

Immunological And Dna Fragmentation Studies On The Protective Effect Of Thyme Against Navelbine Induced Oxidative Stress In Mice

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ABSTRACT: Many types of chemotherapeutic agents including vinorelbine (Navelbine) have been shown to be effective against cancers and tumor cells. *Thymus vulgaris* is recognized as sources of natural antioxidants and thus plays an important role in the chemoprevention of diseases. In this study Thyme oil was orally administered to normal, Navelbine treated, *Pseudomonas aeruginosa* infected and non infected mice at a dose level of 720µg/10 mice. The elevated level of liver mitochondrial DNA fragmentation was profoundly ameliorated as a result of treatment with Thyme oil. With regards to oxidative stress, the increased lipid peroxidation and deteriorated antioxidant defense system in liver Navelpine infected and non infected mice were alleviated. The neutrophil phagocyte activity had potentially increased as a result of Thyme oil administration associated with elevated expression of immunoglobulin G. The Thyme oil ameliorating changes may be mediated via its immunomodulatory effects on phagocyte activity and releasing of immunoglobulins by B cell as well as its effects in suppressing DNA fragmentation and oxidative stress and enhancing the antioxidant defense system. This study concluded that thyme oil could be used as a chemo protective and immune protective agent against Navelbine induced toxicity in infected and non infected mice. [Nature and Science 2010;8(8):136-145]. (ISSN: 1545-0740).

Key Words: (Vinorelbine tartrate) Navelbine, Thyme, *Ps. aeruginosa*, % DNA fragmentation, GSH, MDA and phagocytosis.

INTRODUCTION

Vinorelbine is semi synthetic vinca alkaloid that is effective as monotherapy (Curran and Plosker, 2002). Vinca alkaloids vinblastine and vincristine and some of their derivatives such as vinorelbine are widely used in therapy of leukemia and several solid tumors (Makarov *et al.*, 2007). Vinca alkaloids are used clinically against a variety of hematological and solid tumors (Gonzalez *et al.*, 1999). Moreover, some toxicity studies showed myelosuppression, neutropenia, thrombocytopenia and anemia (Liu *et al.*, 2006). Vinorelbine action is associated with alterations of the mitotic spindle functions that present the cell cycle progression and leads to mitotic block, mitochondrial depolarization responses and then apoptosis (Aggarwal *et al.*, 2008.)

Researches using animals being treated for cancer may use dietary supplements, particularly antioxidants, in the hope of reducing the toxicity of chemotherapy. Antioxidants can protect cells against chemotherapy. It seems likely that they would therefore reduce treatment-related toxicities (Gabriella and Andrea, 2005, Ahmed and Zaki, 2009 and Ahmed, 2010).

Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in chemoprevention of diseases (Faix *et al.*, 2005

and Ahmed and Ali, 2010). Plant volatile oils have an important role to play in animal health. They have antibacterial, antifungal and antioxidant properties (Deans and Svoboda, 1990). *Thymus vulgaris* has essential oils possess wide range spectrum of fungicidal activities, antiviral effect and the most effective antibacterial activity. It was expressed by the essential oil of organo, even on multiresistant strains of *Pseudomonas aeruginosa* and *Escherichia Coli* (Bozin *et al.*, 2006). Thyme has significantly higher superoxide dismutase and glutathione peroxidase activities and total antioxidant status (Youdim *et al.*, 1999 & Clara Gross *et al.*, 2010).

Infections, drugs, chemoattractants, cytokines, interleukins are present in increased amounts both at the site of disease and in the circulation. These molecules play an important regulatory role in the host response to disease and together with endotoxin, they are important modulators of neutrophil phagocyte capacity (Bjerknes, 1992 and Platt *et al.*, 2002).

Since chemotherapeutic treatment is usually associated with immunosuppression response that might be associated to infection especially *Ps. aeruginosa*, therefore it would be considerable to evaluate the effect of *Ps.aeruginosa* infected in different treatments used in the study.

Besides, this study aimed to evaluate the chemoprotective and immunoprotective effect of thyme oil on (Vinorelbine) Navelbine treated-bacterial infected and non-infected mice.

MATERIALS AND METHODS

MATERIALS

Animals:

Animals were obtained from laboratory animal house of the National Organization for Drug Control and Research (NODCR). Animals were kept in cages under standard laboratory conditions of food, water temperature and light throughout the period of study.

