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Histopathological Changes in Kidneys of Developing Chick Embryo on Exposure to Lixisenatide

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Abstract

Background and Objectives- Chick embryo is one of the most commonly used animals to study the adverse effects of various drugs for research purpose. Currently, type 2 diabetes mellitus is treated using the medication lixisenatide. Therapeutically, is thought to be superior to other GLP1 receptor antagonists for the treatment of type 2 diabetes. To understand the adverse effects of lixisenatide on kidney of developing chick embryo.

Materials and Methods- It was an Interventional study conducted at Department of Anatomy, Santosh Medical College, Ghaziabad uttar pradesh India. It is confirmed from literature survey/ pilot study that the expected mean ± SD of parameter of control and experimental groups are 27± 5.32 with 32±5.30, with the help of software G* Power analysis for α 5% and Power (1-β) 95.36 %. The effects size is 1.961 and sample size for each group is 28. So we have taken the total sample size 280. The chick embryos were dissected after being sacrificed, and the kidneys from both side were separated and kept in a 10% formaldehyde solution. Under flowing water, the tissues were cleaned. To learn about the typical histological characteristics of the kidneys, the sections were examined using a light and compound microscope.

Results- Minute vacuolation with or without fat globules in the

cytoplasm of lining epithelium of proximal and distal convoluted tubules were observed in experimental groups C, D and E the lymphocytic infiltration was higher in cortical areas of kidneys. As the dose of Lixisenatide increases with each experimental groups the histopathological changes in kidney also shows more changes with each experimental group and these are statistical significant ($p<0.05$).

Conclusion- The medicine has some negative effects on the kidney of the chick embryo, as evidenced by the observations (vacuolation in DCT, congestion in glomeruli, lymphocytic infiltration, etc.). Therefore, if we tend to use this vital medication carelessly patient can develop renal issues and resistance.

Keywords- Lixisenatide, chick embryo, diabetes mellitus, glomerular degeneration, vacoulation

Introduction-

For ethical reasons, animal models are frequently used in research. This is because chicks have short gestation periods (21 days), their eggs are relatively large and therefore manageable, they are readily available throughout the year, they can be artificially incubated, and their embryology is generally similar to that of humans [1, 2].

Lixisenatide, which acts as a GLP-1 receptor agonist. Lixisenatide is a peptide containing 44 amino acids, which is amidated at the C-terminal amino acid (position 44). The order of the amino acids is given in the figure below. Its molecular weight is 4858.5 and its molecular formula is C215H347N61O65S. [3]

Currently, type 2 diabetes mellitus is treated using the medication lixisenatide. Compared to other GLP1 receptor antagonists, is thought to be the most effective treatment for diabetes mellitus from a therapeutic standpoint. Its mode of action is to delaying the speed of stomach emptying while inhibiting glucagon and increasing insulin secretion. In this way, it both directly affects how the stomach works and helps to reduce blood sugar levels. 20mcg are recommended daily for type 2 diabetes mellitus. [4]

As there is no any clear cut human dose recommendations in respect with age and weight as well as there is no any available data about effect of lixisenatide on kidney. It looks worthwhile to do more work on histopathological effects of lixisenatide for the human welfare.

Materials and Methods-

It was an Interventional study conducted at Department of Anatomy, Santosh Medical College, Ghaziabad uttar Pradesh India. It is confirmed from literature survey[7]/ pilot study that the expected mean ± SD of parameter of control and experimental groups are 27± 5.32 with 32±5.30, with the help of software G* Power analysis for α 5% and Power (1-β) 95.36

%. The effects size is 1.961 and sample size for each group is 28. So we have taken the total sample size 280.

Inclusion criteria - Eggs known to be nutritionally healthy. Proper calcified eggs with intact shell. Eggs having air cell at broader end without any clot.

Exclusion criteria- Eggs with cracked shell due to improper calcification**.** Eggs not having air cell at proper place**.** Eggs having blood clot on air cell.

