



Concurrent Use of Tilmicosine and Thyme Oil in Treatment of *Mycoplasma gallisepticum* in Chickens

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ABSTRACT

The objective of this study is designed to clarify the effect of tilmicosin and thyme oil on treatment of chronic respiratory disease in broiler chickens. Eighty one-day old chicks tested negative *Mycoplasma gallisepticum* (MG). On the 22th day of age the chicks were divided into five equal groups Gp. (1) non infected non treated (control), but chicks in Gp. (2, 3, 4, and 5) at 22th day of age were inoculated with 0.2ml of MG broth into air sac. Gp (1) non infected non treated (control), Gp.(2) infected non treated, Gp.(3) infected treated with thyme oil 200 ppm for 5 successive days, Gp.(4) infected treated with tilmicosin 25 mg/kg for 5 successive days. and Gp.(5) infected treated with both thyme oil and tilmicosin in same dose and period. At 1st and 7th day post treatment all chicks in all groups were weighed and blood samples were collected for hematobiochemical studies, biochemical studies and phagocytic activity. The clinical signs observed in infected broilers chickens were poor appetite, depression, nasal discharge, mild conjunctivitis, sneezing, coughing, gasping, rales and mortalities were 20%. Our results revealed that infected non treated chicks showed significant decrease in body weight gain, RBCs, Hb, PCV, WBCs, T.Protein, albumin and globulin beside significant increase in AST, ALT, creatinine, uric acid, phagocytic activity and phagocytic index at 1st day post treatment. Treated infected broilers chickens with tilmicosin and thyme oil showed improvements in clinical signs, mortalities and body weight beside improvement in hematobiochemical and biochemical parameters and phagocytic activity post treatment. The pathological examination of internal organs of MG infected broiler chickens showed severe pathological lesions in lung, liver, kidney and heart. But infected broiler chickens treated with both thyme oil and tilmicosin is induced marked improvement in the pathological changes. It could be concluded that the concurrent use of both thyme oil and tilmicosin is a useful mean to treat and control CRD in broiler chicks. Thyme oil can be used as an assistant factor to retard CRD in broiler chicks due to its immune enhancer and growth promoter properties.

Keywords: Tilmicosine and Thyme Oil , *Mycoplasma gallisepticum* , Chickens.

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1. INTRODUCTION

In the poultry industry, it is important to stimulate the immune system to decrease or prevent infectious diseases. Numerous factors like inhibition of antibiotics and failure of vaccination can induce immunodeficiency.

The usage of immune enhancers is a key solution to improve immunity and reduce susceptibility to infectious diseases in poultry farms. Phytogetic feed additives, which are rich in flavonoids, extend the biological activity of ascorbic acid, act as antioxidants

and may enhance immune function (Acamovic and Brooker, 2005). Thymol, carvacrol and 6-gingerol possess useful antioxidant properties and may become important in the search for 'natural' replacements for synthetic antioxidant food additives (Aeschbach R et al, 1994). Thyme methanol extract possessed the best antioxidative activity (Mohamed et al, 2013).

Thyme oil induces significant increase in total proteins and globulins (Xiaolei Zhu et. al, 2014). Thyme oil for broiler chicks improves the growth and performance (Saki et. al., 2014). Thyme oil improves antioxidant status and enhancing an immune response in chickens (Placha et al., 2014 and Placha et al., (2013).

Mycoplasma gallisepticum continuous to be of primary concern to the poultry industry worldwide as it commonly affects intensively reared chickens and turkeys causes large economic losses (Evans et al., 2002). *Mycoplasma gallisepticum* is a major pathogen species in poultry which causes chronic respiratory disease in chickens. Economic losses in the poultry industry caused by *Mycoplasma gallisepticum* (Stipkovits and Kempf, 1996). Uncomplicated cases of MG infection don't always cause overt clinical signs or mortality, but can result in sub-optimum production, unacceptable feed conversion ratio and down grading of the carcasses (Charleston et al., 1998).

Tilmicosin is a semisynthetic, broad - spectrum, bacteriostatic macrolide antibiotic synthesized from tylosin for veterinary uses (Kempf et al. 1997). Tilmicosin offers promising prospect of application in clinical veterinary practices. It is a useful drug for treatment and control of respiratory diseases due to its high volume of distribution, long half-life and preferable accumulation in lung. Tilmicosin is used for control and treatment of respiratory diseases caused by *Mycoplasma* spp (Jordan & Horrocks 1996, Abu-Basha et

al. 2007). Chicken must not be slaughtered before 4 days from the stopping of tilmicosin administration (Mossad Elsayed et. al, 2014).

This work focused on the study of the efficacy of thyme oil and tilmicosin either alone or together in treatment CRD infected broiler chickens and studying there effects on clinical symptoms, mortalities, body weight and hematobiochemical parameters.

2. MATERIALS AND METHODS

Drugs:

1-Tilmicosin: Tilmicosin phosphate was obtained as an oral solution from ATCO Pharma for Pharmaceutical Industries, Cairo, Egypt under a trade name tilmicoral®, a 25% oral solution.

2- Thyme oil: Dry thyme plant is obtained from the local market in Ismailia city , Egypt. Using ethanol 75 % as solvent then freeze drying process according (Vamanu Emanuel et. al 2011).

- The dose of thyme oil was used in drinking water equivalent to 200 ppm of feed intake (Adel Feizi et al., 2013; Al-Kassie, 2009 and Mahmoodi, 2014).

