gel ointment 20% and 40% and gentamicin ointment 0.1% at different times of treatment on the 3rd DPT (p<0.05; Table-2), then returned to their normal values from the 7th to 14th DPT when compared to control healthy ones (p>0.05; Table-2).

**Hematological findings between the studied groups of dogs**

The mean values of the red blood cells (RBCs) and hemoglobin (Hb) were significantly decreased in infected control positive dogs on the 3rd-14th day without treatment compared to control negative ones (p<0.05; Table-3). Packed cell volume (PCV) values were significantly decreased in infected control positive dogs on the 3rd-10th day without treatment compared to control negative ones (p<0.05; Table-3), but not on the 14th day without treatment (p>0.05). In A. vera ointment gel 20% treated group, RBCs and Hb values were decreased on the 3rd DPT (p<0.05), then returned to their baseline on the 7th-14th DPT when compared to control ones (p>0.05). No significance was recorded in PCV values between groups treated with A. vera gel ointment 20% and healthy ones (p>0.05). No significant change for RBCs values in A. vera 40% treated group at different time points of treatment.

**Table-2: Clinical examination of dogs. Values expressed as mean with SE.**

| Variables | Body temperature (°C) | Respiratory rate (cycle/min) | Pulse rate (beat/min) |
|-----------|------------------------|-----------------------------|-----------------------|
| Control negative dogs (before induction) (n=20) | 37.96±0.14<sup>a</sup> | 24.6±1.3<sup>a</sup> | 80±1.7<sup>a</sup> |
| Infected dogs without treatment Group 1 (n=5) | | | |
| 3 DWT | 38.9±0.1<sup>b</sup> | 32.5±1.04<sup>b</sup> | 91.25±1.49<sup>b</sup> |
| 7 DWT | 38.4±0.08<sup>b</sup> | 29±1.29<sup>c</sup> | 85±0.4<sup>c</sup> |
| 10 DWT | 38.35±0.2<sup>c</sup> | 27.75±1.03<sup>c</sup> | 84.25±0.85<sup>c</sup> |
| 14 DWT | 38.2±0.85<sup>c</sup> | 26±0.4<sup>c</sup> | 82±1.6<sup>c</sup> |
| Dogs treated with Aloe vera gel ointment 20% Group 2 (n=5) | | | |
| 3 DPT | 38.7±0.24<sup>a</sup> | 28.6±1.8<sup>a</sup> | 85.3±2.06<sup>a</sup> |
| 7 DPT | 38.2±0.1<sup>a</sup> | 25.4±0.8<sup>a</sup> | 83.4±0.8<sup>a</sup> |
| 10 DPT | 38.3±0.4<sup>a</sup> | 26±1.4<sup>a</sup> | 82±1.2<sup>a</sup> |
| 14 DPT | 38.2±0.08<sup>a</sup> | 25.2±1.06<sup>a</sup> | 80.8±1.4<sup>a</sup> |
| Dogs treated with Aloe vera gel ointment 40% Group 3 (n=5) | | | |
| 3 DPT | 38.6±0.2<sup>a</sup> | 29.5±0.6<sup>a</sup> | 86.4±1.8<sup>a</sup> |
| 7 DPT | 38.2±0.14<sup>a</sup> | 24.9±1.54<sup>a</sup> | 82±1.34<sup>a</sup> |
| 10 DPT | 37.8±0.3<sup>a</sup> | 25±0.8<sup>a</sup> | 80.6±0.88<sup>a</sup> |
| 14 DPT | 38±0.2<sup>a</sup> | 23.3±1.6<sup>a</sup> | 81.4±2.08<sup>a</sup> |
| Dogs treated by gentamicin sulfate 0.1% ointment Group 4 (n=5) | | | |
| 3 DPT | 38.6±0.09<sup>b</sup> | 30.2±0.8<sup>b</sup> | 88.4±0.85<sup>b</sup> |
| 7 DPT | 38±0.4<sup>b</sup> | 26.3±1.2<sup>b</sup> | 80.4±2.4<sup>b</sup> |
| 10 DPT | 38.2±0.1<sup>c</sup> | 24±0.8<sup>c</sup> | 82±0.88<sup>c</sup> |
| 14 DPT | 37.8±0.2<sup>c</sup> | 25±1.4<sup>c</sup> | 82.5±1.4<sup>c</sup> |

