Original Article

Gamma Amino Butyric Acid Ameliorates Jejunal Oxidative Damage in Diabetic Rats

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ABSTRACT

Introduction: The small intestine exhibits morphological and functional changes in streptozotocin (STZ)-induced diabetes. This study was carried out to clarify the role of gamma-aminobutyric acid (GABA) in ameliorating the histopathological changes and the oxidative stress in small intestine of the diabetic rats. **Material and Methods:** For this purpose, 40 adult male Swiss albino rats (*Sprague dawley* strain) were divided into 4 groups (10 animals in each group); the control group, GABA treated group, the diabetic group and the fourth group was subjected to induction of diabetes and GABA treatment. All animals were sacrificed and specimens from jejunum were removed and processed for the histopathological examinations, cytomorphometric analysis and oxidative stress assessment. **Results:** In diabetic rats, Jejunal sections from the diabetic rats exhibited pleomorphism of villi, inflammatory cellular infiltration and edema in the villous core with lymphoid hyperplasia, an increase in goblet cells number was also observed with disappearance of Paneth cells or they were degranulated, increased crypt numbers and mucosal height and increased thickness of muscularis layer. Moreover, there was an increase in the oxidant biomarkers with decline in the antioxidant enzymes in Jejunal sections of the diabetic rats. **Conclusion:** Treatment of rats with GABA ameliorated the observed histopathological alterations, morphometric changes and improved the oxidant/antioxidant statusof the jejunum in diabetic rats. Based on these results, it was concluded that GABA administration to rats ameliorated diabetes-induced jejunal histopathological and biochemical alterations.

Key words: Diabetes, Jejunum, Morphometry, Paneth Cells, Gamma-Aminobutyric Acid.

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INTRODUCTION

Diabetes mellitus(DM) is a chronic, heterogeneous and multifactorial disease; it is characterized by hyperglycemia due to impaired insulin secretion and aberrant glucagon secretion resulting from changes in the pancreatic islet cellsfunctions.[1] Most of diabetic patients are non-insulin- dependent while small proportions have insulin-dependent diabetes,[2] type 1 diabetes mellitus (T1DM) is a chronic disease results in a cellular-mediated autoimmune destruction of β-cells of the pancreas, insulin deficiency and hyperglycemia are the main outcomes of it,[3] while in type 2 diabetes or adult-onset diabetes patients have insulin resistance and usually have relative insulin deficiency.[4] Hyperglycemia is responsible for metabolic disturbances including oxidative stress which leads to injuries in the different cellular types of gastrointestinal tract and impair endogenous antioxidant defense system.[5] Oxidative stress due to auto-oxidation of glucose and decreased antioxidant defense is believed to play a role in the onset of DM and its complications.[6]

The small intestine is unique in its variety of cell types including enterocytes (small

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intestinal epithelial cells), goblet cells (for mucus secretion), entero-endocrine cells (for hormone secretion) and Paneth cells which are stem cells differentiate to form enterocytes.[7] Paneth cells located at the baseof the Lieberkühn glands[8] and they synthesize substances with antimicrobial properties such as α-defensins, lysozyme and phospholipase A_2in response to different stimuli.[9] Paneth cells also control the composition of endogenous flora andplay a role in the crypt formation and developmentof phagocytosis, digestion and detoxification processes.[10,11]

Gamma-amino butyric acid (GABA) is one of the main neurotransmitters in the central nervous system. It is produced from glutamate by decarboxylation via glutamic acid decarboxylase (GAD)activity and it creates biological signaling through activation of A and B types of GABA receptors.[12] GABA and GABA-A receptors are found in non-neuronal cells of visceral organs.[13] It is known that GABA is a transmitter of enteric interneurons and it regulates the gastrointestinal tract functions.[14] GABA is also found in the epithelial cells of rat jejunum[15] and it has immune inhibitory effectsthat may protect β-cells of pancreas from autoimmune destruction.[16]

MATERIAL AND METHODS

Streptozotocin treatment

Streptozotocin (STZ) purchased from Sigma-Aldrich, St. Louis, Missouri, USA, within the type of one gram vials. Type 1 diabetes mellitus was induced by single intraperitoneal injection of rats with freshly prepared STZ at a dose of 60 mg/kg dissolved in 0.1M cold citrate buffer pH 4.5.[17,18] Since STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were given 5% glucose for the next 24 hr to prevent hypoglycemia.[19] Blood glucose levels were determined using an Accu-check blood glucose meter (Roche Diagnostics, Basel, Switzerland). Three days following STZ injection the blood was withdrawn fromthe tail vein and glucose level was monitored. Rats with fasting blood glucose levels ≥ 250 mg/dl were considered as diabetic and included in the current study.

Treatment of rats with Gamma amino butyric acid

Gamma amino butyric acid (GABA) purchased from Sigma-Aldrich, St Louis, Missouri, USA in the form of 25 g vials was dissolved in distilled water and administered to rats daily by gastric gavages at doses of 200 mg/Kg body weight/day[20] for successive 3 weeks.

Animals

The present study was conducted on 40 adult male Swiss albino rats (*Sprague dawley* strain) (10 ± 2 weeks old; weighting 120 ± 10 g at the beginning of the experiment) purchased from the Egyptian company for Biological merchandise and Vaccines (Helwan, Cairo, EGYPT). They were kept for about 15 days, before the onset of the experiment under observation to be acclimatized to the laboratory conditions. Animals were housed collectively in plastic cages, maintained under standard conditions of light, ventilation, temperature and humidity and allowed free access of standard pellet diet and tap water. For biochemical analyses animals were sacrificed at 11:00 am ± 1 h. All animal procedures were carried out in accordance with the Ethics Committee of the National Research Centre followed to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health.

