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The Potential Teratogenic Effect of Tilmicosin in Rats: Visceral Malformations and Histomorphological Alterations in Fetal Internal Organs

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ABSTRACT

Potential fetal adverse effects are well-known for several antibacterial classes. The present study was performed to evaluate the potential teratogenic effect of Tilmicosin (TMS) antibiotic in Sprague Dawley rats. TMS was daily administered orally to pregnant rats at two dose levels, 250 or 500 mg/kg bw, on 6-15 days of gestation. The morphological or skeletal malformations were non-significantly different from control values. However, visceral examination revealed elevation in the percentage of intrathoracic and intracranial haemorrhages, and kidney hypoplasia (unilateral or bilateral) in fetuses from dams treated with the high dose. In addition, both dose levels produced fetuses with increased percentages of dilated brain ventricles, heart ventricles and renal pelvis. Histopathologically, both TMS doses induced pathological changes in fetal liver and kidney, in a dose-dependent manner. Liver showed hepatocyte hydropic degeneration, congestion of hepatic blood vessels, engorgement of bile canaliculi and dilatation of hepatic lymphatic vessels. Kidney revealed coagulative necrosis of renal tubules and glomerular tuft, adhesion between the parietal and visceral layers of Bowman's capsule and the glomerular tuft became ring shape with the presence of multiple layer glomerular tufts. In conclusion, TMS antibiotic has the potential to induce teratogenic effects (mainly visceral) and pathological changes in liver and kidneys tissues of rat fetuses when administered during the organogenesis period. This result demonstrated the ability of TMS to pass the placental barrier.

Keywords: *Tilmicosin, teratogenic, malformation, histopathology, rats.*

INTRODUCTION

Drugs or medicinal agents should only be used during pregnancy if they are proven to have benefits to the pregnant dams without inducing any adverse effects on the embryo and fetus. It has been proved that most of medications can cross the placental barrier and enter to fetal circulation. It is believed that every agent given during pregnancy has a tendency to induce some types of structural abnormalities in the fetus until otherwise is proved (Schlegel and Marshall, 1991; Witt *et al.*, 2003)

Sometimes it is necessary to use antibiotics during pregnancy to treat different types of infections in women or female animals to avoid mother death, preterm birth, low birth weight and spontaneous abortion. Antibiotics administration during pregnancy have been associated with both short-term and long-term effects (Bookstaver *et al.*, 2015).

Although it appears that some classes of antibacterials have relatively proven to be used safely during the period of pregnancy, there have been no large-scale studies proving safety or risk from using many classes of antibacterial drugs until now (Briggs *et al.*, 2002), and thus antibiotics are not recommended for women in pregnancy without sure evidence of complete

safety of the recommended antibiotic (Meeraus *et al.*, 2015).

Tilmicosin is semi-synthetic broad-spectrum macrolide antibiotic mainly used as a veterinary medication. It is recommended for treatment and prevention of pneumonia in cattle, sheep and pigs (FAO/WHO, 2008). Also, it is active *in vitro* against numerous gram negative and gram-positive microorganisms, that cause respiratory problems, like K. pneumoniae, Actinobacillus pleuropneumoniae, M. haemolityca, P. multocida, E. coli, E. aerogenes, P. aeruginosa, Salmonella and Mycoplasma (Ziv *et al.*, 1995; Womble *et al.*, 2006). It has advantages of low inhibitory concentration, large distribution volume, good tissue penetration especially in lung, long elimination half-life and slow excretion in milk (Han, *et al.*, 2009; Xie, *et al.*, 2011; Bhavsar *et al.*, 2012).

Modric (1997) revealed that the main component in liver, kidney, and at the injection site was parent TMS, and it is excreted mainly in urine as parent TMS and then in feces as parent and metabolic compounds after its injection in cattle.

Some researchers studied the developmental effects of TMS and proved that its administration to pregnant dams at organogenesis period couldn't induce significant deformities in rat fetuses from dams treated with up to 500 mg/kg bw (Jordan and Higdon, 1988). On contrast, others proved that TMS could induce developmental adverse effects in rabbit and rat fetuses and zebrafish embryos (Noda, 1993; Abo-Kora *et al.*, 2016; Yan *et al.*, 2019).

Therefore, in this study the teratogenic effects of TMS after its administration to pregnant rats at organogenesis period; 6-15 days of gestation was evaluated.

MATERIALS AND METHODS

The tested antibiotic

Pulmotil AC (Tilmicosin phosphate, 250 mg/ml TMS) of Elanco Animal Health Company, USA was used in this experiment. TMS has the chemical formula $C_{46}H_{80}N_2O_{13}$; 20-Deoxo-20-(3,5-dimethyl-1-piperidinyl) desmycosin.

Chemicals

Alizarin red S stain powder (LOBA Chemie PVT. LTD, India); Glycerin, diethyl ether 100%, methyl alcohol and absolute and 95% ethyl alcohol (El-Nasr Pharmaceutical Chemicals Co., Abu Zaabal, Egypt); potassium hydroxide, glacial acetic acid, and neutral buffered formalin solution (ADWIC, Egypt); saturated picric acid solution (Sigma-Aldrich Co. (St Louis, MO, USA) were used, and all other chemicals were of analytical grade.

Animals:

Sexually mature 10 male and 30 female Sprague Dawley rats (180-200 g) were obtained from the local Farm of Faculty of Veterinary Medicine, University of Sadat City. Upon arrival to our lab, the males and females were housed separately in propylene plastic cages with ad libitum access to clean tap water and balanced ration for two weeks for acclimatization before starting the experimental study under standard conditions (12h light/dark period, temperature 23 ± 2 °C, and humidity 50%).

The experiment was performed in strict accordance with the Guides of Laboratory Animals Care and Use that approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City, Egypt.

Experimental design

The possible adverse effects of TMS on developing rat fetuses during the organogenesis period (6-15 days of gestation) were investigated as described by Manson and Kang (1994). After acclimatization period, the female rats were placed into the males' cages for pairing on a ratio of 1:2, usually in the early afternoon and left overnight. Pregnancy was confirmed on the next day morning by presence of intact sperms or sperm remains in the vaginal smear for each female, and this day is considered as the zero-day of pregnancy (Paumgartten *et al.*, 1998).

