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## Mansoura Veterinary Medical Journal

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### SOME PHARMACOLOGICAL STUDIES ON MARBOFLOXACIN AND FLUNIXIN MUGLUMINE COMBINATION DURING TREATMENT OF COLIFORM MASTITIS IN CATTLE

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#### ABSTRACT

The aim of the present work was to study the effect of Marbofloxacin with Flunixin Muglumine combination during treatment of coliform mastitis in cattle. Twenty coliform mastitic cows were selected from 50 mastitic cows and treated by Marbofloxacin and Flunixin muglumine. The results showed that the red blood cell (RBC) count and hemoglobin concentration were significantly decreased. Similar changes were observed with the mean corpuscular volume and total platelets. Meanwhile, White blood cell (WBC) count was significantly elevated. There are no significant changes recorded in serum albumin and globulin with tested drugs throughout the study. However, there is a significant increase in serum total proteins. The result recorded also, a significant increase (in serum aspartate aminotransferase (AST) and serum alkaline phosphatase (ALP)) and a significant decrease in serum alanine aminotransferase (ALT) was recorded.

**Keywords:** cattle, coliform, mastitis, Marbofloxacin, Flunixin Muglumine, RBCs, AST, ALP, ALT and WBCs.

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#### INTRODUCTION

Mastitis is, the most important disease of dairy animals which responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) (Bhikane and Kawitkar, 2000), More over the productive performance of the animal was affected (Bardhan, 2013).

The main causative bacteria include: *S. aureus*, *St. agalactiae* (both of which are contagious), coliforms, Streptococci and Enterococci. All of these pathogens are found in the environment of the animals (water, feed, bedding, manure and soil). Several other pathogens have been isolated from infected mammary glands which include *Actinomyces*

*pyrogenes*, *Cl.perfringens* and other coliforms, such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Pasteurella haemolytica*, among others (Conington *et al.*, 2005).

Coliform infection is highest during two weeks following drying off and in two weeks prior calving (Radostits *et al.*, 2007).

Treatment of mastitis should be based on bacteriological diagnosis and take national and international guidelines on prudent use of antimicrobials into account. In acute mastitis, where bacteriological diagnosis is not available, treatment should be initiated based on herd data and personal experience. Rapid bacteriological diagnosis would facilitate the proper selection of the antimicrobial. Treatment of mastitis during lactation with antibiotics is referred "lactation therapy",

which is used by many producers to reduce the clinical signs of mastitis and bring back the normal milk production of cows. This therapy has proven useful in reducing the SCC in milk and thereby maintains the quality of milk. Bactericidal drugs are preferred. Intramammary therapy achieves high concentrations but an uneven distribution (Pyorala, 2009).

The antibiotic treatment by parenteral route is indicated when some general signs are present, life threatening situations or in the case of chronic infections for which diffusion of the antibiotic into the parenchyma is necessary (Grandemange *et al.*, 2012).

Marbofloxacin is approved for use in the treatment of respiratory, urinary, and dermatological diseases in the cattle and pigs for the treatment of respiratory, soft tissue, and gastrointestinal infectious diseases and also for the treatment of acute coliform mastitis (Schneider *et al.*, 1996).

Marbofloxacin is a bactericidal antibacterial and its mechanism of action is as inhibitor of DNA gyrase (an essential cell enzyme necessary for the supercoiling of DNA), which allows bacterial DNA to fit within the bacterial cell and leads to rapid bacterial cell death (Chu and Fernandes, 1991).

Flunixin meglumine (FM) has anti-inflammatory, anti endotoxin, analgesic and antipyretic properties and has been used extensively to treat a number of conditions in veterinary species, such as fever and mastitis in cows (Rantala *et al.*, 2002).

The flunixin meglumine has been reported to decrease body temperature and udder edema, and restore rumination, dry matter intake (DMI) and milk production in cases of experimentally induced mastitis. The use of flunixin meglumine was evaluated during experimentally induced *E. coli* mastitis. (Yeiser *et al.*, 2012).

