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RESEARCH ARTICLE

BIOEQUIVALENCE STUDY OF TWO STREPTOMYCIN FORMULATIONS (ESTREPTOVALL® AND NILESTREPT®) IN BROILER CHICKENS

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Abstract

The present study was designed to assess the comparative bio-equivalence of Estreptovall® and Nilestrept® in healthy broiler chickens after IM injection of both products in a dose of 25 mg streptomycin base/kg.b.wt. Twenty four broiler chickens were divided into two groups. The first group was designed to study the pharmacokinetics of Estreptovall®, while the 2nd group was designed to study the pharmacokinetics of Nilestrept®. Each broiler chicken in both groups was IM injected with 25 mg streptomycin base/kg.b.wt. Blood samples were obtained from the wing 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 IM injection. The disposition kinetics of Estreptovall® and Nilestrept® following IM injection of 25 mg streptomycin base/kg.b.wt. revealed that the maximum blood concentration [C_{max}] were 25.75 and 23.84 µg/ml and attained at [t_{max}] of 2.50 and 2.51 hours, respectively. In conclusion: Nilestrept® is bioequivalent to Estreptovall®since the ratios of C_{max}, AUC_{0-24} and$AUC_{0-\infty}$ (T/R) were 0.93, 0.92 and 0.92 respectively. These are within the bioequivalence acceptance range. Estreptovall® and Nilestrept®are therefore bioequivalent and interchangeable.

Introduction:-

Aminoglycosides are utilized primarily in the treatment of infections caused by aerobic gram-negative organisms. They are not active against anaerobic organisms. In addition to their strength in the treatment of gram-negative pathogens, aminoglycosides can be effective against some gram-positive organisms, such as *Staphylococcus aureus,* (Calvert et al., 1985; Brown, 1988), some mycobacteria (Malik et al., 1994), and some spirochetes. They are sometimes administered concurrently with other antibacterials for a possible synergistic effect.

However, the use of aminoglycosides in the treatment of infection in animals has been tempered by toxicity considerations in the animal treated (Prescott and Baggot, 1993). Often, systemic use is limited to the treatment of serious gram-negative infections resistant to less toxic medications. Also, local environment at the therapeutic site can affect the efficacy of these drugs, acidic or purulent conditions can hamper their effect (Ziv et al., 1982).

Streptomycin was the earliest aminoglycoside introduced. It is active against mycobacteria, Leptospira,Francisellatularensis, and Yersinia pestis, but only some mycoplasma, gramnegative organisms, and Staphylococcus species (Prescott and Baggot, 1993). Dihydrostreptomycin is chemically very similar to

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streptomycin. The introduction of newer aminoglycosides has eclipsed the significance of dihydrostreptomycin and streptomycin in the face of increasing bacterial resistance although some dosage forms of these medications are still available (Cote et al., 1991; Morishita et al., 1996).

Aminoglycosides are more effective against rapidly multiplying organisms, and they affect and ultimately destroy bacteria by several mechanisms. They need only a short contact with bacteria to kill them and, as such, are concentration dependent in their actions. Their main site of action is the membrane-associated bacterial ribosome through which they interfere with protein synthesis.

Pharmacokinetic studies are used to discover the fate of drug in the body. Knowledge of the route of removal, metabolic paths and the degree of efficiency are vital informations for the right dosage, which protects the body against potential drug toxicity (Anfossi *et al*., 2002; Naoaki *et al*., 2002).

The bioavailability and bioequivalence 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). Bioavailability is defined as the rate and extent to which an active drug ingredient is absorbed and becomes available at the site of drug action. In case of bioequivalence it 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 bioequivalence of two streptomycin soluble powders (Estreptovall® and Nilestrept®) after IM injection of a single dose in broiler chickens.

Materials and Methods:-

Drugs

Estreptovall®: is manufactured by Mevet SA, Spain. It is dispended as soluble powder. Each 1gm contains 626 mg streptomycin sulphate (equivalent to 500 mg streptomycin base) and it was used as reference product.

