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RESEARCH ARTICLE

PHYSIOLOGICAL AND HISTOLOGICAL STUDY OF EXPERIMENTAL DIABETES MELLITUS BY ALLOXAN.

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Abstract

Study included using exogenous alloxan (100 mg for every 1 kg of animal weight) for inducing experimental diabetes mellitus in rats. 46 rats divided for two groups, during the time of study glucose, glycogen levels estimated every week extend for 3 weeks. Also the study included histo-pathological effect on liver and pancreas. The experiment showed an increase in blood glucose, after 7 days, 14 days, 21 days of alloxan injection, values were (11,92), (15,89), (17,31) mmol/Lrespectively, glucose level of control group was (5.84 - 7.44 mmol/L). while glycogen level in liverat the same period was (0,72), (018), (0.075) mg/g. Control group glycogen was (3,18 – 3.65mg/g) during 3 weeks of experiment. Histological examination of liver samples of ratswere traced pronounced signs of toxic hepatitis as a violation of the beam structure of the lobules, necrosis of hepatocytes, fat and protein dystrophy, the presence of infiltration of hematogenous cell clusters.

In the control group was characterized by a uniform distribution with respect to the same hepatocytes for liver slices, hepatocytes and the structure of the test cells corresponded to the ratio of the classical histological characteristics of the active functioning of the liver. The number of β-cells in the islets of sharply reduced, in most of them marked vacuolization of the cytoplasm, reducing the size of the nuclei, chromatin condensation, in some cells - kariopik¬noz. Presence of a lymphocytic infiltrate along the periphery of the islets, interlobular connective tissue edema, congestion of the capillaries; vascular stasis traced.

Introduction:-

Diabetes mellitus has been considered as one of the major health concerns all around the world today (Stolar, et.al., 2008; Kruger, et.al.,2012) Experimental animal models are one of the best strategies for the understanding of pathophysiology of any disease in order to design and develop the drugs for its treatment (Rees DA and Alcolado JC, 2005 ; Chatzigeorgiou et.al., 2009) .Numerous animal model have been developed for the past few decades for studying diabetes mellitus and testing anti-diabetic agents that include chemical, surgical and genetic manipulations(Srinivasan K. and Ramarao P, 2007 ; Etuk EU, 2010). Alloxan-induced diabetes has been comm. Only employed as an experimental model of insulin dependent diabetes mellitus. The mechanism of alloxan action has been thoroughly studied which currently can be characterized quite well. Several experimental studies have demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment(Szkudelski et.al.,1998 ; Lachin et.al., 2012).

Diabetes, it is generally assumed that liver glycogen is low in this condition. However, a review of the available clinical and experimental data reveals that low hepatic glycogen levels in diabetes are the exception rather than the

rule. Whereas liver glycogen levels may be somewhat low in the diabetic when compare with normal, fed animals, they are appreciably higher when compared in the fasted condition (Shanmugasundaram et.al., 1983 ;).

Regulation of glycogen deposition and degradation during the prolonged fasting periods in rats with diabetes1 differs markedly from that of rats with diabetes2 which, in turn, behave similarly to normal healthy rats. There is little information available about the mechanisms underlying these differences, but data available about the influence of glutamine on glycogen syntheses activation during prolonged starvation (Mouterde et al., 1992; Winternitz et.al.,1956)

Methods:-

The objects of study were 46 males laboratory rats (Rattusrattus L.) weighting 200-250 g., 11 months age. Grown in a vivarium with a standard diet. Animals divided into two groups, 23 rats for each group, first group induction of diabetes mellitus by injection intraperitoneally with a 5% solution of alloxan, dose was 100mg per one kg of animal body weight in 0.9% normal saline solution. Second group consider as control, rats injected with normal saline. Animals were grown under normal feeding regime and housing conditions. Glucose and glycogen level estimated in 4 different times (0 day of injected alloxan, then after 7, 14, 21 days) Study including the following objectives:

1. 1-Determination of glucose in blood. Determination of glucose produces Portable blood glucose test device.
2. Determination of glycogen**.** The glycogen content was determined by "direct" method [Seifter, 1950]. 3 rats were killed every weeks to calculated glycogen level in liver .
3. Preparation of material for histo pathological study**.** Organ included liver and pancreas. After cranio-cervical dislocation under ether anesthesia, liver and pancreas tissues taken from experimental animals were fixed in 10% neutral formalin, alcohol dehydrated, paraffin-embedded and the section to mean thickness of 4 μm. The histological examination was evaluated by assessing the morphological changes with Hematoxylin and Eosin (H&E) stains (Humason, 1967).