The females with confirmed pregnancy were classified into 3 main groups, 10 animals for each. Group I (control) received distilled water. Group II (LD-TMS) was administered TMS at 250 mg/kg bw. Group III (HD-TMS) was administered TMS at 500 mg/kg bw. All

treatments were daily administered orally using stomach tube on days 6-15 of gestation.

Scheduled time and procedures of teratological examination:

All treated and control female dams were sacrificed under light anesthesia by diethyl ether on 20th day of gestation**.** The numbers of implantations and resorption sites on two uterine horns, live and dead fetuses were counted for each dam. All fetuses were removed by cesarean section of greater side of the uterine horns and blotted dry. The obtained fetuses and placentae were weighed and fetuses were examined for gross external abnormalities. One fetus/litter was used for histopathological examination. The remaining fetuses were assigned into two sets. One third of fetuses/litter kept for at least one week in Bouin's fixative, after which, fetuses were sectioned using Wilson's free-hand razor blade sectioning technique with recording of internal visceral malformations. The other two thirds/litter kept in 95% ethanol for subsequent preparation for skeletal examination**.**

Histopathological investigation:

Liver and kidneys of fetuses taken for histopathological examination were fixed in 10% neutral formalin and prepared for investigation as described by Bancroft *et al.*, (1996).

Statistical Analysis:

Values are given as percentages and mean ± SE. Statistical significances concerning fetal and placental weights were determined by one-way ANOVA. The comparison of the different morphological, visceral and skeletal anomalies between treated and control groups was done by Chi square test (SPSS: statistical package for social sciences 10.0 for windows) (Alan and Duncan, 2001).

RESULTS

Effects on Maternal and Fetal Indices

The alterations in maternal and fetal indices are illustrated in Table 1 and displayed in Fig. (1). TMS administration at both dose levels induced insignificant elevation in the percentages of early uterine resorption sites compared with control group (Fig. 1). Moreover, no significant changes in the percentages of the live fetuses and the mean fetal and placental weights were recorded between the different groups.

Fetal Morphological Changes:

Regarding the fetal morphological changes recorded in table 2. No external morphological abnormalities were observed in the different groups except insignificant changes in the percentages of dwarf fetuses (2.89%, 2.86%, and 3.08% in the control, LD-TMS, and HD-TMS groups, respectively, Fig. 2).

Fetal Visceral Abnormalities:

The most prominent visceral abnormalities in the fetuses from treated dams were found in nares, brain, heart, and kidneys (Table 3 and Figures 3-6).

As shown in Table 3, rat fetuses from dams treated with low dose of TMS showed insignificant increase in the percentage of intracranial hemorrhage compared with control dams. However, rat fetuses from dams treated with the high dose showed significant increase in the percentage of intracranial hemorrhage compared with those of control dams (Fig. 3).

The low and high dose levels of TMS did not induce any abnormalities in eyes, but induced insignificant elevation in the percentages of dilated nares compared with control group, as showed in Figs. 4B and 4C, respectively.

Significant elevation in the percentages of dilated brain ventricles was recorded in fetuses obtained from LD-TMS (Fig. 4E) and HD-TMS (Fig. 4F) -treated dams compared with control dams (Table 3).

Regarding the examination of transverse sections of chest cavity, there were no lung abnormalities in fetuses of all experimental groups. Fetuses of LD-TMS- treated dams showed insignificant increase in the percentage of intrathoracic hemorrhage (Fig. 5B) and significant increase in the percentage of dilated heart ventricles (Fig. 5E). However, fetuses of HD-TMS- treated dams showed significant increase in the percentage of intrathoracic hemorrhage and dilated heart ventricles (Fig. 5C&5F) versus 0% in control fetuses for both (Table 3).

The main abnormalities in the pelvic cavity of obtained fetuses were unilateral or bilateral

hypoplasia of kidney or dilated renal pelvis as showed in figure (6). Administration of low dose of TMS resulted in insignificant increase in the percentage of unilateral hypoplasia of kidney (Fig. 6B) in the obtained fetuses and significant increase in the percentage of dilated renal pelvis (Fig. 6E) against 0% of control fetuses for both (Table 3). On the other hand, administration of high dose of TMS resulted in significant increase in the percentage of hypoplasia of kidney (Fig. 6C) and dilated renal pelvis (Fig. 6F) against 0% of control fetuses for both (Table 3).

Fetal Skeletal Abnormalities

Fetal skeletal malformations in skull bones, sternum, ribs, phalanges, sacral vertebrae and caudal vertebrae were listed in Table 4. No skeletal abnormalities were observed in fetuses obtained from LD-TMS or HD- TMS groups except induction of insignificant changes in the percentages of wide-open fontanel, incomplete ossification of skull bones and reduction in the numbers of sternebrae compared with those recorded in control group.

Histopathological findings:

The liver sections of fetuses obtained from LD-TMS- treated dams showed hydropic degeneration in hepatocytes and congestion of hepatic blood vessels (Fig. 7B). However widespread hydropic degeneration in hepatocytes, congestion of hepatic blood vessels and dilatation of hepatic lymphatic vessels were observed in liver sections of fetuses obtained from HD-TMS- treated dams (Fig. 7C, 7D).

Many histological alterations in the glomeruli and renal tubules were observed in kidney sections of fetuses of both low and high dose groups. Deformities in renal glomeruli and renal tubules with increase of Bowman's space were observed in kidney of fetuses of LD-TMS group (Fig 8B, 8C). However, widespread structural deformities in the renal glomeruli and tubules along with coagulative necrosis of most of renal tubules were shown in kidneys of fetuses of HD-TMS group (Fig 8D, 8E).

Table 1: Changes in maternal and fetal indices of control and treated groups.

