

## Clinicopathological Studies on the Effect of *Origanum majorana* in Broilers

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### Abstract

The objective of the present study was to evaluate the effect of *E. coli* on serum biochemistry and to determine the preventive effect of adding marjoram as growth promoters in 2 concentrations (0.5% & 1%/kg ration) on serum biochemistry. This study was carried out on 150 one day- old chicks to evaluate the effect of dietary supplementation of 0.5 and 1% *origanum majorana* for 6 weeks on broilers and the possibility of facing the experimental infection with *E. coli* O78. In treated groups with marjoram 1%, biochemical results revealed increase in the total protein and total globulin and decrease in AST, ALT and uric acid in 4<sup>th</sup> and 6<sup>th</sup> weeks of the experiment. In addition to significant decrease in serum cholesterol, triglyceride, LDL and increase in HDL, while, serum albumin showed non-significant changes compared to control group. Regarding to the infected group and group infected and treated with 0.5% marjoram, increased total protein, total globulin, AST, ALT, glucose, cholesterol and uric acid were observed

### Introduction:-

In modern broiler management, preventive measures are taken to control diseases and bacterial enteritis, which reduce feed utilization and live performance. Natural growth promoters feed additives are frequently used for this purpose (Zohair, 2006). The genus *Origanum majorana* L. is an aromatic, perennial, herbaceous plant belonging to the family *Lamiaceae*. The plant has been used as a flavoring and herbal spice from time immemorial. Steam distillation of leaves and flower heads yields a volatile oil, known in the trade as oil of sweet marjoram, widely used in flavoring food and also in

perfumery. Medicinally it is used in both Ayurveda and Yunani system to cure various human ailments (Leeja and Thoppil, 2007). Marjoram (*Origanum majorana*) is a very popular and a common medicinal plant (Ezz El-Arab, 2008). The plant is pungent, bitter, hot, stomachic, anthelmintic, useful in diseases of the heart and blood, fevers and inflammation (Kirtikar and Basu, 1985). It is reported to possess antibacterial activity (Farooqi and Sreeramu, 2004). Different experimental *E. coli* infections have been described: septicemia, enteritis, granuloma, omphalitis, sinusitis, airsacculitis, arthritis, synovitis, peritonitis,

pericarditis, cellulitis, swollen head syndrome, etc. More frequently, *E. coli* disease occurs as a consequence of the adverse influence of factors such as ammonia, moisture, dust, hormones or infectious agents such as viruses and mycoplasmas (*Leitner and Heller, 1992*). The aim of the present work is to investigate the effect of marjoram as growth promoters in 2 doses (0.5 & 1% of ration) on body weight, hematology, blood biochemistry, immunological and pathological lesions which may appear as result of long use in normal and *E.coli* infected broilers.

### Material and Methods

One hundred and fifty one day- old broiler chicks, Isa Hubbard breed were obtained from El-dakhlea Poultry Company, Mit Ghamr City, Egypt. Chicks were reared in litter under standard environmental and hygienic conditions. The temperature was adjusted according to the age (the first week 32°C and decreased 2°C per week), (*Harrison and Harrison, 1986*). Chicks were fed on a balanced commercial ration (basal diet) and water *ad libitum*. All chickens were vaccinated against Newcastle disease at 7, 13 and 26 days old and against Gumboro disease at 15 and 24 days old (*Giambrone and Ronald, 1986*).

### Sampling:

The blood samples were obtained from each bird from the wing vein.

About 3 ml of blood was collected in a plain centrifuge tube without anticoagulant and was used for preparation of serum. After collection of the whole blood, allow the blood to clot by leaving it undisturbed for 15 – 30 minutes at room temperature. Clear serum was separated after centrifugation of clotted blood sample at 1000-2000rpm for 10minutes

It was kept at refrigerator at +4c until biochemical studies.

### Serum biochemical parameters:

**1- Enzymes activities:** The serum alanine aminotransferase and aspartate amino transfrase (ALT and AST) activities were determined calorimetrically according to the method of *Reitmans and Frankel (1957)*.

**2-Total protein (TP):** Serum TP was determined using the Biuret method after *Gronall et al. (1949)*.

**3- Albumin:** Albumin was determined according to *Bablok et al. (1988)*.

**4- Globulin:** Was determined by subtracting the serum albumin from total serum protein according to *Doumas and Biggs (1972)*.

