ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

# EFFECT OF VARYING DOSES OF ACE INHIBITOR DRUG LISINOPRIL ON DEVELOPMENTAL STAGES OF CHICK EMBRYO

**ShaziaPerveen*[1], Kiran Fatima[2], Rabia Sadiq[3], Kainat Sardar[4]**
[1,2,3,4] Department of Zoology, The Women University Mattital campus, Multan.
* Corresponding Author: drshazia.zol@wum.edu.pk

**Abstract**:
Hypertension is common and significant issue experienced by the majority of women during pregnancy. However treating hypertension during pregnancy is very challenging due to the complications caused by the antihypertensive drugs which pose risk on the health of both fetus and mother. Few researches have been carried out to investigate the effects of antihypertensive drugs on the fetus. Controlled studies on Lisinopril drug are not available. Our study aimed to investigate the effect of lisinopril using chick embryo as a model organism. A total of 32 disease free Sussex chicken eggs were selected and categorized into four groups containing one control and three experimental. After 24 hours the eggs were opened by windowing and injected with lisinopril drug of varying doses, and then closed again using sticky tape. On 15$^{th}$ day the eggs were opened and assessed for their morphological development and their also they were accessed for their hatching time. Treatment with lisinopril significantly affected the features and down neural tube development. The intermediate doses showed more significant results than the highest group when compared to control lisinopril had a negative effect on chick development. Further research is needed for identification of safe and effective drug in order to treat high blood pressure during the pregnancy.
**KEYWORDS**: Chick embryo, Hypertension, Pregnancy, Lisinopril, Toxicity

## 1. INTRODUCTION

One of the commonly prescribed drug is lisinopril which belongs to angiotensin-converting enzyme (ACE) inhibitor family. It at measurements of 20 mg to 80 mg once every day is successful in bringing down pulse in hypertension. Around 25 to half of an oral portion of lisinopril is bioavailable in man. Its peak serum level of are reached in at roughly 6 hours. The dose depends on ailment and reaction to treatment. For kids the measurements is likewise founded on weight. Retention is unaffected by sustenance. Lisinopril isn't fundamentally processed in people and assimilated medication is discharged unaltered in urine (Lancaster and Todd, 1988). Hypertension is a genuinely normal condition, assessed to influence somewhere in the range of 6 % and 8 % of pregnancies. Hypertension itself has not been related with innate deformities. The most continuous clinical antagonistic encounters on lisinopril are cerebral pain, discombobulation, looseness of the bowels and cough(Rush and Merrill, 1987). Sudden withdrawal of lisinopril has not been related with a quick increment in pulse or a significant increment in circulatory strain contrasted with pretreatment levels. Two High dosages of lisinopril are more successful than low portions for diminishing the danger of major clinical occasions in patients with heart issues. (Simpson and Jarvis, 2000). ACE inhibitors have been embroiled in fetopathies in people and perinatal mortality in rodents, bunnies, sheep and mandrills. The utilization of angiotensin changing over chemical inhibitors in pregnant ladies has genuine and harmful impacts on fetal advancement including renal disappointment, renal dysplasia, hypotension, oligohydramnios, aspiratory hypoplasia, and hypocalvaria(Quan, 2006).

Specialists hypothesize that diminished fetal pulse and if renal perfusion is not properly controlled it might lead to oliguria in fetus, rounded dysgenesis in the kidneys, and oligohydramnios at times. Low fetal blood pressure, oligohydramnios and poor peripheral perfusion of the superficial tissues might cause poor perfusion of the skull that, in conjunction with increased forces applied by the uterine wall, cause poor ossification in the calvaria region. The mechanism by which kidneys fail to perform function likely is related to low blood pressure in fetus and also if the glomeruli fail to filter for prolonged hours. It has also been manifested that ACE inhibitors oppose the proliferation of smooth muscle cells of fetus in the ductus arteriosus that occasionally leads to patent ductus arteriosus. ACE inhibitors also elevate chances of miscarriages as shown by some studies(Moretti et al., 2012). Significant difference was seen in average gestational age at birth and also birth weight with babies born either to mothers exposed ACEI or to other antihypertensive drugs. The decrease in age at birth likewise as birth weight is according to the findings in ladies with chronic high blood pressure because of placental disfunction and slashed placental blood flow. A high incidence of fetal impediments is related to the use of ACE-1 during

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

three trimesters of pregnancy(Shotan et al., 1994). ACEIs are contraindicated in women who can bear children. There is substantial amount of evidence depicting the high degree morbidity and mortality in fetuses of mothers who took lisinopril during pregnancy (Rosenthal and Oparil, 2002). The detailed mechanism underlying the fetal anomalies can further be studied.

## 2. Material and Methods

*2.1. Experimental Subject*

White chicken Sussex fertilized eggs (8 per group) were obtained from local hatchery. The eggs were made sure of standard weight approximately 60 g.

