Prophylactic and therapeutic evaluation of the phytobiotic (Orego-stim)® in chicken experimentally infected with *E. coli*

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Abstract: The prophylactic and therapeutic effects of the phytobiotic (Orego-stim)® was evaluated in chicken experimentally infected with Novobiocin marked *E. coli O78*. Enrofloxacin (Opitryl)® was used as a standard. The obtained results demonstrated that, birds prophylactically received Orego-stim® showed more favorable clinical signs, mortality rate, P.M. lesions, recovery rate, bacterial reisolation results and growth performance. Both cellular and humeral immunity were enhanced. A decrease in the mean values of serum ALT & AST ,albumin, uric acid and creatinine levels were recorded that may provide evidence for the hepato and renoprotective effects of the essential oils. It could be concluded that, Orego-stim® can be considered a promising mixture of essential oils due to its high efficacy (growth performance, antibacterial and immunomodulating effects) and positive impact on both liver and kidney functions. The study highly recommends the use of Orego-stim® as a prophylactic agent in dealing with *E. coli* infection in chicken however, its concurrent administration with enrofloxacin in treatment of such case revealed the most favorable outcomes.

[Naglaa Z. H. Eleiwa; E. M. El Sayed and A. A. Nazim. **Prophylactic and therapeutic evaluation of the phytobiotic (Orego-stim)® in chicken experimentally infected with *E. coli*.** Journal of American Science 2011; 7(8):91-102]. (ISSN: 1545-1003). http://www.americanscience.org.

Key words: Orego-stim®, essential oils, *E. coli*, efficacy, side effects, chicken

1. Introduction:

In the last few years, frequent outbreaks of resistant bacterial strains in field settings occurred and claims regarding the hazard of multiple resistance to antibiotics used in human and animal medicine increased due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases or as food additives ***(Davis, 1994; Service,1995).***

These concepts have led the European Union to ban the use of most of these additives in poultry feeds. For this reason, many studies have recently been undertaken investigating the use of novel yet promising natural feed-additives "organic acids, probiotics, prebiotics, plant extracts, enzymes and essential oils" ***(Schwarz et al., 2001)***. Herbs, spices and various plant extracts have received increased attention as possible replacements for antibiotic growth promoters ***(Burt, 2004 and Peschel et al., 2006)***.

In this context, aromatic plants, especially species of Origanum genus, have emerged as effective compounds seeking microbiological safety represented by phytobiotic ***(Ipek et al., 2005 and Souza et al., 2007)***.

The genus Origanum (Family: Lamiaceae) comprises about 38 species widespread all over the world. Among them, *Origanum vulgare is* an endemic spontaneous plant, growing in North Africa and used as a medicinal plant against whooping cough, cough, fever and bronchitis ***(Baba Aissa, 1991).***

The notion that, *Origanum species* has antibacterial, antifungal, insecticidal, antioxidant and anti-carcinogenic activities motivated the study of the biological activities of their essential oil contents ***(Ipek et al., 2005)***. In fact, purified essential oils and their fractions; carvacrol, thymol, limonene and cineole; have shown extensively to have antimicrobial properties *in vitro* ***(Ultee et al., 2002 and Faleiro et al., 2003).*** Mode of their antimicrobial action consists of interactions with cell membranes changing the permeability of cations such as H+ and K+ ***(Ultee et al., 1999).***

Orego-stim® is a phytobiotic containing Oreganum aetheroleum as active substance. Oreganum aetheroleum is oregano etheric oil obtained by steam distillation of the leaves and flowers of the plant *Origanum vulgare ssp. hirtum* that has been demonstrated to possess wide-spectrum antibacterial activities and has been extensively used by people for the treatment of bacillary dysentery, enteritis and cholera etc. The principal ingredients are phenolic derivatives, such as carvacrol and thyme camphor, which have strong antibacterial effects ***(Hammer et al., 1999).***

In this spirit, then, the current work was designed to evaluate the prophylactic and therapeutic effects of the phytobiotic (Orego-stim®) in chicken experimentally infected with *E. coli*

2- Material and Methods

2.1. Agents:

2.1.1. Orego-stim®:

Orego-stim®; a product of Meriden Animal Health Co. – UK; is a phytobiotic used as a natural feed/drinking water additive. It contains Oreganum aetheroleum as active substance. Oreganum aetheroleum is oregano etheric oil obtained by steam distillation of the leaves and flowers of the plant *Origanum vulgare ssp. hirtum* and contains many essential oils, mainly *carvacrol* 81.89%, *y- terpinrnr* 5.1%, *p- cymene* 3.76% and *thymol* 2.12%. It was given as oral solution at a dose of 0.3 ml/ liter.

2.1.2. Antibiotic:

Enrofloxacin (Opitryl)®; a product of El-Obour Modern Pharmaceutical Industries Co., Egypt. Each 100ml contains 10gm enrofloxacin.

