

## IMMUNO-PHARMACOLOGICAL EFFECT OF MARBOFLOXACIN IN RABBITS

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### ABSTRACT

*The effect of marbofloxacin on cellular immune response was investigated in twenty Newzealand white rabbits of 3-4 months old (either non-vaccinated or vaccinated with the inactivated rabbit viral hemorrhagic disease (RVHD) vaccine) and weighing about 2-2.5 kg b.wt. The drug was given intramuscularly (2 mg/kg b.wt.) daily for 5 successive days. The total leukocytic count, serum nitric oxide level and serum lysozyme activity were evaluated. It was achieved that the administration of marbofloxacin induced a significant decrease in total leukocytic count (up to 4 weeks), serum nitric oxide level (up to 1 week) and serum lysozyme activity (up to 3 days) in vaccinated group. On the other hand, no significant changes could be detected in total leukocytic count, serum nitric oxide level and serum lysozyme activity of non-vaccinated treated group. It was concluded that administration of marbofloxacin is not recommended to be used in vaccinated rabbits as it possess an immunosuppressive effect but can be used safely in non-vaccinated rabbits.*

### INTRODUCTION

Rabbits are important farm animals raised for a variety of purposes including meat, fur and wool production from some breeds. They are also used as laboratory animals and kept as pets (Okerman, 1988). From the most serious viral diseases attacking rabbit populations is the viral hemorrhagic disease (RVHD) which causes high mortalities reaching 100% especially in adults (Hanaa, et al., 2009). Vaccination of rabbits in industrial rabbitries against RVHD is the only way to control the disease where it is endemic (Taha, et al, 2009).

Antibiotics are widely used in rabbit industry either for prophylaxis or treatment of bacterial diseases affecting rabbits (Hanaa, et al., 2009). Many antibiotics are capable of depressing the immune system even at therapeutic levels. Immunosuppressive properties of some antibiotics are effective in inhibition of both cellular and humoral immune responses to a variety of vaccines (Shalaby, 1989).

Marbofloxacin is a new third generation of fluoroquinolones intended for veterinary use (Ismail and El-Kattan, 2007). Many studies

have shown an immunotropic action of fluoroquinolones; they can either stimulate or inhibit the functions of the immune system (Szczyпка and Obminska-Mrukowicz, 2003).

There have been no published reports on the immuno-pharmacological effect of marbofloxacin in rabbits. Therefore, the present work was aimed to explore the possible effect; if any; of marbofloxacin on cellular immune response of non-vaccinated and RHDV-vaccinated rabbits.

### **MATERIALS AND METHODS**

#### **Drug: Marbofloxacin (Marbocyl® 10%) :**

It is present as injectable vial of 50 ml. It was produced by Vétoquinol SA, France.

#### **Vaccine :**

Inactivated rabbit haemorrhagic disease virus vaccine was used for active immunization of experimental rabbits. It was purchased from Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt.

#### **Animals :**

A total of twenty (20) Newzealand white rabbits of 3-4 months, weighing about 2-2.5 kg were used in this work. They were purchased from a private rabbitry without previous history of RHDV outbreaks or vaccination against RHDV.

#### **Experimental design :**

Rabbits were housed in disinfected metal cages in a well ventilated, well lightened and disinfected room. They received commercial pellet ration and clean water (ad-libitum), and

kept under observation for 1 week before being used. They were classified into 4 groups (each of 5 rabbits) as the following:

- Group (1) was left as control, non-vaccinated non-treated group.
- Group (2) was intramuscularly injected with marbofloxacin 10% at a dose of 2 mg/kg b.wt. (0.05 ml/rabbit) according to **Abo-El-Sooud and Goudah, (2009)** for 5 successive days.
- Group (3) was subcutaneously injected with the inactivated rabbit haemorrhagic disease virus vaccine at a dose of 0.5 ml per rabbit (**Hanaa, et al., 2009**).
- Group (4) was given marbofloxacin 10% (2 mg/kg b.wt.) then vaccinated at the 5th day of treatment.

Each group was housed separately under well hygienic measures with daily observation until the end of the experiment.

#### **Sampling :**

Two blood samples (whole blood and serum) were collected from the ear vein of each rabbit at zero time (prior vaccination and drug administration), and at 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days post vaccination and/or drug administration for studying the cellular immune response.

