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RESEARCH ARTICLE

# Effect of gum arabic and Nigella sativa on T-helper1 and T-helper2 immune response in Wistar rats infected with methicillin-resistant *Staphylococcus aureus*

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## ABSTRACT

**Background:** The herbal medications are widely used in folk medicine for treatment of variable diseases and infection. *Staphylococcus aureus* (Sa) is a Gram-positive bacteria that cause severe organ affection, especially in the lung. Nigella sativa (NS) and gum arabic (GA) are probiotics with multi-beneficial effects for human health. **Aims and Objectives:** The current study was outlined to examine the protective effect of NS and GA against SA induced serum alterations and changes in cytokines and antioxidants expressions. **Materials and Methods:** A total of 42 male Wistar rats were allocated into six groups (6 each). Control group without any treatment; SA group in a dose of $2 \times 10^9$ colony forming units /mL per rat. Rats in 3-6 groups served as SA infected groups and received NS for Group 3 (150 mg/kg b.w. daily), GA plus SA for Group 4 (10% wt/vol. daily), and SA group received a mixture of NS plus GA for Group 5, and SA administered group received *Lactobacillus* for Group 6 as a positive control. NS and GA were administered 2 weeks before SA infection and continued for 1 week. Serum levels of cytokines and oxidative stress biomarkers were measured. Lung tissues were examined at molecular levels for mRNA expression of inflammatory cytokines and antioxidants. **Results:** SA induced a significant decrease in serum levels of interleukin -6 (IL-6), IL-2, and tumor necrosis factor -alpha. NS induced significant decrease on examined cytokines levels in SA ($P < 0.05$). GA did not induce any additive changes in cytokines. When both NA and GA were administered together, NS induced additive inhibitory effect. SA injection induced significant increase in serum levels of catalase and glutathione reductase. NS induced additive significant increase on antioxidant levels in SA injected. GA did not induce any additive increase for examined antioxidant levels. NS decreased total bacterial count increased in SA group more significantly than the effect induced by NS. **Conclusion:** NS has the potential to control the degree of inflammation at the protein and gene levels of examined cytokines (T-helper1/T-helper2) and antioxidants compared to the effect induced by GA.

**KEY WORDS:** Nigella Sativa; Gum Arabic; Lung; *Staphylococcus aureus* Infection; Protection

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## INTRODUCTION

Medicinal plants are widely used in humans for the treatment of several diseases. Nigella sativa (NS), family Ranunculaceae, has pharmaceutical and medical properties such as diuretics, antihypertensive, antibacterial, anthelmintic, antioxidant, anti-inflammatory, and immunopotential activity.[1-4] Many

studies pointed on the effect of NS extracts on the immune system. All of them have shown that the NS extracts inhibit many inflammatory cytokines.[5,6] Another probiotics widely used in human remedies is gum arabic (GA). GA is the dried exudates from *Acacia senegal* stems and branches. It is greatly used in industry as stabilizing and emulsifying substance. In folk medicine, it is used for curing bowel inflammation.[7] GA has strong antioxidant activities. Moreover, it stimulates the growth of normal bowel flora (benefit bacteria). It acts as prebiotic.[8]

*Staphylococcus aureus* (SA) is Gram-positive bacteria capable of causing sepsis condition (septicemia) due to the presence of some components in cell wall as peptidoglycan and lipoteichoic acid.[9] It considered as a potential stimulatory microorganism for the production of inflammatory cytokines such as tumor necrosis factor (TNFα), TNFγ, interleukin -1 (IL), IL-6, IL-12, IL-4, and IL-8 in response to inflammation.[9]

The purpose of the present study aims to investigate the effects of NS extract and GA on rats experimentally infected with SA and evaluates the antioxidant, cytokines production (T-helper1 and T-helper2), and total bacterial count (TBC) in lung tissue. Moreover, this study discussed and concluded the effect of NS and GA on sepsis condition induced by SA.

## MATERIALS AND METHODS

### Animals and Experimental Design

Male Wistar rats ($n = 42$), 3 months old, weighing 180-200 g, were purchased from the King Fahd Institute for Scientific Research, King AbdulAziz University, Jeddah, Saudi Arabia. The rats were subjected to 12 h/12 h daylight with free water and food and handled manually for 7 days to avoid stress effect. All experimental design was approved by the Ethical Committee of Taif University.

