Original Article

The Possible Role of Bee Venom on Gastric Fundic Mucosa in Streptozotocin Induced Diabetes Mellitus in Rats. A Histological Study

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ABSTRACT

Introduction: Diabetes mellitus represents one of the most prevalent chronic diseases and a major public health challenge worldwide. It leads to serious consequences in several tissues including gastrointestinal tract. Bee venom (BV) is a traditional medicine used for treating various diseases.

Aim: To investigate the effects of diabetes on gastric fundic mucosal changes and to evaluate the possible therapeutic role of BV on these changes in a rat model of streptozotocin (STZ) induced diabetes mellitus.

Materials and Methods: Thirty adult male albino Wister rats were divided into four groups. Group I (control), group II (BV): received intraperitoneal injection (IP) of 0.5mg/kg BV twice weekly for four weeks, group III (diabetic): received single IP of 45mg/kg STZ and group IV (diabetic-BV treated): one week after confirmation of diabetes, rats received BV for four weeks (as group II). At the end of the experiment, samples of the fundus of the stomach were collected from all rats. The specimens were processed for light and transmission electron microscopic studies. Morphometric and statistical studies were also performed.

Results: Diabetic rats showed sloughing of fundic epithelium, significant decrease in mucous secreting cells, significant increase in collagen fibers and increase inflammatory cells in lamina propria. Cells of fundic glands were seen with karyolitic nuclei and dilated rough endoplasmic reticulum. Administration of BV showed improvement of histological structural changes induced by diabetes.

Conclusions: BV have relevant therapeutic role in the fundic mucosa induced by diabetes. So, it could potentially provide possible solution for gastric changes associated with diabetes.

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Key Words: Bee venom, diabetes, gastric fundic mucosa, histology, rats.

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INTRODUCTION

Diabetes mellitus (DM) is a global threat in public health nowadays. It is a metabolic disease caused by failure of blood sugar control and it is a very common disorder, with a prevalence of 15.1% of total adult population in Egypt according to the international diabetes federation[1]. It is well known that deficiency of treatment of DM results in critical impairment by causing acute complications (as diabetic ketoacidosis) along with chronic complications including nephropathy, angiopathy, neuropathy, and ophthalmopathy[2]. Moreover, some investigators[3,4] noticed that most diabetic patients suffer one or more gastrointestinal symptoms, which involve abdominal pain, early satiety, constipation, diarrhea, nausea, vomiting, gastric bleeding and fecal incontinence. All these symptoms result in a poor quality of patient's life.

Streptozotocin (STZ) is an antibiotic produced by Streptomyces achromogenes display several biological properties including anti-tumor and diabetogenic activities. It has been widely used for inducing experimental DM in a diversity of animals. It induces degeneration of pancreatic β-cells therefore simulating the naturally occurring DM. Moreover, STZ- induced diabetes may display most of the diabetic complications such as, cardiovascular, nervous, kidney and urinary bladder dysfunction[5].

There is still much to be improved in the treatment of diabetes. An important strategy to fight against diabetes epidemic is to prevent or delay the disease[6]. Regarding the side effects and high prices of blood sugar lowering chemicals, achieving new therapeutic agents with low side effects seems to be necessary. Bee venom (BV) or apitoxin, produced in the venom gland of the bee (Apis mellifera), is a complex mixture of substances with reported biological activity[7,8]. Bee venom therapy is a treatment modality involving the application of live bee stings to the patient's skin or, in more recent years, the injection of BV into the skin with a hypodermic needle[9]. Recently, BV has proven its effectiveness in traditional medicine for treating a variety of disease conditions, as arthritis, back pain, skin diseases, liver disorders, cardiovascular diseases and even cancerous tumors. In addition to its anti-inflammatory

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activity, BV also exhibits antioxidant, antimicrobial, and analgesic effects[10]. Honey BV has been utilized as pain reliever and as treatment against inflammatory diseases since ancient times[11,12]. It is a traditional Korean medicine that has been widely used in the treatment of some immune-related diseases[12]. Bee-venom contains various peptides including mellitin, apamin, adolapin and mast cell degranulation peptide, which have a wide variety of pharmaceutical properties. It also contains enzymes (e.g. phospholipase A2) and non-peptide components (e.g. histamine, lipids and carbohydrates). The two major ingredients of BV are phospholipase A and melittin[13,14,15].