It was given as a 10% oral solution in the recommended therapeutic dose of 10 mg/kg of bird's body weight (1 ml /2 liter) for 5 successive days ***(Luke et al., 2006).***

2.1.3. Bacteria

*E. coli O*78 Kindly supplied from the Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Zagazig University.

2.1.3.1. Preparation of bacterial cultures:

*E. coli O*78 was reconstituted in 5ml nutrient broth and incubated at 37°C for 24hrs, then sub-cultured on MacConkey's agar and incubated at 37°C for 24hrs.

2.1.3.2. Preparation of Novobiocin marked strains:

E. coli O78 resistant strains was prepared by sub-passage of the micro-organism on media contained 1gm Novobiocin /liter after several sub-passage on graded levels of the Novobiocin ***(Barnhart et al., 1999)***.

2.1.3.3. Bacterial titration:

Ten fold dilutions were prepared from 24hrs cultures on peptone water to obtain 10^8 CFU/ml to be used for inoculation of chicks according to ***Sambrook et al., 1989***.

2.2. Experimental chicks:

Sixty, one day old, commercial (Hubbard) broiler chicks obtained from El-Banna Poultry Company were used for the experiment. The chicks were floor reared and fed on a balanced commercial ration free from antimicrobial agents.

2.3. Experimental design:

On the 1st day of age, chicks were divided into 6 equal groups; 10 each.

The 1st group : was not inoculated and left as a control (-ve control).

From the 2nd group through the 6th group, Novobiocin marked *E. coli O78* was inoculated I/nasal at approximately $1x10^8$ CFU /ml on the 5th day of age.

The 2nd group: was kept as +ve control (infected, non-treated).

The 3rd group: was inoculated, treated with the recommended dose of Orego- stim®; 0.3ml / liter orally for 5 successive days.

The 4th group: was inoculated, treated with the therapeutic dose of enrofloxacin (Opitryl)®; 10 mg/kg of body weight of birds (1 ml / 2 liter) orally for 5 successive days.

The 5th group: was inoculated, treated with both Orego-stim® and enrofloxacin (Opitryl)®; at doses as mentioned in the 3rd and 4th groups respectively for 5 successive days.

The 6th group: received Orego-stim® from the 1st day of age throughout the experimental period and inoculated with *E. coli O78* I/nasal at approximately $1x10^8$ CFU /ml on the 5th day of age.

2.4. Samples collection and preservation:

On the 15th, 22nd and 29th and 36th days of age, five chickens from each group were used in each collection .Two blood samples, 2.5 ml each were collected from the wing vein of each chick. The first blood sample was collected in test tube containing 50 I.U. /ml blood heparin as anticoagulant to determine the phagocytic activity and phagocytic index. The second blood sample was collected in a centrifuge tube and left to clot then centrifuged at 2000 rpm for 10 minutes to allow serum separation which then aspirated into cryovials and stored at -20°C for humeral immunity investigation and liver & kidney function tests.

2.5. Analysis:

2.5. 1. Evaluation of Orego-stim® efficacy:

2.5. 1. 1. Clinical signs, P.M. lesions, Mortality rate and bacterial reisolation:

All groups were kept under observation for a week post inoculation, clinical signs; postmortem lesions and mortality rate were recorded.

On the 16th day of age, dropping samples were collected under aseptic conditions for bacteriological investigation. One gram of the sample was weighted and placed in a sterilized glass tube. The initial dilution was made by adding 9 volumes of sterile saline (Nacl solution 0.9%). Further serial 10 fold dilutions using sterile saline solution were made. Following dilution, 0.01ml of the suspension from the dilutions, including the initial dilution, were taken and spread on MacConkey's agar containing 25 µg Novobiocin for reisolation of *E. coli* **(*Edao et al., 1998*).**

2.5.1.2. Growth performance evaluation:

From the 1st day of age throughout the experimental period, body weight (B.W.), feed intake (F.I.) and food conversion rate (F.C.R.) for each group were recorded weekly

2.5.1.3. Immunological response evaluation:

2.5.1.3.1. Cellular immunity:

By determination of Phagocytic activity and phagocytic index ***(Kawahara et al., 1991).***

Phagocytic activity (PA) = percentage of phagocytic cells containing yeast cells.

Phagocytic index (PI) = Number of yeast cells phagocytozed / Number of counted phagocytic cells

2.5.1.3.2. Humeral immunity:

By Quantitative estimation of serum IgM ***(Young, 1997)*** and IgG ***(Friedman and young, 1997).***

2.5.2. Evaluation of Orego-stim® side effects:

2.5.2.1. Effect on Liver functions:

By quantitative estimation of serum total proteins and albumen ***(Weichselbaum, 1946 & Doumas, 1971)*** and liver enzyme activities (ALT & AST) ***(Reitman and Frankel, 1957)***.

5.2.2.2. Effect on kidney functions:

By quantitative estimation of serum uric acid ***(Haisman and Muller, 1977)*** and creatinine ***(young et al., 1975***).

