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Pancrease-Protective Effects of Arabic Gum on Diabetic Type2 Streptozotocin-Induced in Albino Mice

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ABSTRACT

Diabetes mellitus is a worldwide disease, considered one of the major causes of morbidity and mortality. Arabic gum is used in pharmaceutical , cosmetic , food industries and traditional treatments. The present study was designed to investigate the protective effect of Arabic gum on pancrease, its antioxidant and anti-apoptosis effects in diabetic mice type 2. Mice were divided into three groups , first group served as control group , diabetes mellitus type 2 for second and third groups was induced by intraperitoneal injection of 50 mg/kg of STZ for 3 consecutive days . The second group stayed as untreated diabetic mice and the third group contained diabetic mice received daily oral dose of (15% w/v) of Arabic gum for 12 consecutive days . Results showed that treated diabetic mice with Arabic gum revealed decrease in blood glucose level compared to untreated diabetic mice. Histopathological results showed severe alterations in pancrease represented by distorted and minimized islets of Langerhans , severe pathological scors and interlobular fibrosis. Whereas, treated diabetic group with Arabic gum showed less alterations , healthy islets , improved pathological score and minimal fibrosis. Immunhistochemistry results showed slight secretion of insulin in islets of untreated diabetic group , intense reaction for oxidative stress and apoptosis. Whereas, moderate secretion of insulin in islets of treated diabetic group with Arabic gum and negative reaction for oxidative stress and apoptosis. It could be concluded that Arabic gum has hypoglycemic effect ,it could reduce pathological effects , oxidative stress and apoptosis from diabetic pancrease .

Keywords : Pancrease – diabetes – Arabic gum – oxidative stress- apoptosis.

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INTRODUCTION

According to the World Health Organization (WHO), there are approximately 160,000 diabetics world wide, the number of diabetics has double in the last few years and is expected to double once again in the year 2025 (Beretta, 2001). Due to its high prevalence and potential deleterious effect on a patient physical and psychological state, diabetes is a major medical concern (Macedo et al., 2002).The disease remains incurable and can only be controlled with drugs. pathogenesis of type 2 diabetes is complex involving progressive development of insulin resistance in liver and peripheral tissues accompanied by a defective insulin secretion from pancreatic beta cells leading to induce hyperglycaemia (an abnormally high amount of glucose levels in blood) (Cheng,2005) .

The induction of diabetes in the experimental animals using chemicals which selectively destroy pancreatic beta cells is very convenient and simple to use. The most usual substances to induce diabetes in the rat are alloxan and streptozotocin. Streptozotocin (STZ, 2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose) is synthesized by *Streptomycetes achromogenes* and is used to induce both insulin-dependent and non-insulin-dependent diabetes mellitus (Szkudelski,2001). STZ may be given in multiple low doses, such treatment is used predominantly in the mice for induction of diabetes type 2 that mediated by the activation of immune mechanisms (Ziegler et al.,1984& Wright and Lacy 1988) .

Arabic gum (*Gum acacia*) is a dietary fibrous heteropolysaccharide obtained from either Acacia senegal or *Acacia seyal* trees, which are cultivated in the Sudan as a cash crop in agroforestry systems (Lelon et al. 2010). It is a polysaccharide with branched chains of (1-3) linked β-D-galactopyranosyl units containing α-L-arabinofuranosyl, α-Lrhamnopyranosyl, β-D-glucuronopyranosyl and 4-O-methyl-β-D-glucuronopyranosyl units . GA is rich in Ca2+, K+ and Mg2+ (Nasir, 2013). Various Pharmacological activities on Arabic gum exudates offers protection against cyclophosphamide induced urinary bladder cytotoxicity (Adel et al., 2009). Scavenging of nitric oxide by gum arabic has been reported to limit the acetaminophen-induced hepatotoxicity in mice (Gamal El-din et al., 2003). Other studies have documented the antioxidant properties of gum arabic in a variety of animal model system (Rehman et al., 2004) . Antioxidant potential and free radical scavenging activity by pod extracts of Arabic gum (Hooda et al., 2012) and anti-diabetic effect (Faid, 2013).