AIM OF THE WORK

To investigate gastric fundic mucosal structural changes that could occur in STZ- induced diabetes in rats and to evaluate the possible therapeutic role of BV on these changes that might yield promising results and potentially provide a possible solution for this depleting disease.

MATERIALS AND METHODS

A-Animals

Thirty adults male Wistar albino rats weighing 200-250 gm were used in this study. All procedures of animal care and experiments were done according to guideline of animal care and the scientific research ethical committee of the Faculty of Medicine, Ain Shams University. Animals were housed in clean wire mesh cages under standard conditions of illumination and ventilation. They were allowed free access to standard laboratory chow and water. The experiment was performed in the Medical Research Center, Ain Shams University hospitals.

B-Experimental design

Rats were kept for one week before the beginning of the experiment for acclimatization. Before STZ injection, blood glucose level was measured to ensure that rats were normoglycemic. Then they were randomly divided into four groups:

Group I (Control): included five rats which received a single intraperitoneal (IP) injection of 0.1 ml of 0.1 M citrate buffer of PH 4.5, which is the vehicle of the STZ. The animals were then sacrificed after five weeks.

Group II (bee venom): included five rats which received a single (IP) injection of 0.1 ml of 0.1 M citrate buffer as in group I and after one week each rat received IP injection of 0.5 mg/kg BV (dissolved in 1 ml sterile water) twice weekly (3-4 days apart) at fasting condition for four consecutive weeks then the animals were sacrificed)[16]. Bee venom powder was purchased from Department of Allergy and Clinical Immunology, Faculty of Medicine, Ain Shams University.

Group III (diabetic): included ten rats. Each rat received single IP injection of 45mg/kg STZ in 0.1 ml of 0.1 M citrate buffer pH 4.5 (Sigma, USA)[17]. Rats were fed with glycosylated water in the following 24 hours, in order to avoid hypoglycemia resulting from destruction of beta cells. To confirm diabetes, three days after induction, fasting blood glucose level was measured from the tail vein using One Touch Ultra 2 glucose meter and strips (LifeScan, Inc. USA). Animals were considered diabetic with blood glucose level ≥ 250 mg/dl[17]. The animals were then sacrificed after five weeks.

Group IV (diabetic-BV treated): included ten rats. Each rat received single IP injection of STZ as group III. One week after conformation of diabetes[18] rats received BV as group II, (IP injection of 0.5 mg/kg BV twice weekly (3-4 days apart) at fasting condition for four consecutive weeks and then they were sacrificed). All injections were preceded by sterilization of the skin of the rats' abdomen with Betadine antiseptic solution.

C-Blood glucose level analysis

A drop of fresh blood was collected from the animal`s tail using a lancet at fasting conditions. Fasting blood glucose levels were measured in all groups twice weekly using glucometer instrument One Touch Ultra 2 glucose meter and strips (LifeScan, Inc. USA) and statistical analysis were done.

D-Sample collection

At the end of the experiment, rats were sacrificed by cervical dislocation after ether inhalation anesthesia. Laparotomy was done, the stomach was dissected out, split longitudinally with scissors along their greater curvature, and washed thoroughly with saline to remove food residues. Thereafter, fundus specimens were divided into two halves. One half was processed for obtaining paraffin block after being pinned onto a card to avoid rolling of the specimens, while the other half was processed for transmission electron microscope (TEM).

E-Histological study

I-Preparation of paraffin sections

Fundic specimens were fixed in 10% buffered formalin, dehydrated, cleared and embedded in paraffin. Serial 5 μm sections of the specimens were stained with Hematoxylin and Eosin (HandE), Masson`s trichrome stain and combined Alcian blue–PAS staining[19].

II-Preparation of Transmission electron microscope (TEM) study

Small pieces (1mm3) from the fundus were cut and fixed immediately in 2.5% glutaraldehyde solution, followed by 1% osmium tetroxide, dehydrated, and embedded in epoxy resin. Ultrathin sections were collected on copper grids and stained with uranyl acetate and lead citrate[19]. The ultrastructural examination was carried out with a transmission electron microscope (JEM-1010; JEOL, Akishima-Shi, Tokyo, Japan) at the Regional Center for Mycology and Biotechnology, Al Azhar University.