Parameter / Group	No. of pregnant dams	No. of uterine implants	Early resorption sites		Late resorption sites		Live fetuses		Mean fetal weights (g.)	Mean placental weights (g.)
			No.	%	No.	%	No.	%		
Control	**10**	**71**	**2**	**2.82**	**0**	**0**	**69**	**97.18**	**4.10 ± 0.096**	**0.653 ± 0.032**
LD-TMS (250 mg/kg)	**10**	**73**	**3**	**4.11**	**0**	**0**	**70**	**95.89**	**4.12 ± 0.094**	**0.625 ± 0.013**
HD-TMS (500 mg/kg)	**10**	**70**	**5**	**7.14**	**0**	**0**	**65**	**92.86**	**4.22 ± 0.104**	**0.610 ± 0.028**

Data are presented as mean ± standard error and percentages.

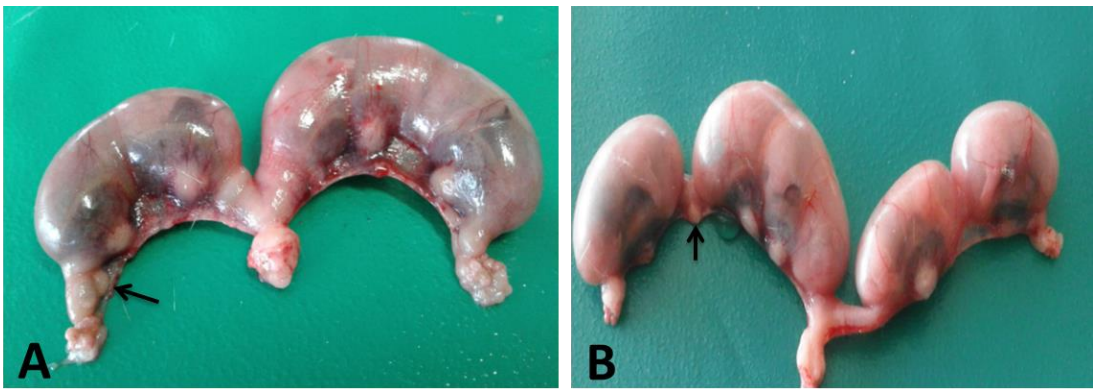

Figure 1: Uteri of pregnant dams treated orally with low, 250 mg/kg (A) and high, 500 mg/kg (B) doses of tilmicosin on days 6-15th of gestation showing resorption site on the left uterine horn.

Table 2: Fetal Morphological abnormalities of rat fetuses obtained from control and treated dams**.**

Parameter / Group	External morphological abnormalities					
	Dwarfism		S/c Hemorrhage		S/C Edema	
	No.	%	No.	%	No.	%
Control	2	2.89	0	0	0	0
LD-TMS (250 mg/kg)	2	2.86	0	0	0	0
HD-TMS (500 mg/kg)	2	3.08	0	0	0	0

Data are presented as mean ± standard error and percentages**.** LD-TMS: low dose-Tilmicosin; HD-TMS: high dose-Tilmicosin.

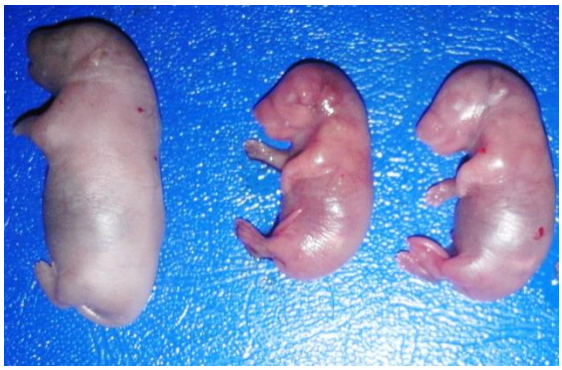

Figure 2: Rat fetuses obtained from control (left), LD-TMS (middle) and HD-TMS (right) -treated dams on days 6-15th of gestation.

Table 3: Visceral abnormalities of rat fetuses obtained from control and treated dams.

Parameter / Group	No. of examined fetuses	Visceral Malformations															
		Head						Chest						Pelvis			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Control	22	0	0	1	4.55	0	0	0	0	0	0	1	4.55	0	0	0	0
LD-TMS (250 mg/kg)	22	2	9.09	2	9.09	10	45.45*	5	22.73*	0	0	2	9.09	2	9.09	6	27.27*
HD-TMS (500 mg/kg)	20	10	50*	4	20	9	45*	7	35*	0	0	6	30*	5	25*	10	50*

Data are presented as percentages of viscerally deformed fetuses in relation to total number of examined fetuses. *: Significant difference between treated and control groups at $p<0.05$. LD-TMS: low dose-Tilmicosin; HD-TMS: high dose-Timicosin.

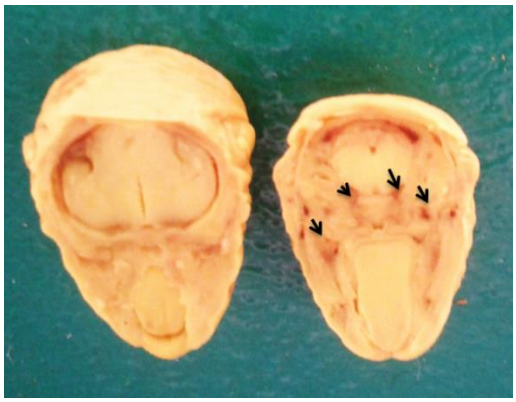

Figure 3: Longitudinal section in the head of rat fetuses of control (left) and HD-TMS (right) groups showing intracranial hemorrhages (arrows).