**5-Glucose:** Serum glucose was determined according to *Werner et al. (1970)*.

### 6-Determination of serum lipid profile:

**A- Total cholesterol (T.C):** Serum cholesterol level was determined according to *Rich mond (1973)*.

**B- Triglycerides:** Serum triacylglycerol level was

determined according to *Fossati and Principe (1982)*.

**C- High density lipoprotein – cholesterol (HDL-C):** High density lipoprotein – cholesterol was determined according to *Burstien et al. (1970)*.

**7- Uric acid:** Serum uric acid was determined according to *Caraway (1963)*.

**Statistical analysis:**

Data collected from the hematological and serum biochemical analysis of treated groups of chicks were statistically analyzed in comparison to control group for the mean and standard error. Means separation and pairwise comparisons were done by Duncan's Multiple Range test. Statistical analyses were conducted by SPSS for windows. *Tamhane and Dunlop (2000)*.

**Table (1) Experimental design:-**

*Chicks were divided into 6 groups as shown in the following table:*

group	Treatment/ 6weeks	Duration and samples collection
<b>Group I</b>	Chicks received a basal diet only and kept as control without any treatment.	The samples were collected from all experimental groups at 2 <sup>nd</sup> , 4 <sup>th</sup> and 6 <sup>th</sup> weeks post treatment. Whole blood sample for complete blood picture investigation. Serum sample for estimation of some biochemical and Immunological parameters.
<b>Group II</b>	Chicks received a basal diet containing <i>Origanum majorana</i> 0.5% in feed all over the experimental period.	
<b>Group III</b>	Chicks received a basal diet containing <i>Origanum majorana</i> 1% in feed all over the experimental period	
<b>Group IV</b>	Chicks received a basal diet and infected with <i>E.coli</i> at 21 days of age.	
<b>Group V</b>	Chicks received a basal diet containing <i>Origanum majorana</i> 0.5% in feed all over the experimental period, then infected with <i>E.coli</i> at 21 days of age.	
<b>Group VI</b>	Chicks received a basal diet containing <i>Origanum majorana</i> 1% in feed all over the experimental period and infected with <i>E.coli</i> at 21 days of age.	

**Results:-****After Four Weeks (1 week post infection)**

Concerning biochemical changes as showed in table (2), serum AST and ALT were significantly increased in groups IV and V compared with the control group, while other groups were non significantly different. Groups IV, V and VI showed significant increases in total protein and globulin compared with the control group. Also, II and III groups showed significant increases in total protein and globulin in comparison with control group but less than the increase of infected groups. But there was non significant difference in serum albumin level between all treated groups. Groups IV and V showed a significant increase in serum glucose level in comparison with the control group, while other groups were non-significantly different in comparison with that of the control group. Groups IV and V showed significant increases in cholesterol, TG and LDL in comparison with control group, while HDL was significantly decreased when compared with the control group. While other groups showed non-significant change in lipid profile when compared with the control group. Serum uric acid revealed a significant increase in groups IV and V while other groups were non-significantly different in comparison with the control group.

**After Six Weeks:-**

Concerning biochemical changes as showed in table (3), serum AST and ALT were significantly increased in groups IV and V when compared to control one, meanwhile the other groups were no significantly changed. Total protein was significantly increased in groups IV and V when compared to control one, while other groups were in significantly different in comparison with the control one. Globulin was significantly increased in groups II, III, IV, V and VI when compared to control one. But there was no significant difference in serum albumin level between all different treated groups. Glucose was significantly increased in groups IV and V when compared to control one, while other groups were non- significantly different in comparison with the control one. Groups IV and V showed significant increases in cholesterol, TG and LDL while a significant decrease in HDL in comparison with control group. While other groups were non-significantly different in comparison with the control one. IV and V groups revealed a significant increase in uric acid compared with the control group. Other groups were non-significantly different in comparison with the control group.

**Table 2:** The effect of different dietary levels of *Origanum majorana* (0.5 and 1%) on some serum biochemical parameters (mean  $\pm$ SE) in normal and *E. coli* experimentally infected chicks after four weeks .