Animal groups

Experimental animals were randomly divided into 4 groups (n=10) treated in parallel and classified as following:

1. Control (C) group: Normal healthy rats administered 1ml distilled water daily during 3 weeks via gastric gavages.
2. GABA (G) group: Normal healthy rats administered GABA (200 mg/Kg body weight/day) daily during 3 weeks via gastric gavages.
3. Diabetic (DM) group: STZ-treated rats administered 1ml distilled water daily during 3 weeks via gastric gavages.
4. DM+GABA group: STZ-treated rats administered GABA (200 mg/Kg body weight/day) daily during 3 weeks via gastric gavages.
5. At the end of the third week rats were sacrificed after a fasting period of 12 hours next day to the last dose of GABA.

Biochemical analysis

Rats were anaesthetized with light ether, sacrificed and underwent laparotomy immediately. One cm segment of jejunum was dissected and washed with saline solution for the histological examinations. Other small segments of jejunum (10% w/v) were rapidly excised, homogenized in saline by using Teflon homogenizer (Glass-Col, Terre Haute, Ind., USA) and after centrifugation at 10 kg for fifteen minutes using refrigerated centrifuge (manufactured by K3 Centurion Scientific, Ltd, London, UK) the supernatant was used for the estimation of markers of oxidative stress. Chemicals and reagents

were purchased from Sigma-Aldrich, St Louis, MO, USA. Measurement of absorbance was accomplished by utilizing a T60 UV/VIS spectrophotometer, PG instruments, London, UK.

Assessment of oxidative stress in jejunal section

The extent of lipid peroxidation was assayed as described by Yoshioka *et al.*,[21] advanced oxidation protein products (AOPPs) was determined according to the method of Witko-Sarsat *et al.*,[22] catalase activity was determined as described by Sinha[23] and glutathione peroxidase (GSH-Px) activity was determined according to the method described by Necheles *et al*.[24]

Histopathologic examination

Tissue samples were fixed in 10% neutral buffered formalin and Carnoy's fixative and embedded in paraffin. The sections were cut at 5 µm and stained with hematoxylin and eosin (H&E) and Masson's trichrome stains for detection of the histological changes,[25] periodic acid Schiff's (PAS) method[26] and Feulgen's reaction.[27] Sections were examined for mucosal injury, inflammation, congestion and graded in a blinded manner by a pathologist. Sections stained with PAS were evaluated for goblet cell prevalence and Masson's trichrome stained sections were used to detect Paneth cells.

Morphometric and Cytometric analysis

For morphometric analysis, four whole circumference sections were examined. Also the number of villi and crypts per circumference, mucosal thickness, muscularis layer thickness and the outer intestinal boundary were estimated. Ten well orientated villi per segment were randomly selected and measured for villus height. Nuclear area, proliferation index and ploidy values of 100-150 cells within random fields were estimated in Feulgen's stained sections. All morphometric and cytometric parameters were done in the Pathology Department, National Research Centre employing the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, England,) that consists of Leica DM-LB microscope provided with JVC color video camera attached to a computer system Leica Q 500IW. The results were recorded as the mean and standard deviation values.

Statistical analysis

The SPSS/PC computer program (version 20.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis of the results. Data were analyzed using one-way analysis of variance (ANOVA) followed by Post Hoc LSD test. The data which obtained were expressed as mean ± standard deviation (SD). Differences were thought of statistically significant once P value less than 0.05.

RESULTS

Biochemical measurements

Changes of oxidant and anti-oxidant biomarkers

The results obtained in the present study showed that the administration of gamma amino butyric acid (GABA) (200 mg/Kg/day) to normal rats (GABA group) for 3 weeks showed insignificant changes in catalase (CAT), glutathione peroxidase (GSH-Px) activities, malondialdehyde (MDA) and advanced oxidation protein products (AOPPs) levels and they were within the normal values in the small intestine tissues Tables 1 and 2. The administration of STZ to rats (DM group) showed a significant increase in AOPPs and MDA values and a significant decrease in CAT and GSH-Px values Tables 1 and 2. Administration of GABA to STZ-treated rats (DM+GABA group) improved the status of the above mentioned parameters Tables 2 and 3 which was evidenced by a significant increase in the antioxidants combined with a significant decrease in the oxidant biomarkers levels in small intestine tissues compared to those of the diabetic group.

Histopathologic observations

Control and GABA treated groups

Light microscopic examination of the control group H&E-stained sections showed that the villi are covered with tall columnar cells (enterocytes) with oval basal vesicular nuclei and connective tissue core. Crypts of Lieberkühn are invaginations of mucosa between the bases of the villi. Goblet cells were seen in the PAS stained sections, they were present in between the enterocytes at intervals. Paneth cells were found at the base of the crypts as pyramidal shaped cells with prominent eosinophilic granules in Masson's trichrome stained sections Figure 1 A-C. The intestinal specimens obtained from the GABA treated group showed normal architecture of the villi and crypts Figure 1 D-F.

