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A POTENTIAL ROLE OF ANTIOXIDANTS (VITAMIN-E) ON STREPTOZOTOCIN-INDUCED PANCREATIC B-CELL DAMAGE IN ADULT MALE ALBINO RATS

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ABSTRACT

Aim: To evaluate the effect of streptozotocin on the endocrinal functions of the pancreatic gland, and the potential protective effects of vitamin-E in adult male albino rat. **Materials and Methods:** This study was conducted between March and May, 2022 at animal house of Medical Physiology Department, Al-Azhar Faculty of Medicine, Cairo. 50 adult male albino rats were distributed randomly into 5 equal groups: Normal control group I, Control group II that received olive oil, Control group III (Vitamin-E-treated group), Group IV (streptozotocin-treated group), and Group V (streptozotocin-vitamin E-treated group). **Results:** Streptozotocin-treated group (group IV) was associated with significant higher levels of serum glucose, MDA, interlukin-1b, and interlukin-6 levels and lowered level of serum insulin and GSH as compared to the control normal groups. this was confirmed by distortion of the normal architecture of the pancreatic tissue with areas of hemorrhage, vacuolation and necrosis in the islets of Langerhans. There were significant amelioration of lipid peroxidation in vitamin-E- streptozotocin -treated group (group V) represented by significant decrease of serum glucose, MDA, interlukin-1b, and interlukin-6 levels, and significant increase of serum insulin and GSH level as compared to the control normal groups.

Histopathological changes were improved after receiving vitamin. **Conclusion:** Vitamin-E could protect the pancreas against streptozotocin -induced alteration in adult male albino rat.

Keywords: Streptozotocin; Vitamin- E; Insulin; Pancreas; Diabetes.

INTRODUCTION

Streptomyces achromogenes is used to produce streptozotocin (STZ). It is a DNA alkylating agent and glucosamine-nitrosourea that only enters cells through the glucose transport protein (GLUT2). The pancreatic islet beta-cells that produce insulin are extremely toxic to the diabetogen streptozotocin. Neuroendocrine tumour cells that are GLUT2 expressing are poisonous to it. For scientific purposes, type I diabetes is induced with streptozotocin. It is applied clinically to treat pancreatic -cell cancer. **[1].**

Vitamin E (x-Tocopherol) is referred to as a membrane antioxidant since it is one of the main fat-soluble antioxidant vitamins found in membrane lipoproteins. Through the scavenging of intermediate peroxyl radicals, it halts the chain reaction of lipid peroxidation. **[2].** In a dose-dependent way, vitamin E supplements may shield the pancreas, liver, kidney, and other organs against environmental toxins including ozone, chemotherapy, and radiotherapy. **[3].**

Inhibiting platelet aggregation, protecting against polyunsaturated fatty acid oxidation, and supporting neurological processes are all actions of vitamin E. **[2].**

The aim of the current research was to assess the potential protective effects of vitamin-E on β-cells injury induced by Streptozotocin in adult male albino rat.

MATERIALS AND METHODS

Vitamin-E was purchased from El Kahira Pharmaceutical Company Cairo, Egypt as a gelatinous capsule. Each capsule contained 600 mg vitamin-E which dissolved in olive oil solvent to obtain a concentration of 6000 mg/100 ml (each 1ml containing 60 mg of vitamin-E). Each rat was administered vitamin-E (600 mg/kg/day) orally through gastric gavage **[4].**

Streptozotocin was purchased from Sigma-Aldrich, USA. Each rat received a single intraperitoneal (IP) injection of STZ (40 mg/kg body weight) **[1].**

Olive Oil was used as a vehicle for vitamin-E (Sekem, Cairo, Egypt).

Isoflurine was purchased from (Nile Pharmaceutical-Egypt) was used for anesthesia .

