Teratogenic effects of Metoprolol in the chick embryos

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Summary

This study was conducted to demonstrate the effect of Metoprolol on chicken embryos during the period of cuddling automated .This study was completed in Babylon hatchery dedicated to the production of chick chickens, which is located in the province of Babylon. One hundred eggs (Belgian origin, CZ 1924) were divided into equal four groups. The first group injected eggs by Metaprolol at dose 10 mg /70kg BW, and the second group eggs was injected by Metoprolol at dose 15 mg /70kg BW, while eggs in the third group was injected by physiological normal saline only and the fourth group did not inject their eggs any material and considered the control group and all the eggs used in this experiment to take on the fourth day of cuddling. The results of this study showed a decrease in the percentage hatching in groups injected with Metoprolol (T1 and T2 groups, 4% and 13%, respectively) compared to the T3 and the control groups. Also showed the results of the current study, no significant difference at the level of ($P < 0.05$) in the weights of embryos after hatching. While histopathological examination showed the presence of pathological lesions in the heart tissue in injected eggs groups by Metoprolol (T1 and T2 groups) and included these changes infiltration of inflammatory cells, thickening in epicardium and the presence of vacuolation in heart tissue with the appearance of edema, heart tissue damage, congestion in the blood vessels and the occurrence of hemorrhage.

Keywords: Metoprolol, Chick embryos, Cuddling period.

Introduction

β-blocker therapy is of proven value in the treatment of arrhythmias and hypertension, ventricular systolic dysfunction, heart failure symptoms, and survival in patients with heart failure as well as for long-term secondary prevention after unstable angina and myocardial infarction (1-3). One of the β-blocker drugs is a Metoprolol which is a β_1-selective beta-adrenergic antagonist (4) that is classified as a class I substance according to the Biopharmaceutics Classification Scheme (5). Metoprolol reduces or inhibits the agonistic effect of catecholamines on the heart. A significant increase in cardiovascular medication use during pregnancy occurred in recent years. Only limited evidence on safety profiles is available and little is known about the mechanisms of adverse effect on the fetus. .As the heart rate is the most important determinant of cardiac output in the embryonic heart effect on the fetus. As the heart rate is the most important determinant of cardiac output in the embryonic. One theory suggests that the drug-induced bradycardia is the leading mechanism of heart failure and embryo demise or developmental toxicity (6). There are very few of information and research on the effects of Metoprolol on embryonic development. Therefore, the present study aimed to demonstrate the effect of this drug on chicken embryos.

