

COMPARATIVE STUDIES BETWEEN PEFLOXACIN AND TIAMULIN AGAINST MYCOPLASMA IN TURKEY

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ABSTRACT

Samples from lungs, trachea and air sacs were collected from 40 turkey poultts suffering from respiratory signs for bacteriological examination for isolation and identification *mycoplasma* spp. The results revealed, 18 (45%) samples were positive (+ve) for *mycoplasma* spp. Antibiogram study of isolates revealed that pefloxacin and tiamulin was effective against *mycoplasma*. A total of 40, one-day old poultts provide free from *mycoplasma* infection were divided into 4 equal groups. 1st group healthy negative (-ve control) 2nd, 3rd & 4th groups were artificially infected with *Mycoplasma gallisepticum* (MG), 2nd group infected non treated (+ve control), 3rd group infected treated with 5 mg pefloxacin / kg bwt in drinking water for 5 successive days and 4th groups was infected treated with 25mg tiamulin/kg bwt in drinking water for 5 successive days, At 1st and 10th days post treatment 5 poultts from each group were sacrificed for record lesion scores % and two blood samples were taken from each poultts for hematobiochemical parameters study. Poultts infected with *Mycoplasma gallisepticum* revealed loss of appetite, depression, sneezing, conjunctivitis, frothy exudation in eyes, air sacculitis 7 (70%), pericarditis 7 (70%), perihepatitis 8 (80%) & mortality rate 4 (40%) beside induces significant increase in WBCs, AST ALT, ALP uric acid, creatinine and significant decrease in weight gain. Phagocytic %, index, killing % IgA, IgG, IgM, T protein, albumin and globulin. Medication *mycoplasma gallisepticum* by tiamulin or pefloxacin revealed disappears of clinical signs, reduced mortality rate to 10%, mild lesion scores re-isolation rate of *Mycoplasma gallisepticum* from poultts treated with pefloxacin was (20%) and (10%) for tiamulin beside improve in hematobiochemical parameters and returned to nearly normal level at 10th day post treatment. It could be concluded that pefloxacin and tiamulin was effective in treatment *Mycoplasma gallisepticum* in poultts and ameliorate severity of its lesions besides improving adverse effect in hematobiochemical parameters.

Keyword: *Mycoplasma gallisepticum*; pefloxacin; tiamulin; turkey; hematobiochemical.

INTRODUCTION

Turkey is considered one of the main sources of proteins. Turkey industry in

Egypt was progressed in last few years and numerous farms had developed different breeds.

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Mycoplasmosis is a contagious disease causing economic losses in poultry (El-Ashram *et al.*, 2021). *MG* infection referred “chronic respiratory disease,” in chickens and infect-ious sinusitis in turkeys as

characterized by nasal discharge, tracheal rale, coughing, and dyspnea (Levisohn and Kleven, 2000). *MG* infection causes economic losses by growth retardation, increased feed conversion rate, increased mortality rate (Kleven, 2008). Controlling *MG* disease on a global level is done by eradication of positive breeder flocks or by vaccination and medication (Raviv and Hey, 2013).

Tiamulin are used to treatment *MG* infection, respiratory and genital-urinary organs mucus are preferred (Bozorgmehri *et al.*, 1998). Tiamulin binds at 50S ribosomal subunit with rRNA in which it prevents correct positioning of tRNA for peptide transferase and subsequent bacterial protein production (Poulsen *et al.*, 2001).

Fluroquinolone compounds are wide spectrum bactericidal activity against mycoplasma (Andon, 1993). Pefloxacin is one of a third generation of fluoroquinolones with broad spectrum activity against many bacterial diseases in poultry as salmonellosis, infectious coryza and avian mycoplasmosis (Mohamed and Dardeer, 2001).

The purpose of this study was to evaluate the efficacy of pefloxacin and tiamulin against *MG* infected turkey poult together with a special reference to the immune response and some biochemical parameters.

MATERIALS AND METHODS

Drug:

1-Tiamulin (Tiamutin®) water soluble granules contain 45g tiamutin hydrogen fumarate. Produced by Sandoz Company, Austria.

