Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2014, 4(2):63-71

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Toxicopathological lesions of fosfomycin in embryonic model

Hadi Tavakkoli[1*], Amin Derakhshanfar[2] and Samaneh Noori Gooshki[3]

[1]*Department of Avian Medicine, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran*
[2]*Center of Comparative & Experimental Medicine, Basic Sciences in Infection Diseases Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran*
[3]*School of Veterinary Medicine, ShahidBahonar University of Kerman, Kerman, Iran*

ABSTRACT

The increasing antimicrobial drug resistance of pathogens, together with the increasing need for new antimicrobial agents, call for re-evaluate old antibiotic compounds. One of the alternative treatments for multidrug-resistant bacterial infections is fosfomycin. Toxicopathological effects of fosfomycin compounds have always been a major concern. There is scant information available about the toxicopathological effects of fosfomycin in the fetus. The objective of this study is to determine the macroscopic and microscopic lesions of various dosages of fosfomycin in the chicken embryo since the embryogenesis in chick is similar to human beings. Forty fertile chicken eggs were divided into five equal treatment groups as follows: Group 1: uninjected group. Group 2: needle-injected group; the needle was inserted into the yolk sac without any injection. Group 3: phosphate buffered saline-*injected group, whereby individuals were injected with* phosphate buffered saline *of 0.3 ml/egg. Groups 4 and 5, individuals were likewise injected with fosfomycin-calcium at dosages of 160 and 320 mg/Kg egg-weight/daily, at days 4, 5 and 6 of incubation, respectively. Macroscopic abnormality in size, color, feathers, limb and other external body features in embryos were accompanied by pathological changes in brain, liver, kidney, heart and lung. Based on macroscopic and microscopic findings, it is concluded that fosfomycin at the above-mentioned concentrations are toxic to the chicken embryo in a dose dependent manner. Further studies are needed to clarify the toxic effects of this drug on the development of a human fetus.*

Keywords: Embryo, Fosfomycin, Human fetus, Toxicopathology

INTRODUCTION

The recent emergence of bacterial strains and increasing considerable problem of antimicrobial resistance lead to the idea of finding alternative solutions. One of the most promising ones is the evaluation of old antibiotic drugs, despite the fact that they were previously thought to be less effective and/or more toxic than newer agents. Thus, a number of antibiotic compounds such as fosfomycin, fusidic acid and polymyxins are re-emerging for the treatment of currently prevalent pathogens [5, 16, 17]. Fosfomycin compounds have been used across the globe for many years. Today, they often dispense in the treatment of urinary tract infections, soft-tissue infections, bacterial spondylodiscitis, osteomyelitis, pediatric cancer and sepsis [1, 10]. Fosfomycin acts inside the bacterial cytoplasm. This drug inhibits bacterial cell wall biosynthesis by acting on the early stages in the synthesis of murein/peptidoglycan procedures.Despite the use of fosfomycin in human and animal medicine, its use is sometimes associated with adverse effects such as rash, headache, abdominal pain, dizziness, nausea, rhinitis and vaginitis [17]. Following administration, fosfomycin crosses from the placental barrier in humans. In this regard, it is necessary to identify the benefits and risks of using fosfomycin during pregnancy especially for the fetus.

Although increasing consumption of fosfomycin compounds are predicted inhuman medicine, there is little information about the teratogenic and toxic effects of these compounds on the fetus. Thus, the current study aimed to determine the macroscopic and microscopic lesions of fosfomycin-calcium in the chicken embryo. Furthermore, this basic embryo-toxicological study was performed in chicken embryo as a model to investigate harm full effect of fosfomycin-calcium for human fetus since the embryogenesis in chick is similar to human beings.

MATERIALS AND METHODS

Hatching eggs

A total of 40 fertile chicken eggs (Marandi breed) with the average egg-weight of 49.5 ± 0.4 g were purchased from a local breeder farm. In this farm, birds were kept and grown up under the standard condition of breeding.

Drugs

Pure fosfomycin-calcium was obtained from Jamedat's pharmaceutical company, Iran.