SE=Standard error, DWT=Day without treatment, DPT=Day post-treatment, n=Number. Means with different letter superscripts in the same column are significantly different at p<0.05.
Table 3: Hematological profile of control and infected dogs. Values expressed as mean with SE.

| Variables | Hb (g/dl) | PCV (%) | MCV (fl) | MCH (pg) | MCHC (%) | WBCs | Neutrophil (%) | Lymphocyte (%) | Monocyte (%) | Eosinophil (%) |
|-----------|-----------|----------|----------|-----------|-----------|------|---------------|---------------|--------------|---------------|
| Control negative dogs (before induction) (n=20) | 5.96±0.15a | 13.5±0.3a | 33.8±0.66a | 56.75±0.7a | 22.7±1.7a | 40.1±1.7a | 18.8±0.5a | 61.75±0.62a | 30.75±0.85a | 5.5±0.28a | 2±0.4a |
| Infected dogs without treatment Group 1 (n=5) | | | | | | | | | | | |
| 3 DWT | 5.5±0.11b | 12.3±0.1b | 31.5±0.6b | 56.8±2.2b | 22.1±0.2b | 39.1±1.2b | 30.47±0.6b | 68.25±0.8b | 26±0.57b | 4.25±0.2b | 1.5±0.28b |
| 7 DWT | 5.6±0.08b | 12.6±0.08b | 32.0±0.4c | 57.1±0.12c | 22.5±0.3c | 39.4±0.5c | 28.4±0.3c | 65.7±0.4c | 28±0.4c | 4.5±0.28c | 1.75±0.25c |
| 10 DWT | 5.6±0.06b | 12.5±0.1b | 32.7±0.4c | 58.2±0.6c | 22.2±0.2c | 38.2±0.3c | 26.39±0.4c | 65±0.4c | 28.5±0.6c | 5±0.0c | 1.5±0.28c |
| 14 DWT | 5.7±0.06b | 12.7±0.09c | 33.0±0.4c | 56.9±1.5c | 21.6±0.4c | 38.4±0.7c | 22.9±0.59d | 63.5±0.6c | 30.5±0.6c | 4.75±0.25c | 1.25±0.25c |
| Dogs treated with Aloe vera gel ointment 20% Group 2 (n=5) | | | | | | | | | | | |
| 3 DPT | 5.5±0.06b | 11.3±0.2c | 33±0.4d | 59.1±0.17b | 21.7±0.5c | 36.7±0.7b | 28.32±0.4c | 66.25±0.47b | 28.25±0.25b | 4.2±0.25b | 1.2±0.25b |
| 7 DPT | 6±0.1a | 13.1±0.16a | 33±1.08a | 55.1±2.52a | 21.2±0.58a | 40±0.9a | 25.15±0.38a | 64.25±0.25a | 29.75±0.25a | 4.5±0.28b | 1.5±0.28b |
| 10 DPT | 6.2±0.1a | 13.47±0.2a | 33.5±0.6a | 54.1±1.82a | 21.75±0.5c | 40.23±0.5a | 23.4±0.35a | 62.75±0.47a | 30.75±0.47a | 5±0.4a | 1.5±0.28b |
| 14 DPT | 6.06±0.05b | 13.07±0.1a | 33.75±0.6a | 54.48±1.4c | 21.1±0.44a | 38.7±0.45a | 20.34±0.57a | 62.75±0.47a | 30.75±0.47a | 5±0.4a | 1.5±0.28b |
| Dogs treated with Aloe vera gel ointment 40% Group 3 (n=5) | | | | | | | | | | | |
| 3 DPT | 6±0.09a | 12.17±0.2a | 32.5±0.9a | 54.1±1.12c | 20.26±1.2a | 37.4±0.4a | 28.2±0.35a | 66.75±0.47a | 27.25±0.25b | 4.7±0.25a | 1.2±0.25b |
| 7 DPT | 6.1±0.09a | 13±0.09a | 33.2±0.62a | 54.5±0.98a | 21.3±0.39a | 39.1±0.86a | 26.75±0.21a | 65±0.47c | 29±0.4d | 4.5±0.28b | 1.5±0.28b |
| 10 DPT | 6.15±0.13a | 13±0.18a | 35.2±0.47a | 57.39±1.56a | 21.17±0.6a | 36.88±0.3a | 24.7±0.44a | 62±0.4a | 31.25±0.47a | 5±0.4a | 1.75±0.25c |
| 14 DPT | 6.25±0.14a | 13.05±0.1a | 33.7±0.85c | 54.4±2.12c | 20.8±0.35a | 38.7±1.3a | 20.95±0.28a | 61.5±0.28c | 31.75±0.25a | 5.25±0.25a | 1.5±0.28b |
| Dogs treated by gentamicin sulfate 0.1% ointment Group 4 (n=5) | | | | | | | | | | | |
| 3 DPT | 5.7±0.19a | 12.17±0.24a | 30.5±0.64c | 53.65±2.7a | 21.36±0.9a | 39.8±0.6a | 30.1±0.38a | 64±0.4d | 30±0.4a | 4.5±0.28b | 1.5±0.28b |
| 7 DPT | 6.1±0.1a | 13.1±0.29c | 32.7±0.47a | 53.7±1.38a | 21.9±0.78a | 40.8±1.2a | 27.6±0.21b | 63±0.4d | 29.75±0.47a | 5.25±0.25a | 2±0.25a |
| 10 DPT | 6.12±0.08a | 12.8±0.17a | 32.25±0.8a | 52.6±0.82a | 21.01±0.2a | 39.9±0.5a | 23.125±0.5a | 61.75±0.6a | 31.25±0.47a | 5.25±0.25a | 1.75±0.25a |
| 14 DPT | 6.35±0.1c | 13.1±0.19a | 31.5±0.64c | 49.6±1.7a | 20.7±0.18a | 41.9±1.3a | 20.9±0.51a | 62.5±0.2c | 30.75±0.47a | 5±0.4a | 1.75±0.25a |