*2.2. Study Design*

Eggs were acclimatized for a day or two and divided into groups. The eggs were washed with 70 % ethanol and labelled. The labelled eggs were divided into four groups with each group containing eight eggs each. Three of experimental groups were subjected to the lisinopril drug 10 mg/body weight to group A, 20 mg to group B 40 mg to group C (Table 1). The drug dose was dissolved into autoclaved water and injected later. Control group received no drug (Kučera and Burnand, 1987). Lisinopril was obtained from local pharmacy under brand name Zestrotic. Windowing was done by using candling. Air sac was located and the point where window was to be made was marked. Window was created through the use of egg driller. Immediately the corresponding doses were injected to the eggs according to the group they belonged to. Window was later closed with the help of sticky tape and eggs were placed with their vegetal ends upwards. Eggs were later placed into the incubator with 56 % of humidity and carefully stacked (Kotwani, 1998). Half of eggs from each group were sacrificed on the $10^{th}$ day of incubation while remaining half were left to check if the hatching delays or not. The physical anomalies were observed. The behavior of live chickens was observed in the latter group.

*2.3. Morphometric Parameters*

The basic morphological characters such as wing length, hind limb length, weight of chicken and length of body were determined by using a Vernier calipers. The physical anomalies were determined in the group sacrificed on $7^{th}$ day.

*2.4. Craniofacial development*

The development of the head and face during embryogenesis is a highly complicated mechanism that involves tissues that are derived from all germ layers, complex pathways of cell migration, broad range of morphogenetic movements and multiples of intercellular signals. This complex procedure is prone to be manipulated by lisinopril through its inactivation of SHH gene. The jaw and head structure was compared to that of in control and observations were noted down. Angiogenesis and amniotic fluid biochemistry was also determined (Levy *et al.,* 2001).

*2.5. Behavioral Patterns:*

Behavioral patterns were observed in group that was sacrificed on $21^{st}$ day or naturally hatched ones. The gait of chickens was observed which was hypothesized to be abnormal. Chicken activity was also determined. If chickens were sluggish or not.

*2.6. Hatchability:*

The time of each egg hatching was determined. The eggs that naturally hatched on $21^{st}$ day were noted down and the ones that were forcefully ruptured were also noted down along with their corresponding groups. The aim was to study if our drug of choice was responsible for delayed hatching or not.

*2.7. Statistical Analysis:*

The recorded results are expressed as mean ± SEM. ANOVA was used to perform the multiple comparison among the four groups that included three experimental and a control. T test was used in order to compare the two means significant value was $P < 0.05$.

## 3. RESULTS

*3.1. Control group*

Two eggs from each group were sacrificed on the tenth day of incubation and $7^{th}$ day of drug dose. In control group the normal growth pattern was noticed (Figure 1). Distal segments in wings were noticed to have become longer. The legs were elongated too. Primordia of claws was also seen. Nostril of chick had slit like appearance. On tip of upper jaw labial groove was also observed in both of embryos as shown in Figure 1 A and B of control group. In A the beak development was better than the embryo B. The head and eye size was nearly the same. The body length of the embryos varied minutely. The limb development was poorly observed in embryo A when compared to B.

*3.2. Experimental groups*

In the group A that was given 10 mg lisinopril, no embryo was developed in eggs. However the signs of initial growth were present such as blood vessels were seen in one of them. Head fold and neural fold were observed which depicted that the growth was normal till the day 3 but later it attenuated. Optical vesicle was noticed in one of the embryos (Figure 2). In group BEmbryos were given 20 mg showed the distinct features with

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

complete growth yet dwarf in size. The embryo had well developed eyes and ellipsoidal shaped eyelids. Phalanges were distinctly observed. Egg tooth was also completely visible. Length of limbs was also short (Figure 3). In group C with 40 mg dose one of zygote got aborted and no growth was observed however in some embryos the distal segment of wings was very short. Legs were also smaller in size compared to other groups. Sclera papilla was not developed fully (Figure 4). On observation at 21st day of incubation in Control group among six eggs four hatched naturally and chicks had normal development and were fully covered with feathers. Two were manually taken out on the 22nd day of incubation. Amniotic fluid was observed in one (Figure 8 A). One died in few seconds (Figure 8 B). In Experimental group A No embryo development was observed in majority of eggs. Few of them had very minute vascularization. The rest didn't have any signs of growth leading us to conclusion that the growth stopped at very early stages. One embryo was prominent among the others but no wings, legs or beak formation was observed.

In Experimental group B Out of six eggs four hatched before the day 21 leading us to conclusion that 20 mg dose reduced the hatching time. Out of four hatched chicks one died in a few hours. Remaining two very sluggish and smaller in size. The weak ones were not able to walk properly (Figure 10). While in Group CNo chicks hatched naturally. On manual hatching it was found that all embryos were dead (Figure 11). The growth and size of three chicks was normal when compared to control however in remaining embryos the yolk had failed to retract. Abdomen region was not covered (Figure 11 A). In some no feathers were observed. Notice that 40 mg and 20mg caused 100 % lethality. The cause of lethality in control group maybe some sort of extraneous variable. The dosage of 20 mg was lethal for less than 50 % of chick embryos (Figure 14).