Experimental protocol

Eggs were incubated at 37.7ºC and 60% relative humidity. The eggs were randomly assigned to five equal treatment groups, 8 eggs each, as follows: Group 1: uninjected group; embryonated eggs did not receive any treatment at all. Group 2: needle-injected group, the needle (22-gauge) was inserted into the yolk sac without any injection. Group 3: phosphate buffered saline injected group, embryonated eggs were injected with sterile phosphate buffered saline of 0.3 ml/egg into the yolk sac. Groups 4 and 5: eggs were treated with fosfomycin-calcium at dosages of 160 and 320 mg/Kg egg-weight/daily, three times at days 4, 5, and 6 of incubation, respectively. Embryos received treatment by direct injection into the yolk sac according to the standard techniques [9, 14]. Embryos were re-incubated post-treatment and allowed to develop until day 18, after which they were collected and examined for macroscopic and microscopic lesions. All treatments and procedures in this study were conducted according to local ethical guidelines, and were approved by the Animal Ethics Committee of the Research Council of ShahidBahonar University, Iran.

Pathological examination

At the end of experiment (on day 18) embryos were humanely killed by placing on ice and then eggs were opened at the wider end [11]. After washing in normal saline solution, embryos were observed under stereomicroscope to study any gross abnormalities on the external body surface. The membranes and yolk sac were also inspected. Then, the tissues of embryos were dissected out and fixed in 10% neutral buffered formalin. Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5 µm thicknesses were cut using a microtome (Slee-Germany) and stained with hemotoxylin and eosin and studied under light microscope.

Statistical analysis

Statistical analysis was performed using SPSS version 20. The Fisher's exact test was used to determine the significant differences in lesion occurrence between experimental groups. Kruskal-Wallis test was also applied to establish whether there were differences betweendosage of fosfomycin-Ca and pathological findings. A p-value of <0.05 was considered as statistically significant.

RESULTS

Gross abnormalities of the external body features

The embryos in group 4 (injected with 160 mg fosfomycin/Kg egg-weight were normal as embryos in groups 1, 2 and 3 and there was not any abnormality in size, color, feather, limb and other external body features (figures 1 to 2). However, the embryos in group 5 (injected with 320 mg fosfomycin/Kg egg-weight) were stunted and under development. In this group, the embryos were red-dark in color and feather formation was not complete and normal. In addition, feet and wings were too small and eyes in comparison to body size were large and bulged (figure 3).

Fig. 1: The chicken embryo treated with phosphate buffered saline of 0.3 ml/egg into the yolk sac. The embryo is normal with no gross lesions of the external body features

Fig. 2: The chicken embryo treated with fosfomycin-calcium at a dosage of 160 mg/Kg egg-weight into the yolk sac. The embryo is normal with no gross lesions of the external body features

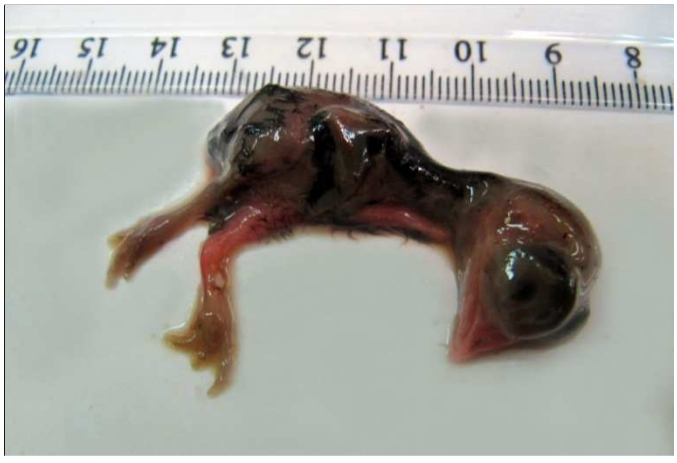

Fig. 3: The chicken embryo treated with fosfomycin-calcium at a dosage of 320 mg/Kg egg-weight into the yolk sac. The embryo is characterized by stunting, impaired feather formation, small feet and wings and dark-bulged eyes

Microscopic findings

Histopathological evaluation has been revealed that all organs were normal in groups 1, 2 and 3. In group 4 (injected with 160 mg fosfomycin/Kg egg-weight), lung, liver and heart were normal while, focal encephalomalacia and mild

tubular epithelial changes were seen in brain and kidney, respectively. In group 5 (injected with 320 mg fosfomycin/Kg egg-weight), the brain was edematous and disorganized. In addition, intensive tubular and glomerular necrosis were seen in the kidney. Heart and liver were affected by massive infarction. Lung was so destructed that the only remnant of its structure was seen (figures 4 to 13).

