Research Article

Effect of leptin on α-cell growth and function in mouse model of streptozocin-induced type I diabetes mellitus

Sameh S. Akkila,[1] Samia A. Eleiwi[2]

[1]MSc Anatomy, Al-Mustansiriyah University, College of Medicine; Department of Anatomy, Histology, and Embryology, Baghdad, Iraq.
[2]Assistant Professor, PhD Anatomy, Al-Mustansiriyah University, College of Medicine, Department of Anatomy, Histology, and Embryology, Baghdad, Iraq.

Iraqi New Medical Journal, 2014;1(1): P 36-40

Abstract

Background: Leptin mainly acts as a satiety signal in the hypothalamus; but it also affects pancreatic function via the adipoinsular axis of hormonal interactions. In type 1 diabetes mellitus, this axis is severely suppressed.

Objectives: to examine the potential benefits of using leptin with and without insulin in treating animal models of type 1 diabetes.

Methods: A laboratory experimental study was conducted at the postgraduate laboratory of the department of anatomy at Al-Mustansiryah University/College of medicine (Baghdad-Iraq) over a period of four weeks in October 2013. Thirty male mice were used. Mice were selected due to the practicability of using low doses of the drugs, only males were used to avoid female cyclic hormonal changes that may affect the results. Type 1 diabetes was induced by a high dose streptozocin injection and the animals were divided into three groups. Group A was treated with insulin and leptin, group B with leptin alone and group C was given placebo saline. Random blood sugar levels were recorded twice weekly and insulin tolerance test was performed before animals were sacrificed and pancreata harvested for histomorphometric analysis. Tissue sections were stained with H&E and modified Gomori's aldehyde fuchsin.

Results: Levels of blood sugar returned to normal in group A and moderately decreased in group B, but remained greatly increased in group C. The insulin sensitivity was greatly enhanced in groups A & B. All pancreata showed total destruction of β-cells with α-cell hyperplasia that was less in leptin treated animals. Islet sizes were significantly greater in group C and smallest in group A.

Conclusion: Leptin prevents α-cell hyperplasia and enhances exogenous insulin action. Leptin monotherapy moderately ameliorates hyperglycemia, but combined with insulin therapy can restore euglycemia.

Key words: Leptin, Insulin, Streptozocin, Diabetes mellitus, Pancreas, α-cells, Random blood sugar, Glucose tolerance test.

INTRODUCTION

The pathogenesis of type 1 diabetes mellitus (DM) involves pancreatic β-cell destruction leading to severe insulin deficiency, a defect that causes hyperglycemia, dyslipidemia, hyperglucagonemia, cachexia and other deadly abnormalities if not treated.[(1)] The disease changed with the discovery of insulin in the early 1920's, from being a fatal condition to a manageable disorder. However, patients with type 1 DM live a life of burdensome daily insulin injections that cannot duplicate the metabolic homeostasis achieved with endogenous insulin.[(2)]

The adipo-cytokine leptin is released from adipose tissue and its plasma concentration is proportional to body fat stores. It mainly acts as a satiety signal in the hypothalamus but it also has functional receptors on pancreatic α- and β-cells.[(3)] This observation led to the concept of an interrelated endocrine insulin-leptin feedback system termed the "adipoinsular axis". In this concept, insulin stimulates adipogenesis and leptin production in adipocytes whereas leptin inhibits the production of insulin in pancreatic β-cells. However, leptin enhances the peripheral sensitivity to endogenous insulin.[(4)]

In untreated type 1 DM, the adipoinsular axis is severely suppressed at both ends. The deficiency of insulin and dyslipidemic state negatively affect leptin release from adipose tissue, an effect that is exaggerated by the loss of fat mass characteristic of type 1 DM. This inhibitory effect of insulin deficiency on leptin release appears to out-weigh the stimulatory effect of hyperglycemia resulting in low levels of serum leptin. Studies of humans with untreated type 1 DM consistently showed that plasma leptin concentrations and leptin mRNA levels are reduced.[(5)] This suppression of the adipoinsular axis is supported by the fact that leptin levels rise with insulin treatment of type 1 DM, especially in the presence of poor glycemic control.[(6)]