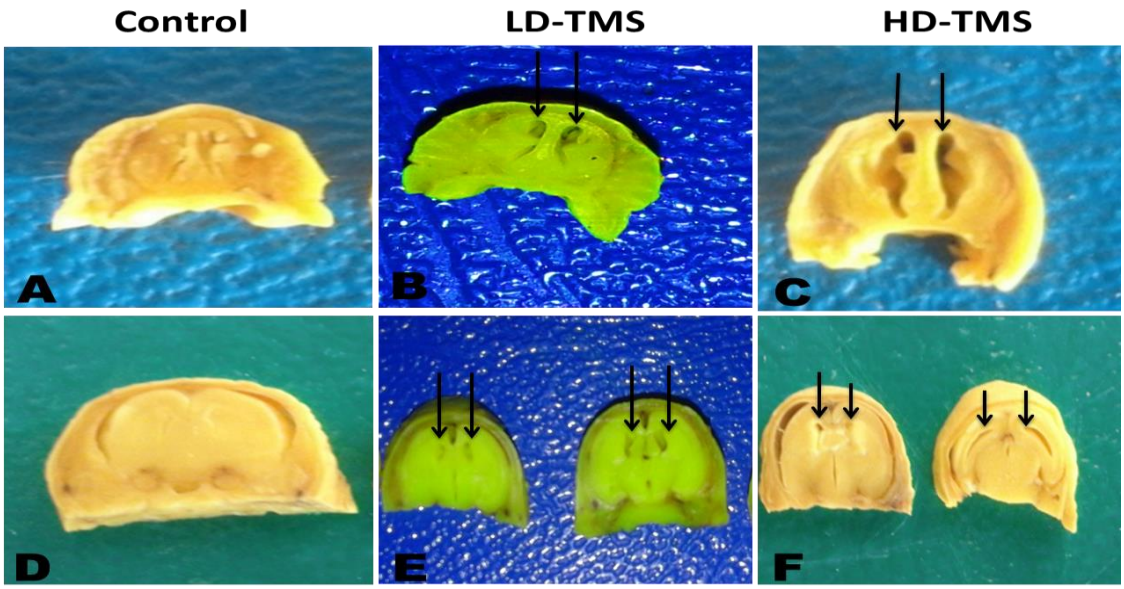

Figure 4: Transverse sections in the head of rat fetuses obtained from control, LD-TMS (250 mg/kg) and HD-TMS (500 mg/kg) -treated dams showing dilated nares (B, C) and different degrees of dilated brain ventricles (E, F), arrows.

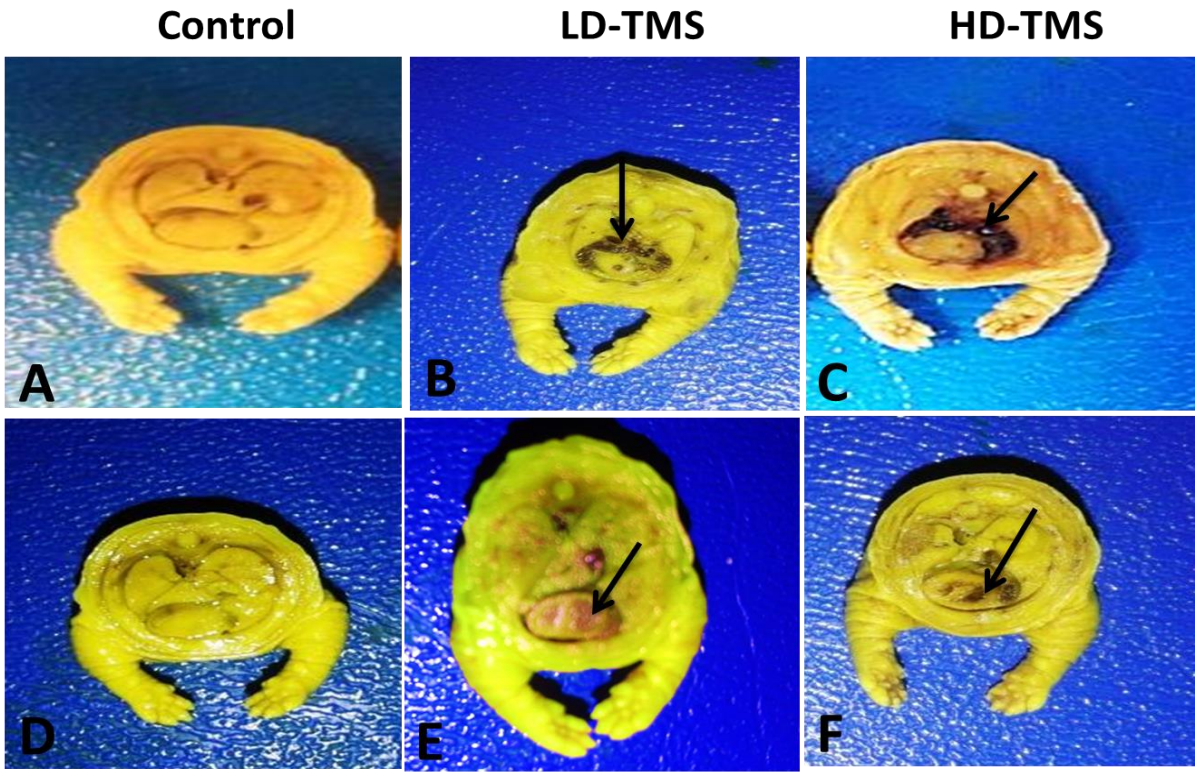

Figure 5: Transverse sections in the chest of rat fetuses obtained from control, LD-TMS (250 mg/kg) and HD-TMS (500 mg/kg) -treated dams showing intrathoracic hemorrhage (B, C) and dilated heart ventricles (E, F), arrows

