National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTILCE

Synergetic effect of probiotic and *Nigella sativa* to control enteric and respiratory infections in an animal model

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Received: April 17, 2016; **Accepted:** May 03, 2016

ABSTRACT

Background: Recently, there is a trend in the world to use natural products to attain wanted therapeutic effects and to avoid unwanted adverse effects. Probiotics are one of the most important of these natural products, which are friendly microbiota, can boost immunity and reduce inflammation and infection. **Aims and Objectives:** Therefore, we examined the protective effect of *Nigella sativa* (NS), gum arabic (GA), and *Lactobacillus* (LA) as a positive control against pathogenicity induced by *Escherichia coli* and *Staphylococcus aureus* infection in Wistar rats at microbiological and molecular levels. **Materials and Methods:** A total of 66 rats were used for this study and allocated into 11 groups. Rats were infected by either *E. coli* or *S. aureus* after administration of NS and/or GA for 2 weeks and continued for 7 days later. Another 2 groups infected *by E. coli* or *S. aureus* were administered LA as a positive control. Kidney, lung, and spleen were collected for the total bacterial count and molecular examination of immune cytokines and oxidative stress expression. **Results:** NS exerted a strongly antioxidant effect in *E. coli* and *S. aureus* infected rats as indicated by the upregulation in mRNA of superoxide dismutase (SOD) and glutathione peroxidase (GPX), whereas GA groups showed downregulation in SOD and GPX expression. NS normalized the inhibitory effect of GA when co-administered together. Immunomodulatory effect of NS and GA showed a clear response for Th-1 more than Th-2. NS significantly increased interferon-gamma (INF-γ) and inhibited *interleukin-4* (IL-4) expression in *E. coli* infected group, while the combination of each gives the lowest results. In *S. aureus* infected rats, the NS extract upregulates the expression of IL-12 and GA downregulated IL-12 either alone or in combination with NS. The bacterial count per gram of organs significantly decreased in NS extract ($P < 0.01$), whereas increased significantly in GA and NS plus GA-treated groups compared to LA administered rats. **Conclusion:** NS possess strong and more antioxidant, immunomodulatory, and antibacterial properties than GA, which showed only an increase in Th-1 response INF-γ.

KEY WORDS: Probiotics; Antioxidants; Cytokines Expression; Pathogenicity; Rats

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2016.6.0410203052016	

INTRODUCTION

The medicinal plants have been used for curing diseases and are promising for the treatment of various diseases such as diabetes, cancer, inflammation, and rheumatoid.[1-3] *Nigella sativa* (Family Ranunculaceae; NS) is a miracle herb as many researchers discovered its broad spectrum

of pharmacological potential. NS is commonly identified as the black seed.[1] NS has been extensively studied for its biological and beneficial effect and shown to have a wide spectrum of activities as diuretic, antidiabetic, antihypertensive, anticancer and immunomodulatory, analgesic, and antimicrobial.[2,3] The antibacterial effect of NS was studied in disc diffusion method. A clear inhibition zone of growth of *Staphylococcus aureus* was observed, this inhibition may be attributed to the main active ingredients of NS.[4] The most valuable extracts were the crude alkaloid and water extracts of NS against Gram-negative bacterial isolates were affected more than the Gram-positive ones.[5] The antibacterial spectrum of NS against clinical isolates of methicillin-resistant *S. aureus* (MRSA) was investigated. All examined strains of MRSA were sensitive to the ethanolic extract of NS at a concentration of 4 mg/disc with an MIC range of 0.2-0.5 mg/mL.[6] The immunomodulatory properties of NS on splenocytes proliferation, natural killer cells, and macrophages activity on BLAB/c were investigated. The results clarified that the aqueous extract of NS significantly enhances splenocyte proliferation in a dose-dependent manner. Moreover, it favors the Th-2 versus Th-1cytokines as the secretion of interleukin-6 (IL-6), tumor necrosis factor-α, and nitric oxide, known pro-inflammatory mediators, is modulated by NS.[7]

Gum arabic (GA) is considered as an edible, dehydrated, sticky exudates excreted from the stems and branches of *Acacia Senegal* and *Acacia seyal*.[8] GA is prebiotic that enhances the growth of intestinal flora,[9] it is similar to breast milk and inulin in its importance.[10,11] It plays an important role in protection against pathogenic microorganisms, growth, and homeostasis of immune cells, digestion of polysaccharides that is indigestible by human enzymes and fat metabolism.[12] Microbiological studies showed that GA has antibacterial activities that attributed to second metabolites in its contents as tannins, carbohydrates, phenols, flavonoids, and glycosides.[13,14]

This study was designed to investigate the effects of ethanolic extract of NS and GA (natural powder) as natural probiotics on the antibacterial and immune-modulator factors on rats after infection by either Gram-negative or Gram-positive bacteria. The gene expression of immune-modulator cytokines was examined after challenge in rats and possible protection by NS and GA were outlined compared to *Lactobacillus* (LA) species bacteria.