Several mechanisms may underlie leptin potential actions in

Corresponding Author: Sameh S. Akkila, Al-Mustansiriyah University, College of Medicine, Department of Anatomy, Histology & Embryology, Baghdad, Iraq. E mail: samakkila@gmail.com. Mobile: 00 964 7901 213 016

insulinopenic and insulin treated animals with type 1 DM. Leptin levels are expected to be lower than normal in insulin-deficient states because leptin release is dependent on insulin-induced uptake of glucose by adipocytes.[7] Leptin deficiency may cause upregulation of its central receptors, rendering the animals quite sensitive to exogenous leptin.[8] Moreover, leptin and insulin share similar intracellular signaling pathways in the hypothalamus. Classically, leptin stimulates the Janus kinase pathway but it can also utilise the phoshphatidylinositol 3-kinase pathway which is activated by the insulin receptor substrates upon insulin binding.[9]

The current study was designed to examine the potential therapeutic effects of leptin in type 1 DM with and without concomitant insulin treatment.

METHODS

The study was performed at the postgraduate lab of the department of anatomy at Al-Mustansiriyah University-College of Medicine for four weeks in October 2013. Thirty male Albino mice aged 6-7 weeks with an average weight of 22.3 grams were used in the study. They were housed under controlled laboratory conditions and were provided with commercially available mouse regular pellet chow and water ad libitum. The animals were left to adapt to lab conditions for one week.

Random blood sugar (RBS) was recorded at the end of the first week of the study as a baseline measurement and twice weekly thereafter (on the 2nd and 5th days of each week). ACCU-CHEK® Compact Plus glucometer (Switzerland) with 1.5 µl sample size strips were used. After restraining the mouse in a plastic container, the lancing device was used to prick the lateral tail vein and the tail was milked for a drop of blood to be tested.

On the second week, the animals were treated with a high dose (150 mg/kg) intraperitoneal streptozocin (STZ) injection to induce type 1 DM according to the Animal Models of Diabetic Complications Consortium Protocols.[10] Moderate to severe hyperglycemia (450-600 mg/dl) developed within 3-5 days of STZ treatment. The animals were then divided into three groups: group A received daily injections of mixtard insulin and leptin for two weeks, Group B received only leptin treatment for two weeks and group C were treated with placebo (normal saline) injections.

Mixtard insulin (Novo Nordisk, Denmark) was given at a dose of 1-1.5 units per mouse after dilution in water for injection.[11] The total animal insulin requirement was given subcutaneously in two divided doses at 8:00 am (2/3 dose) and 6:00 pm (1/3 dose). Recombinant mouse leptin (Sigma-Aldrich, USA), was used in the study. The drug was supplied as a one mg lyophilized powder vial with a reconstitution fluid. The working solution was prepared according to the manufacturer instructions. A single physiological daily intraperitoneal dose of 4 µg/mouse was used.[12]

On the day before euthanasia, animals were subjected to an insulin tolerance test (ITT) according to the Mouse Metabolic Phenotyping Centers Protocols, University of California.[13] A stock solution (100 U/ml) of Actrapid insulin (Novo Nordisk, Denmark) was diluted with saline to a concentration of 0.5 U/mL (1/200 dilution). Mice were fasted for 4 hours before the test. A baseline glucose level was recorded for each animal. An intraperitoneal injection of insulin (0.75 U/kg) was then given to each mouse. Blood glucose was re-measured at 30, 60, 90 and 120 minutes after injection. A blood glucose level <40 mg/dl is considered hypoglycemic in mice.

Animals were euthanized by opening the abdominothoracic cavity and draining blood under anesthesia with chloroform. Each animal was then fixed by formalin using transcardial perfusion. The pancreas was identified by pushing the stomach upwards to reveal the spleen which was then held by a forceps with a little traction. The pancreas extended from the spleen to the duodenum along the greater curvature of the stomach. It was carefully dissected away from these organs and isolated.

The pancreas was fixed in 10% neutral buffered formaldehyde solution and prepared for paraffin sectioning according to Bancroft and Stevens.[14] Pancreatic sections of 5µm thickness taken at a regular interval of 60 µm. Sections were stained with H and E to assess changes in general morphology and with modified Gomori's aldehyde fuchsin stain (direct method) to histochemically visualize the pancreatic islets cells. The adapted protocol was used according to Woods and Ellis.[15] With this method, nuclei are stained blue/black, β-cells blue/purple, α-cells yellow/orange, δ-cells and collagen fibers green. Exocrine cells appear yellowish brown.

