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In Vivo Investigation of the Anti-*Staphylococcus aureus* Impact of *Balanites aegyptiaca* and *Curcuma longa* Ethanol Extracts on Apoptotic Gene Expression and Their Implications on Hemogram and Serum Protein Profiles

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Abstract

WE AIM TO DETERMINE effects of BAF-EE and CLP-EE ethanol extracts on apoptotic-gene expression , hemogram and serum protein profiles in *S. aureus*-infected mice. HPLC was utilized for qualitative estimation of phenolic compounds in both extracts. Sixty mice were divided into non-infected Group (n=15) and a group of 45 mice that were inoculated S/C with 0.1 ml of 2×10^7 CFU of a well-identified isolate of *S. aureus*. On 7th dpi, mice were subdivided into 3 equal groups and were orally dosed with BAF-EE, CLP-EE and Ciprofloxacin, at dosages of 200 mg, 100 mg and 20 mg/Kg BW/daily for 5 days, respectively. All mice were clinically monitored for 4 weeks for local inflammatory reactions and symptoms of sepsis. On days 3, 7, 14, 21, and 28, two blood samples were taken from three mice in each group before being sacrificed. Blood samples were used for hemogram and serum protein assays. The expression of apoptosis-genes in liver cells was investigated via semi-qRT-PCR. The data obtained show that four phenolic compounds were identified in each extract. Erytrogram was elevated after all treatments while, leucocyte counts except monocytes were elevated in mice treated with both extracts. Total protein and globulins increased in ciprofloxacin-treated mice. Treatment with both extracts reduced the expression of pro-apoptotic genes caspase-3, Bax and p53 while, increasing the anti-apoptotic gene Bcl-2, with CLP-EE outperforming BAF-EE. The study concludes that both CLP-EE and BAF-EE seem to have antibacterial capabilities, as evidenced by their protective impact on apoptosis-related genes and blood picture.

Keywords: *Staphylococcus aureus, Balanites aegyptiaca*, *Curcuma longa,* Apoptosis, Blood metabolic profile.

Introduction

Staphylococcus aureus (*S. aureus*) is one of the most significant zoonotic bacterial pathogens, infecting a variety of species, including dairy cows and humans [1]. Recent reports of disease control from the US Centres, recorded that *S. aureus* is now considered the second most common bacterial pathogen after *Escherichia coli* causing disease, and that staphylococcal enterotoxin causes food poisoning [2]**.** *S. aureus* can infect different parts of the body; it may infect superficial skin and soft tissues, and the infections may extend to the blood causing septicaemia threatening the survival of the animal [3, 4, 5]. The courses of infection depend on the strength of the host's immunity and the virulence of *S. aureus* [6].

S. aureus developed a variety of defence mechanisms against antimicrobial agents, and the overuse and misuse of these drugs have contributed to the rise in antimicrobial resistance (AMR). The emergence of AMR of *S. aureus* is a global public health threat worldwide [2]. When bacteria are exposed to antibiotics, resistance can emerge in organisms causing genetic mutations, making treatment challenging [7,8]. Methicillin-resistant *S. aureus* (MRSA) strains carry a mec gene on the bacterial genome, which is part of the wider staphylococcal chromosomal cassette mec (SCC mec) area, conferring resistance to numerous antibiotics [9,10]. Thus, there are growing interests in

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exploring natural products, such as medicinal plants, for their potential antimicrobial activity, as they may offer a source of novel compounds with unique mechanisms of action [11,12].

Many medicinal plants have been traditionally used for their antimicrobial properties and have been shown to be effective against a variety of microorganisms including *Staphylococcus* species [13, 14, 15]. Among these plants, *Balanites aegyptiaca* (*B. aegyptiaca*) and turmeric (*Curcuma longa* L.). *B. aegyptiaca,* also known as 'desert date', is a plant found in Africa and South Asia. Its fruits have been traditionally used in medicine to treat various ailments [16, 17]. The fruits of *B. aegyptiaca* are rich in various compounds with reported antioxidant, anti-inflammatory and antimicrobial properties [15,18 , 19, 20]. *B. aegyptiaca* contained a wide assortment of bioactive compounds which have pharmacological action such as flavonoids, saponins, tannins, phenols, terpenoids and steroids [15, 20]. The hydro ethanolic extracts of *B. aegyptiaca* inhibited the growth of *S. aureus* isolated from wounds [21]. Administration of *B. aegyptiaca* ethanol extract in goats infected with *Haemonchus Contortus* was able to reestablish the diminish in antioxidant enzyme activities [22] with regulating pro-inflammatory cytokines, hence preserving cells from apoptosis [23].

The dried and powdered rhizomes of *Curcuma longa* L. (*C. longa*) belongs botanically to the *Zingiberaceae* family, commonly known as turmeric. Turmeric is generally used as a food-coloring ingredient and has a wide diversity of pharmacological properties [24]. A number of studies approved that turmeric extract is effective and safe for the treatment of autoimmune and inflammatory diseases [25]. It includes a wide antibacterial impact against a variety extend of bacteria, indeed those resistant to antimicrobials [15, 26, 27]. Curcumin has been appeared to be viable against the bacteria that are dependable for surgical contaminations and implant-related bone diseases, essentially *S. aureus* and *E. coli* [26]. It has also a wide range of effects, such as antifungal, antimalarial, antiviral, antioxidant, anti-inflammatory and anti-parasitic properties [15, 28,29]. These medical properties can be attributed to turmeric's high amount of curcuminoids particularly curcumin, which is regarded a chemical mark for that species [26].

Studying the therapeutic benefits of medicinal plants contributed to the identification of biologically active compounds known as polyphenols. They are plentiful in plants and structurally varied [30]. Polyphenols, which comprise phenolic acids, flavonoids, tannic acid, and ellagitannin, are fair a couple of the auxiliary metabolites that medicinal plants possess [31]. Polyphenols have been linked to a variety of biological activities, including immunomodulatory, antioxidant, anti-inflammatory, along with antibacterial and viral capabilities [32,33]. Moreover, through demonstrated anti-inflammatory impacts they can balance apoptotic forms within the vascular endothelium [34]**.** More, common polyphenols act synergistically with clinically affirmed display drugs, making them more productive and can be utilized to plan a potential treatment in combination with existing medicate [35,36].

Apoptosis, a type of programmed cell death, holds significance in bacterial sepsis, with numerous bacterial pathogens triggering host cell death through apoptosis [37]. This process relies on a dynamic cascade of cysteine endopeptidases known as caspases, which form a family of intracellular cysteine proteases. Caspases cleave various cellular proteins, ultimately culminating in the cessation of the cell [38]. The *S. aureus* infection results in apoptosis, particularly when it causes sepsis and atopic dermatitis. During infection, bacterial toxins promote apoptosis, which is important in disease processes [39].

This article sought to determine the therapeutic properties of *B. aegyptiaca* fruits and *C. longa* rhizome powder ethanol extracts on expression levels of apoptosis-related genes, hemogram and serum protein profiles in mice with *S. aureus* experimental infection. Also, some phenolic components in both extracts were identified using HPLC.

