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LIXISENATIDE INDUCED CONGENITAL ANATOMICAL MALFORMATION AND HISTOPATHOLOGICAL CHANGES IN DEVELOPING CHICK EMBRYO

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Abstract

Background- At present Lixisenatide is the drug been used to treat type 2 diabetes mellitus. Therapeutically is been considered to be best for treatment of diabetes mellitus than other GLP1 receptor antagonists

Objectives- To find out gross anatomical malformations due to teratogenic effects of antidiabetic drug Lixisenatide on developing chick.

Methods- It was an Interventional study conducted at Department of Anatomy, Santosh Medical College, Ghaziabad. 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.

Results- Gross anatomical malformation was seen after administration of lixisenatide as compared to control group. The most common anatomical malformation was growth retardation followed by limbs deformity and twisted neck and it was statistically significant ($p<0.05$) significant decrease in the weights of treated chick embryo compared to the control groups ($p<0.05$)

Conclusion- The data generated in this project shows teratogenic effects like anatomical malformations and histopathological changes due to effects of high dose of Lixisenatide on developing chick embryo in liver organ and these teratogenic effects may be extrapolated to human being.

Keywords- Lixisenatide, chick embryo, diabetes mellitus, growth retardation.

Introduction-

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 $C_{215}H_{347}N_{61}O_{65}S$.[1] The only method of attaining a comprehensive understanding of embryological processes is through the study and comparison of

development in various animals and the chick is one of the most satisfactory animal on which embryological laboratory work may be based. The chick serves as an intermediate form bridging the gap between the simpler processes of development in fishes and amphibians and the more complex processes in mammals. The traditional strength of chicken embryos for studying development is that they are easily manipulated. This character of the chicken has led to some major discoveries in embryological science.[2]

It is preferable to study embryology of chick or common fowl (Gallus gallus) because of several advantages. Eggs of chick are large in size, available through the year, can be incubated artificially and are easy to control. More over the process of development has been most thoroughly worked out in fowl.[3,4] A comparative study of embryology of different birds shows that it is essentially in all the birds with only minor unimportant differences. Chicken embryology is much like that of human in general. Development is direct without a larval stage.[5]

At present Lixisenatide is the drug been used to treat type 2 diabetes mellitus. Therapeutically is been considered to be best for treatment of diabetes mellitus than other GLP1 receptor antagonists.[6] Its mode of action is to increase insulin secretion while inhibits glucagon and at the same time it slower down rate of gastric emptying. This way it is directly involved in functioning of stomach as well as it is lowering down the glucose level. As it is advised 20 µg daily in type 2 diabetes mellitus. 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 liver, kidney, stomach and cerebral cortex. It looks worthwhile to do more work on teratogenic effects of lixisenatide for the human welfare.

Materials and Methods-

It was an Interventional study conducted at Department of Anatomy, Santosh Medical College, Ghaziabad. 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 was 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. The toxicity of the drugs was estimated on the basis of hatchability and development of chicks.

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 center of the surface over the air cell. Before hatching the eggs were broken to collect embryos for examination on 18^{th} day of incubation. Gross anatomical malformations was observed with necked eye, magnifying glass, Vernier caliper and radiograph and recorded in all embryos before preserving them in the fixatives. The embryos were preserved in 10% formalin solution after recording their external anomalies. The embryo was dissected and the sections of different organ of the embryos were stained with hematoxylin & eosin and was be studied with light and compound microscope.

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 and independent samples t test for different dependent variables of the study and $p < 0.05$ was considered statistically significant

Results-

Table 1- Gross Anatomical Malformations

S.No.	Abnormalities	Groups									
		A^c	A	B^c	B	C^c	C	D^c	D	E^c	E
1	Growth Retardation	0	3	0	7	1	12	4	9	4	17
2	Macrocephaly (Enlargement of head)	0	0	0	0	0	2	0	3	0	3