Chemicals:

Navelbine was purchased from Pierre Fabre, and given intraperitoneal (i.p.) at dose 10 mg/kg/week, for four weeks. Thyme oil was obtained from Isis Company for industries, Cairo, Egypt. It was received orally at a dose 720µg/10 mice every second day for twenty days.

Microorganisms:

Pseudomonas aeruginosa (clinical isolate) bacterial strain used in the present study was obtained from Pharmacology Department, Medical microbiology and Immunology Unit, National Organization for Drug Control and Research (NODCAR). Bacteria proved to be *Pseudomonas aeruginosa* by Gram stain and biochemical reactions. Bacteria were subjected to growth in nutrient broth (Oxide) for 24 hours at 37°C.

Experimental design:

Male albino mice, weighting between 20-25 g each, were used in the present study. Mice were divided into eight equal groups (30 mice per each) as follows:

Group 1: Control group (non-treated).

Group 2: Bacterially infected group, injected with 100µl/kg.LD₅₀ (ip) of *Ps. aeruginosa* bacterial suspension.

Group 3: Navelbine treated group, received 10 mg/kg/week (i.p.) for four weeks.

Group 4: Thyme oil group, received 720µg/10 mice orally every second day for twenty days.

Group 5: Navelbine treated- *Ps.aeruginosa* infected-group, mice were injected with Navelbine for four weeks followed by LD₅₀ dose of *Ps.aeruginosa* suspension at day 29.

Group 6: Thyme treated-*Ps. aeruginosa* bacterial infected- group, received Thyme oil for 20 days followed by LD₅₀ dose of *Ps.aeruginosa* suspension at a dose level as mentioned before.

Group 7: Navelbine –Thyme treated group, received Thyme oil after the second dose of Navelbine and continued for 20 days at a dose level as mentioned before.

Group 8: Navelbine –Thyme treated - *Ps.aeruginosa* infected group, received Thyme oil after the second dose of Navelbine and continued for 20 days followed by LD₅₀ dose of *Ps.aeruginosa* suspension.

Methods

A. Biochemical parameters

Preparation of mitochondria from mice liver

Mitochondria fraction was isolated from the liver tissues according to **Kimura et al. (1984)**.

A1-Determination of DNA fragmentation

DNA fragmentation was determined by **Diphenylamine (DPA) Colorimetric assay** according to **Shen et al. (1992)**.

The percentage of DNA fragmentation was expressed by the following formula:

$$\% \text{ DNA fragmentation} = \frac{\text{O.D. 600 Supernatant}}{\text{O.D. 600 supernatant} + \text{O. D. 600 pellet}} \times 100$$

A2-Determination of lipid peroxidation levels (MDA)

Lipid peroxides formation was determined in liver homogenate as TBARS substances. It was determined according to the method of **Buege and Aust (1978)**.

A3-Determination of reduced glutathione (GSH) content

Liver GSH was determined according to **Prins and Loose (1969)**.

A4-Determination of total protein

Total protein level was determined in liver homogenate using a test reagent kit according to **Josephson et al. (1957)**.

B-Hematological parameters

Total leukocyte Count was performed according to **Nourbakhsh et al. (1978)** while differential WBCs Count was evaluated by using Leishman stain (**Alpha Plus, Egypt**) (**Hayhoe and Flemans, 1982**).

C-Immunological parameters

C1-Serum IgG and IgM concentrations were determined by using immunodiffusion plate kit four mice obtained from binding site limited, UK (**Fahey and Mckevely, 1995**).

C2-Phagocytosis in vitro test

Neutrophils were obtained conveniently from

peritoneal exudates induced by the injection of sterile saline containing 1 mg/ml of glycogen. In 20 hours after the injection, the exudates was withdrawn by the aid of a syringe containing 5ml 3.8% sodium citrate sterile solution, and centrifuged. The cells were pipette and washed with saline, lastly they were suspended in sterile saline.