MATERIALS AND METHODS

Bacterial Strains

MRSA and *Escherichia coli* were kindly provided from the (Animal Reproduction Research Institute, Department of Udder and Neonates, Egypt). *S. aureus* was grown in nutrient broth for 24 h. The culture was spin at 15,000 × g for 20 min, and the pellet was washed using sterile phosphate buffer saline (PBS). The viable bacterial count was adjusted to approximately 2 × 10^9 colony forming units (CFU)/mL in PBS.[15] *E. coli* was grown in broth, the suspension was spin at 15,000 × g for 20 min, the supernatant was discarded, and then the bacteria were diluted in sterile saline. The viable bacterial count was adjusted to approximately 5 × 10^{10} CFU/mL in PBS.[16]

Preparation of NS and GA for Administration

NS seeds were purchased from a local herb store at Taif area, Saudi Arabia. The plant was identified by botanists in the herbarium of Taif University, and the specimen number of the plant is 293-0303-1. The hydro-ethanolic extract was prepared using a maceration method.[17] GA powder was a generous gift from Dar Savanna Ltd., Khartoum, Sudan (www.ssgums.com). It is a 100% natural extract powder produced mechanically from the wildly 85 grown *A. Senegal* tree with a particle size <210 μm. GA (10% wt/wt) was added to the drinking water, which has previously been shown to be effective.[18,19] Lacteol Fort probiotic composed of LA corresponding to *Lactobacillus delbrueckii* and *Lactobacillus fermentum* 10 billion (one sachet of lacteal fort added to 500 ml of water) daily in a free intake during all period of the experiment.[20]

Animals and Experimental Design

Male Wistar rats (66 rats), aged 3 months and weighing 200-250 g, were purchased from the King Fahd Institute for Scientific Research, King AbdulAziz University, Jeddah, Saudi Arabia). Rats were subjected to 12 h/12 h daylight with free access to food and water. The rats were divided into 11 groups (6 rats per group). The control group was fed normal diet and water, *E. coli* group was injected intraperitoneally (IP) with virulent strain of *E. coli* in a dose of 2 × 10^{10} CFU/ml per rat, and *S. aureus* group was injected IP with virulent strain of *S. aureus* in a dose of 2 × 10^9 CFU/mL per rat. The remaining 8 groups were as following: *E. coli plus* Luria broth (LB) (one sachet of lacteal fort added to 500 ml of water daily) group; *S. aureus* plus LB; *E. coli* plus NS (150 mg/kg bw daily); *E. coli* plus GA (10% wt/wt daily), *S. aureus* group plus NS; *S. aureus* group plus GA; *E. coli* group plus Ns plus GA; *S. aureus* plus NS plus GA. All animals were kept under observation for 1 week after infection. Animals received NS and GA for 2 weeks before infection by *E. coli* and *S. aureus* then continued for 1 week later. At the end of the experimental schedule, the rats were overnight fasted, and inhaled diethyl ether then decapitated. Organs were used for pathogens isolation under aseptic conditions. Kidney, lung, spleen, and intestine tissues were taken a weighted and checked for bacterial isolation and count (CFU/gram tissues for *S. aureus* and *E. coli*), and for RNA extraction for some cytokines.

Total Bacterial Counts

The collected organs were weighted under aseptic condition then minced into small pieces and incubated in broth at 37°C for 24 h. Serial dilution was performed from the broth; each sample has been diluted in six tubes (10× dilution), then cultured on specific media for the pathogen.

RNA Extraction, cDNA Synthesis, and Cytokines Expression in Tissues

Total RNA was extracted from the tissue as previously discussed.[21] 2 µg of total RNA and 0.5 ng oligoDT primer (Qiagen Valencia, CA, USA) were used for cDNA synthesis. For semi-quantitative gene expression and *polymerase chain reaction (*PCR) expression analysis, primers for the examined genes (Table 1) were designed using Oligo-4 computer program (Version 7.0; Molecular Biology Insights, Cascade, CO, USA) according to the nucleotide sequences published in GenBank (http://www.ncbi.nlm.nih.gov/genbank/; Table 1) and were synthesized by Macrogen Korea (Seoul, South Korea). PCR was conducted in a final volume of 25 µl consisting of 1 µl cDNA, 1 µl of 10 pM of each primer (forward and reverse), and 12.5 µl PCR master mix (Promega Corporation, Madison, WI, USA), the volume was adjusted to 25 µl using sterilized, deionized water. PCR was carried out using Bio-Rad T100TM Thermal cycle machine (Bio-Rad Laboratories, Inc.) with the cycle sequence at 94°C for 5 min one cycle, followed by 26 cycles, each consists 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 an additional final extension at 72°C for 7 min. The expression of glyceraldehyde-3-phosphate dehydrogenase mRNA was used as a reference. PCR products were run in 1.5% agarose (Bio Basic, Markham, ON, Canada) gel stained with ethidium bromide in Tris-Borate-EDTA buffer and visualized under UV light gel using an In Genius 3.0 gel documentation system (Syngene, Frederick, MD, USA). Band intensities from the various rats from each group were quantified densitometrically using Image Software Version 1.47 (http://www.imagej.en.softonic.com/).