Material and Methods

Plants

Plant Materials and Extraction

The *B. aegyptiaca* and Rhizome of *C. longa* were purchased from a local market in Halaieb, Upper Egypt, and the Al Hussein area, Cairo, Egypt, respectively. Both plants were identified and authenticated by a Botanist in the Department of Culturing and Production of Medicinal Plants, NRC, Egypt. Ethanol extraction of *B. aegyptiaca* (BAF-EE) and dried Rhizome powder of *C. longa* (CLP-EE) was performed [40]. The fruit's mesocarp and CL powder were macerated many times for one week at room temperature with 70% ethyl alcohol before being filtered. The solvent was subsequently eliminated under vacuum at 40 °C using a rotary evaporator, and the resulting extract was preserved at – 4 °C.

High Performance Liquid Chromatography analysis of phenolic compounds

The distinguishing proof of polyphenols in BAF-EE and CLP-EE were examined employing High Performance Liquid Chromatography System (HPLC) (Shimadzu, Kyoto, Japan) equipped with a LC-20 AD liquid chromatograph, SIL-20A auto sampler, CTO-20AC column oven and an SPD-M20A diode matrix detector. The qualitative

evaluation of five biologically active phenolic ingredients, specifically gallic acid, quercetin, rutin, coumaric acid, and vanillin within the two extracts was conducted. The dried ethanol extracts were dissolved in ethanol (1 mg/mL), filtered, and analysed using a Shimadzu HPLC system, as described by Sabir et al. 2021 [41]. The most ideal separation emerged in 40 minutes utilizing gradient elution of methanol, deionized water, and acetic acid on a Zorbax plus C18 column (4.6 × 100 mm, 3.5 µm) at 25°C. The chromatographic peaks were validated by comparing their retention times to those of reference standards, and by DAD spectra (200-500 nm). Phenol compounds were identified by screening the Dictionary of Natural Products (DNP), validated by their fragmentation patterns, and compared to literature data.

Bacterial strain

The microbial strain of was isolated from bovine skin abscesses infection. The skin swab was aseptically collected utilizing sterile cotton buds, deposited in a test tube filled with nutrient broth, and promptly transported to the laboratory in an ice box. The skin swab collected was enriched in nutrient broth by incubation at 37 °C for 24 hours. Enriched cultures were streaked on different differential and selective culture mediums such as nutrient agar, blood agar, and mannitol salt agar (MSA), according to the methods described by WHO [42]. The identification of the *staphylococcus* isolate was performed using Gram staining and biochemical tests. The bacterial strains were preserved in a broth with 25% (v/v) glycerol (Sigma-Aldrich) at -20 °C until needed.

Molecular characterization of isolates:

The DNA from the *S. aureus* colonies was purified using the Genomic DNA Mini Kit (Qiagen) instructions provided by the manufacturer. The Nano drop System (Thermo-Scientific, UK) was utilized to assess the concentration and virtue of isolated DNAs at an optical density of 260/280 nm.

Thermocycler reaction was carried out under the following conditions: five-minute heating period at 94°C for primary denaturation, there were thirty seconds of 94˚C, one minute of 55˚C, and one minute of 72˚C for denaturation, annealing, and extension, respectively. These thermal cycles were repeated thirty five times. The last extension was then performed for ten minutes at 72C. The PCR was conducted using primers (Willowfort) as shown in table (1) [43,44, 45]. PCRs products were isolated by electrophoresis on 1.5 % agarose gel (Applichem Germany) in 1× TBE buffer at 25°C. The fragment sizes were determined using the Gene-Ruler 100 bp DNA ladder (Fermentas, Germany). The gel was then photographed using a documentation system (BioRad), and the information were analysed using computer program.

Animal Model and Housing

Sixty healthy white Swiss mice (15-20 g body weight) were obtained from the National Research Centre Animal House in Giza, Egypt. They were kept in a well-ventilated animal room at the Animal House. The room had regulated settings of 25°C temperature, 50±5% relative humidity, and a 12-hour light/dark cycle. The mice had access to ad libitum food and water to fulfill their nutritional needs and underwent a 15-day acclimatization period before the study began NRC [46].

Experimental design

Bacterial inoculation

A well-identified isolate of *S. aureus* was prepared for experimental infection in mice according to Kim *et al.* 2014 [47]. The bacterial solution was thawed, cultured overnight in tryptic soy broth with rotation at 37 °C, diluted 1:100 into fresh broth and incubated further to absorbance at 600 nm (A600) then centrifuged, and the pellet washed in PBS. A viable count was employed to assess the quantity of live bacteria in each bacterial solution, determined by enumerating the colony-forming units (CFU).

Experimental treatments

Following the acclimatization period, the mice were divided into two groups. The first group of 15 mice and was injected S/C with 0.1 ml of sterile saline and served as the negative control. The remainder 45 mice were received S/C injections with 0.1 ml of 2×10^7 CFU (colony-forming units) of *S. aureus* bacteria at four different sites on their shaved backs. On the 7^{th} day post-infection (dpi), mice were divided into 3 equal groups of 15 mice and each group was orally administered with a single dose of the selected plant extracts and standard antibiotic, for 5 successive days. The BAF-EE, CLP-EE, and Ciprofloxacin antibiotics were given at dosages of 200 mg [48], 100 mg [49] and 20 mg/kg BWs, respectively.

Clinical and Macroscopic examination (Post-mortem)

All mice were clinically monitored throughout the trial for local inflammatory reactions and symptoms of sepsis. From each group, three mice were anesthetized using Ketamine/ Xylazine Pfizer 0.1 ml/20 gm mouse wt. IP, euthanized and sacrificed on days 3, 7, 14, 21, and 28 of the experiment for post-mortem inspection.

Sampling and analyses

Two blood samples, with and without ethylene diamine tetra-acetic acid (EDTA), were collected by puncturing the retro orbital plexus of the veins of 3 mice from each group before sacrifice on days 3, 7, 14, 21 and 28 of the experiment. Blood samples obtained in vacationer tubes with EDTA as an

anticoagulant were utilized for hemogram investigations, while those collected in plain tubes were separated into clear sera and stored at -20 °C until needed for the protein levels assays. Liver samples kept at -80 °C until utilized to assess expression levels of apoptosis-related genes. The samples obtained on days 3 and 7 before treatment are deemed control positive.

Blood Picture

The erythrogram and leukogram profiles of blood image were assessed employing a hematological analyzer (ExigoVet, Sweden). Erythrogram exhibited the red blood cell count (RBCs), hematocrit (HCT), hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The leukogram involves total white blood cells (WBCs) as well as differential leukocytic counts of lymphocytes, monocytes, and granulocytes (neutrophils + eosinophils + basophils).

Serum protein analysis

Serum levels of total proteins and albumin were measured using kits purchased from Erba, Germany. Total globulins were determined by subtracting the albumin value from the total proteins. The albumin/globulin ratio (A/G) was computed using the obtained values of albumin and globulins.

Expression of pro- and anti-apoptotic gene levels in mice hepatic cells

RNA extraction and purification

Total RNA was isolated from the liver of both treated and control mice groups using the TRIzol® reagent (Invitrogen, USA) according to the manufacturer's instructions. This technique utilizes a monophasic liquid to homogenize tissues and lyse cells, effectively capturing RNA while minimizing degradation. The RNA pellets were stored in diethyl pyrocarbonate (DEPC). To remove any residual genomic DNA, the extracted RNA was treated with RNase-free DNase (Fermentas Inc., Ontario, CA. RNA quality was subsequently assessed by two methods:

Semi-quantitative reverse transcription-polymerase chain reaction (semi-RT-PCR)

Reverse Transcription (RT): 2 µg of isolated RNA from each sample were utilized for cDNA synthesis using the High-Capacity RNA to RT-Premix Kit (iNtRON Biotechnology, Korea). This kit provides a convenient and streamlined approach for reverse transcription reactions. In brief, total RNA was activated at 70 °C for 10 min, and 20 µL reaction mixtures were prepared with 4 µL $MgCl_2$, 2 µL of reverse transcription 10µlbuffer, 2 µL of dNTP mixture (10mM), 0.5 µL of random primers, 0.75 µL of AMV reverse transcriptase enzyme, 1 ng of RNA, and nuclease-free water to a final volume of 20 µL. The reaction was incubated at 45 °C for 60 min, followed by incubation at 95 °C for 5 min. The resulting cDNA was diluted to 100 µL with nuclease-free water for PCR amplification. The generated cDNA was stored at -20 °C for later use or used directly as a template for semi-quantitative PCR.

