



## Some Immunomodulating Effects of Ginger and Thyme oils in Rabbits

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### ABSTRACT

This study was conducted to investigate the immunomodulating effects of the ginger and thyme oils (250 mg/kg BW, each) given orally for 21 successive days to rabbits vaccinated with rabbit hemorrhagic viral disease (RHVD). Two blood samples were collected at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days post drug and vaccine administration to study the effect of ginger and thyme oils on the innate immune response (phagocytic activity, serum lysozyme activity and nitric oxide production) and the humoral immune response (serum total proteins, determination of serum gamma globulins fraction using electrophoretic technique). The results revealed that ginger and thyme oils had immunostimulant effect on both the innate and humoral immune response as evidenced by significant increase in phagocytic activity, lysozyme activity, nitric oxide production, serum total proteins and gamma globulins in rabbits. Therefore, ginger oil and thyme oils are recommended as immunostimulant agents for rabbits in the dose of 250 mg/kg BW.

Key words: Ginger, Thyme, Humoral immunity, Cellular.

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### 1. INTRODUCTION:

Immunity is the defense mechanisms that protect the living body against the damages caused by invading micro – organisms (Roitt et al., 1996). Immunosuppressive properties of some antibiotics suppress both cellular and humeral immune responses to a variety of vaccines (Shalaby, 1989).

No treatments are effective against rabbit hemorrhagic disease virus, so its control depend on general preventive measures including quarantine and

vaccination (Sun et al., 1985).

Ginger (*Zingiber officinal roscoe*) is very important as it improves growth and enhances immunity. Shubha (2015) recorded that, when acetone extract of ginger was added to aquaculture feed, increase in growth rate; feed conversion rate and digestive enzyme activity were noticed. The author also added the ginger rhizome powder as natural antibiotic and found an improvement in the non-

specific immune response against many fish infection.

The active constituents of ginger function as immunomodulators by increasing the body's immunity through their antimicrobial and free radical scavenging properties. Ginger extract raises the thymus index, spleen index, and percentage of phagocytosis significantly, thus improving immunological function (Kathi, 1999; Schitteket, 2001).

Thyme (*Thymus vulgaris*) is very important because of many uses and health benefits. Ali et al. (2011) showed in their study to investigate the effects of adding different levels of essential oils of thyme per kilogram on growth performance and humoral immune response of chicken broilers. The experiment lasted 48 days. For measuring humoral immune response at 8 and 18 days of age, all used chicks were vaccinated against NDV and at 10th day of age for vaccinated against Avian Influenza (AI). The sera were applied to Haemagglutination Inhibition (HI) test to determine Antibody (Ab) to AI and NDV expressed as the highest dilution that displayed HI. The result was that the response anti-AI was not affected but antibody response anti-NDV was affected by dietary essential oil inclusion levels.

The aim of this study was to evaluate the immunomodulatory effect (cellular and humeral immune response) of ginger and thyme oils in vaccinated rabbits with rabbit hemorrhagic viral disease vaccine.

## 2. MATERIALS AND METHODS:

### 2.1. Materials

2.1.1. *Ginger oil* was obtained as a gift from Pharmacognosy Dept., National Research Center El-Dokki, Cairo, Egypt. Dose: 250 mg/kg b. wt. (40%) administered orally once daily for successive 21 days (Minaiyah et al., 2006).

2.1.2. *Thyme oil* was obtained as a gift from Pharmacognosy Dept., National Research Institute, El-Dokki, Cairo, Egypt. Dose: 250 mg/ b.wt. (40%) administered orally once daily for successive 21 days (Bolukbasi et al., 2006).

### 2.1.3. Vaccine:

Inactivated rabbit haemorrhagic disease virus vaccine was used for active (RHVD) immunization of experimental rabbits. It was purchased from Vet.

Serum and vaccine Research Institute, Abbasia, Cairo, Egypt and was given S.C in a dose of 0.5 ml for each rabbit (Hanaa et al., 2009)

### 2.1.4. Rabbits:

Twenty New-Zealand white rabbits of 2-3 months old and weighing about 1.5 - 1.8 kg were used in this study. They were purchased from a private rabbitary. They were housed in disinfected well ventilated metal cage and provided with *ad-libitum* commercial pellet ration and clean water and kept under observation for one week before being used.