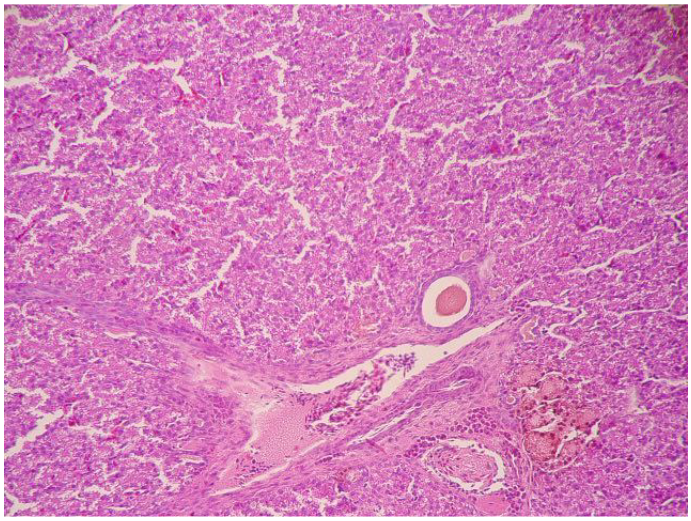

Fig. 4:Photomicrograph of the chicken embryo treated with phosphate buffered saline of 0.3 ml/egg. Normal structure of the liver with one of the portal areas is seen. ×100 H&E

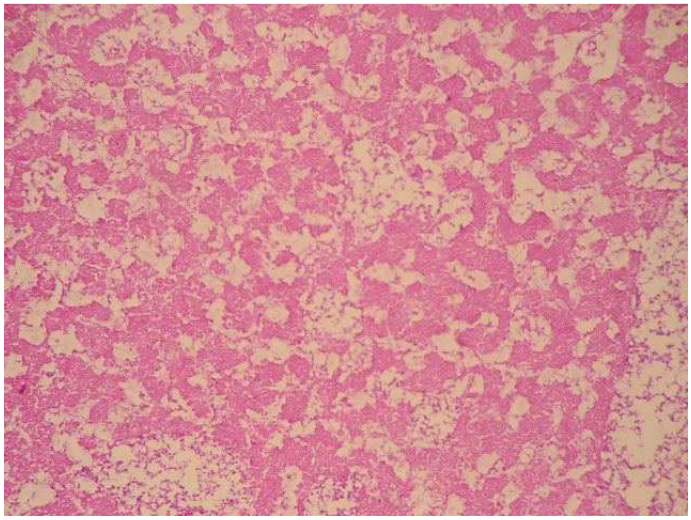

Fig. 5:Photomicrograph of the chicken embryo treated with 360 mg fosfomycin-calcium/Kg egg-weight. Intensive hepatic infarction is seen. ×100 H&E

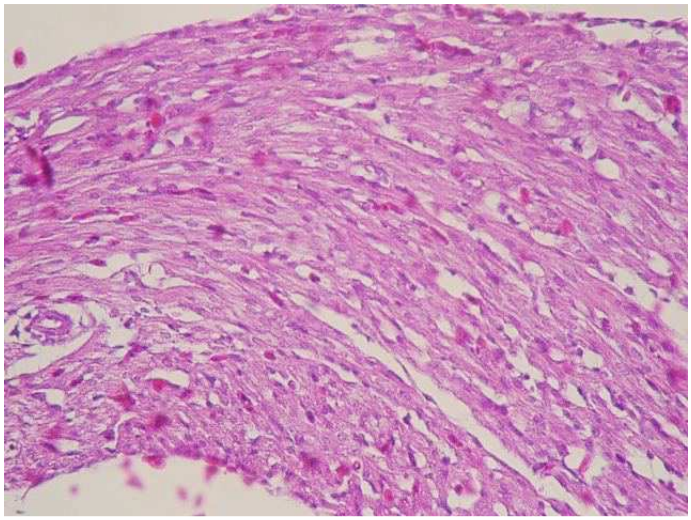

Fig. 6:Photomicrograph of the chicken embryo treated with phosphate buffered saline of 0.3 ml/egg. Note to the normal histological features of the cardiac myocytes. ×200 H&E