2.5.3. Statistical Analysis:

The obtained data were analyzed statistically using an ANOVA test according to ***(SPSS Win, 1995).***

3- Results

3.1. Evaluation of Orego-stim® efficacy:

3.1.1. Clinical signs, P.M. lesions, Mortality rate and bacterial reisolation:

Birds belonged to groups (2); inoculated with Novobiocin marked *E. coli O*78 and received no treatment; showed the severest *E.coli* infection signs compared with other groups. Signs included coughing, sneezing, snicks, rales, ocular and nasal discharge. Diarrhea and dehydration were also noted on clinical examination. Feed intake and growth rate were reduced compared with control –ve chicks (group 1). Occasional birds had a hypopyon and/or hyphema, usually in one eye, which was blind (Panophthalmitis). Mortality rate was 10%. At necropsy, the most pronounced lesions were thickened air sacs with caseous exudates, adhesive fibrinous pericarditis, fibrinous perihepatitis and enteritis with excessive fluid in the intestine. Marked *E. coli O*78 was reisolated from 60% of the birds on the 16th day of age.

Birds belonged to groups 3, 4, 5, 6 were inoculated with Novobiocin marked *E. coli O*78 and treated with Orego-stim® only, Enrofloxacin only, both Orego-stim® and Enrofloxacin, and Orego-stim® from the 1st day of age throughout the experimental period respectively showed the same clinical signs previously recorded in group 2 but with variable milder degrees with special reference to group 6 which displayed the mildest symptoms. Recovery rate was 70%,80%,100% and 90% respectively and marked *E. coli O*78 was reisolated from 30% ,20%, zero% and 10% of the birds respectively on the 16th day of age.

3.1.2. Growth performance evaluation:

Birds belonged to groups (2) demonstrated significant decrease ($P<0.05$) in the mean values of body weight (B.W.) allover the experimental period compared with the –ve control group (Table 1).

Chicks in groups (4, 5, 6) showed significant increase ($P<0.05$) in the mean values of body weight (B.W.) on the 7th, 14th, 35th and 42nd days of age compared with the group (2).

Birds belonged to group (6) displayed significant increase ($P<0.05$) in the mean values of body weight (B.W.) on the 28th, 35th and 42nd days of age compared with the group (3) and birds belong to groups (5) demonstrated a significant increase ($P<0.05$) in the mean values of body weight (B.W.) on the 35th and 42nd day of age compared with the group (4).

The results showed that, chicks in groups (2) showed significant increase ($P<0.05$) in the mean values of feed intake (F.I.) on the 14th ,21st ,28th and 35th days of age compared with the –ve control group (table 1)

Birds in group (6) showed significant decrease($P<0.05$) in the mean values of F.I. on the 7th ,21st ,28th and 35th days of age compared with the

group (3) while chicks in group (5) showed significant increase ($P<0.05$) in the mean values of F.I on the 21st and 28th days of age compared with the group (4).

The obtained results clearly demonstrated that, (group 2) displayed significant increase ($P<0.05$) in the mean values of food conversion rate (F.C.R.) allover the experimental period compared with the control –ve group while chicks in groups (3, 4, 5, 6) showed significant decrease ($P<0.05$) in the mean values of F.C.R. on 21st, 28th, 35th and 42nd day of age compared with the group (2) (Table 1)

Also, birds in group (6) displayed significant decrease ($P<0.05$) in the mean values of F.C.R. on 7th, 28th, 35th and 42nd day of age compared with the group (3) while chicks belonged to group (5) showed significant decrease ($P<0.05$) in the mean values of F.C.R. on 35th and 42nd day of age compared with the group (4).

3.1.3. Immunological response evaluation:

3.1.3.1. Phagocytic activity and phagocytic index:

The results showed that, birds belonged to group (2) demonstrated a significant increase ($P<0.05$) in the mean values of phagocytic activity & phagocytic index on the 15th and 22nd day of age compared with the –ve control group (Table 2).

Birds belonged to groups (3, 5, 6) demonstrated significant increase ($P<0.05$) in the mean values of phagocytic activity & phagocytic index on the 15th, 22nd, 29th and 36th day of age compared with the +ve control group .

On the 22nd, 29th and 36th day of age, birds of group (4) showed significant decrease ($P<0.05$) in the mean values of phagocytic index compared with group (2) values while groups (6&5) showed significant increase in the mean values of phagocytic activity & phagocytic index compared with groups (3 &4) values respectively on the 15th, 29th and 36th day of age.

3.1.3. 2. Quantitative estimation of serum IgM and IgG :

The results showed that, birds belonged to groups (2) demonstrated a significant increase ($P<0.05$) in the mean values of IgM & IgG compared with the –ve control group (Table 2).

Birds belonged to groups (3, 5, 6) demonstrated significant decrease ($P<0.05$) in the mean values of IgM compared with the +ve control group while birds belong to groups (3, 6) showed significant increase ($p<0.05$) in the mean values of IgM compared with control –ve group .There were non significant changes in the mean values of IgG in the birds of groups (3, 4, 5, 6) compared with –ve control group.