## 4. DISCUSSION

Chronic hypertension complicates between 1% and 5% of pregnancies (Haddad B, Sibai BM *et al.*, 2002). Managing hypertension during is very essential as it can have negative impacts on women who are pregnant and their babies. Some of these effects include headache, depression, anxiety and insomnia. Obesity and metabolic syndrome are likely to contribute to hypertension. Several studies have shown that high blood pressure during pregnancy significantly increases the chance of stroke, pre-eclampsia and morbidity in women whose age is older (Yoder SR, Thornburg LL *et al.,* 2000). Pregnant women who experience serious cases of hypertension or pre-eclampsia are at risk of giving birth to premature babies and experiencing abruption of placenta (Williams MA, Peterlin BL *et al.,* 2011). When observed in chicks The drug lisinopril when given at varying doses obtained varying results the prominent differences found in experimental group when compared to control group included the size of chick, body length , beak formation , retraction of yolk and hatching time. The least variations were found in group no 4 however the pre-hatching is striking point in group no 2 that was given intermediate dose of 20 mg. The lowest dose group 10mg of lisinopril significantly stands out as no embryo development was observed at all even at the 22nd day. Maximum before time hatching was observed in group no 2 in which 66 % of hatching occurred before 21st day. The chicks of group C with 40 mg of lisinopril were significantly bigger in size when compared to the controlled. Naturally hatched chicks belonging to experimental groups showed deviation from normal behavior. The still born completely developed embryos in experimental groups in an intriguing point. International guidelines for the treatment of hypertension in pregnancy vary in accordance to thresholds for starting treatment and targeted Blood pressure goals. Thus, there remains a pressing need for safe and effective hypertension treatment to benefit the health of mothers as well as their infants.

## 5. CONCLUSION

The drug dose of 10 mg resulted in complete growth retardation significantly. 20 mg dose resulted in hatching before time and smaller size of embryos though developed fully. 40 mg dose resulted in large sized embryos but fused structures of embryo. Each dose had its effect in different way. Further analysis on amniotic fluid could provide better insight to the mechanism that lead to physical anomalies in chick embryos.

## REFERENCES

BHATT-MEHTA, V. & DELUGA, K. S. 1993. Fetal exposure to lisinopril: neonatal manifestations and management. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy,* 13**,** 515-518.

GOA, K. L., HARIA, M. & WILDE, M. I. 1997. Lisinopril. *Drugs,* 53**,** 1081-1105.

HOELTZENBEIN, M., TISSEN-DIABATE, T., FIETZ, A. K., ZINKE, S., KAYSER, A., MEISTER, R., WEBER-SCHOENDORFER, C. & SCHAEFER, C. 2018. Increased rate of birth defects after first trimester use of angiotensin converting enzyme inhibitors - Treatment or hypertension related? An observational cohort study. *Pregnancy Hypertens,* 13**,** 65-71.

KOTWANI, A. 1998. Use of chick embryo in screening for teratogenicity. *Indian journal of physiology and pharmacology,* 42**,** 189-204.

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

KUČERA, P. & BURNAND, M. B. 1987. Routine teratogenicity test that uses chick embryos in vitro. *Teratogenesis, carcinogenesis, and mutagenesis,* 7**,** 427-447.

LANCASTER, S. G. & TODD, P. A. 1988. Lisinopril. *Drugs,* 35**,** 646-669.

LEVY, B. I., DURIEZ, M. & SAMUEL, J. L. 2001. Coronary microvasculature alteration in hypertensive rats. Effect of treatment with a diuretic and an ACE inhibitor*. *American Journal of Hypertension,* 14**,** 7-13.

MORETTI, M. E., CAPRARA, D., DREHUTA, I., YEUNG, E., CHEUNG, S., FEDERICO, L. & KOREN, G. 2012. The fetal safety of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers. *Obstetrics and gynecology international,* 2012.

QUAN, A. 2006. Fetopathy associated with exposure to angiotensin converting enzyme inhibitors and angiotensin receptor antagonists. *Early human development,* 82**,** 23-28.

ROSENTHAL, T. & OPARIL, S. 2002. The effect of antihypertensive drugs on the fetus. *Journal of Human Hypertension,* 16**,** 293.

RUSH, J. E. & MERRILL, D. D. 1987. The safety and tolerability of lisinopril in clinical trials. *Journal of cardiovascular pharmacology,* 9**,** S99-107.

SHOTAN, A., WIDERHORN, J., HURST, A. & ELKAYAM, U. 1994. Risks of angiotensin-converting enzyme inhibition during pregnancy: experimental and clinical evidence, potential mechanisms, and recommendations for use. *The American journal of medicine,* 96**,** 451-456.

SIMPSON, K. & JARVIS, B. 2000. Lisinopril. *Drugs,* 59**,** 1149-1167.Anti-hypertensive drugs in pregnancy and fetal growth: evidence for "pharmacological programming" in the first trimester?

Bayliss H, Churchill D, Beevers M, Beevers DG Hypertens Pregnancy. 2002; 21(2):161-7 Hypertension in pregnancy and women of childbearing age.Yoder SR, Thornburg LL, Bisognano JD Am J Med. 2009 Oct; 122(10):890-5.