## MATERIAL AND METHODS

**Marbofloxacin** is available as a 10% solution for injection under the trade name of Marbocyl®, obtained from (Laboratoire Veto Quinol, lure, France).

### Structural formula:

The recommended dosage is 2mg/kg in a single daily injection for 3 to 5 successive days by subcutaneous, intramuscular or intravenous routes in cattle (Van huffel and grandemange, 2002).

**Flunixin muglumine** (finadyne) was obtained from MSD Company, France.

### Structural formula:

The recommended dosage is 2.2 mg per kg of body weight every twenty-four hours, or 1.1 mg per kg of body weight every twelve hour by Intravenous, administered slowly, for up to three days (Banamine, 2006).

### Experimental design:

Fifty dairy cattle suffered from clinical mastitis characterized by sudden onset, swelling, and redness of the udder, pain, reduced and altered milk secretion from the affected quarters. The milk may have clots, flakes or of watery in consistency and accompanied by fever, depression and anorexia. Milk samples from the infected quarters were collected for isolation of the causative microorganism.

The drugs under investigation were given according to its dose for all diseased animals, but our experiment was continued on the animals which suffered from coliform mastitis only.

### The Coliform mastitic animals were detected after:-

- Milk samples collection for bacteriological isolation and identification.
- Only twenty Coliform mastitic animals were identified and treated with Marbofloxacin (2mg/kg) and Flunixin meglumine (2.2 mg / kg) combination.

### Samples

#### 1- Blood samples:

Two blood samples (the first sample for hematological studies and the second sample for serological studies) were collected from each animal at zero day, after one week and two weeks post the drugs administration.

- 1- Blood samples were collected on Wassermann tube containing EDTA (0.5 mg ml blood) from jugular vein of all cows for hematological parameters studies (erythrocytic count, leukocytic count, Hb ,PCV, MCV, MCH, MCHC and thrombocytes count).
- 2- The second blood samples were collected in Wassermann tube without anticoagulant from jugular veins of all cows and allowed to clot at room temperature. The serum was separated by centrifugation at 3000 rpm for 15 minutes. The sera were collected in 1.5 ml Eppendroff tubes and kept frozen at  $-20^{\circ}\text{C}$  for biochemical studies (total proteins, albumin, ALT, AST and ALP) ( Stoffregen *et al.*, 1997).

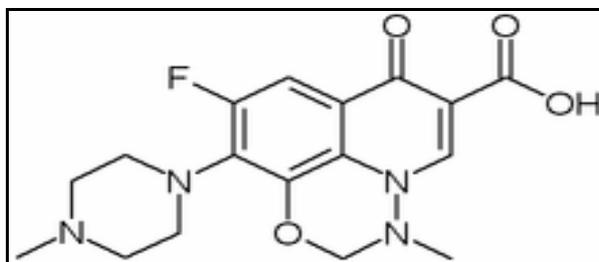


Figure 1: Marbofloxacin formula

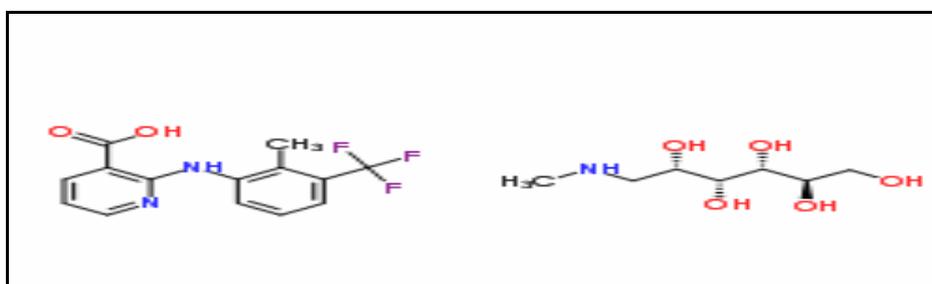


Figure (2) Flunixin –Meglumine formula

**Table (1):** Different bacterial isolates from milk samples.

No of infected cases	Staph	Strept	Staph+ strept	Coli	Coli+strept	Coli+Staph
50	3	5	3	20	10	9