2- Pefloxacin (Peflodad 10 %®) solution was obtained from Dar Al Dawa Vet & Agri Industrial Co. Ltd. Jordan. Each ml contains 100mg of pefloxacin base.

Isolation and identification of *Mycoplasma gallisepticum* isolates:

Aseptic 40 swabs (lungs; trachea and air sacs) were taken from 40 diseased poult suffering from respiratory signs and subjected to bacteriological examination for isolation and identification mycoplasma spp. swabs were immersed into vials containing mycoplasma broth with bacterial inhibitors. Each swab was cultured into pleuropneumonia like organism broth and agar media with inhibitors and indicators for mycoplasma isolation (Adler *et al.*, 1958). Isolates were biochemically identified (Frenske and Kenny 1976).

Birds and experimental design:

About 40, one day-old turkey poult were used in this trial at 14 days of age blood samples were taken from them then subjected to serological examination by serum plate agglutination test to prove their free from *Mycoplasma* infection then poult were divided into four equal groups. 1st group healthy poult (-ve control), 2nd, 3rd and 4th groups were artificially infected with *MG* by inoculating in air sac with a pathogenic strain of *MG* at dose 0.2 ml of 24h broth culture of virulent strain of *MG* containing 2×10^8 (CFU)/ml (Moustafa, 2001), 2nd group was infected, non-treated (+ve control), 3rd group was infected treated with 5 mg pefloxacin / kg bwt in drinking water for 5 days and 4th groups was infected and treated with 25mg tiamulin/kg bwt in drinking water for 5 days, Treatments was start after appear clinical signs (5 day post infection). Poult were individually weighed at 14th day of age (Initial weight) and at 24th day of age (Final weight) for estimation body weight gain and feed conversion rate.

Sampling:

At 1st and 10th days post treatment 5 poult from each group were sacrificed for record Lesion scores % and two blood samples were taken from each group

1st blood sample was taken in tube contain EDTA for estimation leukocytic count

(Jain, 1986), phagocytic%, index and killing % (Wilkinson, 1977 & Lucy and Larry 1982).

2nd sample was taken in tube for obtain clear serum for estimation T. protein (Doumas *et al.*, 1981) albumin (Drupt, 1974) globulin was calculated as difference between total proteins and albumin, AST and ALT (Reitman and Frankel 1957), ALP (John, 1982), creatinine (Henry, 1979), uric acid (James and White, 1971). Serum immunoglobulins (IgA, IgG & IgM) were performed using SANDWICH Elisa (Erhard *et al.*, 1992)

Re-isolation of *Mycoplasma gallisepticum*

Post poult sacrificed Swabs from air-sac and nasal cavity were taken from all poult post treatment for re-isolation of *MG* post treatment then immersed into vials containing mycoplasma broth medium with bacterial inhibitors for culturing.

F. Serological examination:

Blood samples were collected from wing veins of turkey poult just before infection and at 1st day post treatment for rapid serum plate agglutination test for antibodies to *Mycoplasma gallisepticum* according to Kempf *et al.* (1998).

Statistical analysis: The obtained data was analyzed by using computerized SPSS program version 16 according to Tamhane and Dunlop (2000).

RESULTS

Turkey poult infected with *MG* showed clinical sign represented by loss of appetite, depression, sneezing, gasping, conjunctivitis, frothy exudation from eye, decrease body weight, increase in FCR and gross pathological lesions {air sacculitis 7 (70%), pericarditis 9(90%), perihepatitis 8 (80 %)} and mortality rate 4(40%) (Table 2 &3).

Mycoplasma gallisepticum induced significant increase in leukocytic count, serum AST, ALT, ALP, uric acid and cretonne beside significant decrease in phagocytic %, index, killing %, total protein, albumin, globulin coupled with insignificant decrease in IgA, IgG and IgM) allover expermental period post infection (Table 4 & 6).