Experimental birds:

Eighty one-day old apparently healthy chicks (Arbor-Acres) sourced from tanbul For Investment & Poultry Company; five chicks randomly selected, humanly euthanized and tested negative MG by using PCR test. Chicks were fed on commercial unmedicated balanced ration. Chicks were housed under hygienic conditions, feed and water provided ad libtum .

Mycoplasma gallisepticum challenge

Mycoplasma gallisepticum strain that used in this study was obtained from Animal Health Research Institute, El-Dokki, Giza On day 22 of age, 60 experimental infection via air sacs inoculation with 0.2 ml (10⁸ C.F.U.) according to (Ellakany et al., 1997).

Experimental design:

into 5 equal groups each of (15) in separated units, 1st Gp was served as control (non infected non treated), the 2nd Gp infected non treated, 3rd Gp infected treated with 200 ppm thyme oil in drinking water for 12 hours (equivalent to 200ppm in feed for 24 hours).4thGp were infected treated with tilmicosin 25 mg/kg. B.wt for 8 hours for 5 successive days, and 5thGp were infected treated with thyme oil and tilmicosin in same dose and period Clinical findings, mortalities, were recorded also.

Performance parameters.

Broiler chickens were weighted at 22nd day of age and at 1st and 7th day post treatment, feed consumption and weight gain were recorded and feed conversion rate was calculated.

Blood samples

At 1st and 7th day post treatment five chicks from all groups were bled and three blood samples were collected for complete blood picture determination by using automated blood cell analyzer (Sysmex XT-2000iV, Kobe, Japan) (Harvey, 2012).

The third blood samples were taken to obtain clear serum and estimation of AST and ALT according to R according to results of albumin (Artiss 1981).

Creatinine (Henary, 1974).

Bacteriological examination:

At 9th day post infection trachea, lung and air sac were collected for isolation of MG.

Dray swabs from these organ were dipped several times in Frey’s broth and incubated at 37° C (until the color become yellow). By sterile Pasteur pipette 1 ml of the suspension was spread on the surface of the petri-dish plate contain Frey’s media(mycoplasma Selective Agar media) , the suspension was spread in different directions using a platinum loop to wet the whole of its surface and excessive fluid was discarded.

Histopathological examination:

Post chick slaughtered specimens from the lungs, liver, kidneys and heart were collected and fixed in 10% neutral buffered formalin solution. Five-micron thick paraffin sections were prepared and stained by hematoxylin and eosin (H&E.) according to (Bancroft and Stevens 1996).

Statistical analysis:

The obtained data were analyzed using the SPSS software, version 16 for ANOVA (Snedecor and Cochran, 1994).

3. RESULTS

Table (1): Effect of tilmicosin (25 mg / kg BW) and or / thyme oil (200 ppm) each alone and their combination given for 5 successive days on productive performance in treatment of MG- infected broiler chickens.(Mean±S.E) n=5.

Group	Mortality			initial Body weight (on 22 nd day)	Weight post treatment		FCR
	No	No	%		1 st day	7 th day	
GP(1)	15	0	0	0.635±0.01 ^a	1.538±0.02 ^a	2.111±0.06 ^a	2.00
GP(2)	15	3	20	0.613±0.01 ^a	1.300±0.02 ^b	1.399±0.02 ^d	2.67
GP(3)	15	2	13.3	0.615±0.01 ^a	1.485±0.05 ^a	1.795±0.03 ^c	2.30
GP(4)	15	1	6.6	0.612±0.01 ^a	1.500±0.03 ^a	1.948±0.04 ^b	2.15
GP(5)	15	1	6.6	0.618±0.01 ^a	1.510±0.03 ^a	1.979±0.03 ^b	2.13

Means within same column carrying different superscripts are significantly different at (P<0.05).

Table (2): Effect of tilmicosin (25 mg / kg BW) and or / thyme oil (200 ppm) each alone and their combination given for 5 successive days on some hematological parameters in treatment of MG- infected broiler chickens.(Mean±S.E) n=5.

Parameters		GP(1)	GP(2)	GP(3)	GP(4)	GP(5)
RBCs	1 st day	2.213±0.03 ^b	1.846±0.03 ^c	2.437±0.03 ^a	2.163±0.009 ^b	2.257±0.037 ^b
(10 ⁶ /ul	7 th day	2.483±0.31 ^a	2.110±0.14 ^b	2.116±0.03 ^c	2.320±0.13 ^{ab}	2.380±0.15 ^{ab}
HB	1 st day	8.000±0.07	7.133 ±0.07 ^c	9.066±0.09 ^a	8.266±0.27 ^b	8.366±0.32 ^b
(g/dl)	7 th day	9.500 ±0.21	8.233±0.52	8.433±0.21	9.400±0.56	9.600±0.60
PCV	1 st day	32.466±0.44 ^a	27.533±1.59 ^b	28.100±0.10 ^{ab}	31.067±1.89 ^{ab}	31.73±1.97 ^{ab}
(%)	7 th day	32.466±0.44 ^a	27.533±1.59 ^b	28.100±0.10 ^{ab}	31.067±1.89 ^{ab}	31.73±1.97 ^{ab}
WBCs	1 st day	13.133±0.39 ^{bc}	9.167±1.67 ^c	20.633±1.82 ^a	16.733±0.15 ^{ab}	20.433±1.77 ^a
(10 ³ /ul	7 th day	20.633±0.37	13.600±3.04	16.433±1.95	19.800±2.31	20.533±4.12

Means within same column carrying different superscripts are significantly different at (P<0.05).