**Table (2):** Effects of investigated drugs on haematological and biochemical parameters:

Items	Time		
	0	1 <sup>st</sup> week	2 <sup>nd</sup> week
Hemoglobin (gm/dl)	10.74 ± 0.38 <sup>a</sup>	9.3 ± 0.46 <sup>a</sup>	8.92 ± 0.50 <sup>b</sup>
RBC (106/μl)	5.02 ± 0.12 <sup>a</sup>	4.50 ± 0.18 <sup>b</sup>	5.24 ± 0.34 <sup>ab</sup>
Packed cell volume (%)	32.4 ± 1.56 <sup>a</sup>	35. ± 1.87 <sup>b</sup>	31.8 ± 1.46 <sup>b</sup>
WBC (103/μl)	11.4 ± 0.24 <sup>a</sup>	9.6 ± 0.67 <sup>a</sup>	13.4 ± 1.02 <sup>ab</sup>
Platelets(103/μl)	132 ± 3.67 <sup>b</sup>	210. ± 5.82 <sup>a</sup>	200. ± 1.83 <sup>a</sup>
MCV(fl)	63.2 ± 0.86 <sup>b</sup>	71.1 ± 2.39 <sup>a</sup>	71.92 ± 2.39 <sup>a</sup>
Serum albumin (g/dl)	3.64 ± 0.12 <sup>a</sup>	3.38 ± 0.08 <sup>a</sup>	3.38 ± 0.08 <sup>a</sup>
Serum total protein (g/dl)	6.8 ± 0.06 <sup>a</sup>	6.66 ± 0.09 <sup>a</sup>	6.84 ± 0.05 <sup>b</sup>
Serum globulin (g/dl)	3.16 ± 0.02 <sup>a</sup>	3.28 ± 0.04 <sup>a</sup>	3.46 ± 0.04 <sup>a</sup>
ALT activity	27.6 ± 1.40 <sup>a</sup>	20.8 ± 2.87 <sup>b</sup>	25.2 ± 1.01 <sup>a</sup>
AST activity	53.8 ± 5.47 <sup>a</sup>	45.2 ± 6.93 <sup>a</sup>	43.4 ± 5.22 <sup>b</sup>
ALP activity	63.8 ± 1.73 <sup>b</sup>	116.4 ± 6.53 <sup>a</sup>	103.4 ± 2 <sup>a</sup>

## RESULTS & DISCUSSION

### 1- Bacteriological Examination:

Milk samples collected from fifty mastitic Holestien cows for isolation and identification of the causative microorganism as shown in Table (1).

Our results showed that there were different bacterial isolates from milk samples collected from fifty clinical mastitic cows; only twenty coliform mastitis cases.

The present study showed a significant decrease in hemoglobin content at the second week post treatment. **Muhammad et al., (2015)** found a decrease in hematocrit and hemoglobin concentration as well as increases in blood pH, bicarbonate concentration and serum electrolytes with Flunixin meglumine treatment. While, **Markiewicz et al., (2013)** showed that the number of red blood cells and hemoglobin concentration did not change after injection of Flunixin meglumine to coliform mastitis cows. Moreover, **Modi et al., (2013)** showed a non-significant differences in RBCs count and hemoglobin concentration after administration of Marbofloxacin in sheep.

The present study recorded a significant decrease in RBCs count at the first and second weeks post treatment. Also, **Khwanja et al., (2012)** noticed a decrease in RBC count after administration of tolfenamic acid in cats. However, **Hassanpour and Nadalian (2012)** showed a non significant changes in RBCs count and Hb content with Flunixin meglumine injection in horses. Also, **Safarchi et al., (2010)** showed a non significant changes in RBCs count with Flunixin meglumine injection in goat.