Williams MA, Peterlin BL, Gelaye B, Enquobahrie DA, Miller RS, Aurora SK (2011): Trimester-specific blood pressure levels and hypertensive disorders among pregnant migraineurs. Headache 51: 1468-1482.

**Acknowledgement:**
Special Thanks to Dr. shaziaperveen The Women University Multan
**Conflict of Interest**: Authors have no conflict of interest.
**Authors Contribution:**

**Funding Sources**: No funding was received for conducting this study.
**Availability of Data and material**: The datasets generated during and/or analyzed during the current study are available from corresponding Author on reasonable request.
**Code Availability**: The codes used during current study are available from corresponding Author on reasonable request.

**Table 1: Doses of Lisinopril given to Chick Groups**

| GROUP | DOSE |
|---|---|
| CONTROL | 0 mg |
| EXPERIMENTAL A | 10 mg |
| EXPERIMENTAL B | 20 mg |
| EXPERIMENTAL C | 40 mg |

**Table 2: Morphological parameters of embryos sacrificed on the 10$^{th}$ day.**

| Chicks # | Group | Given Dose (mg) | Weight in (g) | Embryo length in mm | Eye size in mm |
|---|---|---|---|---|---|
| 1 | Control | 0 mg | 51.44 g | 40.2 mm | 3.7 mm |
| 2 | Control | 0 mg | 54.87 g | 35.83 mm | 3.6 mm |
| 9 | Experimental A | 10 mg | Aborted | 0 | 0 |

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

| | | | | | |
|---|---|---|---|---|---|
| 10 | Experimental A | 10 mg | Aborted | 0 | 0 |
| 17 | Experimental B | 20 mg | 55.8 g | 25.62 mm | 2.3 mm |
| 18 | Experimental B | 20 mg | 62 g | 32.62 mm | 2.01 mm |
| 25 | Experimental C | 40 mg | 38.01 g | 22.3 mm | 3.1 mm |
| 26 | Experimental C | 40 mg | Aborted | Nill | 0 |

**Table 3: Showing lethal effects induced by the Lisinopril in developing chick embryo**

| Group | Fertilized eggs used | Dead embryos (aborted) | Live embryos | % Age lethality |
|---|---|---|---|---|
| Control group | 6 | 1 | 5 | 16 % |
| Group A | 6 | 6 | 0 | 100 % |
| Group B | 6 | 2 | 4 | 33 % |
| Group C | 6 | 6 | 0 | 100 % |