Gene expression analysis

The sq-PCR was conducted following the methodology outlined previously published methods [50], sq-PCR was performed using the PCR System 9700 thermal cycler (Applied Biosystems, USA). Oligonucleotide PCR primer pairs were designed for *Caspase-3, Bcl2,* and *Bax* genes, referring to the published primer sequences as detailed in Table 2 [51, 52, 53, 54, 55] for internal control and normalization, GAPDH amplification was included using established primer sequences.

The thermal cycling program adopted the following parameters

Initial denaturation: 94°C for 5 minutes, amplification cycles (30 repeats): denaturation: 94°C for 60 seconds, annealing: 52-60°C for 30 seconds (temperature varied depending on the target gene), extension: 72°C for 1-minute, final extension: 72°C for 5 minutes. The thermal cycling parameters involved initial denaturation at 94 °C for 5 min, followed by 30 cycles of amplification at 94 °C for 60 s for DNA denaturation, annealing at 52-60 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min.

Semi-quantitative determination of PCR (semi-qPCR) products

GAPDH amplification served as the internal control for standardizing gene expression levels. Specific primers were utilized for GAPDH amplification alongside those for the target genes listed in Table 1. Following PCR, the ethidium bromide-stained gel bands were scanned and analyzed using the Gel-Pro software (version 3.1 for Windows 3). This software quantified the intensity of each band, revealing the relative levels of target gene and GAPDH amplification products. This ratio, presented in Table 1, facilitated normalization of the initial variation in sample concentrations and controlled for potential differences in reaction efficiency, as previously described by Raben *et al*. 1996 [56].

Statistical analysis

The data was checked for normal distribution by inspection of histogram distribution. Normally distributed continuous data was expressed as mean ± standard error (SE). Statistical significance between the normal control and treated groups was assessed using ANOVA (one-way analysis of variance) followed by Duncan's multiple range test, conducted with the SPSS (Statistical Package for Social Science) Inc., *Chicago, IL*, USA, version 17

computer program. A probability level of $P < 0.05$ was considered indicative of significance.

Results

Identification of some Phenolic compounds using HPLC

As appeared in Fig. 1, four phenolic compounds are recognized in BAF-EE, including gallic acid, catechin, rutin and coumaric acid. Gallic acid showed up at retention time of 6.129 min and area % of 1.97 (peak 1), Catechin at 8.615 min and 14.125 % (peak 2), rutin at 13.16 min and 8.17 % (peak 3) and Coumaric acid at 13.67 min and 24.4 % (peak 4).

Fig. 2 presents the detected polyphenols in CLP-EE, which include Catechin appeared at retention time of 6.129 min and area % of 1.97 (peak 1), rutin at 8.615 min and 14.125 % (peak 2), Coumaric acid at 13.16 min and 8.17 % (peak 3) and Vanillin at 13.67 min and 24.4% (peak 4).

Bacterial isolation and identification

Staphylococcus aureus grew on blood agar and produced β–hemolysis, while on MSA it generated tiny, smooth, circular, yellowish colonies with the change of the medium color from bright red to yellow. Microscopically, using the Gram-stain, the *S. aureus* isolate displayed violet-colored, cocci-shaped organisms arranged in clusters resembling grapes. The biochemical reactions of isolated *S. aureus* showed catalase, coagulase, methyl red, Voges-Proskauer, nitrate reduction, and citrate positive, while oxidase and indole negative.

Molecular characterization of isolates

All isolates were confirmed to be *Staphylococci* by amplification of the 228 bp PCR product of the *Staphylococcal* specific 16SrRNA gene. The isolates were also identified as *S. aureus* by amplifying the 279 bp PCR product of specific thermonuclease gene (nuc). More, amplification of 147 bp fragments, which indicate methicillin-resistant *Staphylococcus aureus* (mecA gene) (Fig. 3).

Clinical and macroscopic assessment

Regardless of the dose used for inoculation, none of the animals given the S/C injection of *S. aureus* had general indications or symptoms of sepsis. After 48 hours, only locations injected with 0.1ml of 2×10^7 CFU of *S. aureus* showed evident inflammation, including skin edema and redness. Macroscopic examination confirmed an inflammatory response within 3 days, with redness and swelling of the skin and internal organs (liver, spleen and lungs). Abscesses appeared in the superficial musculature as well. However, vascular infiltrates were seen surrounding these abscesses as illustrated in Figure 4. Inflammation in the skin of mice in group treated with CLP-EE began to subside faster than in other groups. During the four weeks of the trial, there were no symptoms of toxicity or mortality in the mice that had been treated.

Hematological findings

Erythrogram of mice infected with *S. aureus* was then treated with BAF-EE and CLP-EE and ciprofloxacin (as a standard antibiotic) is illustrated in Table 3. Compared to the negative control, the data revealed that a significant ($P<0.01$) decrease in the levels of RBC, HCT, and Hb was recorded in mice infected with *S. aureus* at the 3rd dpi, while their levels returned to normal values at the 7th dpi. Inversely, the MCV and MCH values were significantly ($P<0.01$) elevated at the third dpi and returned to the normal level at the 7th dpi. The MCHC value was significantly ($P<0.05$) increased at the 7th dpi. Generally, the values of RBC, HCT, and Hb were markedly elevated in mice after treatment with BAF-EE, CLP-EE and ciprofloxacin at the 14th, 21st, and 28th dpi compared to the infected group at the 3rd dpi-infected. It could be stated that RBC, HCT, and Hb levels were reached to their maximum after BAF-EE, CLP-EE and ciprofloxacin at 14 dpi. Also, the MCV and MCH values were reduced ($P<0.05$) after treatment.

The leukogram counts of mice infected with *S. aureus* were then treated with BAF-EE and CLP-EE, and ciprofloxacin (Figure 5). Total leukocytic count decreased ($P<0.01$) significantly at 3 dpi due to reduced monocyte count. At the 7th dpi, the results showed that the WBCs count was markedly ($P<0.05$) elevated due to the increase in the granulocyte counts. Furthermore, the monocyte count was significantly ($P<0.05$) reduced. After treatment, it was observed that the counts of WBCs, lymphocytes and granulocytes were significantly ($P<0.05$) elevated in mice treated with BAE at 21 and 28 dpi compared to the infected group with 7 dpi. On the contrary, they rose after CLP treatment at 21 dpi.

Serum protein profile

Data revealed that serum total proteins and total globulins increased ($P<0.05$) significantly at 7 dpi. However, serum albumin and A/G ratios were not significantly ($P>0.05$) changed. Furthermore, infected mice treated with CLP-EE showed no significant changes in total proteins, albumin, total globulins, or A/G ratios ($P>0.05$). Ciprofloxacin treatment in mice resulted in a significant rise ($P<0.05$) in serum total proteins and total globulins at 14 dpi. After BAF-EE therapy, serum total protein; total globulins were significantly reduced at 21 and 28 dpi (Fig. 6).

