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Original scientific paper

THE EFFECT OF VERAPAMIL ON THE STAGES OF ONTOGENESIS IN COTURNIX JAPONICA QUAIL EGGS

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Abstract

Introduction: Various studies have shown that the effects of the action of drugs, in addition to having positive effects, they can also provoke negative effects in different organs of the body. Verapamil is an L-type calcium channel antagonist with anti-arrhythmic (PR-interval prolongation), angina, and antihypertensive effects. Its mode of action in cardiovascular diseases is somewhat well elucidated. Verapamil has been successfully used for both cardiovascular and non-cardiovascular indications and appears not to have a major effect on blood pressure in non-hypertensive populations. Animal studies show that Verapamil is highly concentrated in tissue, including lung, with concentrations 40-fold or higher than those in plasma.
Verapamil has also neuroprotective effect in several acute neurotoxicity and nephroprotective effect against lead and cadmium toxicity.
Purpose of the paper: This paper aims to study the effects of verapamil as a calcium channel blockerat different stages of embryonic development in Coturnix japonica eggs.
The research of this paper will consist of the toxic effect and macroscopic and microscopic histopathological changes in certain stages of embryonic development of Coturnix japonica eggs.
Material and method: The experiment was conducted in the Scientific Laboratory of Medical Science, Faculty of Medical Science, University of Tetovo, Republic of North Macedonia and in the Institute of Ecological and Technological, University of Tetovo, Republic of North Macedonia.
In the study, 22 fertilized Coturnix japonica eggs were taken and incubated in the incubator for 16 days of incubation. The eggs were treated with Verapamil at certain stages of embryonic development. The eggs hatched on day 17. Using the stereomicroscope with the macroscopic and microscopic method were analyzed ontogenetic changes in certain stages of embryonic development.
Results: The growth development of egg embryos was observed and studied. On the 17th day, the eggs were hatched. In addition to the effect on the weight of the injected eggs, an abnormal embryonic development was observed, with macroscopic defects and in many cases, stagnation of embryotic development.
Conclusion: High injection doses of the antibiotic should not be usedbecause of its harmful effects. The use of antibiotics in Coturnix japonica eggs should be done with caution. The use of Verapamil with the dosage of 0.2mL did not yield successful.

Keywords: dose effect, Verapamil, calcium channel blocker, histopathological changes, teratogenesis, mutagenesis.

1. Introduction

Verapamil is an L-type calcium channel antagonist with anti-arrhythmic (PR-interval prolongation), [1] angina, and antihypertensive effects. Its mode of action in cardiovascular diseases is somewhat well elucidated [2].

It is well known that calcium plays an integrated and crucial role in regulation of cellular movement and transport, electrical activation of excitable cells and various enzymatic reactions throughout the body (Rasmussen, 1986) [3].
Verapamil has been successfully used for both cardiovascular and non-cardiovascular indications [4][5] and appears not to have a major effect on blood pressure in non-hypertensive populations [6]. Animal studies show that verapamil is highly concentrated in tissue, including lung, with concentrations 40-fold or higher than those in plasma [7] [8].
Verapamil has also neuroprotective effect in several acute neurotoxicity (Liu et al., 2011) [9] and nephroprotective effect against lead and cadmium toxicity (Lermioglu and Bernard, 1998, Zhang et al., 2013) [10] [11]. In addition, verapamil has antiproliferative effect on the tumor cell both in vivo and in vitro (Schmidt et al., 1988, Jian et al., 2007) [12] [13]. Moreover, verapamil was recently used in the treatment of waste and surface waters (Khan and Ongerth, 2004, Hummel et al., 2006, Batt et al., 2008) [14] [15] [16].

2. Material and methods:

Incubation and injecton:

The experiment took place on the Scientific Laboratory of Medical Science, Faculty of Medical Science, University of Tetovo, Republic of North Macedonia and in the Institute of Ecological and Technological, University of Tetovo, Republic of North Macedonia.
A total of 22 country Coturnix japonica eggs were weighed and incubated with INCUBATOR Voltage: AC 195-245V at 37ºC and with humidity of 75%, and then were divided into 2 control eggs and 20 experimental eggs. Using the stereomicroscope NIKON SMZ 1500 with the macroscopic and microscopic method were analyzed ontogenetic changes in certain stages of embryonic development.
On the 11th day of incubation the eggs were grouped into 3 groups (2 eggs Group 1; 20 eggs Group 2):

- Group 1: control eggs; were not injected
- Group 2: injected with 0.2 mLVerapamil

On days 10-16 of incubation, the development ofembryos from all three groups was studied after each egg was hatched. Many things were studied, such as the weight of embryo and the size, organ development, formation of head, beak, eyes, limbs, feathers.
The injection method used for this study was injection in the air chamberat the bottom of the egg, where hole was punched with a needle, then 0.2mL of Verapamil was injected. The injection area was then disinfected and sealed with tape. Control and Experiment eggs were kept in the same incubator (the same environment, temperature and conditions).