The focus of this study has been systematically determine whether Arabic gum can protect pancrease against STZ destruction , activate β cells to secrete insulin , hence reduce resulted hyperglycemia and evaluate the antioxidant effect of Arabic gum in pancrease.

MATERIAL AND METHODS

Animals and experimental design:

Thirty male Swiss albino mice (25 ± 30 g) were randomly divided into three groups, ten mice per each group. Mice were housed in polypropylene cages inside a well-ventilated room at 22 ± 1 °C and 12-h periods of light and dark, with free access to clean water and commercial mice food. All mice were fasted for 20 h before experimental induction of diabetes. The first group received cold citrate buffer (pH 4.5) and served as control. Experimental diabetes was induced in the second and third groups via intraperitoneal injection of 50 mg/kg streptozotocin for 3 consecutive days(Sigma Chemicals Co., St. Louis, MO, USA) dissolved in cold 0.01 M citrate buffer, pH 4.5. The experiments were approved by state authorities and followed Saudi Arabian rules for animal protection. The induced diabetes was confirmed via colorimetric detection of blood glucose levels after three days of last STZ injection in blood collected from tails of study animals. After three days of induction of diabetes, animals within the third group received a daily dose of Arabic gum (15% w/v) orally for twelve consecutive days. At the end of experimental period, animals were sacrificed.

Biochemical analysis:

Blood was collected from the heart of animals into centrifuge tubes, samples were centrifuged at 3000 g for separation of serums and stored at −8°C until assay.. Plasma was assayed for levels of glucose.

Histological examination and pancrease injury:

Pieces of pancrease were freshly prepared, fixed in 10% neutral buffered formalin, and then embedded in paraffin. Sections were cut and stained with hematoxylin and eosin and Masson's trichrome

The stained tissue sections were scanned for pancrease tissue abnormalities.The islets areas were measured with Motic 200 image analyzer (Hong Kong, China).

Pathological scoring system of pancrease:

Pancrease sections stained with HE and M.Tr. were examined for pathological score of the following criteria: edema, inflammation, hemorrhage, necrosis in acini and interlobular fibrosis . Scoring values registered according to table 1.

Table-1 Scoring criteria for the assessment of histopathological changes in pancrease

Item	Score
Edema	0= no edema 1= focal edema 2= diffuse edema 3= focal and diffuse edema
Inflammation	0= no inflammatory cells 1= 2-10 leucocytes / 400 hpf 2= 11-20 leucocytes / 400 hpf 3= 21-30 leucocytes / 400 hpf 4= > 30 leucocytes / 400 hpf
Hemorrhage	0= no hemorrhage 1= present
Degeneration	0 = no degeneration 1 =periductal parenchymal destruction 2 = focal parenchymal degeneration 3 = diffuse loss of lobules 4 = severe loss of lobules
Fibrosis	0= no fibrosis 1= minimal fibrosis 2= mild fibrosis 3= moderate fibrosis 4= extensive fibrosis

Immunohistochemistry:

Paraffin embedded pancrease sections mounted on coated slides were deparaffinized in xylene and rehydrated in descending grades of alcohol and finally distilled water. Sections then were heated in citrate buffer (pH 6) within microwave for 5 min. After that sections were washed with PBS buffer for 5 min and incubated in peroxidase blocking solution for 10 min. Sections were incubated overnight at 4 °C in diluted primary antibody (anti-insulin ab6995, anti-caspase3ab13585, anti-malondialdehyde ab194225) then incubated in biotinylated goat anti-mouse (ab128976) as secondary antibody for 30 min, followed by incubation in avidin–biotin complex for 30 min, then incubated in DAB (ab64238) as chromogenic substrate for ten minutes. The stained sections were counter stained with Mayer's hematoxylin, and dehydrated within ascending grades of alcohol and cleared with two changes of xylene, mounted with cover slip based on DPX mountant. All reagents were purchased from Abcam company (Cambridge, UK). Pancrease sections were examined under microscope for brown immunoreactivity color and photos at magnification of x 400. Reaction for gene –expressions scored as, negative, slight, moderate and intense.