Diabetic group

Examined sections of this group showed villous and crypt hypertrophy, disturbed villous architecture was seen with some thick and other conical villi; other villi showed loss of epithelial coverings. Many of these villi appeared twisted or collapsed and others showed separation of the epithelium from the underlying connective tissue core. Inflammatory cellular infiltration and edema of the villous core were seen with lymphoid hyperplasia.

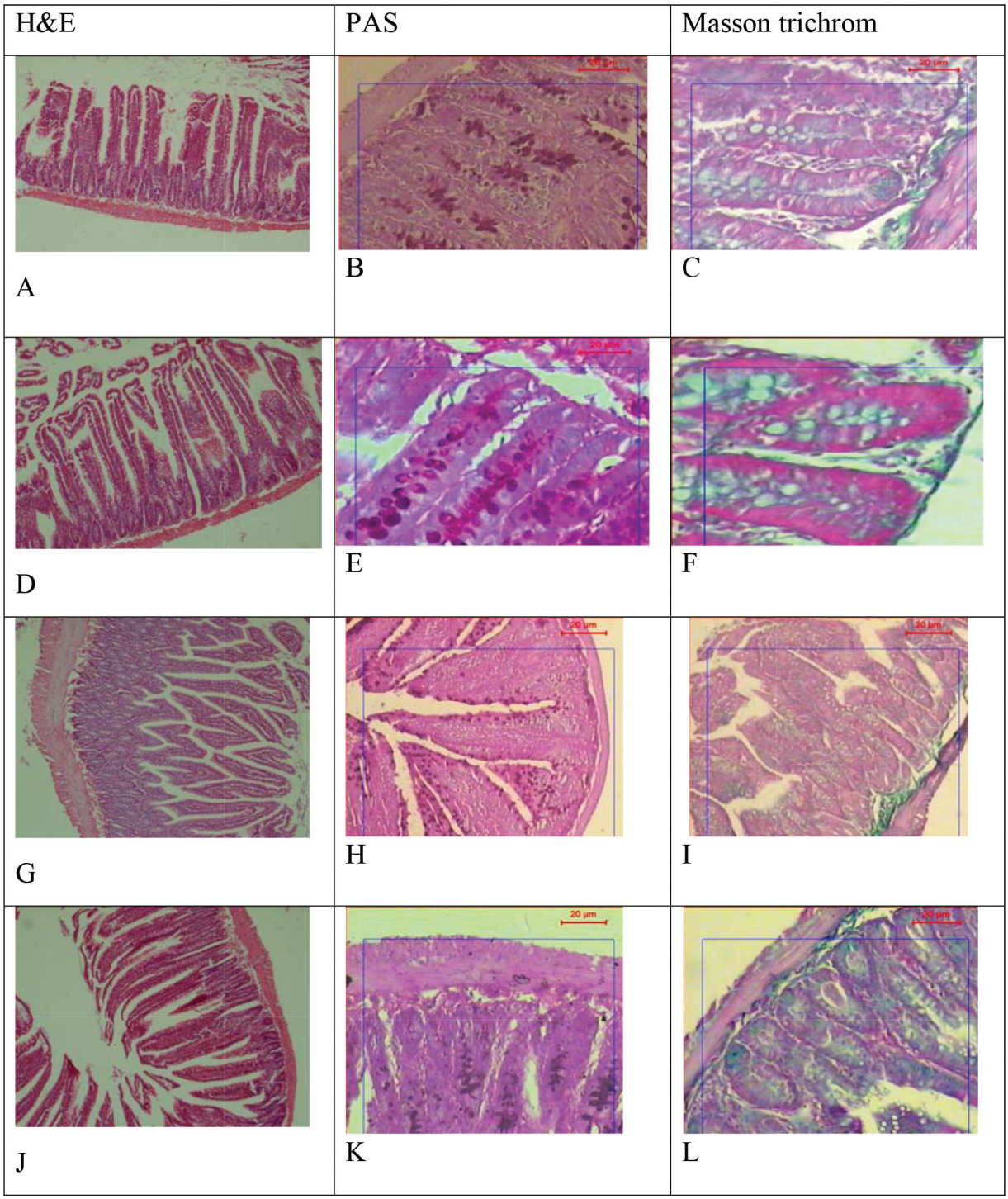
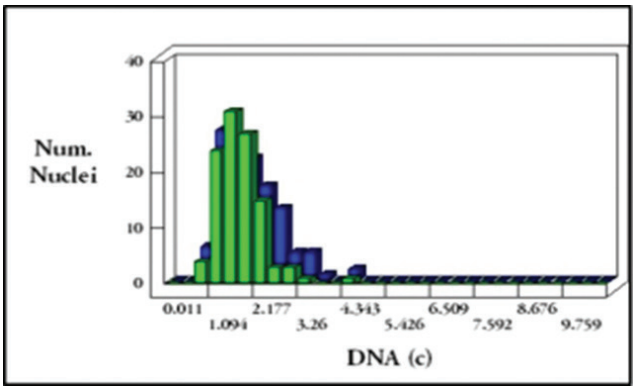