Data collection

Incubation, injection and processing of the data for this experiment was done in the period from June to July 2024. The incubation of the Coturnix japonica eggs lasted 16 days. Data was gathered, comparing the results with the embryonic growth from the Control group and with reference to the Hamburg Hamilton Stages [3].

Figure 1. Coturnix japonica eggs during incubation process.

Figure 2. Coturnix japonica eggs on different monitoring days.

Figure 3. Coturnix japonica eggs during incubation.

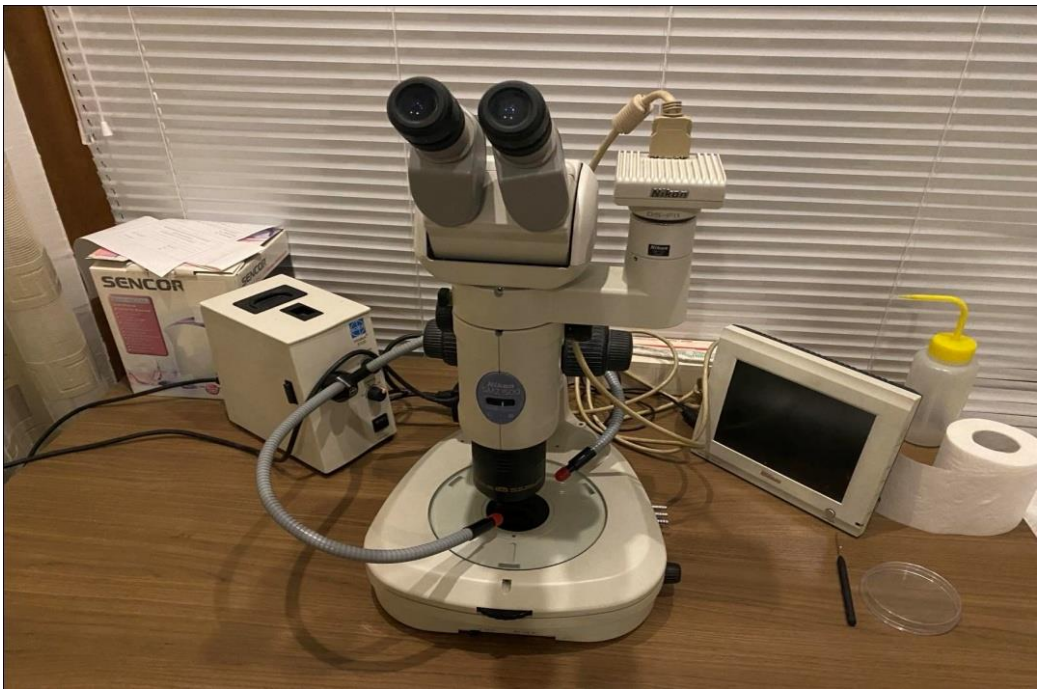

Figure 4. Using the stereomicroscope NIKON SMZ 1500 with the macroscopic and microscopic method were analyzed ontogenetic changes in certain stages of embryonic development.

3. Results

Normal embryonic development

Normal embryonic development in this type of animal lasts 16 days, also at this time of 16 days' experimental eggs are incubated.
Hamburger and Hamilton (1992) described a series of stages encompassing the entire period of Coturnix japonica incubation (16 days) based on the external features. They defined 3 main phases: early, middle, and late stages.

The formation of organs and systems occur during the first 2 stages, followed by the growth of organs and maturation of systems during the late stage. Knowledge of the normal stages in Coturnix japonica embryonic development gives a deeper understanding of the developmental process and how embryonic growth and the hatching process can be influenced and manipulated by both endogenous and exogenous factors. Although all vertebrate embryos are influenced by external environmental conditions, embryonic growth and development is influenced by both endogenous and exogenous factors.
The development of organs starts early in Coturnix japonica embryonic development, and all functional organs are well developed before hatch. Initial development of the Coturnix japonica embryo is rapid: by h 44 of incubation, the heart is beating and vascular systems are linked. By the end of the third day of incubation, limb buds for the wings and legs are visible and the auditory pit is established. Formation of reproductive organs, differentiation of sex, and beginning of voluntary movements take place on day 5 and 6. By day 7, the heart is completely enclosed in the thoracic cavity and feather buds are forming. By day 10, the beak hardens and toes are fully formed (stage 36 of HH when the length of beak and third toe are started to use as an indicator for embryonic staging). On embryonic day 14, the embryo is moving into position for hatching and turns its head toward the large end of egg.

Embryonic development of experimental eggs (injected with Verapamil)

During the incubation of the eggs, their weight was measured at different stages of embryonal growth (**Table 1**).