Pro- apoptotic gene expression levels

The effects of BAF-EE or CLP-EE on the expression levels of pro-apoptotic; caspase3, Bax and p53 gens, which is targeted by a wide range of agents in apoptosis, were carried out in liver tissue samples by semi-qPCR analysis. As shown in Figure 7 A-C, *S. aureus* significantly increased the expression of the genes tested. Supplementation with BAF-EE or CLP-EE after *S. aureus* injection significantly inhibited

elevated expression of caspase3, Bax and p53 induced by pathogens. Furthermore, CLP-EE supplementation produced the best outcomes, restoring the expression of measured genes to the same levels as in the control group.

Anti- apoptotic gene expression levels.

S. aureus, on the other hand, had a substantial negative influence on the expression of anti-apoptotic Bcl-2 gene in liver tissue of all infected groups as compared to the negative control. Treatment with BAF-EE or CLP-EE resulted in considerably higher regulation in both groups, as seen in Figure 7 D. Meanwhile, the group supplemented with CLP-EE outperformed BAF-EE and was able to elevate Bcl-2 expression to near-normal levels, similar to the normal control group.

Discussion

S. aureus evolved a number of defence mechanisms against antibiotics, and improper use of these medications has contributed to the establishment of antimicrobial resistance. This creates a global public health issue that gradually reduces the efficacy of currently used drugs, causing treatment failure of infections. The treatment remains challenging due to the emergence of multi-drug resistant strains such as MRSA (Methicillin-Resistant *S. aureus*). Therefore, natural products, such as medicinal plants, are increasingly being investigated for their potential antibacterial activity. They might provide a source of new molecules with distinct modes of action [10].

In this study, the MRSA *S. aureus* isolates from bovine skin infection were recognized based on the morphology and cultural characteristics of grown colonies in expansion to the results of standard biochemical identification testing that produced positive coagulase and catalase, but negative to oxidase. These findings were similar to other studies [9, 10, 57]. The high specificity of the nuc gene in *S. aureus* detection suggests molecular assaying targeting this gene is effective. These findings were similar to other researchers [43-58]. According to molecular characterization of *S. aureus* results showed that *S. aureus* strains isolated from bovine skin infection were methicillin resistant carrying the mecA gene. These findings were compatible with the findings of other studies showing a high prevalence of *S. aureus* in cattle with various abscesses infections [45, 58].

Agreeing to the literature [15,18,28] numerous polyphenolic substances were found in *B. aegyptiaca* and *C. longa* ethanol extracts. Using HPLC, gallic acid, catechins, rutin**,** coumaric acid and Vanillin are the phenolic compounds that are detected within ethanol extracts of studied plants. Vanillin was missing in *B. aegyptiaca* while, gallic acid was absent in *C. longa.* They are flavonoid glycosides present in a broad variety of plants with several therapeutic properties such as anti-inflammatory, antioxidant, anti-microbial, anti-allergic, anti-cancer and so on. They are employed as an active ingredient for many herbal medications [59,60 ,61,62].

From our previous report, CLP-EE and BAF-EE were approved for antibacterial activities against a variety of bacteria including *S. aureus*, with CLP-EE surpassed BAF-EE [15], which is compatible with other research [26, 27]. In the present study, irritation within the skin of infected mice treated with CLP-EE began to diminish more quickly than in other groups as observed in Fig. 4. CLP-EE's superior antibacterial properties might be due to its content of Vanillin (4-hydroxy-3-methoxybenzaldehyde) belonging to a phenolic aldehyde that exhibiting antibacterial and antioxidant properties. According to Martelli and Giacomini 2018 [12], several vanillin-imines are active *against E. coli, S. aureus*, and *P. aeruginosa*. Besides, it was proved to have antioxidant, DNA, and cellular protective capabilities [62]. Vanillin was found to have a synergistic impact when combined with several antibacterial medications such as gentamicin and imipenem against *S. aureus* and *E. coli* strains, but not with norfloxacin, tetracycline, or erythromycin [63]. Rutin and its derivatives which show in both extracts are one of the most prevalent flavonol glycosides, and have been widely examined to demonstrate its many bioactivities, including antibacterial characteristics, which have offered some scientific basis for the traditional usage to cure infections **[**64]**.** It is considered as one of the strongest herbal antioxidants in the identified natural class [61].

The data of hemogram revealed that the count of RBCs, HCT and Hb contents were significantly elevated after BAF-EE and CLP-EE administration. This may be due to the richness of extracts with several phytochemical ingredients including phenolic acids, quinones, flavonoids, flavones, flavanols, tannins, coumarins, saponins, tannins, terpenoids, essential oils, and alkaloids, all of which have a wide variety of physiological activities, as previously mentioned [15,18,19,20].

The leukogram of the current study showed that white blood cells increased significantly after *S. aureus* infection as a result of elevated lymphocyte counts. Chakraborty et al. [65] stated that lymphocytes were associated in host immune defense against *S. aureus* infection in mice by determined some immune mediators produced by lymphocytes. Therefore, the increase in lymphocytic count in this study was explicable. Macrophages that are derived from circulating monocytes are professional phagocytes capable of locating and destroying *S. aureus*. Macrophage-deficient mice have increased susceptibility to *S. aureus* infection [66]. Despite this, some *S. aureus* is capable of evading mature macrophages and preventing their recruitment. Although monocytes were not necessary as an early

immune response during *S. aureus* skin infection, inflammatory monocytes were recruited to the site of infection to contribute to the expansion and renewal of dermal macrophages (Mφ) [67]. This may be explained by the significant decrease of monocyte counts after infection. On the other hand, polymorphonuclear leukocytes (PMN) especially neutrophils play a critical role in innate immunity against *S. aureus* infection. Local infection with *S. aureus* can stimulate neutrophil mobilization from the bone marrow pool and infiltration to the site of infection by eliciting cytokines such as IL-1 and IL-17 [68, 69] which explained the increase in granulocyte counts in the present study. Kim et al., [68] showed that there is a close correlation between changes in the circulating neutrophil count and its influx to the wound area at the initial 2 days.

Curcumin is a polyphenolic compound of turmeric that mediated the inhibition of a wide spectrum of bacterial infections, especially *S. aureus,* with different mechanisms [29]. One of these mechanisms is by targeting some membrane proteins of *S. aureus* such as α-hemolysin and peptidoglycan [68, 69, 70]. It is traditionally known to have anti-inflammatory and antioxidant properties. In addition, curcumin has an immunostimulant activity. Li and Liu [71] showed that a low dose of curcumin (0-50 μmol/l) enhanced the proliferation of murine spleen lymphocytes and peritoneal macrophage phagocytosis *in vitro*. In addition, curcumin treatment stimulates the proliferation of rat spleen lymphocytes [72, 73] and increased total white blood cell count [69, 73, 74]. This is consistent with the current data, showed that the counts of white blood cells, lymphocytes and granulocytes increased significantly at 21 dpi after *C. longa* treatment.

On the other side, Singla et al. [59] illustrated that treating emphysematous mice with gallic acid, one of the detected phenolic chemicals in BAF-EE, diminishes the inflammatory and oxidative harm. They affirmed that gallic acid treatment diminishes IL-6, and TNF-α gene expression levels within the lung tissue, as well as neutrophil and macrophage levels in bronchoalveolar lavage fluid.