The opsonophagocytic test was carried out according to Hank's technique (Kwapinski, 1972) in which a mixture of equal volumes of a serum and a leucocyte suspension was incubated with growth bacterial suspension of *E.Coli* in 10X 75 mm sterile tubes, which were then sealed with paraffinized cork stoppers and shacked then incubated for 60 minutes at 37C, to investigate the values of phagocyte percentage. Smears from these mixtures were made before and after incubation, dried and stained with Giemsa stain. One hundred neutrophil leucocytes were examined under the high power oil emersionx100 (cider oil were obtained from ADWIC, EGYPT) of the microscope; the number of active leucocytes and the total number of bacteria found in 100 leucocytes were recorded. Results of the test were expressed in terms of:

$$\text{Percentage phagocytosis} = \frac{\text{No. of granulocytes containing bacteria}}{\text{Total No. of granulocytes counted}} \times 100$$

$$\text{Phagocytic Index} = \frac{\text{Total No. of bacteria}}{100}$$

$$\text{Opsonophagocytic index} = \frac{\text{phagocyte index with the test serum}}{\text{Phagocyte index with control serum}}$$

STATISTICAL ANALYSIS

The obtained results were presented as mean \pm standard error. For statistical evaluation, significance of all values was examined by ANOVA test (Kirkwood 1988, Gennaro 2000) and $P < 0.05$ was considered statistically significant. The statistical significance of means between different groups was analyzed using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test.

RESULTS

Compared with control group, all test groups had significantly higher percentage of DNA fragmentation ($P < 0.001$), except Thyme oil group which showed a significant decrease ($P < 0.01$), but when compared to Navelbine treated group, Thyme treated- bacterial infected and Navelbine-Thyme treated bacterially infected groups were significantly lower ($P < 0.001$) (Table 1).

The total protein in liver homogenate had significantly decreased in Navelbine treated animals while thyme oil treatment caused a returning back to its normal values.

Table 2, showed increased MDA in *Ps.*

aeruginosa infected group ($P < 0.001$), and all Navelbine treated groups when compared with control group. On the other hand when compared with Navelbine treated group, MDA had significantly decreased in Navelbine-Thyme treated group ($P < 0.001$) and Navelbine-Thyme treated- *Ps. aeruginosa* infected group ($P < 0.001$). However GSH was statistically lower in *Ps. aeruginosa* group ($P < 0.01$), and Navelbine treated-Thyme treated-*Ps. aeruginosa* infected group ($P < 0.01$) than the control group.

As we expected, immunization with *Ps. aeruginosa* up regulate IgG antibody response in bacterial infected group ($P < 0.01$). At the same time compared to control group, IgG antibody response elevated significantly in both Thyme –treated group ($P < 0.001$) and combined groups ($P < 0.01$) as shown in Table 3. A significant increase in IgG antibody response was detected in Navelbine-thyme treated group ($P < 0.001$) compared to Navelbine infected group. Regarding IgM antibody response, it was statistically lower in Navelbine treated group ($P < 0.001$) and all bacterial infected groups than control group. On the other hand a significant increased in IgM antibody response ($P < 0.001$) was detected in Navelbine-thyme treated group when compared to Navelbine treated group.

Ps. aeruginosa infection was associated with a significant elevation in total leukocyte count ($P < 0.001$), While Navelbine treated group showed a statistical reduction ($P < 0.001$). Treatment of thyme oil led to obvious ameliorative effect returning back of leucocytes count to its normal value. Regarding Neutrophils percentage, it was significantly higher in Thyme treated group ($P < 0.01$) and was significantly lower in Navelbine treated group ($P < 0.01$) than the normal group. Comparing to Navelbine group, neutrophil percentage showed a statistically elevated value ($P < 0.001$) after treatment of Thyme with infection of *Ps.aeruginosa* (Table 4). Administration of Thyme oil caused a significant decreased in lymphocytic percentage ($P < 0.01$), while Navelbine treatment was associated with a significant increase ($P < 0.01$). This was also observed in Navelbine-Thyme treated group when compared to control group. Lymphocytic percentage was statistically lower in Navelbine-Thyme treated-*Ps.aeruginosa* infected group ($P < 0.001$) than Navelbine group (Table 4).

In *in vitro* study, incubation of equal volumes of serum and neutrophil suspension with bacterial growth suspension showed a significant increased in phagocytosis percentage in all main groups except Navelbine treated group which showed a significant decrease. The same results were observed in

phagocyte index and opsonophagocytic index in *Ps. aeruginosa* infected group which significantly increased (Table 5). Phagocyte percentage increased significantly when opsonin was obtained from Navelbine treated mice, comparing to main Navelbine treated group.

Opsonin obtained from *Ps. aeruginosa* infected group increased significantly phagocyte percentage, phagocyte index and opsonophagocytic index when compared to main control group while Phagocyte index was statistically higher than main bacterial infected group. Neutrophils obtained from *Ps. aeruginosa* infected group showed a significantly higher phagocyte percentage and phagocyte index than the main control group. While decreased phagocyte percentage and opsonophagocytic index were observed when compared with *Ps. aeruginosa* infected group.