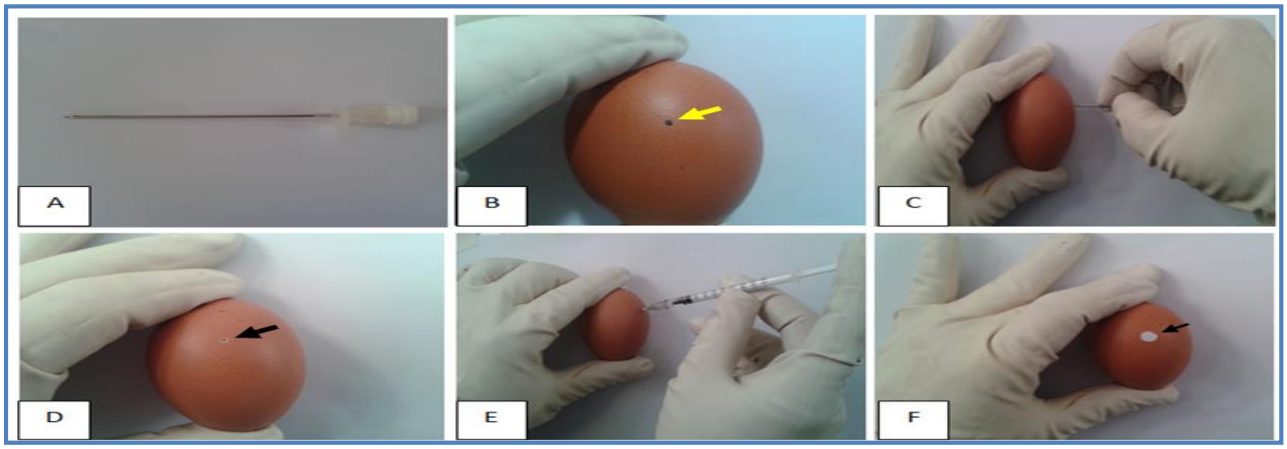
Materials and Methods

Metoprolol tartrate (Betaloc® ampoule was purchased from local pharmacy in Babyloon province) is a selective β_1-adrenoreceptor blocking agent, available in five ml ampoule for intravenous administration. Each ampoule contains a sterile solution of Metoprolol tartrate five mg and sodium chloride and water for injection and it made of by AstraZeneca Company. In this study used the lowest therapeutic dose (10 mg daily) and the highest therapeutic dose (15 mg daily) for Metoprolol a dose intended for intravenous injection in humans, which requires treatment with this drug (7). The dose was calculated by the weight of a chicken egg to typical human weight (70 kg B.W) (8).

Stock solution was prepared by taken three ml from Betaloc® and then complete the total volume to 30 ml by adding a physiological normal saline and mix well (Each one ml of stock solution contains 100 µg). Four experiments were designed to study the effect

of Metaprolol on the check embryo during hatching period (Each group contains 25 eggs) as follow: T_1 group eggs were injected with Metoprolol at 0.15µg per 1gm of egg weight (10 mg /70kg BW). T_2 group eggs were injected with Metoprolol at 0.22µg per 1gm of egg weight (15 mg/70kg BW). T_3 group eggs were injected with vehicle (Physiological normal saline). C group eggs were not injected.This experiment was conducted at the Hatchery Babylon in Babil province. Fertile eggs (Belgium origin, CZ 1924) were used for this study, and it incubated at 37.5°C and a relative humidity of 55 to 60% during day 1-18 and at 36 °C with relative humidity of 60-65% during day 19-21. Average egg weight was approximately 50-55 gm. Eggs were chosen on the fourth day of cuddling through examination by a candle light to make sure the presence of the vital fetus through the appearance of small dark spot with network of blood vessels branching out from it. If the egg is infertile, the egg contents will appear clear or devoid of any evidence of development. Eggs showing rings or steaks of blood contain embryos that have died early in the incubation period (9). To deliver Metoprolol solution into the yolk by injection without the risk of hitting the embryo or any major blood vessel it was necessary to candle each egg to locate the embryo, which appeared as a dark floating silhouette and the head as a dark spot (10). The injection method was done according to Lioyd method (11) and modified by researcher using cannula needle, (Fig. 1) instead of a special hole device and other tools to hole eggshell.

Eggs were taken from hatching machine after four days of cuddling and transferred to a warm room sterile. Sterilization of the area to be drilled with a solution of iodine 10% and then leave the area until dry. Drawing by pencil mark on the area that will pierce. Prove the egg by the left hand and holding the cannula needle by the right hand. Sharp end of the cannula needle was put on the mark previously existing on eggshells and then rotate the needle cannula slowly in a clock wise and counter-clockwise direction even pierce the eggshells. Metoprolol was injection by using insulin syringe through the hole in the eggshell. After the completion of the process of drug injection, the hole was closed by the use of glue paper. Egg returned back to the hatching machine.



Figure, 1: Route of eggs- Chicken injection. (A) Cannula needle 18 G: (B) Site of eggshell puncture: (C) Puncture of eggshell by cannula needle: (D) Show eggshell puncture: (E) Injection Metoprolol drug by insulin syringe through eggshell to yolk sac: (F) Closed eggshell puncture by using glue paper.