Results:-

Dynamics of glucose and glycogen in the blood of rats in normal and diabetes. Alloxan injection the test animals to an increase in blood glucose concentration to 15.8 mmol / l after 10 days after injection. Carrying out these analyzes in dynamics for 21 days revealed certain patterns. The maximum value of this parameter in the blood of experimental rats was 17.31 mmol / l in comparison with control samples. High levels of glucose was typical for two weeks, with a sharp increase in this indicator was observed from the seventh day of the experiment. At the same time the quantitative analyzes were performed on the glycogen content in the liver of control and experimental animals. Analysis of the data obtained (Table. 1) indicates that during a three-week experiment, the concentration of this major reserve polysaccharide ranged from 2.98 to 3.56 mg/ g in the control animals. Dynamics of glycogen in the experimental samples was of a different nature. The amount of the substance during the experiment was reduced, and on day 21 was 0.075 mg /g. Consequently, in experimental diabetes mellitus, mobilization of endogenous sugars, which are represented in the rat liver glycogen

Table 1: Blood glucose level and glycogen in the liver of normal rats and in experimental diabetes (n = 5, p <0.05)

Day	Option	Glucose mmol / l	Glycogen / mg/g of liver
0	**control group animals**	**4,71 ± 0,09**	**2,98 ± 0,12**
	diabetic animals	**4,66 ± 0,1**	**3,11 ± 0,09**
	The control group animals	**5,84 ± 0,12**	**3,18 ± 0,14**
7	**diabetic animals**	**11,92 ± 0,13**	**0,72 ± 0,8**
	The control group animals	**7,68 ± 0,11**	**3,44 ± 0,11**
14	**diabetic animals**	**15,89 ± 0,14**	**0,18 ± 0,03**
	The control group animals	**7,44 ± 0,11**	**3,56 ± 0,15**
21	**diabetic animals**	**17,31 ± 0,14**	**0,075 ± 0,004**

Histology of liver and pancreas in rats AT alloxan diabetes:-

Morphological and histological features of the organization of the rat liver during alloxan diabetes. The liver of animals Contact Group of dark red color, smooth, elastic consistency, with shares of the correct form, visceral sharp edges. In histopathological examination of liver slices polygonal shape, well structured, located in the center, clearly visible nucleoli, size and number of which varies greatly. Cytoplasmic homogeneous, with a high content of RNA as basophilic granules. On the periphery of liver lobules are often met with giant cell nuclei. In the border region

segments are distinctively interlobular excretory bile ducts, which, together with the ramifications of the portal vein and the hepatic artery form a triad between the hepatic lobules (Fig 1).

A B

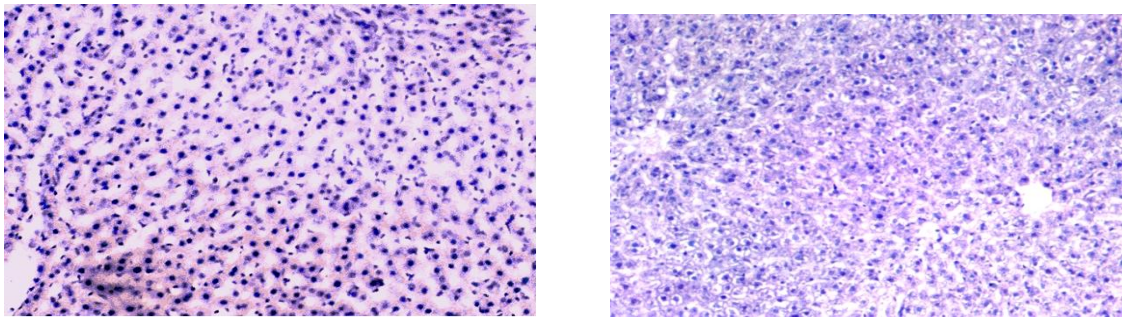

Fig. 1: A fragment of liver lobules animal contact group (A) and with alloxan diabetes (B). H and E. x10.

Analysis of the data of sociological studies of rat liver samples of experimental groups testified that the use of alloxan caused induction of diabetes, appears to change the morphological features of tissues examined organ. These changes were characterized by significant deviations from the control sample in the composition and physical characteristics, as well as size. In general, in experimental alloxan diabetes in liver parenchyma test animals were traced pronounced signs of toxic hepatitis as a violation of the beam structure of the lobules, necrosis of hepatocytes, fat and protein dystrophy, the presence of infiltration of hematogenous cell clusters.

Morphological and histological features of the organization of the pancreas in alloxan diabetic rats:-

Morphological changes in pancreatic tissue of rats after the administration of alloxan characterized most pronounced degenerative changes in the central regions of the islets of Langerhans (Fig. 2). The number and size of islets umen¬sheny shape them wrong. The number of β-cells in the islets of sharply reduced, in most of them marked vacuolization of the cytoplasm, reducing the size of the nuclei, chromatin condensation, in some cells - kariopik¬noz. Presence of a lymphocytic infiltrate along the periphery of the islets, interlobular connective tissue edema, congestion of the capillaries; vascular stasis traced.

