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Attenuation of the cytotoxic, nephrotoxic and hepatotoxic effects of streptozotocin-induced diabetic rats by aqueous extract of *Lepidium sativum* L. (Brassicaceae)

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Abstract
Diabetes mellitus is a health condition whose major feature is hyperglycemia that arises from various etiologies. Diabetes mellitus is connected with constraints in the metabolism of proteins and lipids which leads to complications such as hepatopathy, nephropathy, retinopathy and vasculopathy. Plant resources are utilized for better beneficial curative molecules, minimal side effects and cost. *Lepidium sativum* (garden cress) is a plant whose parts have been reportedly employed in the treatment of conditions like inflammation, diabetes, pyretism, hemorrhage and hypertension. This histopathological study sought to investigate the effect of the aqueous extract of *L. sativum* on the tissues of the pancreas, kidney, and liver in streptozotocin-induced diabetic rats. Haematoxylin and Eosin Staining method of histopathology was employed and the cytotoxic, nephrotoxic and hepatoxic effects on the pancreatic Islet cells, kidney and liver were attenuated on treatment with different doses of aqueous extracts of *L. sativum* plant.

Keywords: Diabetes mellitus, cytotoxic, nephrotoxic, hepatotoxic and lepidium sativum

Introduction
Diabetes mellitus is a health condition whose major feature is hyperglycemia that arises from various etiologies. The β-Langerhans islet cells of the pancreas produce insulin a hormone that stimulates the liver for glucose metabolism to reduce blood sugar levels to a normal range. Diabetes mellitus is connected with constraints in the metabolism of proteins and lipids which leads to complications such as hepatopathy, nephropathy, retinopathy, and vasculopathy [1, 2]. Streptozotocin (STZ) exists in nature and is a cytotoxic chemical that is particularly harmful to the β-cells of the pancreas [3]. It causes harm by damaging the deoxyribonucleic acid (DNA) [4]. STZ consists of glucose and a very reactive methyl nitrosourea moiety. The glucose moiety leads STZ to the pancreatic β cells allowing it to be taken up into the cells by the transport protein GLUT 2 while the methyl nitrosourea moiety deploys its cytotoxic effects [5]. The presence of GLUT 2 in the kidney and liver of mammals also allows STZ to undermine their functions [19]. When taken in, STZ is biotransformed in the liver and removed from the body by the kidneys [7], however, where there is observable functional damage or disorder to the liver or kidney after the excretion of STZ, hyperglycemia may be responsible thereby providing a bedrock for research on the mechanisms of STZ diabetic issues in the liver or kidney [8].
The treatments of Diabetes mellitus with different medications have encountered limitations such as drug resistance (reduction of efficiency), side effects, and even toxicity and considering the multifaceted presentation of diabetes and its impact on different body organs [9], there is the utilization of plant resources for better beneficial curative molecules, minimal side effects, and cost. *Lepidium sativum* (garden cress) is a plant whose parts have been reportedly employed in the treatment of conditions like inflammation, diabetes, pyretism, hemorrhage, and hypertension [10, 11]. This histological sought to investigate the effect of the aqueous extract of *L. sativum* on the tissues of the pancreas, kidney, and liver in streptozotocin-induced diabetic rats.

Materials and Methods
Plant: *Lepidium sativum* plant was collected from a location near the permanent site of the University of Jos, Jos Nigeria, validated by the Federal College of Forestry, Jos, Nigeria, and given the Voucher number (FHJ 221). The plant was air-dried at room temperature for about 21 days, pounded and sieved to collect the fine powder, which was stored in an air-tight container for subsequent use.

The plant extraction was carried out by the method of maceration as employed in [11].

Animals

Male Wister albino rats with body masses between 185 -205 g were purchased from the animal house of the University of Jos, Jos Nigeria. They were fed, had access to water, and were kept in standard laboratory conditions to acclimatize for two weeks. Ethical considerations were in place in the use of the animals with an approval reference F17-00379 from the department of Pharmacology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria.

Thirty male adult rats were used for this study. Diabetes was induced in rats of groups B to E by the intraperitoneal injection of 55 mg/kg streptozotocin in 0.1 M fresh cold citrate buffer at pH 4.5. After 3 days, the blood glucose levels of the rats were determined using the On Call Plus Glucometer [12]. The rats whose fasting blood glucose levels were equal to or greater than 200 mg/dl were taken as diabetic [13].