Ten sections from the pancreas were examined for each animal. Digital images were acquired using Micros microscope (Austria) with built in Micros Camera using the provided NMS Software (v 1.5.3.291) running on Microsoft Windows CE (v 5.00, build 1400). Images were properly scaled using Adobe Photoshop CC14 (Adobe Systems, USA, v14.1) analysis command menu options and scales were loaded into Image J software (version 1.48, National Institute of Health, USA, 2014). Scaling was done using digital images of a stage micrometer. For each animal 10 islets from each section were randomly selected for calculations. The cross sectional surface area of each pancreatic islet was calculated using Adobe Photoshop CC14 analysis tool. Islets that were less than 300 µm2 (<5 cells) were excluded from analysis to distinguish islets from scattered endocrine cells in the parenchyma and to avoid biased calculation of islets that were sectioned at their ends.

Inter-group data comparisons were performed by student t-test using PASW Statistics Software (version 18.0.0, 2009). Data were presented as measures of mean ± standard deviation, at 95% confidence interval.

RESULTS

All animals had an average normal baseline RBS of less than 130 mg/dl until the time of DM induction, after which all animals developed moderate to severe hyperglycemia of more than 420 mg/dl. During the treatment weeks, animals treated with leptin and insulin (group A) showed a marked and continuous decline in RBS to below 250 mg/dl. Animals treated with leptin only (group B) showed a slower decline to a plateau near the 300mg/dl. In group C animals (placebo control), the hyperglycemia became so severe and reached levels of more than 580 mg/dl. As shown in **figure 1**, the difference in RBS at the end of the study was highly significant ($P<0.01$) for all groups.

The ITT curves differed in their amplitudes among the three groups **(figure 2)**. In groups A and B there was a smooth decline towards a short period of hypoglycemia at 90 minutes.

This was followed by a terminal rise in RBS to levels <120 mg/dl. The initial drop was greater in group B than in A. In group C, RBS dropped acutely during the first hour of the test but the drop nearly plateaued over the second hour. The curve of this group did not reach hypoglycemic levels.

The pancreata of all groups showed marked disarrangement of islet structure and exocrine tissue as well. The changes were most pronounced in group C sections where there was a heterogeneous picture of islet changes. In some sections, islets were markedly diminuted but other islets were irregularly enlarged in other sections. Diminuted islets were irregular in shape with scarcity of endocrine cells and clear infiltration with inflammatory cells. There was no clear demarcating layer between the endocrine and exocrine components **(figure 5)**. On modified Gomori's aldehyde fuchsin sections, all islets were exclusively positive for α-cells and no β-cells were recognized. Groups A and B **(figures 3, 4)** also showed many disrupted islets with no clear surrounding sheaths but the sizes were smaller and the shapes were more regular than in group C. Inflammatory cells could still be seen but to a lesser degree among both endocrine and exocrine parts. In all islets, there were α-cells dominance with total destruction of β-cells. On H and E sections, the exocrine tissue close to the islets showed areas of increased acidophilia **(figure 4)**.

There was a highly significant difference in islet surface area among the three groups of animals with type 1 DM **(figure 6)**. Group A pancreata showed the greatest degree of islet shrinkage, while group B pancreata showed relatively larger, more uniform and α-cell-packed islets. The largest islets were seen in group C pancreata.