Table (3): Effect of tilmicosin (25 mg / kg BW) and or / thyme oil (200 ppm) each alone and their combination given for 5 successive days on liver enzymes, uric acid and creatinine in treatment of MG- infected broiler chickens.(Mean±S.E) n=5.

Parameters		GP(1)	GP(2)	GP(3)	GP(4)	GP(5)
AST	1 st day	24.00±2.05 ^d	212.80±7.19 ^a	102.40±4.45 ^c	121.8±10.38 ^b	131.2±5.42 ^b
(U/L)	7 th day	29.00±2.77 ^c	178.60±9.15 ^a	143.0±22.44 ^a	73.2±16.31 ^{bc}	126.6±21.6 ^{ab}
ALT	1 st day	25.20±3.07 ^b	69.00±9.74 ^a	67.80±5.71 ^a	63.00±12.68 ^a	50.0±11.90 ^{ab}
(U/L)	7 th day	26.60±3.35 ^b	57.00±5.79 ^a	43.20±5.78 ^{ab}	43.20±6.54 ^{ab}	41.80±8.61 ^{ab}
Cratnine	1 st day	0.840±0.05 ^c	1.120±0.06 ^a	1.00±0.09 ^{ab}	0.95±0.05 ^{bc}	0.94±0.05 ^{bc}
(mg/dL)	7 th day	0.840±0.09 ^b	1.224±0.19 ^a	0.98±0.09 ^{ab}	0.98±0.08 ^{ab}	1.04±0.07 ^{ab}
Uric acid	1 st day	5.820±0.51 ^b	7.860±0.59 ^a	6.146±0.19 ^b	6.200±0.36 ^b	6.44±0.17 ^b
(mg/dl)	7 th day	6.800±0.24	7.020±0.258	5.20±1.16	5.84±0.35	6.64±0.22

Means within same column carrying different superscripts are significantly different at (P<0.05).

Table (4): Effect of tilmicosin (25 mg / kg BW) and or / thyme oil (200 ppm) each alone and their combination given for 5 successive days on protein picture in treatment of MG- infected broiler chickens.(Mean±S.E) n=5.

Parameters		GP(1)	GP(2)	GP(3)	GP(4)	GP(5)
T.Protein	1 st day	7.460±0.08 ^a	6.160±0.72 ^b	7.160±0.12 ^a	7.400±0.01 ^a	7.36±0.10 ^a
(g/dl)	7 th day	7.320±0.12 ^a	6.520±0.43 ^b	7.240±0.11 ^a	7.360±0.12 ^a	7.560±0.04 ^a
Albumin	1 st day	4.220±0.07 ^a	3.560±0.41 ^b	4.020±0.15 ^{ab}	4.02±0.05 ^{ab}	4.18±0.05 ^{ab}
(g/dl)	7 th day	4.220±0.11	3.900 ±0.12	4,100±0.07	4.36±0.68	4.20±0.29
Globuln	1 st day	3.240±0.18 ^a	2.600±0.35 ^b	3,140±0.10 ^{ab}	3,38±0.10 ^a	3,18±0.19 ^a
(g/dl)	7 th day	3.10±0.11 ^{ab}	2,620±0.34 ^b	3,120±0.07 ^{ab}	3,0±0.14 ^{ab}	3,36±0.29 ^a
A/G	1 st day	1.3096±0.07	1.37±0.11	1.2860±0.08	1.192±0.04	1.32±0.07
ratio	7 th day	1.3680±0.07	1.6032±0.23	1.314±0.03	1.468±0.08	1.3080±0.18

Means within same column carrying different superscripts are significantly different at (P<0.05).

Table (5): Effect of tilmicosin (25 mg / kg BW) and or / thyme oil (200 ppm) each alone and their combination given for 5 successive days on phagocytic activity and phagocytic index in treatment of MG-infected broiler chickens.(Mean±S.E) n=5.

Groups	phagocytic activity		phagocytic index	
	1 st day	2 nd day	1 st day	2 nd day
GP(1)	56.2±1.1 ^a	60.0±1.4 ^{ab}	2.2 ±0.09 ^a	2.4±0.05 ^b
GP(2)	25.2±1.9 ^c	32.0±1.4 ^d	0.7±0.04 ^c	0.9±0.04 ^d
GP(3)	48.00±1.7 ^b	58.0±1.5 ^b	1.5±0.04 ^b	2.9±0.04 ^a
GP(4)	27.4±1.7 ^c	42.6±1.7 ^c	0.8±0.04 ^c	1.4±0.09 ^c
GP(5)	53.2±2.1 ^a	64.0±1.4 ^a	2.0±0.09 ^a	2.9±0.09 ^a

Means within same column carrying different superscripts are significantly different at (P<0.05).