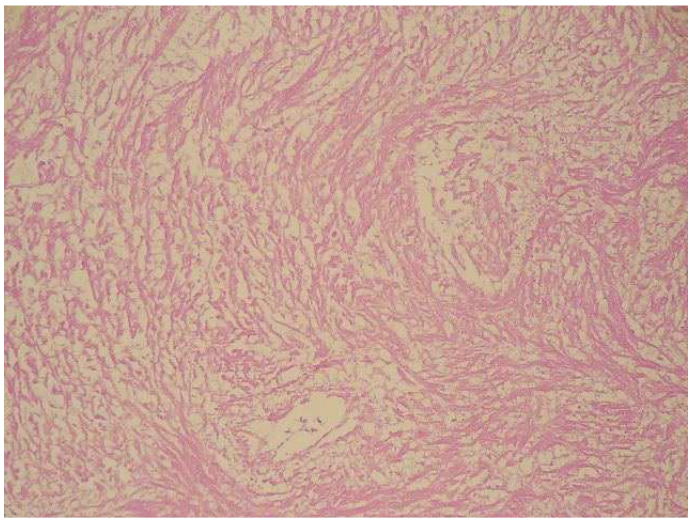

Fig. 7:Photomicrograph of the chicken embryo treated with 360 mg fosfomycin-calcium/Kg egg-weight. Massive cardiac infarction is seen. ×100 H&E

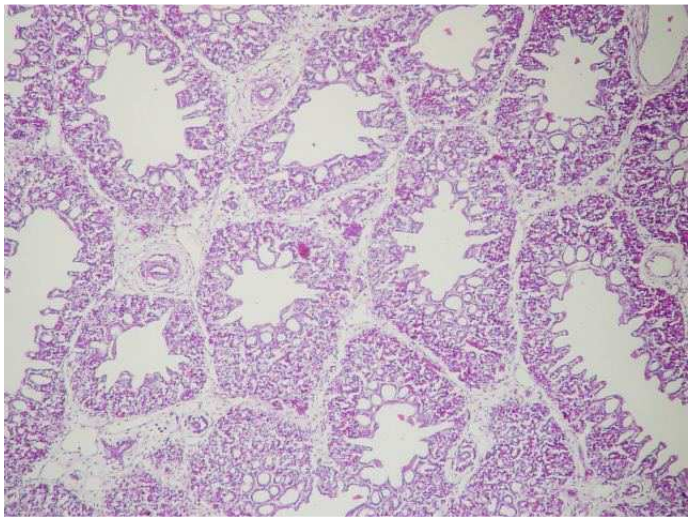

Fig. 8:Photomicrograph of the chicken embryo treated with phosphate buffered saline of 0.3 ml/egg. Normal lung in the chicken embryo is seen. ×40 H&E

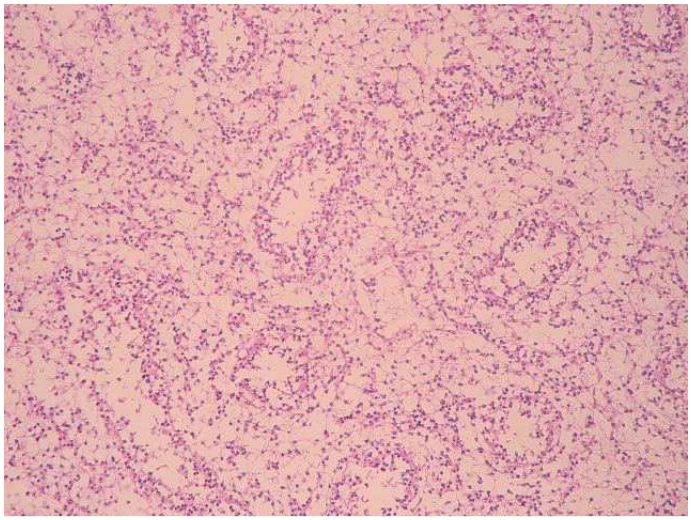

Fig. 9:Photomicrograph of the chicken embryo treated with 360 mg fosfomycin-calcium/Kg egg-weight. The only remnant of abnormal lung is seen. ×100 H&E