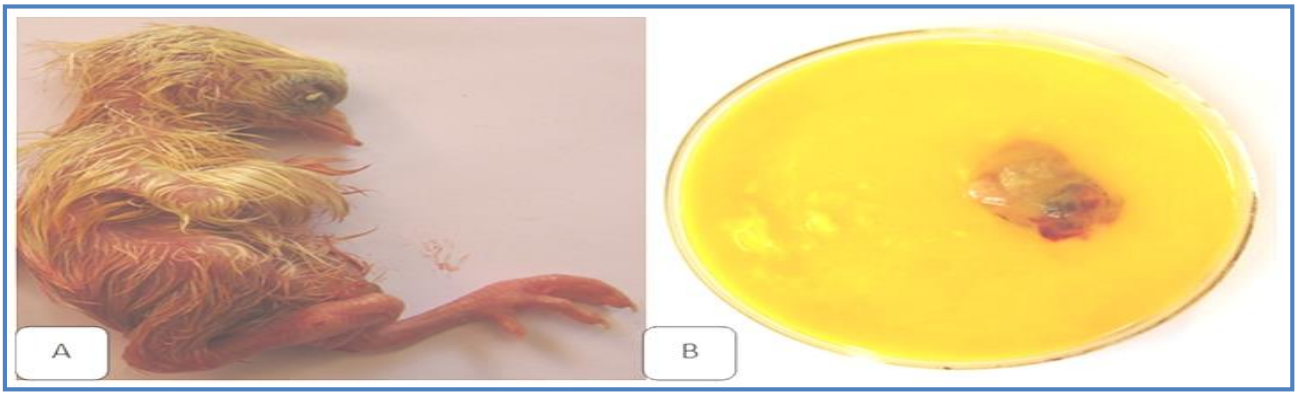
The specimens of heart were fixed by 10% neutral buffered formalin solution till the preparation of histological sections. Tissues were embedded in paraffin and several tissue sections were prepared for histopathological sections and stained with Hematoxylin-Eosin (H and E) Stain (12). Hatchability: (Percentage of live chick embryo after hatching). Body weight changes of chicks hatched. Histopathological examination of the heart of the chicks hatched. Statistical analysis of the experimental results were conducted according to Statistical Package for the Social Sciences (SPSS) version 13.00 where one way ANOVA was used to assess the significance of changes between control and treated eggs. The data were expressed as Mean ± Standard Errors (SE) and P-value <0.05 was considered

statistically significance. LSD was carried out to test the significance levels among means of treatments (13).

Results and Discussion

The current study showed that the percentage of hatching in the T2 group were lower than the T1 group, and both are less percentage of hatching in comparison with T3 and the control group, while no significant change (P>0.05) were present between T3 and control group, as (Table, 1) and (Fig. 2).

The results obtained in this study are in agreement with those found by (6) who showed a significant increase in cardiovascular medication use during pregnancy occurred in recent years. Only limited evidence on safety profiles is available, and little is known about the mechanisms of adverse effect on the fetus. Embryo toxicity was tested in ovo after administration of various doses of Metoprolol, Carvedilol, or ivabradine. Embryonic day (ED) 4, chick embryos were studied by video microscopy and ultrasound biomicroscopy ex. ovo after intraamniotic injection of the drug for a period of 30 min. Significant dose-dependent mortality was achieved in embryos injected with Carvedilol and ivabradine. In ED4 embryos, Metoprolol, Carvedilol, and Ivabradine reduced the heart rate by 33%, 27%, and 55%, respectively, compared with controls (6%). Researchers (14) found that the 96-h LC_{50} of propranolol in the zebrafish larvae was 2.48 mg/L, whereas 50 mg/L Metoprolol did not result in death. Both β-blockers decreased the heart rate and hatching rate and increased the mortality of the zebrafish embryos. Among these indicators, the heart rate was the most sensitive. Heart starts work in chicken embryos on the third day of cuddling, and is pumping blood through the blood vessels to all parts of the fetus to the transfer of materials required for the development of the embryo (9) and the decline in the ratio of hatching in current study in the T1 and T2 groups in comparison with the T3 and control groups may be attributed to the influence of Metoprolol which act directly on the β1 receptors in myocardial cells, causing the bradycardia and then to a hypotension and thus led to a decrease of blood flow in the fetus, which led to his death (6). No significant changes (P>0.05) were noticed in body weight change percent between different experimental groups (Table, 2).



Figure, 2: Chick embryo death. (A) T_1 group eggs were injected with Metoprolol at 0.15µg per 1gm of egg weight: (B) T_2 group eggs were injected with Metoprolol at 0.22µg per 1gm of egg weight.

