Evaluation of Teratogenic Potentials and Tissue Residues of Tiamulin in Albino Rats

Mossad, G. A. El-Sayed, Ashraf, A.A. El-Komy and Amany, A. A. El-Meleh

Department of pharmacology, Faculty of Veterinary Medicine, Benha University, Egypt
amani.mailto: amani.mleeh@fvtm.bu.edu.eg.

Abstract: Drugs or medicinal agents should only be used in pregnancy if there is proven benefit to the mother and no potential teratogenic risks. The present study was performed to evaluate the teratogenic potentials of tiamulin in albino rats and its residues in tissues. The pregnant rats were divided into five groups, group (1) behaved as control received normal saline from 6th to 9th day of pregnancy and from 6th to 15th day of pregnancy. Group (2) received 36 mg/kg b.wt of tiamulin orally daily from 6th to 9th day of pregnancy and group (4) from 6th to 15th day of pregnancy. Group (3) received 72mg/kg b.wt of tiamulin orally daily from 6th to 9th day of pregnancy and group (5) from 6th to 15th day of pregnancy. There was no death or abortions, treated groups showed significant decrease in litter size, weight, length and retarded growth in fetuses. Fetal resorption was significantly increased in treated groups. The incidences of skeletal and visceral anomalies were increased in treated groups. Following oral administration of both dose of tiamulin from 6th to 9th day of pregnancy and from 6th to 15th day of pregnancy revealed distribution of the drug in tested tissues (brain, lung, heart, liver, spleen, kidney, thigh, and thoracic muscles, fat, skin and whole fetuses).

[Mossad, G. A. El-Sayed, Ashraf, A.A. El-Komy and Amany, A. A. El-Meleh. **Evaluation of Teratogenic Potentials and Tissue Residues of Tiamulin in Albino Rats.** *Nat Sci* 2014;12(1):100-105]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 16

Keywords: tiamulin, pregnancy, anomalies, residues.

Introduction

Most therapeutic agents cross placental barrier **(Schlegel and Marshall., 1991; Hikkinen *et al*., 2000 and Witt *et al*., 2003)** and enter fetal circulation. Every agent given during pregnancy has tendency to produce some sort of structural abnormalities in the neonate. The most critical period for malformation to take place is the period of organogenesis. Drugs given during this period are more likely to cause birth defects. This critical time of fetal developments in rats and mice is from 6-12 days of their gestation gestation **(Somer., 1962; Farris., 1967 and Petrova and Savitskaia., 1988)**. Tiamulin is a semisynthetic derivative of pleuromutilin antibiotic produced by pleuromutilus; active against mycoplasmas; gram positive and gram negative germs **(Crivineanu., *et al*; 1999; Marne and Alexander., 2003)**.

The aim of present study to investigate and compare the effect of tiamulin administration during gestation on albino rats in term of, litter size, weight, length, resorptions and detection of any gross malformation as skeletal and visceral anomalies. As well as tissue residues of drug in both female rats and their fetuses.

2- Material and methods

2.1.1. Animals:

The experiment was carried out on Wister albino rats. The used rats were 8-10 months and 210-250 gram, obtained from animal house colony of faculty of Veterinary Medicine Benha University. Rats were kept under hygienic conditions fed on standard balanced diet and water free from antimicrobials to withdraw any antibacterial residues. Female rats were examined periodically using vaginal smear technique to ensure that they were in regular estrous cycle (Cahen, 1966). Each female in estrous phase was paired with a male of proven fertility in a separate cage. In the morning, vaginal smear was taken to verify day of pregnancy. Presence of spermatozoa in the obtained vaginal smear suspected pregnancy (Barcellona et al., 1977).

2.1. 2.Drug:

Tiamulin (Tiamutin®) in the form of water soluble granules contain 45 g tiamutin hydrogen fumerate=14-desoxy-14(2-diethylaminoethyl) mercapto acetoxy)-mutilin-hydrogenfumerate, corresponding to 36.45g tiamutin base. It produced by Sandoz GmbH A-6250 Kundl, Austria.

2.2. Experimental design:

Group (1): Ten pregnant female rats were used as control group and received normal saline according to the method of administration used.

Group (2): Ten pregnant female rats were given therapeutic dose (36 mg/kg b.wt) of tiamulin orally from 6th to 9th day of pregnancy, and sacrificed at 10th day of pregnancy to detect number of resorption sites, percentage of fertility and tissue residues of the drug.