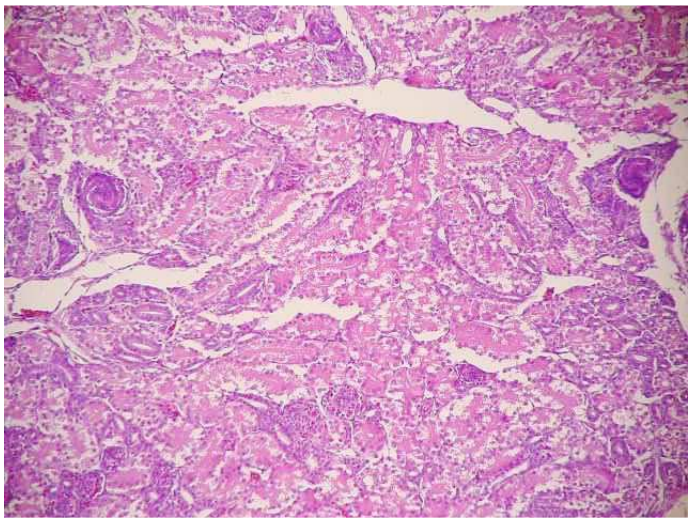

Fig. 10:Photomicrograph of the chicken embryo treated with phosphate buffered saline of 0.3 ml/egg. An entire lobe of the normal kidney is seen. ×40 H&E

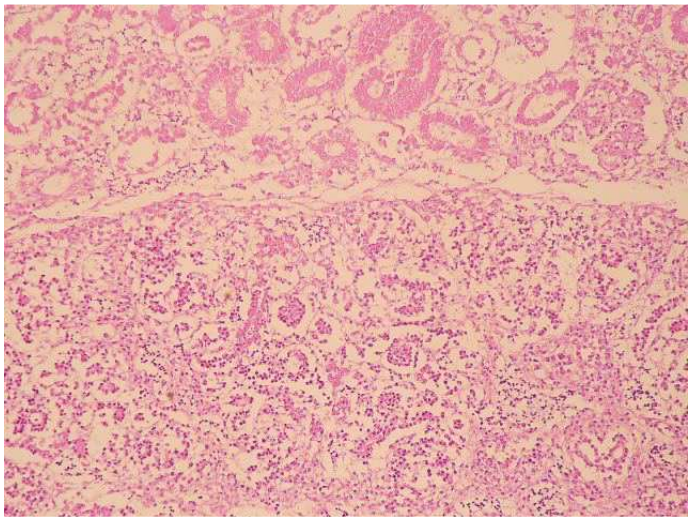

Fig. 11:Photomicrograph of the chicken embryo treated with 360 mg fosfomycin-calcium/Kg egg-weight. Distorted and disorganized kidney with no characteristic normal microscopic structure. ×40 H&E

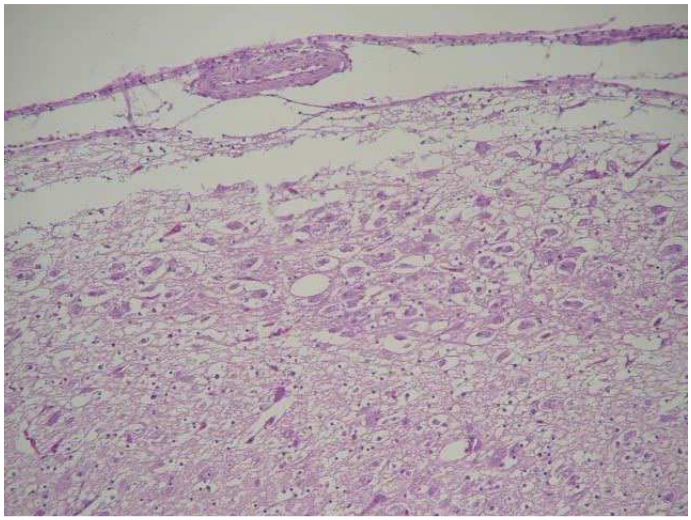

Fig. 12:Photomicrograph of the chicken embryo treated with phosphate buffered saline of 0.3 ml/egg. A portion of normal cerebrum and related meninges is seen. ×100 H&E