Statistical Analysis

Data are represented as means±standard error of means for 3 different independent experiments. Data were analyzed using analysis of variance (ANOVA) (one-way ANOVA) by SPSS software version 11.5 for Windows (SPSS, IBM, Chicago, IL, USA). Values with $P < 0.05$ are considered statistically significant.

RESULTS

Effect of NS and GA on *E. coli* and *S. aureus* Counts in Wistar Rats

The total bacterial count for *E. coli* showed that NS strongly and significantly ($P < 0.05$) inhibited bacterial growth. In contrast, without explanation GA increased alone or in combination *E. coli* growth. Of note, LB increased also the pathogens growth (Table 2). For *S. aureus* growth, the total bacterial count was decreased significantly ($P < 0.01$) when NS administered 2 weeks before *S. aureus* infection. In parallel, same results with less significance ($P < 0.05$) for GA were obtained. When both co-administered together, additive inhibition for the bacterial count ($P < 0.01$) was reported as seen in Table 3. LB induced more inhibition for *S. aureus* count.

Protective Effect of NS and GA on Superoxide Dismutase (SOD) Activity on Ileum and Spleen after Infection by *E. coli* and *S. aureus* in Wistar Rats

Induction of pathogenicity by *E. coli* in ileum and *S. aureus* in spleen downregulated mRNA expression of SOD as seen in Figure 1a. Administration of NS alone normalized and upregulated SOD mRNA expression (Figure 1a). GA alone

Table 1: Primer sequence of examined genes and PCR conditions

Gene	Product size (bp)	Annealing (°C)	Direction (5'-3')	Sequence
SOD	410	55	Forward	AGGATTAACTGAAGGCGAGCAT
			Reverse	TCTACAGTTAGCAGGCCAGCAG
GPX	407	57	Forward	AAGGTGCTGCTCATTGAGAATG
			Reverse	CGTCTGGACCTACCAGGAACTT
IL-4	327	57	Forward	AGGTCAACACCACGGAGAAC
			Reverse	AGGACATGGAAGTGCAGGAC
IFN-γ	321	58	Forward	AGGAAAGAGCCTCCTCTTGG
			Reverse	TCTACCCCAGAATCAGCACC
G3PDH	309	52	Forward	AGATCCACAACGGATACATT
			Reverse	TCCCTCAAGATTGTCAGCAA

SOD: Superoxide dismutase, PCR: Polymerase chain reaction, GPX: Glutathione peroxidase, IL-4: Interleukin-4, IFN-γ: Interferon-gamma G3PDH: Glyceraldehyde-3-phosphate dehydrogenase

Table 2: Effect of different treatments on *E. coli* count in organs (kidney and spleen) of Wistar rats after *E. coli* challenge

Group	Control	*E. coli*	*E. coli*+NS	*E. coli*+gum	*E. coli*+(NS+gum)	*E. coli*+LA
Means	00	76.33×10^{10}	45.83×10^{10}	170.17×10^{10}	619.0×10^{10}	635.83×10^{10}
SEM	00	4.19×10^{10}	3.5×10^{10}	19.8×10^{10}	88.8×10^{10}	143.7×10^{10}
P- value			$P<0.05$	$P<0.05$	$P<0.05$	$P<0.05$

Values are means (M)±standard error (SE) for 6 different rats. SEM: Standard error of the mean, *E. coli: Escherichia coli*, NS: *Nigella sativa*, LA: *Lactobacillus*

Table 3: Effect of different treatments on *S. aureus* count in organs (lung and spleen) of Wistar rats after *S. aureus* challenge

Group	Control	*S. aureus*	*S. aureus*+NS	*S. aureus*+gum	*S. aureus*+(NS+gum)	*S. aureus*+LA
Means	00	412.7×10^{10}	54.25×10^{10}	331.3×10^{10}	115.65×10^{10}	29.38×10^{10}
SEM	00	168.5×10^{10}	22.14×10^{10}	135.25×10^{10}	47.2×10^{10}	11.99×10^{10}
P- value			$P<0.01$	$P<0.05$	$P<0.01$	$P<0.05$