Group (3): Ten pregnant female rats were given double therapeutic dose (72 mg/kg b.wt) of tiamulin orally from 6th to 9th day of pregnancy and sacrificed at 10th day of pregnancy to detect number of resorption

sites, percentage of fertility and tissue residues of the drug.

Group (4): Twenty pregnant female rats were given therapeutic dose (36 mg/kg b.wt) of tiamulin orally from 6th to 15th day of pregnancy, ten rats were sacrificed at 16th day of pregnancy to detect tissue residues and the other ten rats were sacrificed at 20th day of pregnancy to detect tetratogenic effects and tissue residues of the drug.

Group (5): Twenty pregnant female rats were given double therapeutic dose (72 mg/kg b.wt) of tiamulin orally from 6th to 15th day of pregnancy, ten rats were sacrificed at 16th day of pregnancy to detect tissue residues and the other ten rats were sacrificed at 20th day of pregnancy to detect teratogenic effects and tissue residues of the drug.

2.3. Teratological design:

Pregnant female rats of groups one, four and five were sacrificed at the 20th day of gestation and dissected to examine the effect of the administered drugs on fetal development by morphological, visceral and skeletal examinations according to Hayes. (1986).

2.4. Analytical procedure:

Tiamulin was assayed in serum and tissues bymicrobiological method According to Grove. and Randall (1955) and Arret et al (1971)

using *Micrococcus Luteus* as tested organisms, which was obtained from microbiological Department (Animal Health Institute, Dokky, Giza, Egypt).

2.5. Statistical analysis:

The data were calculated as mean ± standard error. All statistical analysis was carried out manually according to Snedecor and Cochrohn. (1967).

3. Results

The obtained results indicated that, oral administration of tiamulin in therapeutic dose (36 mg/kg b.wt) and double therapeutic dose (72 mg/kg b.wt) orally from 6th to 9th day of pregnancy to female pregnant rats produced significant increase in number of resorbed fetuses per mother as recorded in table (1). Also there was significant increase in number of resorbed fetuses were recorded following administration of both doses of tiamulin from 6th to15th of pregnancy as recorded in table (1). Highly significant decrease in number of fetuses table (2), fetal body weight and length were significantly decreased as table (3).

Visceral examination of examined fetuses following administration of therapeutic dose of tiamulin (36 mg/kg b.wt) resulted in diverticulum dilation of the brain in 31.03%, thymus hypoplasia in17.24 %, pulmonary hypoplasia in 34.48%, cardiac enlargement in 31.03%, hepatomegaly in 51.72%, kidney hypotrophy with dilation of renal pelvis either unilateral or bilateral in 44.83% and small size of suprarenal gland in 10.34%. Oral administration of double therapeutic dose of tiamulin (72 mg/kg b.wt) to pregnant rats from 6th to 15th day of pregnancy induced diverticulum dilation of the brain in 37.04%, thymus hypoplasia in 33.33%, pulmonary hypoplasia in 51.85%, cardiac enlargement in 40.74%, hepatomegaly in 59.26%, and kidney hypotrophy in 66.67% (Table 4).

Skeletal examination of fetuses obtained from mothers given orally therapeutic dose of tiamulin (36 mg/kg b.wt) from 6th to 15thday of pregnancy showed impaired ossification of the skull in 30.77%, absence of sternbra in 26.92%, or small size of sternbra. Absence of digital bone of fore and hind limb were recorded in 38.46%, absence of some metatarsal bone in 30.77% and some metacarpal bone in 34.62%, absence of caudal vertebrae in 46.15% of examined fetuses. Administration of double therapeutic dose of tiamulin (72 mg/kg b.wt) to pregnant rats resulted in impaired ossification of the skull in 52.17%, absence of sternbra in 59.56%, also complete absence of digits bone of fore and hind limb were recorded in 56.52%, absence of some metatarsal bone in 47.83% and some metacarpals bone in 43.48%, absence of caudal vertebrae in 69.57% of examined fetuses as shown in table (5).