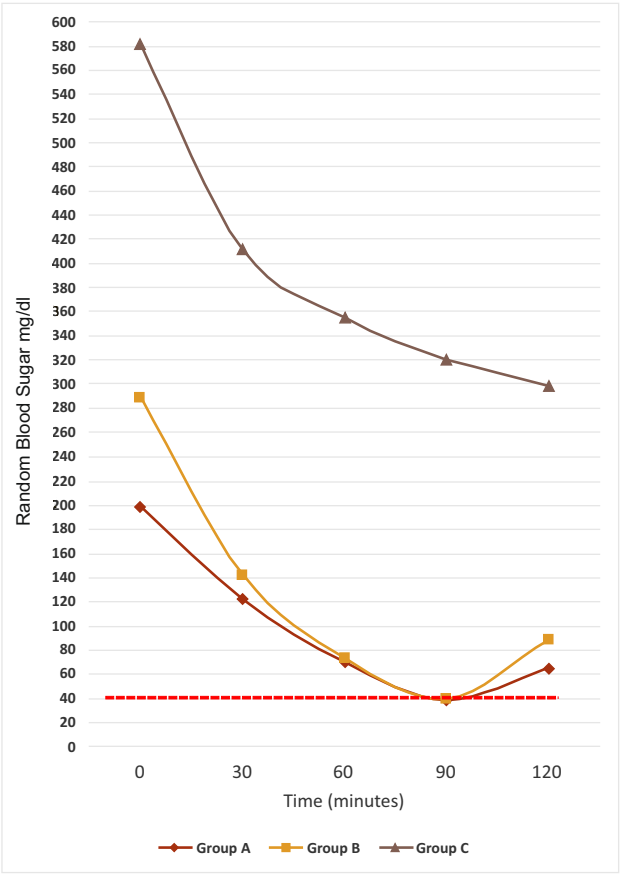
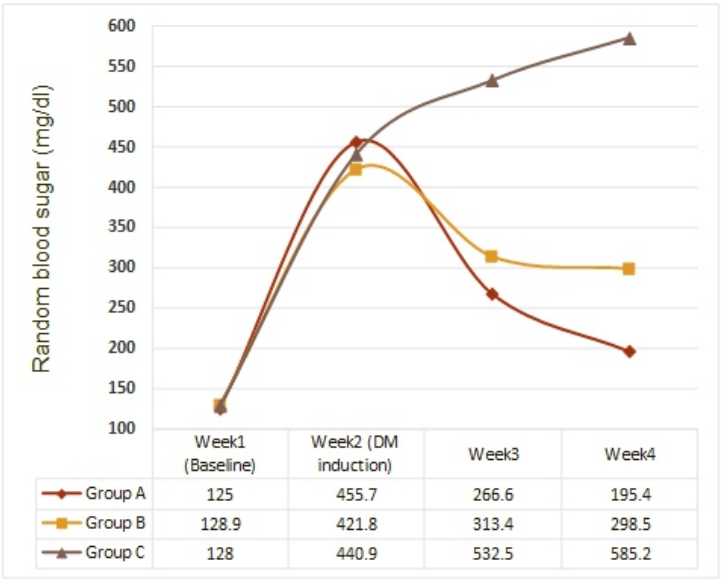


Figure 2: Insulin tolerance test curves in animals with type 1 DM. Values represent the mean. (The dotted red line indicates the level of hypoglycemia).



	Week1 (Baseline)	Week2 (DM induction)	Week3	Week4
Group A	125	455.7	266.6	195.4
Group B	128.9	421.8	313.4	298.5
Group C	128	440.9	532.5	585.2

Figure 1: Timeline changes in mean RBS values in animals with type 1 DM. Values represent the mean.

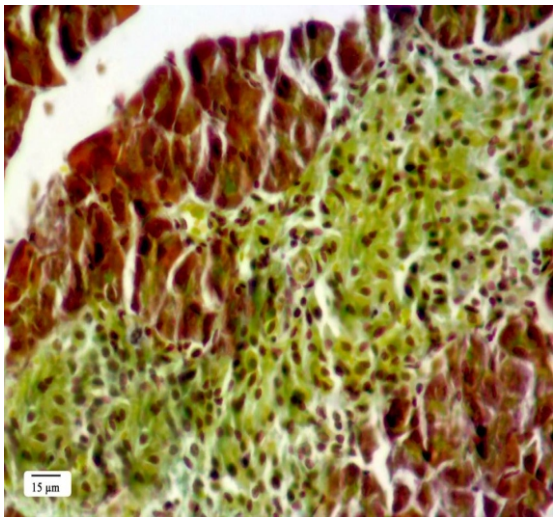


Figure 3: Pancreas of group A showing irregular small islet with abundant α-cell signal (yellowish-brown color). (Modified Gomori's Aldehyde Fuchsin, X400)