Figure1 –A-C Photomicrographs of jejunum sections from control rat showed that the villi are covered with tall columnar cells (enterocytes) with oval basal vesicular nuclei and connective tissue core. Crypts of Lieberkühn are invaginations of mucosa between the bases of the villi. Goblet cells were seen in the PAS stained sections, they were present in between the enterocytes at intervals. Paneth cells were found at the base of the crypts as pyramidal shaped cells with prominent eosinophilic granules in Masson's trichrome stained sections, (D-F) Photomicrographs of jejunum sections from GABA treated rat showed normal architecture of the villi and crypts, (G-I) Photomicrographs of jejunum sections from diabetic rat showed villous and crypt hypertrophy, disturbed villous architecture was seen with some thick and other conical villi; other villi showed loss of epithelial coverings. Many of these villi appeared twisted or collapsed and others showed separation of the epithelium from the underlying connective tissue core. Inflammatory cellular infiltration and edema of the villous core were seen with lymphoid hyperplasia. Goblet cells increased in number and Paneth cells disappeared or appeared degranulated, (J-L) Photomicrographs of jejunum sections from GABA treated diabetic rat slight villi hypertrophic changes with moderate lymphocytic infiltration. Goblet cells appeared normal and Paneth cells were of normal count and shape. (H&E, x400)



Graph 1: Cytometric analysis of the diabetic group sections showed that the majority of epithelial cell nuclei have diploid DNA values with medium proliferation index and minor population of tetraploid cells comparable to the control and GABA treated groups.

Goblet cells increased in number and Paneth cells disappeared or appeared degranulated Figure 1 G-I.

GABA treated diabetic group

Sections of diabetic and GABA treated rats showed slight villi hypertrophic changes with moderate lymphocytic infiltration. Goblet cells appeared normal and Paneth cells were of normal count and shape Figure 1 J-L.

Morphometric and Cytometric analysis

Measured morphometric parameters of the examined sections showed a significant increase in the number of villi and crypts in the diabetic group. The mucosal height, the muscle layer thickness and the outer intestinal circumference increased significantly in the diabetic model compared to both the control and GABA groups demonstrating the effects of diabetes on the intestinal mucosa. The number of villi and crypts approximate somewhat near to the control value in the GABA treated diabetic group. Also, the mucosal height, the muscle layer thickness and the outer intestinal circumference were significantly lower than those of the diabetic group Table 3. Cytometric analysis of the diabetic group sections showed that the majority of epithelial cell nuclei have diploid DNA values with medium proliferation index and minor population of tetraploid cells comparable to the control and GABA treated groups. However, GABA treated diabetic group showed that the majority of epithelial cell nuclei have diploid DNA values with low proliferation index Table 4 and graph 1. The mean nuclear area was lower in GABA treated diabetic group as compared to the other groups.

Table 1: Influence of GABA administration to different animal groups on the oxidant biomarkers values in the small intestine tissues of STZ-treated rats

Parameter / Groups	AOPPs (µmol/L)	MDA (nmol/g)
Control	164.90±6.59	112.50±7.65
GABA	158±5.19	103.80±11.63
	-8%	-8%
P1	NS	<0.05
DM	176.80±8.23	131.50±4.99
	7%	17%
P1	<0.01	<0.001
DM+GABA	135.80±10.14	107.30±6.80
	-18	-5%
P1	<0.001	NS
P2	<0.001	<0.001

Values are expressed as Means ± Standard Deviation (n=10). Values between brackets show percentage of change from Control. P1: significance vs control. P2: significance vs respective group (DM) not receiving GABA. NS: non-significant. P < 0.001 highly significant

Table 2: Influence of GABA administration to different animal groups on the antioxidants biomarkers values in the small intestine tissues of STZ-treated rats

Parameter / Groups	GSH-Px (GSH /min/g fresh tissue)	Catalase (µmol of H_2O_2 consumed/min/mg protein)
Control	16.62±0.37	13.37±0.74
GABA	16.50±0.46	13.66±1.38
	-0.7%	2%
P1	NS	NS
DM	13.30±0.95	4.36±0.43
	-20%	-67%
P1	<0.001	<0.001
DM+GABA	16.88±0.60	5.68±0.85
	2%	-58%
P1	NS	<0.001
P2	<0.001	<0.001

Values are expressed as Means ± Standard Deviation (n=10). Values between brackets show percentage of change from Control. P1: significance vs control. P2: significance vs respective group (DM) not receiving GABA. NS: non-significant. P < 0.001 highly significant

DISCUSSION

Diabetes is one of the leading causes of death worldwide. It is a group of metabolic diseases characterized by hyperglycemia. Diabetes results from disorders in insulin production and/or insulin action and impaired operate within the metabolism of carbohydrates, lipids and proteins which lead to long term health complications.[28] Type 1 diabetes mellitus is an autoimmune disease characterized by pancreatic islets infiltration by T lymphocytes, macrophages and other immune cells and loss of β-cells.[29]

Table 3: Morphometric parameters in the studied groups

Parameter / Groups	No. of villi	No. of crypts	Mean mucosal thickness (µm)	Mean musclosa thickness (µm)	Outer intestinal circumference (µm)
Control	50	135	56.35±16.54	7.72±0.76	1379.46
GABA	45	130	50.22±15.23	7.13±0.75	1334.52
DM	65*	153*	100.86±4.35*	19.53±2.13*	1477.99*
DM + GABA	50	120	67.44±7.73	11.32±1.54	1399.46

Values are expressed as Means ± Standard Deviation (n=10). (*) significantly different from the control at P< 0.05

Table 4: DNA cytometry parameters in the studied groups

Parameter / Groups	Diploid value %	Proliferation index	Tetraploid value %	Aneuploid value %	Nuclear area(µm²)
Control	75.676 %	18.018 %	6.306 %	0.0 %	27.74
GABA	80.237 %	15.961 %	4.402 %	0.0 %	25.23
DM	83.019 %	15.094 %	1.887 %	0.0%	25.97
DM + GABA	93.660 %	6.422 %	0.917 %	0.0 %	24.70