Values are means (M)±standard deviation (SD) for 6 different rats. SEM: Standard error of the mean, NS: *Nigella sativa*, *S. aureus: Staphylococcus aureus*, LA: *Lactobacillus*

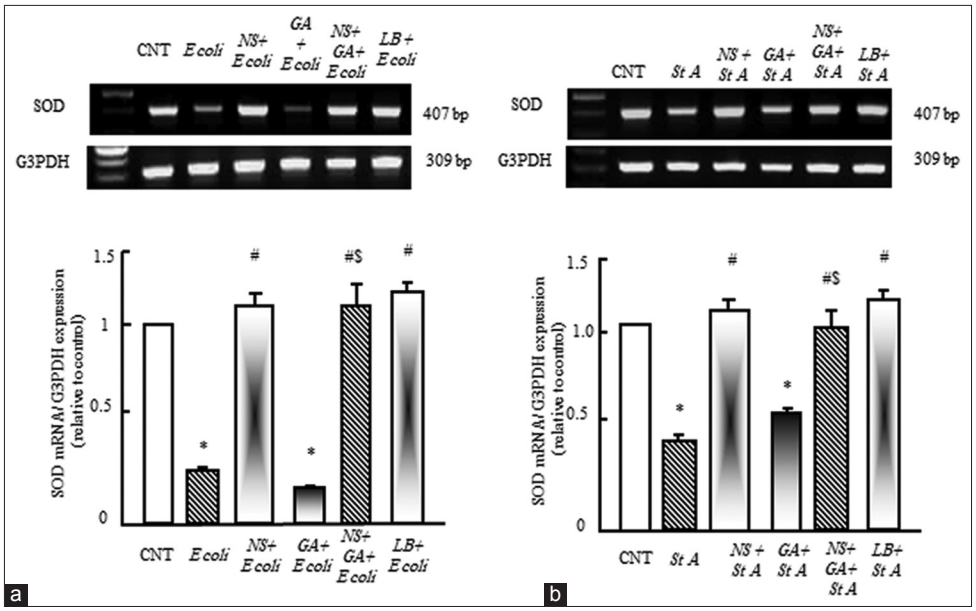

Figure 1: (a and b) Protective effect *Nigella sativa* and gum arabic (GA) on superoxide dismutase (SOD) mRNA on ileum and spleen after infection by *Escherichia coli* and *Staphylococcus aureus* in Wistar rats. RNA was extracted and reverse transcribed (2 mcg) and reverse transcription polymerase chain reaction analysis was carried out for SOD expression as described in materials and methods. Densitometric analysis (down columns) was carried for 3 different experiments. Data are means±standard error of the mean for 3 independent experiments. Values are statistically significant at **P*<0.05 versus control, $^{\#}P$<0.05 versus either *E. coli* or *S. aureus* and $^{\$}P$<0.05 versus GA plus either *E. coli* or *S. aureus*

did not normalize the downregulation in SOD expression and combination with NS did not induce any effect. The normalized effect seen in Figure 1a is due to NS action. Administration of LA to *E. coli* infected rats upregulated SOD expression compared to *E. coli* administered rats. In Figure 1b, SOD mRNA expression was examined after infection of rats with *S. aureus*. The same pattern of SOD mRNA expression reported for *E. coli* in ileum is the same for SOD in *S. aureus* in spleen (Figure 1b) as NS was stimulatory while GA is inhibitory when both co-administered the effect is due to NS action.

Protective Effect of NS and GA on Glutathione (GHS) Peroxidase (GPx) Activity on Ileum and Spleen after Infection by *E. coli* and *S. aureus* in Wistar Rats

In ileum, infection of rats by *E. coli* induced downregulation in mRNA expression of GPX (Figure 2a). Administration of NS to infected rats normalized the downregulation in GPX mRNA while GA did not show any alteration in GPX expression and remained downregulated. When NS co-administered with GA, NS normalized the inhibitory effect of both *E. coli* and GA. As seen LB normalized GPX mRNA expression that is downregulated in *E. coli*. *Staph aureus* induced partial

(15%) downregulation in GPX expression (Figure 2b). NS administration normalized and upregulated mRNA expression of examined GPX (Figure 2b). GA induced additive downregulation in GPX mRNA expression (Figure 2b). Co-administration of NS with GA did not show any additive or suppressive effect to their effect alone on GPX mRNA expression. However, the prominent effect is the suppressive action of GA (Figure 2b) on the stimulatory effect of NS.

