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The Potential Antioxidant And Anti-Inflammatory Impact Of Oregano Extract In Rabbit Model

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Abstract:
Background*: Oregano (Origanum syriacum L.) is an aromatic plant of the Mediterranean flora widely used as spices and also for medicinal purposes. The influence of aqueous extracts of oregano on the physiological performance in rabbit model was assayed via dietary nourishment.*
Materials and Methods*: An experimental design was conducted on thirty mature males New Zealand white 6 months old sole housed rabbits, and randomly assigned into three dietary groups for 21 days. Two groups fed with 50 mg/kg and 100 mg/kg of oregano (Origanum syriacum L.) extract versus the third one was kept as control.*
Results*: Oregano supplementation resulted in a significant decrease in nitric oxide, myeloperoxidase activity, oxygen free radicals and interleukin -2, while caused a significant increase in lysozyme activity.*
Conclusion: *The obtained data declared the antioxidant and anti-inflammatory effect of oregano besides its non-specific immune-stimulant activity.*
Key Word*: Oregano – Interleukin-2 – Nitric oxide –Myeloperoxidase – ROS- lysozyme activity.*

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I. Introduction

Rabbit husbandry is prevalent in the Middle East region because of it is simply growing and eco-friendly breeding on farms and in backyards. Furthermore, rabbit's meat is a commonly likable food, and considered convenient for all nutritional patterns of human consumers [1].

Nowadays, natural components such as medicinal plants and phytogenics fed additive have been used as alternative approach instead of synthetic drugs as metabolic enhancers, weight gain promoters and immune-stimulants as well as stress antagonism [2].

Oregano (*Origanum syriacum* L.) is an aromatic plant of the Mediterranean flora widely used as spices and also for medicinal purposes. It offered an antioxidant and antimicrobial performances and some reports accord with its antimutagenic and anticarcinogenic impacts. *Origanum* essential oil is obtained by steam distillation and its prime components are carvacrol and thymol. *Origanum* essential oil is known to possess antimicrobial, antifungal, and antioxidant activities [3].

Reactive oxygen species (ROS), nitric oxide (NO) myeloperoxidase (MPO) are prime intermediates in the oxidative process, and so on, when produced in surplus as in inflammation, they can harm cells through lipids peroxidizing and perturbing cell molecules [4-6].

The use of phytogenic components has earned importance and given notice in nutritional scope because of their wide antioxidative behavior as well as their growth and immune promoting actions [7].

So the target of this experiment is the assessment of the effect of oregano diet additives on rabbit immune response as laboratory animal model.

II. Material And Methods

Ethical approval

All experimental procedures were carried out according to the following protocol approved by the Medical Research Ethics Committee, National Research Centre, (1179102021).

Plant

Dried leaves of oregano (*Origanum syriacum* L.) were grounded into powder using an electric grinder. Fifteen grams of the fine powder of oregano were subjected to extraction with one hundred ml of boiling water

in a covered flask and left for thirty minutes. After that, the extract was cooled and filtered by means of Whatman No.1 filter paper to remove the particulate material. The dose and duration of extract treatment were according to Rababa et al. [8]

Animals

Thirty mature New Zealand white males rabbits, 6 months old with initial weight 2.4 kg were used, sole housed in cages and weighed weekly throughout the experimental period. Food and water were provided as necessary desired.

Animals were randomly assigned to 3 groups (10 rabbits in each) as follows:

1) Control group: healthy rabbits that were not exposed to plant treatment.
2) Oregano extract low-dose group: (50 mg/kg) was given by oral daily for 3 weeks,
3) Oregano extract high-dose group: (100 mg/kg) was given by oral daily for 3 weeks

Blood sample collection

Blood samples were gathered from the marginal ear vein of the control and treated groups of rabbits at 4,7,14, and 21 days after treatment. Blood samples were transmitted to plain tubes for serum separation. All tubes were instantly kept at 4°C and then centrifuged (3,000×g for 10 min); the obtained serum was separated and all samples transmitted to the laboratory and stored at –20 °C until analyses.

Measurement of nitrite concentration

The nitrite accumulated in serum samples of all groups was estimated as an indicator of nitric oxide production, according to (Rajaraman *et al.*1998) [9]. On details, one hundred microlitters of serum samples was incubated with an equal volume of Griess reagent into flat bottom 96 well plate at 25ºC for ten minutes in a dark place. The absorbance was assessed at 540nm by universal microplate reader ELx 800 UV (Bio-Tek), and the concentration of nitrite was estimated from the sodium nitrite standard curve.