The data of the present study revealed that RBCs count, HCT, Hb and all leukogram parameters were significantly elevated after BAF-EE administration. This may be due to the richness of extract with saponins, tannins and anthraquinones which are precursors of steroidal substances with a wide variety of physiological activities [19].

Regarding the serum protein profile, the present study revealed that a significant elevation of total proteins and globulin levels was observed in *S. aureus* infected mice. This is attributed to the increased level of acute phase proteins, which was approved to increase during infection and inflammation. The serum albumin level was not significantly altered. This could be due to the fact that albumin is considered a negative acute phase protein and its level did not change during infection [75]. From previous reports, CLP-EE and BAF-EE were approved for antibacterial activities against *S. aureus* [15,26,27]. For that reason, serum total proteins and globulins were expected to decrease after treatment with CLP-EE and BAF-EE. However, ciprofloxacin has been widely used for the treatment of a wide range of bacterial pathogens. The results on its effect on serum proteins were contradictory. Ismail [76] revealed that protein contents in the liver decreased markedly in pregnant rats and their fetuses after administration of 57 and 114 mg/kg bwt. Ciprofloxacin. The author explained that ciprofloxacin inhibits protein synthesis by interfering with DNA and RNA replication. On the other hand, Kaita et al. [77] and Laulund et al. [78] showed that ciprofloxacin administered at dose 100 mg/kg bwt had stimulating activity on liver regeneration and DNA synthesis in rat model with liver failure. This may be explained by the exponential increase in serum protein levels after the administration of 200 mg/kg bwt of *ciprofloxacin* in this study. This increase in serum total proteins was due to the increased level of serum total globulins that agree with the immunomodulatory effect of ciprofloxacin [79, 80].

In terms of the expression of apoptotic genes in the present study, showed that *S. aureus* induced a significant up-regulation of pro-apoptotic genes caspase3, Bax, and p53 expression levels, while significantly decreasing the expression level of the anti-apoptotic Bcl-2 gene in infected mice's liver tissue. Treatment of *S. aureus* infected mice with CLP-EE and BAF-EE was able to decrease the expression of pro-apoptoticcaspase3, Bax and P53 gens levels while increasing anti-apoptotic Bcl-2 gene expression as compared to the control group. Meanwhile, CLP-EE showed better results than BAF-EE. Several research findings have highlighted the significance of apoptosis in certain diseases triggered by *S. aureus,* such as atopic dermatitis (AD) and sepsis [81]. In the course of infection, *S. aureus* has the capability to induce cell apoptosis through diverse pathways [39]. The majority of apoptotic processes in cells caused by *S. aureus* result from the action of various secreted toxins. Among the recognized toxins of *S. aureus*, α-toxin, Panton-Valentine leucocidin producing *S. aureus* (PVL-SA), and secreted enzymes such as staphylococcal cysteine protease staphopain B (SSPB) and coagulase enzyme are identified for their role in stimulating apoptosis [82,83,84], although the intricate underlying mechanisms are acknowledged. Consistent with the results obtained, a study was performed by [24, 85]. Administration of BA-EE proved effective antioxidant and anti-inflammatory activities, therefore preserving cells from apoptosis in goats experimentally infected with *H. Contortus* [23]. This aligns with the

established understanding that the diverse phytochemical constituents found in many medicinal plants contribute to their antimicrobial properties [86]. Numerous reports in the literature highlight the antibacterial activity of crude extracts derived from plants [28]. In the present study, all of the detected phenolic compounds have antibacterial, antioxidant and anti-inflammatory actions that protect cells from apoptosis as previously stated, More, coumaric acid is demonstrated to avoid lipid peroxidation and cell passing in endothelial cells beneath oxidative stress [87]**.** Catechin has the ability to scavenge radicals through electron transfer mechanisms, and have immune-modulating potential [60].

Conclusions

The experimental findings suggest that *C. longa* and *B. aegyptiaca* ethanol extracts exhibit potential antibacterial capabilities linked with better host immune response and hemogram profile, as well as a protective impact on apoptosis-related genes.

The results indicate that *C. longa* rhizome and *B. aegyptiaca* can be considered as potential sources of biologically active compounds. This clearly suggested that they have the capacity to be selected as an alternative medicinal plant used in pharmaceuticals, with CLP-EE outperforming BAF-EE.

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Declaration of Conflict of Interest

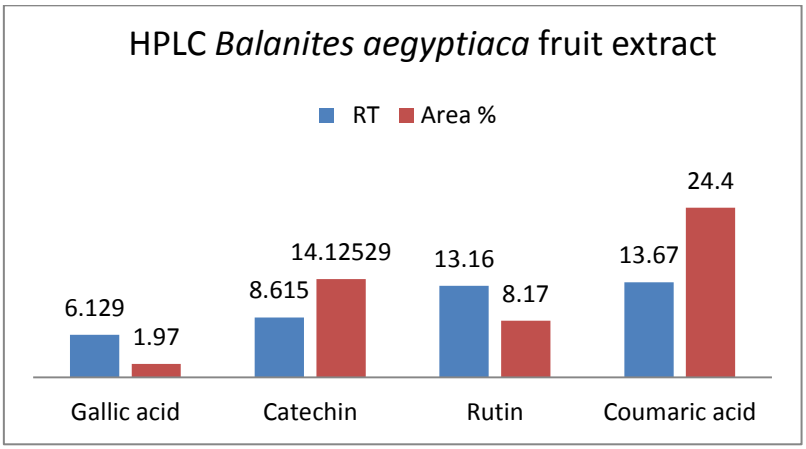
The authors declare that there is no conflict of interest.

Ethical of approval

This research adhered to the protocols outlined by the Institutional Animal Care and Use Committee at the National Research Centre, Giza, Egypt, under Approval Protocol No. 16229. All animal processes during this study adhered to the ethical guidelines set by the institution or practice where the research was carried out.

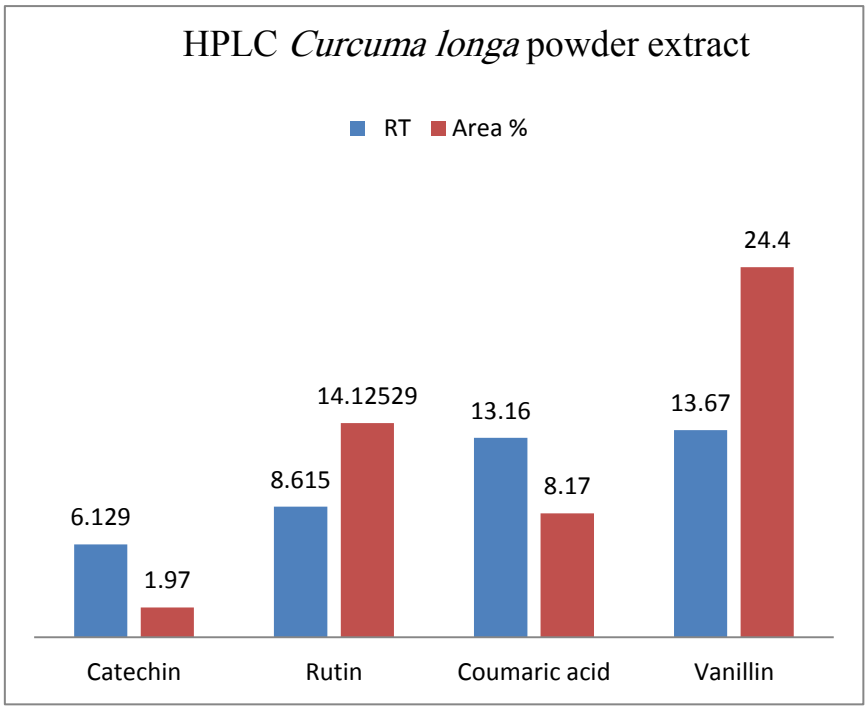
TABLE 1. Oligonucleotide primers sequences for molecular characterization of isolates