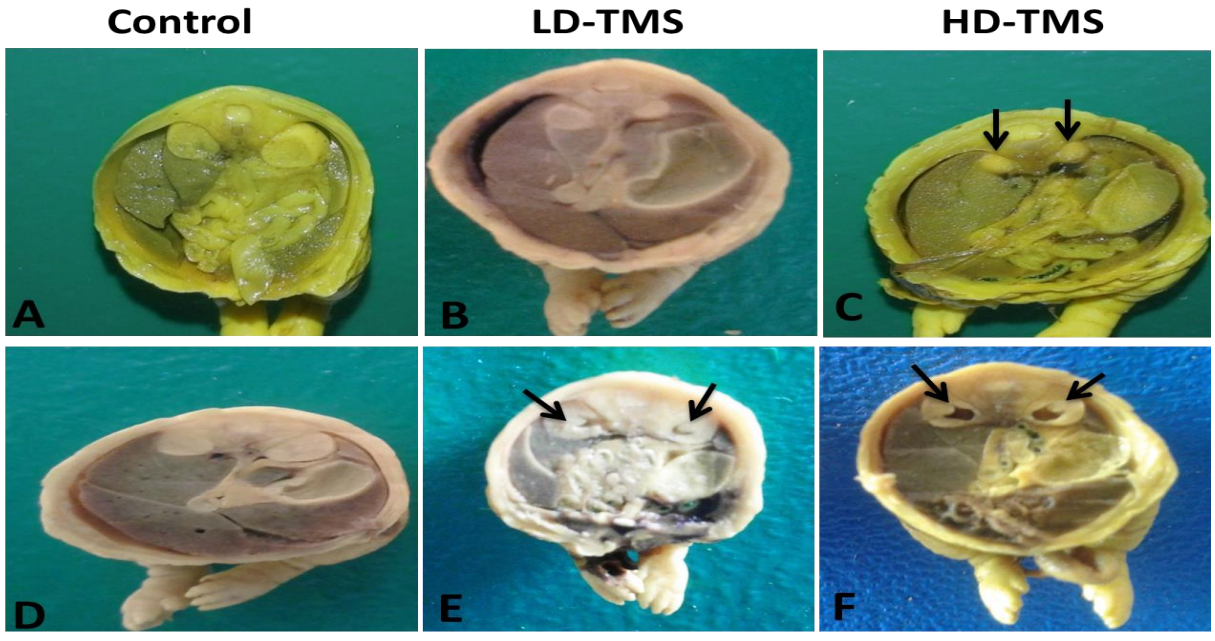

Figure 6: Transverse sections in the pelvis of rat fetuses obtained from control, LD-TMS (250 mg/kg) and HD-TMS (500 mg/kg)- treated dams showing unilateral (B) and bilateral (C) renal hypoplasia; bilateral dilated renal pelvis (E,F), arrows.

Table 4: Skeletal abnormalities of rat fetuses obtained from control and treated dams.

Parameters / Group	No. of examined fetuses	Skeletal Malformations					
		Skull Bones				Reduced No. of Sternbrae	
		Wide open fontanel		Incomplete ossification of parietal and/or interparietal bones			
		No.	%	No.	%	No.	%
Control	42	1	2.38	1	2.38	0	0
LD-TMS (250 mg/kg)	43	1	2.33	1	2.33	1	2.33
HD-TMS (500 mg/kg)	40	1	2.5	1	2.5	2	5

Data are presented as percentages of skeletal deformed fetuses in relation to total number of examined fetuses. LD-TMS: low dose-Tilmicosin; HD-TMS: high dose-Tilmicosin.

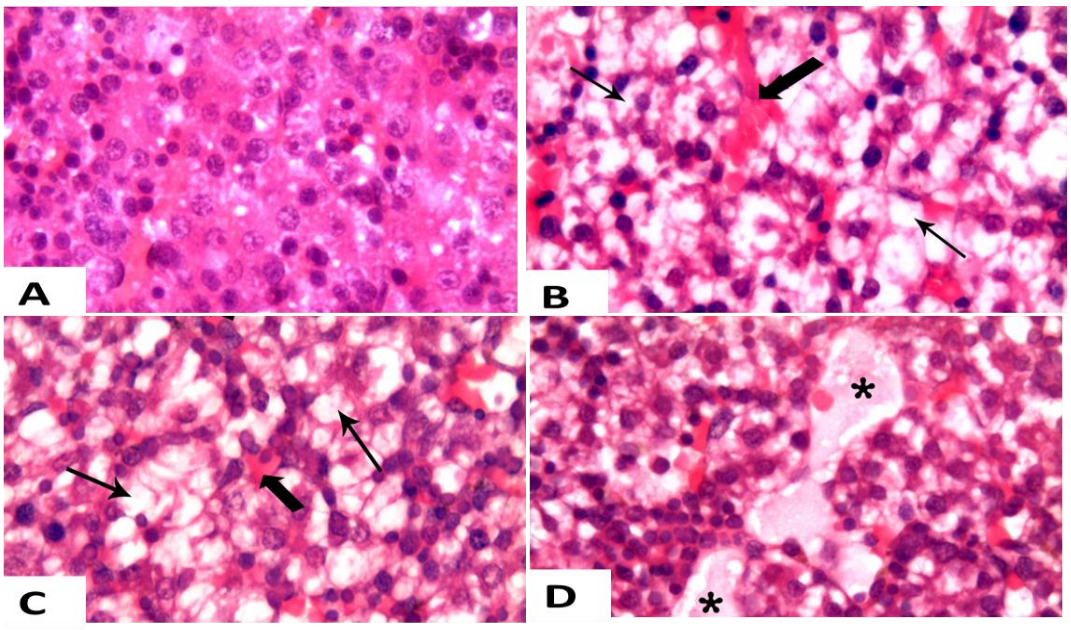

Figure 7: Representative photomicrographs of histopathological alterations in liver sections of fetuses from different groups (H&E stain 40; thin arrow: hydropic degeneration in hepatocytes, thick arrow: congestion of hepatic blood vessels, star: dilatation of hepatic lymphatic vessels). A: Control group showing normal histological architecture. B: LD-TMS group showing hydropic degeneration in hepatocytes and congestion of hepatic blood vessels. C, D: HD-TMS group showing widespread hydropic degeneration in hepatocytes and congestion of hepatic blood vessels (C) and dilatation of hepatic lymphatic vessels (D).