The diabetic animals were randomly placed in groups B-D while all rats of group A were not induced. The administration of the aqueous extract of *L. sativum*, standard drug (glibenclamide), and food happened in 28 days.

Group A: Non-diabetic and non-treated rats

Group B: Diabetic Control Rats that were not treated with the aqueous extract of *L. sativum.*

Group C: Diabetic Rats that were treated with 100 mg/kg aqueous extract of *L. sativum.*

Group D: Diabetic Rats that were treated with 200 mg/kg aqueous extract of *L. sativum.*

Group E: Diabetic Rats that were treated with Glibenclamide 2 mg/kg.

Samples collection

The rats were sacrificed by inhalation method after one day fast. The liver, kidney, and pancreas were excised from the rats according to their groups and fixed in labeled bottles containing 10% neutral buffered formalin for up to 22 hours at room temperature for the histopathological studies.

Chemicals and Reagents

These were acquired from Sigma Aldrich St. Louis, USA.

Histopathological study

Tissue Processing and Embedding

Procedure: Following fixation, tissue samples were passed through the following procedure to prevent excessive tissue hardening and brittleness. Samples were immersed for fifteen minutes in ascending grades of alcohol as follows: Eighty percent (80%); 95% with 2 changes; 100% with 2 changes. The samples were also immersed in Xylene solvent with 2 changes, then in molten paraffin wax at 60 °C with 3 changes, were tagged and submerged in paraffin wax as blocks.

A microtome was employed to separate them into 5 µm thick sections and a section from each tissue block was collected, placed on a slide, and stained with Haematoxylin and Eosin (H & E) [14].

Haematoxylin and Eosin Staining

De-paraffinization was conducted by heating sections in the oven at 80 °C for 60 minutes. The sections were immersed in 2 successive changes of xylene. After de-waxing, tissue sections were treated with lowering grades of ethyl alcohol (100%, 95%, 80% and 70%, for 2 minutes each), washed under indirect tap water for 15 minutes, stained with Harris's Haematoxylin for 5 minutes, washed again with running tap water for 3 minutes, immersed several times (4-7) in 1% acid alcohol (clearing) and immersed several times (4-7) in blueing solution. The tissue sections were stained with eosin for 45 seconds, dried in an oven at 80 °C for 1-2 minutes, cleared in xylene, with (1-2) drops of Disteren Plasticizer Xylene (DPX) added, quickly covered with coverslips and left to dry overnight. The slides were then examined with a light microscope and photomicrographs were taken using X 40 objective [14].

Results

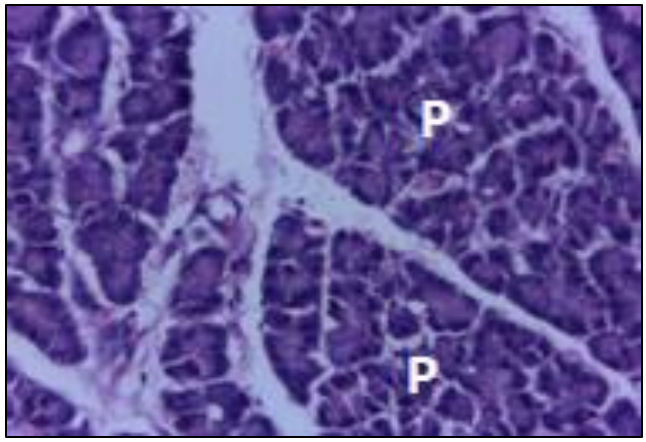

Plate 1: Section of Pancreas of Group A. Showing Normal Architecture of Parenchyma (P) (H and E, X 40)

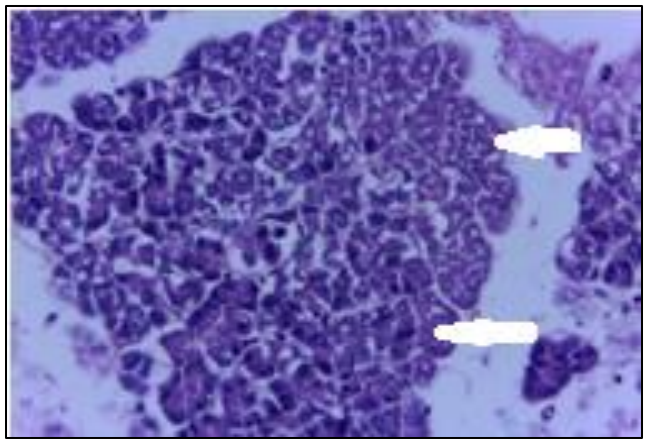

Plate 2: Section of Pancreas of Group B Showing the Necrosis of the Parenchyma (Arrow). (H and E, X40)