Group Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
AST(U/I)	31.20 <sup>b</sup> $\pm$ 0.58	30.20 <sup>b</sup> $\pm$ 0.58	29.90 <sup>b</sup> $\pm$ 0.37	41.60 <sup>a</sup> $\pm$ 0.51	40.00 <sup>a</sup> $\pm$ 0.44	29.99 <sup>b</sup> $\pm$ 0.37
ALT(U/I)	8.60 <sup>b</sup> $\pm$ 0.17	8.46 <sup>b</sup> $\pm$ 0.07	8.56 <sup>b</sup> $\pm$ 0.01	11.20 <sup>a</sup> $\pm$ 0.58	10.26 <sup>a</sup> $\pm$ 0.17	8.63 <sup>b</sup> $\pm$ 0.09
Total protein (gm /dl)	4.19 <sup>b</sup> $\pm$ 0.01	5.08 <sup>a</sup> $\pm$ 0.01	5.16 <sup>a</sup> $\pm$ 0.02	5.37 <sup>a</sup> $\pm$ 0.01	5.26 <sup>a</sup> $\pm$ 0.01	5.20 <sup>a</sup> $\pm$ 0.01
Albumin (gm/dl)	1.09 <sup>a</sup> $\pm$ 0.01	1.08 <sup>a</sup> $\pm$ 0.01	1.09 <sup>a</sup> $\pm$ 0.01	1.06 <sup>a</sup> $\pm$ 0.01	0.96 <sup>a</sup> $\pm$ 0.01	1.13 <sup>a</sup> $\pm$ 0.01
Globulin (gm/dl)	3.10 <sup>b</sup> $\pm$ 0.01	4.00 <sup>a</sup> $\pm$ 0.10	4.07 <sup>a</sup> $\pm$ 0.03	4.31 <sup>a</sup> $\pm$ 0.01	4.30 <sup>a</sup> $\pm$ 0.01	4.07 <sup>a</sup> $\pm$ 0.01
Glucose (mg/dl)	207.00 <sup>b</sup> $\pm$ 1.04	200.00 <sup>b</sup> $\pm$ 0.95	199.80 <sup>b</sup> $\pm$ 0.95	306.80 <sup>a</sup> $\pm$ 1.93	299.07 <sup>a</sup> $\pm$ 0.95	199.40 <sup>b</sup> $\pm$ 0.51
Cholesterol (mg/dl)	148.80 <sup>b</sup> $\pm$ 1.12	140.20 <sup>b</sup> $\pm$ 0.80	145.40 <sup>b</sup> $\pm$ 1.07	164.60 <sup>a</sup> $\pm$ 1.21	161.60 <sup>a</sup> $\pm$ 0.58	144.20 <sup>b</sup> $\pm$ 1.07
Triglycerides (mg/dl)	91.60 <sup>b</sup> $\pm$ 0.60	89.80 <sup>b</sup> $\pm$ 0.66	85.80 <sup>b</sup> $\pm$ 0.80	122.20 <sup>a</sup> $\pm$ 0.86	120.60 <sup>a</sup> $\pm$ 0.51	89.99 <sup>b</sup> $\pm$ 0.51
HDL (mg/dl)	84.40 <sup>a</sup> $\pm$ 0.60	82.00 <sup>a</sup> $\pm$ 0.84	84.20 <sup>a</sup> $\pm$ 0.58	59.80 <sup>b</sup> $\pm$ 0.58	60.00 <sup>b</sup> $\pm$ 0.94	83.00 <sup>a</sup> $\pm$ 0.95
LDL(mg/dl)	46.08 <sup>b</sup> $\pm$ 1.45	40.24 <sup>b</sup> $\pm$ 1.14	44.04 <sup>b</sup> $\pm$ 1.22	80.36 <sup>a</sup> $\pm$ 1.37	77.48 <sup>a</sup> $\pm$ 1.46	43.20 <sup>b</sup> $\pm$ 0.86
Uric acid(mg/dl)	5.86 <sup>b</sup> $\pm$ 0.11	4.88 <sup>b</sup> $\pm$ 0.07	4.99 <sup>b</sup> $\pm$ 0.04	9.46 <sup>a</sup> $\pm$ 0.06	8.99 <sup>a</sup> $\pm$ 0.11	5.20 <sup>b</sup> $\pm$ 0.10

Within the same row means with different superscripts are highly significantly differ ( $P \leq 0.01$ ).