Plat (1) :

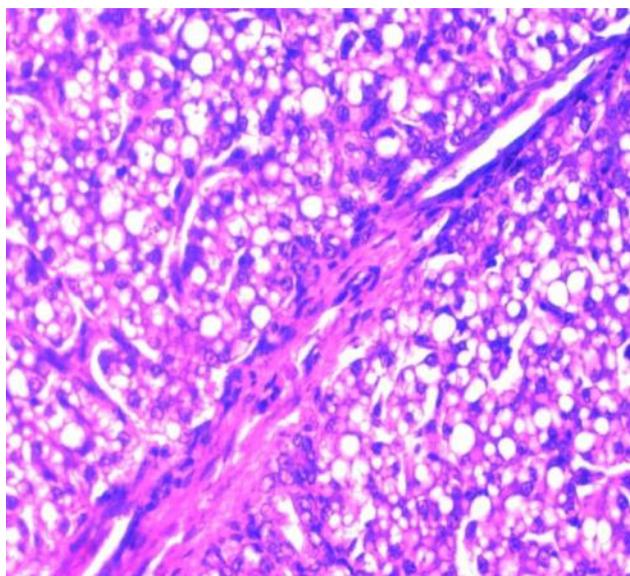


Image A

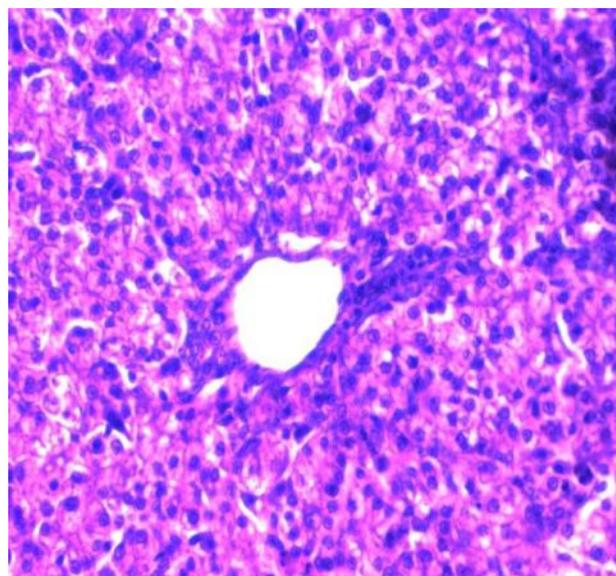


Image B

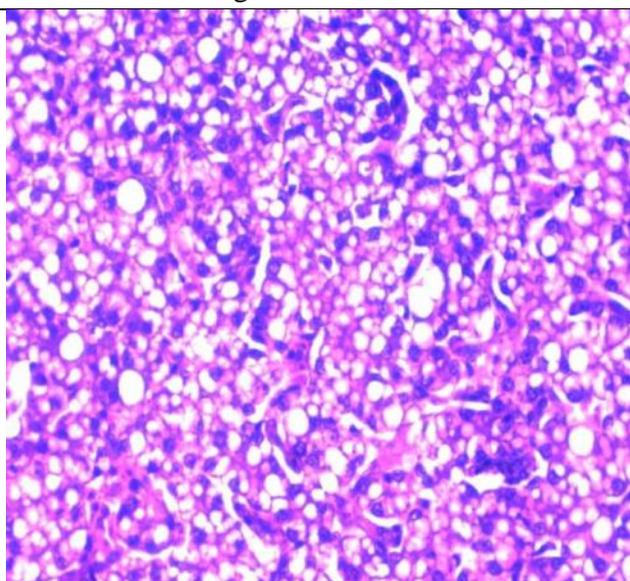


Image C

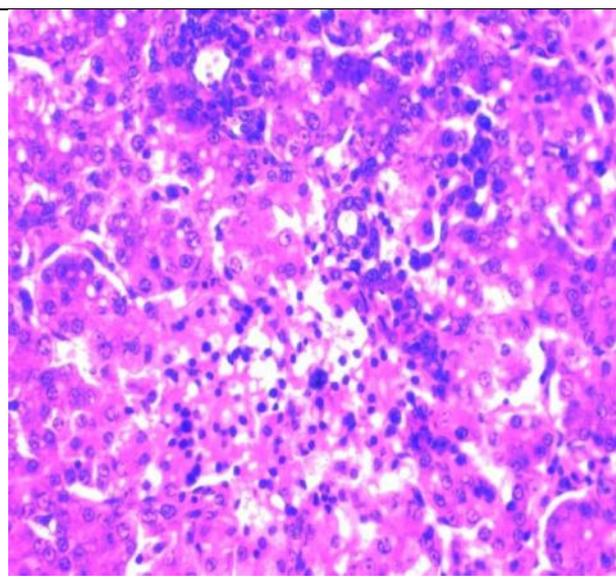


Image D

Image A : liver tissue of broiler chicken infected with mycoplasma showing severe fatty change of the lymphocytes and aggregation of inflammatory cells in the portal tract (H & E X 200) .

Image B : liver tissue of broiler chicken infected with mycoplasma and treated with tilmicosin showing mild fatty change and presence of mild aggregation of inflammatory cells around central vein (H & E X 200) .

Image C : liver tissue of broiler chicken infected with mycoplasma and treated with thyme oil showing severe fatty change of lymphocytes and aggregation of inflammatory cells around central vein (H & E X 200).

Image D : liver tissue of broiler chicken infected with mycoplasma and treated with tilmicosin and thyme oil showing return of the liver cells to its normal state and presence few scattered inflammatory cells (H & E X 200) .