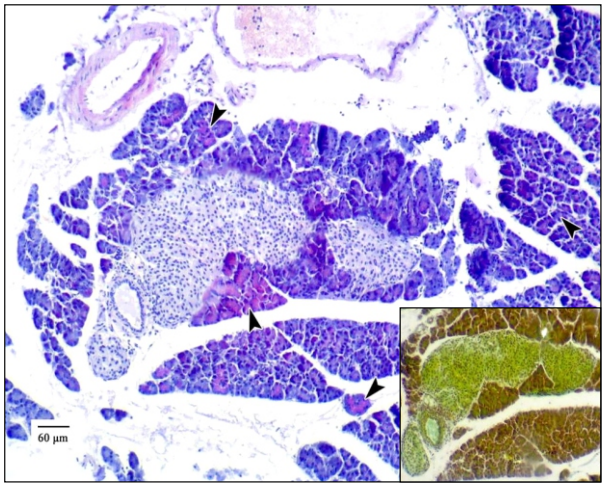


Figure 4: Pancreas of group B showing three fused islets with no clear limiting sheath. Black arrows indicate areas of exocrine tissue with increased acidophilia. (H&E, X100). The inset to the lower right shows the same islets stained with modified Gomori's aldehyde fuchsin, the predominant yellow-brown color indicates α-cells dominance.

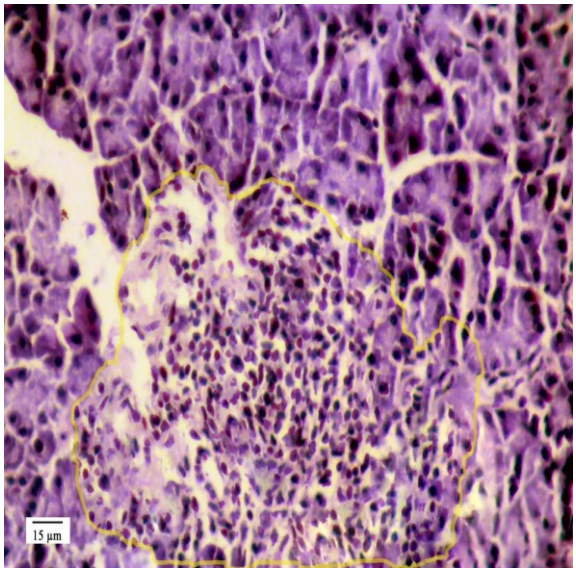


Figure 5: Pancreas of group C showing an irregular large islet with many pyknotic and inflammatory cells. The approximate outline of the islet is highlighted by pale yellow (loss of limiting sheath). (H&E, X400)

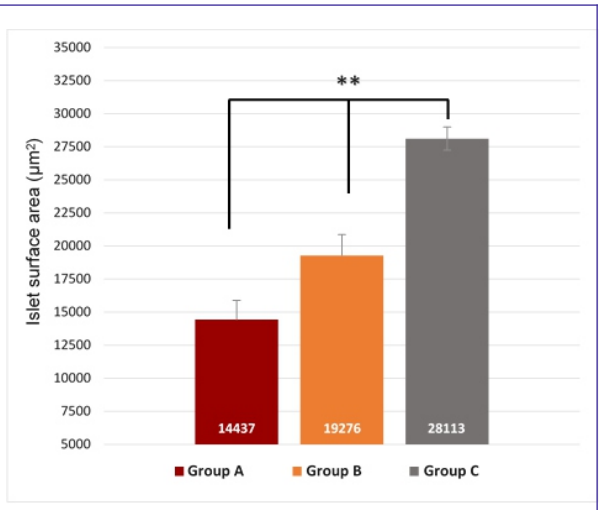


Figure 6 :Pancreatic islet surface area in animals with type I DM) .Values represent mean, error bars represent standard deviation = ** ,high statistical