**Figure 1: 10th day developed in control group**

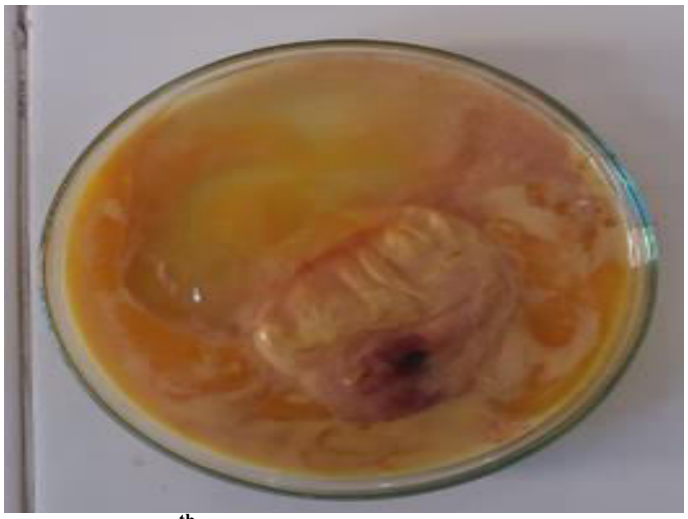

**Figure 2: 10th day developed in experimental group A**

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

**Figure 3:10th day development in experimental group B**

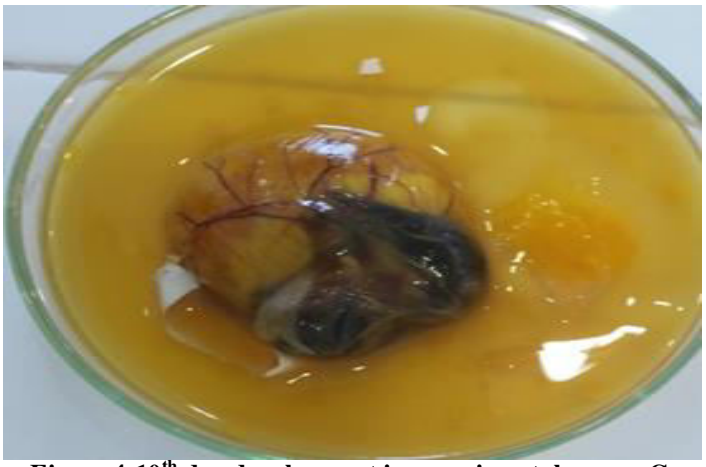

**Figure 4:10th day development in experimental group C**

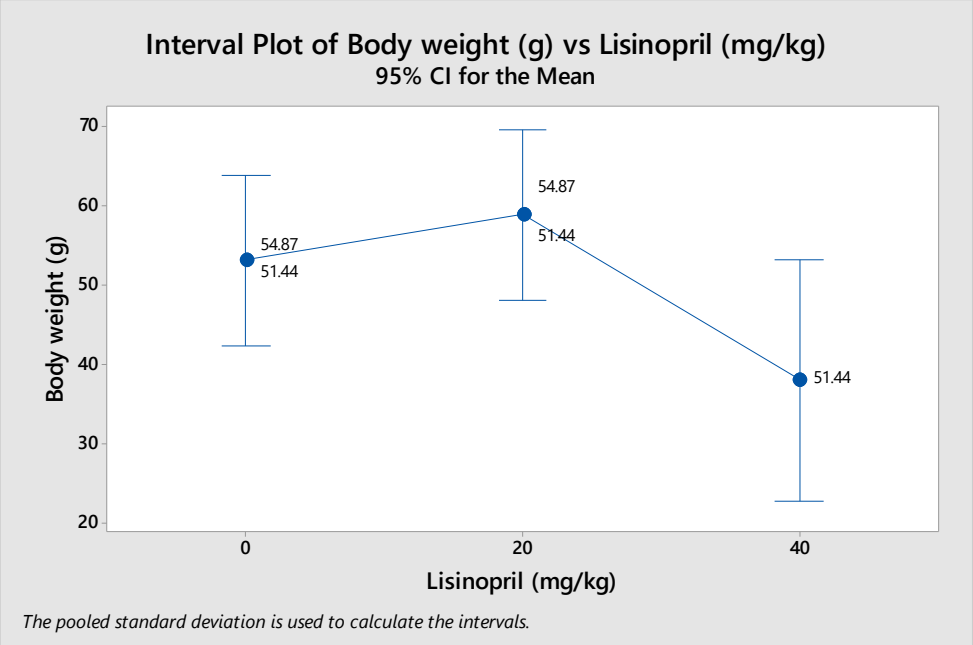