Nilestrept®: is manufactured by Boston Company, Khayrat El- Nile Division, Egypt, as soluble powder. Each 1gm contains 626 mg streptomycin sulphate (equivalent to 500 mg streptomycin base) and it was used as test product.

Broiler Chickens and Experimental Design

Twenty four healthy broiler chickens (40 - 45 days old and weighing 2 - 2.40 kg) were obtained from Benha private poultry farm, Egypt. They were kept individually in cages, within a ventilated, heated room (20˚C), and 14 hours of day light. They received a standard commercial ration free from any antibiotics for 15 days before starting the experiment to insure complete clearance of any anti-bacterial substances from their bodies. Water was offered *ad-libitum.*

Bioequivalence Study:

Broiler chickens were used to study the bio-equivalence of Estreptovall® and Nilestrept® after IM injection. Broiler chickens were divided into two groups. The 1st group (12 broiler chickens) was used to study the pharmacokinetics of Streptomycin®. The 2nd group (12 broiler chickens) was used to study the pharmacokinetics of Nilestrept®. Broiler chickens in the 1st group were IM injected with Estreptovall® in a dose of 25 mg streptomycin base/kg.b.wt, while broiler chickens in the 2nd group were IM injected with Nilestrept® in a dose of 25 mg streptomycin base/kg.b.wt.

Blood Samples

Blood samples were obtained from the wing vein (1 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 IM injection (groups 1 and 2). Samples were centrifuged at 3000 rpm for 10 minutes and the obtained sera were used for the estimation of streptomycin 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 streptomycin in small volumes of blood by using Bacillus subtilis (ATCC 6633) (Arret *et al*. 1971).

Fresh stock solutions of streptomycin at 1,000 µg/ml were made up in 0.1 M phosphate 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 0.25 and 100 µg/mL using blank chickens 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 streptomycin serum concentrations versus diameters of inhibition zone was linear with a correlation coefficient of 0.976. Serum concentrations of streptomycin 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 streptomycin contained pores on nutrient agar media was 0.10 µg/ml.

Pharmacokinetics and Statistical Analysis

Serum concentrations of streptomycin 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 streptomycin 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 bioequivalence evaluation, the ratios of C_{max} (T/R), AUC_{0-24} (T/R) and $AUC_{0-\infty}$ (T/R) were calculated. Values within the bioequivalence acceptable range at 90% confidence interval, 0.80 – 1.25 were considered for accepting the null hypothesis of bioequivalence between the reference and the test brands (EMEA, 2002, 2006).

Results:-

The mean serum concentrations of streptomycin in Estreptovall® and Nilestrept® following IM injection of 25 mg streptomycin base/kg.b.wt. in broiler chickens are shown (Table 1 and Figure 1).

The mean pharmacokinetic parameters of streptomycin in Estreptovall® and Nilestrept® following IM injection of 25 mg streptomycin base/kg.b.wt. in broiler chickens are shown (Table 2). The disposition kinetics of streptomycin in Estreptovall® and Nilestrept® following IM injection of 25 mg streptomycin base/kg.b.wt. in broiler chickens revealed that the maximum blood concentration [C_{max}] were 25.75 and 23.84 µg/ml and attained at [T_{max}] of 2.50 and 2.51 hours, respectively. The mean ratio of C_{max} and AUC of the reference and tested formulations were within bioequivalence range and summarized in Table (3).All the experimental chickens remained healthy during and after IM injection in this study.

Discussion:-

The objective of the present study was to investigate the pharmacokinetics of streptomycin in the chickens following a single IM injection. This information becomes important when considering the potential use of streptomycin as a therapeutic agent in chickens.

The effectiveness of a drug is partly dependent on its formulation, route of administration and metabolic pattern (Alvinerie *et al*., 1999). These factors determine the plasma concentration-time profile of the drug. Following a single IM injection (25 mg/kg b.wt) of streptomycin formulations to healthy broiler chickens, therapeutic concentration were achieved 5 minutes post injection in all the chickens. The concentration was detected up to 24 hours in the serum of chickens given the (Estreptovall® as a reference product and Nilestrept® as a tested product).

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 (Rowlan and Tozer, 1989).