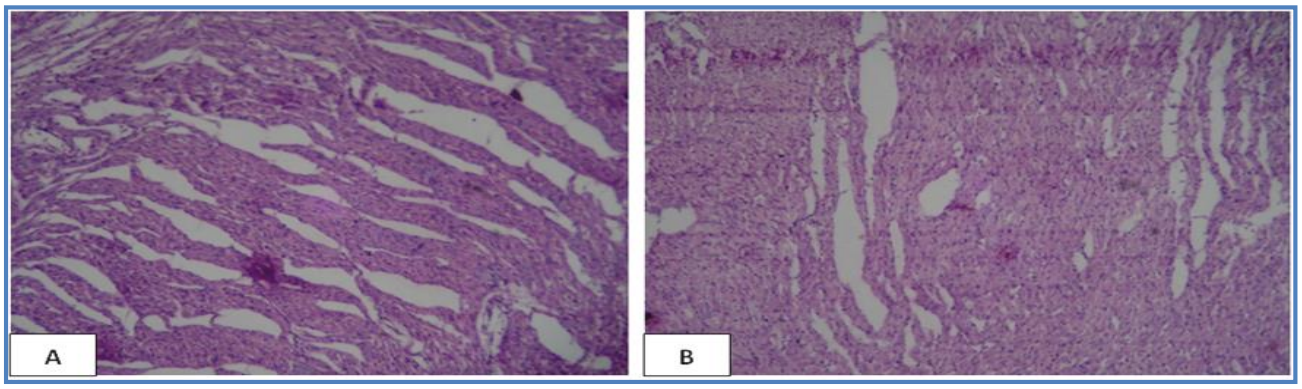
Table, 1: Hatchability of chick embryos after 21 days of cuddling for experimental groups.

Groups	Number of chick eggs	Number of died chick embryos	Percentage of chick embryos died
T 1 0.15µg Metoprolol per 1gm of egg weight	25	1	4%
T 2 0.22µg Metoprolol per 1gm of egg weight	25	3	13%
T 3 Physiological normal saline	25	0	0
Control	25	0	0

Table, 2: Body weight changes of chick embryos after 21 days of cuddling for experimental groups.

Group	Number of live of Chick embryos after hatching	Body weight M±SE
T1 Metoprolol (0.14 mg /kg)	24	40.64±0.37 A
T2 Metoprolol (0.21 mg /kg)	22	40.36±0.41 A
T3 Physiological normal saline	25	39.56±0.40 A
Control	25	40.04±0.41 A