**Figure 5: One-way ANOVA analysis between dosage and body weight of Chicks on 10th day**

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

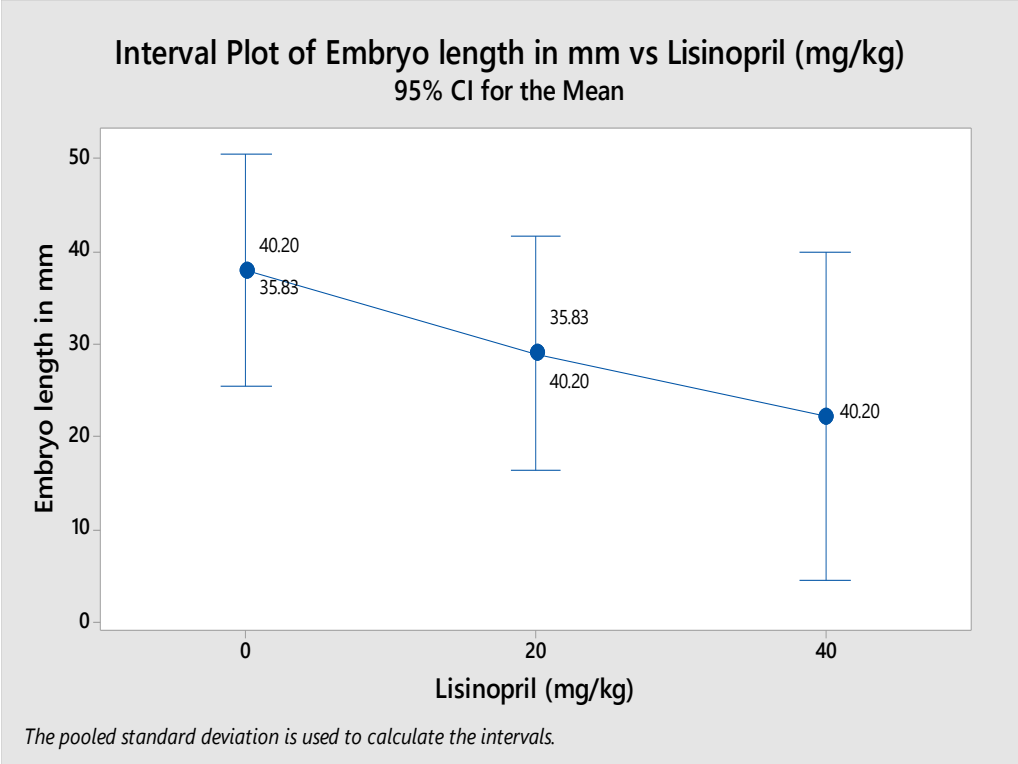


**Figure 6: One-way ANOVA analysis between dosage and Length of Chicks on 10th day**

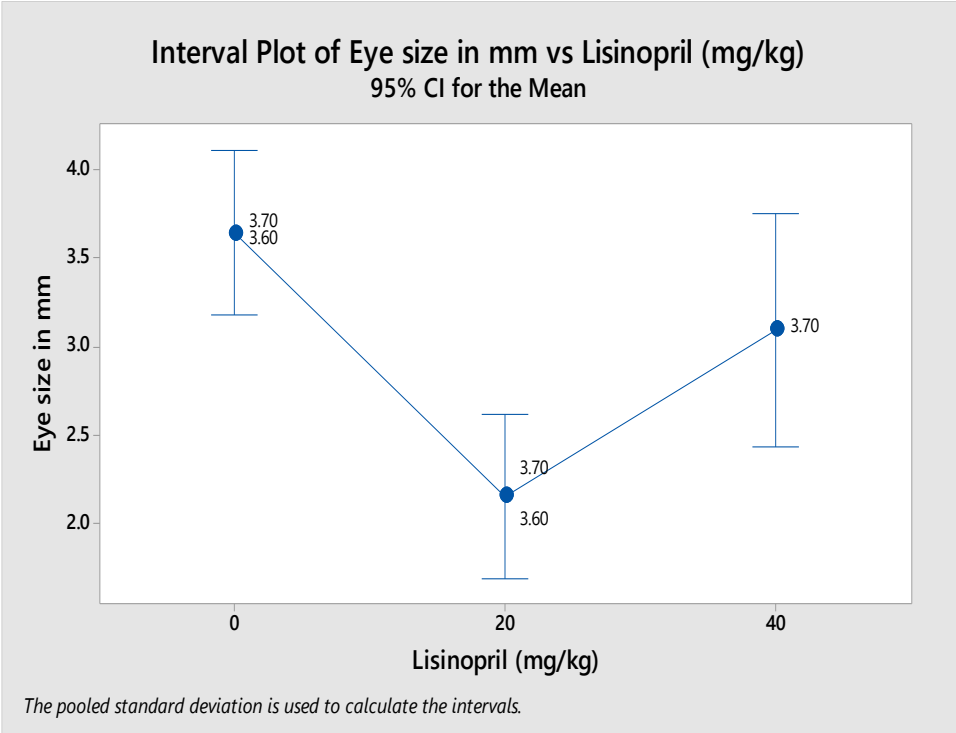


**Figure 7: One-way ANOVA analysis between dosage and Eye size of Chicks on 10th day**

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