Opsonin of Thyme treated showed a significant increase in phagocyte index, while neutrophils obtained from Thyme treated increased significantly both phagocyte percentage and phagocyte index compared to main control and main Thyme treated groups.

Opsonin obtained from Navelbine treated-*Ps. aeruginosa* infected group exhibited a remarkable phagocyte percentage, phagocyte index and opsonophagocytic index when compared to main control group and main Navelbine treated group. In comparison to main *Ps. aeruginosa* infected group, phagocyte index decreased while opsonophagocytic index increased significantly.

Compared with main control group, all of phagocyte percentage, phagocyte and

opsonophagocytic index had remarkably elevated in case of neutrophil of Navelbine-*Ps. aeruginosa* group. But when compared to main *Ps. aeruginosa* infected group, phagocyte percentage and phagocyte index were significantly reduced.

Opsonin and neutrophils obtained from Navelbine treated-Thyme protective produced a significant amelioration of phagocyte percentage and phagocyte index when compared with main Navelbine treated group. But opsonin of the same groups exhibited a marked increase in phagocyte percentage and phagocyte index. While Opsonin obtained from Navelbine - Thyme treated - *Ps. aeruginosa* infected group showed lower phagocyte percentage and phagocyte index than main *Ps. aeruginosa* infected group with increased phagocyte percentage comparing to control group.

Neutrophils obtained from Navelbine -Thyme treated-*Ps. aeruginosa* infected group produced remarkable phagocyte percentage and phagocyte index when compared to main control group and main *Ps. aeruginosa* infected group. Also, neutrophils and opsonin obtained from Navelpine-Thyme treated - *Ps. aeruginosa* infected group exhibited a significant amelioration in phagocyte percentage when compared to main control group and main Navelbine group (Table5).

Since chemotherapeutic treatment is usually associated with immunosuppression response that might be associated to infection especially *Ps. aeruginosa*, therefore it would be considerable to evaluate the effect of *Ps. aeruginosa* infection in different treatments used in the study.

Table 1: Level of DNA and total protein in liver homogenate of control, treated and protective animal groups

Animal group	Parameters	DNA Fragmentation	Total protein gm/dl
Control		9.38±0.18	7.59±1.33
<i>Ps.aeruginosa</i>		58.55±2.42 ^a	10.07±0.56 ^a
Navelbine		68.30±3.62 ^a	4.48±0.71 ^a
Thyme oil		6.10±0.19 ^a	7.82±0.40
Navelbine+ <i>Ps.aeruginosa</i>		68.77±2.31 ^a	7.92±1.22
Thyme+ <i>Ps.aeruginosa</i>		25.87±1.16 ^{ab}	8.11±1.32 ^b
Navelbine +Thyme		26.72±0.72 ^{ab}	7.51±1.46 ^b
Navelbine+Thyme+ <i>Ps.aeruginosa</i>		29.32±1.14 ^{ab}	5.79±1.30 ^a

Value are expressed as mean ± SE. n= 30, ANOVA test

a Significant at P<0.05 when compared to control group.

b Significant at P<0.05 when compared to Navelbine treated group.

Table 2: Level of MDA and GSH in liver homogenate of control treated and protective animal groups

Parameters	MDA Level (nmol/ml)	GSH Level (mg %)
Animal group		
Control	7.41±0.14	0.25±0.01
<i>Ps.aeruginosa</i>	19.52±0.12 ^a	0.11±0.01 ^a
Navelbine	17.13±0.34 ^a	0.20±0.01
Thyme oil	7.82±0.40	0.28±0.01
Navelbine+ <i>Ps.aeruginosa</i>	18.95±0.42 ^a	0.20±0.02
Thyme+ <i>Ps.aeruginosa</i>	9.49±0.11 ^a	0.22±0.0
Navelbine +Thyme	10.37±0.37 ^{ab}	0.19±0.01
Navelbine+Thyme + <i>Ps.aeruginosa</i>	13.10±0.10 ^{ab}	0.16±0.02 ^a

Value are expressed as mean ± SE. n= 30, ANOVA test

a Significant at P<0.05 when compared to control group.

b Significant at P<0.05 when compared to Navelbine treated group.