Plat (2)

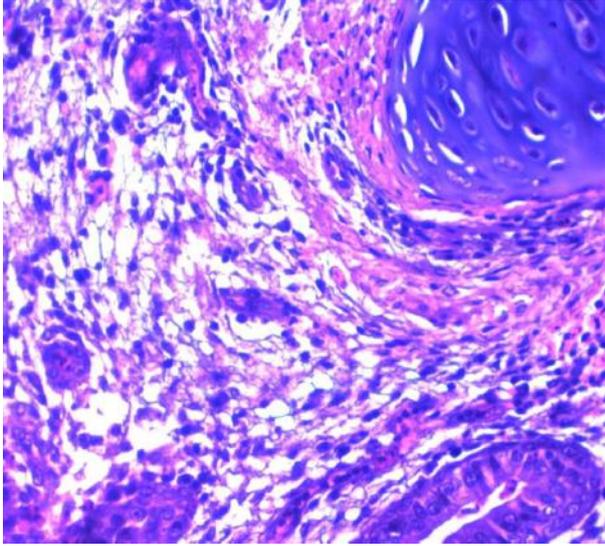


Image A

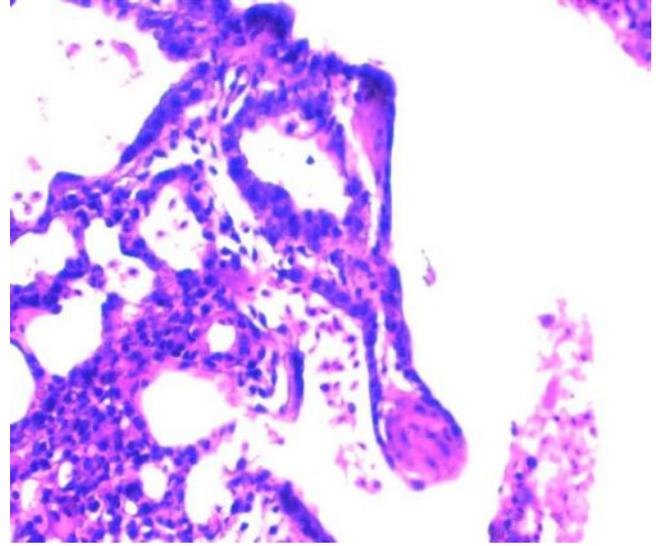


Image B

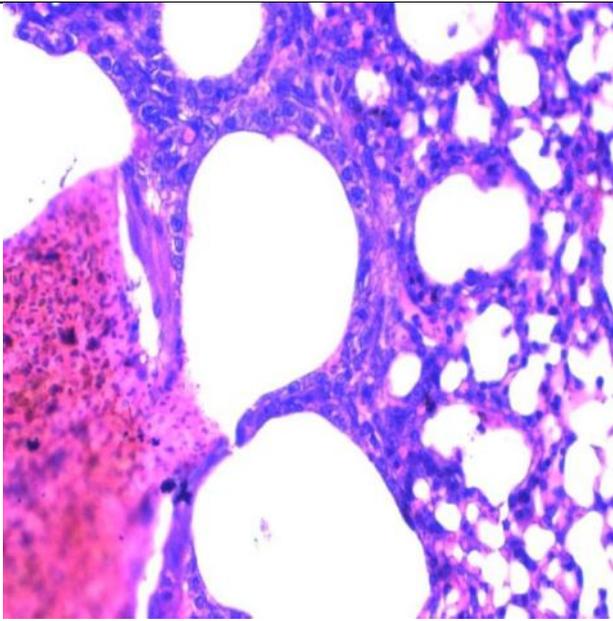


Image C

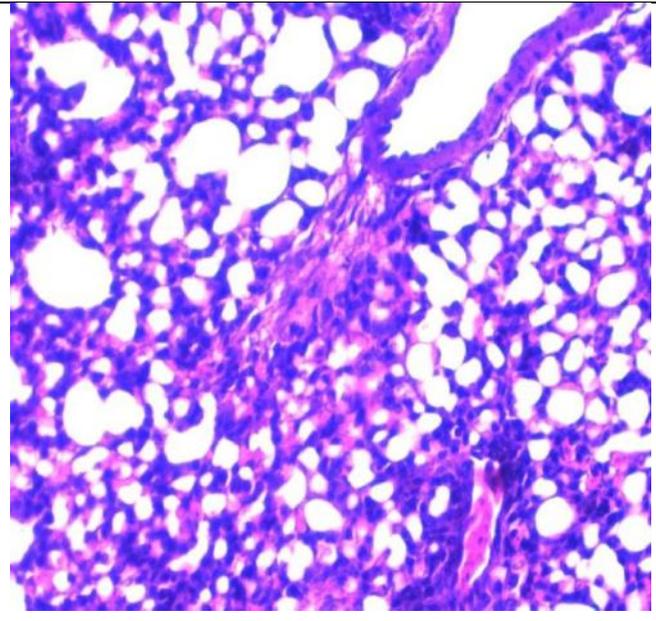


Image D

Image A : Lung tissue of broiler chicken infected with mycoplasma showing heavy aggregation of inflammatory cells and area of hemorrhage in interstitial tissue of the lung (H&E X 200).

Image B : Lung tissue of broiler chicken infected mycoplasma and treated with telmicosin showing mild aggregation of inflammatory cells in the lung alveoli (H&E X 200).

Image C : Lung tissue of broiler chicken infected with mycoplasma and treated with thymus showing area of hemorrhage and aggregation of inflammatory cells around the alveoli and bronchi (H & E X 200).

Image D : Lung tissue of broiler chicken infected with mycoplasma and treated with tilmicosin and thymus showing normal lung alveoli and bronchi (H & E X 200).