The results reflected a significant decrease in PCV level at the first and second weeks post treatment. However, **Safarchi et**

**al., (2010)** showed a non significant changes in PCV level with Flunixin meglumine injection in goat. Also, **Modi et al., (2013)** showed a non-significant differences in Packed Cell Volume (PCV), total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) after administration of Marbofloxacin in sheep.

The present study showed a significant increase in total leukocytic count at the first and second weeks post treatment. This result agree with that of **Markiewicz et al., (2013)** who recorded a significant increase of WBC count after injection of Flunixin meglumine to coliform mastitis cows. In contrast, **Naglaa et al., (2014)** reported a significant decrease in TLC, neutrophils and monocytes levels with significant increase in the lymphocytes counts post-treatment with Flunixin meglumine in calves. However, **Azza (2015)** reported a significant decrease in total Leukocytic count after administration of Tolfenamic acid in sheep.

Our data reflected a significant decrease in total Platelets count in treated cows at first day of treatment.

Our data reflected a significant decrease in Mean corpuscular volume at first day of treatment while, at the first and second weeks post treatment there was a significant increase. While, **Modi (2009)** mentioned that Marbofloxacin evoked non-significant differences in hematological parameters between pre and post drug treatment period in sheep. **Sadariya et al., (2010)** found a non-significant changes in Hematological parameters (Haemoglobin, RBC, WBC, MCV, MCH, MCHC, HCT and DLC) with Marbofloxacin injection in rats.

### 3- Effects on liver function tests:

There are no significant changes recorded in serum albumin and globulin with tested drugs in all times. However, there is a significant decrease in serum total proteins at the second week post treatment. However, **Hassanpour and Nadalian (2012)** recorded a significant increase in serum albumin, globulins and total proteins with Flunixin muglumine injection in horses. **Safarchi et al., (2010)** showed a non significant changes in total proteins and albumin with Flunixin muglumine injection in goat. Also, **Sadariya et al., (2010)** showed a non significant changes in total proteins, globulin and albumin with Marbofloxacin injection in rats.

The obtained data showed a significant decrease in serum alanine aminotransferase (ALT) activity at the first week post treatment. However, **Azza (2015)** observed a significant increase in serum alanine aminotransferase (ALT) activity with tolfenamic acid treatment in sheep. Also, **Hassanpour and Nadalian (2012)** showed a significant increase in ALT activity with Flunixin muglumine injection in horses.

The present study showed a significant increase in serum aspartate aminotransferase (AST) activity at the first day and first week post treatment. This agrees with those of **Muammer et al., (2008)** and **Hassanpour and Nadalian (2012)** who showed a significant increase in AST activity with Flunixin muglumine injection in horses.

Our results showed a significant increase in serum alkaline phosphatase (ALP) activity at the first and second weeks post treatment. This agree with those of **Hassanpour and Nadalian (2012)** who showed a significant increase in ALP activity with Flunixin muglumine injection in horses but disagree with those of **Mukesh and Suresh (2015)** and **Maden et al., (2001)**.

From this study we could be concluded that the using of antibacterial drug (Marbofloxacin) in combination with anti-inflammatory drug (Flunixin muglumine) on mastitic cattle have highly significant effect on blood picture ,liver function tests of mastitic cattle and play an important role in treatment and control of mastitis.

### REFERENCES

- Azaa, A.I. (2015):** Pharmacological studies on some non-steroidal anti-inflammatory in sheep. Master degree, Pharmacology department, Fac. Vet. Med., Mansoura University.
- Banamine (2006):** injectable solution product information (Schering- Plough—US), Rev 3/03. Available at [www.spah.com](http://www.spah.com). Accessed on December 27, 2006.
- Bardhan, D. (2013):** Estimates of economic losses due to clinical mastitis in organized dairy farms, *Indian J. Dairy Science*. Vol. 66, No 2, pp 168-172.
- Bhikane, A.V. and Kawitkar, S.B. (2000):** Hand Book for Veterinary Clinician. Venkatesh Books. Udgir, India.
- Chu, D.T.W., and Fernandes, P.B. (1991):** Recent development in the field of quinolone antibacterial agents. *Adv Drug Res*. 21: 39-144.
- Conington J., Cao G.Q., Stott A. & Büniger L. (2005):** Towards a better understanding of using breeding to control mastitis in sheep and cattle. Scottish Agricultural College (SAC) Technical Note. Sustainable Livestock Systems Group, SAC,
- Grandemange E., Fournel S., Giboin H., and Woehrle F. (2012):** Efficacy and