Myeloperoxidase activity

The assay was determined in *vivo*, according to (Quade and Roth1997) [10] using 3,3′,5,5′-Tetramethylbenzidine (TMB, Roth). On detailed, ten microlitters serum samples was combined with 80 µl 0.75 mM H2O2(Sigma) and 110 µl TMB solution (2.9 mM TMB in 14.5% DMSO (Sigma) and 150 mM sodium phosphate buffer at pH 5.4), and the plate was incubated at 37°C for five minutes. The reaction was ceased by adding fifty µl 2 M H2SO4, and the absorbance was estimated at 450 nm and MPO activity was assessed via the standard curve.

Reactive oxygen species (ROS)

The assay was performed following the (Anderson and Siwicki 1995) [11] protocol, via colorimetric estimation of the ROSs output by the macrophage respiratory burst, which enhances reduction of nitroblue tetrazolium (NBT, Bio Basic, Ontario, Canada Inc.) into dark blue precipitate inside the phagocyte, called formazan granules.

In detailed, one hundred microlitters of serum sample was added to one hundred microlitters of 0.2% NBT solution (prepared in phosphate buffered saline, pH 7.4) and incubated for thirty minutes at 25 °C. After incubation, fifty microlitters from the combined solution were added to one ml of N, N-dimethyl formamide (DMF, Sigma, St. Louis, MO, USA). The new solution was centrifuged at 3000 g for five minutes, then the supernatants were transmitted to final plate, and the absorbance was estimated at 540nm using the standard curve.

Determination of Interleukin-2

The mouse IL-2 ELISA kit (Koma Biotech, Inc.) was used for the quantitative measurement of IL-2 in serum samples of the five experimental groups followed the manufacture guidelines.

Lysozyme activity assay

One hundred microlitters of tested sera were added to two millilitters of a suspension of *Micrococcus lysodeikticus* ATCC 4698 Sigma (0.2 mg/ml) in a 0.05 M sodium phosphate buffer (pH 6.2). The reactions were performed at a 20°C, and optical density at 530 nm was determined between five and twenty minutes on a spectrophotometer. A lysozyme activity unit was determined as the amount of enzyme producing a reduction in optical density of 0.001/min against standard curves [12].

Statistical analysis

Data for immunological parameters were analyzed and main effects were discussed if $P < 0.01$, and were presented as means ± SE for the indicated number of independently performed experiments. Statistical significance (≤ 0.01) was assessed by t-test.

III. Result

The data obtained in figure (1) exhibited a significant decrease in nitrite concentration indicated decreasing in NO production from macrophages in groups treated with oregano and proposed directed to increasing in the doses.

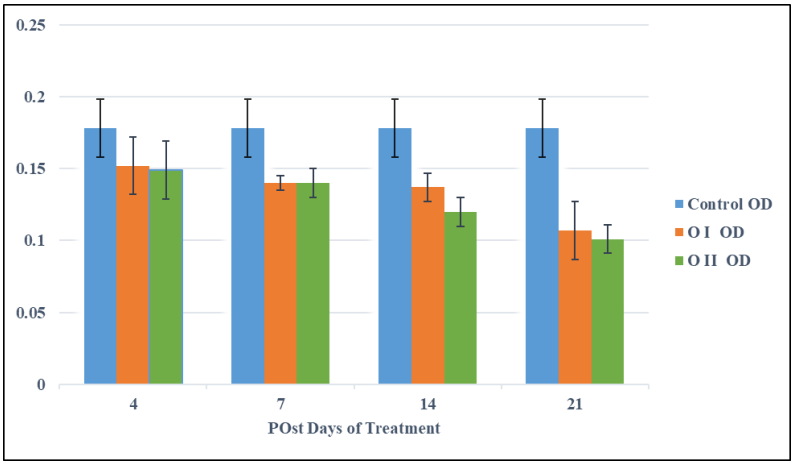

Figure 1: Determination of nitric oxide production through nitrite concentration by Griess assay
O1- (50 mg/kg of Oregano extract) O2- (100 mg/kg of Oregano extract) significant decrease P < 0.01

The results obtained from figure (2) indicated the significant decrease in MPO activity in the treated oregano samples by increasing sampling days but not significantly affected by increasing the treatment dose.