Target Gene	Primer Sequence 5' -3'	Amplified product (bp)	Reference
nuc-F	5' GCGATTGATGGTGATACGGTT 3'	279 bp	Brakstad *et al.* [43]
nuc-R	5' AGCCAAGCCTTGACGAACTAAAGC 3'		
16SrRNA-f	5'GTAGGTGGCAAGCGTTATCC 3'	228 bp	Monday and Bohach [44]
16SrRNA-r	5' CGCACATCAGCGTCAG3'		
mecA-F	5' GTGAAGATATACCAAGTGATT 3'	147 bp	Zhang *et al.* [45]
mecA-R	5' ATGCGCTATAGATTGAAAGGAT 3'		



RT retention time

Fig. 1. Some identified phenolic compounds in *B. aegyptiaca* fruit ethanol extract using High Performance Liquid Chromatography (HPLC).



RT retention time

Fig. 2. Some identified phenolic compounds in *C. longa* powder ethanol extract using High Performance Liquid Chromatography (HPLC).

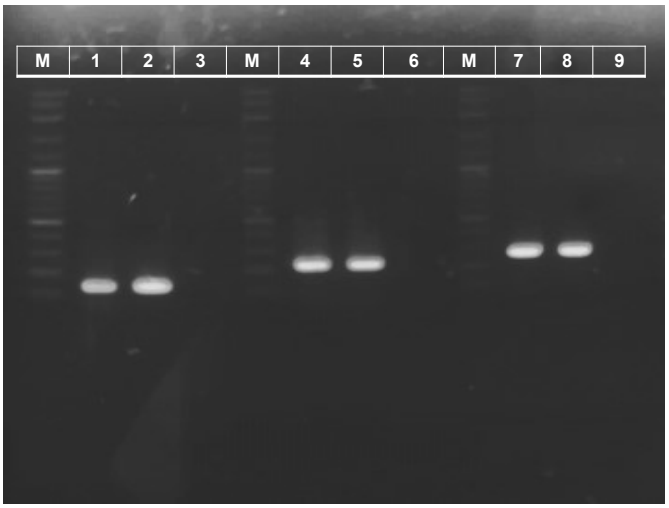


Fig.3. Agarose gel electrophoresis of PCR products amplified from positive samples of *S. aures* spp. Lanes (M): 100 bp DNA ladder, Lanes (1-2): showing 147 bp fragments indicative for mecA gene . Lanes (4-5): showing 228 bp fragments indicative for16SrRNA gene. Lanes (7-8): showing 279 bp fragments indicative for nuc gene. Lanes (3-6-9): negative control.

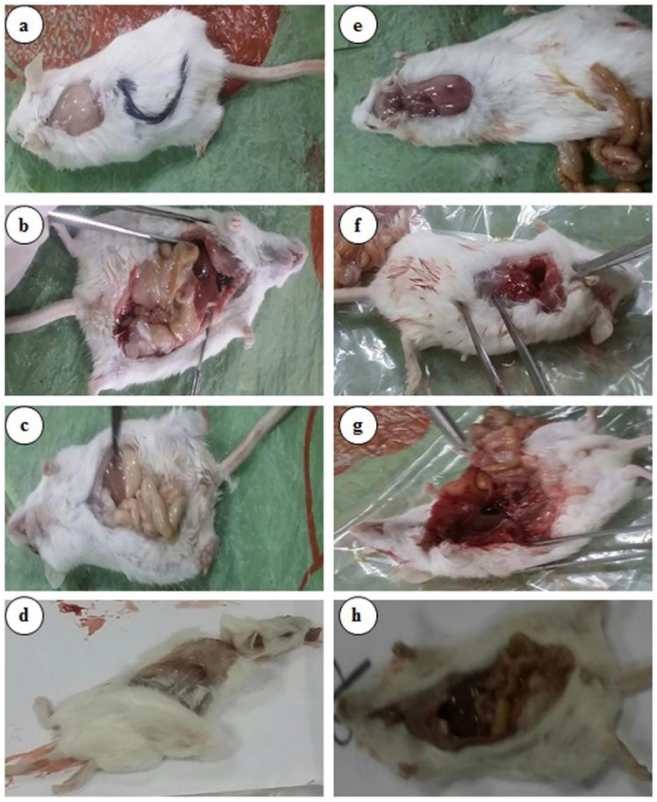


Fig. 4. Macroscopic observations in the skin and internal organs of mice: (a, b, c, d) normal control non-infected mice and (e, f, g, h)infected mice S/C with 0.1 ml of 2 ×10^7 colony-forming units (CFU) of *Staphylococcus aureus*.

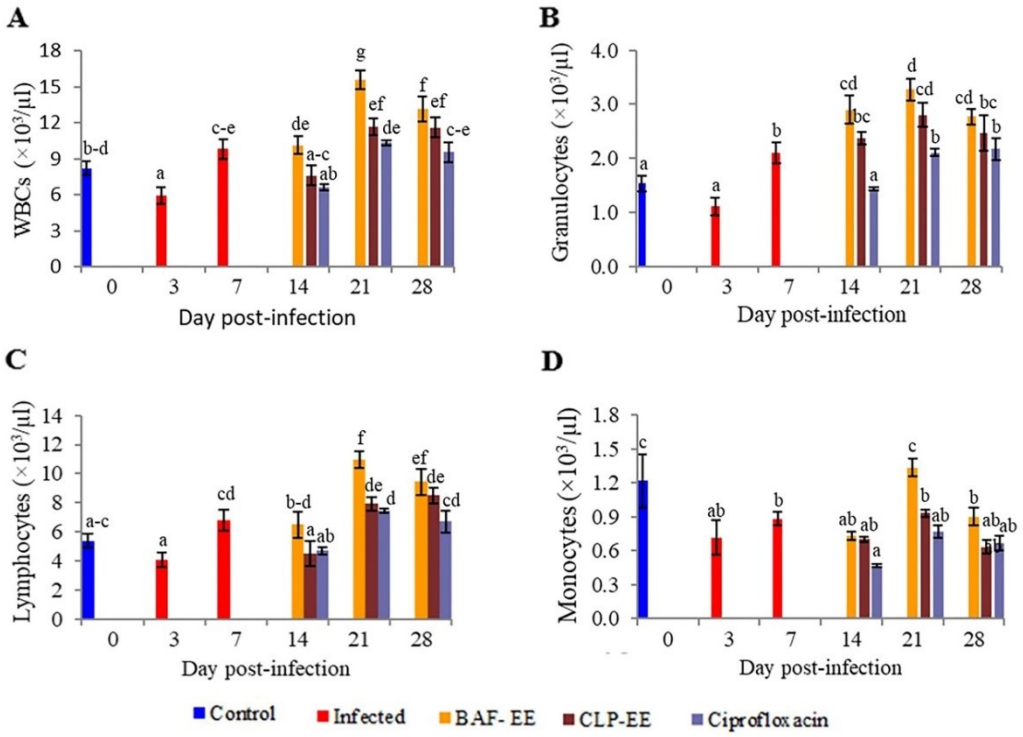


Fig. 5. Leukogram of experimental mice:(A) total white blood cell counts (WBC), (B) Granulocytes, (C) lymphocytes, and (D) monocytes in different groups; normal control, infected with *Staphylococcus aureus*, infected then treated with *Balanites aegyptiaca* fruits ethanol extract (BAF-EE),*Curcuma longa* powder ethanol extract (CLP-EE), and Ciprofloxacin as standard drug during experimental periods. (Mean ±SE). Means of different letters in the same parameter are significantly different at *P*<0.05. (Mean ±SE).