Table 3: Serum immunoglobulin G (IgG) and immunoglobulin M (IgM) in control and main treated animal groups

Parameters	IgG mg/LX10 ³	IgM mg/L
Animal group		
Control	12.5±0.1	469±17.5
<i>Ps.aeruginosa</i>	17.1±0.8 ^a	358±17.5 ^a
Navelbine	10.1±0.1	229±70.1 ^a
Thyme oil	17.5±0.5 ^a	370±17.1 ^a
Navelbine+ <i>Ps.aeruginosa</i>	12.4±1.1	228±64.4 ^a
Thyme+ <i>Ps.aeruginosa</i>	18.4±2.7 ^a	573±50.1 ^a
Navelbine+Thyme	15.7±0.6 ^{ab}	402±17.2 ^b
Navelbine+Thyme + <i>Ps.aeruginosa</i>	16.6±3.6 ^{ab}	208±11.9 ^a

Value are expressed as mean ± SE. n= 30, ANOVA test

a Significant at P<0.05 respectively when compared to control group.

b Significant at P<0.05 respectively when compared to Navelbine treated group.

Table 4: Peripheral blood total and differential leukocyte counts in control and main treated animal groups.

Parameters	Total Leukocyte count x10 ³ /Cmm	Neutrophil %	Lymphocyte %
Animal group			
Control	5.13±0.33	51.6±2.20	45.3±2.18
<i>Ps.aeruginosa</i>	7.35±0.08 ^a	58.2±1.45	38.6±1.34
Navelbine	3.11±0.21 ^a	44.1±1.71 ^a	54.2±1.46 ^a
Thyme oil	4.18±0.07	86.1±1.19 ^a	29.5±1.26 ^a
Thyme+ <i>Ps.aeruginosa</i>	4.18±0.39	50.8±1.38	47.9±1.16
Navelbine+ <i>Ps.aeruginosa</i>	6.23±0.11	47.3±3.51	50.4±3.55
Navelbine+Thyme	5.3±0.39 ^b	48.4±0.53	49.5±0.54 ^a
Navelbine+ Thyme+ <i>Ps.aeruginosa</i>	2.92±0.11 ^a	54.8±1.45 ^b	42.8±1.62 ^b

Value are expressed as mean ± SE. n= 30, ANOVA test

a Significant at P<0.05 respectively when compared to control group.

b Significant at P<0.05 respectively when compared to Navelbine treated group.

Table 5: Opsonophagocytic test results as estimated in control and main treated animal groups.

Neutrophils	Opsonin	Phagocytosis Test %	Phagocytic Index	Opsonophagocytic Index
Main control	Main control	64.1±5.8	3.40±0.41	2.74±0.25
Main <i>Ps.aeruginosa</i>	Main <i>Ps.aeruginosa</i>	93.8±1.9 ^a	15.88±2.48 ^a	4.45±0.16 ^a
Main Navelbine	Main Navelbine	51.8±3.9 ^a	2.45±0.53 ^a	2.37±0.12
Main Thyme	Main Thyme	83.6±3.2 ^a	6.06±1.19 ^a	2.71±0.28
Control	<i>Ps.aeruginosa</i>	96.8±1.1 ^{ab}	30.31±3.02 ^{ab}	6.55±0.69 ^{ab}
<i>Ps.aeruginosa</i>	Control	88.3±5.83 ^{ab}	12.22±4.63 ^a	2.45±0.46 ^{ab}
Control	Navelbine	62.07±1.94 ^d	3.96±0.41	2.62±0.38
Navelbine	Control	63.56±6.53	3.06±0.74	2.19±0.87
Control	Thyme	70.89±3.04	6.01±1.06 ^a	1.00±0.09
Thyme	Control	90.67±1.05 ^{ac}	13.44±1.03 ^{ac}	2.95±0.22
Control	Navelbine+ <i>Ps.aeruginosa</i>	90.6±2.82 ^{ad}	7.09±1.54 ^{abd}	7.09±1.54 ^{abd}
Navelbine+ <i>Ps.aeruginosa</i>	Control	67.3±13.12 ^{ab}	6.52±0.08 ^{ab}	5.67±1.09 ^a
Control	Thyme+ <i>Ps.aeruginosa</i>	85.62±1.06 ^a	12.50±1.06 ^{ac}	1.92±0.22
Thyme+ <i>Ps.aeruginosa</i>	Control	75.00 ±5.94	2.99±0.89 ^c	2.08±0.28
Control	Navelbine+ Thyme	92.2±1.08 ^{ad}	12.88±1.02 ^{ad}	2.45±0.12
Navelbine+ Thyme	Control	65.8±6.21 ^d	4.64±0.98 ^d	3.12±0.09
Control	Navelbine+ Thyme+ <i>Ps.aeruginosa</i>	78.7±4.09 ^{ab}	3.03±0.21 ^b	3.41±0.26
Navelbine+ Thyme+ <i>Ps.aeruginosa</i>	Control	97.1±1.21 ^{ab}	7.79±0.92 ^{ab}	1.59±0.19
Navelbine+ Thyme+ <i>Ps.aeruginosa</i>	Navelbine + Thyme+ <i>Ps.aeruginosa</i>	79.33±3.06 ^{ad}	2.74±0.05 ^{bc}	2.16±0.20