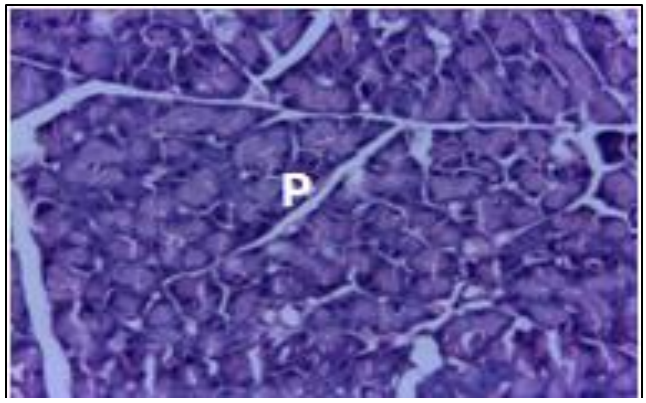

Plate 3: Section of Pancreas treated with 100 mg/kg *L. sativum* (Group C) Showing Partial Recovery of Parenchyma (P). (H and E, X 40)

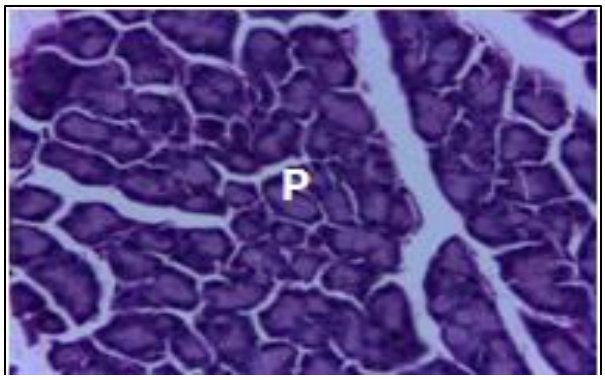

Plate 4: Section of Pancreas of *L. sativum* Treated Group D with 200 mg/kg Showing Prominent Recovery of the Parenchyma (P). (H and E, X 40)

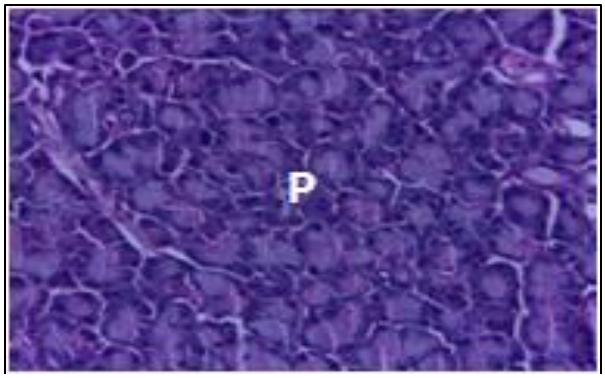

Plate 5: Section of Pancreas of Glibenclamide Treated Group E Showing Recovery of the Parenchyma (P). (H and E, X 40)

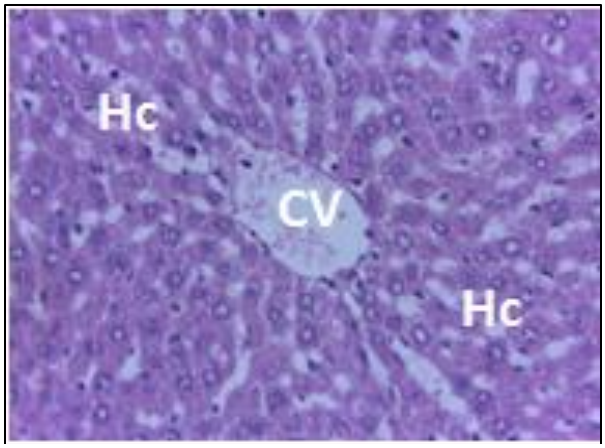

Plate 6: Section of Liver from Group A Showing Normal Architecture of Liver; Central Vein (CV) and Hepatic Cells (Hc) (H and E, X 40)