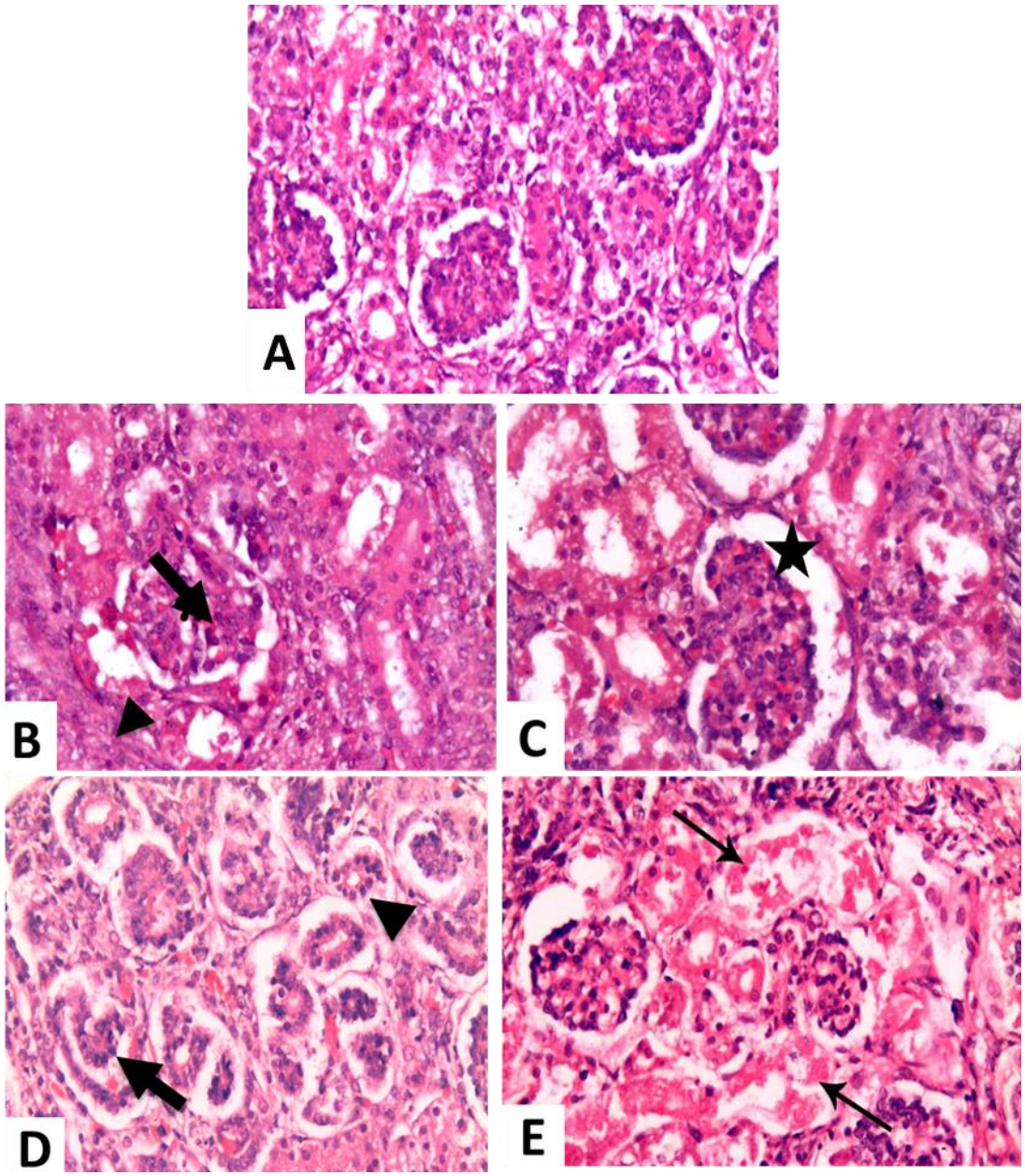

Figure 8: Representative photomicrographs of histopathological alterations in kidney sections of fetuses obtained from different groups (H&E stain X20); Thick arrow: deformities in glomeruli, arrow head: deformities renal tubules, star: Bowman's space, thin arrow: coagulative necrosis of renal tubules). A: Control group showing normal histological architecture. B, C: LD-TMS group showing deformities in renal glomeruli and renal tubules (B) and increase of Bowman's space (C). D, E: HD-TMS group showing widespread structural deformity in the renal glomeruli and tubules (D) and widespread coagulative necrosis of renal tubules (E).

DISCUSSION

In the present work, tilmicosin was daily administered orally by gavage to pregnant female rats at dose levels of 250 and 500 mg/kg during the period of organogenesis. TMS at both dose levels induced insignificant elevation in the percentages of early resorptions, with insignificant reduction in the number of the live fetuses and mean fetal and placental weights. In addition, insignificant elevation in the percentages of the dwarfed fetuses was recorded.

Our results agree with the study of Dearlove *et al.*, (1987) in which female rats were given TMS by gavage at doses of 0, 50, 125, 250, 500 or 750 mg/kg bw per day. Treatment started 14 days before mating with untreated males and continued until the end of experiment on post-partum day 4. The gestation duration, litter weight and size, and pup survival and weight gain to post-partum day 4 showed no significant changes. Similarly, Jordan and Higdon (1988)

reported that fetuses from pregnant rats treated with TMS by gavage at doses of 10, 70 or 500 mg/kg bw per day on gestation days 6 to 15 showed no alteration in the numbers of resorptions, live fetuses, fetal weight and sex ratio.

In a multigeneration study, rats received TMS at 10, 45 or 200 mg/kg bw per day by gavage. There were no effects on pregnancy rates, mating performance, gestation duration, litter size and weight, and the offspring weight gain (Christian and Hoberman, 1989). Contrary to our results, Abo-Kora *et al.*, (2016) have shown that TMS administered orally to pregnant female rats at a dose of 100 or 200 mg/kg/day from the 6th to 15th day of gestation significantly increased the number of resorbed fetuses and reduced the number of viable fetuses and fetal growth.

The results of the present study revealed that both doses of TMS induced insignificant increase in the percentages of dilated nares. In addition, significant elevations were recorded in the percentages of intracranial hemorrhage (high dose), dilated brain ventricles (both dose levels), dilated heart ventricles (both dose levels) and intrathoracic hemorrhage (high dose), unilateral or bilateral hypoplasia of kidney (high dose) and dilated renal pelvis (both dose levels). This indicates that the effects of TMS were dose-dependent, reflecting that TMS is teratogenic at the selected dose levels.

In the same line histomorphological examination of fetal liver and kidneys revealed presence of many pathological and structural changes at both dose levels of tilmicosin.