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Statistical Analysis:

The data were expressed as mean±SEM (standard error of mean). Statistical significance of the control and experimental groups was evaluated by SPSS16.0. Comparison was made between control and experimental groups.

RESULTS

Untreated diabetes mice group showed significance decrease in weights compared to control group . Whereas, treated diabetes mice group with Arabic gum showed significant weight increase compared to untreated diabetes mice group and insignificant difference compared to control group (table 2). Untreated and treated diabetic groups showed significant glucose level increase compared to control group. However , treated diabetic group with Arabic gum showed significant glucose level decrease compared to untreated diabetic group (table 2).

Table 2 : showed weight, glucose levels and islets area in control , untreated diabetes and treated diabetes with Arabic gum mice groups

Item	Control	Diabetes	Diabetes+Arabic Gum
Weight (g)	29±2.7	18.4±0.17^{*a}	27±1.5^{*b}
Glucose(mg/dl)	112±0.2	444±2.4^{*a}	148±0.8^{*a,b}
Islets area (µm^3)	1.6±0.3	0.3±0.06^{*a}	1.2±0.2^{*b}

Data = mean ± standard error

^{*a} = significant difference between control and experimental group

^{*b} = significant difference between diabetes and experimental group

Control pancrease section showed normal architecture of exocrine acini, small intralobular ducts are scattered throughout. Larger interlobular ducts are surrounded by dense connective tissue. Endocrine Islets of Langerhans are the lighter staining areas. The islets contain alpha cells secreting glucagon, beta cells secreting insulin, and delta cells secreting somatostatin (Fig.1).

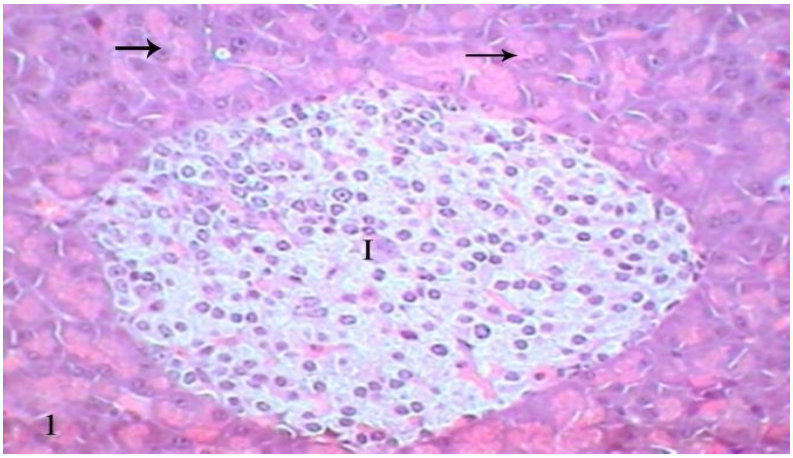

Fig. 1: Control pancrease showed normal structure (I) healthy islet of Langerhans and (arrows) normal pancreatic acini. (HX& E x Mag -400).

Pancreatic sections of untreated diabetic mice showed severe alterations manifested by distorted and minimized islets (area= 0.3µm^3) compared to control group (table 2), focally edema (scored =3) , presence of hemorrhage (scored=1) (table 3 & fig. 2a). Moreover, severe leucocytic inflammation (scored=4) , focal degeneration in parenchymal acini (scored=2) and necrotic foci in islets (table 2 & fig. 2b). Untreated diabetic pancreatic section stained with M.Tr revealed depositions of concentric layers of blue collagenous fibers (scored=3) moderate interlobular fibrosis (fig. 2c).