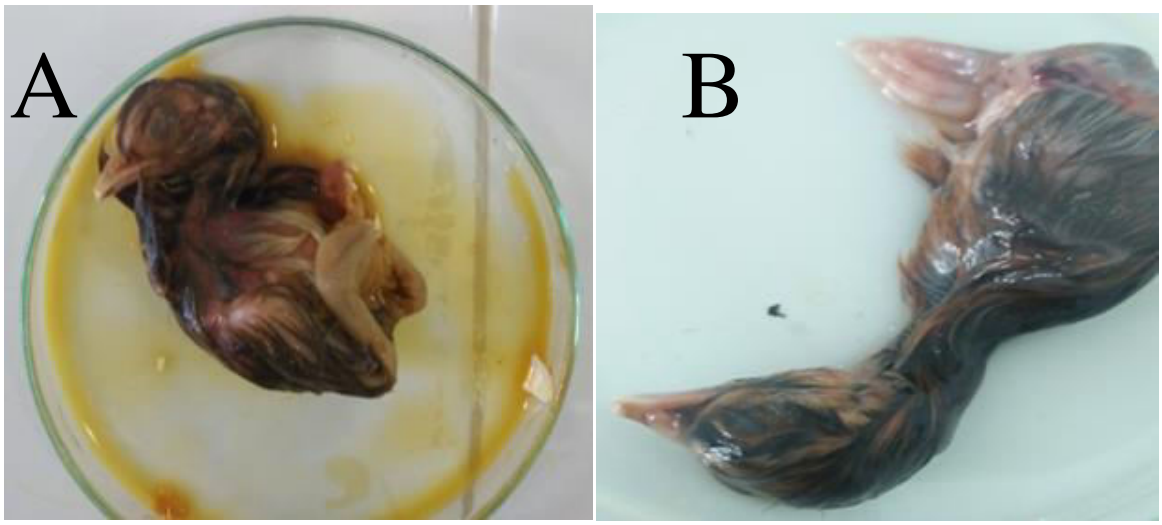


**Figure 8:22nd day in control group**

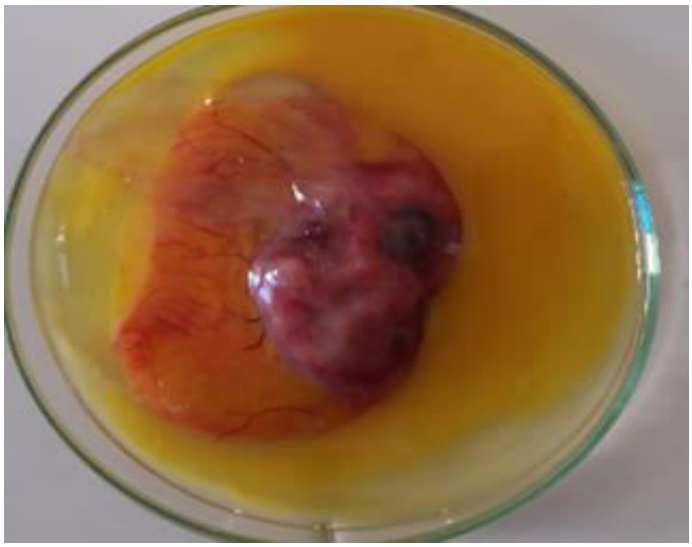

**Figure 9:22nd day in experimental group A**

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

**Figure 10: 21st day in experimental group B**

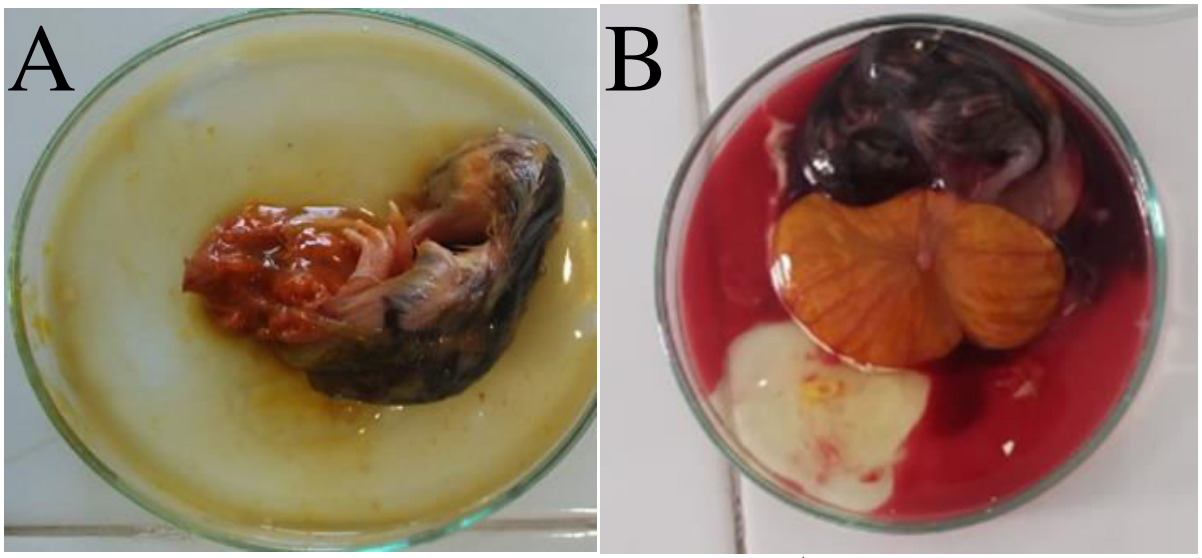


**Figure 11:A): Yolk Sac present outward B) Poor yolk Retraction (21st day development in experimental group C (manually hatched)**