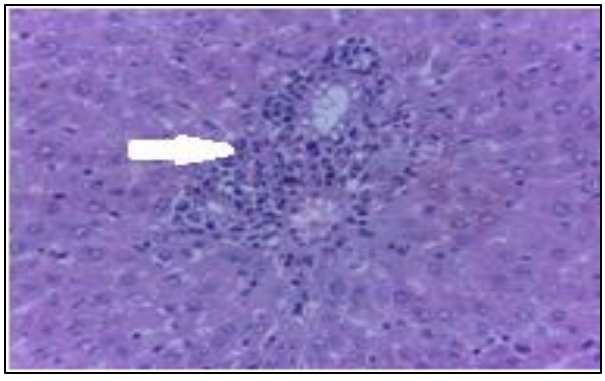

Plate 7: Section of Liver from Group B Showing Portal Inflammation (Arrow). (H and E, X 40)

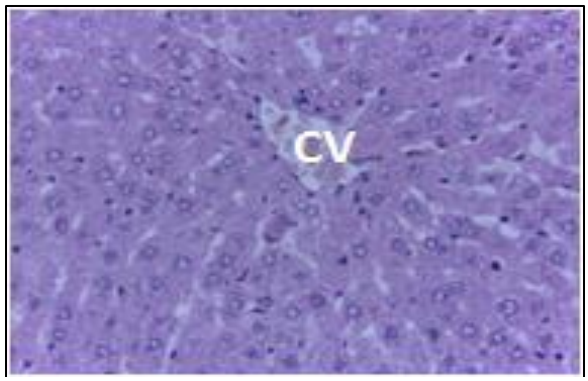

Plate 8: Section of Liver Administered 100 mg/kg *L. sativum* Group C Showing Prominent Recovery of Liver Architecture (central vein, CV) (H and E, X 40)

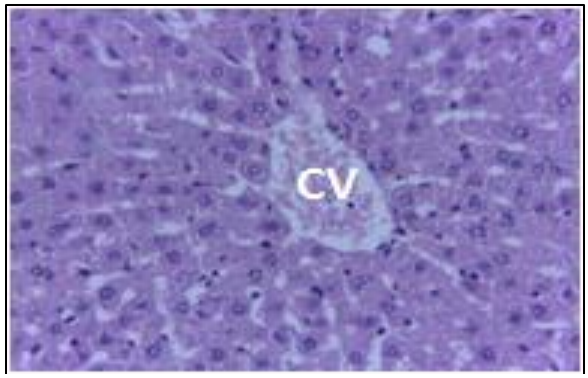

Plate 9: Section of Liver Administered 200 mg/kg of *L. sativum* Group D Showing Prominent Recovery of Liver Architecture (central vein, CV) (H and E, X 40)

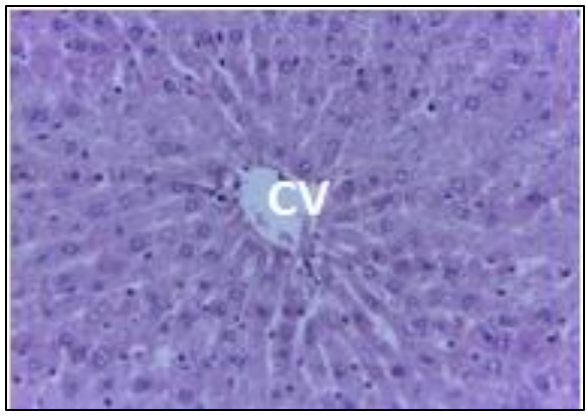

Plate 10: Section of Liver of Glibenclamide treated Group E showing recovery of liver central vein (Arrow). (H and E, X 40)

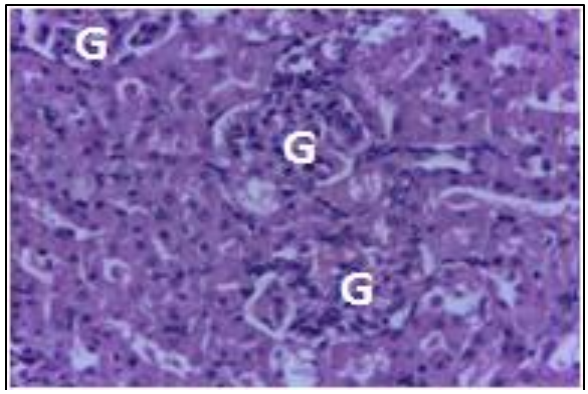

Plate 11: Section of Kidney from Group A Showing Normal Architecture of Kidney Glomerulus (G). (H and E, X 40)