SE = Standard error, DWT = Day without treatment, DPT = Day post-treatment, n = Number. Means with different letter superscripts in the same column are significantly different at p < 0.05.
treatment when compared with healthy ones before induction (p<0.05). In A. vera 40% treated group, Hb values were decreased on the 3rd DPT, while PCV values were elevated on the 10th DPT (p<0.05), then returned to their normal values on the 14th DPT (p>0.05). In gentamicin 0.1% treated group, no significant change for RBCs values at different time points of treatment when compared with healthy ones (p>0.05), but Hb and PCV values were decreased on the 3rd DPT than healthy ones (p<0.05), then returned to their normal values when compared to control ones (p<0.05; Table-3). Moreover, white blood cells (WBCs) and neutrophils were significantly increased in infected dogs on the 3rd-14th day without treatment, while in dogs treated with A. vera gel ointment 20%, WBCs and neutrophils were elevated on the 3rd, 7th, and 10th DPT (p<0.05) and returned to their normal values on the 14th day when compared to healthy ones (Table-3). In dogs treated with A. vera gel ointment 20% and gentamicin ointment 0.1%, WBCs were increased on the 3rd, 7th, and 10th DPT (p<0.05) and returned to their normal values on the 14th day, except for neutrophils which were elevated on the 3rd and 7th day (p<0.05) then returned to their normal values on the 10th and 14th DPT when compared to healthy ones. Lymphocytes, monocytes, and eosinophils were decreased in all treated groups (p<0.05) and returned to their baseline on the 14th DPT when compared to healthy groups (p>0.05; Table-3). Furthermore, mean corpuscular volume, mean corpuscular hemoglobin (MCH), and MCH concentration values were not significantly different between all groups except on the 3rd DPT at which these values were decreased when compared with control ones (p<0.05; Table-3).