Table 1. Determination of weight, day of hatching and status of embryonal growth of 22 Coturnix japonica eggs.

Nr.	Egg	Weight before incubation (gr)	Weight on day 8 of incubation (gr)	Temperature of incubation	Humudity during incubation
1	1K	11.48	9.58	37°C	75%
2	2K	10.7	7.6	37°C	75%
3	1E	11.4	8.0	37°C	75%
4	2E	12.3	11.0	37°C	75%
5	3E	10.7	7.5	37°C	75%
6	4E	9.8	8.5	37°C	75%
7	5E	10.9	7.4	37°C	75%
8	6E	11.3	7.8	37°C	75%
9	7E	12.00	10.00	37°C	75%
10	8E	11.2	9.3	37°C	75%
11	9E	10.1	9.0	37°C	75%
12	10E	9.02	6.12	37°C	75%
13	11E	10.02	9.32	37°C	75%
14	12E	11.40	9.4	37°C	75%
15	13E	9.80	7.6	37°C	75%
16	14E	9.77	7.17	37°C	75%
17	15E	10.00	8.7	37°C	75%
18	16E	11.48	7.38	37°C	75%
19	17E	9.90	8.4	37°C	75%
20	18E	11.59	9.99	37°C	75%
21	19E	11.00	9.00	37°C	75%
22	20E	12.07	10.97	37°C	75%

Table 1 shows data such as the number of eggs, control eggs, experimental eggs, egg weight before incubation, egg weight after incubation, incubation temperature and incubation humidity. These parameters are optimal parameters for egg incubation during the stages of embryonic development.

Normal weight loss can be observed in the first week, and then its gradual increase in the following stages of embryonic growth. The injections with Verapamil and sempervivum tectorum tea were carried out on the 11^{th} day.

On days 10-16 of incubation, the development of egg embryos from each group was studied. Embryo weight, organ formation, embryo size, formation of beak, eyes, feathers were monitored.

Group 1 eggs hatched on day 14 (Fig.1).

As expected, normal embryonic development is clear and distinct macroscopically.

On eggs of Group 2, the development of the embryos has been stagnated. The dose of 0.2mL of Verapamil has been lethal to most of the embryos, with extreme cases such as 3E, 4E, 5E, 17E (Fig. 2).

Figure 5. Embryonic development of Group 1 eggs on different monitoring days.

Figure 5 shows control eggs of the Coturnix japonica quail that are not treated with Verapamil, so the trends of embryonic development stages throughout the ontogenesis period are clearly observed.