Effect of NS and GA on IL-4 and Interferon-gamma (INF-γ) on Ileum after Infection by *E. coli* in Wistar Rats

As seen in Figure 3, *E. coli* significantly upregulated IL-4 expression; this suppression was inhibited by NS as it induced downregulation in IL-4 mRNA. GA induced more inhibition for *E. coli* suppressive effect on IL-4 mRNA ($P < 0.05$). Co-administration of NS plus GA showed inhibition of IL-4 mRNA (Figure 3a). LB showed upregulation of IL-4 expression. INF expression in Figure 3b showed upregulation by *E. coli* and administration of NS to *E. coli* infected rats showed additive stimulatory effect ($P < 0.05$). GA showed inhibition for upregulated expression induced by *E. coli*. Moreover, GA inhibited the additive stimulatory effect of both *E. coli* and NS (Figure 3b).

Effect of NS and GA on IL-12 Spleen after Infection by *S. aureus* in Wistar Rats

Finally, we examined the expression of IL-12 in the spleen of rats after *S. aureus* challenge. As seen in Figure 4, *S. aureus* infection downregulated mRNA expression of IL-12. In contrast, NS administration into infected rats normalized and upregulated IL-12 expression in examined groups. GA induced downregulation of IL-12 mRNA and co-administration of GA with NS inhibited NS induced upregulation of IL-12 mRNA. IL-12 expression was downregulated in spleen after administration of LB to *S. aureus* infected rats.

DISCUSSION

GA has been widely used as an emulsifier and stabilizer agent basically in food industry.[22] In Falk medicine, GA is used for management of inflammatory bowel diseases. Some recent reports explained that GA has antioxidant and nephron protected property.[23] NS has been employed for many years as potential medicinal properties; the main constituent of seeds includes thymoquinone, thymol, dithymoquinone, carvacrol, nigellicine, and alpha-hedrin.[24] Immunomodulatory and therapeutic properties of NS as antibacterial, antifungal, and cytotic effect have been reported.

In this study, the antioxidant effect of GA and NS in Wistar rats infected with *E. coli* and *S. aureus* either in ilium or spleen tissue. *E. coli* and *S. aureus* infection downregulated the expression of SOD and GPX. NS administration alone normalized and upregulated mRNA of SOD and GPX compared to control. While GA alone did not normalize the downregulation in case of *E. coli* infection and somewhat more than *S. aureus* but less than normal and in combination

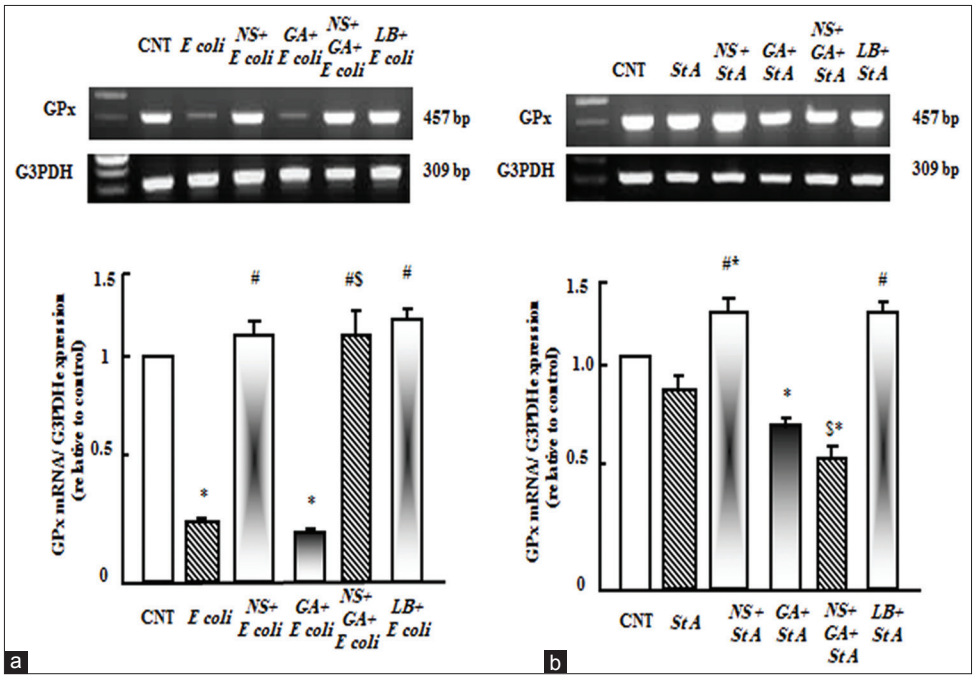

Figure 2: (a and b) Protective effect of *Nigella sativa* and gum Arabic (GA) on glutathione peroxidase (GPX) mRNA on ileum and spleen after infection by either *Escherichia coli* or *Staphylococcus aureus* in Wistar rats. RNA was extracted and reverse transcribed (2 mcg) and reverse transcription polymerase chain reaction analysis was carried out for GPX expression as described in materials and methods. Densitometric analysis (down columns) was carried for 3 different experiments. Data are means±standard error of the mean for 3 independent experiments. Values are statistically significant at *P<0.05 versus control, $^{\#}P$<0.05 versus either *E. coli* or *S. aureus* and $^{\$}P$<0.05 versus GA plus either *E. coli* or *S. aureus*