Rats were divided into six equal groups (each 7 rats). The control group was fed normal diet and water. SA methicillin-resistant SA (MRSA) groups were injected intraperitoneally with virulent strain of MRSA in a dose of $2 \times 10^9$ colony forming units (CFU)/mL per rat.[10] The remaining four groups were divided as following: SA plus NS (SA + NS) group was administered water-alcohol extract of NS in a dose of 150 mg/kg b.w. daily.[11] SA plus GA group was administered GA dissolved in water in a dose of 10% wt/vol. daily.[12] SA group plus Ns plus GA in drinking water. Positive probiotic control group taken one sachet of lacteal fort (*Lactobacillus* spp.) added to 500 mL of water and infected by SA.[13] All rats were kept under observation for 1 week after infection with SA. Animals received NS, GA, and *Lactobacillus* spp. for 2 weeks before experimental infection and continued for 7 days later. At the end of the experimental schedule, the rats were overnight fasted, and inhaled diethyl ether then decapitated. Blood was taken for serum cytokines and antioxidants measurements; lungs were taken aseptically, weighted and cultured for SA isolation and counting (CFU/g tissues), and for RNA extraction for cytokines of T-helper 1 and T-helper 2 and antioxidant mRNA expression.

*Staph aureus* (MRSA) strain was kindly provided from the Animal Reproduction Research Institute (Al-Haram, Egypt). The culture of SA was grown in tryptic broth and incubated for 24 h. The culture was then centrifuged at 15,000 × g for 20 min, and the pellet was washed with sterile phosphate buffer saline. The viable bacterial count was adjusted approximately to $2 \times 10^9$ CFU/mL.

### Serum Chemistry and Cytokines Measurements

Serum IL-6, IL-2, and TNF-α levels were measured using commercial kits imported from abcam, Co, USA. Antioxidants (glutathione reductase [GSH-R] and catalase) were measured spectrophotometrically using commercial ELISA kits based on manufacture instruction manual of Biodiagnostic Company, Dokki, Giza, Egypt.

### *Staph aureus* Counting

Lungs were weighted under aseptic condition then grinded homogenate was incubated in nutrient broth at 37°C for 24 h. Tenfold serial dilution was performed; each sample has been diluted in normal saline for five dilution then cultured on mannitol salt agar media for 24 h at 37°C then count the separate colony as formula (count of colony × dilution factor/gram tissues).

### Gene Expression and Reverse Transcription Polymerase Chain Reaction (RT-PCR)

For preparation of total RNA, lung samples (100 mg per sample) were collected from rat lungs, frozen in liquid nitrogen and subsequently stored at −80°C in 1 mL Qiazol. Frozen samples were homogenized using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY). Then, 0.3 mL chloroform was added to the homogenates. The mixtures were shaken for 30 s followed by centrifugation at 4°C and 12.500 rpm for 20 min. The supernatant layer was transferred to a new set of tubes, and an equal volume of isopropanol alcohol will be added to the samples, shacked for 15 s and centrifuged at 4°C and 12.500 rpm for 15 min. The RNA pellets were washed with 70% ethanol, briefly dried up then, dissolved in diethylpyrocarbonate (DEPC) water. The prepared RNA integrity was checked by electrophoresis. RNA concentration and purity were determined spectrophotometrically at 260 nm. For cDNA synthesis, mixture of 3 µg total RNA and 0.5 ng oligo dT primer in a total volume of 11 µL sterilized DEPC water were incubated in the Pe× 0.5 thermal cycler (PCR machine Bio-Rad) at 70°C for 5 min for denaturation. Then, 4 µL of