### 2.2.1. Experimental Design:

Rabbits were classified into 4 groups (each of 5 rabbits) as following; the first group was non-vaccinated non-treated and was kept as a negative control; the second group was vaccinated (0.5 mL RHVD S.C) non-treated and was kept as a positive control. The 3<sup>rd</sup> group was

treated by ginger oil (250mg /kg b. wt.) (40%) given orally for 21 successive days then vaccinated at 21<sup>st</sup> of treatment with RHVD (0.5 mL S.C). The 4<sup>th</sup> group was treated by thyme oil (250mg /kg b. wt.) (40%) given orally for 21 successive days then vaccinated at 21<sup>st</sup> of treatment with RHVD (0.5 mL S.C). Two blood samples were collected from the ear vein of 5 rabbits/group at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> days post vaccination for studying both the innate and humoral immune responses. The first sample was 2-3 mL of blood collected in a sterile Wasserman tube containing heparin (50 IU/mL) to measure the phagocytic activity (Lee and Baccon,1982). The second sample was 3-5 mL of blood, collected in a sterile Wasserman tube without an anticoagulant. These samples were allowed to clot, and the serum was separated by centrifugation at 865.33 g. for 15 min and stored at -20° C

### 3. RESULTS:

The results of the present work reported that oral administration of ginger and thyme oils for successive 21 days in vaccinated rabbits illustrated a significant increase ( $p < 0.001$ ) in the mean value of phagocytic activity percent and phagocytic index on the 3<sup>rd</sup> day post-vaccination. as shown in table 1 and Fig. 1.

In the present study, oral administration of ginger and thyme oils to vaccinated rabbits display a significant increase ( $p < 0.001$ ) in serum

Estimation of lysozyme concentration (Mohrig and Messner 1968), nitric oxide production (Rajarman et al., 1998), total serum proteins (Gornal et al., 1949) and serum, gamma globulins (Davis and Ornstein, 1964).

#### 2.2.2. Statistical Analysis:

Data were statistically analyzed for descriptive measurements including arithmetic mean and standard error using SPSS program version 23. The one-way ANOVA test was performed for comparison between the different groups (i.e. Control, Vaccinated, Vacc.+ ginger oil and Vacc.+ thyme oil) and followed by post hoc test using Duncan multiple range (DMR) test for comparisons between means of groups. The means followed by the same letter in each column are not significantly different from each other at the 0.05 probability level. (Tamahane, and Dunlop, 2000).

nitric oxide production on the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day post-vaccination as shown in table 2 and Fig. 2.

Our results showed a significant increase ( $p < 0.001$ ) in lysosomal activity at 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day post-vaccination in vaccinated non-treated group compared with non-vaccinated non-treated group. Also, a significant increase ( $p < 0.001$ ) in lysosomal activity in the vaccinated groups treated with ginger oil and thyme oil at the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> day post-vaccination as shown in table3 and fig. 3.

Table (1): Effect of oral administration of ginger and thyme oils (250 mg/kg b.wt of each) /once daily for 21 successive days on phagocytic index and phagocytic % in vaccinated rabbits (means  $\pm$  SE, n=5).

Groups	Phagocytic %	Phagocytic index
	Days post-vaccination	
	3 <sup>rd</sup>	
Control	74.80 $\pm$ 2.23 <sup>c</sup>	6.14 $\pm$ 0.100 <sup>d</sup>
Vaccinated	87.44 $\pm$ 0.42 <sup>b</sup>	10.48 $\pm$ 0.20 <sup>c</sup>
Vacc. + ginger oil	96.56 $\pm$ 0.50 <sup>a</sup>	12.24 $\pm$ 0.31 <sup>a</sup>
Vacc. + thyme oil	93.14 $\pm$ 3.53 <sup>ab</sup>	11.53 $\pm$ 0.22 <sup>b</sup>
P value	***	***

Notes: The means followed by the same letter in each column are not significantly different from each other at the 0.05 probability level (Duncan's multiple range test). \*, \*\* and \*\*\* refers to p values  $\leq$  0.05, 0.01 and 0.001 respectively. ns refer to there is no significance.