Plat (3)

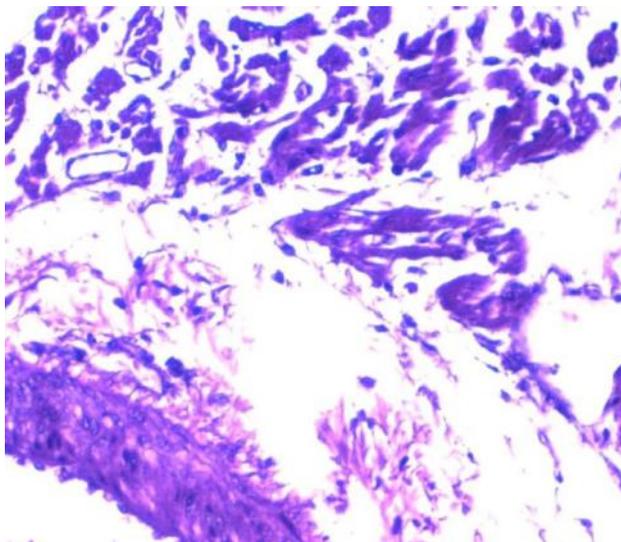


Image A

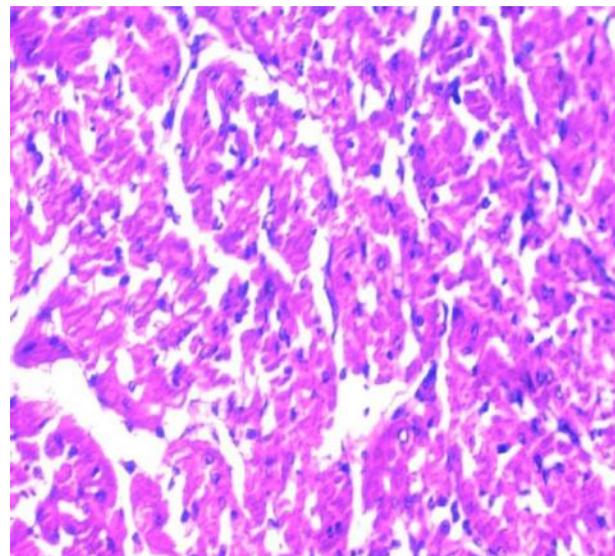


Image B

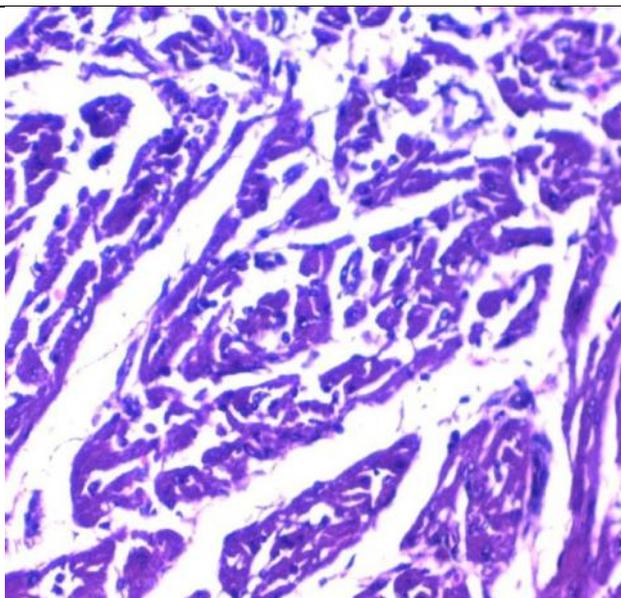


Image C

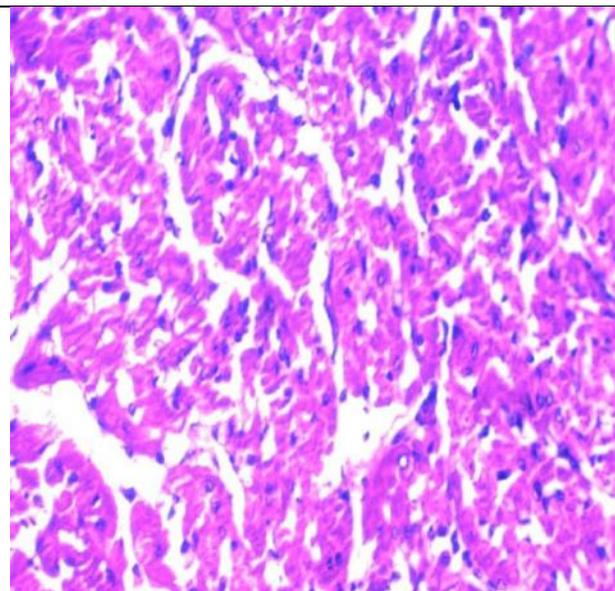


Image D

Image A : Cardiac tissue of broiler chicken infected with mycoplasma showing atrophy and necrosis of the Cardiac muscles and increase aggregation of inflammatory cells (H and E X 200).

Image B : cardiac tissue of broiler chicken infected with mycoplasma and treated with tilmicosin show regeneration of cardiac muscles and mild aggregation of different inflammatory cells (H & E X 200).

Image C : Cardiac tissue of broiler chicken infected with mycoplasma and treated with thymus show atrophy of the Cardiac muscles and increase aggregation of inflammatory cells(H&EX 200).