#### **a) Whole blood sample :**

Blood sample (2-3 ml) was collected from the ear vein in a sterile Wasserman tube containing EDTA (0.5 mg/ml of blood) to be used for determination of total leukocytic counts.

#### **b) Serum sample :**

In a sterile Wasserman tube, 3-5 ml of blood was collected without an anticoagulant.

The sample was allowed to coagulate, and then the serum was separated by centrifugation at 3000 r.p.m. for 10 minutes and stored at -20°C until assayed.

**Assessment of cellular immune response:**

- 1- Determination of Total leukocyte count was carried out according to **Schalm, et al., (1975)** using Turk's solution as a diluent for white blood cells.
- 2- Measurement of serum nitric oxide level in serum was measured according to **Ramadan and Attia, (2003)**.
- 3- Measurement of lysozyme activity by agarose gel cell lysis assay was carried out according to the method described by **Schltz, (1987)**.

**Statistical analysis:**

The obtained data in the present study were statistically analyzed for analysis of variance (ANOVA) and least significant difference (LSD) as described by **Snedecor and Cochran, (1981)** by using computerized SPSS (1996) version 10.0.

**RESULTS AND DISCUSSION**

In veterinary practice, antibiotics are involved in the treatment of bacterial infection. Many of these antibiotics are capable of modifying the immune system even at therapeutic levels. The present work was aimed to explore the possible effect of using marbofloxacin on cellular immune response of both non-vaccinated and vaccinated rabbits (rabbit viral hemorrhagic disease). The total leukocyte count, serum nitric oxide level and serum lysozyme activity were evaluated.

**1- Effect of marbofloxacin on total leukocytic count:**

The present study showed that intramuscular injection of marbofloxacin (2 mg/kg b.wt.) for 5 successive days in vaccinated group evoked a significant decrease in total leukocytic count during the entire period of the study (4 weeks) in comparison with vaccinated non-treated group. Meanwhile, no significant changes were observed between non-vaccinated treated group and non-vaccinated non-treated group (Table 1). These results were in agreement with that of **Zahra and Abd El-Azem, (2003)** who found that when marbofloxacin was given simultaneously with the vaccine and one week post vaccination at therapeutic and double therapeutic doses it revealed a significant decrease in total leukocytic count.

Similar results were obtained by **Jimenez-Valera et al., (1995)** who observed that leukopenia was induced in ciprofloxacin treated mice. On a similar ground, **Yamaoka et al., (1991)** concluded that ofloxacin decreased the leukocytic count in aged patients suffering from lower respiratory tract infections. Furthermore, **Blum et al., (1994)** stated that decreased WBC counts, Hb concentration and PCV ratio were reported in humans receiving fluoroquinolones.

**2- Effect of marbofloxacin on serum nitric oxide level:**

Nitric oxide (NO) is a product of macrophages activated by cytokines, microbial compounds or both. It is derived from the amino acid L-arginine by the enzymatic activity of inducible nitric oxide synthase (iNOS) and functions as a tumoricidal and antimicrobial mole-

cule in vitro and in vivo (Nathan, 1992).

The obtained results concerning the effect of marbofloxacin on serum nitric oxide level in vaccinated and non-vaccinated rabbits were illustrated in Table (2). Nitric oxide level was significantly decreased in vaccinated treated group at 1 day, 3 days, 1 and 2 weeks of the experiment when compared with vaccinated non-treated group. The decrease of nitric oxide level in vaccinated marbofloxacin treated group reflects the depression of macrophage activity in response to marbofloxacin administration. Meanwhile, the non-vaccinated group showed a non significant change in nitric oxide level in response to marbofloxacin treatment in comparison with the non-vaccinated non-treated group. This was in full agreement with **Szczyпка et al., (2004)** who found that marbofloxacin administered intramuscularly five times at 24-h intervals at a dose of 2 mg/kg decreased the production of nitric oxide by peritoneal macrophages in non-infected and *E. coli*-infected mice. Similar finding was recorded by **Kolios et al., (2008)** who stated that ciprofloxacin inhibits cytokine-induced nitric oxide production in human colonic epithelium. Furthermore, **Chin-Dustin et al., (1997)** stated that norfloxacin can block the effect of nitric oxide that produced during endotoxemia in patients with hepatic cirrhosis.

### **3- Effect of marbofloxacin on serum lysozyme activity:**

Lysozyme is a natural enzyme with anti-

ral, antibacterial and immunomodulating actions that acts as a non-specific defense mechanism and reflects the activities of macrophages (**El-Sayed and Manal, 2007**).