Following oral administration of therapeutic dose of tiamulin (36mg/kg b.wt) once daily revealed distribution of the drug in tested tissues (brain, lung, heart, liver, spleen, kidney, thigh, and thoracic muscles, fat, skin and whole fetuses). The liver and kidney contained the highest concentrations during concentrations (0.43 ± 0.010 and 0.34± 0.003 mg/g respectively), in rats slaughtered at 10th of pregnancy. On other hand, the lowest concentrations were recorded in fat and skin (0.07±0.006 and 0.13±0.005 µg/g respectively). The data reported for the rats slaughtered at 16th day of pregnancy (24/hours after last dose administration) reported that the liver, kidney, spleen, lung and heart contained the highest concentrations (0.45±0.005, 0.51±0.145, 0.32±0.011, 0.31±0.008 and 0.31±0.006 µg/g respectively). Fat and skin contained the lowest concentration of tiamulin (0.10 ± 0.006 and 0.16±0.007 mg/g respectively).The data resulted from the rats slaughtered at 20th day of pregnancy (5 days post drug administration, the drug cannot be assayed in all tissues except liver and kidney which contained low concentrations 0.05± 0.006 and 0.04± 0.008 µg/g respectively. high concentrations of the drug after double therapeutic administration were assayed in liver, kidney and spleen of slaughtered rats at 10th day of pregnancy (0.62± 0.012, 0.39 ± 0.021 and 0.34±0.012 µg/g respectively) while brain and fat contained the lowest concentrations. The results from the rats slaughtered at 16th day of pregnancy (24 hours past dray administration) revealed that the liver, kidney, spleen, lung and muscle (thoracic and thigh)

and heart contained highest concentrations (1.33±0.008, 0.84±0.012, 0.44±0.011, 0.36±0.008, 0.34±0.005, 0.33±0.009. and 34±0.006 µg/g. respectively). The data reported from the rats slaughtered at 20th day of pregnancy (5 day post drug administration) recorded that the drug was disappeared from all tissue, except liver, kidney, spleen which contained 0.11± 0.012, 0.05± 0.009 and 0.04, and 0.006 µg/g respectively as shown in table (6).

Table (1): Effect of tiamulin on the number of resorbed fetuses N=10.

Animal group	Number of resorbed fetuses X ± S.E
Group (2)	2.00±0.831*
Group (3)	1.50±0.640*
Group (4)	2.80±1.00**
Group (5)	2.80±0.680**

Table (2): Effect of tiamulin on the number of viable and dead fetus obtained from rats administered 36 and72 mg/kg b.wt. of tiamulin orally daily from 6th to 15th day of pregnancy. (n=10)

Animal group	Number of fetus X ± S.E	Number of viable fetus X ± S.E	Number of dead fetus X ± S.E
control	8.40 ± 0.221	5.40± 0.221***	-
Group (4)	7.80 ± 0.133*	7.60± 0.164**	-
Group (5)	6.60± 0.163***	5.40± 0.221***	-

Table (3): Effect of tiamulin on fetal body weight and length. (n=10)

Animal group	Fetal body length (cm)	Fetal body weight (g)
Control	3.74±0.0642	4.40±0.047
Group (4)	3.42±0.017***	4.14±0.036***
Group (5)	2.27±0.051***	3.47±0.020***

Table (4): Visceral abnormalities in fetuses obtained from rats administered tiamulin (36 and72mg/kg b.wt.) orally once daily from 6th to 15th day of pregnancy.

Abnormalities in	Tiamulin	
	(36mg/kg b.wt.) n=29	(72mg/kg b.wt.) n=27
Brain	31.03 **%**	37.04 **%**
Thymus	17.24 **%**	33.33 **%**
Heart	31.03 **%**	40.74 **%**
Lungs	34.48 %	51.85 %
Liver	51.72 **%**	59.26 **%**
Kidney	44.83 %	66.67 **%**

Table(5): Skeletal abnormalities in fetuses obtained from rats administered tiamulin (36 and 72mg/kg b.wt orally, once daily from 6th to 15th day of pregnancy.

Abnormalities in	Tiamulin	
	(36mg/kg b.wt) n=26	(72mg/kg b.wt) n=23
Skull	30.77 %	52.17 %
Sternbrea	26.92 %	59.56 %
Ribs	-	-
Digital bone	38.46 %	56.52 %
Metatarsal bone	30.77 %	47.83 %
Metacarpal bone	34.62 %	43.48 %
Caudal vertebrae	46.15 %	69.57 %

Table (6): Serum (µg/ ml) and tissue (µg/g) concentrations of tiamulin in pregnant rats. (n=3).