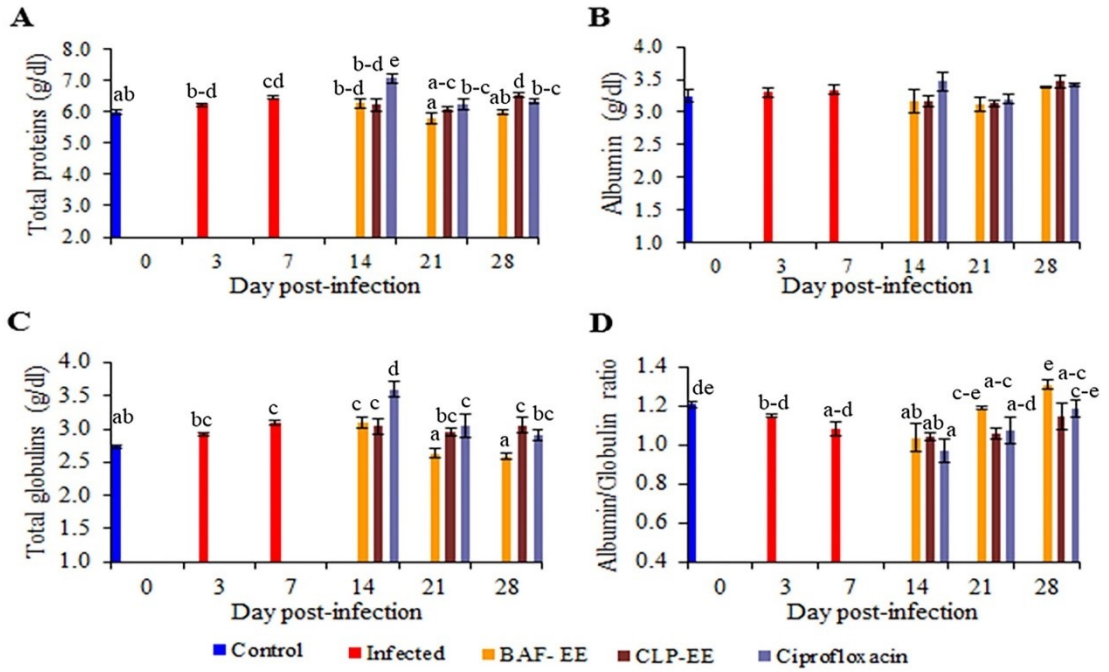


Fig. 6. Serum protein profile of mice; (A) total proteins, (B) albumin, (C) total globulins and (D) albumin globulin ratio (A/G), in different groups; normal control, infected with *Staphylococcus aureus*, infected then treated with *Balanites aegyptiaca* fruits ethanol extract (BAF- EE), *Curcuma longa* powder ethanol extract (CLP- EE), and Ciprofloxacin as standard drug during experimental periods. Means of different letters in the same parameter are significantly different at *P*<0.05. (Mean ±SE).

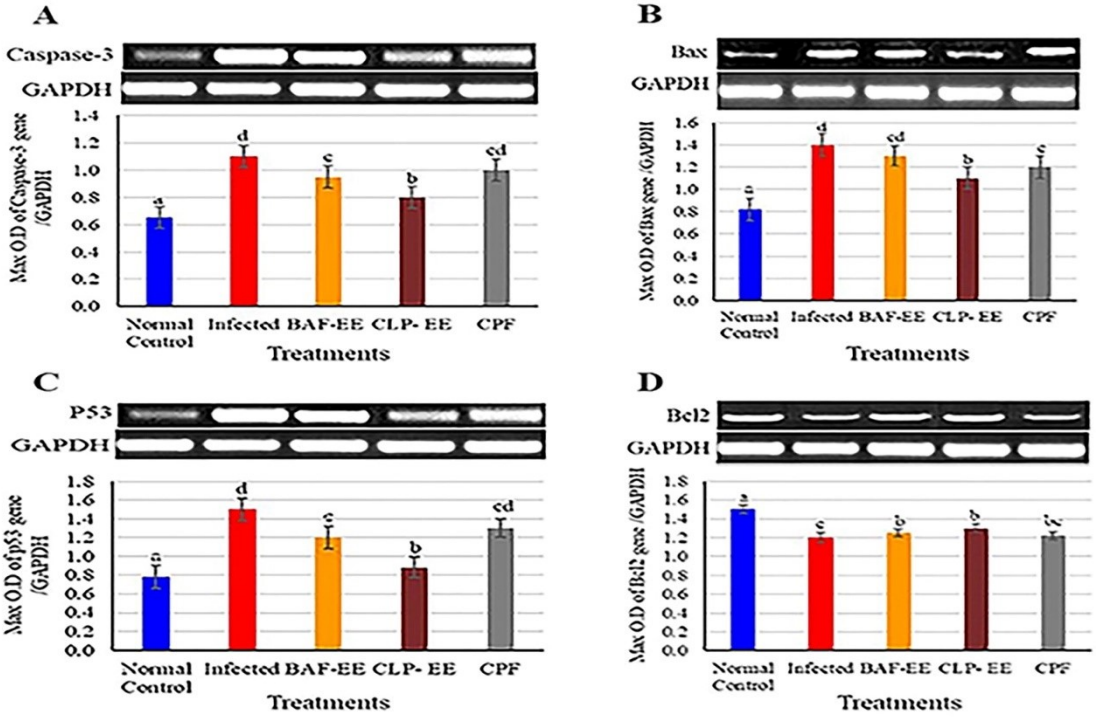


Fig. 7. Effect of *Balanites aegyptiaca* fruits ethanol extract (BAF-EE),*Curcuma longa* powder ethanol extract (CLP-EE) and Ciprofloxacin (CPF) on expression levels of apoptotic gene markers; (A) Caspase-3, (B) Bax (C) p53 (D) Bcl2 in liver tissue of mice infected with *Staphylococcus aureus*. The results illustrated are normalized to the level of GAPDH level and the data are the mean of intensity for each gene divided by that for GAPDH. Means with different letters in the same parameter are significantly different at *P*<0.05. (Means± SE)

TABLE 2. Primer sequences and polymerase chain reaction (PCR) conditions of the genes analyzed.