3	Microcephaly (Small size of head)	0	0	0	0	0	3	7	4	4	8
4	Exopthalmia (Bulging eye)	0	0	0	0	0	0	0	0	0	2
5	Micropthalmia (Small eye)	0	0	0	0	0	0	0	0	0	0
6	Narrow neck	0	0	0	0	0	0	0	0	0	2
7	Twisted neck	0	2	1	7	4	9	3	7	6	12
8	Subcutaneous Hemorrhage (Hematomas)	0	2	0	4	0	3	0	2	1	4
9	Limbs deformity	0	2	1	7	2	11	1	12	4	11

As per table 1 gross anatomical malformation were seen after administration of lixisenatide as compared to control group. The most common anatomical malformation was growth retardation followed by limbs deformity and twisted neck and it was statistically significant ($p<0.05$).

Table 2- Summary of chick embryos found with histopathological changes 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	19	9	

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

Figure 1-Histological Section of Liver in Chick Embryo

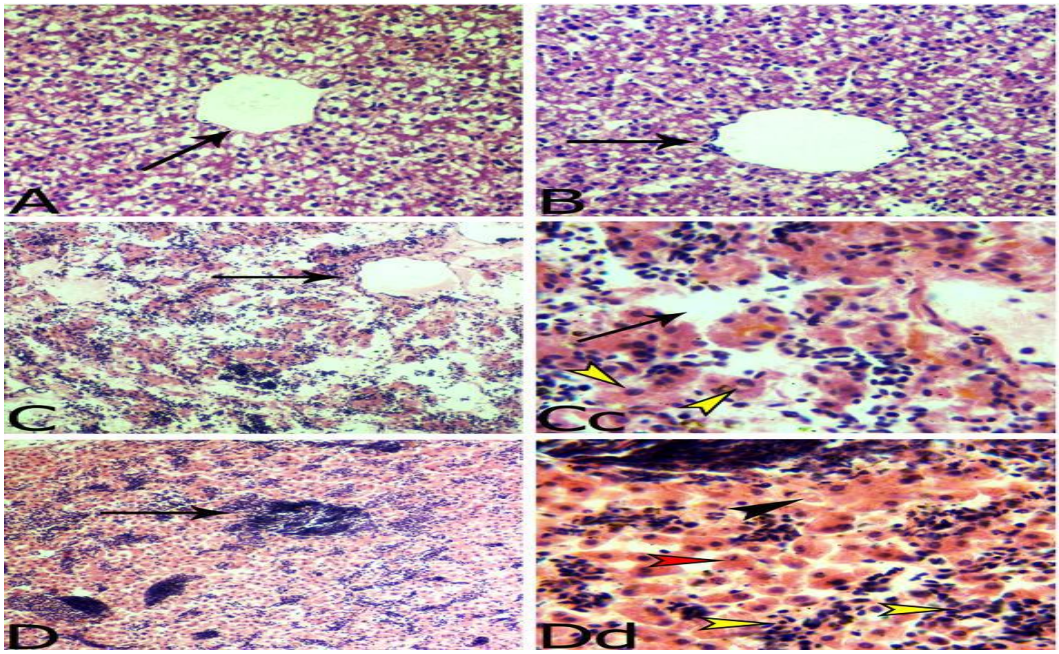
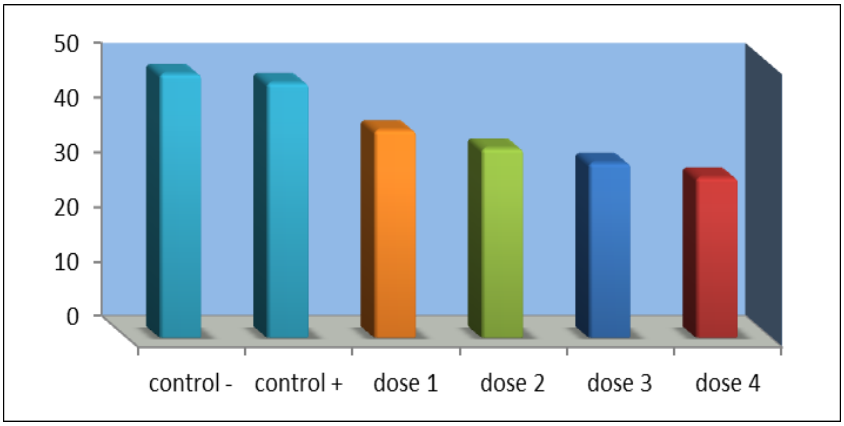