3.2. Evaluation of Orego-stim® side effects:

3.2.1. Effect on Liver functions:

The results revealed that, birds belonged to groups (2) demonstrated significant increase ($P<0.05$) in the mean values of total proteins on the 15th day of age compared with the control –ve group (Table 3).

Birds belonged to groups (3, 5, 6) demonstrated significant increase ($P<0.05$) in the mean values of total proteins on the 15th day of age compared with the -ve control group. Chicks in group (5) showed significant increase ($P<0.05$) in the mean values of total proteins on the 15th day of age compared with group (4).

Concerning albumin level, birds in group (2) demonstrated significant decrease ($P<0.05$) in the mean values of albumin on the 15th day of age compared with the -ve control group. Chicks in groups (3, 4, 5, 6) showed significant increase ($P<0.05$) in the mean values of albumin on the 15th day of age compared with group (2) and non significant changes compared with control –ve group .

The results clearly demonstrated that, birds in group (2) showed significant increase ($P<0.05$) in the mean values of serum ALT & AST levels on the 15th and 22nd day of age compared with the -ve control group . Chicks in groups (3, 4, 5, 6) showed significant decrease ($P<0.05$) in the mean values of serum ALT & AST levels on the 15th and 22nd day of age compared with group (2) .

Birds belonged to groups (6) demonstrated significant decrease ($P<0.05$) in the mean values of serum ALT & AST levels on the 15th and 22nd day of age compared with group (3) and chicks in group (5) showed significant decrease ($P<0.05$) in the mean values of serum AST level on the 22nd day of age compared with group (4).

3.2.2. Effect on Kidney functions:

The obtained results clearly demonstrated that, on the 15th and 22nd day of age, control +ve chicks (group 2) displayed significant increase ($P<0.05$) in the mean values of uric acid & creatinine compared with the control –ve group (Table 4).

Birds belonged to groups (3, 4, 5, 6) demonstrated significant decrease ($P<0.05$) in the mean values of uric acid and creatinine compared with the +ve control group. Chicks in group (6) displayed significant decrease ($P<0.05$) in the mean values of uric acid & creatinine compared with those in group (3) and birds of group (5) showed significant decrease ($P<0.05$) in the mean values of uric acid & creatinine compared with group (4).

4- Discussion

The present study was designed to evaluate the prophylactic and therapeutic effects of the phytobiotic mixture of essential oils (Orego-stim®) in chicken experimentally infected with *E. coli*.

The results clearly demonstrated that birds received Orego-stim® pre infection (group 6) showed more favorable clinical signs, mortality rate, P.M. lesions , recovery rate and bacterial reisolation results compared with those treated with it post infection (group 3) or infected and none treated at all (group 2).

Orego-stim® is a phytobiotic contains Oreganum aetheroleum as active substance. Oreganum aetheroleum is oregano etheric oil obtained by steam distillation of the leaves and flowers of the plant *Origanum vulgare ssp. hirtum* and contains many essential oils, mainly *carvacrol* 81.89%, *y- terpinrnr* 5.1%, *p- cymene* 3.76% and *thymol* 2.12% to which the broad spectrum antimicrobial activity of *Origanum* oil is attributed ***(Knobloch et al., 1989; Bendahou et al., 2008)***. Its antimicrobial activities against bacteria, yeast and fungi such as, *E. coli*, *Pseudomonas aeruginosa*, *Staph. aureus*, *Enterococcus hirae*, *Candida albicans* and *Candida tropicalis* was proved ***(Sari et al., 2006)*** and recognized since antiquity ***(Leung and Fostere , 1996)***.

It has been suggested that phenolic derivatives can cause membrane-disturbing activities that change the permeability of cations such as H^+ and K^+ (***Ultee et al., 1999; Ipek et al., 2005)***. Their hydrophobic characters 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).***

Hydrophobicity of carvacrol, the essential oil of the highest amount in *O. vulgare*, and its mode of action suggest the partition in the cytoplasm membrane ***(Ultee et al., 2002)***. Exposure of bacterial cells to carvacrol increases the membrane fluidity causing leakage of protons and potassium ions, leading to a decrease in pH gradient across the cytoplasm membrane, a collapse of the membrane potential, an inhibition of ATP synthesis and ultimately the cell death ***(Ultee et al., 1998; Ultee et al., 2000)***. Addition of carvarcrol to bacterial cell suspensions causes a dose-related extension of the lag-phase, a lower maximum specific growth rate, and a lower final population density ***(Lambert et al., 2001)***.

Sublethal injury of microbial cell membrane provided by subinhibitory concentrations of antimicrobial compounds may alter their permeability and affect the ability of the membrane to osmoregulate the cell adequately or to exclude toxic material ***(Carson et al., 2002)***. The loss of salty tolerance could reveal membrane damage in sublethally injured *Staph. aureus* cells caused by *O. vulgare* essential oil.

Concerning the effect of Orego-stim® on F.I., B.W. and F.C.R., our results revealed that, birds belonged to groups (6) and (5) showed more favorable results compared with those belonged to groups (3) and (4) respectively.