5× RT-buffer, 2 µL of 10 mM dNTPs, and 100 U moloney murine leukemia virus reverse transcriptase were added in a total volume of 20 µL by DEPC water. The mixture was reincubated in the thermal cycler at 37°C for 1 h, then at 90°C for 10 min to inactivate the enzyme. Specific primers for genes were designed using Oligo-4 computer program and synthesized by Macrogen (Macrogen Company, GAsa-dong, and Geumcheon-gu, Korea), as shown in Table 1. PCR were conducted in a final volume of 25 µL consisting of 1 µL cDNA, 1 µL of 10 picomolar of each primer (forward and reverse), and 12.5 µL PCR master mix (Promega Corporation, Madison, WI). The volume was brought up to 25 µL using sterilized deionized water. The cycle sequence of PCR reaction was carried out at 94°C for 5 min one cycle, followed by 28 cycles each of which consisted of denaturation at 94°C for 1 min, annealing at the specific temperature corresponding to each primer (Table 1) and extension at 72°C for 1 min with additional final extension at 72°C for 7 min. As a reference, expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA as housekeeping gene was expressed. PCR products were electrophorized on 1% agarose gel stained with ethidium bromide in Tris-Borate-EDTA buffer. PCR products were visualized under ultraviolet light and subsequently photographed using an In Genius 3.0 gel documentation system (Syngene, Frederick, MD, USA). The band intensities were densitometrically quantified and calculated using ImageJ software version 1.47 (http://imagej.en.softonic.com/).

### Statistical Analysis

The data are presented as the mean ± standard error of the mean. Analysis of variance and Fisher *post hoc* descriptive test were used to analyze the data using SPSS software version 11.5 for Windows (SPSS, Inc., Chicago, IL, USA). Using the same software, regression analysis was performed. Values with $P < 0.05$ were considered statistically significant.

## RESULTS

### Effects of NS and or GA on SA Infection Induced Alterations in Serum Cytokines

As seen in Table 2, SA infection induced significant decreases in serum levels of IL-6, IL-2, and TNF-alpha. Prior administration of NS induced additive significant decrease on cytokines levels in SA ($P < 0.05$), on same time GA did not induce any additive decrease for examined cytokines levels and the effect is only for SA. When both NA and GA were administered together the clear effect is for NS, as NS induced additive inhibitory effect. As a positive control, *Lactobacillus* showed a highly significant decrease on cytokines levels as reported for NS alone (Table 2).

### Effects of NS and or GA on SA Infection Induced Alterations on Serum Antioxidant Levels

As seen in Table 3, SA infection induced significant increase in serum levels of catalase and GSH-R. Prior administration of NS induced additive significant increase on antioxidant levels in SA injected rats ($P < 0.05$). On contrast, GA did not induce any additive increase for examined antioxidant levels, and the effect is for SA only. When both NA and GA were administered together, NS induced an increase in catalase and GSH-R as reported for NS alone. As a positive control *Lactobacillus* showed a significant increase in antioxidant levels as reported for NS alone compared to SA injected rats (Table 3).

### Effects of NS and or GA on TBC in Lung

As shown in Table 4, NS administration showed a significant reduction in the number of TBC. GA showed moderate decrease in TBC. Coadministration of NS plus GA did not induce any additive reduction compared to the effect of each of them separately. Of note, LB induced a great reduction in TBC.

**Table 1:** PCR conditions for examined genes in lung tissue

| Gene | Product size (bp) | Annealing (°C) | Direction | Sequence (5'-3') |
|---|---|---|---|---|
| SOD | 410 | 55 | *Sense* | AGGATTAACTGAAGGCGAGCAT |
| | | | *Antisense* | TCTACAGTTAGCAGGCCAGCAG |
| GST | 575 | 55 | *Sense* | GCTGGAGTGGAGTTTGAAGAA |
| | | | *Antisense* | GTCCTGACCACGTCAACATAG |
| IL-1 | 497 | 61 | *Sense* | ATGGCAACCGTACCTGAACCCA |
| | | | *Antisense* | GCTCGAAAATGTCCCAGGAA |
| IL-12-α | 447 | 57 | *Sense* | GCTTACCACTGGAACTCCACA |
| | | | *Antisense* | TCCTACAGGAGCTGAAGGTCA |
| GAPDH | 457 | 60 | *Sense* | ATGTACGTAGCCATCCAGGC |
| | | | *Antisense* | TCCACACAGAGTACTTGCGC |

PCR: Polymerase chain reaction, SOD: Super oxide dismutase, GST: Glutathione-S-reductase, IL: Interleukin, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase