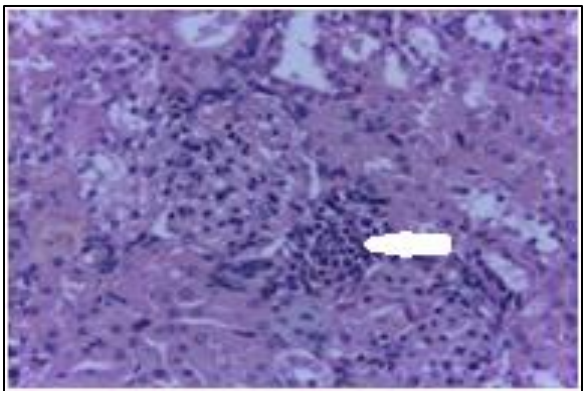

Plate 12: Section of Kidney from Diabetic Control Rat Group B showing inflammation of the Cortex (Arrow). (H and E X 40)

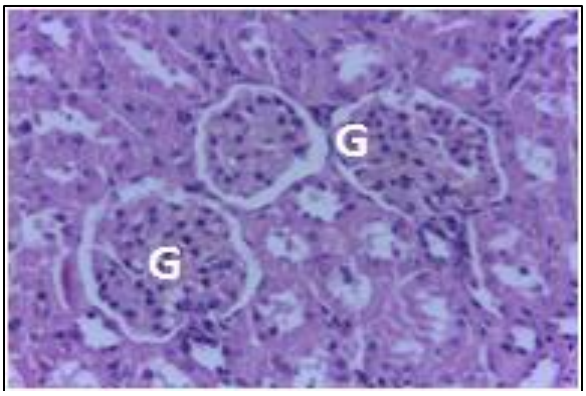

Plate 13: Section of Kidney from Group C Administered 100 mg/kg of *L. sativum* showing prominent recovery of Kidney glomerulus. (H and E, X 40)

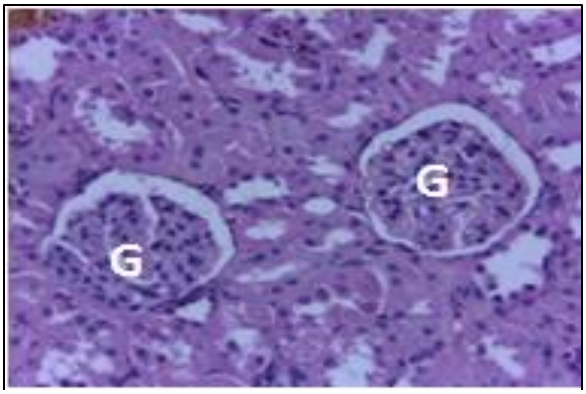

Plate 14: Section of Kidney from Group D Administered 200 mg/kg of *L. sativum* showing Prominent Recovery of Kidney Glomerulus. (H and E, X 40)

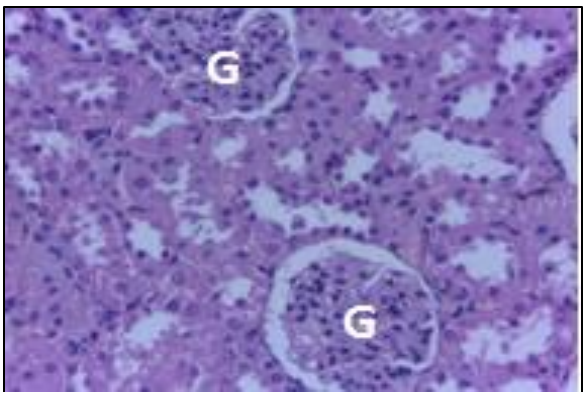

Plate 15: Section of Kidney from Glibenclamide Group E Showing Prominent Recovery of Liver Architecture/glomerulus. (H and E, X 40)

Discussion

The microphotographs of hematoxylin and eosin staining of the pancreas, kidney, and liver tissues revealed the degenerative changes due to cytotoxic, nephrotoxic, and hepatotoxic effects of STZ-induced diabetes. In addition to the harmful effect of streptozotocin, intracellular reactive oxygen species (ROS) have been reported to be a major contributor to DNA fragmentation and give rise to unfavorable changes in the pancreatic β-cells [15, 16]. The damage to the liver in diabetics is linked to oxidative stress due to hyperglycemia and the activation of inflammation [17]. Diabetes mellitus has also been identified as a cause of kidney failure condition where the kidney undergoes changes in structure and function [18]. These cytotoxic, nephrotoxic, and hepatoxic effects on the pancreatic Islet cells, kidney, and liver were attenuated on treatment with 100 and 200 mg/kg aqueous extracts of *L. sativum* plant as presented in the photomicrographs.