Animals: The Medical Physiology Department of the Al-Azhar Faculty of Medicine in Cairo conducted an experimental investigation there between March and May of 2022. 50 mature male albino rats of a local strain weighing between 90 and 110 grams (average weight: 100 g) served as the study's animal model. The animals were kept in appropriate cages (40 x 32 x 40 cm for every 5 rats) that met normal environmental requirements and had wide-meshed raised flooring to

prevent coprophagia. In order to acclimate, the rats were housed for 10 days on ad libitum food and tap water at room temperature.. Animals were distributed randomly and equally into 5 groups:

Group I (Negative control Group) Each rat received normal saline intraperitoneally (i.p.), equivalent amounts to STZ-treated group.

Group II (Positive control Group): Each rat received only a single oral dose of 0.7 ml of oily solvent as a vehicle for vitamin-E daily for 4 weeks **[1].**

Group III (Vitamin-E-treated group): Each rat received vitamin-E at a single oral dose of 600 mg/kg body weight dissolved in olive oil by a gastric tube daily for 4 weeks **[4].**

Group IV (Streptozotocin-treated group): Each rat received a single intraperitoneal (IP) injection of STZ (40 mg/kg body weight **[1].** Blood glucose level was measured after 3 days. Animals with fasting blood glucose level above 250 mg/dL were considered diabetic and were used in the experiment.

Group V (**Streptozotocin-Vitamin E-treated group):** each rat received both streptozotocin and vitamin-E daily for 4 weeks in the same pervious doses.

Blood sampling and biochemical estimation: The retro-orbital plexus was punctured with a heparinized capillary tube (0.75-1.0 mm internal diameter) and blood was drawn from each site (approximately 3.5 ml of blood total). Blood was drawn into a dry, clean, graduated glass centrifuge tube in order to obtain serum. It was quickly sent to centrifuge for 15 minutes at 5000 rpm. A little less than half of the supernatant serum was drawn out into Eppendorf tubes and kept frozen at -20°C until it was utilised to measure the serum levels of interlin-1B, interlukin-6, MDA, and GSH-peroxidase, as well as serum insulin and serum glucose.

Histopathological examination: The pancreas was removed for histological research at the end of the fourth week. Tissue samples were quickly removed, stained with Hematoxylin and Eosin (H and E), and viewed under a light microscope. **[5, 6].**

Ethical Approval.

Study protocol was submitted for approval by Institution Research Board (IRB00012367-22-010-005) of Damietta faculty of medicine – AL-Azhar University

Statistical Analysis: The obtained results were collected, charted, statistically analyzed and represented graphically. Values were presented as mean, Standard Deviation (SD) and confidence intervals values. Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that data were normally distributed (parametric data), therefore one-way analysis of variance (ANOVA) and Tukey's post hoc tests were used for comparison. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with SPSS 22.0 for Windows.

RESULTS

The mean ± standard deviation of fasting serum glucose levels were 116.17±0.73, 118.11±0.65, 110.14±0.44, 267.93±5.75 and 149.56±3.83 mg/dl in groups I, II, III, IV and V respectively. Group II and III showed insignificant changes in respect to the group I (control group). Streptozotocin-treated group resulted in a significant elevation in the levels of fasting serum glucose (FSG) in group IV in respect to control group I, while the treatment with Vitamin-E reduced the elevated fasting serum glucose in group V in respect to untreated streptozotocin-treated group, but still significantly higher than that of the control groups I, II and III (Table 1).

The mean ± standard deviation of serum insulin was 2.48±0.02, 2.88±0.01, 2.43±0.05, 1.68±0.026 and 2.52±0.04 mg/dl in groups I, II, III, IV and V respectively. Group II and III showed insignificant changes in respect to the group I (control group). Streptozotocin -treated group resulted in a significant reduction in the levels of serum insulin in group IV in respect to control group I, while the treatment with Vitamin-E elevated the serum insulin in group V in respect to untreated streptozotocin-treated group (Table 1).

