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RESEARCH ARTICLE

BIO-EQUIVALENCE STUDY OF TWO INJECTABLE TILMICOSIN FORMULATIONS (MICOTIL 300® AND COZINA 300®) IN CALVES

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Abstract

The present study was designed to assess the comparative bio-equivalence of Micotil 300® and Cozina 300® in healthy Calves after SC injection of both products in a dose of 10 mg tilmicosin base/kg.b.wt. Ten calves were divided equally into two groups (5 calves for each group). The first group was designed to study the pharmacokinetics of Micotil 300®, while the 2nd group was designed to study the pharmacokinetics of Cozina 300®. Each calf in both groups was SC injected with 10 mg tilmicosin/kg.b.wt. Blood samples were obtained from the jugular vein and collected immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single SC injection. The disposition kinetics of Micotil 300® and Cozina 300® following SC injection of 10 mg tilmicosin/kg.b.wt. revealed that the maximum blood concentration [C_{max}] were 4.01 and 3.53 µg/ml and attained at [t_{max}] of 1.82 and 1.83 hours, respectively. In conclusion: Cozina 300® is bioequivalent to Micotil 300®since the ratios of C_{max}, AUC_{0-24} and$AUC_{0-\infty}$ (T/R) were 0.88, 0.87 and 0.89 respectively. These are within the Bio-equivalence acceptance range. Micotil 300® and Cozina 300®are therefore bioequivalent and interchangeable.

Introduction:-

Tilmicosin injection is administered as a single subcutaneous injection at a dose rate of 10 mg/kg body weight. Tilmicosin has proven particularly effective in the treatment of bovine respiratory disease (BRD) (Scott, 1995; Musser et al., 1996). While work undertaken in healthy animals can give a guide to the distribution of an antibiotic in the body, most cattle receive tilmicosin are clinically ill ones with pneumonia. The role of alveolar macrophages in the clearance of bacteria from lungs has recently attracted renewed interest (Thompson et al., 1994).

Macrolide antibiotics are known to accumulate in leukocytes, bronchial secretions and penetrate into the subcellular compartments (lysosomes), thus contribute to the efficacy of the antibiotic (Thompson et al., 1994). Tilmicosin has an in vitro antibacterial spectrum that is primarily against Gram-positive, Pasteurella and Mycoplasma (Hartman and Geryl, 1993).

Tilmicosin is a macrolide antibiotic that has been available in the United States since 1992. It has been approved for treatment of the bovine respiratory disease (BRD) in beef cattle and nonlactating dairy cattle associated with

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Pasteurella and Mycoplasma species (Crosier et al., 1996), and in swine as a feed additive for the control of respiratory disease associated with Actinobacillus pleuropneumoniae and P. multocida (Federal Register, 1996).

Macrolide antibiotics are considered to be among the safest anti-infective drugs in clinical use, with severe adverse reactions being rare (Periti et al., 1993). Although adverse effects of several macrolides on the cardiovascular system have been reported (Wakabayashi & Yamada, 1972; Tamargo et al., 1982; Freedman et al., 1987), they have been at doses much greater than therapeutic, and/or the effects were seen in subjects with already compromised cardiac status or impaired renal function. Some reported cardiovascular toxicity of tilmicosin involved large doses given by routes other than the labelled subcutaneous injection (Jordan et al., 1993).

The Bio-equivalence studies play an important role in determining therapeutic efficacy to register the generic drug products according to the Food and Drug Administration (FDA) regulations (Chen et al., 2001). Bio-equivalence is defined as statistically equivalent bioavailability between two products at the same molar dose of the therapeutic moiety under similar experimental conditions (Chen et al., 2001; Toutain and Bousquet-Melou, 2004). The drug products are said to be bioequivalent if they are pharmaceutical equivalents or pharmaceutical alternatives and if their rate and extent of absorption do not show a significant differences statistically according to the FDA regulations (Chen et al., 2001).

The aim of this study is to evaluate Bio-equivalence of two injectable tilmicosin formulation (Micotil 300® and Cozina 300®) after SC injection of a single dose of 10 mg tilmicosin/kg b.wt. in calves.

Materials And Methods:-

Drugs

Micotil 300®: is manufactured by Elanco-Animal Health, Gmbh, Germany). It is dispended as injectable solution. Each 1ml contains 300 mg tilmicosin (as phosphate) and it was used as reference product.

Cozina 300®: is manufactured by Boston Company, Pharma Cure Division, Egypt, as injectable solution. Each 1ml contains 300 mg tilmicosin (as phosphate) and it was used as test product.

Calves and Experimental Design

Ten Holstein calves, weighing 150-170 kg (8-10 months of age) were obtained from Faculty of Veterinary Medicine, Benha University, Egypt. Each calf was housed in an individual well-ventilated hygienic pen during the entire period of the experiment. Calves were fed with antimicrobials free diet ad-libitum with drinking water freely available throughout the experimental period.