Medication infected poult using tiamulin or pefloxacin showed disappear of clinical signs, reduced mortality rate to 10%, Resolution of *MG* from infected poult treated with pefloxacin or tiamulin were (20%) and (10 %) respectively in comparison with (100 %) of infected non treated poult coupled with mild lesion scores, improve body weights and FCR post treatment (Table, 1, 2 & 3).

leukocytic count, phagocytosis%, phagocytosis index, killing %, serum total protein, albumin, globulin, IgA, IgG and IgM, AST, ALT, ALP, uric acid, creatinine and returned to nearly normal levels at 10th day post treatment (Table 4,5 & 6).

Table 1: Incidence of the isolated mycoplasma from turkey poult.

Source of swabs	Total No. of swabs	+ve swabs		-ve swabs	
		No	%	No	%
Air sac	15	8	53.33	7	48.66
Lungs	15	6	40	9	60
Trachea	10	4	40	6	60
Total	40	18	45	22	55

Table 2: Effect of mycoplasma pefloxacin and tiamulin on mortality rate and bacteria re-isolation of turkey poult.

Parameters Groups	Total No	Mortality		<i>mycoplasma re-isolation</i>	
		No	%	No	%
GP 1	10	00	00	00	00
GP 2	10	4	40	6	100
GP 3	10	1	10	2	20
GP 4	10	1	10	1	10

Table 2: Effect of mycoplasma, pefloxacin and tiamulin on pathological lesions, mortality rate and bacteria re-isolation of turkey poult.

Parameters Groups	GP 1		GP 2		GP 3		GP 4	
	No	%	No	%	No	%	No	%
Total No	10		10		10		10	
A.sacculitis	00	00	7	70	2	20	1	10
Pericarditis	00	00	9	90	1	10	1	10
Perihepatitis	00	00	8	80	2	20	1	10

Table 3: Effect of pefloxacin and tiamulin on body weight of turkey poult (n=5).

Parameters and Groups	GP 1	GP 2	GP 3	GP 4
IBW (gm) (14 th day of age)	240.13±2.18 a	241.24±1.96 a	245.21±1.86a	243.25±1.64 a
FBW (gm) (24 th day of age)	558.98±4.32 a	515.34±2.76 b	555.55±4.76 a	551.21±5.65 a
Weight gain (gm)	318.85±4.84 a	274.10±5.72 b	310.34±3.49 a	307.96±3.64 a
Feed consumption	518.35	509.28	515.34	508.04
Feed conversion ratio	1.63	1.86	1.66	1.65

Means with different superscripts of the same raw indicate significant difference at P < 0.05

Table 4: Effect pefloxacin and tiamulin on WBCs, Phagocytosis% and Killing% of poult (n=5).

period	Parameters and Groups	GP 1	GP 2	GP 3	GP 4
1 st day	WBCs X103/μl	13,51±0,24 b	15,83±0,58 a	14,94±0,35 a	14,89±0,49 a
	Phagocytosis	54.89±1.21 a	49.21±1.23 b	51.07±1.12 b	51.12±1.06 b
	Phagocytic index	6.81±0.55 a	4.51±0.28 b	4.89±0.33 b	4.99±0.24 b
	Killing%	68.41±0.96 a	63.03±0.89 b	64.85±0.72 b	65.16±0.55 b
10 th day	WBCs X103/μl	13,28±0,47 b	15,58±0,57 a	14,23±0,41 a	14,11±0,69 a
	Phagocytosis	54.96±1.42 a	49.13±1.13 b	53.43±1.57 a	53.38±1.45 a
	Phagocytic index	6.70±0.48 a	4.43±0.39 b	5.57±0.58 a	5.63±0.43 a
	Killing%	68.73±0.89 a	63.21±0.78 b	66.79±0.84 a	66.90±0.93 a

Means with different superscripts of the same raw indicate significant difference at P < 0.05

Table 5: Effect of pefloxacin and tiamulin on liver and kidney function of turkey poult (n=5).