Groups / **Parameters**	**Control normal (Group I)**	**Control group received oil (Group II)**	**Vitamin-E treated group (Group III)**	**Streptozotocin-treated group (Group IV)**	**Streptozotocin-Vitamin-E treated group (Group V)**
Fasting serum glucose (mg/dl)	116.17±0.73	118.11±0.65	110.14±0.44	267.93±5.75	149.56±3.83
		P > 0.05*	P < 0.05* P < 0.05®	P < 0.05* P > 0.05≠ P > 0.05Ω	P < 0.05* P > 0.05¶ P < 0.05@
Insulin (mIU/ml)	2.48±0.02	2.88±0.01	2.43±0.05	1.68±0.026	2.52±0.04
		P > 0.05*	P < 0.05* P < 0.05®	P < 0.05* P > 0.05≠ P > 0.05Ω	P < 0.05* P > 0.05¶ P < 0.05@
HOMA- IR	0.71	0.83	0.66	1.11	0.93

Table (1): Serum glucose, insulin and HOMA-IR levels among different studied groups (Mean ±SD).

N Number of rats in each group = 10.

® Groups III was compared to group II.

@ Groups V were compared to group III

¶ Groups V was compared to group IV

*All groups were compared to control group I. Ω Groups IV was compared to group III.

The mean ± standard deviation of MDA was 0.42± 0.05, 0.51± 0.07, 0.39± 0.03, 2.09 ± 0.26 and 0.49 ±0.07 nmol/L in groups I, II, III, IV and V respectively. Group II and III showed insignificant changes in respect to the group I (control group). Streptozotocin-treated group resulted in a significant elevation in the levels of serum MDA in group IV in respect to control group I, while the treatment with Vitamin-E reduced the elevated MDA in group V in respect to untreated streptozotocin-treated group (Table 1).

The mean ± standard deviation of serum GSH was 1.37±0.997, 1.29±0.779, 1.46±0.988, 0.57±0.076 and 1.02±0.075 nmol/L in groups I, II, III, IV and V respectively. Group II and III showed insignificant changes in respect to the group I (control group). Streptozotocin -treated group resulted in a significant elevation in the levels of serum GSH in group IV in respect to control group I, while the treatment with Vitamin-E reduced the elevated serum GSH in group V in respect to untreated streptozotocin -treated group, but still insignificantly lower than that of the control groups I, II and III (Table 2)

Groups / Parameters	**Control normal (Group I)**	**Control group received oil (Group II)**	**Vitamin-E treated group (Group III)**	**Streptozotocin - treated group (Group IV)**	**Streptozotocin-Vitamin E treated group (Group V)**
MDA(nmol/L)	0.42± 0.05	0.51± 0.07	0.39± 0.03	2.09 ± 0.26	0.49 ±0.0
		P > 0.05*	P < 0.05* P < 0.05®	P < 0.05* P > 0.05≠ P > 0.05Ω	P < 0.05* P > 0.05¶ P < 0.05@
GSH (nmol/L)	1.37±0.997	1.29±0.779	1.46±0.988	0.57±0.076	1.02±0.075
		P > 0.05*	P < 0.05* P < 0.05®	P < 0.05* P > 0.05≠ P > 0.05Ω	P < 0.05* P > 0.05¶ P < 0.05@

Table (2): Serum MDA and GSH enzymes among different studied groups (Mean ±SD)

N Number of rats in each group = 10. ® Groups III was compared to group II.

@ Groups V were compared to group III ¶ Groups V was compared to group IV

*All groups were compared to control group I. Ω Groups IV was compared to group III

The mean ± standard deviation of Interleukin-1B was 22.82±6.04, 20.74±5.05, 19.99±7.07, 92.80±8.29, and 37.90±8.74 pg/ml in groups I, II, III, IV and V respectively. The mean ± standard deviation of Interleukin-6 was 16.42±4.17, 17.22±3.15, 16.33±5.13, 75.12±12.08, and 36.42±12.73 pg/ml in groups I, II, III, IV and V respectively Group II and III showed insignificant changes of both intrlulin-1B and 6 in respect to the group I (control group). Streptozotocin -treated groups resulted in a significant elevation in the levels of intrlulin-1B and 6 in group IV in respect to control group I, while the treatment with Vitamin-E reduced the elevated intrlulin-1B and 6 in group V in respect to untreated streptozotocin-treated group, but still significantly higher than that of the control groups I, II and III (Table 3).