In the current study, a single intraperitoneal injection of 60 mg/kg body weight of STZ to male albino rats induced hyperglycemia, and insulin deficiency (similar to type 1 DM) that was proved by our previous study.[18] The decrease of insulin might result from hyperglycemia-induced beta-cell toxicity[30] and degeneration of Langerhans islet beta cells.[19]

STZ, an antibiotic produced by *Streptomyces chromogenes*, is the most commonly used agent in experimental diabetes.[31,32] It was demonstrated that STZ induced damage to pancreatic cell membranes and evoked oxidative stress in islet cells.[33] In addition, STZ has been shown to induce DNA strand breaks and DNA alkylation in pancreatic islet cells.[34] Experimental studies demonstrated that STZ induces Type 1 DM or Type 2 DM, depending on the dose administered.[35]

Administration of STZ to rats induced oxidative stress indicated by a significant increase in the oxidant biomarkers values (AOPP and MDA) and a significant decrease in the antioxidants (catalase and GSH-Px) activities, such observations come in coincidence with those reported by Eltahawy *et al*.[18] Oxidative stress plays an important role in cellular injury following hyperglycemia where high glucose level could stimulate free radical production process whereas weak body defense system becomes unable to counteract the enhanced reactive oxygen species (ROS) generation resulting in imbalance between ROS and their protection which finally lead to oxidative stress process.[36,37] Several mechanisms have been reported to be responsible for oxidative stress in diabetes including excessive oxygen free radicals production from auto-oxidation of glucose, glycated proteins and glycation of antioxidative enzymes which inhibit their capacity to detoxify oxygen free radicals in addition to high glucose levels stimulation of cytochrome P_{450}-like activity by excessive nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) resulting from glucose metabolism and ketosis, a hallmark of T1DM, which may increase oxygen free radicals production.[38]

Results of the current experiment showed numerous histopathological changes in jejunum of STZ-induced diabetic rats illustrated by villous and crypt hypertrophy, disturbed villous architecture of some villi and loss of epithelial covering of others. Inflammatory cellular infiltration and edema of the villous core with lymphoid hyperplasia were also depicted with increased number of goblet cells whereas Paneth cells disappeared or appeared degranulated. The majority of epithelial cell nuclei of the diabetic group had diploid DNA values with medium proliferation index. Such histopathologic findings come in collaboration with those observed by Abo Gazia and Hasan.[39] Mucosal hyperplasia may be contributed to hyperphagia, overproduction of gastrin, growth hormone and increased level of glucagons-like peptide-2, up-regulation of cell proliferation and inhibition of cell death via apoptosis.[40] In addition, surface epithelium sloughing and the necrotic cells appearance in some villi may be due to decreased blood supply resulted from the diabetic microangiopathy.[41] Moreover, villous inflammatory cellular infiltration of the diabetic rats may be attributed to bacterial overgrowth and parasitic infestation of the small intestine.[42] As well as

many environmental toxins such as concomitant exposure to aluminum and ionizing radiation increased detrimental structural changes in Paneth cells.[43]

In the past five decades, the function of GABA in the CNS has been well documented. However, the presence of a GABAergic system within the pancreas as a potential target for treatment diabetes mellitus appeared only recently. In α-cells, GABA induces membrane hyperpolarization and inhibits glucagon secretion. In β-cells, GABA induces membrane depolarization and improves insulin secretion. GABA also has valuable effects on β-cell survival and regeneration, which results in enlarged β-cell mass. Moreover, GABA inhibits insulitis and systemic inflammatory cytokine production.[44]

The current experiment showed that GABA administration to normal rats for three weeks induced non-significant effect on the studied parameters which corroborate previous report of Yoshikuni.[45]

According to the results of the current work, administration of GABA (200 mg/Kg/day) daily during a period of 3 weeks to STZ-treated rats lead to the significant improvement in all the studied parameters could be attributed to the role of GABA in improving insulin and glucose level as shown by our previous study[18] that attributing to GABA treatment increased β-cell proliferation, while decreasing apoptosis, leading to enhanced β-cell mass**.** In addition, the activation of GABA-A receptors in islet β-cells increases insulin release.[46-48]

Nevertheless, according to the results of the current work, the significant improvement in all the studied parameters could be attributed to the role of GABA in attenuating oxidative stress through increasing endogenous superoxide dismutase (SOD) and CAT activities, decreasing lipid peroxidation[49] due to its free radicals scavenging activities,[50] its effectiveness in inhibiting the formation of reactive carbonyl intermediates and to react with MDA, increased in diabetic subjects as a side effect of increased protein glycation, to form different conjugated complexes[51] thus protect the tissue against active carbonyl damage.