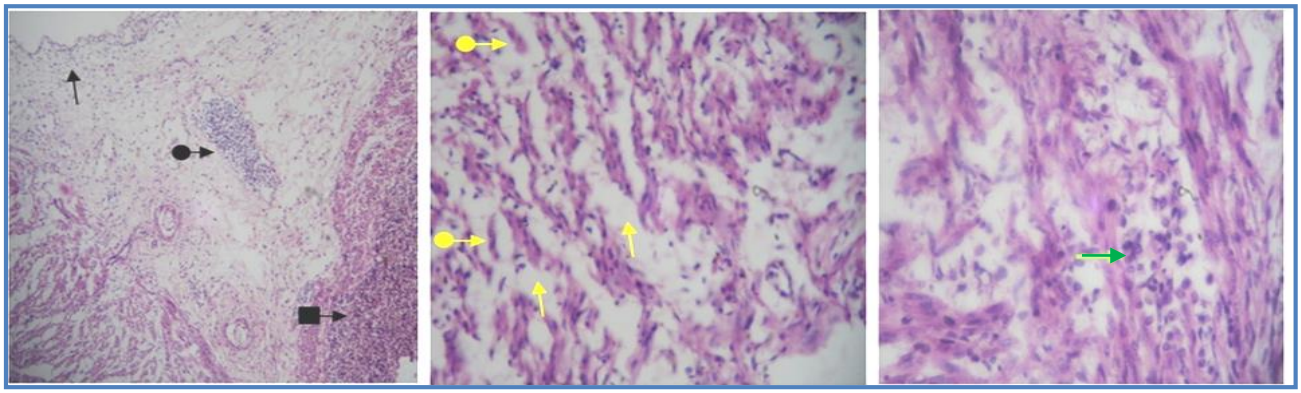
Metoprolol, a cardioselective β-adrenergic blocking agent, has been used during pregnancy for the treatment of maternal hypertension and tachycardia (15 and16). The results obtained in this study are disagreement with those found by (15 and 16) who conducted a study in 1978 on 101 hypertensive pregnant patients treated with Metoprolol alone (57 patients) or combined with hydralazine (44 patients) compared with 97 patients treated with hydralazine alone. The duration of pregnancy at the start of antihypertensive treatment was 34.1 weeks (range 1341 weeks) for the Metoprolol group and 32.5 weeks (range 1240 weeks) for the hydralazine group. The Metoprolol group experienced a lower rate of perinatal mortality (2% vs. 8%) and a lower incidence of intrauterine growth retardation (11.7% vs. 16.3%). No significant change in body weight of chick embryos in this study after hatching (Of chick embryos that remained alive after hatching), may result from two possible reason, the first one, until decrease in body weight occurs by Metapolol needs to doses higher than the therapeutic dose, and this is agreement with (17) who have found no evidence of impaired fertility or teratogenicity during their studies on the reproductive system in rats and mice when treated with Metoprolol, and when they increase the dose of the metaprolol to more than 55.5 times the maximum daily human dose noticed in fetal loss and decreases in neonatal survival and this explains the absence of body weight changes after hatching in this study because the dose that were given were within the therapeutic dose only. Secondly, it may needs long period's treatment during the embryonic stage configuration which cause interference with embryonic mass and thus will lead to a body weight changes. While the histological sections of control group showed normal histological structure, (Fig. 3).



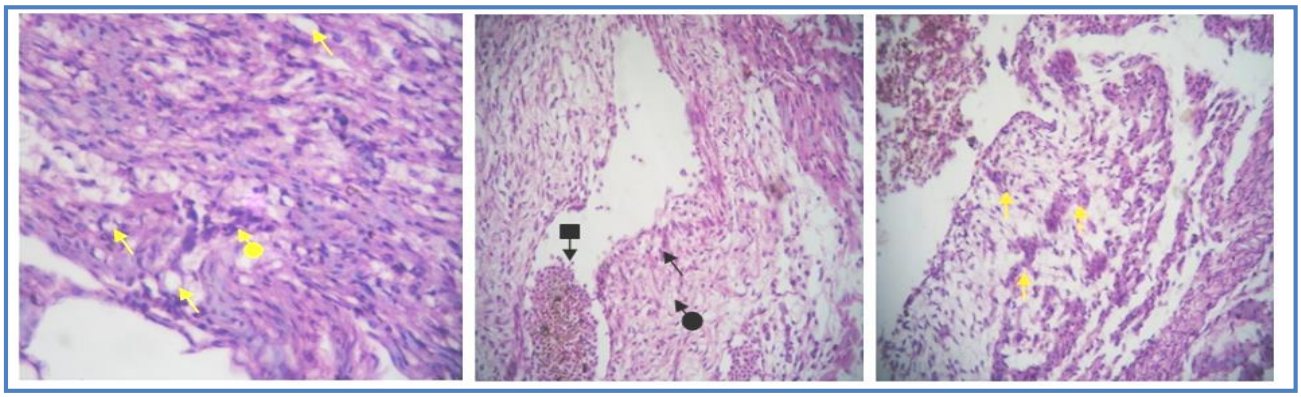
Figure, 3: Histological section of chick embryo heart (After one day of hatc hing): (A) T3 group eggs were injected with vehicle (normal saline): (B) Control group eggs were not injected (H and E stain 40X).