Serum or tissue	Tiamulin (36 mg/kg b.wt) from 6th to 15th day of pregnancy (X ± S.E)			Tiamulin (72 mg/kg b.wt) from 6th to 15th day of pregnancy (X ± S.E)		
	Time of slaughter			Time of slaughter		
	10th day	16th day	*20th day*	10th day	16th day	*20th day*
Serum	0.17±0.003	0.23±0.005	-	0.21±0.008	0.27±0.003	-
Fat	0.07±0.006	0.1±0.006	-	0.13±0.005	0.18±0.003	-
Skin	0.13±0.005	0.16±0.007	-	0.23±0.007	0.28±0.003	-
Brain	0.15±0.003	0.21±0.005	-	0.17±0.006	0.26±0.003	-
Fetus	0.21±0.006	0.24±0.012	-	0.25±0.007	0.28±0.003	-
Heart	0.18±0.003	0.31±0.006	-	0.23±0.006	0.34±0.006	-
Liver	0.43±0.010	0.45±0.005	0.05±0.006	0.62±0.012	1.33±0.008	0.11±0.012
Kidney	0.34±0.003	0.51±0.145	0.04±0.008	0.39±0.021	0.84±0.012	0.05±0.009
Spleen	0.29±0.012	0.32±0.011	-	0.34±0.012	0.44±0.011	0.04±0.006
Lung	0.26±0.003	0.31±0.008	-	0.29±0.006	0.36±0.008	-
Thigh Muscle	0.22±0.006	0.30±0.006	-	0.28±0.003	0.34±0.005	-
Thoracic Muscle	0.24±0.007	0.27±0.005	-	0.24±0.012	0.33±0.009	-

4. Discussion

The current investigation aimed to study teratogenicity of tiamulin in rats. Oral administration of tiamulin by using stomach tube in therapeutic and double therapeutic dose (36 and72mg/kg b.wt) to female pregnant rats induced marked and significant decrease in the number of fetuses / mother when compared with that recorded value of the control group without any fetal death. This result was consistent with the data reported by **Kitajima *et al.* (1987)** after administration of rokitamycin to rabbits lead to decrease in number of the fetuses. The decrease in the number of fetuses per mother might be attributed to the direct toxic action of the tested drug on the early developed fertilized ovum or the lack of oval production or of the basic cell constituent by the mother **Tuchmann-Duplessis, 1975.** Tiamulin in therapeutic and double therapeutic dose resulted in marked increase in the number of resorbed fetuses either early or late. This result was similar to that reported by **Farley *et al.* 1991** following administration of low dose of tacrolimus to pregnant mice resulted in a higher number of resorptions and following administration of Bryostatin-1 a macrolide antibacterial agent to pregnant rats **Jiangbo *et al.* 2010** The increase in the number of resorbed fetuses in the present study might be attributed to the interference of the tested drug with the placental transmission of leucin amino acid and magnesium as deficiency of leucin or magnesium produced high incidence of fetal resorptions **Tuchmann-Duplessis, 1975.** Administration of tiamulin in therapeutic and double therapeutic dose to female pregnant rats during the period of organogenesis produced significant decrease in both weight and length of fetuses. This result was consistent with that reported by **Furuhashi *et al.* 1989** following administration of miporamicin to rats, following administration of tilmicosin in rabbits **Noda (1993)**. there was significant decrease in all growth and developmental parameters following administration of macrolides in cultured rat embryos dose dependently **Karabulut *et al.* 2008**.The recorded reduction in fetal weight and length which resulted after oral administration of tested drug might be attributed to the disturbance in metabolism of some minerals as magnesium and zinc in fetus, or to the interference of the drug to the placental transmission of magnesium and zinc from the mother to the fetus **Tuchmann-Duplessis, 1975** as deficiencies of magnesium and zinc induced retardation of fetal growth, increasing fetal resorption and high rate of embryonic death. Administration of therapeutic and double therapeutic dose of tiamulin to female pregnant rats during the period of organogenesis induced many fetal visceral abnormalities as diverticulum dilatation in the brain of fetuses. The obtained result was consistent with those reported by **Karabulut *et al.* 2008.** Also another study **Moriyama *et al.* 2011** concluded that treatment with rapamycin induced enormous influences on early developmental period. This lesion might be attributed to the transfusion of arginine amino acid caused by this drug or to the disturbance of the metabolism of arginine in fetus **Tuchmann-Duplessis, 1975** proved that, deficiency of arginine in pregnant rat or mice resulted in brain abnormalities such as diverticulum dilatation due to inhibition of cell differentiation. Tiamulin in the therapeutic and double therapeutic dose resulted in hypoplasia of thymus gland, which was a dose-dependent. This result was similar to that reported by **Jordan and Higdon 1988** after administration of tilmicosin to rats induced both total visceral and skeletal anomalies and with **Petrova and Savitskaia (1988)** following administration of oleandomycin in rats. Also **Takai *et al.* 1990** recorded that; Tacrolimus might impair thymic microenvironment and disturb the thymocyte maturation. Other investigators as **Moriyama *et al.* 2011**, mentioned that rapamycin