safety of a single injection of marbofloxacin in the treatment of bovine acute *E. coli* mastitis in a European field study. Proceedings of the 27th World Buiatrics Congress, 2012, Lisbon, Portugal.

- Hassanpour A, and Nadalian, M. G. (2012):** Long term use of Flunixin meglumine and Ketoprofen in Arabian Horses and their digestive and cardiac injures, Journal of Animal and Veterinary Advances 11(6): 750-755.
- Khwanjai V., Chuthatep S., Durongphongtorn S. and Yibchok-Anum S. (2012):** Evaluating the effect of 14 day oral vedaprofen and Tolfenamic acid treatment on renal function, hematological and biochemical profiles in healthy cats. J. Vet. Pharmacol. Therap.; 35: 13-18.
- Maden M., Tras B., A.L. Bas, M. Elmas , E. Yazar and F.M. Bridane (2001):** Pharmacology Investigation of biochemical and haematological side-effects of cefquinome in healthy dogs, Veterinary Quarterly, 23:1, 32-34.
- Markiewicz H., W. Krumrych and M. Gehrke (2013):** The effect of supportive *E. coli* mastitis treatment on PMN chemiluminescence and subpopulations of T lymphocytes, Polish Journal of Veterinary Sciences Vol. 16, No. 4 (2013), 671–677.
- Modi F.D. (2009):** Studies on pharmacokinetics of moxifloxacin in sheep. Sardarkrushinar Dantiwada Agriculture University. Sardarkrushinar. India.
- Modi CM, Mody SK and Patel HB (2013):** Hematological Alteration Induced After Intramuscular Administration of Long Acting Moxifloxacin in Sheep. Biochem Physiol 2: 111. doi:10.4172/2168-9652.1000111.
- Muammer Elmas , Enver Yazar, Kamil Uney, Ays e Er (Karabacak) and Bu'nyamin Tras (2008):** Pharmacokinetics of enrofloxacin and flunixin meglumine and interactions between both drugs after intravenous co-administration in healthy and endotoxaemic rabbits, The Veterinary Journal 177 (2008) 418–424.
- Muhammad Arif Zafar, Ghulam Muhammad, Zafar Iqbal, Arfan Yousaf, Asif Riaz and Murtaz-ul Hasan (2015):** Evaluation of Relative Resuscitative Effects of Hypertonic and Isotonic Saline Solutions as an Adjunct to Ceftiofur HCl in Bovine Neonatal Diarrhea Associated with *Escherichia coli*, international journal of agriculture and biology, 14–774/2015/17–5–953–960.
- Mukesh Mangal and Suresh Kumar Sharma (2015):** Effect of Repeated Administration of Cefquinome on Biochemical and Hematological Parameters in Buffalo Calves. Toxicol Int. 22(1): 110–113.
- Naglaa Z.H. Eleiwa, Gehan N.A. Gad and Amal I.A.El-Shorbag (2014):** Effect of a combined Oxytetracycline HCl / Flunixinmeglumine therapy on undifferentiated respiratory disease in calves, International Journal of Advanced Research (2014), Volume 2, Issue 3, 387-398.
- Pyorala, S. (2009):** treatment of mastitis during lactation. Irish Veterinary Journal 62 Supplement 40-44.
- Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2007):** Veterinary Medicine, 10th Edition. Saunders, Philadelphia.