Bio-equivalence Study:

Calves were used to study the bio-equivalence of Micotil 300® and Cozina 300® after SC injection. Calves were divided into two groups. The 1st group (5 calves) was used to study the pharmacokinetics of Micotil 300®. The 2nd group (5 calves) was used to study the pharmacokinetics of Cozina 300®. Calves in the 1st group were SC injected with Micotil 300® at a dose of 10 mg tilmicosin/kg.b.wt, while calves in the 2nd group were SC injected with Cozina 300® at a dose of 10 mg tilmicosin/kg.b.wt.

Blood Samples

Blood samples were obtained from the Jugular vein (2 ml) and collected in test tubes immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single SC injection (groups 1 and 2). Samples were centrifuged at 3000 rpm for 10 minutes and the obtained sera were used for the estimation of tilmicosin concentration. The serum samples were stored at −20˚C until analysis, and the assay was performed within a week of obtainment.

Analytical Procedure

Rapid agar-diffusion assay for the quantitative determination of tilmicosin in small volumes of blood by using Bacillus subtilis (ATCC 6633) as a test organism (Arret et al. 1971). Fresh stock solutions of tilmicosin at 300 µg/ml were made up in 0.1 M buffer (pH 6.0) for each set of assays. About 1 ml of the suspension of Bacillus subtilis (was added to 100 ml agar at 55-60 ^{o}C. The mixture was shaken thoroughly till complete mixing of the test organism with agar. Petri dishes (20 cm x 20 cm) were used; about 25 ml of inoculated medium were poured to each dish by using sterile cylinder. After complete solidification, six wells were made on the surface of inoculated agar using stainless

steel cylinder. The wells of each plate were filled with the serum sample. The plates were incubated at 37 ˚c for 16-18 hours. The diameter of each inhibition zone was measured.

The calibration curves of serum were prepared with different concentrations between 0.1 and 100 µg/mL using blank calves serum. Thereafter, the diameters of inhibition zones were measured with the aid of a transparent rule to the nearest millimeter. Each sample was replicated three times and analyzed similarly. The plot of tilmicosin serum concentrations versus diameters of inhibition zone was linear with a correlation coefficient of 0.971. Serum concentrations of tilmicosin were determined by comparing the zone of inhibition diameters with the standard curve. The absence of interfering endogenous compounds was demonstrated in antibacterial-free plasma obtained at time 0 (pretreatment) which showed no visible zone of inhibition around the impregnated disks. The limit of quantification (LOQ) defined visually as the smallest amount of drug that still produced a clearly distinguishable inhibition zone around the edges of tilmicosin contained pores on nutrient agar media was 0.20 µg/ml.

Pharmacokinetics and Statistical Analysis

Serum concentrations of tilmicosin versus time data obtained during the study were utilized for calculating various pharmacokinetic variables using a compartmental and non-compartmental analysis using computerized program, WinNonline 4.1 (Pharsight, USA).

The peak concentrations (C_{max}) and time to peak (T_{max}) were obtained from the serum concentration-time data directly. The areas under the serum concentration of tilmicosin time curves from time 0 to the last sample collected (AUC_{0-24}) were calculated using linear trapezoidal method (Baggot, 2001). While $AUC_{0-\infty}$ was derived from AUC_{0-24} + $AUC_{24-\infty}$, where $AUC_{24-\infty} = C_{24}/ß$. For Bio-equivalence evaluation, the ratios of C_{max} (T/R), AUC_{0-24} (T/R) and $AUC_{0-\infty}$ (T/R) were calculated. Values within the Bio-equivalence acceptable range at 90% confidence interval, 0.80 – 1.25 were considered for accepting the null hypothesis of Bio-equivalence between the reference and the test brands (EMEA, 2002, 2006).

Results:-

The mean serum concentrations of tilmicosin in Micotil 300® and Cozina 300® following SC injection of 10 mg tilmicosin/kg.b.wt. in calves are shown (Table 1 and Figure 1).

The mean pharmacokinetic parameters of tilmicosin in Micotil 300® and Cozina 300® after SC injection of 10 mg tilmicosin/kg.b.wt. in calves are shown (Table 2).

The disposition kinetics of tilmicosin in Micotil 300® and Cozina 300® following SC injection of 10 mg tilmicosin/kg.b.wt in calves revealed that the maximum blood concentration [C_{max}] were 4.01 and 3.53 µg/ml and attained at [T_{max}] of 1.82 and 1.83 hours, respectively. The mean ratio of C_{max} and AUC of the tested and reference formulations were within Bio-equivalence range and summarized in Table (3).