induced enormous influences on early developmental period.

Administration of tiamulin in therapeutic and double therapeutic dose to female pregnant rats produced cardiac hyperplasia which was a dose-dependent. These fetal abnormalities similar to that reported by **Jordan and Bernard (1989)** following administration of tilmicosin to female rats and by **Källén *et al.* 2005** reported that erythromycin induced congenital malformation.

Administration of tiamulin in therapeutic and double therapeutic dose to female pregnant rats from 6th to 15th day of pregnancy induced pulmonary hypoplasia which was dose -dependent. Similar results were obtained by **Karabulut *et al.* 2008** reported that, there was significant decrease in all growth and developmental parameters following administration of macrolides in cultured rat embryos dose dependently.

Administration of tiamulin produced hepatomegaly. This result agrees with **Jordan and Higdon 1988** after administration of tilmicosin to rats induced both total visceral and skeletal anomalies. Administration of tiamulin in therapeutic and double therapeutic dose to female pregnant rats induced hypoplasia of one or both kidneys with unilateral or bilateral dilatation of renal pelvis. This result was dose-dependent. The same result was recorded by **Nielson *et al.*1995** following administration of tacrolimus to pregnant rats.

Administration of tiamulin in therapeutic and double therapeutic dose produced some fetal skeletal malformations such as impaired ossification of skull, absence of sternbrae, reduction or absence of caudal vertebrae, absence of digit's bone of fore and hind limb and absence of some metacarpal and metatarsal bone. This result agree with that reported by many investigators as **Phornphutkul *et al.* 2009 and Noda (1993)**.The reported bone lesion might be attributed to deficiency of functionally available magnesium **Menschik *et al.* 1997**.

Estimation of serum and tissue concentration of tested drug in rats during period of organogenesis revealed that; Liver and kidney contain the highest concentration in rats slaughtered at 10th day of pregnancy while fat and skin contain the lowest concentrations. The liver, kidney, slaughtered at 16th day of pregnancy contain the highest concentration but fat and skin contain the lower concentration. The drug was not be assayed in all tissue except liver and kidney at 20th day of pregnancy (5 days post drug administration). This result was agreed with **Locke *et al* 1982** following administration of tylosin to some avian species and tylosin residues were not detected in any pig tissue after two days after stop of drug administration **Moats *et al.* 1985**. Also **Burrows *et al.* 1986** found that, the highest concentration of tylosin in cavles kidney while lowest concentrations were recorded in muscle and cerebrospinal fluid**.** Residues of tiamulin were detected only in liver and kidney of pigs fed tylosin in diet for 17 days **Lauridsen *et al.* 1988.** Tissue residues of tylosin in kidney persisted 7 days post treatment **Moran *et al.* 1990**. High tissue level of spiramycin observed in most tissues of rats such as, liver, spleen, stomah, intestine, and lung **Shi *et al.*2004**. **Maria *et al.* 2008** found that, withdrawal time of tiamulin in pork 6 days and after 5 days for pig liver after stop of drug administration. The highest concentration of tylosin were detected in liver, kidney and muscle of sheep **Samia *et al.* 2008**. Also **Zhang *et al.* 2011**found that, the extensive distribution of valnemulin in swine lung and liver. This could be due to the fact that, the macrolide antibiotics are weak organic bases and are distributed throughtout most tissue. The high tissue levels create a reservoir of drugs that could be slowely released into systemic circulation and allow long duration of action from multiple administration of drug.