Groups / Parameters	Control normal (Group I)	Control group received oil (Group II)	Vitamin-E treated group (Group III)	Streptozotocin-treated group (Group IV)	Streptozotocin-Vitamin-E treated group (Group V)
Interleukin-1B (pg/ml)	22.82±6.04	20.74±5.05	19.99±7.07	92.80±8.29	37.90±8.74
		P > 0.05*	P < 0.05* P < 0.05®	P < 0.05* P > 0.05≠ P > 0.05Ω	P < 0.05* P > 0.05¶ P < 0.05@
Interleukin-6 (pg/ml)	16.42±4.17	17.22±3.15	16.33±5.13	75.12±12.08	36.42±12.73
		P > 0.05*	P < 0.05* P < 0.05®	P < 0.05* P > 0.05≠ P > 0.05Ω	P < 0.05* P > 0.05¶ P < 0.05@

Table (3): Serum interleukin-1B and interleukin-6 of among different studied groups (Mean ±SD

N Number of rats in each group = 10.

® Groups III was compared to group II.

@ Groups V were compared to group III

¶ Groups V was compared to group IV

*All groups were compared to control group I.

Ω Groups IV was compared to group III.

Light microscopically examination of pancreatic tissue in control groups (groups I, II, and III) showed normal pancreatic structure in the form of normal islets of Langerhans surrounded by the pancreatic acini with normal interlobular connective tissue (Figure 1a and 1b).

Pancreatic sections from streptozotocin-treated group (group IV) showed displayed degeneration of cells of acini with widening of the interlobular spaces with blood vessels and infiltrated by inflammatory cells. Cells of islet of Langerhans are degenerated (Figure 2).

Vitamin-E treatment caused partial improvement in the histopathological alterations as there was minimal inflammatory reaction and mild degeneration of acinar cells. Cells of islet of Langerhans showed mild degenerative change and still widening of intercellular septa (figure 3).

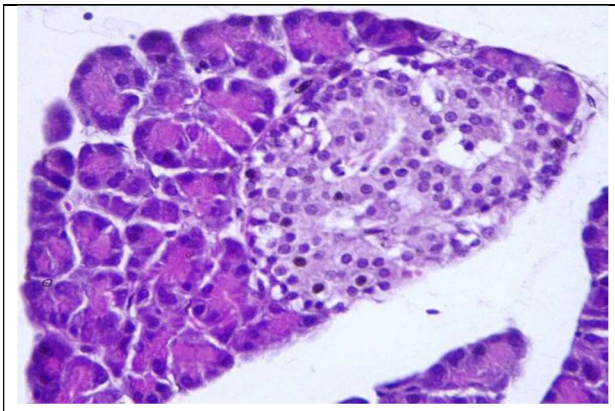

Fig. (1a): control groups (I, II, and III) showed normal pancreatic architectures; lobules of closely packed acini separated by thin interlobular septa and islets of Langerhans (Hx & E, 400X).

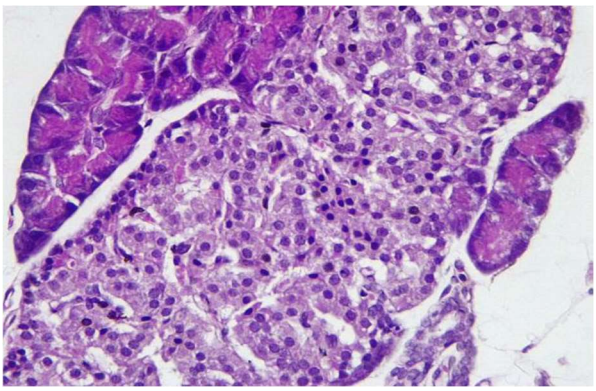

Fig. (1b): control groups (I, II, and III) another section showed also normal pancreatic architectures; lobules of closely packed acini separated by thin interlobular septa and islets of Langerhans (Hx & E, 400X).