F-Morphometric measurements

Samples were analyzed using an image Leica Q win V.3 program installed on a computer in the Histology and cell biology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM2500 microscope with built-in camera (Leica Microsystems GmbH, Ernst-Leitz-StraBe, Wetzlar, Germany). Five specimens from five different rats of each group were examined (n=5). From each specimen, five different captured non-overlapping fields were taken. Five different readings from every captured photo were counted and the mean was calculated for each specimen. Measurements were taken by an independent observer blinded to the specimens' details to perform an unbiased assessment.

The following parameters were measured

1. Mean thickness of the gastric fundic mucosa was measured as the perpendicular distance between the gastric mucosal surface and the muscularis mucosa X100
2. The mean area percentage of collagen fibers stained by Masson trichrome stain X400
3. The mean of area percentage of different types of mucous secreting cells stained by combined Alcian blue–PAS. X200

G-Statistical analysis

All data were collected, revised, and subjected to statistical analysis using one-way analysis of variance (ANOVA) performed with SPSS.21 program (IBM Inc., Chicago, Illinois, USA) and post-Hoc least significant difference. The significance of the data was determined by the *P value. P values* greater than 0.05 were considered non-significant, and *P values* equal or less than 0.05 were considered significant. Summary of the data was expressed as mean ± standard deviation (SD).

RESULTS

Mortality rate

Two rats had died in diabetic group (group III) during the experiment with mortality rate of 20%. No further deaths were recorded in the other groups.

Histological results

In the present study, examination of the structure of fundus of the stomach of rats of group I (control group) and group II (BV group) revealed nearly similar histological structure in all different histological methods.

A) Light microscopic results

Histological examination of HandE stained sections of group I (control) and group II (BV) showed the wall of the gastric fundus formed of mucosa, submucosa, muscularis externa and serosa. The mucosa was composed of regularly arranged tightly packed tubular fundic glands surrounded by connective tissue lamina propria and basally bounded by smooth muscle of mascularis mucosa (Figure 1A). The lining epithelium of apical part of fundic gland was composed of surface columnar mucous secreting cells having basal rod -shaped nuclei and apical vacuolated cytoplasm. The neck of fundic glands contained large parietal cells with acidophilic cytoplasm and central rounded vesicular nuclei (Figures 1B and 1D). Moreover, the basal part of the fundic gland was lined by many low columnar chief cells with basal nuclei and basal basophilic cytoplasm. Few parietal cells with acidophilic cytoplasm and groups of mucous neck cells having flattened basal nuclei and vacuolated cytoplasm were detected (Figures1C and 1E). Many chief cells were also noticed in group II (Figure 1E).

Meanwhile, examination of group III (diabetic) showed significant ($P \leq 0.05$) thinning of the fundic mucosa compared to all other groups (Table 1), focal epithelial cell depletion and ulceration of the fundic glands was also noticed (Figure 2A). The apical parts of the fundic glands were distorted and most of the epithelial lining was sloughed. Some cells appeared with vacuolated cytoplasm and pale stained nuclei. Parietal cells were frequently seen sloughed (Figure 2B). In addition, the basal part of the fundic gland displayed focal sloughing of epithelial lining. Different types of cells had deeply stained pyknotic nuclei. Chief cells in the base of the gland sometimes appeared cubical with pale cytoplasm and large nuclei. Congested blood vessel and inflammatory cells are seen in the lamina propria between widely spaced fundic glands (Figure 2C).

Examination of group IV (diabetic- BV treated) showed the fundic mucosa is nearly comparable to the control group. The fundic mucosa was significantly ($P \leq 0.05$) increased compared to group III (Table 1). The fundic glands were regularly arranged with short narrow pits (Figure 2D). The apical parts of the fundic glands were lined by closely packed surface mucous cells with oval vesicular nuclei. Many mucous neck cells with foamy cytoplasm and basal flattened nuclei and some parietal cells with acidophilic cytoplasm were noticed (Figure 2E). In addition, the basal parts of the fundic glands were lined by many Chief cells and few parietal cells (Figure 2F).