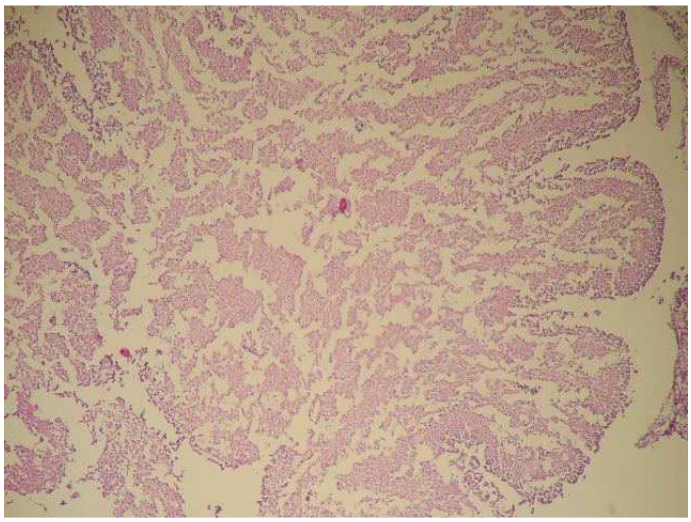

Fig. 13:Photomicrograph of the chicken embryo treated with 360 mg fosfomycin-calcium/Kg egg-weight. Disorganized cerebellum. No normal structural layers are seen. ×40 H&E

DISCUSSION

Fosfomycin compounds have an increasing role as the therapeutic agents against multidrug-resistant pathogens. They have a rapid bactericidal effect and a wide antibacterial spectrum [8]. In many countries such as Europe, Austria, Germany, Spain, France, Brazil, South Africa and Japan fosfomycin has been used successfully for several decades. The Food and Drug Administration (FDA) has approved oral fosfomycin only for the treatment of urinary tract infections because of limited fosfomycin-related clinical research. Nevertheless, considering the adverse effects of fosfomycin on animal and human health still needs to be justified.Determining the side effects of drugs on the development of chicken embryo is a useful method for studying the biological properties of drugs. There is scant information available about the toxicopathological effects of fosfomycin in the fetus. The current study is focused on the lesions induced by administration of fosfomycin-Ca in chicken embryo. This research can further help us to investigate the toxic potential of fosfomycin compounds in the human fetus.Our results showed gross and microscopic alteration in chicken embryos exposed to various dosages of fosfomycin-CA. The lesions showed a dose-dependent relationship in the fosfomycin-injected groups ($P<0.05$). The worth condition was seen in the embryos of group 5 (320 mg fosfomycin/kg). In this group red-dark color, stunting, impaired feather formation, small feet and wings and dark-bulged eyes were observed. Our study established that fosfomycin-related toxicity on the chicken embryo is directly related to the dosage of administration.

In the present study, microscopic lesions were seen in brain, kidney, heart, lung and liver of embryos. In human model, it was showed that fosfomycin distributes well into the various tissues such as lung, eye, muscle, bone, heart,

gall bladder, lymph, ascitic and cerebrospinal fluid [13]. Based on our results, it was concluded that fosfomycin-Ca candistribute tobrain, kidney, heart, lung and liver in chicken embryos. Further studies still need to be undertaken to determine the pharmacokinetic of fosfomycin-Ca in human fetus. In the brain, focal encephalomalacia, edema and disorganization were seen. The mechanism behind fosfomycin-induced brain lesions in embryo is not exactly known. Neurotoxic effects of fosfomycin compounds, such as headache, dizziness, migraine, somnolence, and nervousness have previously reported [16, 17]. These alterations may be due to cytotoxic properties and adverse effect of fosfomycinon the central nervous system (CNS). Fosfomycin, due to its small molecular weight and low protein binding, can cross the meninges and reach significant concentrations in the CNS [13, 15, 18].

The pathological changes observed in lung were prominent in embryos given the highest dosage of fosfomycin but in other groups, lungs were observed normal with no sign of lesions. It was showed that fosfomycin can penetration into the pulmonary tissues [12].Our study show that heart lesions can be occur in embryos that treated with fosfomycin-Ca at a dosage of 320 mg/Kg egg-weight/daily, three times. Fosfomycin has been utilized for cardiac surgery in human, because following cardiac surgery many pathogens can cause infections. Fosfomycin penetrates into the valvular, myocardial, muscle and surrounding adipose tissue [13, 18]. Further studies are needed to evaluate the cardiotoxic activity of fosfomycin in human during surgery and especially pregnancy.This study showed hepatotoxic activity of fosfomycin-Ca in chicken embryos. Fujii et al reported an increase in the levels of SGOT and/or SGPT, common serum liver enzymes, administered with oral form of fosfomycin[7]. Our findings are comparable to previous report for fosfomycin drug known to have hepatotoxic potential in human [6].In the current study, fosfomycin produced histopathological alterations such as tubular epithelial change and necrosis in the renal elements. Little is known about the nature of the renal damage induced by fosfomycin in embryo and no well-described experimental model exists. However, in various clinical experiments fosfomycin was administrated for urinary tract infections [2-4, 19]. It was reported that large amount of fosfomycinadministered orally is absorbed from the gastrointestinal tract and distributed to different tissues. Its low molecular weight gives fosfomycin highly permeability. Fosfomycin is essentially eliminated in active form through the kidney.