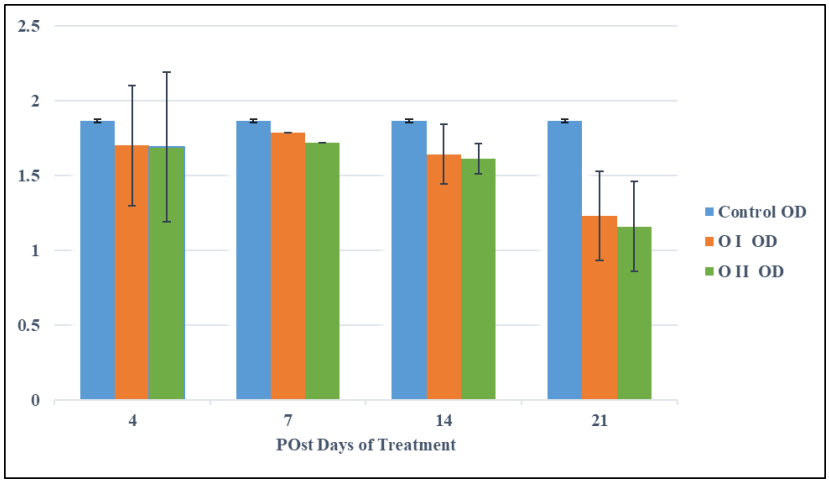

Figure 2: Determination of Myeloperoxidase activity
O1- (50 mg/kg of Oregano extract) O2- (100 mg/kg of Oregano extract) significant decrease P < 0.01

The data recovered from figure (3) displayed a much significant decrease in ROS production with the addition of oregano directly proposed to increasing in both dose and treatment days.

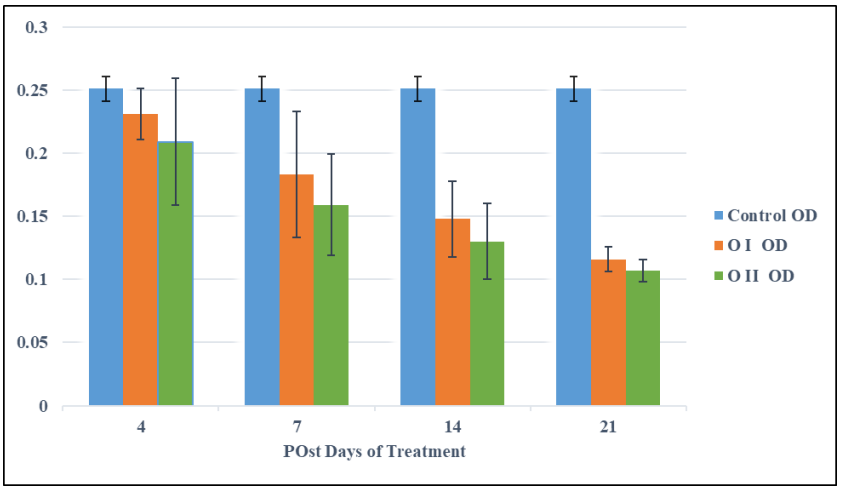

Figure (3): Determination of respiratory oxygen species (ROS) activity
O1- (50 mg/kg of Oregano extract) O2- (100 mg/kg of Oregano extract) significant decrease P < 0.01

The results obtained from figure (4) indicated a decrease in IL-2 production in the oregano treated groups and seemed to be an insignificant difference due to concentration.

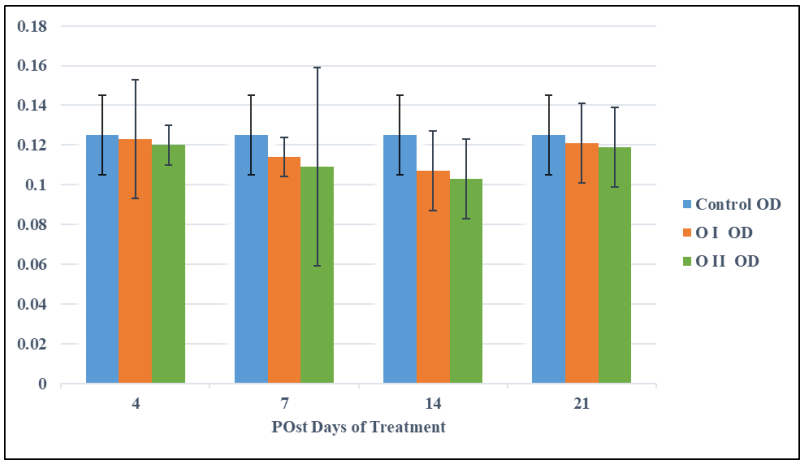

Figure (4): Determination of IL-2 production
O1- (50 mg/kg of Oregano extract) O2- (100 mg/kg of Oregano extract) significant decrease P < 0.01

The data obtained from figure (5) showed a great significant increase in lysozyme activity with the addition of oregano and directly proposed to increasing in treatment days till day 14 then slightly declined but not affected by increasing the dose.