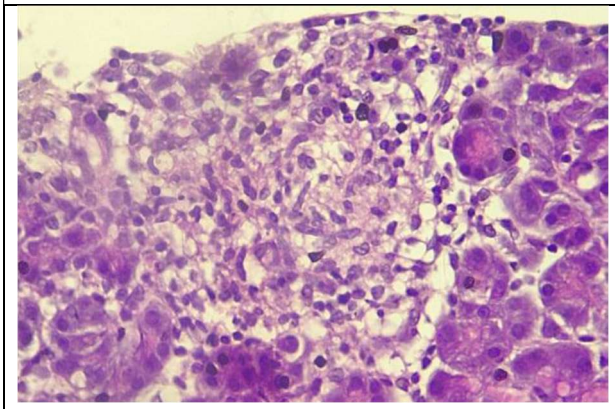

Fig.(2): group IV showed degeneration of cells of acini, widening of the interlobular spaces and infiltrated inflammatory cells. (Hx & E, 400X).

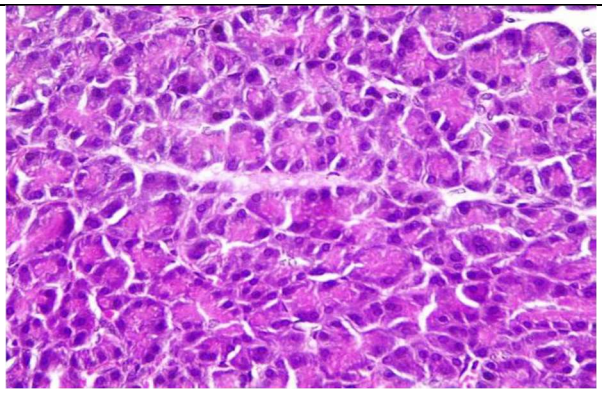

Fig. (3): group V showed partial improvement in the histopathological alterations with minimal inflammatory reactions. Cells of islet of Langerhans showed mild degenerative change and still intercellular widening of intercellular septa (Hx & E 400X).

DISCUSSION

The goal of the current study was to establish how damage caused by STZ affected pancreatic beta-cells. A naturally occurring nitrosamide called streptozotocin is used to cause cytotoxicity in pancreatic beta-cells, possibly by producing excessive reactive oxygen species (ROS) and lipid peroxides, interfering with the glucose transporter GLUT2, and damaging DNA either through alkylation or peroxynitrite formation. **[7].** Streptozotocin's DNA strand breaking activates poly ADP-ribose polymerase (PARP), which depletes ATP and causes cell death and a reduction in insulin levels. **[8].**

The current study made it clear that streptozotocin significantly changed the pancreatic shape and had negative histological effects on the pancreas of adult albino rats. Alteration of the pancreatic tissue's normal architecture due to areas of haemorrhage, vacuolation, and Langerhans islet necrosis.

These findings are in accordance with those of other studies who noted pancreatic vacuolar degeneration following streptozotocin administration. **[9].**

Treatment with vitamin-E in group V of the current study led to a significant decrease in fasting blood glucose (FSG) and associated significant elevation of serum insulin level in comparison with streptozotocin-untreated group IV. These findings could be attributed to improved hepatic insulin sensitivity resulting in decreased hepatic glucose production [**10].** Vitamin E may improve insulin sensitivity or release, and it may protect more pancreatic beta-cells by increasing the availability of insulin. By enhancing membrane mobility, vitamin E supplementation may modify insulin receptors in muscle or adipose tissue. Additionally, vitamin E might improve the diaphragm's ability to absorb glucose. [**10, 2, 3].** Oxidant stress markers which are critical factors increases by inflammatory mechanisms of injury in the pancreas [**11].** Vitamin-E has antioxidant activity because it contains three classes of phytochemicals as flavonoides (quercetin and kaempferol) and phenolic acids **[2].**

Normal rats treated with vitamin-E showed insignificant changes in the serum levels of both MDA and GSH.