Methodology-

In the present study fertilised eggs of white leg horn chicken (Gallas domasticus) were obtained from S. P. Hatchery and Poultry, bhaupur Saharanpur and Venkey's Hatchery, shakumbhari devi road, bhaguwala, Saharanpur Uttar Pradesh. Eggs were taken from stock known to be nutritionally healthy. Eggs to be injected first are candled in order to discard those which are defective. For this purpose a specially made wooden box was procured. This box has a connection for a bulb and was painted black from inside. The slots for the chick eggs were made in the top. Against the light interior of the eggs was scanned to look for any abnormality also through this procedure air cells within the egg were located.

Drug Administration-

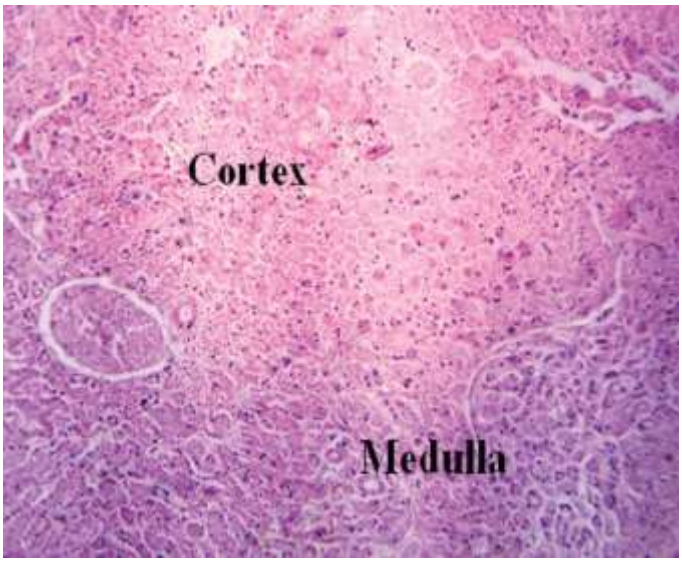
The starting dose of lixisenatide is 10 mcg subcutaneously daily for 14 days. Increase the dose to maintenance dose of 20 mcg daily from day 15. 50 mcg/ ml in 3 ml solution in a green single patient use prefilled pen (for 14 doses; 10 mcg/ dose) 100 mcg / ml in 3 ml single patient use prefilled pen (for 14 doses; 20 mcg/ dose). As 1(0.2ml) shot of 100mcg/ml of 3ml prefilled pen lixisenatide delivers 20 mcg of drug.

Five shots i.e. 1 ml of drug containing 100 mcg was added to 9 ml of distilled water. This way 10 ml of solution containing 100 mcg i.e. 10 mcg/ ml. Then solution was further diluted to obtain desired amount of concentration of drug. The weight of new born chicks was be measured and an average weight was calculated and dose to be injected was calculated as per kg of weight of egg with respect to recommended human dose.

On day 5 of incubation the different concentration of drugs solution was made at volume up to 0.5ml. Control eggs were injected with equal volume of distilled water. On day 5 of incubation the drugs was injected into the eggs. The broad ends of selected eggs were wiped with a sterile gauge pad moistened with 70% alcohol solution. After wiping with alcohol, a hole was drilled in the shell in centre of the surface over the air cell. Before hatching the eggs were broken to collect embryos for examination on 18^{th} day of incubation. The chick embryos were dissected after being sacrificed, and the kidneys from both were separated and kept in a 10% formaldehyde solution. Under flowing water, the tissues were cleaned. To learn about the typical histological characteristics of the kidneys, the sections were examined using a light and compound microscope.