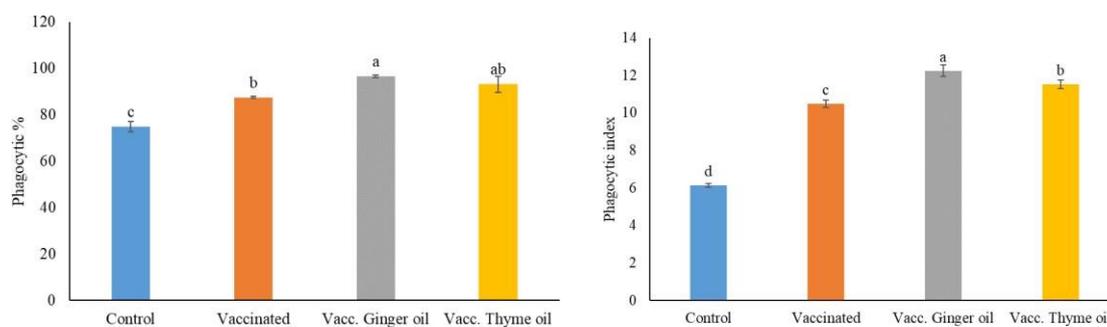


Fig. (1): Effect of oral administration of ginger and thyme oils (250 mg/kg b.wt of each) / once daily for 21 successive days on phagocytic index and phagocytic % in vaccinated rabbits (means  $\pm$  SE, n=5).

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Table (2): Effect of oral administration of ginger and thyme oils (250 mg/kg b. wt of each) / once daily for 21 successive days on serum nitric oxide level (mg/ml) in vaccinated rabbits (means  $\pm$  SE, n=5).

Groups	Serum nitric oxide level		
	Days post – vaccination		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Control	27.00 $\pm$ 0.22 <sup>d</sup>	27.48 $\pm$ 1.00 <sup>d</sup>	29.24 $\pm$ 0.39 <sup>d</sup>
Vaccinated	42.95 $\pm$ 0.26 <sup>c</sup>	41.77 $\pm$ 0.55 <sup>c</sup>	36.29 $\pm$ 0.46 <sup>c</sup>
Vacc. + ginger oil	51.68 $\pm$ 0.29 <sup>a</sup>	53.70 $\pm$ 0.75 <sup>a</sup>	49.29 $\pm$ 0.70 <sup>a</sup>
Vacc. + thyme oil	45.78 $\pm$ 0.23 <sup>b</sup>	47.34 $\pm$ 0.39 <sup>b</sup>	43.25 $\pm$ 0.40 <sup>b</sup>
P value	***	***	***

Notes: The means followed by the same letter in each column are not significantly different from each other at the 0.05 probability level (Duncan's multiple range test). \*, \*\* and \*\*\* refers to p values  $\leq$  0.05, 0.01 and 0.001 respectively. ns refer to there is no significance.

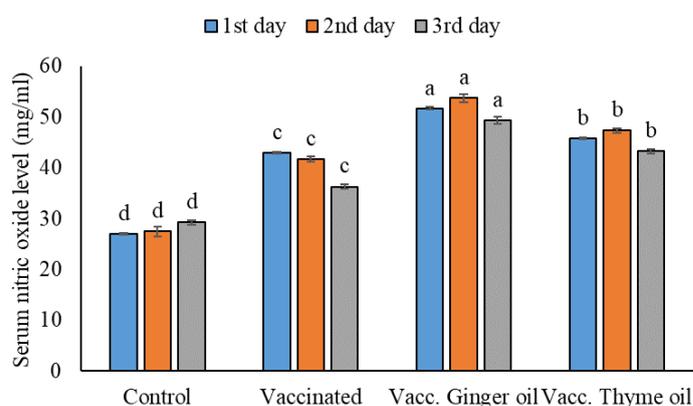


Fig (2): Effect of oral administration of ginger and thyme oils (250 mg/kg b. wt of each) / once daily for 21 successive days on serum nitric oxide level (mg/ml) in vaccinated rabbits (means  $\pm$  SE, n=5).

Table (3): Effect of oral administration of ginger and thyme oils (250 mg/kg b. wt of each) / once daily for 21 successive days on serum lysozyme activity level (mg/ml) in vaccinated rabbits (means  $\pm$  SE, n=5).