The antioxidant that is easily available is GSH. It participates in the catalytic sequences of antioxidant enzymes, acting as an antioxidant either directly or indirectly. Glutathione's thiol group releases the hydrogen atom from free radicals, protecting the cell membrane **[11].** A marker of oxidative stress, serum GSH, was reported to be lowered by streptozotocin treatment **[12].** The MDA in the untreated streptozotocin rat increased dramatically **[13],** and the GSH activity significantly decreased in comparison to the control groups **[14].**

Improvement in pancreatic structure was evident in group V, possibly as a result of vitamin E's antioxidant properties, which reduce oxidative stress and shield pancreatic cells from the creation of free radicals while promoting cellular regeneration and proliferation **[15].** The body naturally produces antioxidants that are needed for defence against free radicals and protection from oxidative stress, and vitamin-E was thought to augment these levels **[3].** Other harmful stimuli including hypoxia, TNF-, and oxidative stresses like nitric oxide, hydrogen peroxide, and superoxide can also induce apoptosis, which antioxidants can prevent **[16].** Antioxidants' therapeutic role in the reduction of inflammatory cytokines in necrotizing tissues **[17].**

In the current study, group IV demonstrated a considerable rise in pancreatic IL-1B and IL-6 levels, indicating a marked inflammatory cellular infiltration. This increase was caused by the action of streptozotocin, which increased the release of all inflammatory mediators and produced targeted inflammatory responses that released TNF, IL-1, IL-6, and IL-10, indicating that pancreatic acinar cells are the primary source of proinflammatory and anti-inflammatory cytokines. [**18].**

Streptozotocin regulates transcription factors for inflammatory regulation such as nuclear factor κB, activator protein-1, inducing pancreatitis, and infiltration of inflammatory cells to the lymphocytes which played the key role in the progression of pancreatic damage [**19, 20].**

In the present study, group V showed marked reduction of serum IL-1B and IL-6 levels. Antioxidants are beneficial in inhibition of the proinflammatory cytokines such as TNF-α, IL-1B, IL-6, and IL-1 **[15].**

Conclusions: It could be concluded that vitamin-E has significantly ameliorated Streptozotocin-induced alterations in the pancreas of adult male Albino rats.

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Author contributions: All authors contributed equally in producing the work. They designed and performed the research. They analyzed and wrote the paper.

REFERENCES

1- Ping S, Gabriela O, Yan T, Qian H, Peter F, Jurgen D, Angelika G, Schmitt B, et al. Streptozotocin Impairs Proliferation and Differentiation of Adult Hippocampal Neural Stem Cells in Vitro-Correlation With Alterations in the Expression of Proteins Associated With the Insulin System. Diabetes. Frontiers in Aging Neuroscience, 2018; 118: 765-7772.

2- Shadia A, Sayeda M, Hala H, et al. The Possible Protective Effect of Vitamin E on Pancreas of Adult Male Albino Rats Treated with Bleomycin Sulfate. Med. J. Cairo Univ., 2021; 89 (1): 363-372.

3- Heba N, Eman E, Ahmed K. Osama A, et al. Study of the toxic effect and safety of vitamin E supplement in male albino rats after 30 days of repeated treatment. Heliyon J., 2019: 5 (10): 1-7.

4- Amany R, Ragab M, Nabila M.A., Elham A, et al. The effect of lead toxicity on male albino rats reproduction with ameliorate by vitamin E and pumpkin seeds oil. Benha Medical Journal. 2019: 28 (1) 43-52.

5- Masamune A, Watanabe T, Kikuta K, Satoh K, Shimosegawa T. NADPH oxidase plays a crucial role in the activation of pancreatic stellate cells. Am. J. of Physiol. Gastrointest. Liver Physiology. 2008; 294: 99-108.