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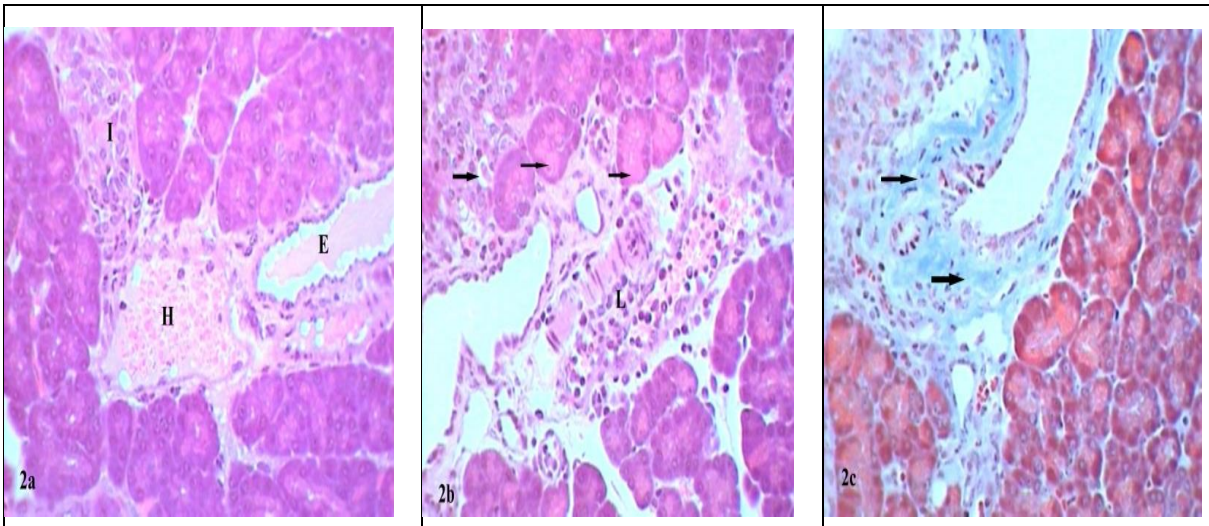

Fig. 2: Diabetic pancrease STZ- induced , (2a) showed edema (E), haemorrhage in dilated duct (H) and distorted islet (I). (2b) showed degenerated acini (arrows) , leucocytic infiltration (L) and necrosis in distorted islet (arrow). (HX& E x Mag -400). (2c) showed concentric layers of blue stained collagenous fibers . (M.tr. x Mag-400).

Pancreatic sections of treated diabetic mice with Arabic gum showed marked improvements compared to untreated diabetic mice represented by healthy islets (area=1.2 μm^3), focal edema (scored =1) and without hemorrhage and degeneration in acini (scored = 0) (table 2 & fig. 3a). Sections stained with M.Tr. showed slight inflammation (scored=1) and minimal fibrosis (scored=1) (table 2& fig. 3b).

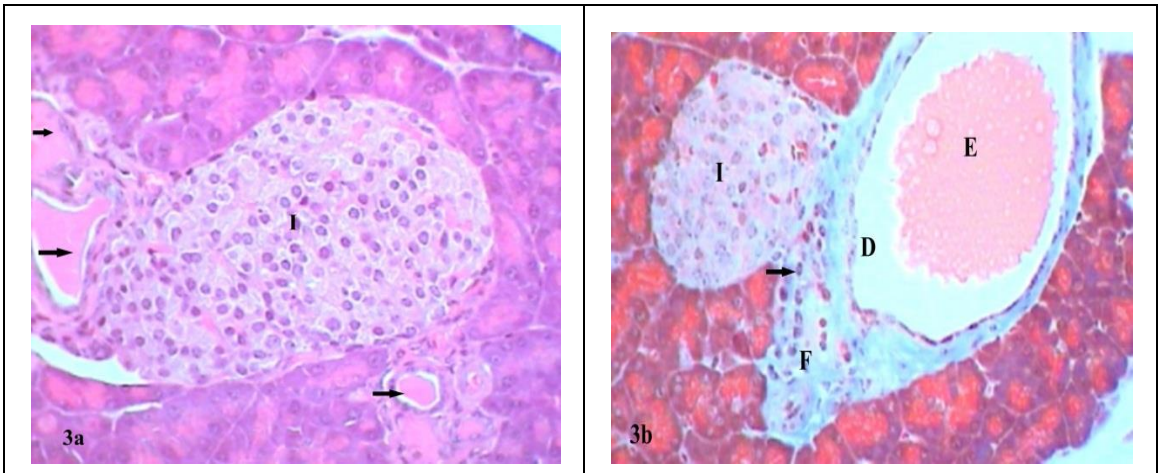

Fig. 3: Diabetic pancrease STZ-induced treated with Arabic gum, (2a) showed healthy islet (I) , edema (arrows) and normal acini. (HX& E x Mag -400).(2b) showed minimal fibrosis (F) , edema (E) in duct (D) , healthy islet (I) and a few number of inflammatory cells (arrow). (M.tr. x Mag-400).