In T_1 group eggs were injected with Metoprolol at 0.15µg per 1gm of egg weight, showed thickening of epicardium tissue due to sever deposition of fibernous exudates associated with cellular aggregate and sever dilatation blood vessels with congestion, as well as sever cellular infiltration and hemorrhage in the muscular tissue mainly in the subepicardial portion. In other section showed odemtus areas between cardiac muscle

with inflammatory reaction restricted mainly in epicardial tissue. There are other lesions seen such as mononuclear cells infiltration between muscles bundles that appear fragmented and speared with interstitial tissue associated with polymorphonuclear cells, as (Fig. 4). In T_2 group eggs were injected with metoprolol at 0.22µg per 1gm of egg weight. The main histopathological lesions in the chick embryo heart (After one day of hatching) include sever mononuclear cells infiltration in the cardiac muscle bundles. In addition sections showed variable sizes of vacuolations between cardiac tissues. In other section showed massive shortening and fragmentation of cardiac muscle fibers associated with sever hemorrhage. In the other field showed thickness in the epicardial tissue associated slight fiberous proliferation and vascular congestion as well as noticed sever distortion of muscle bundles that appear irregular shapes and variable in sizes, (Fig. 5).



Figure, 4: Histopathological section of chick embryo heart (After one day of hatching) for T_1 group eggs were injected with Metoprolol at 0.15µg per 1gm of egg weight. Shows thickening of epicardium (⟶) associated with cellular aggregate and blood vessels congestion (●⟶), as well as sever inflammatory cells infiltration and hemorrhage in the muscular tissue mainly in the subepicardial portion (■⟶), in other section cardiac cells showed slight abnormality (●⟶) also shows interstitial odema (⟶) with mononuclear cells infiltration (⟶) (H and E stain 40X).



Figure, 5: Histopathological section of chick embryo heart (After one day of hatching) for T_2 group eggs were injected with Metoprolol at 0.22µg per 1gm of egg weight. Shows mononuclear cells infiltration (●⟶) in the muscle fibers as well as variable degree of vacuolations (⟶) in other site showed thickness in the epicardium tissue (●⟶) with fiberous proliferation (⟶) and blood vessels congestion with sever distortion and fragmentation of muscle bundles (■⟶) (H and E stain 40X).

The study results showed that the current histopathological analysis that the doses used have caused lesions in heart tissue in the experimental groups (T1 and T2) that injected Metoprolol, and this may explain why the incidence of death in chicken embryos in the final days of hatching. While the reason for the survival of the others chicken embryos alive after hatching is that the effect of Metoprolol was not enough for the occurrence of death and it is possible that the chicks are perish after a period of her life. As a result of the lack of studies on Metoprolol on heart tissue, so you are relying on the results reached in interpreting the results of this study.

In conclusions, the doses used had a deadly effect on chicken embryos by 4-13%, while the doses used in this study did not affect on body weight after hatching, the doses used

caused histopathological lesions in the heart muscle chicken embryos.