CONCLUSION

Results of the present study describe different pathological alterations induced by the administration of fosfomycin-Ca at different dosages in chicken embryos. These alterations occurred in a dose-related manner. The current study also advises caution in the extended use of fosfomycin compounds. Further studies are needed to clarify the toxic effects of this drug on the development of a human fetus.

Acknowledgment
The authors wish to thank Mr. S. Hasanzadeh for his kind cooperation in slide preparation.

REFERENCES

[1] O. Baylan. *Mikrobiyol. Bul*., **2010**, 44, 311.
[2] E. Calderón-Jaimes, G. Casanova-Román, A. Galindo-Fraga, P. Gutiérrez-Escoto, S. Landa-Juárez, S. Moreno-Espinosa, F. Rodríguez-Covarrubias, L. Simón-Pereira, R. Valdez-Vázquez. *Bol. Med. Hosp. Infant. Mex*., **2013**, 70, 3-10.
[3] E. De Vecchi, S. Sitia, C. L. Romanò, C. Ricci, R. Mattina, L. Drago. *J. Med. Microbiol*., **2013**, 62, 859.
[4] M. E. Falagas, K. P. Giannopoulou, G. N. Kokolakis, P. I. Rafailidis. *Clin. Infect. Dis.*, **2008**, 46, 1069-1077.
[5] M. E. Falagas, A. C. Kastoris, D. E. Karageorgopoulos, P. I. Rafailidis. *Int. J. Antimicrob. Agents*, **2009**, 34, 111-120.
[6] R. Ferreira, J. Torres, J. Raposo, M. Ferreira, S. Mendes, C. Agostinho, M. J. Campos. GE Jornal Português de *Gastrenterologia*, **2012**, 19, 263-266.
[7] R. Fujii. *Chemotherapy*, **1977**, 23 234-246.
[8] M. Grabe, T. Bjerklund-Johansen, H. Botto, M. Çek, K. Naber, R. Pickard, P. Tenke, F. Wagenlehner, B. Wullt. *European association of urology*, **2010**, 110, 225-241.
[9] V. Hamburger, A manual of experimental embryology, University of Chicago Press Chicago, **1942**.
[10] N. Hepping, A. Simon. *Int. J. Antimicrob. Agents*, **2009**, 33, 389.
[11] I. D. Jacobsen, K. Große, B. Hube; Embryonated Chicken Eggs as Alternative Infection Model for Pathogenic Fungi, in Host-Fungus Interactions. Springer,**2012**,487-496.
[12] V. Matzi, J. Lindenmann, C. Porubsky, S. A. Kugler, A. Maier, P. Dittrich, F. M. Smolle-Jüttner, C. Joukhadar. *J. Antimicrob. Chemother*., **2010**, 65, 995-998.
[13] A. S. Michalopoulos, I. G. Livaditis, V. Gougoutas. *Int. J. Infect. Dis*., **2011**, 15, 732-739.
[14] Y. Ohta, M. Kidd. *Poult. Sci*., **2001**, 80, 1425-1429.

[15] B. Pfausler, H. Spiss, P. Dittrich, M. Zeitlinger, E. Schmutzhard, C. Joukhadar. *J. Antimicrob. Chemother*., **2004**, 53, 848-852.
[16] M. Popovic, D. Steinort, S. Pillai, C. Joukhadar. *Eur. J. Clin. Microbiol. Infect. Dis.*, **2010**, 29, 127-142.
[17] R. Raz. Clin. Microbiol. Infect., **2012**, 18, 4-7.
[18] N. Roussos, D. E. Karageorgopoulos, G. Samonis, M. E. Falagas. *Int. J. Antimicrob. Agents*, **2009**, 34, 506-515.
[19] G. C. Schito, K. G. Naber, H. Botto, J. Palou, T. Mazzei, L. Gualco, A. Marchese. *Int. J. Antimicrob. Agents*, **2009**, 34, 407-413.