- Rantala M., Kaartinen L., Valimaki E., Stryman M., Hiekkaranta M., Niemi A., Saari L., and Pyorala S (2002):** Efficacy and pharmacokinetics of flunixin meglumine for treatment of cows with enrofloxacin and experimentally induced *Escherichia coli* mastitis. *J Vet Pharmacol Therap*, 25, 251-258.
- Sadariya K.A., Gothi,A.K. Patel, S.D. Bhavsar, S.K. and Thaker, A.M. (2010):** Safety of Moxifloxacin following repeated intramuscular administration in Wistar rats, *Veterinary World*, 2010, Vol.3 (10):449-452.
- Safarchi R., A.A. Mozaffari, A. Derakhshanfar and O. Amiri Marvili (2010):** Evaluation of the Effects of Flunixin Meglumine, Ketoprofen and Phenylbutazone Administration on the Brain, Renal and Hepatic Functions in Iranian Cross-Breed Goats, *Journal of Biological Sciences*, 10: 170-173.
- Schneider M, Thomas V, Boisrame B. and Deleforge J (1996):** Pharmacokinetics of marbofloxacin in dogs after oral and parenteral administration. *J Vet Pharmacol Ther*19: 56-61.
- Stoffregen, D.A., Wooster, G.A., Bustos, P.S. and Bowser, P.R. (1997):** Multiple route and dose pharmacokinetics of ciprofloxacin in Juvenile Atlantic salmon *J. Vet.Pharmacol. Ther.* ;20 (2):111-123.
- Van huffel B. and Grandemange E (2002):** Treatment of BRD in cattle: comparison of 2 antibiotic treatments. XXII World Buiatrics Congress, Hannover, 18-23.
- Yeiser EE, Leslie KE, Mcgilliard ML and Petersson-Wolfe CS (2012):** The effects of experimentally induced *Escherichia coli* mastitis and flunixin meglumine administration on activity measures, feed intake, and milk parameters. *J Dairy Sci.*, 95(9):4939-4949.

## المخلص العربي

### بعض الدراسات الدوائية على الماربوفلوكساسين والفلونكسين ميجلومين أثناء العلاج من التهاب الضرع القولوني في الماشية

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يهدف هذا العمل إلى دراسة تأثير الماربوفلوكساسين (2mg/kg) مع فلونيكسين ميجلومين (2.2mg/kg) أثناء العلاج من التهاب الضرع القولوني في الماشية. وقد تم تحديد عشرين بقرة مصابة بالتهاب الضرع القولوني من أصل خمسين بقرة مصابة بالتهاب الضرع وتم استخدام عقاري الماربوفلوكساسين والفلونيكسين ميجلومين في علاج هذه الحالات المصابة. وأظهرت النتائج انخفاض ملحوظ في عدد خلايا الدم الحمراء وتركيز الهيموجلوبين. وقد لوحظت تغييرات مماثلة مع MCV وإجمالي الصفائح الدموية. في الوقت نفسه لوحظ ارتفاع ملحوظ في عدد خلايا الدم البيضاء ولا توجد تغييرات كبيرة في نسبة الزلال والجلوبولين. ومع ذلك، وجد أن هناك زيادة كبيرة في البروتين الكلي. وأظهرت النتائج أيضاً، زيادة كبيرة في نشاط انزيم الفوسفاتيز القاعدي (ALP) ، في حين هناك انخفاض كبير في نشاط انزيم الالانين امينو ترانسفيريز (ALT) ونشاط انزيم الاسبرتات امينو ترانسفيريز (AST).

ومن هذه الدراسة نستخلص أن : استخدام المضاد البكتيري الماربوفلوكساسين بالاشتراك مع مضاد الالتهاب الفلونكسين ميجلومين في علاج الابقار المصابة بالتهاب الضرع القولوني له تأثير كبير على صورته الدم الكامله واختبارات وظائف الكبد للابقار المصابة بالتهاب الضرع وكذلك لعب دور مهم في العلاج والسيطره على مرض التهاب الضرع في الابقار.