**Oxidant and antioxidant concentrations between dogs’ studied groups**

Serum MDA concentrations were higher in infected dogs on the 3rd-14th day without treatment compared to control ones (p<0.05; Table-4), while dog treated with A. vera gel ointment 20% and 40% and gentamicin 0.1%, serum MDA concentrations were elevated on the 3rd and 7th DPT (p<0.05) and returned to its baseline on the 10th and 14th DPT compared to control ones (p<0.05; Table-4). Serum MDA concentrations were a diagnostic biomarker for Staphylococcus pyoderma in dogs (area under the curve=0.92; p<0.05; Figure-4). Serum TAC and catalase concentrations were decreased in infected dogs on the 3rd-14th day without treatment compared to control group (p<0.05). In dogs treated with A. vera gel ointment 20%, serum TAC and catalase concentrations were decreased on the 3rd and 7th DPT (p<0.05) and returned to their normal concentrations on the 10th and 14th DPT compared to healthy ones (p>0.05). In dogs treated with A. vera gel ointment 40%, serum TAC and catalase concentrations were decreased on the 3rd DPT (p<0.05) and returned to their baseline from the 7th to 14th DPT when compared to healthy ones (p>0.05). In dogs treated with gentamicin 0.1%, serum TAC concentrations were decreased on the 3rd and 7th DPT (p<0.05) and returned to their baseline on the 10th and 14th DPT when compared to healthy ones (p>0.05; Table-4).

**Acute-phase proteins and cytokines concentrations between studied groups of dogs**

There was a significant increase in the concentrations of acute-phase proteins (CP, SAA, and HP) and ILs (IL-1, IL-6, IL-10, and TNF-α) in infected dogs on the 3rd-14th day without treatment and in dogs treated with A. vera gel 20% and 40% and gentamycin ointments 0.1% on the 3rd and 7th DPT when compared to the control negative ones (p<0.05; Table-5), then started to return to their baseline on the 14th DPT in dogs treated with A. vera gel ointment 20% and 40% and gentamicin 0.1% when compared to control ones (p>0.05). SAA, HP, and TNF-α concentrations were significant biomarkers for Staphylococcus pyoderma in dogs (p<0.05; Figure-4). Serum HP and TNF-α concentrations were significantly lower on the 10th and 14th DPT in dogs treated with A. vera gel 20% and 40% compared to dogs treated with gentamicin 0.1% (p<0.05; Table-5). Dogs’ pyoderma treated with A. vera 20% and 40% were more likely to have low HP and TNF-α concentrations than gentamicin ([OR=4.6; 95% CI=1.31-17.40; p<0.05]; [OR=5.2; 95% CI=1.04-2.23; p<0.05]), respectively, as shown in Table-6.

**Discussion**

In the present study, the antibacterial effect of A. vera gel ointments 20% and 40% against dogs’ staphylococcal pyoderma had been evaluated in vitro. A. vera extract showed clear inhibition zone for S. aureus growth in well diffusion agar test. The inhibition zone of A. vera extract 40% was wider than the ones that caused by A. vera extract 20% and gentamicin 0.1%. Our results were in the same line with previous studies that recorded the antibacterial effect of A. vera extract against S. aureus [21,22]. The antibacterial activity of A. vera could be attributed to its components that include p-coumaric, ascorbic, pyrocatechol, and cinnamic acid that could be used as an alternative herbal antibacterial therapy to subside the antibiotic resistance and reduce wound healing time [22,23].