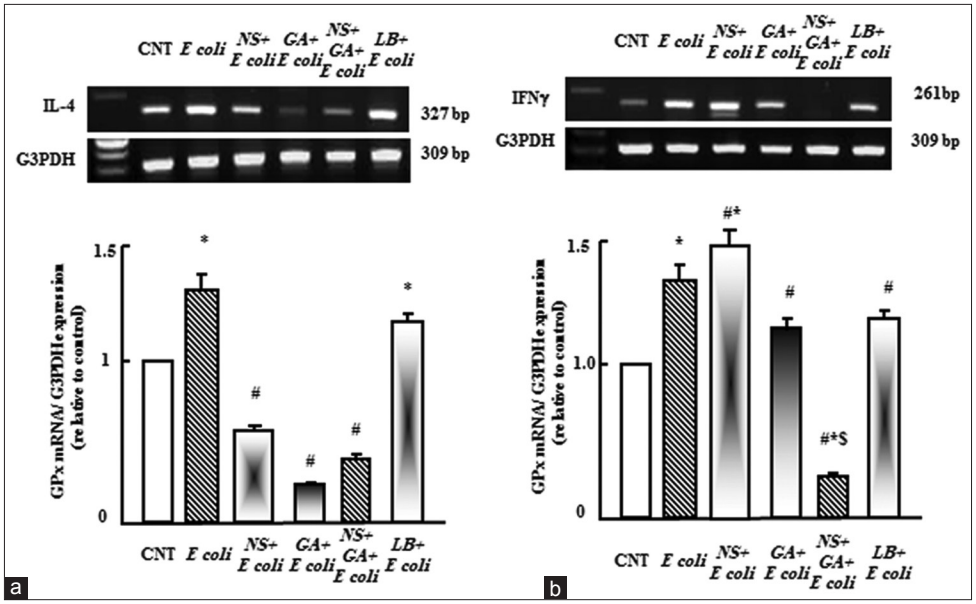

Figure 3: (a and b) Effect of *Nigella sativa* and gum Arabic (GA) on interleukin-4 (IL-4) and interferon-gamma (IFN-γ) on ileum after infection by *Escherichia coli* in Wistar rats. RNA was extracted and reverse transcribed (2 mcg) and reverse transcription polymerase chain reaction analysis was carried out for IL-4 and IFN-γ expression as described in materials and methods. Densitometric analysis (down columns) was carried for 3 different experiments. Data are means±standard error of the mean for 3 independent experiments. Values are statistically significant at **P*<0.05 versus control, $^{\#}P$<0.05 versus *E. coli* and $^{\$}P$< 0.05 versus GA plus *E. coli*

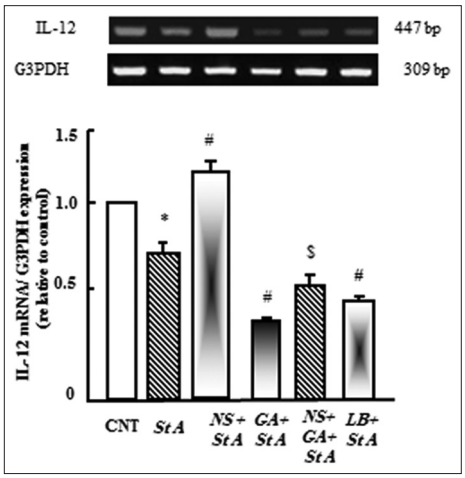

Figure 4: Effect of *Nigella sativa* and gum Arabia (GA) on interleukin-12 (IL-12) on spleen after infection by *S. aureus* in Wistar rats. RNA was extracted and reverse transcribed (2 mcg) and reverse transcription polymerase chain reaction analysis was carried out for IL-12 expression as described in materials and methods. Densitometric analysis (down columns) was carried for 3 different experiments. Data are means±standard error of the mean for 3 independent experiments. Values are statistically significant at **P*<0.05 versus control, $^{\#}P$<0.05 versus *S. aureus* and $^{\$}P$<0.05 versus GA plus *S. aureus*

with NS induce changes related to the co-administrated effect of NS. The combination of NS and GA in *E. coli* infected rats gives more expression than *S. aureus* in GPX. In contrast, LB upregulated SOD mRNA. These results agree as reported that GA in a dose of 2.5%, 5%, and 10% in drinking water did not significantly alter either the concentration of free radicals scavenger, reduced GPX, GHS, ascorbic acid, and SOD and there is no evidence that GA has strongly antioxidant effects.[25] The antioxidant potential of NS which indicating a higher free radical scavenger activities.[26]