Corresponding author
Amany, A. A. El-Meleh
Department of pharmacology, Faculty of Veterinary Medicine, Benha University, Egypt
amani.mailto:amani.mleeh@fvtm.bu.edu.eg.

References:

1. Arret, B.; Johnson, D.P. and Kirshbaum, A.(1971): Outline of details for microbiological assays of antibiotics 2nd revision. J. Pharm. Sci, 60 (11), 1689-1694.
2. Barcellona, P.S.; Fanelli, O. and Compana ,A: Teratological study of etoperidone in the rat and rabbits. Toxicology (1977); 8, 87-94.
3. Burrows G.E., Barto P.B. and Martin B. (1986). Antibiotic disposition in experimental pneumonic pasteurellosis: gentamicin and tylosin. Can. J. Vet. Res. 50, 193-199.
4. Cahen, R.C: Experimental and clinical chemoteratogensis. Adv.Pharm(1966); 263-334.
5. Crivineanu; Maria; Statescu, C; Crivineanu, V.(1999): Farmacoterapie; Farmacotoxicologie; farmacovigilnă. Ed. Romana de Maine; Bucuresti.
6. F.; Shelby, J.; Alexander, D. and Scott, J.R: The effect of two new immunosuppressive agents, FK506 and didemnin B, in murine pregnancy. Transplantation. 1991 Jul;52(1):106-10.
7. Farris, EJ: The care and breeding of laboratory animals.7 Ed.(1967). New York:John Willy and sons.
8. F.; Suda, H.; Sasaki, M.; Nakajima, M. and Yamamoto, H. 1989: Oral dosage study of miporamicin administered during the period of fetal organogenesis in rats.
9. Grove, D.C. and Randall, W.A. (1955): Assay methods of antibiotics: A laboratory manual medical encyclopedia. Inc, pp 34-36.