After single IM injection of streptomycin at a dose of 25 mg/kg B.W, the $t_{1/2\beta}$ (3.50 and 3.48 h) for both Estreptovall® and Nilestrept® , respectively. These results was longer than gentamicin at a dose of 5 mg/kg bw, IM, the $t_{1/2\beta}$ (2.87 h) in broiler chickens (Abu-Bash et al., 2007), gentamicin in turkey (Haritova *et al.*, 2004) and higher than those reported for gentamicin in eagles (Bird *et al.*, 1983) and roosters (Pedersoli *et al.*, 1990). The short elimination half-life in chickens suggests that streptomycin was rapidly eliminated from the body.

Our results showed that streptomycin is rapidly absorbed after IM injection, with a peak plasma concentration (C_{max}) of 25.75 and 23.84 µg/ml at 2.50 and 2.51 h for both Estreptovall® and Nilestrept® , respectively. These results were higher than gentamicin in broiler chickens (C_{max}) of 11.73 µg/ml at 0.55 h (Abu-Bash et al., 2007).

Concentration of streptomycin in serum from 5 min up to 24 h exceeds the MIC against sensitive micro-organisms. The concentration was detected up to 24 hours in the serum of chickens (The MIC of aminoglycosides for *Escherichia coli* strains was 0.215 µg/ml; Jakobsen et al., 2007).

Bioequivalence study is a test to assure the clinical efficacy of a generic versus brand drugs (Chen *et al*., 2001). Bioequivalence 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 (Alvinerie *et al*., 1999). This study shows that the bioequivalence ratio for mean C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ (T/R) of Nilestrept® versus the reference products (Streptomycin®) were 0.93, 0.92 and 0.92 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 formulations of streptomycin tested in this experiment could therefore be considered bioequivalent.

Table 1:- Mean (X ± S.E) serum concentrations (µg/ml) of streptomycin in **Estreptovall®** and **Nilestrept®** following IM injection of 25 mg streptomycin base/kg.b.wt. in broiler chickens (n = 12).

Time post Administration (hour)	Mean serum concentration (µg/ml)	
	Estreptovall®(Reference)	**Nilestrept®** (Test)
0.08	0.26±0.01	0.23±0.01
0.16	0.97±0.05	0.84±0.03
0.25	2.74±0.11	2.15±0.06
0.5	6.98±0.26	5.82±0.12
1	18.67±0.63	16.94±0.78
2	30.68±0.78	28.76±0.91
4	21.89±0.72	20.89±0.49
6	13.61±0.81	12.43±0.74
8	8.38±0.36	7.83±0.31
12	3.11±0.12	2.67±0.09
24	0.42±0.02	0.39±0.01

Table 2:- Mean (X ± S.E) pharmacokinetic parameters of streptomycin in **Estreptovall®** and **Nilestrept®** following IM injection of 25 mg streptomycin base/kg.b.wt. in broiler chickens (n = 12).

Parameter	Unit	**Estreptovall®**(Reference)	**Nilestrept®** (Test)
K_{ab}	h^{-1}	0.40 ± 0.002	0.39 ± 0.002
K_{el}	h^{-1}	0.197 ± 0.001	0.199 ± 0.001
$t_{1/2(ab)}$	h	1.73 ± 0.04	1.74 ± 0.07
$t_{1/2(el)}$	h	3.50 ± 0.14	3.48 ± 0.16
C_{max}	µg ml^{-1}	25.75 ± 0.49	23.84 ± 0.78
t_{max}	h	2.50 ± 0.11	2.51 ± 0.14
AUC	µg $ml^{-1}h^{-1}$	188.86 ± 4.68	174.26 ± 6.87
AUMC	µg $ml^{-1}h^{-2}$	1067.74 ±78.13	973.71 ±73.17
MRT	h	5.63 ± 0.12	5.58 ± 0.25

k_{ab}; K_{el} absorbtion and elimination rate constant after IM injection; $T_{1/2(ab)}$ absorbtion half life after IM injection; $T_{1/2(el)}$ elimination half life after IM injection; C_{max} maximum plasma concentration; T_{max} time to peak plasma