DISCUSSION

Concomitant leptin and insulin treatment was very effective in restoring euglycemia and the insulin sensitivity remained high until the time of the ITT. This effect may have resulted from central and peripheral insulin sensitizing effects of leptin as described by earlier studies.[7] Leptin treatment alone was successful in reducing RBS levels but not to euglycemic levels. A possible mechanism is related to the reduced α-cell hyperplasia in group B pancreata, which entails a reduction in glucagon levels and amelioration of hyperglycemia. However, other counter-regulatory hormones of diabetes (e.g. growth hormone, glucocorticoids) could have sustained moderate hyperglycemia in the absence of insulin. The interaction with counter-regulatory hormones is apparent in the insulin tolerance test. The principle of the insulin tolerance test is to provide an exogenous dose of insulin that results in hypoglycemia to examine both insulin sensitivity and the body response to hypoglycemic stress. Normally, the hypoglycemic stress stimulates the hypothalamic release of growth hormone and adrenocorticotropic hormone with subsequent release of corticosterone from the adrenal. Growth hormone and corticosterone act as counter-regulatory hormones that elevate blood glucose levels. At the same time, pancreatic α-cells respond to low glucose levels by releasing glucagon, which stimulates glycogenolysis and hepatic glucose output. Therefore, in addition to examining insulin sensitivity, the test provides information about pancreatic function and the integrity of the hypothalamic-pituitary-adrenal axis.[16] The sharpest initial drop in RBS seen in group B supports the insulin-sensitizing effect of leptin for animals that were not exposed to exogenous insulin and, therefore, are expected to have uprrgulated insulin receptors. On the contrary, group A animals, already treated with insulin sowed a less acute initial drop, possibly due to receptor adaptation to previous treatment with exogenous insulin. In group C animals, failure of the ITT to induce hypoglycemia indicates the severity of activation of the hypothalamic pituitary adrenal axis and extent of hyperglucagonemia secondary to marked α-cell hyperplasia. Both effects may be related to insulinopenia and hypoleptinemia.

Several other studies showed that leptin treatment alone could restore euglycemia in STZ-induced type 1 DM. The main difference from the current study is that leptin was given either centrally (intracerebroventricular infusion) in low doses [17] or peripherally at very high doses (>10 times physiological dose).[18] This indicates that physiological doses of leptin can regulate some counter-regulatory hormone levels to improve insulin resistance; but cannot restore euglycemia.

All pancreata showed inflammatory and necrotic changes in the islets with complete loss of β-cell due to the alkylating action of STZ. In group C, there was a regional heterogeneity in islet changes. Some were greatly diminuted while others were relatively large, irregular and formed of hyperplastic α-cells. The murine pancreas is described in three regions:

duodenal, gastric and splenic. Different embryological origins and different responses of each region to stressful stimuli may explain the heterogeneity in islet changes. The islets may be intrinsically different or they may receive different extrinsic signals from their environment.[19] Accordingly, it is possible that smaller islets in certain regions underwent near total destruction and diminution while larger islets in other regions maintained their α-cell population that later on proliferated and relatively enlarged the islet size.

Type 1 DM is characterized by α-cell dysfunction. The hyperglucagonemia of diabetes may be caused by the loss of β-cell suppression on α-cells after β-cell destruction. It may also result from inability of α-cells to detect high circulating levels of glucose due to insulin deficiency. Severe hyperglycemia and inflammatory cytokines (especially Interleukin-6) are thought to be the cause of α-cell proliferation and hyperglucagonemia, as seen in group C.[20]

In groups A and B the islet size and α-cell hyperplasia were markedly reduced. This may be attributed to the improved glycemic control and inhibitory effects of leptin and insulin on α-cells. These inhibitory effects may be at the level of α-cell gene expression causing reduction of glucagon synthesis [21] or at the cell membrane level causing reduction of release.[22]

CONCLUSION

The current work shows a potential benefit of physiological doses of leptin in correcting or ameliorating hyperglycemia in animal models of type 1 DM. It can also prevent α-cell hyperplasia. This action is of greater value when leptin is used in combination with insulin. However, care should be taken to avoid hypoglycemia that may result from enhancement of peripheral insulin actions by concomitant leptin therapy.