References

1. Ruch W, Zumsteg U. Diabetes or hyperglycemia? Schweizerische medizinische Wochenschrift. 1988;118(8):264-266.
2. Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabetic medicine. 1997;14:S5.
3. Brentjens R, Saltz L. Islet cell tumors of the pancreas: the medical oncologist's perspective. Surg Clin. North Am. 2001;81:527-542.
4. Schnedl WJ, Ferber S, Johnson JH, Newgard CB. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. Diabetes. 1994;43:1326-1333.
5. Johansson EB, Tjalve H. Studies on the tissue-disposition and fate of streptozotocin with special reference to the pancreatic islets. Acta Endocrinol. 1978;89(2):339-351.
6.
7. Karunanyake EH, Hearse DJ, Mellows G. The synthesis of C14Streptozotocin and its distribution and excretion in the rat. Biochem J. 1974;142:673-683.
8. Tesch GH, Allen TJ. Rodent models of streptozotocin-induced diabetic nephropathy. Nephrology. 2007;12(3):261-266.
9. Kumar S, Pandey AK. Antioxidant, lipo-protective and antibacterial activities of phytoconstituents present in *Solanum xanthocarpum* root, International Review of Biophysical Chemistry. 2012;3(3):42-47.
10. Divanji M, Viswanatha GL, Nagesh S, Vishal J, Shivaprasad HN. Review Article ethnopharmacology of *Lepidium Sativum* Linn (Brassicaceae): International Journal of Phytothearpy Research. 2012;2(1):1-7
11. Olotu IA, Luka CD, Olotu PN. Evaluation of the Effects of Aqueous Whole Plant Extract of *Lepidium sativum* L. (Brassicaceae) on some Biochemical and Hematological Parameters in Streptozotocin-Induced Diabetic Rats. Scholars Research Library. J Nat. Prod. Plant Resour 2018;8(1):19-25.
12. King S, Austin A. Animal Modes of Type 1 and Type 2 Diabetes Mellitus. In Animl Dodels for the Study of Human Disease. Second Edition. Elsevier Inc.; c2017. p. 245-265.
13. Pouya P, Rajab-Ali S, Shapour H, Azadeh F. An Investigation on body weights, blood glucose levels and pituitary-gonadal axis hormones in diabetic and

metformin-treated diabetic female rats. Veterinary Research Forum. 2012;3(2):79-84.
14. Egbujo EC, Adisa OJ, Yahaya AB. A study on the staining effect of Roselle (*Hibiscus sabdariffa*) on the histologic section of the testis. Int. J Morphol. 2008;26(4):927-930.
15. Takasu N, Komiya I, Asawa T, Nagasawa Y, Yamada T. Streptozocin-and alloxan-induced H_2O_2 generation and DNA fragmentation in pancreatic islets. H_2O_2 as mediator for DNA fragmentation. Diabetes 1991;40(9):1141-5.
16. Bedoya FJ, Solano F, Lucas M. N-monomethyl-arginine and nicotinamide prevent streptozotocin-induced double strand DNA break formation in pancreatic rat islets. Experientia. 1996;52(4):344-347.
17. Yazdi HB, Hojati V, Shiravi A, Hosseinian S, Vaezi G, Hadjzadeh MA. Liver Dysfunction and Oxidative Stress in Streptozotocin-Induced Diabetic Rats: Protective Role of *Artemisia* Turanica. Journal of pharmacopuncture 2019;22(2):109-114.
18. Snehal NM, Jayesh BD, Sangita BK, Archana RJ. Attenuation of diabetic nephropathy in streptozotocin-induced diabetic rats by *Punica granatum* Linn. Leaves extract. J Tradit Complement Med. 2017;7(3):273-280.
19. Bouwens L, Rooman I. Regulation of pancreatic beta-cell mass. Physiol. Rev. 2005;85(4):1255-1270.