<i>period</i>	Parameters and Groups		GP 1	GP 2	GP 3	GP 4
<i>1st day</i>	Liver Enzymes (U/L)	AST	79.43±1.0c	85.34±1.16a	84.19±1.21b	84.03±1.22 b
		ALT	23.38±0.71c	27.04±0.68a	25.13±0.27 b	25.28±0.28 b
	Kidney Function (gm/dl)	ALP	108.72±1.17c	115.29±1.61a	113.21±1.5b	113.28±1.49 b
		Uric acid	4.14±0.20c	5.41±0.18a	4.82±0.14 b	4.88±0.18 b
<i>10th day</i>	Liver enzymes (U/L)	Creatinine	1.22±0.15c	2.04±0.17a	1.79±0.13 b	1.77±0.11 b
		AST	78.52±1.5b	85.13±1.30a	80.13±1.52 b	80.17±1.21 b
		ALT	29.43±0.6 b	31.3±0.19 a	30.21±0.37 b	30.42±0.54 b
	Kidney Function (gm/dl)	ALP	112.16±1.1b	117.06±1.23a	113.78±1.9 b	114.21±1.98 b
		Uric acid	4.06±0.39 b	5.43±0.20 a	4.21±0.34 b	4.22±0.25 b
		Creatinine	1.23±0.32 b	2.15±0.12 a	1.68±0.12 b	1.70±0.14 b

Means with different superscripts of the same raw indicate significant difference at P < 0.05

Table 6: Effect of pefloxacin and tiamulin on humeral immunity of turkey poult (n=5)

<i>period</i>	Parameters and Groups		GP 1	GP 2	GP 3	GP 4
<i>1st day</i>	Protein Picture (gm/dl)	T.protein	5.50±0.28 a	4.60±0.15 b	4.70±0.20 b	4.75±0.19 b
		Albumin	3.08±0.14 a	2.45±0.14 b	2.40±0.23 b	2.50±0.19 b
		Globuline	2.42±0.10 a	2.05±0.11 b	2.30±0.14 b	2.25±0.10 b
		A/G ratio	1.27±0.11 a	1.20±0.14 a	1.04±0.10 a	1.11±0.15 a
	Immuno-globulin (gm/100ml)	IgA	3.82±0.41 a	2.89±0.53 a	3.21±0.81 a	3.64±0.47 a
		IgM	5.68±0.22 a	4.48±0.72 a	5.32±0.59 a	5.58±0.38 a
		IgG	9.58±0.37 a	8.32±0.69 a	9.17±0.48 a	9.38±0.70 a
<i>10th day</i>	Protein Picture (gm/dl)	T.protein	5.63±0.25 a	4.71±0.21 b	5.70±0.22 a	5.73±0.19 a
		Albumin	3.03±0.14 a	2.60±0.12 b	2.88±0.19 a	2.90±0.21 a
		Globuline	2.60±0.16 a	2.11±0.11 b	2.82±0.21 a	2.83±0.19 a
		A/G ratio	1.17±0.15 a	1.23±0.12 a	1.02±0.13 a	1.3±0.11 a
	Immuno-globulin (gm/100ml)	IgA	3.79±0.55 a	2.80±0.69 a	3.62±0.43 a	3.76±0.48 a
		IgM	5.89±0.48 a	4.59±0.58 a	5.77±0.39 a	5.82±0.61 a
		IgG	9.47±0.60 a	8.44±0.72 a	9.28±0.38 a	9.40±0.55 a

Means with different superscripts of the same raw indicate significant difference at P < 0.05

DISCUSSION

Poults infected with *MG* showed clinical signs represented by depression, loss of appetite, sneezing, gasping, conjunctivitis with frothy exudation in the eyes and decreased body weight beside gross pathological lesions as air sacculitis 7(70%), pericarditis 9(90%), perihepatitis 8 (80%) and mortality rate 4 (40%). Same clinical signs and mortality rate were observed by Saif *et al.* (2007) in broilers

suffering from mycoplasmaosis. Same clinical signs were observed by Hany *et al.* (2019) in turkey suffering from mycoplasmosis and Andrea *et al.* (2020) in broilers infected with *mycoplasma*. Avian mycoplasmosis induced decrease in weight gain (Jay *et al.*, 2021).