Treatment of the diabetic rats with GABA reduced the severity of changes in the intestinal epithelial cells where the villi appeared slightly hypertrophic with moderate lymphocytic infiltration in the connective tissue core. Both goblet and Paneth cells were of normal count and shape. The majority of epithelial cells nuclei had diploid DNA values with low proliferation index, the mean nuclear area was lower in GABA treated diabetic group as compared to the other groups. In addition, treatment with GABA showed a significant increase in the antioxidants content associated with a significant decrease in the oxidant biomarkers values in the small intestine tissues indicating that GABA ameliorated the intestinal changes in STZ-induced diabetic rats. GABA exert pancreatic β-cell regenerative effects as it protects β-cells against apoptosis and supported anti-inflammatory and immunoregulatory activities.[44] Moreover, it exerted anti diabetic effects by acting on both the islet of β-cells and immune system, therapy preserving β-cell and preventing the development of T1DM. Moreover, it could suppress insulitis and inflammatory cytokines production[16] Furthermore, GABA has been shown to diminish inflammation by inhibition of activated T lymphocytes.[52]

CONCLUSION

Oxidative stress plays an important role in progression and development of the different types of diabetes in addition to associated complications and its complications. The current results showed that gamma aminobutyric acid (GABA) ameliorated most of the degenerative changes in STZ-induced diabetes in rat's small intestine which may be attributed to its free radicals scavenging activities, its antioxidant properties and its role in regulating insulin level.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

ABBREVIATIONS USED

STZ: Streptozotocin; **GABA:** Gamma aminobutyric acid; **DM:** Diabetes mellitus; **GAD:** Glutamic acid decarboxylase; **AOPPs:** Advanced oxidation protein products; **GSH-Px:** Glutathione peroxidase; **MDA:** Malondialdehyde; **CAT:** catalase; **PAS:** Periodic acid Schiff's; **ROS:** Reactive oxygen species; **CNS:** Central Nervous System.

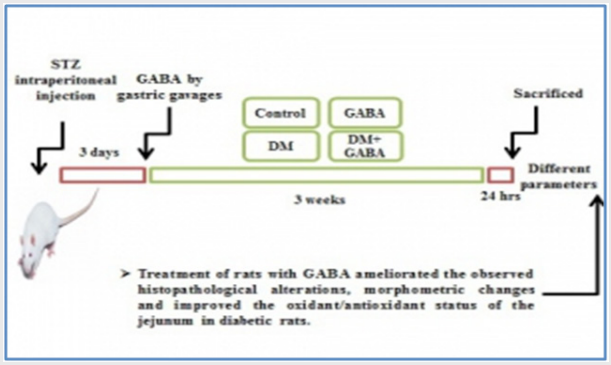
REFERENCES

1. Brereton MF, Iberl M, Shimomura K, Zhang Q, Adriaenssens AE, Proks P, *et al*. Reversible changes pancreatic islet structure and function produced by elevated blood glucose. Nat in Commun. 2014;22(5):1-10. https://doi.org/10.1038/ncomms5639.
2. Rochette L, Zeller M, Cottin Y, Vergely C. "Diabetes, oxidative stress and therapeutic strategies," Biochimica et Bio physica Acta—General Subjects. 2014;1840(9):2709–29.