**Table 3:** The effect of different dietary levels of *Origanum majorana* (0.5 and 1%) on some serum biochemical parameters (mean  $\pm$ SE) on normal and *E. coli* experimentally infected chickens after six weeks

Group Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
AST(U/I)	32.60 <sup>b</sup> $\pm$ 0.51	31.90 <sup>b</sup> $\pm$ 0.51	33.09 <sup>b</sup> $\pm$ 0.71	54.00 <sup>a</sup> $\pm$ 0.32	50.40 <sup>a</sup> $\pm$ 0.51	32.80 <sup>b</sup> $\pm$ 0.71
ALT(U/I)	7.90 <sup>b</sup> $\pm$ 0.12	7.87 <sup>b</sup> $\pm$ 0.07	7.80 <sup>b</sup> $\pm$ 0.04	13.60 <sup>a</sup> $\pm$ 0.51	12.68 <sup>a</sup> $\pm$ 0.09	7.66 <sup>b</sup> $\pm$ 0.08
Total protein (gm/dl)	5.09 <sup>b</sup> $\pm$ 0.03	5.90 <sup>b</sup> $\pm$ 0.01	6.00 <sup>b</sup> $\pm$ 0.02	6.94 <sup>a</sup> $\pm$ 0.01	6.32 <sup>a</sup> $\pm$ 0.01	5.90 <sup>b</sup> $\pm$ 0.01
Albumin (gm/dl)	1.09 <sup>a</sup> $\pm$ 0.01	1.08 <sup>a</sup> $\pm$ 0.01	1.06 <sup>a</sup> $\pm$ 0.01	0.99 <sup>a</sup> $\pm$ 0.01	1.06 <sup>a</sup> $\pm$ 0.01	0.99 <sup>a</sup> $\pm$ 0.01
Globulin (gm /dl)	4.00 <sup>b</sup> $\pm$ 0.02	4.82 <sup>a</sup> $\pm$ 0.01	4.94 <sup>a</sup> $\pm$ 0.02	5.95 <sup>a</sup> $\pm$ 0.01	5.26 <sup>a</sup> $\pm$ 0.01	4.91 <sup>a</sup> $\pm$ 0.01
Glucose (mg/dl)	206.60 <sup>b</sup> $\pm$ 0.68	204.60 <sup>b</sup> $\pm$ 0.51	200.80 <sup>b</sup> $\pm$ 0.58	300.00 <sup>a</sup> $\pm$ 0.45	300.20 <sup>a</sup> $\pm$ 0.58	200.60 <sup>b</sup> $\pm$ 1.02
Cholesterol (mg/dl)	182.60 <sup>b</sup> $\pm$ 0.51	179.20 <sup>b</sup> $\pm$ 0.96	176.40 <sup>b</sup> $\pm$ 0.74	213.40 <sup>a</sup> $\pm$ 0.51	209.00 <sup>a</sup> $\pm$ 0.54	181.40 <sup>b</sup> $\pm$ 1.14
Triglycerides (mg/dl)	95.80 <sup>b</sup> $\pm$ 0.58	93.00 <sup>b</sup> $\pm$ 0.94	94.60 <sup>b</sup> $\pm$ 0.67	183.00 <sup>a</sup> $\pm$ 0.71	179.80 <sup>a</sup> $\pm$ 0.58	93.80 <sup>b</sup> $\pm$ 0.37
HDL (mg/dl)	91.99 <sup>a</sup> $\pm$ 0.37	91.80 <sup>a</sup> $\pm$ 0.96	92.80 <sup>a</sup> $\pm$ 0.58	69.40 <sup>b</sup> $\pm$ 0.51	63.60 <sup>b</sup> $\pm$ 0.51	90.94 <sup>a</sup> $\pm$ 0.51
LDL (mg/dl)	71.45 <sup>b</sup> $\pm$ 0.49	68.80 <sup>b</sup> $\pm$ 0.00	64.68 <sup>b</sup> $\pm$ 0.37	107.40 <sup>a</sup> $\pm$ 0.32	109.44 <sup>a</sup> $\pm$ 0.93	71.70 <sup>b</sup> $\pm$ 0.58
Uric acid (mg/dl)	5.00 <sup>b</sup> $\pm$ 0.07	4.36 <sup>b</sup> $\pm$ 0.06	4.38 <sup>b</sup> $\pm$ 0.11	10.62 <sup>a</sup> $\pm$ 0.12	9.90 <sup>a</sup> $\pm$ 0.09	4.95 <sup>b</sup> $\pm$ 0.07

Within the same row means with different superscripts are highly significantly differ ( $P \leq 0.01$ ).