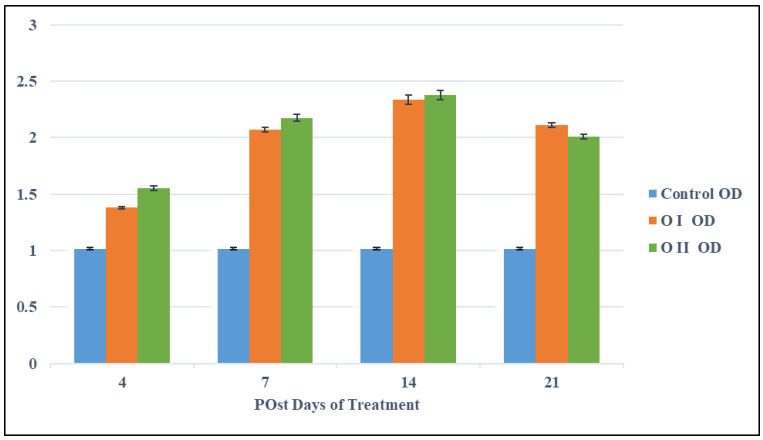

Figure (5): Determination of lysozyme activity
O1- (50 mg/kg of Oregano extract) O2- (100 mg/kg of Oregano extract) significant decrease P < 0.01.

IV. Discussion

Oregano, is a bushy, everlasting flavoring herb, native to Europe and Central Asia, is a broadly used among the world to flavor different foods and processed products [13]. Essential oils and active components extracted from the leaves and flowers of oregano have been demanded to have numerous useful biological performances [14].

Oxidative stress is the trouble in the pro-oxidant-antioxidant balance, driving to prospect damage; and is commonly induced by the attack of reactive radicals, upon the components of living organisms [15]. Oxidative stress can induce an elevation in cell proliferation, DNA damage, aging and cell death. Thus, numerous studies have positively correlated oxidative stress with the pathogenesis of illnesses, such as chronic-inflammation, arthritis, atherosclerosis Parkinson's disease, Alzheimer's disease and some types of diabetes, cancer, and, among others [16-18].

Results mentioned in figures (1-3) revealed that the oregano addition to diet resulted in a significant decrease in output of nitric oxide, myeloperoxidase activity, free oxygen radicals exhibited antioxidant plus anti-inflammatory behavior. Regarding to these data corresponded with many investigations; (Tan 2015) [19] announced that the supplementation of ration with *O. vulgare* subsp. *hirtum* EO was able to reduce serum of reactive substances levels in sows' sera. It was mentioned that leaf-flower oils showed the best antioxidant activity [20]

High antioxidant activity of four varied types of oregano from central and southern regions of Argentina was declared via the ABTS assay Asensio et al. [21]

Furthermore, different concentrations of EOs of fifty one wild plants of *Origanum* ssp. gathered from varied areas of Sicily prohibited 50% (IC50) of the UV radiation-induced peroxidation in liposomal membranes and exhibited a good radical scavenging activity [22]. Sarfaraz et al [23] determined the high antioxidant performance of oregano and marjoram extract using two food model systems, constituting DPPH and β-carotene bleaching.

Interleukin-2 (IL-2) is one of important signals produced by activated T cells and a variety of immune disorders have been associated to imbalanced output of these cytokines as inflammatory response [24].

Results obtained from this study revealed decreasing in IL -2 and coincided with that stated by (Gholijani et al.2015) [25] who added carvacrol "the main component of oregano" at 25 µg/ml to human Jurkat T Cells in vitro, the addition demonstrated a significant decrease in IL-2 output from 119.4 ± 8pg/ml in control cells to 32.3 ± 3.6pg/ml. Different studies have reported anti-inflammatory merits of various species of oregano [26, 27]

Ocaña-Fuentes et al [28] mentioned a reduction in the synthesis of pro-inflammatory cytokines, as well as an elevation in the output of anti-inflammatory cytokine from macrophages treated with EO from *O. vulgare*.

During the early stages of the illness, neutrophils and macrophage infiltration was directed to combat microorganism, by many approaches as enhancement of lysozymes activity. Lysozyme is a mucolytic enzyme able to exert damage of peptidoglycan, so it is highly efficient, particularly versus Gram-positive bacteria [29, 30].

Our results that obtained from figure 5 declared significant increasing in lysozyme activity, this data were coincided with other previous studies; lysozyme activity in rainbow trout plasma was significantly higher in fish fed diet containing 3.0 mL kg−1 essential oil of O. onites ($P < 0.05$) [31]. Volpatti et al. (2013) [32] reported a similar trend in the level of lysozymes.

V. Conclusion

Oregano supplementation diminished the deleterious free radicals, and therefore, it could be used as a natural antioxidant and stress antagonist. Additionally, this merit could be of a valuable regard, as protect meat which featured by the existence of high amount of unsaturated fatty acids from oxidation leading to prolonging in meat shelf life. On the other hand, addition of oregano resulted in minimizing the inflammation incidence (anti-inflammatory) agent, so it is helpful in treatment of many inflammatory cases. Furthermore, it was declared that oregano and its extracts and oils have potential innate immune-stimulant activity.

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