References

1. Ali Ahmed. (2003). Myocardial β1-adrenoceptor down-regulation in aging and heart failure: implications for beta-blocker use in older adults with heart failure. The Europ. J. of Heart Failure, 5:709-715.
2. Yusuf, S.; Peto, R.; Lewis, J.; Collins, R. and Sleight, P. (1985). Beta-blockade during and after myocardial infarction: an overview of the randomized trials. Prog. Cardiovasc. Dis., 27: 335–71.
3. The Task Force on Beta-blockers of the European Society of Cardiology. (2004). Expert consensus document on -adrenergic receptor blockers. Eur. Heart J., 25: 1341–1362.
4. Kurt, S.; Gergana, K.; Robert, Z.; Robert, M.; Norbert W.; Friedrich, M. F. and Werner, K. (2001). Differing beta-blocking effects of Carvedilol and Metoprolol. Europ. J. Heart Failure, 3(3): 343-349.
5. Guidance for Industry. (2000). Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. U.S Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) Rockville, MD.
6. Radka, K.; Jarmila, S.; Jiri, N.; Lucie, H.; Bohuslav, O. and Davd, S. (2013). Heart rate changes mediate the, embryotoxic effect of antiarrhythmic drugs in the chick embryo. Amer. J of Physiol., 304**:** 895-902.
7. Product information. Betaloc, AstraZeneca Company, 2013.
8. Walpole, S. C.; Prieto-Merino, D.; Edwards, P.; Cleland, J.; Stevens, G. and Roberts, I. (2012). "The weight of nations: an estimation of adult human biomass" . BMC Public Health, 12: 439.
9. Albert, W. A. and Marior, E. J. (1979). Incubating chicken eggs. Kansas state University, Mnhattan. Cooperative extension service, Manhattan, Kansas .File code: 4.H and Youth 2.
10. Lizza, M. Macalintal. (2012). in ovo Selenium (Se) injection of incubating chicken eggs: effects on embryo viability, tissue se concentration, lipid peroxidation, immune response and post hatch development, Ph.D Dissertation, Animal and Food Sciences, University of Kentucky, Lexington.UK.
11. Lioyd, F. K. (1989). Techniques for preparing bird eggs and nests. Los Angles country Museum of Nature History and Western Foundation of Vertebrate Zoolology, from workshop on bird specimen preparation held at the Carnegie museum of nature history in conjunction with the 107th stated meeting of the American ornithologists, union.
12. Luna, G. L. and Lee, A. A. (1968). Manual of Histological staining methods of the armed forces. Institute of pathology, (3rded.). McGraw-Hill Book Company, New York: USA.
13. Joda, M. (2008). The progressive statistical analysis by using SPSS. (1sted.) Churchill livingstone .Edinburgh.
14. Sun, L.; Xin, L.; Peng, Z.; Jin, R.; Jin, Y. and Qian H, Fu Z. (2013). Toxicity and enantiospecific differences of two β-blockers, propranolol and metoprolol, in the embryos and larvae of zebrafish (Danio rerio). Environ Toxicol. 10. doi: 10.1002/tox.21867.
15. Sandstrom, B. (1978). Antihypertensive treatment with the adrenergic beta-receptor blocker Metoprolol during pregnancy. Gynecol Invest., 9: 195-204.
16. Lundborg, P.; Agren, G.; Ervik, M.; Lindeberg, S.; Sandstrom, B. (1981). Disposition of Metoprolol in the newborn. Br J. Clin. Pharmacol., 12:598-600.
17. Product information. Lopressor. CibaGeneva Pharmaceuticals, 1997.

التأثيرات الماسخة لعقار الميتوبرولول في اجنة الدجاج

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الخلاصة

اجريت هذه الدراسه لبيان تأثير عقار الميتوبرولول على اجنة الدجاج خلال فترة الحضن الالي حيث انجزت هذه الدراسة في مفقس بابل لانتاج افراخ الدجاج الواقع في محافظة بابل، استخدمت 100 بيضة (بلجيكية المنشأ، سي زت 1924) وقسمت الى اربع مجاميع متساوية. المجموعة الاولى حقن البيض فيها بعقار الميتوبرولول بجرعة 10 ملغم/ 70 كغم من وزن الجسم و المجموعة الثانية حقن بيضها بعقار الميتوبرولول بجرعة 15ملغم/ 70 كغم من وزن الجسم والمجموعة الثالثة حقن بيضها بالمحلول الملحي الفسيولوجي فقط و المجموعة الرابعة لم يحقن بيضها باي مادة و اعتبرت مجموعة سيطرة وجميع البيض المستخدم في هذه التجربه اخذ في اليوم الرابع من الحضن. اظهرت نتائج هذه الدراسة انخفاض في نسبة الفقس في المجاميع المحقونه بعقار الميتوبرولول (المجموعة الاولى والثانية 4% و 13% على التوالي) مقارنة بالمجموعة الثالثة و مجموعة السيطرة. كذلك اظهرت نتائج الدراسة الحالية عدم وجود فرق معنوي على مستوى ($P<0.05$) في اوزان الاجنة بعد الفقس، بينما اظهر الفحص النسجي المرضي وجود تغيرات مرضية في نسيج القلب في المجاميع التي حقن بيضها بعقار الميتوبرولول (المجموعة الاولى والثانية) وشملت هذه التغيرات ارتشاح للخلايا الالتهابية وتثخن في جدار الغشاء المحيط بالقلب و وجود تفجي في النسيج القلبي مع ظهور الوذمه و تلف نسجي قلبي و احتقان في الاوعية الدموية و حدوث نزف دموي.

الكلمات المفتاحية: ميتوبرولول، أجنة الدجاج، فترة الحضن.