Primer Name (cDNA) of genes	Accession Number	Primer sequence 5' to 3'	R^2	PCR Efficiency (%)	SE	PCR amplification size	References
Cas-3	NM_012922.2	F: AAATTCAAGGGACGGGTCAT R: ATTGACACAATACACGGGATCTGT	0.972	91.28	0.007	205bp	Liu et al. [51]
p53	NM_030989.3	F: GCACAAACACGCACCTCAAAGC R: CTTGCATTCTGGGACAGCCAAG	0.926	100.79	0.010	494bp	Yonguc et al. [52]
Bax	NM_017059.2	F: AGGATGATTGCTGATGTGGATAC R: CACAAAGATGGTCACTGTCTGC	0.996	101.35	0.014	300bp	Aboshanab et al. [53]
Bcl-2	NM_016993.2	F: GCTACGAGTGGGATACTGGAGA R: AGTCATCCACAGAGCGATGTT	0.960	93.43	0.012	446bp	Hassan et al.[54]
GAPDH	NM_017008.4	F: CAAGGTCATCCATGACAACTTTG R: GTCCACCACCCTGTTGCTGTAG	0.998	97.63	0.008	496bp	Abdel-Wahhab et al., [55]

Cas-3: Caspase-3. p53: p53 protein. Bax: B-cell lymphoma-2 protein-associated X protein. Bcl-2: B-cell lymphoma-2 protein.
GAPDH: Glyceraldehyde-3- phosphate dehydrogenase.SE: Standard error. R^2: coefficient of determination.

TABLE 3. Erytrogram of experimental mice in different groups; normal control, infected with *Staphylococcus aureus*, then treated with *Curcuma longa* powder ethanol extract (CLP- EE), *Balanites aegyptiaca* fruits ethanol extract (BAF- EE) and Ciprofloxacin (CPF) as standard drug during the experimental periods. (Mean ±SE)

Parameters	Normal control	Infected		Infected then treated								
		3 dpi	7 dpi	14 dpi			21 dpi			28 dpi		
				BAF- EE	CLP- EE	CPF-EE	BAF- EE	CLP- EE	CPF-EE	BAF- EE	CLP- EE	CPF-EE
RBCs ($\times 10^6$)	8.18^{b} ±0.06	6.92^{a} ±0.65	8.89^{b-d} ±0.17	9.52^{e} ±0.17	9.31^{c-e} ±0.15	9.44^{de} ±0.08	9.23^{c-e} ±0.23	8.45^{b} ±0.07	8.88^{b-d} ±0.08	8.49^{b} ±0.12	8.61^{ab} ±0.06	8.78^{bc} ±0.08
Hematocrit (%)	38.57^{b} ±0.27	34.33^{a} ±2.96	41.03^{b-d} ±1.21	45.80^{f} ±0.86	44.27^{d-f} ±0.48	45.17^{ef} ±0.36	42.30^{c-e} ±0.35	39.10^{bc} ±0.92	40.30^{bc} ±0.30	40.50^{bc} ±0.83	41.03^{b-d} ±0.29	38.53^{b} ±0.97
Hemoglobin (g/dl)	13.23^{b} ±0.06	11.68^{a} ±0.98	14.33^{b-e} ±0.44	15.60^{f} ±0.27	15.10^{d-f} ±0.15	15.40^{d-f} ±0.14	14.83^{c-f} ±0.16	13.80^{bc} ±0.27	14.43^{cd} ±0.13	14.00^{b-d} ±0.25	14.37^{b-e} ±0.11	13.67^{bc} ±0.42
MCV (fl)	47.20^{bc} ±0.18	49.97^{d} ±0.57	46.17^{bc} ±1.22	48.07^{c} ±0.16	47.57^{c} ±0.23	47.83^{c} ±0.01	46.03^{bc} ±0.86	46.20^{bc} ±0.88	45.37^{ab} ±0.09	47.67^{c} ±0.30	47.67^{c} ±0.60	43.80^{a} ±0.84
MCH (pg)	16.17^{ab} ±0.06	17.05^{c} ±0.27	16.10^{ab} ±0.42	16.40^{bc} ±0.04	16.20^{ab} ±0.11	16.30^{b} ±0.04	16.13^{ab} ±0.30	16.33^{b} ±0.23	16.23^{b} ±0.01	16.50^{bc} ±0.08	16.67^{bc} ±0.14	15.50^{a} ±0.40
MCHC (g/dl)	34.30^{ab} ±0.07	34.10^{a} ±0.19	35.00^{cd} ±0.08	34.13^{a} ±0.05	34.13^{a} ±0.08	34.17^{a} ±0.10	35.07^{cd} ±0.06	35.40^{de} ±0.16	35.80^{e} ±0.07	34.63^{bc} ±0.13	35.00^{cd} ±0.24	35.43^{de} ±0.23

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دراسة التأثير المضاد للمكورات العنقودية الذهبية لمستخلصي الايثانولي لبلح الصحراء والكركم واثارها على التعبير الجيني للموت المبرمج للخلايا وصورة الدم وبروتينات المصل في الفئران

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الملخص

تهدف هذة الدراسة إلى تحديد تأثيرات مستخلصات الإيثانول لبلح الصحراء والكركم على التعبير الجيني للموت المبرمج للخلايا، وصورة الدم والبروتين بمصل الدم في الفئران المصابة تجريبياً ببكتيريا المكورات العنقودية الذهبية. وقد تم استخدام جهاز HPLC للتقدير النوعي للمركبات الفينولية في كلا المستخلصين. إجريت التجربة على عدد ستون فأرًا، حيث تم تقسيمها إلى مجموعة غير مصابة (العدد = 15) ومجموعة مكونة من 45 فأرًا تم عمل عدوى لهم باستخدام 0.1 مل من 2×10^{7} CFU من عزلة معرفة جيدا من البكتيريا. وفي اليوم السابع بعد العدوى تم تقسيم هذة الفئران إلى 3 مجموعات متساوية، وتم إعطاؤها عن طريق الفم جرعات من مستخلصات الإيثانول لبلح الصحراء و الكركم و سيبروفلوكساسين، بجرعات 200 مجم و100 مجم و20 مجم/كجم من وزن الجسم/يوميًا لمدة 5 أيام على التوالي. تم رصد جميع الفئران سريريا لمدة 4 أسابيع للتفاعلات الالتهابية البكتيرية . وفي الأيام 3 و7 و14 و21 و28، تم أخذ عينات دم وكبد من ثلاثة فئران من كل مجموعة لكل فترة من فترات التجربة، وذلك لفحص صورة الدم وتقدير البروتينات في المصل. وقياس التعبير الجيني لجينات موت الخلايا المبرمج في خلايا الكبد بواسطة qRT-PCR. أظهرت البيانات التي تم الحصول عليها أنه تم تحديد أربعة مركبات فينولية في كل مستخلص.، ولقد ارتفع مستوى صورة الدم الاحمر بعد كل العلاجات بينما ارتفع عدد كريات الدم البيضاء باستثناء خلايا المونوسيت في الفئران المعالجة بكلا المستخلصين، كما لوحظ زيادة في مستويات البروتين والجلوبيولين الكلي في الفئران المعالجة بالسيبروفلوكساسين. وقد أوضحت النتائج أن العلاج بكل من المستخلصين أدى إلى تقليل التعبير الجيني للجينات المسببة لموت الخلايا المبرمج (Caspase-3 ، Bax ، p53)، بينما زاد التعبير عن الجين المضاد للموت المبرمج للخلايا Bcl-2، وقد أظهرت النتائج تفوق مستخلص الكركم على مستخلص بلح الصحراء. وخلصت الدراسة إلى أنه يبدو أن كلا المستخلصين من بلح الصحراء والكركم يتمتعان بقدرات مضادة للبكتيريا، كما اتضح من تأثيرهما الوقائي على الجينات المرتبطة بموت الخلايا المبرمج وعلى صورة الدم.

الكلمات الدالة: المكورات العنقودية الذهبية، بلح الصحراء، الكركم، موت الخلايا المبرمج، صورة الأيض للدم