Value are expressed as mean ± SE. n= 30, ANOVA test

a Significant at P<0.05 when compared to control.

b Significant at P<0.05 when to compared *Ps. aeruginosa* groups

c Significant at P<0.05 when to compared Thyme

d Significant at P<0.05 when to compared Navelbine

DISCUSSION

Several studies have previously reported on the effect of Navelbine, contribute to their superior antitumor efficacies. Vinorelbine monotherapy, Vinorelbine-based chemotherapy is considered as a new treatment option for patients with metastatic or relapsed breast cancer (Ito and Kobayashi, 2009; Watanabe *et al.*, 2010).

The results of the experimental research obtained a significant increased in DNA fragmentation in Navelbine treated animals. These results are confirmed by Candelaria *et al.* (2007) who noticed that vinorelbine-induces DNA fragmentation in which apoptosis takes place through

caspases-3 and-9 and then causes a reduction in global DNA methylation, histone deacetylase activity, and promoter demethylation. According to Zhou *et al.* (2003) mice that were challenged directly with bacteria, the liver impairment might be the critical cause of the lethality while apoptotic characteristic of vital organs like liver was associated with DNA fragmentation. Our results are in agreement with these findings where liver DNA fragmentation was significantly increased in case of bacterially infected group. Besides, this study showed statistically elevated DNA fragmentation in Navelbine treated-*Ps. aeruginosa* infected animals. These results could be explained by Hu and Cheung

(2009) who mentioned that combination of Navelpine with rMETase; an enzyme isolated from *Pseudomonas* species; it showed synergistic DNA damaging effect in all cell lines. Concerning with apoptotic activity of Navelbine which associated with enhancing DNA fragmentation, we expected its influence on hematological and immunological parameters. In the current study, total leukocyte count was profoundly reduced in Navelbine treated mice. This change was associated consequently with marked reduction in neutrophils and lowering concentrations of both IgG and IgM antibody response. Semrau (2007) obtained similar results in his work; he noticed the hematological side effects of Navelbine including granulocytopenia and leukocytopenia.

Thyme as a natural product is effective in inhibiting tumor growth in mice and subsequently in delaying animal mortality; it has a damaging effect in all cell lines (Barek *et al.*, 2007). Plant volatile compounds appear to accumulate in the cell membrane and increase permeability, resulting in leakage of enzymes and metabolites (Tsai *et al.*, 2007). Eva Horvathova *et al.* (2006) noticed the DNA-protective effects of essential oils on hepatoma HepG2. They concluded that the thyme reduced the level of DNA damage induced by hydrogen peroxide (H₂O₂) associated with their antioxidant activity. Our results were in agreement with these findings where DNA fragmentation was significantly decreased in thyme oil treated-navelbine treated group.

Aromatic plants and their essential oils can be used as antibacterial and hepatoprotective supplement in the developing countries towards the development of new therapeutic agents (Suvajdzic *et al.*, 2006; Sylvestre *et al.* 2006). Oils were found to be inhibiting both gram-positive and gram-negative bacteria including *Ps. aeruginosa* (Seenivasan, 2006). Hydrophobicity of essential oils and their components enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable (Tepe *et al.*, 2004). This explains the significant decreased of DNA fragmentation in thyme oil treated-*Ps. aeruginosa* infected group.