In the present study, RBCs, Hb, PCV and WBCs were returned to their normal values after topical application of different concentrations of A. vera gel ointments 20% and 40% on dogs’ pyoderma thus could be explained by A. vera may increase erythropoiesis process and also increase leukocyte infiltration as a part of immunomodulation [24].

We have shown that the concentrations of acute-phase proteins are in positive correlation with the severity of dog’s pyoderma that could be attributed to increase in their synthesis and release from the liver in response to tissue injury of skin [25]. Furthermore, we
have documented that ILs were elevated in infected dogs than control ones. It is possible that pyoderma is suggested to increase an inflammatory response by releasing cytokines into the bloodstream [26,27]. Interestingly, the serum concentrations of MDA were significantly increased, while the TAC and catalase concentrations were decreased in infected dogs with staphylococcal pyoderma compared to control ones. It has been reported that dogs with skin pathological conditions have significant oxidative stress with elevated MDA and decreased TAC and catalase concentrations as reported in our study [28-30].

In the current study, topical application of A. vera gel ointment 20% and 40% modulates the inflammatory response.
Table-5: Acute-phase protein biomarkers and cytokines expression in control and infected dogs. Values expressed as mean with SE.

| Variables                        | Ceruloplasmin (µg/mL) | Serum amyloid A (g/mL) | Haptoglobin (µg/mL) | Interleukin 1 (pg/mL) | Interleukin 6 (ng/L) | Interleukin 10 (ng/L) | Tumor necrosis factor-α (ng/L) |
|----------------------------------|-----------------------|------------------------|--------------------|-----------------------|----------------------|----------------------|-------------------------------|
| Control negative (before induction) (n=5) | 25.8±0.24<sup>a</sup> | 0.62±0.04<sup>a</sup> | 67.25±2.2<sup>a</sup> | 59.3±0.6<sup>a</sup> | 29.5±0.8<sup>a</sup> | 40.5±0.4<sup>a</sup> | 12.04±0.2<sup>a</sup> |
| Infected dogs without treatment Group 1 (n=5) |                       |                        |                    |                       |                      |                      |                               |
| 3 DWT                            | 57.38±0.2<sup>b</sup> | 1.8±0.8<sup>b</sup> | 118.8±2.2<sup>b</sup> | 88.8±0.8<sup>b</sup> | 38.2±1.4<sup>b</sup> | 54.3±1.4<sup>b</sup> | 18.2±0.4<sup>b</sup> |
| 7 DWT                            | 48.45±0.6<sup>c</sup> | 1.4±0.2<sup>c</sup> | 70.6±1.1<sup>c</sup> | 60.3±1.2<sup>c</sup> | 30.8±0.6<sup>c</sup> | 41.8±0.5<sup>c</sup> | 12.5±0.2<sup>c</sup> |
| 10 DWT                           | 44.54±0.5<sup>d</sup> | 0.8±0.06<sup>d</sup> | 72.2±1.8<sup>d</sup> | 64.5±0.6<sup>d</sup> | 32±0.8<sup>d</sup> | 42.5±0.8<sup>d</sup> | 13.2±0.8<sup>d</sup> |
| 14 DPT                           | 28.12±0.54<sup>a</sup> | 0.68±0.1<sup>a</sup> | 70.6±1.1<sup>a</sup> | 60.3±1.2<sup>a</sup> | 30.8±0.6<sup>a</sup> | 41.8±0.5<sup>a</sup> | 12.5±0.2<sup>a</sup> |
| Dogs treated with Aloe vera gel ointment 20% Group 2 (n=5) |                       |                        |                    |                       |                      |                      |                               |
| 3 DPT                            | 52.4±0.3<sup>c</sup> | 1.4±0.1<sup>c</sup> | 115.4±2.4<sup>c</sup> | 85.2±0.95<sup>c</sup> | 38±0.38<sup>c</sup> | 55.3±0.7<sup>c</sup> | 19.2±0.5<sup>c</sup> |
| 7 DPT                            | 44.48±0.38<sup>d</sup> | 1.08±0.06<sup>d</sup> | 80.7±1.2<sup>d</sup> | 73.6±0.8<sup>d</sup> | 32±0.8<sup>d</sup> | 42.8±0.3<sup>d</sup> | 15.7±0.2<sup>d</sup> |
| 10 DPT                           | 30.2±0.4<sup>c</sup> | 0.8±0.0<sup>c</sup> | 73.8±0.8<sup>c</sup> | 65.2±0.4<sup>c</sup> | 30.4±0.4<sup>c</sup> | 42±0.6<sup>c</sup> | 13.6±0.8<sup>c</sup> |
| 14 DPT                           | 28.7±0.6<sup>c</sup> | 0.66±0.04<sup>c</sup> | 70.85±1.2<sup>c</sup> | 61.4±1.2<sup>c</sup> | 30±1.3<sup>c</sup> | 40±0.9<sup>c</sup> | 12.2±0.6<sup>c</sup> |
| Dogs treated with Aloe vera gel ointment 40% Group 3 (n=5) |                       |                        |                    |                       |                      |                      |                               |
| 3 DPT                            | 58.12±0.2<sup>a</sup> | 1.28±0.1<sup>c</sup> | 116.6±1.4<sup>a</sup> | 87.9±0.4<sup>a</sup> | 39.2±0.6<sup>a</sup> | 55.8±0.6<sup>a</sup> | 18.4±0.2<sup>a</sup> |
| 7 DPT                            | 44.2±0.4<sup>a</sup> | 1.18±0.06<sup>c</sup> | 112.9±0.8<sup>a</sup> | 74.8±1.2<sup>a</sup> | 38.5±0.8<sup>a</sup> | 45.8±0.8<sup>a</sup> | 16.2±0.3<sup>a</sup> |
| 10 DPT                           | 42.42±0.5<sup>a</sup> | 0.85±0.02<sup>a</sup> | 111.4±1.2<sup>a</sup> | 68.4±0.58<sup>a</sup> | 32±0.5<sup>a</sup> | 40.6±1.08<sup>a</sup> | 19.6±0.4<sup>a</sup> |
| 14 DPT                           | 30.4±0.6<sup>a</sup> | 0.7±0.04<sup>a</sup> | 112.4±2.2<sup>a</sup> | 63.42±0.6<sup>a</sup> | 31.4±1.4<sup>a</sup> | 40.2±0.7<sup>a</sup> | 19.8±0.2<sup>a</sup> |