Concerning the skeletal malformations, TMS at both dose levels produced no skeletal malformations, except induction of insignificant changes in the percentages of rat fetuses that had wide open fontanel, incomplete ossification of skull bones and reduction in the numbers of sternebrae compared with those of control group.

Our results are consistent with the study of Yan *et al.*, (2019) in TMS – treated zebrafish embryos. They reported anomalies in the form of pericardial edema and spinal curvature. In addition, our findings partially agree with the study of Noda (1993) in rabbits, who reported visceral abnormalities in the form of open eyelids, cleft palate or club foot. However, contrary to our findings, they recorded retardation of skeletal development of fetuses at the 2 high dose levels but they attributed it to dam malnutrition.

The results of the current investigation partially agree with the study performed previously by Abo-Kora *et al.*, (2016) who treated pregnant rats orally with TMS at 20, 100 or 200 mg/kg/day from the 6th to 15th day of gestation. They recorded visceral abnormalities, in a dose-dependent manner, including pulmonary hypoplasia with cardiac enlargement diverticulum dilatation and hypoplasia in thymus. However, dissimilar to our data, they reported skeletal deformities in the form of incomplete ossification of skull, absence of caudal vertebrae, small sized sternebrae, and absence of digital bone of fore limbs.

Contrary to our findings, Jordan and Higdon (1988) demonstrated that administration of TMS to pregnant rats on gestation days 6-15 at 10, 70 or 500 mg/kg did not significantly increase the incidences of total skeletal and visceral anomalies and there was no dose-response relationship and the findings were within the historical control values.

Herein, dilated renal pelvis of fetuses from TMS-treated dams reflects fetal hydronephrosis. Fetal hydronephrosis may occur due to mechanical impairment in the urinary flow (generally in the uretero-pelvic regions or vesico-ureteral) or in the absence of any overt obstruction (Khera, 1981).

Development of embryo is a complex process including various cellular events such as proliferation and differentiation which take place in a harmony and any disturbance in these actions can induce defective developmental outcomes. Cellular redox states control in part the proliferation, differentiations and apoptosis processes, and thus oxidative stress with redox imbalance can cause disturbance in these processes. The most vulnerable periods to redox imbalance are that periods of transition from an important developmental event to another (e.g. proliferation/ differentiation) in developing target organs and systems (Schafer and Buettner, 2001; Hansen *et al.*, 2018).

Yan *et al.*, (2019) demonstrated that the developmental toxicity induced by TMS in zebrafish embryos was associated with

triggering oxidative stress including elevated SOD activities and increased MDA.

Other studies demonstrated occurrence of oxidative stress in TMS intoxicated animals in various organs as in heart (Aboubakr *et al.*, 2020; Awad *et al.*, 2020; Khalil *et al.*, 2020) and liver (Farag *et al.*, 2019).

Many cytotoxic agents and teratogens produce birth defects through apoptosis induction. Excessive death of embryonal cells by apoptosis is undoubtedly one of the major pathways causing the occurrence of visceral and skeletal abnormalities in fetuses (Brill *et al.*, 1999). The teratogenic effect of TMS may be, at least in part, due to the potential of the drug to induce apoptosis in fetuses, as Yan *et al.*, (2019) demonstrated that TMS produced developmental toxicity in zebrafish embryos accompanied with up-regulation of apoptosis associated genes such as p53, bcl-2, bax, caspase-3 and caspase-9.

CONCLUSION

Tilmicosin administration to pregnant rats during organogenesis period could induce some visceral malformations in head, chest and pelvis but the number of resorption sites; live fetuses and dwarfed fetuses and also skeletal malformations were within normal limits. The occurrence of visceral malformations was on the same line with occurrence of pathological alterations in fetal liver and kidneys, reflecting transplacental passage of tilmicosin in pregnant dams.

REFERENCES

Abo-Kora, S., El-Meleh, A., & Aboubakr, M. (2016). Effect of Tilmicosin on Fetal Developments in Pregnant Female Albino Rats. *Pharmacology & Pharmacy*, *7*(4), 147-152

Aboubakr, M., Elsayd, F., Soliman, A., Fadl, S. E., El-Shafey, A., & Abdelhiee, E. Y. (2020). L-Carnitine and vitamin E ameliorate cardiotoxicity induced by tilmicosin in rats. *Environmental Science and Pollution Research*, 1-9.

Alan, B. and Duncan, C. (2001): Quantitative data analysis with SPSS Relase 10 for windows (chapter 2): Analysing Data with Computers, First steps with SPSS 10 for windows.

Awad, A., Khalil, S. R., Hendam, B. M., Abd El-Aziz, R. M., Metwally, M. M., & Imam, T. S. (2020). Protective potency of Astragalus polysaccharides against tilmicosin-induced cardiac injury via targeting oxidative stress and cell apoptosis-encoding pathways in rat. *Environmental Science and Pollution Research*, 1-15.

Bancroft, J.D.; Stevans, A. and Turner, D.R. (1996): Theory and practice of histopathological techniques, fourth edition. Churchill Livingstone, Edinburgh, London, Melbourne, New York.

Bhavsar, S. K., & Thaker, A. M. (2012). Pharmacokinetics of antimicrobials in food producing animals. In Readings in advanced pharmacokinetics-theory, methods and applications. IntechOpen

Bookstaver, P. B., Bland, C. M., Griffin, B., Stover, K. R., Eiland, L. S., & McLaughlin, M. (2015). A review of antibiotic use in pregnancy. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *35*(11), 1052-1062.

Briggs, G. G., Freeman, R. K., & Yaffe, S. J. (2002). Azathioprine. *Drugs in Pregnancy and Lactation. 6th ed. Philadelphia, PA: Lippincott Williams & Wilkins*, 113-116.

Brill, A., Torchinsky, A., Carp, H., & Toder, V. (1999). The role of apoptosis in normal and abnormal embryonic development. *Journal of assisted reproduction and genetics*, *16*(10), 512-519.

Christian, M.S. & Hoberman, A.M. (1989). Reproductive effects of EL-870 administered orally via gavage to Crl:COBS CD(SD) BR rat for two generations, with two litters per generation. Unpublished study No. 112-001 from Argus Research Laboratories. Submitted to WHO by Lilly, Basingstoke, UK. Cited by FAO/WHO. Joint FAO/WHO Expert Committee on Food Additives; WHO Food Additive Series 38: Toxicological Evaluation of Certain Veterinary Drug Residues in Food: Tilmicosin (1996).