On either side of the vertebral column, directly beneath the lungs, on the dorsal wall of the chicken's trunk, are its brownish-colored kidneys. The ureters, which discharge into the cloaca, are fed by the collecting tubules. The reproductive and digestive tracts both empty into the cloaca, a shared vestibule. There are three different forms of nephrons in chicken kidneys, including multilobulated, looped, and loopless nephrons. There are more loop-less (reptilian) nephrons than looped (mammalian) nephrons. While looped nephrons reach up to the medulla, loop less nephrons only exist in the cortex. Another intermediate kind of nephron was discovered in addition to the reptilian and mammalian types. These nephrons are structurally in-between those of reptiles and mammals. The Bowman's capsule and glomerulus are found in both the large and small renal corpuscles that make up the chicken kidneys. The basic cuboidal epithelium that lines the PCT, DCT, and collecting ducts is present. Simple cuboidal epithelium lines both the thick and thin portions of the loop of Henle. [5]

Figure 1- Transverse section of chick embryo showing cortex and medulla

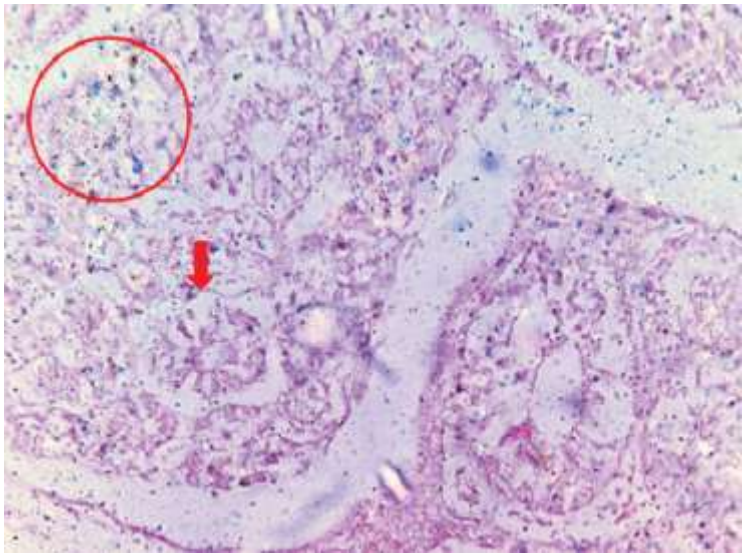


Statistical Analysis-

Data so obtained were subjected to statistical analysis. Data analysis was done by SPSS software ® version 22.0. Descriptive statistical analysis, which included frequency and percentages, was used to characterize the data. Inferential statistics included chi-square test for different dependent variables of the study and $p < 0.05$ was considered statistically significant.

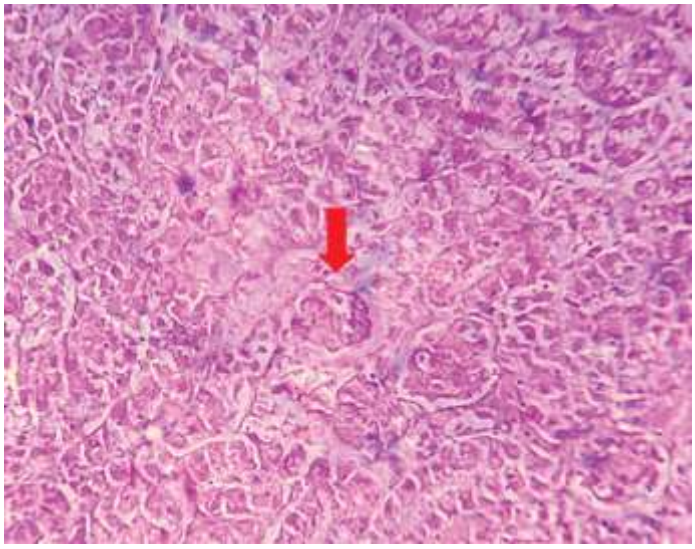
Results-

Figure 2- TS of kidney of chick embryo showing vacuolation in DCT (arrow) and mild lymphocytic infiltration (circle) in experimental group E



As per figure 2 as the dose of Lixisenatide increases to highest dose mild to moderate tubular fatty degenerative changes were found in all experimental groups except experimental groups A and B. Minute vacuolation with or without fat globules in the cytoplasm of lining epithelium of proximal and distal convoluted tubules were observed in experimental groups C, D and E the lymphocytic infiltration was higher in cortical areas of kidneys.