Several researchers showed that, supplementation of some essential oils stimulates the animal digestive systems ***(Ciftci et al., 2005)*** to increase production of digestive enzymes and improve utilization of digestive products through enhanced liver functions ***(Hermandez et al., 2004)***. Thus**,** improves feed intake, feed conversion ratio ***(Alcicek et al., 2003; Halle et al., 2004; Cross et al., 2007)***, promoting a better sedimentation of muscle protein ***(Zheng et al., 2009)***, and so improves the live body weight ***(Denli and Uluocak, 2004)***.

On the other hand, it was found that the probable beneficial effect produced by thymol essential oil was its nutrient digestibility ***(Langhout, 2000).*** Likewise ***Hernandez et al. (2004)*** suggested that plant extract supplementation improved apparent whole- tract and ileal digestibility of nutrients.

In addition to the antimicrobial activity of essential oils ***(Valero and Salmeron, 2003)*** they posses biological activities such as that of antioxidants ***(Miura et al., 2002; Zheng et al., 2009)*** and as hypocholesterolemics ***(Craig, 1999)*** that enhances growth performances.

Our findings fit in with the results obtained by ***Alcicek et al. (2003)*** who found that, supplementation of Orego-stim® improved the F.C.R. in broilers which may attributed to the stimulation of digestion induced by Orego-stim® .Other study reported that, essential oils derived from different aromatic plants improved weight gain and F.C.R. ***(Giannenas et al., 2003; Jamroz et al., 2005).***

Regarding the effect of Orego-stim® on the immune response, the results showed that, birds belonged to groups (6, 5) showed significant increase in the mean values of phagocytic activity & phagocytic index compared with those belonged to groups (3, 4) respectively on the 15th, 29th and 36th day of age and birds belonged to groups (3, 6) showed significant increase in the mean values of IgM compared with control –ve group.

As a matter of fact, however plants and their bioactive components, when known, are very diverse and their potential to enhance animal health and immunity has only been scarcely evaluated *in vivo*. Mixtures of essential oils based on thymol and carvacrol, whose major sources are thyme and oregano respectively ***(Burt, 2004)*** seem promising

due to their potential immunomodulatory properties ***(Woollard et al., 2007; Gabor et al., 2010)***.

It was found that, extract of *Origanum vulgare*, enriched with thymol and carvacrol in similar proportions, was reported to protect animals from diseases. That health benefit was associated with an increased proportion of CD4+, CD8+ and double positive T cells in peripheral blood and mesenteric lymph nodes ***(Walter and Bilkei, 2004).*** Thymol used alone enhances total IgA and IgM serum levels and exhibits some local anti-inflammatory properties, as indicated by a reduction in TNF-mRNA in the stomach of post-weaned pigs ***(Trevisi et al., 2007).***

In this frame of references, it is fitting to mention that, 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)***.

Table (1): Effects of oral administration of Orego-stim®; 0.3ml / liter and Enrofloxacin (Opitryl®; 10 mg/kg kg B.W. of birds (1 ml / 2 liter) on growth performance (Body weight "B.W.", Feed Intake "F.I." and Food conversion rate " F.C.R.") in chicks experimentally infected with *E. coli*.