Groups	Serum lysozyme activity level (mg/ml)		
	Days post – vaccination		
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>
Control	167.68 $\pm$ 0.92 <sup>c</sup>	179.78 $\pm$ 3.43 <sup>b</sup>	153.73 $\pm$ 1.20 <sup>d</sup>
Vaccinated	240.49 $\pm$ 3.92 <sup>b</sup>	239.28 $\pm$ 5.03 <sup>ab</sup>	179.54 $\pm$ 3.09 <sup>c</sup>
Vacc. + ginger oil	343.91 $\pm$ 21.46 <sup>a</sup>	276.83 $\pm$ 61.36 <sup>a</sup>	279.06 $\pm$ 10.0 <sup>a</sup>
Vacc. + thyme oil	260.9 $\pm$ 12.55 <sup>b</sup>	269.64 $\pm$ 4.73 <sup>ab</sup>	236.46 $\pm$ 5.58 <sup>b</sup>
P value	***	ns	***

Notes: The means followed by the same letter in each column are not significantly different from each other at the 0.05 probability level (Duncan's multiple range test). \*, \*\* and \*\*\* refers to p values  $\leq 0.05$ , 0.01 and 0.001 respectively. ns refer to there is no significance.

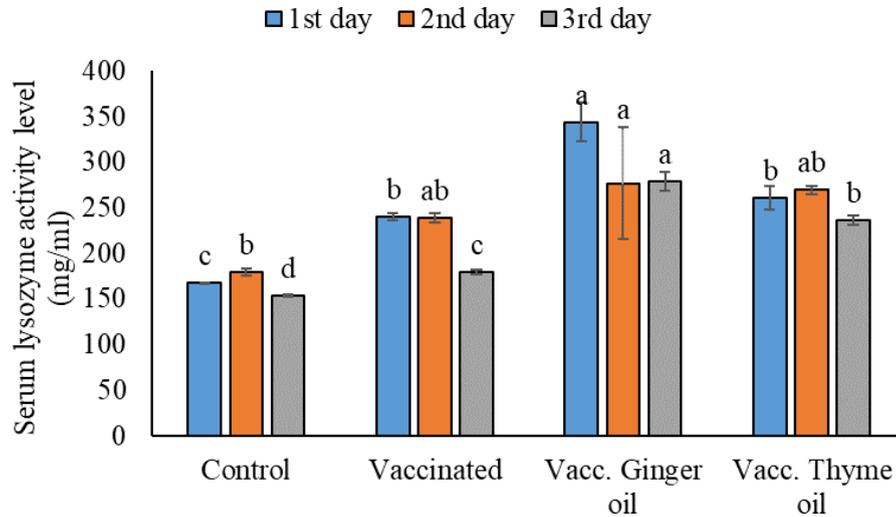


Fig. (3): Effect of oral administration of ginger and thyme oils (250 mg/kg b. wt of each) / once daily for 21 successive days on serum lysozyme activity level (mg/ml) in vaccinated rabbits (means  $\pm$  SE, n=5).

Table (4): Effect of oral administration of ginger and thyme oils (250 mg/kg b. wt of each) / once daily for 21 successive days on total serum protein level (g/dl) and gamma-globuline level (%) in vaccinated rabbits in vaccinated rabbits (means  $\pm$  SE, n=5).

Groups	Total serum protein level (g/dl)			Serum gamma-globuline level (%)		
	Weeks post – vaccination					
	1st	2nd	3rd	1st	2nd	3rd
Control	7.46 $\pm$ 0.140 <sup>c</sup>	7.40 $\pm$ 0.18 <sup>d</sup>	8.51 $\pm$ 0.47 <sup>d</sup>	17.77 $\pm$ 0.33 <sup>c</sup>	16.99 $\pm$ 0.42 <sup>c</sup>	18.11 $\pm$ 0.34 <sup>c</sup>
Vaccinated	10.94 $\pm$ 0.04 <sup>b</sup>	10.40 $\pm$ 0.34 <sup>c</sup>	11.41 $\pm$ 0.33 <sup>c</sup>	19.07 $\pm$ 0.32 <sup>b</sup>	19.55 $\pm$ 0.39 <sup>b</sup>	19.82 $\pm$ 0.44 <sup>b</sup>
Vacc. + ginger oil	12.86 $\pm$ 0.34 <sup>a</sup>	15.69 $\pm$ 0.43 <sup>a</sup>	18.08 $\pm$ 0.34 <sup>a</sup>	20.72 $\pm$ 0.36 <sup>a</sup>	21.84 $\pm$ 0.58 <sup>a</sup>	22.37 $\pm$ 0.60 <sup>a</sup>
Vacc. + thyme oil	11.36 $\pm$ 0.33 <sup>b</sup>	14.03 $\pm$ 0.29 <sup>b</sup>	16.24 $\pm$ 0.44 <sup>b</sup>	21.48 $\pm$ 0.62 <sup>a</sup>	20.53 $\pm$ 0.47 <sup>ab</sup>	20.93 $\pm$ 0.38 <sup>b</sup>
P value	***	***	***	***	***	***

Notes: The means followed by the same letter in each column are not significantly different from each other at the 0.05 probability level (Duncan's multiple range test). \*, \*\* and \*\*\* refers to p values  $\leq 0.05$ , 0.01 and 0.001 respectively. ns refer to there is no significance.