## Discussion

Regarding the serum biochemical study, serum AST and ALT activities are good indicators of the liver function and health. The increased activities of these enzymes mainly reflect liver damage and hepatocellular degeneration with leakage of enzymes into blood stream, this result agreed with that reported by *Kubena et al. (1995)*. The result showed that, there were significant increase in the levels of AST and

ALT in chicken infected with *E. coli*, these results came in agreement with *Manimaran et al. (2003)* who found that the levels of serum enzymes like AST and ALT were significantly increased at 3<sup>rd</sup> week post infection as compared to control. These elevations might be due to hepatic injury during the endotoxin of *E. coli* bacterial toxin (*Marcel, 1994*). On the other hand, the improvement in levels of AST and ALT activities which observed in infected treated chicks compared

to the infected non-treated (IV) might be due to the presence of isoflavones, polyphenols as antioxidant in marjoram (*EL-Ashmawy et al.*, 2005). Supplementation of *O.majorana* did not significantly change in the AST and ALT values compared to the control group and this was similar to finding of *Mona et al.* (2010). Data of plasma protein results revealed a significant increase in total proteins of *E. coli* infected chicks and in infected and treated chicks at the 4<sup>th</sup> and 6<sup>th</sup> weeks. This may be due to increased level of globulin not albumin, this suggestion came in agreement with *Wafaa and Ismail* (2013) who found a significant increase in total protein and globulin levels in chickens challenged with *E. coli*. Also, *O.majorana* treated groups showed a significant increase in serum total protein and globulin. This globulin increasing level was suggested to be used as an indicator of immune responses and source of antibody production, this result came in harmony with the result of *Abdel-Azeem et al.* (2005). Incontrast, *Soliman* (2003) mentioned that feeding broiler chickens different feed additives did not significantly affect blood constituents. The result pointed out that, no significant variation in serum albumin levels between treated and control group. This result disagreed with that of *Ali* (2014) who reported that a significant decrease in albumin of

marjoram treated groups compared to control group. This may be due to the active components of the marjoram that may increase globulin level in the blood and decrease albumin and albumin/globulin.

Hyperglobulinemia in infected groups may be attributed to increase of acute-phase proteins, nephritic syndrome, active liver disease and increase of immunoglobulins due to antigenic stimulation (*Thrall, 2004*). Glucose is supplied to tissue either by intestinal absorption of dietary glucose or by hepatic glucose production from its precursors (*Kaneko et al., 1997*). *E.coli* infected non treated group showed a significant increase in serum glucose level compared with the control group especially at 4<sup>th</sup> and 6<sup>th</sup> weeks, This increase was due to stress due to *E. coli* infection (*Kaneko et al., 1997*). Meanwhile *O.majorana* treated group (1%) showed a significant decrease in glucose level in comparison with the infected group especially at 4<sup>th</sup> and 6<sup>th</sup> weeks of age, this result agreed with *Mohamed et al.* (2014). The glucose lowering effect of *O.majorana* may be due to alteration of genes related to hepatic glucose production (*Eddouks et al., 2003*) and stimulation of glucose utilization by peripheral tissues by stimulating the expression of adiponectin and glucose transporter-2 in liver and peripheral tissues (*Ismail et al., 2013*). Marjoram