Infected poults treated with tiamulin or pefloxacin showed disappear of clinical signs, reduced mortality rate to 10% beside mild lesion scores, improve body weights

and feed conversion rate. This improvement in body weights and feed conversion rate post treatment may be due to bactericidal effect of used drug on *MG*. Fluoroquinolones was effective in *MG* in broilers (Kempf *et al.*, 1998). Pefloxacin treated *MG* revealed disappear clinical signs and reduced mortality rate (Mohamed and Mona 2002). treatment infected broilers by tiamulin reduced clinical signs, lesion scores and mortality (Zakeri and Kashefi 2011). Tiamulin effective against *MG* in broilers (Farran *et al.*, 2018).

Re-isolation rate of *MG* from poult treated with pefloxacin was (20%) and (10 %) for tiamulin. Pefloxacin have bactericidal activity against *MG* infection and reduced re-isolation *MG* (El Sayed and Mahmoud 2003). Laying hens infected with mycoplasma treated with tiamulin reduced *MG* re-isolation (Masour *et al.*, 2017).

In the present study, *MG* infection induces leukocytosis beside Significant decrease in phagocytic %, index and killing %. Broilers infected with *MG* showed leukocytosis, decrease in killing % and phagocytosis (Avakian and Ley 1993). Mycoplasma infection revealed leucocytosis and decrease phagocytosis (Malhat *et al.*, 2005). *MG* induce leukocytosis and decrease in phagocytosis and killing % (Mohamed *et al.*, 2007).

Medication *MG* infected poult by tiamulin or pefloxacin revealed improved in WBCs, phagocytosis and killing % and reterened to normal levels at 10th day post treatment. Our results were agreed with those previously reported by Wieliczko *et al.* (1988) in broilers infected with *MG* and treated by taimolin. Pefloxacin have good efficacy in treatment *MG* and improve phagocytosis and killing % (Mohamed and Mona 2002).

Our findings revealed, significant increase in AST, ALT, ALP, uric acid and cretonne allover experimental period post infection of poult with *MG*. These finding may be

due to damage of hepatocyte by bacterial toxin (Coles, 1986). These results come in harmony with results reported by Abdalla and Adayel (2006) stated that *MG* infection in broiler induce elevation in liver enzymes, uric acid and creatinine in chickens experimentally infected with *MG* Infected poult treated with tiamulin or pefloxacin showed improved in liver enzymes, uric acid and cretonne. Same results were reported Mohamed and Mona (2002) stated that chickens infected with *MG* treated with pefloxacin revealed improved in liver enzymes and kidney function (Mohamed and Mona (2002). Tiamulin effective against *MG* in broilers and improved in AST, ALT, ALP, uric acid and cretonne (Bastamy *et al.*, 2021).

In the present work, serum total protein, albumin and globulin significantly decreased in poult infected with *MG*. Reducton in total protein and albumin may be due to necrotic effect of *Mycoplasma* toxin on hepatocytes which are the main site of albumin synthesis (Sorkar *et al.*, 2005). *Mycoplasma gallisepticum* induce significant reduction in total protein, albumin and globulin in broilers (Malhat *et al.*, 2005 and Mohamed *et al.*, 2007). Laying hens infected with mycoplasma gallisepticum showed decrease in serum total protein, albumin and globulin (Masour *et al.*, 2017).

Infected poult treated by tiamulin or pefloxacin revealed improved in protein profile and reterened to normal levels at 10thday post treatment. Tiamulin has good effect in treatment *mycoplasma gallisepticum* infected broilers and improved protein profile (Zakeri and Kashefi 2011). Also, Masour *et al.* (2017) stated that taimulin treatment infected broilers by *mycoplasma gallisepticum* showed improved protein picture.

Infected poult with *MG* revealed insignificant decrease in IgA, IgG and IgM allover experimental period post infection Mycoplasma infection induced reduction in

immune-globulin (Romero *et al.*, 2001). Same change in immunoglobulin was reported by Wijesurendra *et al.* (2017) in poult infected with *MG*.