Immunohistochemistry:

Control pancreatic sections showed intense immunoreaction for anti-insulin, negative immunoreaction for anti-caspase and anti-MDA (figs.4a,5a and 5c). Whereas, untreated diabetic sections showed slight immunoreaction for anti-insulin , intense immunoreaction for anti-caspase3 and anti-MDA compared to control section (figs. 4b,5b and 6b). However, treated diabetic pancreatic sections showed moderate immunoreaction for anti-insulin and negative immunoreaction for anti-caspase3 and anti-MDA (figs. 4c, 5c and 6c) (table 3).

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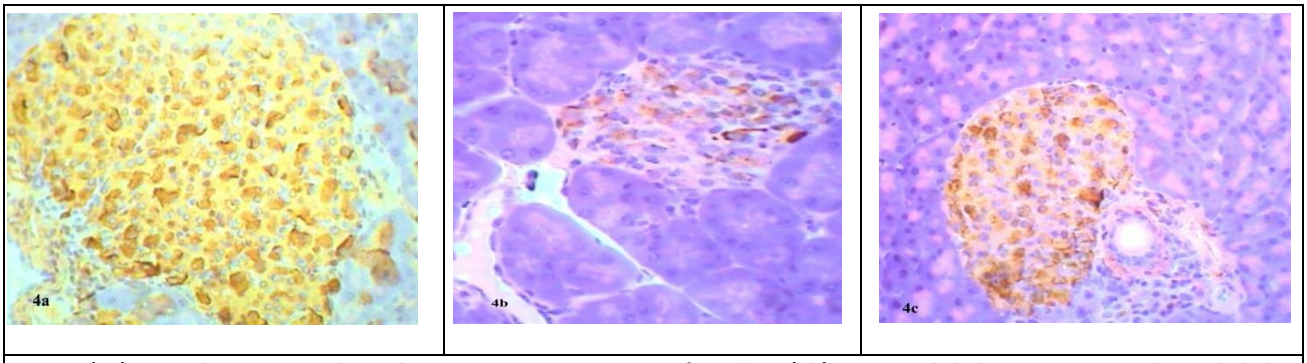

Fig. 4: (4a) control pancrease showed intense immunoreaction of insulin , (4b) untreated diabetic pancrease showed slight immunoreaction of insulin , (4c) treated diabetic pancrease with Arabic gum showed moderate immunoreaction of insulin (ABC immunohistochemistry method , Mag-400).