**Table 2:** Effect of different treatments on SA infection-induced changes in cytokines levels

| Parameter | IL-6 (U/mL) | IL-2 (U/mL) | TNF- alpha (U/mL) |
|---|---|---|---|
| Control | 19.8±0.9 | 1.89±0.1 | 84.05±4.4 |
| SA | 6.2±0.2* | 0.57±0.05* | 25.6±2.2* |
| SA+NS | 3.6±0.1*# | 0.39±0.04*# | 17.6±1.8*# |
| SA+GA | 5.6±0.2* | 0.55±0.02* | 24. 5±0.9* |
| SA+NS+GA | 4.4±0.4*# | 0.41±0.04* | 20.02±1.9*# |
| SA+LB | 3.4±0.1*# | 0.33±0.01*# | 14.9±0.7*# |

Values are means±standard error mean (SEM) for seven different rats per each treatment. Values are statistically significant at *$P$<0.05 versus control, #$P$<0.05 versus SA injected rats. IL: Interleukin, TNF: Tumor necrosis factor, SA: *Staphylococcus aureus*, NS: Nigella sativa, GA: Gum arabic, LB: Lactobacillus

**Table 3:** Effect of different treatments on SA infection-induced changes in antioxidant levels

| Parameter | Catalase (U/mL) | GSH-R |
|---|---|---|
| Control | 13.1±0.57 | 34.6±1.7 |
| SA | 26.3±2.2* | 69.3±4.02* |
| SA+NS | 31.9±1.2*# | 83.6±4.3*# |
| SA+GA | 26.3±1.3* | 68.3±2*# |
| SA+NS+GA | 30.6±1.4*# | 81.9±2.8*#$ |
| SA+LB | 33.4±0.8*# | 89.5±1.3*# |

Values are means±standard error mean (SEM) for seven different rats per each treatment. Values are statistically significant at *$P$<0.05 versus control, #$P$<0.05 versus SA injected rats and $$P$<0.05 versus SA plus gum arabic administered rats. SA: *Staphylococcus aureus*, NS: Nigella sativa, GA: Gum arabic, GSH-R: Glutathione reductase

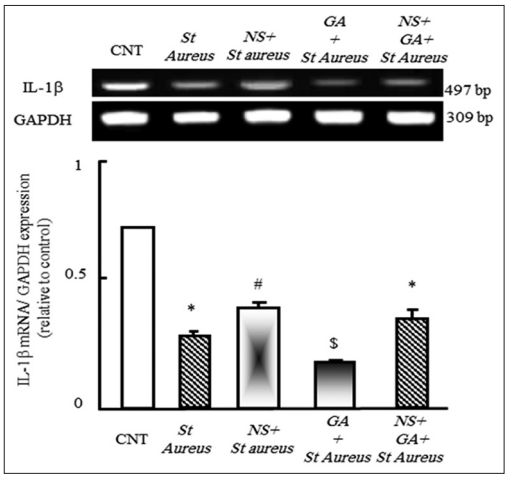


**Figure 1:** Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of interleukin −1β in lung tissues after injection of *Staphylococcus aureus* (SA) in Wistar rats and possible protection by Nigella sativa and gum arabic. Total RNA was extracted and reverses transcribed (3 μg) as mentioned in methods section, and RT-PCR analysis was performed for examined gene. *$P$ < 0.05 versus the control group, #$P$ < 0.05 versus SA, $$P$ < 0.05 versus SA plus NS, glyceralde-hyde-3-phosphate dehydrogenase

### Effects of NS and or GA on SA Induced Alterations IL-1 and IL-12 mRNA Expression

SA induced a significant decrease in mRNA expression of IL-1. Prior administration of NS partially restored SA down-regulation but still low compared to control rats. Unlike NS effect, GA showed more additive inhibitory effect on IL-1 expression (Figure 1). When both NS and GA administered together, NS protected lung from the inhibitory effect of GA. Unlike IL-1 expression, SA induced upregulation in IL-12-α expression and NS induced additive upregulation in IL-12-α (Figure 2). GA inhibited IL-12 expression and coadministration of NS with GA inhibited the down-regulation of IL-12 expression induced by GA and partially normalized it.