Table (3) showed that administration of marbofloxacin to vaccinated group elicited a significant decrease in serum lysozyme level at the 1<sup>st</sup> and 3<sup>rd</sup> days of the study in comparison with vaccinated non-treated group. The decrease of lysozyme activity in vaccinated marbofloxacin treated group reflects the depression of macrophage activity in response to marbofloxacin administration. While, the drug administration in non-vaccinated rabbits induced non-significant changes when compared with non-vaccinated non-treated control group. Similar results were obtained by **Bradford and Rubin (1986)** who found that the quinolone nucleus-containing nedocromil sodium produced small but significant inhibition of FMLP-induced lysozyme secretion by about 25% in rabbits. The results also agree with that of **Webber et al., (1997)** who mentioned that nedocromil sodium inhibited the increase in lysozyme produced by platelet activating factor of ferret's trachea.

It was concluded that marbofloxacin has a suppressive effect on cellular immune response of vaccinated rabbits, so it is not recommended to be used during vaccination programs but can be used safely in non-vaccinated rabbits.

Table (1): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on total leukocytic count ( $\times 10^3$  cells/ml) in vaccinated and non-vaccinated rabbits. (Mean  $\pm$  S.E.) n=5.

Group	Time post vaccination and/or treatment					
	Zero time	3 <sup>rd</sup> day	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
G1 (Non-vacc. Non-treat.)	7.38 $\pm$ 0.42 <sup>a</sup>	6.78 $\pm$ 0.54 <sup>b</sup>	7.48 $\pm$ 0.50 <sup>b</sup>	7.67 $\pm$ 0.57 <sup>b</sup>	7.31 $\pm$ 0.45 <sup>b</sup>	6.57 $\pm$ 0.44 <sup>b</sup>
G2 (marbofloxa cin treated)	7.68 $\pm$ 0.58 <sup>a</sup>	6.62 $\pm$ 0.59 <sup>b</sup>	6.84 $\pm$ 0.74 <sup>b</sup>	7.58 $\pm$ 0.66 <sup>b</sup>	7.75 $\pm$ 0.46 <sup>b</sup>	6.44 $\pm$ 0.97 <sup>b</sup>
G3 (RHDV vaccinated)	7.60 $\pm$ 0.61 <sup>b</sup>	9.27 $\pm$ 0.81 <sup>a</sup>	9.50 $\pm$ 0.19 <sup>a</sup>	11.19 $\pm$ 0.86 <sup>a</sup>	9.82 $\pm$ 0.28 <sup>a</sup>	9.15 $\pm$ 0.52 <sup>a</sup>
G4 (vaccinated treated)	7.02 $\pm$ 0.81 <sup>a</sup>	6.21 $\pm$ 0.73 <sup>b</sup>	7.52 $\pm$ 0.21 <sup>b</sup>	8.38 $\pm$ 0.72 <sup>b</sup>	7.85 $\pm$ 0.78 <sup>b</sup>	5.71 $\pm$ 0.58 <sup>b</sup>

Means within the same column bearing different superscripts are significant at ( $p < 0.05$ ).

Table (2): Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on serum nitric oxide level ( $\mu\text{g/ml}$ ) in vaccinated and non-vaccinated rabbits. (Mean  $\pm$  S.E.) n=5.

Group	Time post vaccination and/or treatment				
	Zero time	1 <sup>st</sup> day	3 <sup>rd</sup> day	1 <sup>st</sup> week	2 <sup>nd</sup> week
G1 (Non-vacc. Non-treat.)	17.61 $\pm$ 0.95 <sup>a</sup>	17.88 $\pm$ 0.97 <sup>b</sup>	18.44 $\pm$ 1.11 <sup>b</sup>	18.17 $\pm$ 0.84 <sup>b</sup>	17.72 $\pm$ 1.50 <sup>b</sup>
G2 (marbofloxa cin treated)	18.59 $\pm$ 1.687 <sup>a</sup>	18.46 $\pm$ 1.66 <sup>b</sup>	20.00 $\pm$ 0.84 <sup>b</sup>	18.25 $\pm$ 0.65 <sup>b</sup>	18.44 $\pm$ 1.22 <sup>b</sup>
G3 (RHDV vaccinated)	18.97 $\pm$ 1.45 <sup>b</sup>	28.40 $\pm$ 1.38 <sup>b</sup>	28.94 $\pm$ 1.30 <sup>b</sup>	24.59 $\pm$ 1.72 <sup>a</sup>	22.32 $\pm$ 1.56 <sup>a</sup>
G4 (vaccinated treated)	18.65 $\pm$ 1.47 <sup>a</sup>	20.48 $\pm$ 1.22 <sup>b</sup>	21.90 $\pm$ 0.99 <sup>b</sup>	19.63 $\pm$ 1.44 <sup>b</sup>	18.21 $\pm$ 0.98 <sup>b</sup>