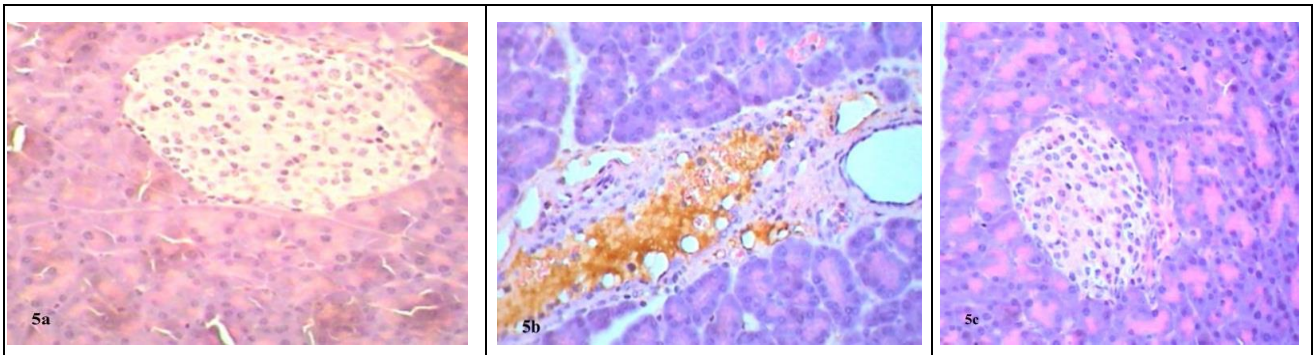

Fig. 5: (5a) control pancrease showed negative immunoreaction of Caspase 3 , (5b) untreated diabetic pancrease showed intenseimmunoreaction of Caspase 3, (5c) treated diabetic pancrease with Arabic gum showed negative immunoreaction of Caspase 3 (ABC immunohistochemistry method , Mag-400).

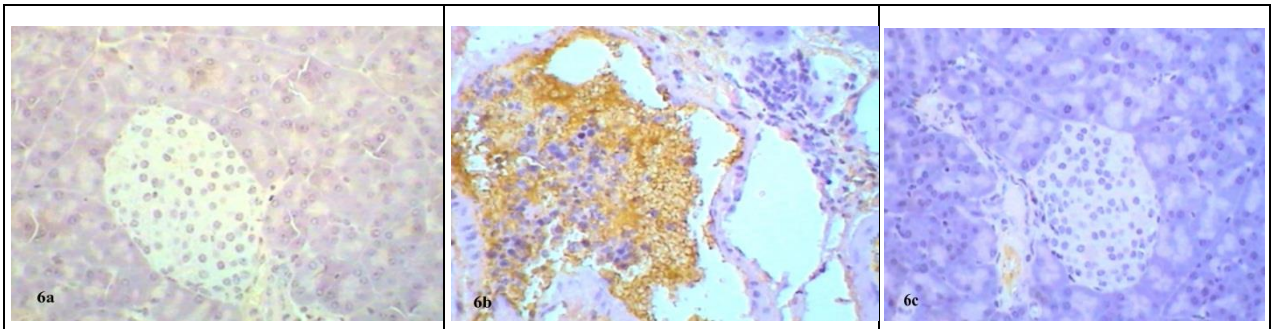

Fig. 6: (6a) control pancrease showed negative immunoreaction of MDA , (6b) untreated diabetic pancrease showed intenseimmunoreaction of MDA, (6c) treated diabetic pancrease with Arabic gum showed negative immunoreaction of MDA (ABC immunohistochemistry method , Mag-400).

Table 3 : showed pathological score of pancrease in control, untreated diabetes and treated diabetes with Arabic gum

Items	Control	Diabetes	Diabetes + gum
Edema	0±0	3±0.1	1±0.1
Hemorrhage	0±0	1±0	0±0.1
Inflammation	0±0	4±0.2	1±0.5
Degeneration	0±0	2±0	0.8±0.3
Fibrosis	0±0	3±0.1	1±0.2

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DISCUSSION

Diabetes mellitus is a serious metabolic disorder of endocrine system effecting considerable population. Diabetes is a disorder indicating insufficient insulin production or increasing resistance to its performance (Singh et al., 2011 & Joseph and Jini, 2011). Pharmacologically, streptozotocin and alloxan are widely used for the induction of diabetes in the experimental models. These drugs selectively destroy pancreatic beta cells while leaving the remaining pancreatic function intact and are used in small animals (mice, rabbits and cat) (Lasker and Raihan, 2010).

Low doses of STZ (35-50 m/kg) for 3-4 consecutive days induced diabetes type 2 in experimental animals ensured by blood glucose level increase (Srinivasan et al., 2005 & Srinivasan and Ramarao, 2007). In agreement with the previous studies , the present work showed that injection of 50 mg/kg for 3 consecutive days caused diabetes type 2 in mice induced elevating of glucose level.