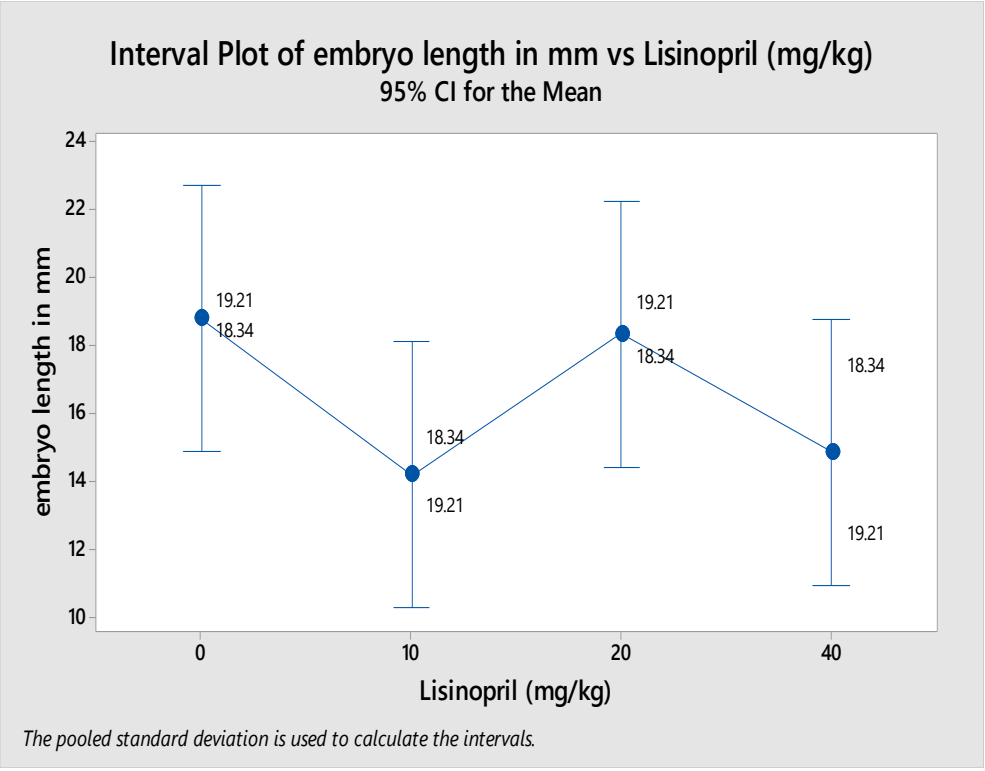


**Figure 12: One-way ANOVA analysis between dosage and Embryo length of Chicks on 21st day**

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 04, 2021

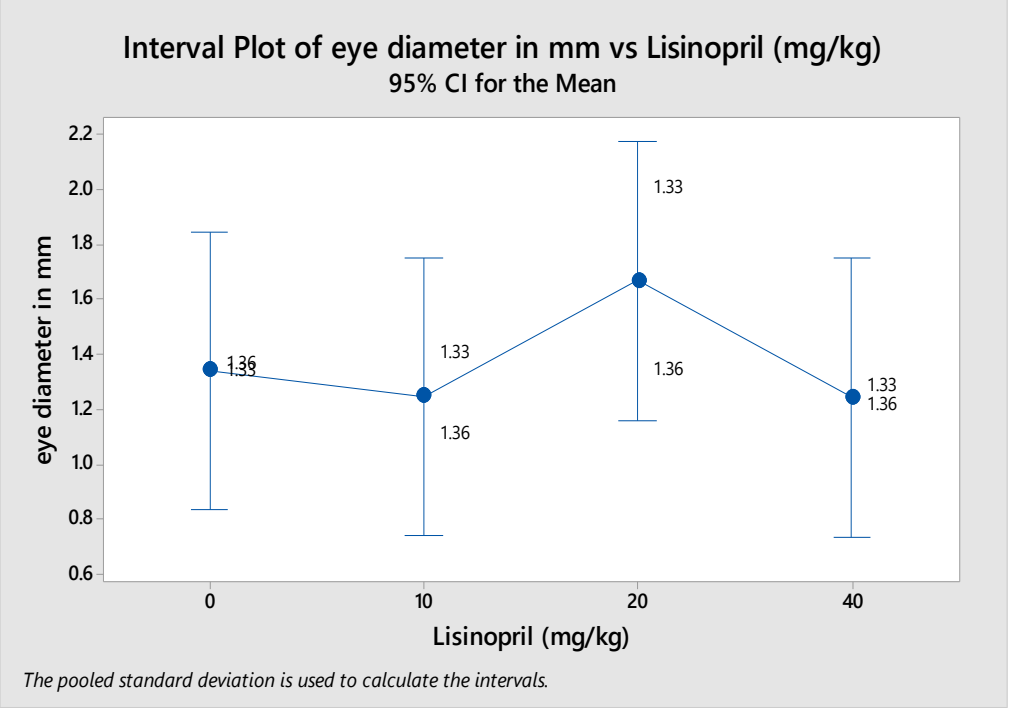


**Figure 13: One-way ANOVA analysis between dosage and Eye size of Chicks on 21st day**

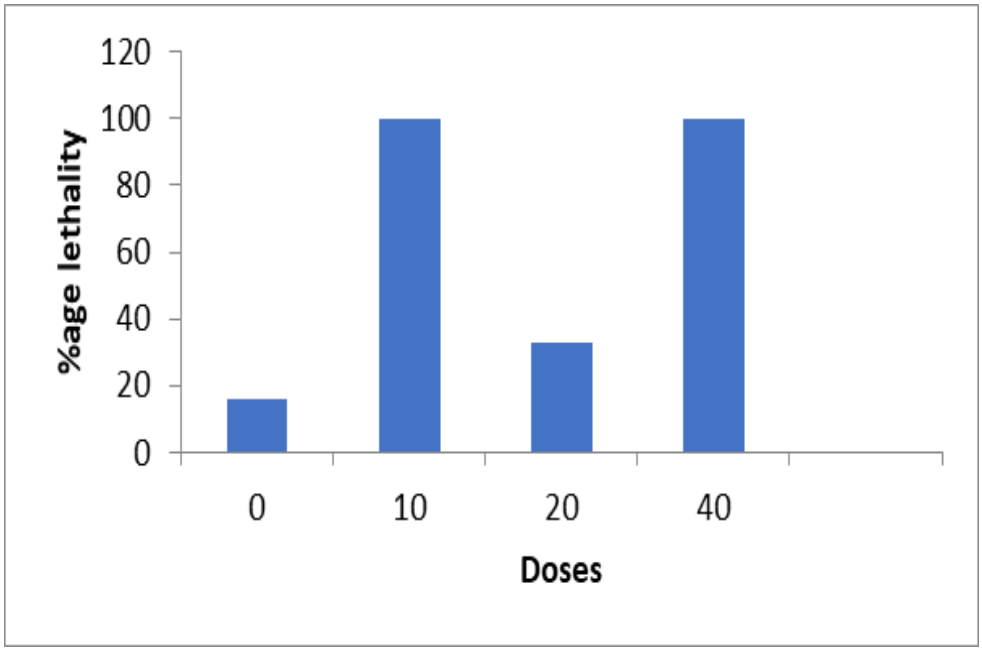


**Figure 14:Percentage lethality among different groups**