plant also improves the liver functions and reduces the serum cholesterol, triglycerides, LDL and increase HDL. (*Shreen et al., 2015*). In contrast, *Ezz El Arab (2008)* reported that, adding the grounds of rosemary, marjoram and sweet basil did not significantly affect any of blood cholesterol and triglycerides. Dietary cholesterol is present in both free and esterified forms, but non esterified cholesterol is only absorbed. After absorption of non-esterified cholesterol, it transferred by the way of lymph to general circulation (*Kaneko et al., 1997*). The present results revealed hypercholesterolemia in the *E. coli* infected non treated group at 4<sup>th</sup> and 6<sup>th</sup> weeks and thus proved by *Coles (1986)* who, reported significant increase in cholesterol in high fat diet and in hepatobiliary diseases. These increases in serum total cholesterol, HDL-cholesterol and triglycerides concentrations observed in infected group could be suggestive of hepatic injury (*Kaneko et al., 1997*), which was supported by the observed hepatic necrosis in these groups due to *E. coli* infection. In contrast to *Fatma (2005)* who reported hypercholesterolemia in infected non-treated group. Regarding *O. majorana* treated groups a significant decrease in cholesterol and TG level were observed. The hypocholesterolemic effect of marjoram could be attributed to

presence of flavonoids, saponins, glycosides, tannins and phenolics and their free radical scavenger activity which prevent intestinal absorption of cholesterol by competition for its absorption sites (*Rang and Dale, 1991*), interfere with lipoprotein production, increased expression of hepatic LDL receptors leading to an increase removal of LDL-cholesterol from the blood and its increased degradation and catabolism of cholesterol from the body (*Amarowicz et al., 2008*). These results also in accordance with *Nagm (2002)* who found that marjoram extract lead to significantly decrease in TG compared to control group. This effect of lowering TG may be due to lower fatty acids synthase activity in hepatocytes (*Fiordaliso et al., 1995*). Also, the significant effect of marjoram on lipid profile in the present study may be due to the inhibitory effect of marjoram on lipid metabolism through interfering with micelles solubilization of cholesterol in digestive tract which in turns decreased cholesterol absorption and increased the excretion of fecal bile acid cholesterol. (*Yang and Koo, 2000*). This result came in harmony with the result of *Shreen et al. (2015)*. In contrast, *Ali (2014)* found non-significant changes in cholesterol level of treated groups compared to control group. And this difference may be due to difference in dose or duration or route of administration.



Concerning the results of renal function test, there was a significant increase in the level of uric acid in *E. coli* infected chicks at 4<sup>th</sup> and 6<sup>th</sup> weeks (1 and 3 weeks post infection) when compared to the control group, these results agree with **Hanan (2002)** who reported that, the experimental infection of chicks with *E.coli* cause an elevation of serum uric acid and this may be attributed to increase in the breakdown of plasma proteins. While *O.majorana* treated groups showed decrease in uric acid level compared to the infected group. These results agree with the findings of **Lobna et al. (2014)** who found a significant decrease in serum uric acid with marjoram treatment (0.5 % powder, 5 % oil kg diet) compared to control group, this may be due to its flavonoids, polyphenols and other antioxidants contents. Flavonoids have been reported as potential inhibitors of xanthine oxidase and so reduce uric acid production (**Costantinoi et al., 1992**).

### Conclusion

**From the result of the present work, we can conclude that:**

*O.majorana plant* has marked growth promoter as well as immune-modulatory effects in broilers.

*O.majorana* 1% has better effect in poultry industry than 0.5% *O.majorana* has a role for controlling of *E.coli* infection.

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### الملخص العربي

استهدف هذا العمل تأثير استخدام مسحوق نبات البردقوش كإضافات أعلاف على معدل النمو والمناعة في بدارى التسمين في الحالات الطبيعية والمصابة تجريبيا بالميكروب القولوني. تم استخدام 150 ككتوت لإجراء هذه الدراسة تم تقسيمهم إلى 6 مجموعات متساوية كل منها 25 ككتوت. أظهرت الفحوصات الكيميائية زيادة نشاط الإنزيمات الخاصة بوظائف الكبد و بروتينات الدم و مستويات الدهون والسكر في الدم مع حدوث زيادة في نسبة حمض اليوريك (الذي يعتبر المؤشر الحساس لخلل وظائف الكلى) في المجموعات المصابة بالميكروب القولوني والمجموعات المصابة بالمعالجة بالبردقوش 0,5% وقد انخفضت هذه الزيادة في المجموعات المصابة والمعالجة بالبردقوش 1%.