In the current study , histological observations showed marked alterations in the untreated diabetic pancrease tissue represented by diminished islets and high scoring pathological system with existence of interlobular fibrosis , these findings agreed with (Nirmala et al., 2009; Ozdemir et al.,2009; Elkordy and Alshahrani,2015) who ensured that low doses for 3 consecutive of STZ caused damaged and shrunken islets with marked vacuolated cytoplasm and pyknotic nuclei in most of its endocrine cells. Many studies provide the evidence that STZ is a nitric oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, it was proposed that this molecule contributes to STZ-induced DNA damage Pancreatic B cells exposed to STZ manifested changes characteristic for NO action, i.e. increased activity of guanylyl cyclase and enhanced formation of cGMP . STZ is, however, not a spontaneous nitric oxide donor . This molecule is liberated when STZ is metabolized inside cells, but NO synthase is not required for this effect. However, the results of several experiments provide the evidence that NO is not the only molecule responsible for the cytotoxic effect of STZ. It was found to generate reactive oxygen species, which also contribute to DNA fragmentation and evoke other deleterious changes in the cells(Szkudelski, 2001).

Many studies proved that STZ- induced marked decrease in insulin antibody positivity in islets with increasing of free radical formation resulted in decrease in both antioxidant capacity and insulin activity (Lai, 2008; Hamdene et al., 2011). In agreement with the previous studies , immunohistochemistry of the current work proved that STZ caused decrease in insulin activity manifested by slight immunoreaction for insulin antibody , moreover , increasing of oxidative stress and apoptosis represented by intense reaction for MDA and Caspase 3 antibodies respectively.

(Grover et al., 2002 & Pal et al., 2013) revealed that Arabic gum exerted a significant hypoglycemic effect by initiating the release of insulin from pancreatic beta cells , that agreed with the present findings that treated diabetic mice with Arabic gum showed significant glucose level decrease. Moreover, the current work proved that arabic gum treatment of diabetic mice reduced histopathological alterations STZ-induced in pancrease represented by healthy islets and decreasing in pathological score system compared to untreated diabetic group.

(Pal et al., 2013) added that in order to protect the body from reactive oxygen stress (ROS) effects there are two defense mechanisms against ROS, primary defense by antioxidant enzymes that capable of catalytically removing the ROS and the second mechanism by free radical scavengers and hence suggested that Arabic gum could be improving the availability of insulin and reveal primary and secondary defense against ROS. The present results revealed that treated diabetic mice with Arabic gum showed negative immunoreaction for MDA and Caspase 3 that give an evidence that Arabic gum has antioxidant and anti-apoptic effects that agreed with the previous study.

Allover results concluded that consecutive 3 low doses of STZ caused partial destruction of beta cells in islets of langerhas that induced diabetes type 2, hence , decrease of insulin secretion resulted in increase of blood glucose level . In addition , STZ increased oxidative stress and apoptosis in pancrease tissue . Arabic gum is a natural product can activate beta cells , improve islets , reduce pathological changes and hence, reduce hyperglycemia . In addition Arabic gum has antioxidant and anti-apoptic effects.

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REFERENCES

[1] Abd-Allah A., Al- Yahya A., Al-Majed A., Gado A., Daba M., Al-Shabanah O., and El-Azab A. 2009. Acacia senegal gum exudate offers protection against cyclophosphamide induced iurinary bladder cytotoxicity. Oxidative Medicine and Cellular Longevity, 2: 207-213.

[2] Beretta A. 2001. Campanha de prevencao e diagnostic do diabetes realizada pela UNIARARAS e prefeitura municipal na cidade de Araras. Laes and Haes, 22: 188-200.

[3] Cheng D.2005. Prevalence, predisposition and prevention of type II diabetes. Nutr Metab, 18 : 2-29.

[4] El-Kordy E., and Alshahrani A. 2015 . Effect of genistein, a natural soy isoflavone, on pancreatic beta -cells of streptozotocin-induced diabetic rats: Histological and immunohistochemical study. Journal of Microscopy and Ultrastructure, 3 :108–119.

[5] Faid S. 2013. Biological Study on the Beneficial Effects of Arabic Gum on Biological Parameters of Hyperglycemic Albino Rats. Life Science Journal, 10:3570-3579.