### Effects of NS and or GA on SA Induced Alterations on Super Oxide Dismutase (SOD) and GSH-R mRNA Expression in Wister Rats

SA infection induced significant decrease in SOD and glutathione-S-reductase (GST) mRNA expression. Prior administration of NS inhibited the decrease in SOD and GST expression induced by SA (Figures 3 and 4). GA also to a lesser extent counteracted the inhibition in mRNA expression of SOD but not GST. When both given together, NS normalized the expression of SOD but not GST in SA injected rats.

## DISCUSSION

Sepsis is a severe condition that accompanied by great changes in the concentrations and types of inflammatory cytokines and other parameters in blood plasma.[14] In this study, septicemia induced by SA in Wistar rats followed by reduction of some measured serum cytokines, (IL-6, IL-2, and TNF-α). Moreover, NS SA induced more reduction of inflammatory cytokines. GA and *Lactobacillus* induced a significant reduction of the measured cytokines. These findings are attributed mainly to SA infection in mice significantly reduced the production of inflammatory cytokines such as TNF -α, IL-1β, IL-6, TNF, IL-12, and IL-18.[15] NS has an anti-inflammatory effect and inhibits cytokines production.[5,6] GA reduced inflammatory cytokines such as TNF- α up to 50% in another study.[16]

**Table 4:** Effect of different treatment on TBC in lungs of Wistar rats after SA challenge

| Group | SA | NS+SA | GA+SA | NS+GA+SA | *Lactobacillus*+SA |
|---|---|---|---|---|---|
| Mean±SEM | $92\times10^4\pm14.47\times10^4$ | $39\times10^4\pm3.48\times10^4$ | $52\times10^4\pm4.4\times10^4$ | $47\times10^4\pm19.01\times10^4$ | $22\times10^4\pm14.74\times10^4$ |
| *P* value | | *P*<0.05 | *P*<0.05 | *P*<0.05 | *P*<0.05 |

Values are means (M)±standard error (SE) for seven different rats. SEM: Standard error of the mean. TBC: Total bacterial count, SA: *Staphylococcus aureus,* NS: Nigella sativa, GA: Gum arabic

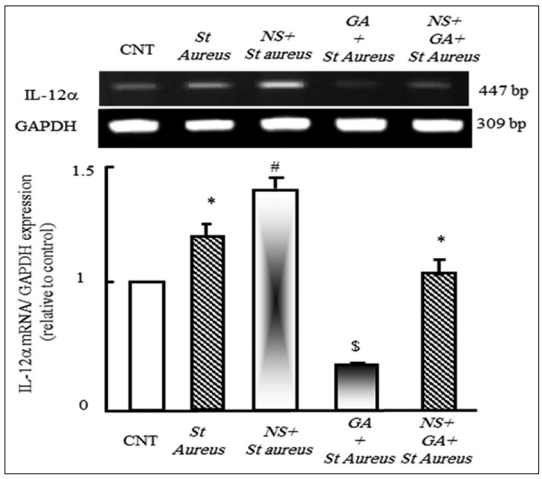


**Figure 2:** Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of interleukin −121α in lung tissues after injection of *Staphylococcus aureus* (SA) in Wistar rats and possible protection by Nigella sativa and gum arabic. Total RNA was extracted and reverses transcribed (3 μg) as mentioned in methods section, and RT-PCR analysis was performed for examined gene. **P* < 0.05 versus the control (CNT) group, $^{\#}P$ < 0.05 versus SA, $^{\$}P$ < 0.05 versus SA plus NS, glyceralde-hyde-3-phosphate dehydrogenase

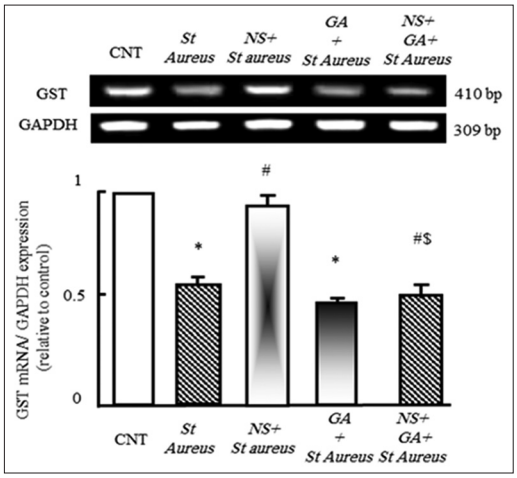


**Figure 3:** Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of superoxide dismutase in lung tissues after injection of *Staphylococcus aureus* (SA) in Wistar rats and possible protection by Nigella sativa and gum arabic. Total RNA was extracted and reverses transcribed (3 μg) as mentioned in methods section, and RT-PCR analysis was performed for examined gene. **P* < 0.05 versus the control (CNT) group, $^{\#}P$ < 0.05 versus SA, $^{\$}P$ < 0.05 versus SA plus GA, glyceralde-hyde-3-phosphate dehydrogenase