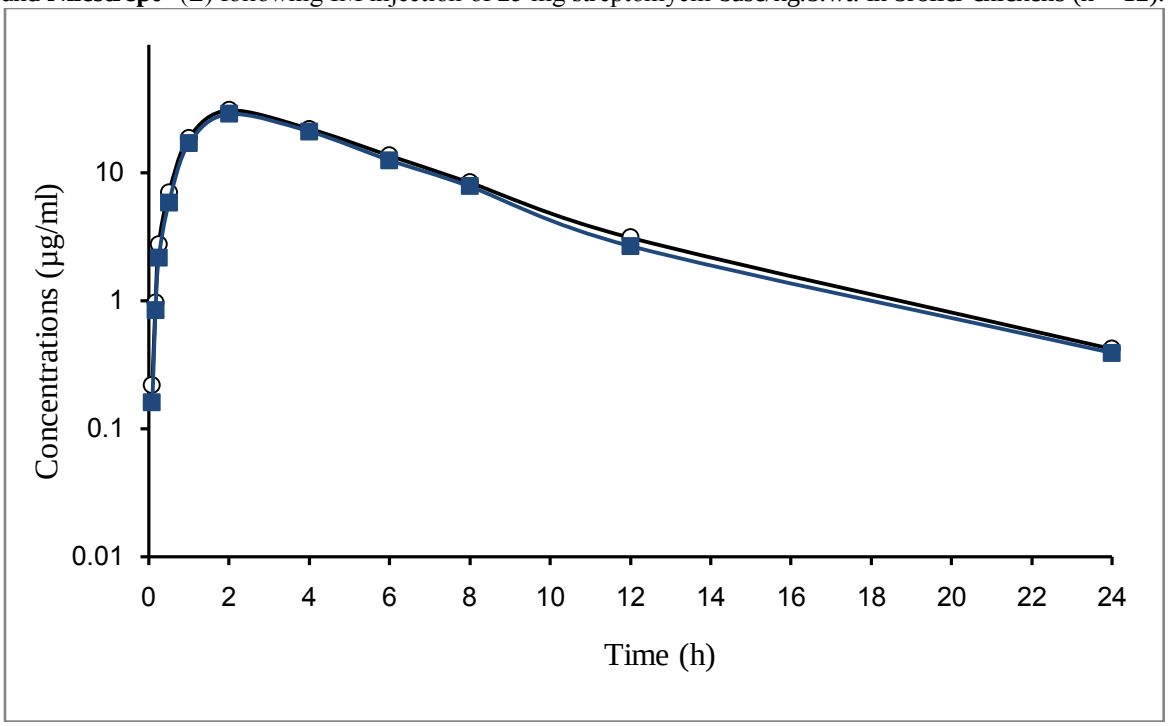
concentration; AUC; area under serum concentration-time curve; AUMC area under moment curve; MRT mean residence time.

Table 3:- Bioequivalence between Estreptovall® (reference) and Nilestrept® (test) formulations.

Bioequivalence	C_{max}	AUC_{0-24}	$AUC_{0-\infty}$
Estreptovall® (Reference)	25.75 ± 0.49	186.74±8.14	188.86 ± 4.68
Nilestrept® (Test)	23.84±0.78	172.30±6.83	174.26 ± 6.87
Point estimate	0.93	0.92	0.92
Acceptable range	0.80-1.25	0.80-1.25	0.80-1.25
Conclusion	**BE**	**BE**	**BE**

BE-Bioequivalence

Figure 1:- Semilogarthimic plot showing the serum concentrations-time profile of streptomycin in **Estreptovall®** (○) and **Nilestrept®** (■) following IM injection of 25 mg streptomycin base/kg.b.wt. in broiler chickens (n = 12).



Conclusions:-

Based on the above pharmacokinetic and statistical results that calculated in the current study, we concluded that Nilestrept® which manufactured by Boston Company, Khayrat El- Nile Division, Egypt, was bioequivalent to Estreptovall® which manufactured by Mevet SA, Spain, and both products can be used as interchangeable drug in veterinary medicine practice especially in poultry.

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