REFERENCES

1. Daneman D. Type 1 diabetes. ***Lancet*** 2006;367(9513):847-858.
2. Arora S, Ohaj SK & Vohora D.Characterisation of streptozotocin induced diabetes mellitus in swiss albino mice. ***Glob J Pharmacol*** 2009;3(2):81-84.
3. Morton G & Schwartz M. Leptin in the central nervous system control of glucose metabolism. **Physiol Rev** 2011;91:389-411.
4. Vickers MH, Reddy S, Ikenasio BA & Breier BH. Dysregulation of the adipoinsular axis-a mechanism for the pathogenesis of hyperleptinemia and adipogenic diabetes induced by fetal programming. ***J Endocrinol*** 2001; 170: 323-332.
5. Kirel B, Doğruel N, Korkmaz U, Kiliç FS, Ozdamar K & Uçar B. Serum leptin levels in type 1 diabetic and obese children: relation to insulin levels. ***Clin Biochem*** 2000;33(6):475-80.
6. AL-Suhaimi EA, AL-Kulaifi FM, Ravinayagam V & Al-Qahtani MH. Serum adipocytokines, metabolic and immunological correlations in type 1 diabetes mellitus children. ***Open Endocrinol J*** 2012;6:110-116.
7. Tsai M, Asakawa A, Amitani H & Inui A. Stimulation of leptin secretion by insulin. ***Ind J Endocrinol Metab*** 2012;16(9): 543-548.
8. Lin CY, Higginbotham DA, Judd RL & White BD. Central leptin increases insulin sensitivity in streptozotocin-indiced diabetic rats. Am J Phy***siol Endocrinol Metab 2002***; 28(2):84-91.
9. Fujikawa T, Chuang JC, Sakata I, Ramadori G & Coppari R. Leptin therapy improves insulin defecient type 1 diabetes by CNS-dependent mechanisms in mice. ***Proc Natl Acad Sci*** (USA) 2010;107(40):391-396.
10. Brosius F (Ed.). High-Dose Streptozotocin Induction Protocol (Mouse)/Low-Dose Streptozotocin Induction Protocol (Mouse). Animal Models of Diabetic Complications Consortium (AMDCC) Protocols (The University of Michigan Medical Center). 2009; 1: 1-3.
11. Grant CW, Duclos SK, Moran-Paul CM et al. Development of standardized insulin treatment protocols for spontaneous rodent models of type 1 diabetes. ***Comp Med*** 2012;62(5): 381-390.
12. Harris RB, Zhou J, Redmann SM et al. A leptin dose-response study in obese (ob/ob) and lean (+/-) mice. Endocrinol. 1998; 139(1): 8-19.
13. Haj FG. Intraperitoneal insulin tolerance test. Mouse metabolic phenotyping centers (MMPC) protocols, UC-Davis. 2012; 1:1-2.
14. Bancroft JD & Stevens A. Theory and practice of histological techniques. 2nd ed. London: Churchill Livingstone. 1982:188-190, 482-502.
15. Woods AE & Ellis RC (eds.). Laboratory histopathology: a complete reference Special tissues. 2nd eds. New York: Churchill Livingstone; 1994. Chapter 10: Siebert R & Stirling J., Cell Organelles and Inclusions. p. 235-249.
16. Pfeifer M, Kanc K, Verhovec R & Kocijancic A. Reproducibility of the insulin tolerance test (ITT) for assessment of growth hormone and cortisol secretion in normal and hypopituitary adult men. ***Clin Endocrinol*** (Oxf)2001; 54(1):17-22.
17. Denroche HC, Levi J, Wideman RD et al. Leptin therapy reverses hyperglycemia in mice with streptozotocin-induced diabetes, independent of hepatic leptin signaling. ***Diabetes*** 2011; 60(5):1414-23.
18. Meek TH & Morton GJ. Leptin, diabetes and the brain. ***Ind J Endocrinol Metab*** 2013; 16(3): 534-542.
19. Ellenbroek JH, Tons HA, de Graaf N et al. Topologically heterogeneous beta cell adaptation in response to high-fat diet in mice. PLoS One. 2013;8(2):DOI: 10.1371/journal.pone.0056922.
20. Ellingsgaard H, Ehses JA, Hammar EB et al. Interleukin-6 regulates pancreatic alpha-cell mass expansion. ***Proc Natl Acad Sci*** (USA) 2008; 105(35):131-138.
21. Marroquı´ L, Vieira E, Gonzalez A, Nadal A & Quesada I. Leptin downregulates expression of the gene encoding glucagon in alpha TC1-9 cells and mouse islets. ***Diabetol*** 2011; 54(4):843-51.
22. Tudurí E, Marroquí L, Soriano S et al. Inhibitory effects of leptin on pancreatic α-cell function. ***Diabetes*** 2009;58(7): 1616–1624.