Mak *et al.* (1996) reported that the GSH antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. MDA has been used as the primary indicator of oxidative stress (Navarro *et al.*, 2002). Aydin *et al.* (2005) observed that Thyme oil possess useful antioxidant properties and may

become important in the search for natural and antioxidant food additives. Besides, antioxidant nutritional support proved to be beneficial to improve patients receiving chemotherapy (Yin S *et al.*, 2006). Eva Horvathova *et al.* (2006) assumed that the observed DNA- protective effects of volatiles can be associated with their antioxidant activity. Our results support this suggestion, they showed a significant decrease in MDA and a significant increase in GSH in Thyme oil treated- Navelbine treated group when compared with Navelbine treated group.

Elswaifi *et al.* (2009) improved that numerous pathogenic agents have the ability to produce free radicals and damage the cells of the immune system. *Pseudomonas aeruginosa* is a bacterium that produces the toxin pyocyanin, which induces cell damage and compromises the immune system through production of reactive oxygen species (ROS). In contrast, nanoparticle antioxidants, by virtue of their anti-inflammatory activity, may blunt a host's normal immune defenses to certain microorganisms. This review will address this emerging double-edged sword for nanomedicine and its potential role in controlling infectious disease and will address future directions for research in this emerging frontier. Besides, Gusarov *et al.* (2009) observed that the inhibition of NOS activity may increase the effectiveness of antimicrobial therapy. Therefore, our results showed a significant decrease in MDA and a significant increase in GSH in *Ps.aeruginosa* infected group treated with Thyme oil. Vigo *et al.* (2004), noticed that *Thymus vulgaris* extracts significantly inhibit the enhanced production of NO induced by LPS in a dose-dependent manner. These effects on immune system may explain our findings in which a significant increase in IgG antibody response in bacterial infected group, Thyme oil treated group and Thyme oil treated-*Ps.aeruginosa* infected group. Besides, a significant decrease in IgG was detected in Navelbine treated-*Ps.aeruginosa* infected group when compared with *Ps.aeruginosa* group. As regards immunoglobulin M, a significant decrease was detected in Navelbine treated animal group and all *Ps.aeruginosa* bacterial infected groups except the one treated with Thyme oil when compared with control group. This decrease in IgM in *Ps.aeruginosa* infected animals could be explained by Elswaifi *et al.* (2009) who mentioned that *Pseudomonas aeruginosa* is a bacterium that produces the toxin pyocyanin, which induces cell damage and compromises the immune system.

Neutrophils are highly specialized for their primary function, that is, recognition, phagocytosis and destruction of microorganisms. The interaction

between microorganisms and neutrophils induces complex and concerted structural and metabolic alterations of the neutrophils, essential for normal function (Bjerknes, 1998). Toshifumi *et al.* (1995) determined phagocyte percentage, phagocyte index as parameters of phagocyte function.

Navelbine treated groups showed significantly decreased phagocyte parameters. Navelbine suppressed mainly the neutrophil phagocyte function. This finding was supported by Delozier *et al.* (2006) and Douillard *et al.* (2006) who mentioned that Navelbine caused neutropenia. This is explained by one obvious strategy in defense against phagocytosis which is direct attacked by the bacteria upon the professional phagocytes. Any of the substances that pathogens produce that cause damage to phagocytes have been referred to as aggressions. Most of these are actually extracellular enzymes or toxins that kill phagocytes. Phagocytes may be killed by a pathogen before or after ingestion.

The results of experimental research confirm those finding, while a significant increase in phagocyte percentage, phagocyte and opsonophagocytic index in bacterial infected groups. *Ps.aeruginosa* infection stimulated both neutrophilic activity and opsonin. Neutrophilic activity is less active than that of opsonin. These parameters are significantly stimulated by Thyme. Thyme could counteract the suppressive effect of navelbine, both on the level of neutrophilic phagocyte function and the opsonin but it was more predominant as regards the neutrophilic phagocyte activity. This finding is explained by Farinacci *et al.* (2008) who mentioned that *Thymus vulgaris* modulate the neutrophils immune function.

From the present study it would be concluded that the opsonophagocytic test is a quite sensitive test for evaluating the immune system and the immune response to different factors that might affect the immune function. It illustrates the importance of the use of different immunostimulation factors (drugs, antibodies) to overcome the different forms of bacterial resistance such as bacterial avoidance of recognition, decrement of chemotaxis, inhibition of attachment, ingestion and intercellular killing as well as escape from phagosome and killing of the phagocytes.

In conclusion, it can be suggested that *Thymus vulgaris* might be conveniently exploited to be good immune stimulatory modifiers and had the potential to apply in the tumor therapy.

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