Thus, cytotoxic effects of alloxan and insulin deficiency caused pathological changes in the insular part of the pancreas, bearing destructive character, and toxic effects are most exposed to β-cells and components of the microvasculature.

A B

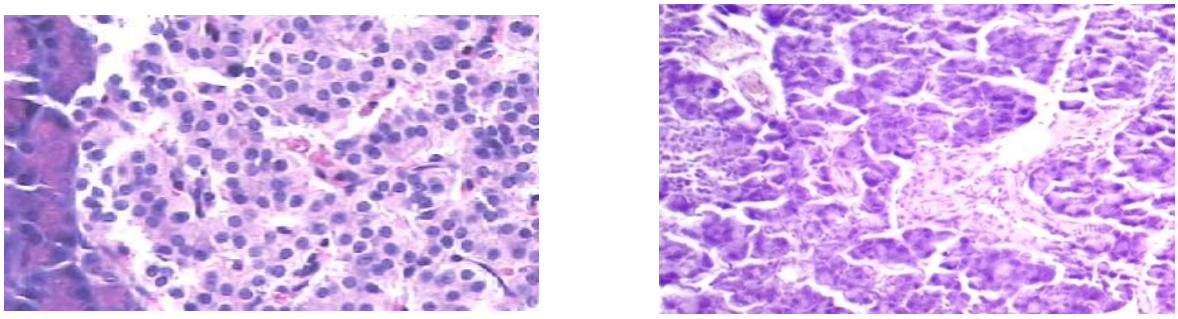

Fig. 2: islet apparatus of pancreas rat contact group (A) and after the administration of alloxan (B). H and E. x20.

Discussion:-

Alloxan, as a thiol reagent, selectively inhibits glucose-induced insulin secretion through its ability to inhibit β-cell glucose sensor, glucokinase, an essential rate-limiting step glucose metabolic enzyme (Lenzen, 2008). The alloxan treated animal sex hibited a decrease in hepatic glycogen content which may be due to enhancements in the glucose-6-phosphatase activity and deactivation/inhibition of glucokinase activity (Shirwaikar et. al., 2004).

Alloxan is the next most commonly used chemical for induction of diabetes mellitus, it is a well- known diabetogenic agent widely used to induce Type 2 diabetes in animals (Viana et al., 2004). Alloxan is a ureaderivative which causes selective necrosis of the pancreatic islet β-cells, It is used to produce experimental diabetes in animals such as rabbits ,rats, mice and dogs, with this agent it is possible to produce different grades of severity of the disease by varying the dose of alloxan used classified by measuring fasting blood sugar (FBSe. g. in rabbits moderate diabetes has been defined as an FBS level of 180 – 250 mg/dl, and severe diabetes as an FBS level of above 250mg/dl(Huralikuppi, 1991).Thus alloxan induced diabetes mellitus served as a pathological bio model for testing a substance withsupposed antioxidant activities in vivo (Bartosikova et.al., 2003). One of the targets of the reactive oxygen species is DNA of pancreatic islets, Its fragmentation takes place in beta cells exposed to alloxan (Takas et. al., 1991).

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted(Kruger, et.al.,2012) .Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas (Prince and Menon 2000 ; Jelodar et.al., 2003). Alloxan causes a massive reduction in insulin release by the destruction of b-cells of the islets of langerhans,thereby inducing hyperglycaemia (Grover et.al., 2000) Insulin deficiency leads to various metabolic alterations in the animals vizin creased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases (Shanmugasundaram et.al., 1983 ; Begum and Shanmugasudnaram, 1978)

previous study it has been established that alloxanrat developed atrophy of pancreatic islets and pyknosis of islets cells (Guria et.al., 2012). In this study treatment with alloxan caused central vein congestion of liver with significant dilatation of sinusoidal spaces, pyknosis of nuclei of hepatocytes. In alloxan treated rat increments of blood glucose levels were observed after GTT and the hyperglycemia persisted even 24 h after glucose load. Moreover, in contrast to unusually high level of glucose, the hepatic glycogen content in alloxan-induced diabetic rat was not increased compared to control rat. The lack of increase in glycogen levelsmay be to decreased glucokinase activity in the liver and altered cytomorphology of liver parenchymatous tissues (Bollen et.al., 1998). All a previous studies indicated increase in the level of blood glucose and decrease of liver glycogen, with toxic effect of alloxan for hepatocytes and pancreatic cells which corresponding with our results.

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