Age day	Group 1			Group 2			Group 3			Group 4			Group 5			Group 6		
	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.	B.W. (gm)	F.I. (gm)	F.C.R.
7	135.0± 2.88^{A}	123.0± 1.73^{C}	0.91± 0.02^{C}	110.0± 2.88^{B}	109.0± 2.30^{D}	0.99± 0.005^{ABC}	136.6± 3.17^{A}	145.0± 2.88^{A}	1.06± 0.005^{A}	129.0± 5.19^{A}	130.0± 5.77^{BC}	1.006± 0.003^{ABC}	129.0± 10.96^{A}	134.0± 2.30^{B}	1.050± 0.08^{AB}	128.0± 4.61^{A}	120.0± 2.88^{C}	0.93± 0.008^{BC}
14	323.8± 1.73^{BC}	380.0± 2.30^{C}	1.17± 0.005^{C}	288.0± 1.73^{D}	403.2± 1.84^{B}	1.36± 0.03^{B}	317.5± 4.33^{C}	305.0± 2.88^{D}	0.96± 0.005^{D}	337.0± 4.04^{A}	460.0± 2.88^{A}	1.36± 0.01^{B}	330.5± 3.17^{AB}	470.0± 5.77^{A}	1.41± 0.01^{A}	315.5± 2.74^{C}	305.0± 2.88^{D}	0.96± 0.003^{D}
21	686.0± 1.15^{A}	841.0± 1.73^{C}	1.22± 0.003^{D}	623.8± 1.73^{C}	1035.5± 2.88^{A}	1.65± 0.003^{A}	690.0± 2.88^{A}	755.0± 1.73^{E}	1.09± 0.003^{E}	644.7± 1.90^{B}	870.0± 5.77^{C}	1.34± 0.01^{C}	646.2± 3.40^{B}	890.0± 2.30^{B}	1.37± 0.006^{B}	630.0± 5.77^{C}	680.0± 8.66^{F}	1.07± 0.01^{E}
28	913.3± 1.55^{A}	1530± 4.61^{B}	1.67± 0.003^{B}	872.2± 4.15^{AB}	2023.7± 5.05^{A}	2.32± 0.005^{A}	850.0± 5.77^{D}	1363± 1.73^{E}	1.60± 0.011^{C}	856.3± 3.29^{BC}	1438± 4.61^{D}	1.68± 0.005^{B}	878.5± 4.90^{B}	1460± 2.88^{C}	1.65± 0.008^{B}	874.6± 10.91^{BC}	1293± 1.73^{F}	1.47± 0.01^{D}
35	1283.3± 1.70^{B}	2752.2± 1.30^{B}	2.14± 0.003^{C}	1137.8± 4.5^{E}	2958± 4.56^{A}	2.59± 0.008^{A}	1146.0± 3.46^{E}	2585± 2.88^{E}	2.25± 0.01^{B}	1189.0± 5.19^{D}	2690± 5.77^{C}	2.26± 0.005^{B}	1312.0± 1.15^{A}	2665± 2.88^{D}	2.03± 0.003^{D}	1239.0± 5.19^{C}	2495± 2.88^{F}	2.01± 0.005^{E}
42	1650.0± 5.77^{A}	4139.7± 5.08^{A}	2.50± 0.008^{E}	1400.0± 2.88^{E}	4032± 4.04^{B}	2.88± 0.005^{A}	1380.0± 11.54^{F}	3680± 5.77^{E}	2.66± 0.02^{C}	1420.0± 2.88^{D}	3865± 2.30^{C}	2.72± 0.005^{B}	1490.0± 1.15^{B}	3765± 2.88^{D}	2.52± 0.003^{DE}	1444.4± 2.66^{C}	3756± 0.63^{D}	2.56± 0.03^{D}

Group (1) Non infected, non treated chicks (-ve control) Group (2) *E. coli* infected chicks (+ve control)

Group (3) Infected chicks & treated with the Orego-stim® Group (4) Infected chicks& treated with the therapeutic dose of enrofloxacin (Opitryl)®

Group (5) Infected chicks& treated with Orego-stim® and therapeutic dose of enrofloxacin (Opitryl)®

Group (6) Chicks given Orego-stim® from the 1st day old throughout the experimental period and infected with *E. coli* on the 5th day of age

Means carrying different superscripts in the same column are significant at $p< 0.05$

In this context, ***Toshifumi et al. (1995)*** determined phagocyte percentage, phagocyte index as parameters of phagocyte function. They recorded that neutrophilic activity is less active than that of opsonin. These parameters are significantly stimulated by Thymol, 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 *Origanum vulgaris* modulate the neutrophils immune function.

The current work demonstrated that, chicken in groups (3, 4, 5, and 6) showed significant decrease in the mean values of serum ALT & AST , uric acid and creatinine levels compared with group (2). Birds belonged to groups (6) demonstrated significant decrease in the mean values of serum ALT & AST, uric acid and creatinine levels compared with group (3) and chicks in group (5) showed significant decrease in the mean values of serum AST; uric acid and creatinine levels compared with group (4).

Generally AST and ALT considered as liver enzyme which increased with liver damage (heptatocellular degeneration), so the decrease in AST and ALT may provide evidence for the occurrence of hepatoprotective effect of the essential oils ***(Hermandez et al., 2004)***.

Table 2: Effects of oral administration of Orego-stim®; 0.3ml / liter and Enrofloxacin (Opitryl)®; 10 mg/kg B.W. of birds (1 ml / 2 liter) on Phagocytic (activity & index) and serum levels of IgM & IgG (mg/dl) in chicks experimentally infected with *E. coli*.