Infected poult treated with tiamulin or pefloxacin showed improved in IgA, IgG, and IgM and returned to nearly normal levels at 10th days post treatment. Improved in IgA, IgG and IgM post treatment may be due to antibacterial effect of used drugs against *MG* (Abd El-Aziz 2002).

It could be concluded that pefloxacin and tiamulin was effective in treatment of *MG* in turkey and ameliorate severity of lesions besides improve adverse effect in biochemical and immunological parameters of the infected turkey poult.

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دراسات فارماكولوجية على البيفلوكساسين والتيمولين في بدارى تسمين الرومى

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تم تجميع مسحات من الرئتين ، القصبة الهوائية والحويصات الهوائية من 40 طائر رومى مريضة تعاني من امراض تنفسية للفحص البكتيريولوجى لعزل وتصنيف البكتيريا المسببة للامراض التنفسية. وتم عزل الميكوبلازما فى عدد 18(45%) وبعمل اختبار الحساسية للمعزولات وجد انها حساسة للتيمولين والبيفلوكساسين . لذلك اجريت هذه الدراسة لمعرفة تأثير التيمولين والبيفلوكساسين على الميكوبلازما وتأثير الميكوبلازما على كرات الدم البيضاء وبعض الوظائف البيوكيميائية والمناعية. اجريت الدراسة على عدد 40 كتكوت رومى عمر يوم واحد وعند اليوم الرابع عشر من العمر يتم تقسيمهم إلى 4 مجموعات متساوية (10 كتكوت بكل مجموعة). المجموعة الأولى مجموعة ضابطة ، المجموعات الثانية ، الثالثة والرابعة تم عدوتهم عدوى اصطناعية بالميكوبلازما جليسيبتكم. المجموعة الثانية تم تركها بدون علاج (مجموعة مصابه وغير معالجة)، المجموعة الثالثة مصابة علاجها بالبيفلوكساسين بجرعة 5 محم / لتر من مياة الشرب يوميا لمدة 5 أيام متتالية والمجموعة الرابعة مصابة وتم علاجها بالتيمولين بجرعة 25 محم / لتر من مياة الشرب يوميا لمدة 5 أيام متتالية عند اليوم الاول والعاشر من نهايه العلاج تم أخذ عينتين دم من وريد الجناح العينة الأولى على هيبارين لدراسة التأثيرات على كرات الدم البيضاء وقوة اللتهام والأخرى لفصل المصل وذلك لقياس بعض المؤشرات البيوكيميائية والمناعية .

الإصابة بالميكوبلازما جليسيبتكم في كتاكيت الرومى ادت إلى ظهور أعراض مرضية وأدت إلى زيادة نسبة الوفيات (40%) ومعدل التحويل الغذائى ونقص فى وزن الجسم ووزن الجسم المكتسب وأدت إلى وجود زيادة معنوية في كرات الدم البيضاء ، انزيمات الكبد (ALT-AST والفوسفاتيز القاعدي) ، اليوريا والكرياتين ونقص معنوي فى ، قو اللتهام والقتل، IgA , IgG and IgM البروتين الكلى، الجلوبيولين، الزلال .

وقد أتضح من هذه الدراسة أن عقارى البيفلوكساسين والتيمولين أدى إلى اختفاء الأعراض وقلل نسبة الوفيات وتحسنت وزن الجسم المكتسب ومعدل التحويل الغذائى والوظائف البيوكيميائية والمناعية وكان التيمولين ذو كفاءة عالية عن البيفلوكساسين.

من مجموع ما تقدم من نتائج نستخلص أن الإصابة بالميكوبلازما جليسيبتكم في كتاكيت الرومى تؤدي إلى حدوث تغيرات في بعض الوظائف البيوكيميائية والمناعية ولكن عقارى البيفلوكساسين والتيمولين لهم تأثير جيد فى عاج الميكوبلازما لذلك ينصح باستخدام البيفلوكساسين واتيمولين فى علاج الإصابة بالميكوبلازما فى بدارى تسمين الرومى بكل امان.