[6] Gamal El-din M., Mostafa M., Al- Shabanah A., Al-Bekairi M. and Nagi N. 2003. Protective effect of arabic gum against acetaminophen-induced hepatotoxicity in mice. Pharmacol Res.,48: 631-365.

[7] Grover J. , Yadav S, Vats V. 2002. Medicinal plants of India with anti-diabetic potential. Journal of Ethnopharmacology,81:81-100.

[8] Hamdena K., Jaouadib B., Carreauc S., Aouidetd A., Elfekia A.2011. Therapeuticeffects of soy isoflavones on a-amylase activity, insulin deficiency,liver–kidney function and metabolic disorders in diabetic rats. NatProd Res, 25:244–55.

[9] Hooda S, Pal R., Bhandari A. and Singh J. Antioxidant potential and free radical scavenging activity by pod extracts of Acacia Senegal. Int. J Phar Chem and Biological Sciences,2: 500-506.

[10] Joseph B. and Jini D. 2011.Insight into the hypoglycaemic effect of traditional indian herbs used in the treatment of diabetes. Res. J. Med. Plant, 5:352-376.

[11] Lai H.2008. Antioxidant effects and insulin resistance improvement ofchromium combined with vitamin C and E supplementation for type2 diabetes mellitus. J Clin Biochem Nutr,43:191–8.

[12] Lasker P. and Raihan R. 2010. Animal Research In Diabetes And Ethical Consideration. Bangladesh Journal of Bioethics, 1: 20-23.

[13] Lelon K., Jumba O., Keter K., Chemuku W., Oduor O.2010. Assessment of physical properties of gum Arabic from Acacia senegal varieties in Baringo District, Kenya. Afr J Plant Sci, 4: 95-98.

[14] Macedo S, Capelletti M., Mercadante S., Padovani R., Spadella T. 2002. Role of metabolic control on diabetic nephropathy. Acta Cir Bras, 17: 37 –5.

[15] Nasir O. 2013 . Renal and Extrarenal Effects of Gum Arabic (Acacia Senegal) – What Can be Learned from Animal Experiments? Kidney Blood Press Res ,37:269-279.

[16] Nirmala A., Saroja S., Vasanthi R., Lalitha G.2009. Hypoglycemic effect of Basella rubra in streptozotocin induced diabetic albino rats. J Phar-macogn Phytother,1:25–30.

[17] Ozdemir O., Akalin P., Baspinar N., Hatipoglu F.2009. Pathological changesin the acute phase of streptozotocin-induced diabetic rats. Bull VetInst Pulawy ,53:783–90.

[18] Pal R,. Hooda M., Bhandari A., Singh J. 2013. Antidiabetic Activity of Acacia Senegal Pod Extract in Streptozotocin-Induced Diabetic Rats. International Journal of Indigenous Medicinal Plants,46: 1400-1404.

[19] Rehman U., Codipilly N. and Wapnir A.2004. Modulation of small intestinal nitric oxide synthase by gum arabic. Exp Biol Med., 229: 895-901.

[20] Singh U., Kochhar A., and Singh S. 2011.Blood glucose lowering potential of some herbal plants. Journal of Medicinal Plant Research, 5:4691-4695.

[21] Srinivasan k., and Ramarao p. 2007. Animal models in type 2 diabetes research: An overview . Indian J Med Res., 125: 451-472.

[22] Srinivasan K. , Viswanad V. , Asrat L. , Kaul C. , Ramarao P. 2005. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacological Research, 52: 313–320.

[23] Szkudelski T. 2001. The Mechanism of Alloxan and Streptozotocin Action in B Cells of the Rat Pancreas. Physiol. Res., 50: 536-546.

[24] Wright R., Lacy E.1988.Synergistic effects of adjuvants, endotoxin, and fasting on induction of diabetes with multiple low doses of streptozotocin in rats. Diabetes, 37: 112-118.

[25] Ziegler M., Ziegler B., Hehmke B.1984. Severe hyperglycaemia caused autoimmunization to beta cells in rats. Diabetologia, 27: 163-165.