Means within the same column bearing different superscripts are significant at ( $p < 0.05$ ).

**Table (3):** Effect of marbofloxacin (2 mg/kg b.wt.) given intramuscularly for 5 successive days on serum lysozyme concentration (ug/ml) in vaccinated and non-vaccinated rabbits. (Mean  $\pm$  S.E.) n=5.

Group	Time post vaccination and/or treatment				
	Zero time	1 <sup>st</sup> day	3 <sup>rd</sup> day	1 <sup>st</sup> week	2 <sup>nd</sup> week
G1 (Non-vacc. Non-treat.)	109.76 $\pm$ 7.19 <sup>a</sup>	111.79 $\pm$ 6.04 <sup>b</sup>	120.61 $\pm$ 5.40 <sup>b</sup>	103.13 $\pm$ 3.98 <sup>b</sup>	103.13 $\pm$ 3.98 <sup>a</sup>
G2 (marbofloxacin treated)	113.97 $\pm$ 8.11 <sup>a</sup>	113.99 $\pm$ 5.40 <sup>b</sup>	116.19 $\pm$ 7.48 <sup>b</sup>	102.94 $\pm$ 2.21 <sup>b</sup>	109.78 $\pm$ 7.19 <sup>a</sup>
G3 (RHDV vaccinated)	111.77 $\pm$ 6.05 <sup>a</sup>	169.15 $\pm$ 5.40 <sup>a</sup>	160.33 $\pm$ 8.25 <sup>a</sup>	120.20 $\pm$ 4.41 <sup>a</sup>	107.55 $\pm$ 7.37 <sup>a</sup>
G4 (vaccinated treated)	105.35 $\pm$ 4.25 <sup>a</sup>	127.09 $\pm$ 11.25 <sup>b</sup>	133.85 $\pm$ 9.88 <sup>b</sup>	118.61 $\pm$ 5.40 <sup>a</sup>	109.58 $\pm$ 6.43 <sup>a</sup>

Means within the same column bearing different superscripts are significant at (  $p < 0.05$  ).

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## الملخص العربى

### التأثير الفارماكولوجى المناعى للماريفلوكساسين فى الأرانب

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تم دراسة تأثير الماريفلوكساسين على المناعة الخلوية فى عدد ٢٠ أرنب نيوزيلاندى (سواء تم تحصينها بلقاح النزف الدموى الفيروسى أو لم تحصن)، يتراوح عمرهم بين ٣-٤ شهور وأوزانهم بين ٢-٢.٥ كجم. تم إعطاء الدواء عن طريق الحقن العضلى بجرعة قدرها ٢مجم/كجم من وزن الجسم ولمدة ٥ أيام متتالية، تم تقييم العدد الكلى لكرات الدم البيضاء، مستوى أكسيد النيتريك ونشاط الليزوزيم فى المصل، أوضحت نتائج الدراسة أن إعطاء الماريفلوكساسين فى الأرانب المحصنة أدى إلى نقص معنوى فى العدد الكلى لكرات الدم البيضاء لة ٤ أسابيع، مستوى أكسيد النيتريك فى المصل لمدة إسبوع واحد ونشاط الليزوزيم فى المصل لمدة ثلاثة أيام، على الجانب الآخر لم يكن هناك تغيرات معنوية فى كلاً من العدد الكلى لكرات الدم البيضاء، مستوى أكسيد النيتريك ونشاط الليزوزيم فى المصل فى الأرانب الغير محصنة التى تم إعطائها الماريفلوكساسين، ونستخلص من هذه الدراسة أنه لاينصح باستخدام الماريفلوكساسين فى الأرانب المحصنة حيث أن له تأثير مثبط للمناعة ولكن يمكن استخدامه بصورة آمنة فى الأرانب الغير محصنة.