The Bio-equivalence ratio for mean C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ (T/R) of Cozina 300® versus the reference product (Micotil 300®) were 0.88, 0.87 and 0.89 respectively. These values were within the recommended range at the level of 90% confidence interval, 0.80 – 1.25 (U.S. Food and Drug Administration, 2003). The two injectable formulations of tilmicosin (Micotil 300® and Cozina 300®) in this experiment could therefore be considered bioequivalent.All the experimental calves remained healthy during and after the study.

Discussion:-

The clinical efficacy of an antimicrobial is determined not only by its activity against infective organisms but also by its ability to reach the site of infections and its persistence within tissues. Pharmacokinetic variables such as plasma concentration, half life, bioavailability, rate of elimination are important considerations for rational use of antimicrobial agents.

The value of C_{max} determined in this study after an SC dose of 10 mg tilmicosin/kg b.wt. (4.01 and 3.53 µg/mL for Micotil 300® and Cozina 300®, respectively. This finding was higher than that recorded for tilmicosin in cattle (0.87 µg/ml; Mordic et al. 1998), beef cattle (0.71 µg/ml; Lombardi et al. 2011), calves (0.97 and 1.33 µg/ml; Dimitrova et al. 2012; Soliman ana Ayad, 2014), respectively. On the other hand, time to peak serum concentration was (1.82 and 1.83 h, for Micotil 300® and Cozina 300®, respectively). This result was longer to that recorded for tilmicosin in cattle (0.5 h; Mordic et al. 1998), beef cattle (0.57 h; Lombardi et al. 2011), calves (1 h; Dimitrova et al. 2012). While it was shorter than the result recorded for tilmicosin in calves (7.21 h; Soliman ana Ayad, 2014).

The elimination half-lives of tilmicosin ($t_{0.5el}$) were 9.67 and 10.13 h, for Micotil 300® and Cozina 300®, respectively. These results are longer than that recorded for tilmicosin in broiler chickens (7.30 h; El-Hewaity, 2016) and shorter than that recorded for tilmicosin in cattle (29.4 h; Mordic et al. 1998), beef cattle (31.15 h; Lombardi et al. 2011), calves (32.33 and 24.60 h; Dimitrova et al. 2012; Soliman ana Ayad, 2014), respectively.

Concentration of tilmicosin in serum from 10 min up to 24 h exceeds the MIC against sensitive micro-organisms. The concentration was detected up to 24 hours in the serum of calves given (Micotil 300® as a reference product and Cozina 300® as a tested product) above the MIC for P. haemolytica (0.78 µg/ml; Hartman and Geryl, 1993).

The area under the curve (AUC) estimation, using the method of trapezoids, is the critical step in the calculation of pharmacokinetic estimations using non-compartmental analysis (Rowland and Tozer, 1989).

Bio-equivalence study is a test to assure the clinical efficacy of a generic versus brand drugs (Chen et al., 2001). Bio-equivalence refers to a comparison between generic formulations of a drug, or a product in which a change has been made in one or more of the ingredients or in the manufacturing process, and a reference dosage form of the same drug. This study shows that the Bio-equivalence ratio for mean C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ (T/R) of Cozina 300® versus the reference product (Micotil 300®) were 0.88, 0.87 and 0.89 respectively. These values were within the recommended range at the level of 90% confidence interval, 0.80 – 1.25 (U.S. Food and Drug Administration, 2003). The two injectable formulations of tilmicosin (Micotil 300® and Cozina 300®) in this experiment could therefore be considered bioequivalent.

Table 1:-Mean (X ± S.E) serum concentrations (µg/ml) of tilmicosin in Micotil 300® and Cozina 300® following SC injection of 10 mg tilmicosin/kg.b.wt. in calves (n = 5).

Time post Administration (hour)	Mean serum concentration (µg/ml)	
	Micotil 300® (Reference)	**Cozina 300®** (Test)
0.08	0.45±0.02	0.37±0.01
0.16	0.92±0.02	0.83±0.03
0.25	1.89±0.07	1.56±0.08
0.5	2.52±0.15	2.11±0.16
1	3.42±0.13	3.07±0.12
2	4.29±0.27	3.85±0.25
4	3.35±0.25	2.82±0.19
6	2.67±0.11	2.07±0.14
8	1.98±0.98	1.63±0.09
12	1.34±0.09	1.02±0.07
24	0.86±0.04	0.80±0.03

Table 2:- Mean (X ± S.E) pharmacokinetic parameters of tilmicosin in **Micotil 300®** and **Cozina 300®** SC injection of 10 mg tilmicosin/kg.b.wt. in calves (n = 5).