Groups	Phagocytic activity (%)				Phagocytic Index (P.I)				IgM	IgG
	Age in days				Age in days				Age in days	
	15th	22nd	29th	36th	15th	22nd	29th	36th	15th	22nd
1	8.82± 0.3^{E}	8.65± 0.41^{E}	8.08± 0.42^{D}	7.63± 0.15^{C}	0.92± 0.02^{D}	1.01± 0.06^{E}	0.91± 0.03^{D}	0.86± 0.03^{CD}	5.06± 0.52^{CD}	42.9± 1.0^{BC}
2	12.68± 0.59^{D}	11.44± 0.35^{D}	8.49± 0.55^{D}	7.03± 0.07^{C}	1.40± 0.12^{C}	1.26± 0.04^{C}	0.84± 0.06^{D}	0.74± 0.01^{D}	13.43± 0.61^{A}	49.2± 0.43^{A}
3	20.86± 2.43^{B}	23.33± 0.59^{B}	23.65± 1.2^{B}	19.68± 0.92^{B}	1.75± 0.05^{B}	1.86± 0.08^{AB}	1.81± 0.04^{B}	1.51± 0.03^{B}	10.13± 0.18B	43.4± 1.2^{BC}
4	9.08± 0.63^{DE}	8.28± 0.57^{DE}	8.57± 0.3^{D}	8.68± 0.32^{C}	1.23± 0.13^{C}	1.19± 0.16^{D}	1.43± 0.10^{C}	1.10± 0.08^{C}	4.26± 0.86^{D}	39.1± 0.52^{C}
5	16.51± 0.68^{C}	16.78± 0.21^{C}	17.63± 0.57^{C}	17.95± 1.03^{B}	1.46± 0.10^{C}	1.57± 0.07^{B}	1.59± 0.07^{BC}	1.48± 0.01^{B}	6.10± 0.75^{C}	42.8± 1.9^{BC}
6	29.34± 1.24^{A}	29.66± 0.36^{A}	29.9± 0.84^{A}	30.64± 1.23^{A}	2.07± 0.06^{A}	2.09± 0.12^{A}	2.25± 0.16^{A}	2.43± 0.23^{A}	9.06± 0.40^{AB}	46.8± 3.1^{AB}

Group (1) Non infected, non treated chicks (-ve control) Group (2) *E. coli* infected chicks (+ve control)

Group (3) Infected chicks & treated with the Orego-stim® Group (4) Infected chicks& treated with the therapeutic dose of enrofloxacin (Opitryl)®

Group (5) Infected chicks& treated with Orego-stim® and therapeutic dose of enrofloxacin (Opitryl)®

Group (6) Chicks given Orego-stim® from the 1st day old throughout the experimental period and infected with E. coli on the 5th day of age

Means carrying different superscripts in the same column are significant at $p< 0.05$.

Eva et al. (2006) noticed the DNA-protective effects of essential oils on hepatoma HepG2. They reduced the level of DNA damage induced by hydrogen peroxide (H_2O_2) associated with their antioxidant activity.

In the face of the fact that, plant volatile compounds appear to accumulate in the cell membrane and increase permeability, resulting in leakage of enzymes and metabolites ***(Tsai et al., 2007)***. In addition, they posses biological activities such as that of antioxidants ***(Miura et al., 2002)*** enhanced liver and kidney functions ***(Hermandez et al., 2004)***.

In this context, it is akin to mention that, aromatic plants and their essential oils can be used as antibacterial and hepato and renoprotective supplement in the developing countries towards the development of new therapeutic agents ***(Suvajdzic et al., 2006; Sylvestre et al. 2006)***.

Table (3): Effects of oral administration of Orego-stim®; 0.3ml / liter and Enrofloxacin(Opitryl)®;10 mg/kg B.W. of birds (1 ml / 2 liter) on serum total proteins, albumin, ALT and AST levels (IU/L) in chicks experimentally infected with *E. coli*.

Groups	Total Protein				Albumin				ALT				AST			
	Age in days				Age in days				Age in days				Age in days			
	15th	22nd	29th	36th	15th	22nd	29th	36th	15th	22nd	29th	36th	15th	22nd	29th	36th
1	2.64± 0.15^{C}	2.83± 0.14^{A}	2.33± 0.08^{A}	2.63± 0.08^{A}	1.42± 0.01^{A}	1.39± 0.03^{A}	1.45± 0.02^{A}	1.45± 0.02^{A}	27.3± 0.33^{D}	27.6± 1.45^{C}	26.6± 1.45^{A}	27.6± 0.33^{A}	67.3± 0.88^{D}	68.6± 1.85^{D}	64.0± 2.08^{A}	68.3± 1.20^{A}
2	3.82± 0.09^{A}	3.60± 0.17^{A}	2.43± 0.33^{A}	2.90± 0.23^{A}	1.00± 0.05^{B}	1.41± 0.01^{A}	1.42± 0.06^{A}	1.41± 0.02^{A}	41.6± 0.88^{A}	41.3± 1.85^{A}	28.0± 1.52^{A}	25.3± 0.66^{A}	86.3± 2.18^{A}	91.3± 1.85^{A}	64.3± 1.20^{A}	69.3± 1.45^{A}
3	3.00± 0.05^{B}	3.13± 0.18^{A}	2.40± 0.23^{A}	2.60± 0.11^{A}	1.38± 0.04^{A}	1.38± 0.04^{A}	1.42± 0.01^{A}	1.44± 0.02^{A}	35.6± 1.76^{B}	33.6± 1.85^{B}	25.6± 0.88^{A}	26.3± 0.88^{A}	76.6± 1.20^{B}	85.0± 0.57^{B}	65.0± 3.05^{A}	69.0± 4.93^{A}
4	1.93± 0.06^{D}	2.53± 0.29^{A}	2.23± 0.14^{A}	2.53± 0.14^{A}	1.40± 0.05^{A}	1.38± 0.03^{A}	1.43± 0.03^{A}	1.44± 0.03^{A}	32.0± 1.52^{C}	33.0± 0.57^{B}	25.3± 1.45^{A}	26.3± 1.45^{A}	73.6± 1.8^{BC}	79.6± 1.45^{C}	63.3± 2.84^{A}	70.0± 2.88^{A}
5	3.23± 0.08^{B}	2.96± 0.26^{A}	2.33± 0.17^{A}	2.40± 0.25^{A}	1.42± 0.01^{A}	1.40± 0.005^{A}	1.44± 0.03^{A}	1.43± 0.01^{A}	29.0± 0.5^{CD}	30.6± 0.3^{BC}	25.3± 2.40^{A}	28.0± 1.52^{A}	71.3± 1.8^{CD}	72.3± 1.45^{D}	64.6± 1.45^{A}	68.0± 2.30^{A}
6	3.27± 0.03^{B}	3.20± 0.35^{A}	2.40± 0.17^{A}	2.46± 0.26^{A}	1.42± 0.01^{A}	1.39± 0.02^{A}	1.44± 0.03^{A}	1.44± 0.02^{A}	28.6± 0.3^{CD}	31.0± 0.5^{BC}	27.0±2.08^{A}	28.3± 1.66^{A}	69.3± 0.3^{CD}	71.0± 1.15^{D}	65.3± 0.66^{A}	69.3± 4.17^{A}