Many researchers reported the immunomodulatory effect of NS on the immune system and the immunoprotective role against tumors.[27] In our study, cytokines expression were ameliorated by NS administration and are in parallel with other reports[28] as the aqueous extract of NS favors the secretion of pro-inflammatory cytokines and increase the secretion of INF-y as protective effect.[28] The co-administration of NS and GA reduced each other that may be attributed to the interaction of the two plants ingredients. The two plants (GA and NS) increased INF-γ more than IL-4. It has been shown that NS favors the secretion of Th-2 versus Th-1. On the same time, cytokines profile of rats infected with *S. aureus* regulated the secretion of IL-12. IL-12 play a role in Th-1 response, especially on macrophages function and regulate Th-1 response by regulating the secretion of IL-4, 10, and IL-13.[29]

The antibacterial effect of NS components (thymoquinone, thymol, dithymoquinone) found to possess an inhibitory response against both Gram-positive and Gram-negative bacteria.[30] In the current study, the higher antibacterial effect (lowest bacterial growth) reported was found in the NS-treated group ($p < 0.01$) followed by the LA-treated group, followed by GA group then GA plus NS combination. On the other hand, *E. coli* infection showed lower bacterial count in NS-treated group compare with the non-treated group, followed by GA group, GA plus NS group, and LA group. It has been confirmed that GA suppresses the

macrophages activities of rats to produce superoxide anion and capable for almost blocking completely macrophages function, especially in chronic liver diseases.[31]

CONCLUSION

From the data obtained in this study, we concluded that GA has weak antioxidant activities as it downregulates the two genes expressed (SOD and GPX). Moreover, GA increases both bacterial counts of each Gram-positive and Gram-negative bacterium in the spleen, lung tissues; on the contrary, NS very strong antioxidant, reduce bacterial count, and modulate the immune response through activation of Th-1 cytokines. The combination between NS and GA is not recommended and GA not suitable prebiotic in the case of bacterial infection.

ACKNOWLEDGMENTS

This study was supported by a grant in aid of the Deans of Scientific Research Affairs, Taif University, Saudi Arabia (# 4344-1436-1).