Examination of Masson's trichrome stained section of group I (control), group II (BV group) and group IV (diabetic- BV treated) showed few collagenous fibers within the lamina propria, mostly between the bases of the fundic glands (Figures 3A, 3B and 3D). Meanwhile, examination of group III (diabetic) showed significant increase ($P \leq 0.05$) in the collagen fibers in the lamina propria of fundic glands compared to the other groups (Figure 3C, Table 1).

Examination of combined Alcian blue–PAS stained section of group I (control) and group II (BV group) showed the surface columnar mucous-secreting cells with magenta PAS-positive reaction, whereas the mucous neck cells showed strong Alcian blue-positive reaction. A faint

Alcian blue-positive reaction was noticed over scattered basal cells (Figures 4A and 4B). However, examination of group III (diabetic) showed significant decrease ($P \leq 0.05$) of both of PAS and Alcian blue - reaction in most fundic gland cells. Sloughed out fundic glands could also be seen (Figure 4C). Examination of group IV (diabetic-BV treated) showed significant increase ($P \leq 0.05$) of both of PAS and Alcian blue - positive reaction of the mucous cells of the fundic glands compared to group III (Figure 4D, Table 1).

B) Transmission electron microscopic results

Ultrastructure examination of sections of group I (control) showed the surface mucous secreting cells with numerous apical electron dense mucous granules and basal rod shaped euchromatic nuclei (Figure 5a). Mucous neck cells had numerous apical electron lucent mucous granules and basal euchromatic nuclei (Figure 5b). Parietal cells showed rounded euchromatic nuclei and prominent nucleoli. The cytoplasm had numerous mitochondria and intracellular canaliculi (Figure 5c). Other Parietal cells showed intracellular canaliculi with many microvilli (Figure 5d). Chief cells showed some apical electron dense granules, basal oval shaped euchromatic nuclei and basal rough endoplasmic reticulum (Figure 5e). Enteroendocrine cells had euchromatic nuclei and numerous small mostly basal electron-dense secretory granules (Figure 5f).

Meanwhile, examination of group III (diabetic) showed an apparent decrease height of surface mucous secreting cells compared to control group. Most cells were seen with karyolitic nuclei (chromatin dissolution). Cytoplasmic vacuolations were also seen between few apical electron dense granules (Figure 6a). Mucous neck cells had basal euchromatic nuclei with prominent nucleoli. They showed numerous apical electron lucent mucous granules with central electron dense core (Figure 6b). Parietal cells with rounded karyolitic nuclei and cytoplasmic vacuolations were frequently seen (Figure 6c). Immature chief cells appeared with large apical electron lucent granules. Most chief cells were seen with prominent dilated basal rough endoplasmic reticulum (Figure 6d). Enteroendocrine cells appeared with cytoplasmic vacuolations and they were located near blood capillary in the lamina propria (Figure 6e). Moreover, some eosinophils were detected, they showed characteristic oval granules with a crystalline body core. Chief cells with prominent dilated rough endoplasmic reticulum could be noticed (Figure 6f).

However, examination of group IV (diabetic-BV treated) showed the surface mucous secreting cells had numerous apical electron dense mucous granules and irregular euchromatic nuclei with prominent nucleoli (Figure 7a). Mucous neck cells appeared with numerous apical electron lucent mucous granules and basal rod shaped euchromatic nuclei (Figure 7b). Parietal cells had rounded euchromatic nuclei and their cytoplasm showed numerous mitochondria and characteristic tubulovesicular system (Figure 7c). Few transitional cells had some apical variable size electron dense granules and basal oval shaped euchromatic nuclei were detected (Figure 7d). Moreover, some chief cells had some apical variable size electron lucent granules, basal oval shaped euchromatic nuclei and prominent basal rough endoplasmic reticulum were noticed (Figure 7e). Enteroendocrine cells appeared with euchromatic nuclei and numerous small electron-dense secretory granules (Figure 7f).

C) Morphometric and statistical results (Table 1)

Non-significant changes ($P > 0.05$) were detected between group I (control) and group II (BV) in all the statistical parameters measured in this study.