In this study, SA induced a significant increase in serum levels of catalase and GSH-R. Prior administration of NS induced additive significant increase on antioxidant levels in SA infected rats ($P < 0.05$). Of note, GA did not induce any additive increase for examined antioxidant levels, and the effect is for SA only. When both NA and GA were administered together, NS induced an increase in catalase and GSH-R as reported for NS alone. As a positive control, *Lactobacillus* showed significant increase on antioxidant levels as reported for NS alone compared to SA.

Increased antioxidant enzymes in serum during sepsis condition are attributed to acute stress.[17] GA did not induce any additive effect, but it has an antioxidant effect because it increased antioxidant enzymes such as SOD, catalase, and glutathione peroxidase.[18] NS essential oils have prominent antioxidant properties as confirmed in our results and others.[5,6,19]

In this study, TBC in lungs showed the high count in untreated group then reduced significantly in *Lactobacillus* group followed by NS group and combination group (NS + GA). The GA treated group given the high count of all treated groups. NS has reduced the TBC in lungs as it has an antibacterial effect.[20] In another study, the antimicrobial activity of ethanolic extract of gum acacia had wide spectrum; it could be detected only in 2 of the 17 samples of gum acacia and one of the seven samples of gum chironji. Therefore, edible gums may have little utility as antimicrobials in therapeutics, might be containing some antibacterial component(s) which needs to be identified.[21]

IL-12 considered the key immunoregulatory cytokine that is produced mainly by antigen-presenting cells and macrophages. The expression of IL-12 during infection regulates the innate immune response and determines the type of acquired immune responses. IL-12 induces production of interferon-γ (IFN-γ) and triggers CD4+ T cells to differentiate into Type 1 T- helper cells.[22]

In this study, SA induced a significant decrease in SOD and GST. Prior administration of NS inhibited the decrease in SOD and GST expression induced by SA. GA also to a lesser extent inhibited the decrease in expression of SOD but not GST. When both given together, NS normalized the expression of SOD but not GST in SA injected. These results may be due to the acute inflammation induced by SA.

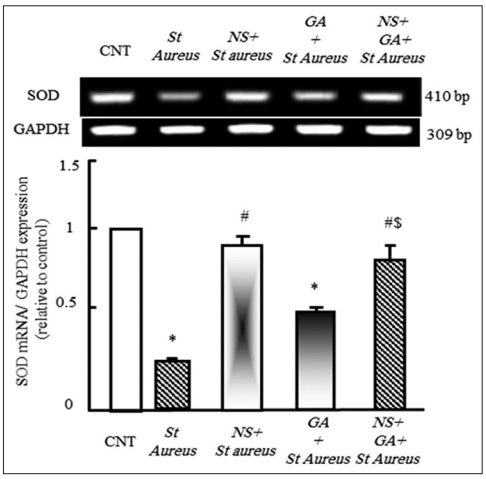


**Figure 4:** Semiquantitative reverse transcription polymerase chain reaction (RT-PCR) analysis of glutathione-S-reductase in lung tissues after injection of *Staphylococcus aureus* (SA) in Wistar rats and possible protection by Nigella sativa and gum arabic. Total RNA was extracted and reverses transcribed (3 µg) as mentioned in methods section, and RT-PCR analysis was performed for examined gene. $^{*}P < 0.05$ versus the control (CNT) group, $^{\#}P < 0.05$ versus SA, $^{\$}P < 0.05$ versus SA plus NS, glyceralde-hyde-3-phosphate dehydrogenase

## CONCLUSION

In sepsis condition, the changes in cytokines and antioxidant genes may be modified using medicinal plants such as NS to regulate inflammatory cytokines and reduce bacterial count. GA has a little antibacterial effect but strong antioxidant compared to NS.

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