SE=Standard error; n=Number. Means with different letter superscripts in the same column are significantly different at p<0.05
response through reduction of acute-phase proteins and IL concentrations and enhances skin healing of dogs’ Staphylococcus pyoderma. The previous studies documented that topical application of different doses of A. vera extract on injured skin resulting in reduction of the acute-phase response [31,32]. Moreover, the positive effect of A. vera gel on oxidant and anti-oxidant status has been shown in this study through elevation TAC and catalase concentrations and reduction of MDA concentrations that could be explained potentially by A. vera had antioxidant effects through glutathione peroxidase activity [33]. From that prospective, one could speculate that A. vera gel ointment has anti-inflammatory and antioxidative roles against dogs’ staphylococcal pyoderma.

Conclusion
Increased acute-phase proteins, cytokines, and oxidants and reduced antioxidant biomarkers were frequent findings in dog’s pyoderma. Furthermore, topical application of A. vera gel ointments 20% and 40% had antibacterial, anti-inflammatory, and antioxidative actions against dogs’ staphylococcal pyoderma that will support its further use rather than antibiotics in the health arena.

Authors’ Contributions
HH designed the experiment. AA, AhE, AE, and HK performed the experiment. AK performed the statistical analysis of results. HH, AK, and AA drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing Interests
The authors declare that they have no competing interests.

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