Image D : cardiac tissue of broiler chicken infected with mycoplasma and treated with tilmicosin show regeneration of cardiac muscles and mild aggregation inflammatory cells (H & E X 20

Plate (4)

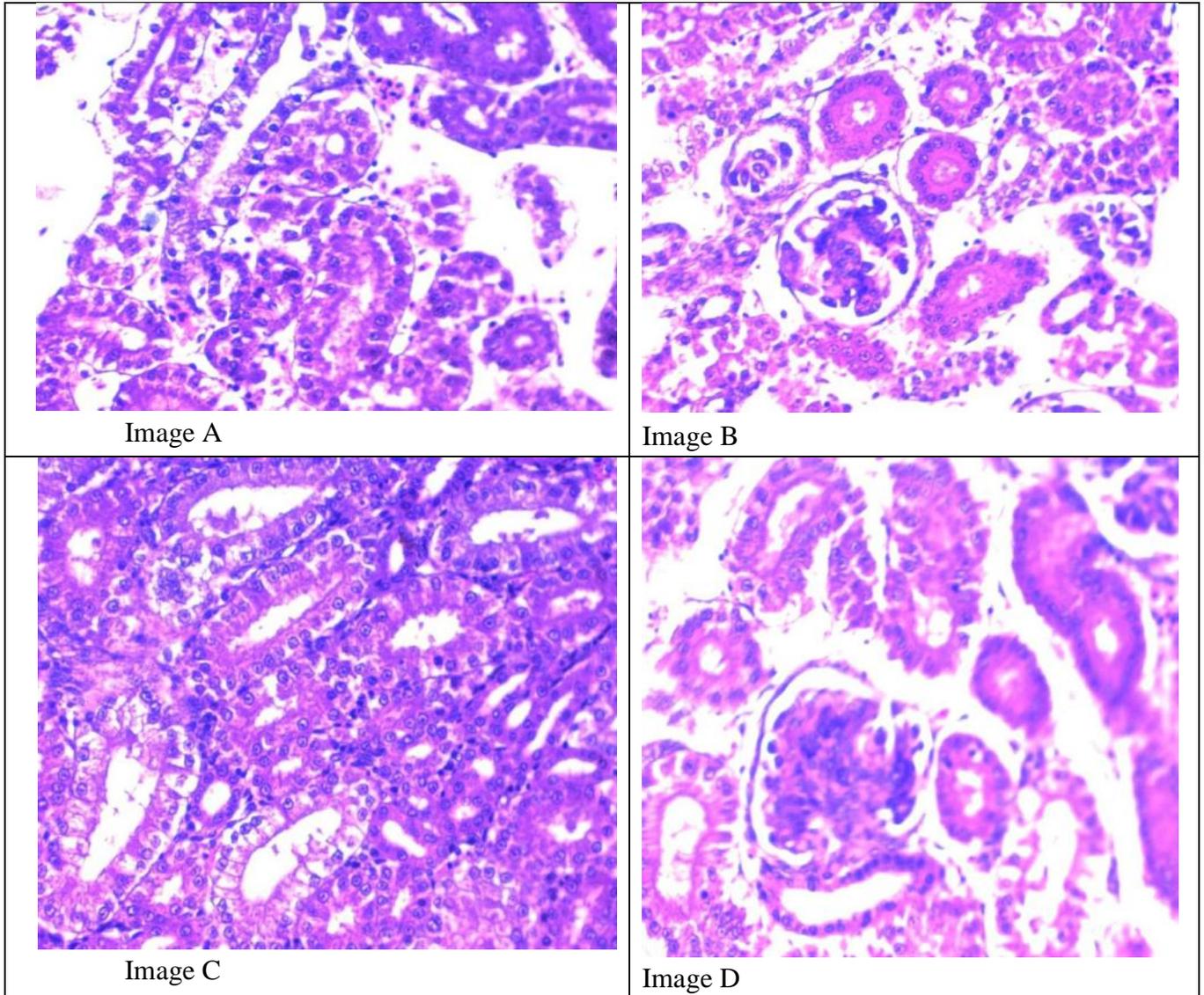


Image A: Renal tissue of broiler chicken infected with mycoplasma showing hydroptic degeneration and sloughing of renal tubular epithelial cells with aggregation of inflammatory cells in the connective tissue stroma (H & E X 200).

Image B: Renal tissue of broiler chicken infected with mycoplasma and treated with telmicosin showing normal glomeruli and tubules with few scattered inflammatory cells (H & E X 200).

Image C: Renal tissue of broiler chicken infected with mycoplasma and treated with thymus showing hydroptic degeneration of renal tubular epithelial cells with aggregation of inflammatory cells (H & E X 200).

Image D: Renal tissue of broiler chicken infected with mycoplasma and treated with telmicosin and thymus showing normal glomeruli and tubules and few scattered inflammatory cells (H & E X 200).

4. DISCUSSION

The clinical signs observed in infected broilers chickens were poor appetite,

depression, nasal discharge, mild conjunctivitis, sneezing, coughing, gasping,

rales, and mortalities. Similar findings were obtained by (Jordan , 1997, Nunoya et al., 1997, Mohamed et al., 2007)

Treated infected broilers chickens with tilmicosin and thyme oil either alone or in combination showed improvements in clinical signs, mortalities and body weight. The best results were showed in broilers treated with thyme oil and tilmicosin due to the antimycoplasmal effect of tilmicosin (Abd El-Samie, 2014) , the antioxidant effects of thyme oil (Placha et. al 2013 and Placha et. al 2014) and also thyme oil growth promoter effects (Saki et. al 2014). Besides, Mohamed and Mohamed (2007) and Abdel-Ghany, (2009) reported that tilmicosin is successful for treatment chickens infected with MG with reduction in clinical signs, mortalities and improved body weight gains.