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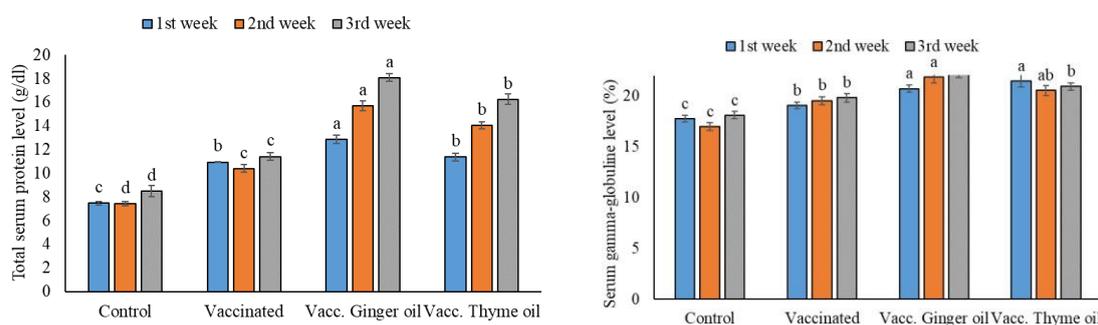


Fig (4): Effect of oral administration of ginger and thyme oils (250 mg/kg b. wt of each) / once daily for 21 successive days on total serum protein level (g/dl) and gamma-globulin level (%) in vaccinated rabbits.

### 4. DISCUSSION:

Our results agreed with those reported by (Salah, 2016) who found that RHDV vaccine stimulate phagocytic activity and phagocytic index in rabbits.

Elevation in macrophage activity in response to vaccination with hemorrhagic disease virus vaccine only or receiving ginger and thyme oils lead to increase of nitric oxide level (Bryan and Grisham, 2007).

This finding was supported by (MacMicking et al., 1997) as they stated that the increase in nitric oxide production by macrophages is believed to be due to increase nitric oxide synthase and secretion.

Our results were supported by the results recorded by *Salah (2016)* who found that lysosomal activity was increased post-vaccination by rabbit hemorrhagic disease virus vaccine.

Our findings are supported by Abdallah (2015) who reported a significant increase ( $p < 0.001$ ) of lysozymes post-vaccination. Our observed data fit with those reported by El-Sissi et al. (2011) who showed a significant increase ( $p < 0.001$ ) in

lysozyme post-vaccination with another viral vaccine (New castle disease virus and infectious bronchitis virus vaccine). Serum total proteins and its fractions (albumin and globulin) are a major constituent of plasma. Immunoglobulins are the main fractions of blood protein those playing on important role in host defenses against pathogens and in immunity as the whole (*Abdallah, 2015*). Globulins act as antibodies because it's the main precursors for the synthesis of immunoglobulins which are principal components responsible for immunity (*Eckersall, 2008*).

Our results revealed that the vaccinated non-treated group in comparison with non-vaccinated non-treated rabbits and the vaccinated groups treated with ginger and thyme oils in comparison with vaccinated non-treated rabbits elicited a significant increase ( $p < 0.001$ ) in serum total proteins and gamma globulins as shown in table 4 and fig. 4.

It had been shown by Ahamed (1991) that vaccination leads to increase

in globulin fraction leading to increase in serum total protein.

Our results agreed with Nabeel (2013) and Abdallah (2015) who recorded a significant increase ( $p < 0.001$ ) in total protein and globulins due to vaccination of rabbits with rabbit hemorrhagic disease virus vaccine.

A significant increase ( $p < 0.001$ ) in the level of gamma globulins have been shown in vaccinated rabbits and the groups which were vaccinated and treated with ginger and thyme oils when compared with regarded controls. Elevation of serum gamma globulins level was parallel to the elevation in serum antibody activity (Khare, et al., 1975).

### **Conclusion**

It could be concluded that ginger oil and thyme oil have a beneficial immunostimulant response.

It could be advised to use both oils as a prophylactic against immunosuppressant diseases.

### **Conflict of interest**

The authors declare no conflict of interest.

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