Dearlove, G.H., Hoberman, A.M., & Christian, M.S. (1987). Dosage-range study of EL-870 administered orally via gavage to Crl:COBS CD(SD) BR rats (Pilot study). Unpublished study No. 112-001P from Argus Research Laboratories. Submitted to WHO by Lilly, Basingstoke, UK. Cited by FAO/WHO. Joint FAO/WHO Expert Committee on Food Additives; WHO Food Additive Series 38: Toxicological Evaluation of Certain Veterinary Drug Residues in Food: Tilmicosin (1996).

FAO/WHO (2008). Evaluation of certain veterinary drug residues in food: Tilmicosin. Joint FAO/WHO Expert Committee on Food Additives. Meeting (70th: 2008: Geneva, Switzerland). (WHO technical report series; no. 954).

Farag, M. R., Elhady, W. M., Ahmed, S. Y., Taha, H. S., & Alagawany, M. (2019). Astragalus polysaccharides alleviate tilmicosin-induced toxicity in rats by inhibiting oxidative damage and modulating the expressions of HSP70, NF-kB and Nrf2/HO-1 pathway. *Research in veterinary science*, *124*, 137-148.

Han, C., Qi, C. M., Zhao, B. K., Cao, J., Xie, S. Y., Wang, S. L., & Zhou, W. Z. (2009). Hydrogenated castor oil nanoparticles as carriers for the subcutaneous administration of tilmicosin: in vitro and in vivo studies. *Journal of veterinary pharmacology and therapeutics*, *32*(2), 116-123.

Hansen, Jason & Jacob, Benjamin & Piorczynski, Ted. (2017). Oxidative stress during development: Chemical-induced teratogenesis. Current Opinion in Toxicology. 7. 10.1016/j.cotox.2017.11.003.

Jordan, W.H. & Higdon, G.L. (1988). A teratology study of tilmicosin (EL-870, compound 177370) administered orally to CD rats. Unpublished study No. RI3387 from Lilly Research Laboratories. Submitted to WHO by Lilly, Basingstoke, UK. Cited by FAO/WHO. Joint FAO/WHO Expert Committee on Food Additives; WHO Food Additive Series 38: Toxicological Evaluation of Certain Veterinary Drug Residues in Food: Tilmicosin (1996).

Khalil, S. R., Abdel-Motal, S. M., Abd-Elsalam, M., Abd El-Hameed, N. E., & Awad, A. (2020). Restoring strategy of ethanolic extract of Moringa oleifera leaves against Tilmicosin-induced cardiac injury in rats: Targeting cell apoptosis-mediated pathways. *Gene*, *730*, 144272.

Khera, K.S. (1981). Common fetal aberrations and their teratologic significance: a review. Fund. Appl. Toxicol., 1: 13-18.

Manson, J.M. and Kang, Y.J. (1994). Test methods for assessing female reproductive and developmental toxicology. In principles and methods of toxicology; 2 nd Ed: by A.Wallace Hayes, Ravenpress, ltd., New York.

Meeraus, W. H., Petersen, I., and Gilbert, R. (2015). Association between antibiotic prescribing in pregnancy and cerebral palsy or epilepsy in children born at term: a cohort study using the health improvement network. PLoS One, 10(3), e0122034.

Modric, S. (1997). Pharmacokinetic and pharmacodynamic properties of tilmicosin in sheep, cattle, and rats. A dissertation presented to the graduate school of the university of florida in partial fulfillment of the requirements for the degree of doctor of philosophy university of florida.

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 and Toxicology, Japan. Submitted to WHO by Lilly, Basingstoke, UK. Cited by FAO/WHO. Joint FAO/WHO Expert Committee on Food Additives; WHO Food Additive Series 38: Toxicological Evaluation of Certain Veterinary Drug Residues in Food: Tilmicosin (1996).

Paumgartten FJR, De-Carvalho RR, Souza K, Madi CAM and Chahoud I (1998). Study of the effectsof β-myrcene on rat fertility and general reproductive performance. Braz. J. Med. Biol. Res. 31, 955-965. https://doi.org/10.1590/S0100-879X1998000700012

Schafer, F. Q., & Buettner, G. R. (2001). Redox environment of the cell as viewed through

the redox state of the glutathione disulfide/glutathione couple. *Free radical biology and medicine*, *30*(11), 1191-1212.

Schlegel, P. N., Chang, T. S., & Marshall, F. F. (1991). Antibiotics: potential hazards to male fertility. *Fertility and sterility*, *55*(2), 235-242

Witt, A., Sommer, E. M., Cichna, M., Postlbauer, K., Widhalm, A., Gregor, H., & Reisenberger, K. (2003). Placental passage of clarithromycin surpasses other macrolide antibiotics. *American journal of obstetrics and gynecology*, *188*(3), 816-819.

Womble, A., Giguère, S., Murthy, Y. V. S. N., Cox, C., & Obare, E. (2006). Pulmonary disposition of tilmicosin in foals and in vitro activity against Rhodococcus equi and other common equine bacterial pathogens. *Journal of veterinary pharmacology and therapeutics*, *29*(6), 561-568.

Xie, S., Wang, F., Wang, Y., Zhu, L., Dong, Z., Wang, X. & Zhou, W. (2011). Acute toxicity study of tilmicosin-loaded hydrogenated castor oil-solid lipid nanoparticles. *Particle and fibre toxicology*, *8*(1), 33

Yan, Z., Huang, X., Xie, Y., Song, M., Zhu, K., & Ding, S. (2019). Macrolides induce severe cardiotoxicity and developmental toxicity in zebrafish embryos. *Science of the total environment*, *649*, 1414-1421.

Ziv G, ShemTov M, Glickman A, Winkler M, Saran A. (1995). Tilmicosin antibacterial activity and pharmacokinetics in cows. J. Vet. Pharmacol. Ther. 18:340–345