3. Sheweita SA, Mashaly S, Newairy AA, Abdou HM, Eweda SM. Changes in Oxidative Stress and Antioxidant Enzyme Activities in Streptozotocin-Induced Diabetes Mellitus in Rats: Role of *Alhagi maurorum* Extracts. Oxid Med Cell Longev. 2016;1-8. https://doi.org/10.1155/2016/4970906;https://doi.org/10.1155/2016/2026582; https://doi.org/10.1155/2016/5264064; PMid:26885249 PMCid:PMC4739472.
4. Talaei A, Mohamadi M, Adgi Z. The effect of vitamin D on insulin resistance in patients with type 2 diabetes. Diabetol Metab Syndr. 2013;26;5(1):8
5. Tangvarasittichai S. Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. World J Diabetes. 2015;6(3):456–80.https://doi.org/10.4239/wjd.v6.i3.456; PMid:25897356 PMCid:PMC4398902
6. Wang JY, Zhu C, Qian TW, Guo H, Wang DD, Zhang F, *et al*. Extracts of black bean peel and pomegranate peel ameliorate oxidative stress-induced hyperglycemia in mice. Exp Ther Med. 2015;9(1):43-8.PMid:25452774.
7. Sahoo S, Thiele I. Predicting the impact of diet and enzymopathies on human small intestinal epithelial cells. Hum Mol Gen. 2013;22(13):2705–22.https://doi.org/10.1093/hmg/ddt119; PMid:23492669 PMCid:PMC3674809.
8. Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, *et al*. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. Nature. 2011;469(7330):415–8.https://doi.org/10.1038/nature09637; PMid:21113151 PMCid:PMC3547360.
9. Porter EM, Bevins CL, Ghosh D, GanzT. The multifaceted Paneth cell. Cell Mol Life Sci. 2002;59(1):156-70.https://doi.org/10.1007/s00018-002-8412-z; PMid:11846026.
10. Bevins CL. Paneth cell and the innate immune response. Curr Opin Gastroenterol.2004;20(6):572-80.https://doi.org/10.1097/00001574-200411000-00012; PMid:15703685
11. Ouellette AJ. Paneth cell a defensins: peptide mediators of innate immunity in the small intestine. Springer Semin Immunopathol. 2005;27:33-146.https://doi.org/10.1007/s00281-005-0202-x. PMid:15931529.
12. Watanabe M, Maemura K, Kanbara K, Tamayama T, Hayasaki H. GABA and GABA receptors in the central nervous system and other organs. Int Rev Cytol. 2002;213:1–47.https://doi.org/10.1016/S0074-7696(02)13011-7.
13. Dong H, Kumar M, Zhang Y, Gyulkhandanyan A, Xiang YY, Ye B, *et al*. Gamma aminobutyric acid up and down regulates insulin secretion from beta cells in concert with changes in glucose concentration. Diabetologia 2006;49(4):697–705.https://doi.org/10.1007/s00125-005-0123-1; PMid:16447058.
14. Krantis A. GABA in the mammalian enteric nervous system. News Physiol Sci. 2000;15(6):284–90; PMid:11390928.
15. Wang FY, Watanabe M, Zhu RM, Maemura K. Characteristic expression of gamma aminobutyric acid and glutamate decarboxylase in rat jejunum and its relation to differentiation of epithelial cells. World J Gastroenterol. 2004;10(24): 3608–11.https://doi.org/10.3748/wjg.v10.i24.3608; PMid:15534915 PMCid:PMC4612001
16. Soltani N, Qiu H, Aleksic M, Glinka Y, Zhao F, Liu R, *et al*. GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. Proc Natl Acad Sci USA. 2011; 108(28):11692-7. https://doi.org/10.1073/pnas.1102715108; PMid:21709230 PMCid:PMC3136292.
17. Erejuwa OO, Sulaiman SA, Wahab MSA, Sirajudeen KNS, Salleh MSM, Gurtu S. Glibenclamide or metformin combined with honey improves glycemic control in streptozotocin-induced diabetic rats. Int J Biol Sci. 2011;7(2):244-52. https://doi.org/10.7150/ijbs.7.244; PMid:21448302 PMCid:PMC3065678.
18. Eltahawy NA, Saada, HN, Hammad, AS. Gamma Amino Butyric Acid Attenuates Brain Oxidative Damage Associated with Insulin Alteration in Streptozotocin-Treated Rats. Ind J Clin Biochem. 2016:1-7
19. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A, Allah A, *et al*. Induction of Diabetes by Streptozotocin in Rats. Indian J Clin Biochem. 2007;22(2)60-64.https://doi.org/10.1007/BF02913315; PMid:23105684 PMCid:PMC3453807.
20. Nakagawa T, Yokozawa T, Kim HJ, Shibahara N. Protective effect of gamma aminobutyric acid in rats with streptozotocin-induced diabetes. . J Nutr Sci Vitaminol. 2005;51(4):278-82. https://doi.org/10.3177/jnsv.51.283; https://doi.org/10.3177/jnsv.51.278; PMid:16262002
21. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated oxygen toxicity in the blood. Am J Obstet Gynecol. 1979;135(3):372-6. https://doi.org/10.1016/0002-9378(79)90708-7.
22. Witko-Sarsat V, Friendlander M, Capelliere-Blandin C, Nguyen-Khoa T, Zing J, Jungers P, *et al*. Advanced oxidation protein products as a noval marker of oxidative stress in uremia. Kidney Int. 1996;49(5):1304-13. https://doi.org/10.1038/ki.1996.186; PMid:8731095.
23. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;(47)2:389-94. https://doi.org/10.1016/0003-2697(72)90132-7.
24. Necheles TF, Boles TA, Allen DM. Erythrocyte glutathione-peroxidase deficiency and hemolytic disease of the newborn infant. J Pediatr. 1968;72(3):319-24.https://doi.org/10.1016/S0022-3476(68)80202-1.
25. Bancroft J, Gamble A. Theory and Practice of Histology Techniques. 6th ed., Churchill Livingstone. New York and London: 83-92;2008.
26. Drury R, Wallington E. Carleton's Histological Technique. 4th ed., Oxford. Univ. Press, New York; 1980.
27. Kiernan JA. Histological and Histochemical Methods, Theory and Practice. Pergamon Press. New York, USA; 1981.
28. Oktayoglu GS, Basaraner H, Yanardag R, Bolkent S. The effects of combined treatment of antioxidants on the liver injury in STZ diabetic rats. Diges Dis Sci. 2009;54(3):538-46.https://doi.org/10.1007/s10620-008-0381-0; PMid:18712602.
29. Lehuen A, Diana J, Zaccone P, Cooke A. Immune cell crosstalk in type 1 diabetes. Nat Rev Immunol. 2010;10(7):501–13.https://doi.org/10.1038/nri2787; PMid:20577267.
30. Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T, Fujitani Y, Umayahara Y, *et al*. Beneficial effects of antioxidants in diabetes. Diabetes. 1999;48(12):2398-406. https://doi.org/10.2337/diabetes.48.12.2398; PMid:10580429.
31. King AJF. The use of animal models in diabetes research. Br J Pharmacol. 2012;166(3):877-94.https://doi.org/10.1111/j.1476-5381.2012.01911.x; PMid:22352879 PMCid:PMC3417415.
32. Li P, Zhang L, Tian X, Xing J. Docosahexaenoic acid has an anti-diabetic effect in streptozotocin-induced diabetic mice. Int J Clin Exp Med. 2014;7(9):3021. PMid:25356177 PMCid:PMC4211827.
33. Lenzen S. Oxidative stress: The vulnerable beta-cell. Biochem Soc Trans. 2008;36(3):343-7.https://doi.org/10.1042/BST0360343; PMid:18481954.
34. Nieman KM, Schalinske KL. Insulin administration abrogates perturbation of methyl group and homocysteine metabolism in streptozotocin-treated type 1 diabetic rats. Am J Physiol Endocrinol Metab. 2011;301: E560–E565.https://doi.org/10.1152/ajpendo.00105.2011; PMid:21730260.
35. Ventura-Sobrevilla J, Boone-Villa VD, Aguilar CN, Román-Ramos R, Vega-Ávila E, Campos-Sepúlveda E, *et al*. Effect of varying dose and administration of streptozotocin on blood sugar in male CD1 mice. Proc West Pharmacol Soc 2011; 54: 5-9.PMid:22423571.
36. Halliwell B, Gutteridge. Free Radicals in Biology and Medicine, Oxford University Press, New York, NY, USA, 4th edition; 2007.
37. Pandey KB, Mishra N, Rizvi SI. Protein oxidation biomarkers in plasma of type 2 diabetic patients. Clin Biochem. 2010; 43(4):508–511. https://doi.org/10.1016/j.clinbiochem.2009.11.011; PMid:19941844.
38. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The Role of Oxidative Stress and Antioxidants in Diabetic Complications. SQU Med J. 2012;12(1):5-18.
39. Abo Gazia MM, Hasan NM. Effect of Glabridin on the Structure of Ileum and Pancreas in Diabetic Rats: A histological, Immunohistochemical and Ultrastructural Study. Nature and Science. 2012;10:78-90.
40. Sen S, Roy M, Chakraborti AS. Ameliorative effects of glycyrrhizin on streptozotocin-induced diabetes in rats. J Pharm Pharmacol. 2011;63(2):287-96. https://doi.org/10.1111/j.2042-7158.2010.01217.x; PMid:21235594.
41. De-Las-Casas LE, Finley JL. Diabetic micro-angiapathy in the small bowel. Histopathology. 1999;35(3):267-70.https://doi.org/10.1046/j.1365-2559.1999.00702.x; PMid:10469219.
42. Virally-Monod M, Tielmans D, Kevorkian JP, Bouhnik Y, Flourie B, Porokhov B, *et al*. Chronic diarrhea and diabetes mellitus: prevalence of small intestinal bacterial overgrowth. Diabetes Metab. 1998;24(6):530-6.PMid:9932220.
43. Eltahawy NA, Sarhan OM, Hammad AS, Abu-nour S. Effects of combined exposure to aluminum chloride and g-radiation on histological and ultrastructure of intestinal Paneth cells. J Radiat Res Appl Sci. 2016;9(4):400-8. https://doi.org/10.1016/j.jrras.2016.05.007.
44. Wan Y, Wang Q, Prud'homme GJ. GABAergic system in the endocrine pancreas: A new target for diabetes treatment. Diabetes Metab Syndr Obes. 2015;8:79–87.PMid:25678807 PMCid:PMC4322886