Figure 1 showing histological sections in the liver of chick embryos on 21-22 day of incubation. (A and B) sections of control groups B, injected with distilled water. The control groups show normal structure of the hepatocytes around the central vein (black arrow). (C and Cc) sections magnification (10X and 40X respectively) in the liver treated with Lixisenatide on ED 21 show dilatation in sinusoids with bleeding (Black arrow) and vacuolization (yellow head arrow). (D and Dd) sections magnification (10X and 40X respectively) in the liver treated with higher dose of lixisenatide on ED 21 observe congestion of central vein with infiltration of inflammatory cells (black arrow). It can be also seen dilatation in blood sinusoid with necrosis (red head arrow) vacuolization (black head arrow) and infiltration of inflammatory cells (yellow head arrow). (Section stained by Haematoxylin and Eosin).

Figure 2- Effect on Weight due to Lixisenatide



As per figure 2 Demonstrates a significant decrease in the weights of treated chick embryo compared to the control groups ($p<0.05$)

Discussion-

Lixisenatide in combination with basal insulin was shown to be an effective treatment strategy for patients with type 2 diabetes, controlling HbA1c levels by reduction of PPG excursions during the whole day. Once-daily lixisenatide significantly improved glycemic control, with a pronounced postprandial effect, without significant increase in symptomatic/severe hypoglycemia risk and with weight loss over 24 weeks. lixisenatide effectively alleviated amyloid β protein (A β) 25-35-induced working memory impairment, reversed A β 25-35-triggered cytotoxicity on hippocampal cell cultures, and prevented against A β 25-35-induced suppression of the Akt-MEK1/2 signaling pathway.[8] Lixisenatide also reduced the A β 25-35 acute application induced intracellular calcium overload, which was abolished by U0126, a specific MEK1/2 inhibitor. Results further confirmed the neuroprotective and cytoprotective action of lixisenatide against A β -induced impairments, suggesting that the protective effects of lixisenatide may involve the activation of the Akt-MEK1/2 signaling pathway and the regulation of intracellular calcium homeostasis.[9]

The liver is an effective organ in removing the toxic effect of many substances and particles that enter the body, as well as removing vital detoxification and xenobiotic of organs.[10] Exposure to lixisenatide affects the liver function and activities enzyme Alanine

aminotransferase (ALT), Aspartate amino -transferase (AST) which leads to liver tissue damage. A high level of ALT and AST enzyme in serum or plasma causes hepatotoxicity and leakage of lysosome enzyme. The elevation in the level of ALT, AST is associated with histopathological lesions like degeneration.[11,12]

Other studies investigated that lixisenatide probably delay the development, failure of retraction yolk sac with bleeding and hematomas. These results are in agreement with findings of the current work during treatment with Lixisenatide. From a review of previous literature, it still unknown the effect of lixisenatide on Ach E activity in the brain of chick embryo during the development. The current study, therefore, investigated that Lixisenatide causes significant decrease in Acetyl cholinesterase activity at 74- 97 % in the brain of chick embryo. Study showed low activity of Ach E in rabbit's brain to 92% when they treated with Malathion insecticide.[13]

A study observed a decrease in the percentage inhibition of Ach E to 40.6% and 69% in brain tissue of chick embryo Gallus gallus treated with mixed anti-diabetic mediations at dose 4, 8 ppm, respectively these interfere with physiological chemical processes and leading to morphological changes.[14,15]

Conclusion-

The data generated in this project shows teratogenic effects like anatomical malformations and histopathological changes due to effects of high dose of Lixisenatide on developing chick embryo in liver organ and these teratogenic effects may be extrapolated to human being. The results of the study may help the clinician to develop more novel therapeutic strategies for the management of diabetes mellitus worldwide.

Conflict of Interest- None declared

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