Blood parameters are good indicators of physiological, pathological and nutritional status of an animal (Ganong, 1999). Our study revealed a significant increase in RBCs count Hb content and significant leukocytosis in thyme oil treated chicks, this result is compatible with that of Al-Kassie, (2009) and Hassan et al.(2004) they found that was a significant increase in HB and RBC count post using thyme. Our result is agree with Youssef et al. (2017) who reported that thyme oil induced elevation in WBC count. The protective effects of thyme oil on body cells like RBCs or the hemobiotic system may due to its protective antioxidant proprieties (Farag et al., 1989, Aeschbach et al. 1994, Cuppett and Hall, 1998 and Sylvestre et al., 2006).

Infected chicks with MG revealed leukocytopenia at 1st and 7th day post treatment meanwhile treatment infected chick showed improvement in leukocytic count. Our results are similar to Mohamed et. al (2007) reported who that MG is immunosuppressant agent, and Abdel-Samie(2014) concluded that tilmicosin phosphate was efficient enough to control the

pathogenicity of MG and thus leading to immune stats enhancement. Also Sande et al. 1985 and Khalifeh et. al, (2009) reported that tilmicosin had beneficial impacts in control the MG infection and improve cell-mediated immune response. Saki et al., (2014) reported that oral administration of thyme oil at level of 2 mL/L could ameliorate the immune response as a health status.

The results of this study showing increase in liver enzymes in MG infected chickens with some improvement in infected treated chickens similar to these results Mossad et. al (2017) who reported an increasing in levels of liver enzymes in MG infected broiler chickens with improvement of liver enzyme concentration post treatment with tiamulin (a macrolide antibiotic). Thyme oil treated chickens induced temporarily improvement of liver enzyme, this reflect that thyme oil did not kill MG but ameliorate the liver condition. Our results agreed with Xiaolei Zhu et al.(2014) who reported that thyme essential oil significantly decreased liver enzyme activity in broiler chickens.

Our results showed that infected chicks with MG revealed significant decrease in serum total protein, albumin and globulin. Treatment of infected broiler chickens with tilmicosin_or thyme oil improved serum protein level. The observed decrease in serum total protein, and albumin in MG-infected chicks may be due to the necrotic effect of MG on the hepatocytes which are the main site of protein synthesis or related to loses of albumin through the damaged renal tubules (Sorkar et al 2005). Moreover, the decreased serum globulin was related to the destructive effects of MG on the immune- producing cells of birds (Warner et al 1992 and Javed et al 2005).

Our results showed that Tilmicosin and thyme oil treated chickens revealed that improved serum total protein and globulin values than infected non-treated chicks

showed improvement in serum protein values, this results due to its antimycoplasmal effect and good distribution of tilmicosine Mohamed and Mohamed (2017). These results come in compatibility with Xiaolei Zhu *et. al* (2014) who mentioned that dietary thyme oil in broilers chicks induce increase serum total proteins and globulins.

Our study revealed that MG infection induced elevation in creatinine and uric acid. This elevation may be due to MG induced renal tissue damage and reduced renal excretion (Coles 1986). Also such result is in agreement with Mohamed *et. al* (2007) and Kempf *et al* (1988).

Our results displayed that phagocytic activity was lowered in infected chickens with MG this due to the immunosuppressant action of *Mycoplasma* on immune system. Same results were reported by Mohamed and Mohamed (2007) who noticed lower values of phagocytic activity in mycoplasma infected birds.

The observed elevation in phagocytic activity in treated broiler chickens especially chickens treated with thyme oil because thyme oil can stimulate non-specific immune system (Langhout, 2000).

Infected broiler chickens with MG showed gross lesion in trachea, lungs, air sacs, heart and liver Bajwa *et al.*, (1992) and Saif *et at.*(2003). Also, Our results showed serious lesions in these mentioned visceral organs.

Microscopical examination of broiler chickens infected with MG revealed that lungs are showing heavy aggregation of inflammatory cells and area of hemorrhage in the interstitial tissue of the lung, with congestion. Liver in broiler chickens infected with *Mycoplasma gallisepticum* revealed fatty change of the lymphocytes and aggregation of inflammatory cells in the portal tract, heart revealed that pericardium was thickened with atrophy and necrosis of the Cardiac muscles

and increase aggregation of inflammatory cell, kidneys showed hydroipic degeneration and sloughing of renal tubular epithelial cells with aggregation of inflammatory cells in the connective tissue stroma, Same pathological lesion was reported by (Amir *et. al* 2011 Saif *et al.* 2003 and Mohamed *et. al* 2007). The improvement in microscopical picture in mentioned visceral organs after usage of thyme oil and tilmicosin is due to thyme oil constituents can reach plasma, gut and other edible tissues in broiler chickens such as liver and flesh (Alexander *et. al*, 2015). Tilmicosin Peak plasma concentration was 1.25 ± 0.09 $\mu\text{g/mL}$ and achieved at 3 hours, also it has good distribution and penetration in lung, liver, kidney and muscle (Mohamed and Mohamed (2017).

5. CONCLUSION

It could be concluded that the concurrent use of both thyme oil and tilmicosin is a useful mean to treat and control CRD in broiler chicks. Thyme oil can be used as an assistant factor to retard CRD in broiler chicks due to its immune enhancer and growth promoter properties.

6. REFERENCES

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