45. Yoshikuni Y. FDA GRAS Notice for gamma-amino butyric acid (GABA). Pharma Foods International Co., Ltd 2008.PMCid:PMC4030648
46. Bansal P, Wang S, Liu S, Xiang YY, Lu WY, Wang Q. GABA coordinates with insulin in regulating secretory function in pancreatic INS-1 β-cells. PLoS One. 2011;6(10):26225.https://doi.org/10.1371/journal.pone.0026225; PMid:22031825 PMCid:PMC3198728
47. Tian J, Dang H, Chen Z, Guan A, Jin Y, Atkinson MA, Kaufman DL. γ-Aminobutyric acid regulates both the survival and replication of human β-cells. Diabetes. 2013;62(11):3760–5.https://doi.org/10.2337/db13-0931; PMid:23995958 PMCid:PMC3806626
48. Purwana I, Zheng J, Li X, Deurloo M, Son DO, Zhang Z, *et al*. GABA promotes human β-cell proliferation and modulates glucose homeostasis. Diabetes. 2014 Jul 9.https://doi.org/10.2337/db14-0153 ; PMid:25008178
49. Sasaki S, Yokozawa T, Cho EJ, Oowada S, Kim M. Protective role of gamma-aminobutyric acid against chronic renal failure in rats. J Pharm Pharmacol. 2006;(11):1515-25.https://doi.org/10.1211/jpp.58.11.0013 ; PMid:17132215
50. Hou CW. Pu-Erh tea and GABA attenuates oxidative stress in kainic acid-induced status epilepticus. J Biomed Sci. 2011;18(1):75.https://doi.org/10.1186/1423-0127-18-75PMid:22014163 PMCid:PMC3217899
51. Deng Y, Wang W, Yu P, Xi Z, Xu L, Li X, *et al*. Comparison of taurine, GABA, Glu, and Asp as scavengers of malondialdehyde *in vitro* and *in vivo*. Nanoscale Res Lett 2013;8(1):190.https://doi.org/10.1186/1556-276X-8-190; PMid:23618076 PMCid:PMC3637245
52. Mendu SK, Akesson L, Jin Z, Edlund A, Cilio C, Lernmark A, *et al*. Increased GABA(A)channel subunits expression in CD8(+) but not inCD4(+) T cells in BB rats developing diabetescompared to their congenic littermates. Mol Immunol. 2011;48(4):399-407.https://doi.org/10.1016/j.molimm.2010.08.005; PMid:21112637

PICTORIAL ABSTRACT



SUMMARY

- This study was planned to investigate the role of GABA in ameliorating the histopathological changes and the oxidative stress in small intestine of the diabetic rats.
- Rats were injected with STZ to induce Diabetes and induce oxidative damage.
- Rats were treated with GABA to ameliorate damage that occurred in small intestine of the diabetic rats.
- GABA administration to rats ameliorated diabetes-induced jejunal histopathological and biochemical alterations.

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