Figure 3- TS of kidney of chick embryo showing congestion in glomerulus in experimental group D & E



As per figure 3 Congestion in glomeruli was found in experimental group D and E. Some nephrons in experimental group D and E showed haemorrhage in urinary space.

Table 1- Summary of chick embryos found with histopathological changes in Kidney and no histopathological changes in each Control group

Name of the groups	Total number of chick embryos in a group (Group size)	Number of chick embryos found with no histopathological changes	Number of chick embryos found with histopathological changes	p-value
A^c	28	28	0	NS
B^c	28	28	0	
C^c	28	28	0	
D^c	28	28	0	
E^c	28	28	0	

As per table 1 as the dose of Lixisenatide increases with each control groups the histopathological changes shows no changes and these are not statistical significant ($p>0.05$).

Table 2- Summary of chick embryos found with histopathological changes in Kidney and no histopathological changes in each experimental group

Name of the groups	Total number of chick embryos in a group (Group size)	Number of chick embryos found with no histopathological changes	Number of chick embryos found with histopathological changes	p-value
A	28	26	2	0.01*
B	28	24	4	
C	28	23	5	
D	28	21	7	
E	28	18	10	

As per table 2 as the dose of Lixisenatide increases with each experimental groups the histopathological changes in kidney also shows more changes with each experimental group and these are statistical significant ($p<0.05$).

Discussion-

It has been demonstrated that combining lixisenatide with basal insulin is a successful treatment plan for those with type 2 diabetes, lowering HbA1c levels by reducing PPG excursions during the day. Over the course of 24 weeks, once-daily lixisenatide considerably improved glycemic control, had a noticeable postprandial effect, did not significantly raise the risk of symptomatic or severe hypoglycemia, and caused weight reduction. Lixisenatide successfully reduced the working memory impairment brought on by amyloid protein (A) 25-35, reversed A 25-35-triggered cytotoxicity on hippocampus cell cultures, and protected against A 25-35-induced inhibition of the Akt-MEK1/2 signaling pathway. [6]

Teratogens are any chemicals that are delivered when development is taking place and have the potential to interfere with embryonic development [7]. A teratogen affects the developing tissues of chick embryos and causes morphological or functional abnormalities. A teratogen's metabolites or the teratogen itself may cause the first event in teratogenesis. A teratogen can affect an organ primordium that would develop into a defective organ later on, as well as other embryonic tissues, maternal tissue, and the placenta [8].

The symptoms seen after exposing a chicken embryo to various teratogens were comparable to those discovered in the current investigation in tests carried out by other researchers including acute kidney failure and hemoglobinuria [8], glomerulus degeneration [9], and lymphoid infiltration in the kidney of a chick embryo [10].

Additional immunohistochemistry research can be done to gain a deeper understanding of the symptoms seen. For a greater knowledge of the potential of lixisenatide, additional research can be done, such as looking at how it affects other organs in chicken embryos. Our study has few limitations as the study was performed on chick embryo animal model. Although the embryology of chick embryos is somewhat similar to mammal including humans, it only gives the idea regarding the toxicity of the drug. Therefore, the study must be performed in mammal research models for better understanding of effects of Lixisenatide in humans.

Conclusion-

There is little research on lixisenatide is quickly acquiring resistance to it. Currently, lixisenatide is the medicine of choice for treating type 2 diabetes mellitus. The medicine has some negative effects on the kidney of the chick embryo, as evidenced by the observations (vacuolation in DCT, congestion in glomeruli, lymphocytic infiltration, etc.). Therefore, if we tend to use this vital medication carelessly patient can develop renal issues and resistance. As a result, the medication must be administered appropriately and only as needed.

Conflict of Interest- None declared

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