6- Shalbueva N, Mareninova O.A, Gerloff A, et al. Effects of oxidative alcohol metabolism on the mitochondrial permeability transition pore and necrosis in a mouse model of alcoholic pancreatitis. Gastroenterology. 2013; 144:437-446.

7- Khadijah S. Balamash, Huda M. Alkreathy , Elham H. Al Gahdali et al., Comparative biochemical and histopathological studies on the efficacy of metformin and virgin olive oil against streptozotocin-induced diabetes in sprague-dawley rats. Journal of Diabetes Research. 2018; 4692197.

8- Ghada Z A. Effect of vitamin c and/or vitamin e on kidney, liver and brain functions of streptozotocin-induced diabetic rats. The Egyptian Journal of Hospital Medicine. 2013; 53: 799-808. DOI: 10.12816/0001642.

9- Ioanna A, Ioanna E, Anastasios T, Chrysi K, Ourania A. Nikolaos T. et al. Effect of Oxidative Stress and Antioxidant Therapies on Pancreatic β-cell Dysfunction: Results from in Vitro and in Vivo Studies. Biochemistry & Molecular Biology J., 2021: 28 (7): 1328:1346..

10- Ayman A, El-Enei S, Mohamed E, et al. Can vitamin-E protect the pancreas against ethanol-induced alterations in adult male albino rats? A histo-morphometric and immune-histo-chemical study. Kasr Al Ainy Medical Journal. 2021; 23: 43-53.

11- Akbarzadeh A, Norouzian A.D, Mehrabi M.R, et al. Induction of diabetes by streptozotocin in rats. Indian Journal of Clinical Biochemistry J., 2007; 22 (2): 60-64.

12- Periyar S, Palanisamy A, Subban K, Sharida F, Murugesan K, et al. Protective Nature of Mangiferin on Oxidative Stress and Antioxidant Status in Tissues of Streptozotocin-Induced Diabetic Rats. Pathophysiology, ISRN pharmacology J., 2013; 1-10.

13- Marjan K, Masoumeh A, Morteza A, et al. Efficacy of melatonin in restoring the antioxidant status in the lens of diabetic rats induced by streptozotocin. Journal of Diabetes and Metabolic Disorders, 2019; 8(2): 543-549.

14- Serino A and Salazar G. Protective role of polyphenols against vascular inflammation, aging and cardiovascular disease, J. Functional Food. 2018; 11 (1): 103662-73.

15- Ergul B K. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress. Kurutas Nutrition Journal, 2016; 15: 71.

16- Wallert M, Bormel L, Lorkowski S, et al. Inflammatory diseases and vitamin E - what do we know and where do we go? Mol. Nutr Food Res. 2021; 65: 20009-200020.

17- Liu W, Jiang H.L, Cai L.L, Yan M, Dong S.J, and Mao B, et al. Tanreqing Injection Attenuates Lipopoly-saccharide-Induced Airway Inflammation through MAPK/NF-kB Signaling Pathways in Rats Model. Evidenced Based Complement Alternat. Med. J., 2016; 15: 346-360.

18- Mangan M.S, Olhava E.J, Roush W.R, Seidel H.M, Glick G.D, Latz E, et al. Targeting the NLRP3 inflammasome in inflammatory diseases. Nature Review Drug Discovery. 2018; 17: 588–606.

19- Melanie Z, Maria W, Stefan L, Karlheinz P, et al. Cardiovascular and Metabolic Protection by Vitamin E: A Matter of Treatment Strategy. MDPI Journal. 2020; 9: 935-939.

20- Zhu B.T. Pathogenic Mechanism of Autoimmune Diabetes Mellitus in Humans: Potential Role of Streptozotocin-Induced Selective Autoimmunity against Human Islet *β*-Cells. Cells j., (2022) ; 11(3): 492. Doi: 10.3390/cells11030492.