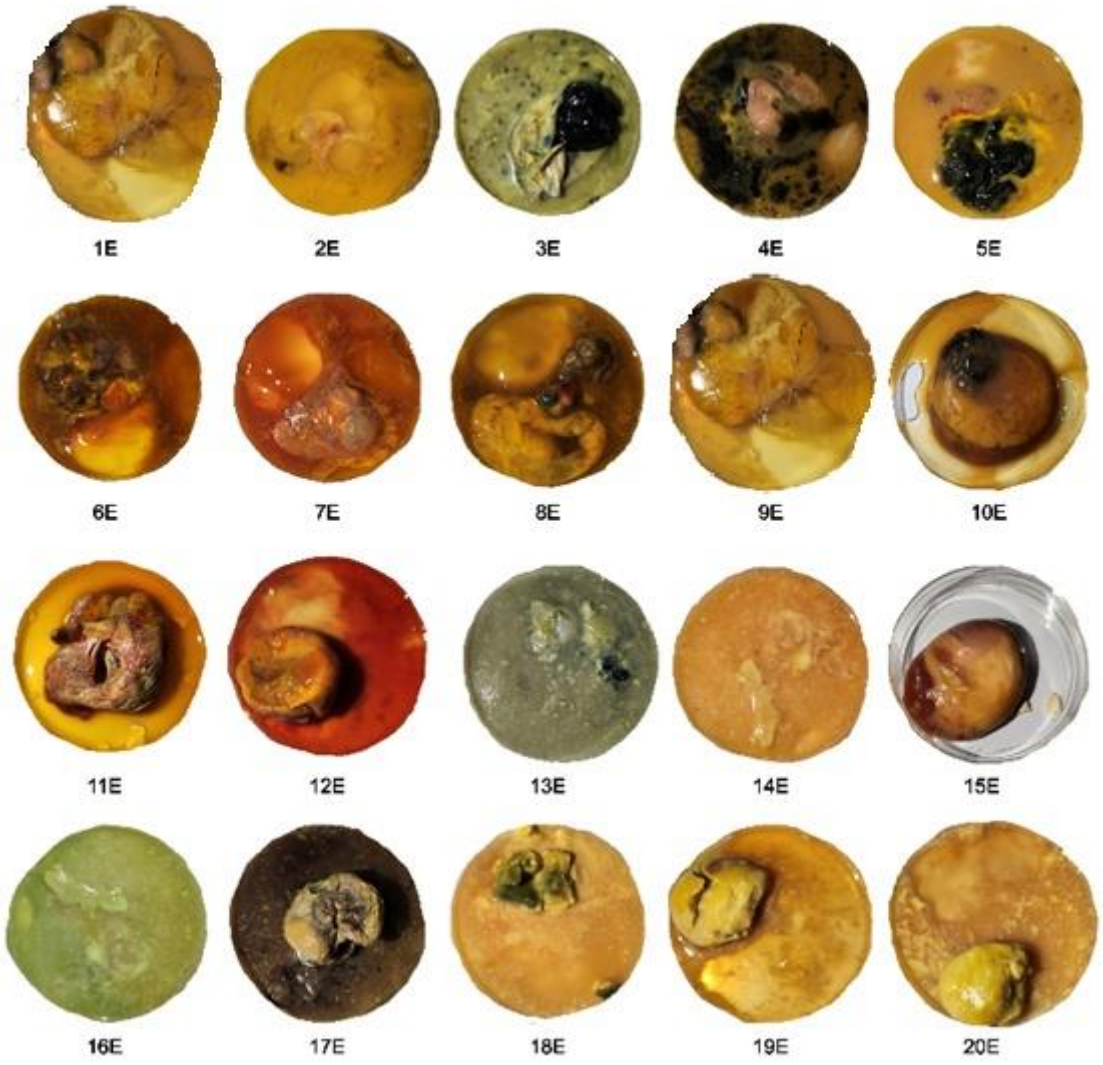

Figure 6. Embryonic development of Group 2 eggs on different monitoring days.

Figure 6 shows the experimental eggs of the Coturnix japonica quail treated with a dose of 0.2mL Verapamil and it was observed that the given dose of 0.2mL Verapamil stagnated almost all stages of embryonic development at certain stages of ontogenesis. This implies that the dose of 0.2mL for the eggs of the Coturnix japonica quail can be calculated as a lethal dose, therefore the results have resulted negatively at certain stages of embryonic development of the eggs of the Coturnix japonica quail.

4. Discussions

Calcium channel blockers have been shown in animal experiments to induce teratogenic effects and to increase the incidence of embryolethality in mammalian animals (Lee and Nagele, 1986, Stein et al., 1990, Robert et al., 2011, Uslu et al., 2013) [17] [18]. In addition, verapamil causes malformations, edemas and reduced heart rate in the embryos, larvae and adult fish (Rottbauer et al., 2001, Shin et al., 2010, Steinbach et al., 2013) [19]. Verapamil inhibits glucose uptake in insulin-sensitive tissues such as adipocyte skeletal myocytes and cardiac myocytes (Khil et al., 1997, Whitehead et al., 2001, TenHarmsel et al., 2005) [20] [21] [22]. Verapamil also inhibits the glucose transport activity of GLUT 1 in a dose-dependent manner (Larry et al., 2010) [23].

Immunohistochemical investigation revealed the expression of the pro-apoptotic factors (cytochrome c and caspase-3) in normal development of the retina at pre-natal stages at E14, E17 of gestation. Immunoexpression of these two factors reflect their role in normal development regardless the effect of verapamil on calcium uptake of the developing retina cells. Also, the expression of BAK and TNFα R2 in normal retinal differentiation however was restricted to the differentiated nerve fibers of the ganglion neural layer and also reflects their role in normal development regionally in contrast to the expression of the other two factors that coincides with the different regions of the developing retina [24].
This research also corresponds with our work on Gallus domestica chicken eggs that were also treated with a dose of 0.2mL of Verapamil and this dose also showed a negative effect on almost all stages of embryonic development in Gallus domestica chicken eggs (Beadini et al., 2019) [25].

5. Conclusions

- This paper aims to follow the stages of embryonic development of the embryo during the stages of ontogenesis and the treatment of Coturnix japonica eggs with Verapamil at certain doses at certain stages of embryonic development.
- The given dose of 0.2ml of Verapamil has caused stagnation in embryonic development, causing a total stagnation. On some of the eggs, the effect of Verapamil is stronger, seen in cases such as egg 3E, 13E, 16E.
- This indicates that the use of Verapamil for embryonic growth is not successful. Further research and data is necessary. Lowering the dose of injection or diluting the dose could yield positive results.
- Verapamil, as an L-type calcium channel antagonist, does not have any negative effects in normal doses; however, administering this drug to overweight individuals may have negative effects and stagnation during ontogenesis or at certain stages of embryonic development.
- The results obtained will be able to provide a valuable contribution to the field of embryology (gynecology) in particular and the field of cardiology in general.

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