Parameter	Unit	**Micotil 300®** (Reference)	**Cozina 300®** (Test)
K_{ab}	h^{-1}	1.57± 0.06	1.44 ± 0.04
K_{el}	h^{-1}	0.071 ± 0.001	0.068 ± 0.001
$t_{1/2(ab)}$	h	0.44 ± 0.01	0.48 ± 0.02
$t_{1/2(el)}$	h	9.67 ± 0.31	10.13 ± 0.47
C_{max}	µg ml^{-1}	4.01 ± 0.25	3.53 ± 0.18
t_{max}	h	1.82 ± 0.06	1.83 ± 0.06
AUC	µg $ml^{-1}h^{-1}$	56.24 ± 1.38	50.55 ± 1.94
AUMC	µg $ml^{-1}h^{-2}$	830.25 ± 15.18	765.48 ±31.73
MRT	h	14.76 ± 0.53	15.36 ± 0.68

k_{ab}; K_{el} absorbtion and elimination rate constant after SC injection; **$T_{0.5(ab)}$** absorbtion half life after SC injection; **$T_{0.5(el)}$**elimination half life after SC injection; **C_{max}** maximum plasma concentration; **T_{max}** time to peak plasma

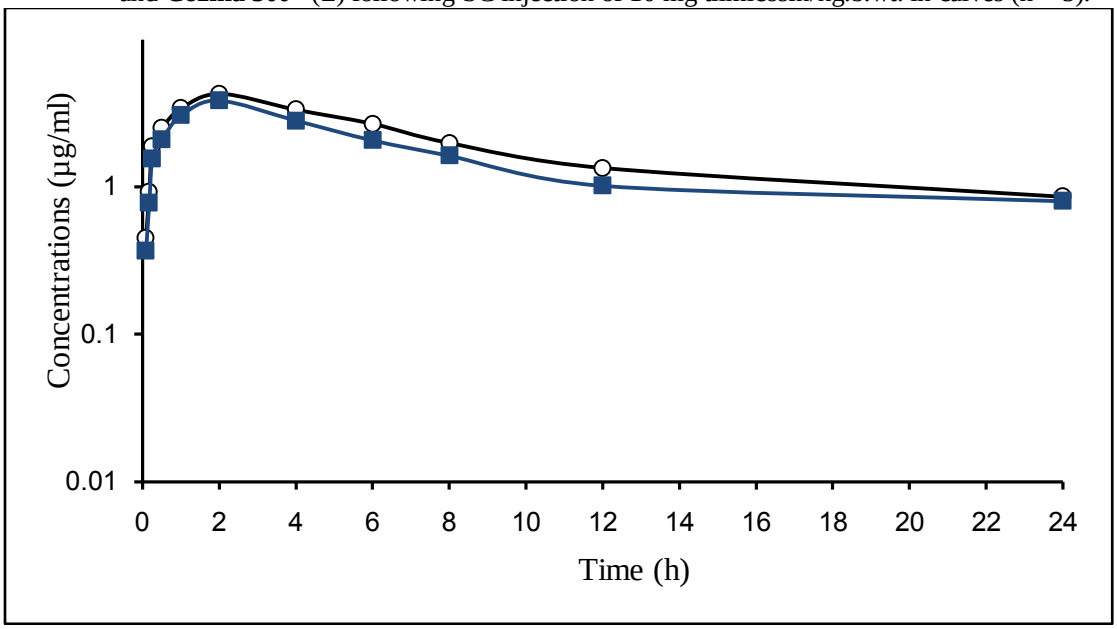
concentration; **AUC**; area under serum concentration-time curve; **AUMC** area under moment curve; **MRT** mean residence time.

Table 3:- Bio-equivalence between **Micotil 300®**(reference) and **Cozina 300®**(test) formulations.

Bio-equivalence	C_{max}	AUC_{0-24}	$AUC_{0-\infty}$
Micotil 300®(reference)	4.01±0.25	44.24±1.37	56.24±1.38
Cozina 300® (test)	3.53±0.18	38.85±2.04	50.55±1.94
Point estimate	0.88	0.87	0.89
Acceptable range	0.80-1.25	0.80-1.25	0.80-1.25
Conclusion	**BE**	**BE**	**BE**

BE-Bio-equivalence

Figure 1:- Semilogarthimic plot showing the serum concentrations-time profile of tilmicosin in **Micotil 300®** (○) and **Cozina 300®** (■) following SC injection of 10 mg tilmicosin/kg.b.wt. in calves (n = 5).



Conclusions:-

Based on the above pharmacokinetic and statistical results that calculated in the current study, we concluded that Cozina 300® which manufactured by Boston Company, Pharma Cure Division, Egypt, is bioequivalent to Micotil 300® which manufactured by Elanco-Animal Health Gmbh, Germany) and both products can be used as interchangeable drug in veterinary medicine practice especially in calves.

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