REFERENCES

1. Khare CP. Encyclopedia of Indian Medicinal Plants. New York, Berlin Heidelberg: Springer-Verlag; 2004.
2. Abel-Salam BK. Immunomodulatory effects of black seeds and garlic on alloxan-induced diabetes in albino rat. Allergol Immunopathol (Madr). 2012;40(6):336-40.
3. Goreja WG. Black Seed: Nature's Miracle Remedy. New York, NY: Amazing Herbs Press; 2003.
4. Bakathir HA, Abbas NA. Detection of the antibacterial effect of *Nigella sativa* ground seeds with water. Afr J Tradit Complement Altern Med. 2011;8(2):159-64.
5. Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta Microbiol Pol. 2000;49(1):63-74.
6. Hannan A, Saleem S, Chaudhary S, Barkaat M, Arshad MU. Anti-bacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. J Ayub Med Coll Abbottabad. 2008;20(3):72-4.
7. Majdalawieh AF, Hmaidan R, Carr RI. Nigella sativa modulates splenocyte proliferation, Th1/Th2 cytokine profile, macrophage function and NK anti-tumor activity. J Ethnopharmacol. 2010;131(2):268-75.
8. Williams PA, Phillips GO, editors. Gum Arabic. Handbook of Hydrocolloids. Boca Raton, FL: CRC Press; 2000. p. 155-68.
9. da Silva ST, dos Santos CA, Bressan J. Intestinal microbiota; relevance to obesity and modulation by prebiotics and probiotics. Nutr Hosp. 2013;28(4):1039-48.
10. Calame W, Weseler AR, Viebke C, Flynn C, Siemensma AD. Gum Arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. Br J Nutr. 2008;100:1269-75.
11. Gerstenzang J, Edmund S. Impact of Bush's Sudan Sanctions Doubted. California: Los Angeles Times; 2007.
12. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends Microbiol. 2011;19(7):349-59.
13. Banso A. Phytochemical and antibacterial investigation of bark extract of *Acacia nilotica*. J Med Plant Res. 2009;3(2):82-5.
14. Ajsybhan P, Navneet, Chauhan A. Evaluation of antimicrobial activity of six medicinal plants against dental pathogens. Rep Opin. 2010;2(6):37-42.
15. Hari Prasad O, Navya A, Vasu D, Chiranjeevi T. Protective effects of *Prosopis juliflora* against *Staphylococcus aureus* induced hepatotoxicity in rats. Int J Pharm Biomed Res. 2011;2(3):172-8.
16. Cirioni O, Giacometti A, Ghiselli R, Bergnach C, Orlando F, Silvestri C, et al. LL-37 protects rats against lethal sepsis caused by gram-negative bacteria. Antimicrob Agents Chemother. 2006;50(5):1672-9.
17. Ibrahim ZS1, Ishizuka M, Soliman M, ElBohi K, Sobhy W, Muzandu K, et al. Protection by *Nigella sativa* against carbon tetrachloride induced downregulation of hepatic cytochrome P450 isozymes in rats. Jpn J Vet Res. 2008;56(3):119-28.
18. Nasir O, Artunc F, Saeed A, Kambal MA, Kalbacher H, Sandulache D, et al. Effects of gum Arabic (*Acacia senegal*) on water and electrolyte balance in healthy mice. J Ren Nutr. 2008;18:230-8.
19. Nasir O, Umbach AT, Rexhepaj R, Ackermann TF, Bhandaru M, Ebrahim A, et al. Effects of gum Arabic (*Acacia senegal*) on renal function in diabetic mice. Kidney Blood Press Res. 2012;35:365-72.
20. Huang HY, Korivi M, Tsai CH, Yang JH, Tsai YC. Supplementation of *Lactobacillus* plantarumK68 and fruit-vegetable ferment along with high fat-fructose diet attenuates metabolic syndrome in rats with insulin resistance. Evid Based Complement Altern Med. 2013;2013:943020.
21. Soliman MM, Hassan MY, Mostafa SA, Ali HA, Saleh OM. Protective effects of camel milk against pathogenicity induced by *Escherichia coli* and *Staphylococcus aureus* in Wistar rats. Mol Med Rep. 2015;12(6):8306-12.
22. Lelon JK, Jumba TO, Keter JK, Chemuku W, Oduor FD. Assessment of physical properties of gum Arabic from *Acacia senegal* verities in Baringo District Kenya. Afr J Plant Sci. 2010;4(4):95-8.
23. Ali BH, Al-Husseni I, Beegam S, Al-Shukaili A, Nemmar A, Schierling S, et al. Effect of gum Arabic on oxidative stress and inflammation in adenine-induced chronic renal failure in rats. PLoS One. 2013;8(2):e55242.
24. Ahmad KM, Muneer IA, Tamimi IA, Chang ME, Ata MO, Yusuf N. Thymoquinone suppresses metastasis of melanoma cells by inhibition of NLRP3 inflammasome. Toxicol Appl Pharmacol. 2013;270(1):70-6.
25. Ali BH, Does GA. Does gum Arabic have an antioxidant action in rat kidney? Ren Fail. 2004;26(1):1-3.
26. Panahi M, Namjoyan F, ShakerinZ. Evaluation of antioxidant effects of *Nigella sativa* extract on the ultra structure of neural tube defects in diabetic rats's off spring. Jundishapur J Nat Pharm Prod. 2011;6(1):16-23.
27. Sheir SK, Maghraby OA, Mohamed AH, Osman GY, Al-Qormuti SA. Immunomodulatory and ameliorative role of *Nigella sativa* oil on schistosoma mansoni-infected mice. Canadian J Pure Appl Sci. 2015;9(2):3345-55.

28. El Gazzar MA, El Mezayen R, Nicolls MR, Dreskin SC. Thymoquinone attenuates proinflammatory responses in lipopolysaccharide-activated mast cells by modulating NF-kappaB nuclear transactivation. Biochim Biophys Acta. 2007;1770:556-64.
29. Hamza T, Barnett JB, Li B. Interleukin 12 a key immunoregulatory cytokine in infection applications. Int J Mol Sci. 2010;11(3):789-806.
30. Kamal A, Ahmad IZ. Alteration in antibacterial potential of *Nigella sativa* L. Seed during different phases of germination. Int J Curr Microbiol Appl Sci. 2014;3(3):268-82.
31. Ali BH, Ziada A, Blunden G. Biological effects of gum Arabic: A review of some recent research. Food Chem Toxicol. 2009;47(1):1-8.

How to cite this article: Abd-El-Hafez SM, Ismael AB, Nasir O, Soliman M, Mohamed EH, Kafaween IK. Synergetic effect of probiotic and *Nigella sativa* to control enteric and respiratory infections in an animal model. Natl J Physiol Pharm Pharmacol 2016;6(5):399-406.

Source of Support: Nil, **Conflict of Interest:** None declared.