10. Hayes, A.W. (1986): Principles and methods of toxicology. Raven Press. New York.
11. Heikkinen, K.; Laine, P.J.; Neuvonen, U. and Ekblad. (2000): The transplacental transfer of the macrolide antibiotics erythromycin, roxithromycin and azithromycin. BJOG an international journal of obstetrics and gynaecology 2000; Volume: 107, Issue: 6, Pages: 770-775.
12. J.; Xuying, W.; Yuping, Z.; Xili, M.; Yiwen, Z. and Tianbao, Z. Toxicity of bryostatin-1 on the embryo-fetal development of Sprague-Dawley rats. Birth Defects Res B Dev Reprod Toxicol 2010 Jun; 89(3):171-4.
13. Jordan, W.H. and Bernard, N.R. (1989). Chronic toxicity study in beagle dogs given oral doses of tilmicosin. Upublished study No. Do7187 from Lilly Research Laboratories. Submitted from Lilly, Basingstoke, UK.
14. Jordan, W.H and Higdon, G.L : A teratological study of tilmicosin adminstered orally to rats. Submitted to WHO 1988.
15. K.; Otterblad, O. P. and Danielsson, B.R. Is erythromycin therapy teratogenic in human therapy? Reprod Toxicol 2005. Jul-Aug; 20(2):209-14.
16. Karabulut, A.K.; Uysal, I. I.; Acar, H. and Fazliogullari. Z. Investigation of Developmental Toxicity and Teratogenicity of Macrolide Antibiotics in Cultured Rat Embryos. Histologia, Embryologia 2008; Volume 37, Issue 5, pages 369–375.
17. K.; Koda, S.; Kobayashi, Y. and Hayano, K.(1987): Reproduction study of rokitamycin. Teratological study in rabbits. Jpn J Antibiot.;40(3):602-7.
18. Lauridsen M.G., Lund C. and Jacobsen M. (1988). Determination and depletion of residues of carbadox, tylosin, and virginiamycin in kidney, liver, and muscle of pigs in feeding experiments. J. AOAC 71, 921-925.
19. Locke, D., Bush, M. and Carpenter, J.W. (1982). Pharmacokinetics and tissue concentrations of tylosin in selected avian species. Am. J. Vet. Res. 43, 1807-1810.
20. Maria, C.; Trifan, V.; Paraschiv, G. And Crivineanu, D. (2008): Tiamulin residue depletion in pork and pig liver. Bulletin Veterinary medicine, 65 (1).
21. Marne, G. and Alexander, S.M. (2003): Macrolide Antibiotics: Binding Site, Mechanism of Action, Resistance. Medicinal Chemistry, 3, 949-961.
22. Menschik, M.; Neumuller, J.; Steiner, C.W.; Erlacher, L,; Koller, M.; Ullrich, R.; Graninger, W.; and Graninger, W.B. (1997): Effect of ciprofloxacin and ofloxacin in adult human cartilage in vitro.. Antimicrob. Agents Chemother., 41, 2562-2565.
23. Moats , W.A.; Harris, E.W. and Steele, N.C. (1985). Comparison of liquid cheromatography and bioassay procedures for determining depletion of intramuscular injected tylosin. J. Assoc. Anal Chem., 68: 413-416.
24. Moran, J.W.; Colman, M.R.; Thomson, T.D. and Cochrane, R.L (1990).The determination of tylosin residues in tissue following administration of tylan by intramuscular injection to weaned calves. Lilly Research Laboratories, Green Field, Indiana.
25. M.; Ohata, Y.; Mori, S.; Matsukawa, S.; Michiue, T.; Asashima, M and Kuroda, H. (2011): Rapamycin treatment causes developmental delay, pigmentation defects, and gastrointestinal malformation on Xenopus embryogenesis. 404(4):974-8. Biochem Biophys Res Commun.
26. Nielson, F.T.; Leyssac, P.P.; Kemp, E.; Starklint, H. and Dieperink, H. Nephrol Dial Transplant. 1995;10(3):334-40. Nephrotoxicity of FK-506 in the rat. Studies on glomerular and tubular function, and on the relationship between efficacy and toxicity.
27. Noda, A. (1993): Teratogenicity study of EL-870 (tilmicosin aqueous) in rabbits by gavage. Unpublished study No. 91-001 from Research Institute for Animal Science in Biochemistry andToxicology, Japan. Submitted to WHO by Lilly, Basingstoke, UK.
28. P. and Savitskaia, T.N. (1988): Prenatal effect of oleandomycin on the development of the immunogenesis organs. Farmakol Toksikol.;51(3):84-6.
29. Phornphutkul, C.; Lee, M.; Voigt, C.; Wu, K.Y.; Ehrlich, M.G.; Gruppuso, P.A. and Chen, Q. The effect of rapamycin on bone growth in rabbits. J Orthop Res. 2009 Sep;27(9):1157-61.
30. Samia, A.A.; Hassan.; Sania, A.I.; Shaddad, I.B.; EL Tayeb, M.A.; Omer, M.H.; AL Nazawi. And Homeida, A.M. (2008). Detection of tylosin residue levels following intramuscular injection in desert sheep. Research. Journal of Pharmacology 2(1): 1-3.
31. Schlegel, P.N.; Chang, T.S. and Marshall, F.F. Antibiotics: potential hazards to male fertility. Fertil Steri, 1991, vol. 55, p. 235-242.
32. Shi, X.G.; Sun, Y.M.; Zhang, U.F. and Zhong, D. (2004): Tissue distribution of bitespiramycin and spiramycin in rats. Acta Pharmacol Sin .; 25 (11): 1396-1401
33. Snedecor, G.W. and Cochrohn. W.G. (1967): Statistical methods. 6th Ed. Low state.Univ. Press. Amer. USA.
34. Somer, G.F. Thalidomide and congenital abnormalities. Lancet. 1962, vol. 1, p. 912-913.
35. T, K., Jojima, K., Sakatoku, J. and Fukumoto, T: Effects of FK506 on rat thymus: time-course analysis by immunoperoxidase technique and flow cytofluorometry. Clin Exp Immunol. 1990 December; 82(3): 445–449.
36. Tuchmann-Duplessis, 1975: Drug effect on the fetus. ADIS press, New York, USA.
37. W.; Sommer, E.M.; Cichna, M.; Postlbauer, K.; Widhalm, A.; Gregor, H. and Reisenberger, K. (2003): Placental passage of clarithromycin surpasses other macrolide antibiotics. Am J Obstet Gynecol.;188(3):816-9.
38. Zhang, Z.; Zhang, C.Y.; Guo, J.P.; zhu, L.X.; Luo, X.Y.; Wang, R. and Liu, L.H. (2011). Pharmacokinetics and lung tissue concentration of valnemulin in swine. Journal of animal and Veterinary Advances 10 (14): 1824- 1828.

12/28/2013