Group (1) Non infected, non treated chicks (-ve control) Group (2) *E. coli* infected chicks (+ve control)

Group (3) Infected chicks & treated with the Orego-stim® Group (4) Infected chicks& treated with the therapeutic dose of enrofloxacin (Opitryl)®

Group (5) Infected chicks& treated with Orego-stim® and therapeutic dose of enrofloxacin (Opitryl)®

Group (6) Chicks given Orego-stim® from the 1st day old throughout the experimental period and infected with E. coli on the 5th day of age

Means carrying different superscripts in the same column are significant at $p< 0.05$

Table (4): Effects of oral administration of Orego-stim®; 0.3ml / liter and Enrofloxacin(Opitryl)®; 10 mg/kg of B.W. of birds1 ml / 2 liter) on serum uric acid and creatinine levels (mg/dl) in chicks experimentally infected with *E.coli*.

Groups	Uric A				Creatinine			
	Age in days				Age in days			
	15th	22nd	29th	36th	15th	22nd	29th	36th
1	8.63± 0.3^{CD}	8.26± 0.14^{C}	7.60± 0.30^{A}	7.50± 0.28^{A}	0.45± 0.01^{D}	0.48± 0.01^{D}	0.54± 0.02^{A}	0.53± 0.02^{A}
2	12.5± 0.81^{A}	12.6± 0.37^{A}	7.36± 0.32^{A}	7.53± 0.35^{A}	0.97± 0.06^{A}	0.90± 0.02^{A}	0.52± 0.03^{A}	0.54± 0.01^{A}
3	10.3± 0.6^{BC}	10.5± 0.28^{B}	7.60± 0.55^{A}	7.63± 0.40^{A}	0.68± 0.03^{C}	0.64± 0.03^{C}	0.52± 0.01^{A}	0.53± 0.02^{A}
4	11.5± 0.5^{AB}	11.2± 0.14^{B}	7.83± 0.57^{A}	7.73± 0.61^{A}	0.80± 0.02^{B}	0.71± 0.005^{B}	0.52± 0.04^{A}	0.53± 0.008^{A}
5	8.56± 0.3^{CD}	8.36± 0.18^{C}	7.46± 0.26^{A}	7.43± 0.40^{A}	0.52± 0.01^{D}	0.48± 0.01^{D}	0.55± 0.01^{A}	0.53± 0.02^{A}
6	8.26± 0.68^{D}	8.66± 0.44^{C}	7.50± 0.17^{A}	7.16± 0.38^{A}	0.48± 0.02^{D}	0.48± 0.01^{D}	0.53± 0.02^{A}	0.52± 0.03^{A}

Group (1) Non infected, non treated chicks (-ve control) Group (2) *E. coli* infected chicks (+ve control)

Group (3) Infected chicks & treated with the Orego-stim® Group (4) Infected chicks& treated with the therapeutic dose of enrofloxacin (Opitryl)®

Group (5) Infected chicks& treated with Orego-stim® and therapeutic dose of enrofloxacin(Opitryl)®

Group (6) Chicks given Orego-stim® from the 1st day old throughout the experimental period and infected with *E. coli* on the 5th day of age Means carrying different superscripts in the same column are significant at $p< 0.05$.

Conclusion

From the obtained results it could be concluded that, Orego-stim® can be considered a promising mixture of essential oils due to its high efficacy (growth performance, antibacterial and immunomodulating effects) and positive impact on both liver and kidney functions that may provide evidence for the hepato and renoprotective effects of the essential oils. The study highly recommend the use of Orego-stim® as a prophylactic agent in dealing with *E. coli* infection in chicken however, its concurrent administration with enrofloxacin in treatment of such case revealed the most favorable outcomes .

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7/20/2011