1. The mean of blood glucose levels showed significant increase in group III compared with the other groups. Meanwhile, there was a significant decrease in group IV compared with group III.
2. The mean thickness of the gastric fundic mucosa showed significant decrease in group III compared with the other groups. However, there was a significant increase in group IV compared with group III.
3. The mean area percentage of collagen fibers stained by Masson trichrome stain showed significant increase in group III compared with the other groups. Meanwhile, there was a significant decrease in group IV compared with group III.
4. The mean area percentage of different types of mucous secreting cells stained by combined Alcian blue–PAS showed significant decrease in group III compared with the other groups. However, there was a significant increase in group IV compared with group III.

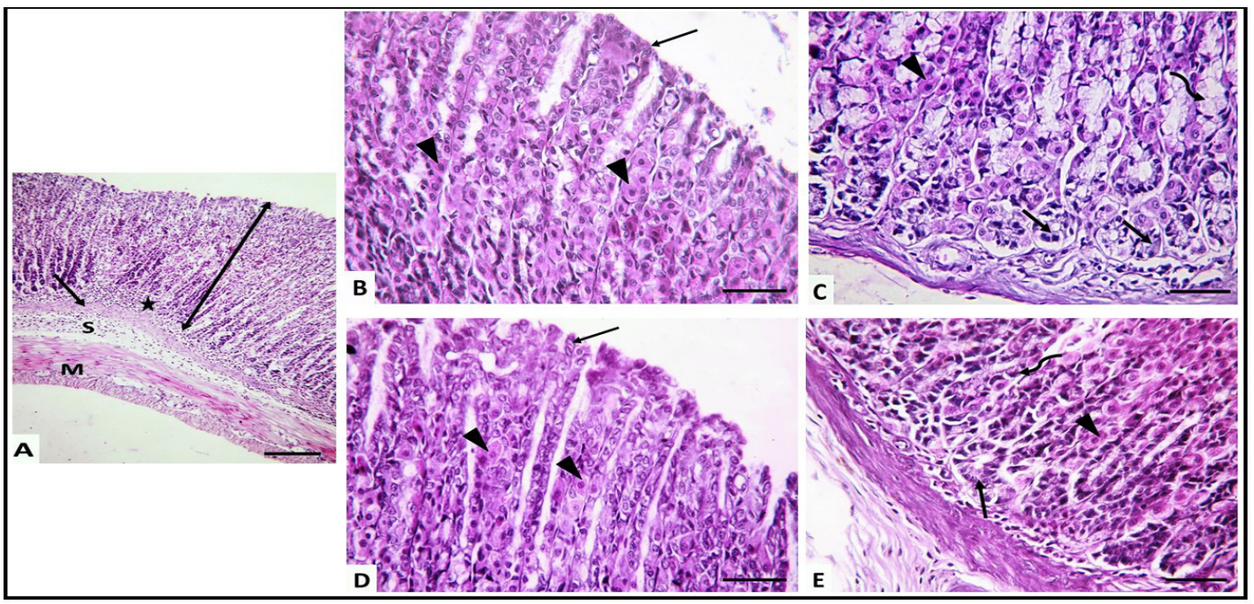

Fig. 1: photomicrographs of the fundus from group I (control) and group II (bee venom) [A-C] Group I: [A] the fundic wall is formed of regularly arranged tightly packed tubular fundic glands (↕), lamina propria (*), mascularis mucosa (↑), submucuosa (S) and musclaries externa (M). [B] the apical part of fundic glands are lined by surface mucous cells (↑) and large parietal cells (▲) with acidophilic cytoplasm and central rounded vesicular nuclei. [C] the basal part of fundic gland is lined by many low columnar chief cells with basal nuclei and basal basophilic cytoplasm (↑). Few parietal cells with acidophilic cytoplasm (▲) and groups of mucous neck cells having flattened basal nuclei and vacuolated cytoplasm (curved arrow) are noticed. [D, E] Group II: [D] the apical part is formed of fundic glands with surface mucous cells (↑) and large parietal cells (▲). [E] many chief cells are noticed in the basal part of the glands (↑). Some mucous neck cells (curved arrow) and few parietal cells (▲) are also seen. HandE [A X 100, Scale bar: 200 µm] [B - E X 400, Scale bar: 50 µm]

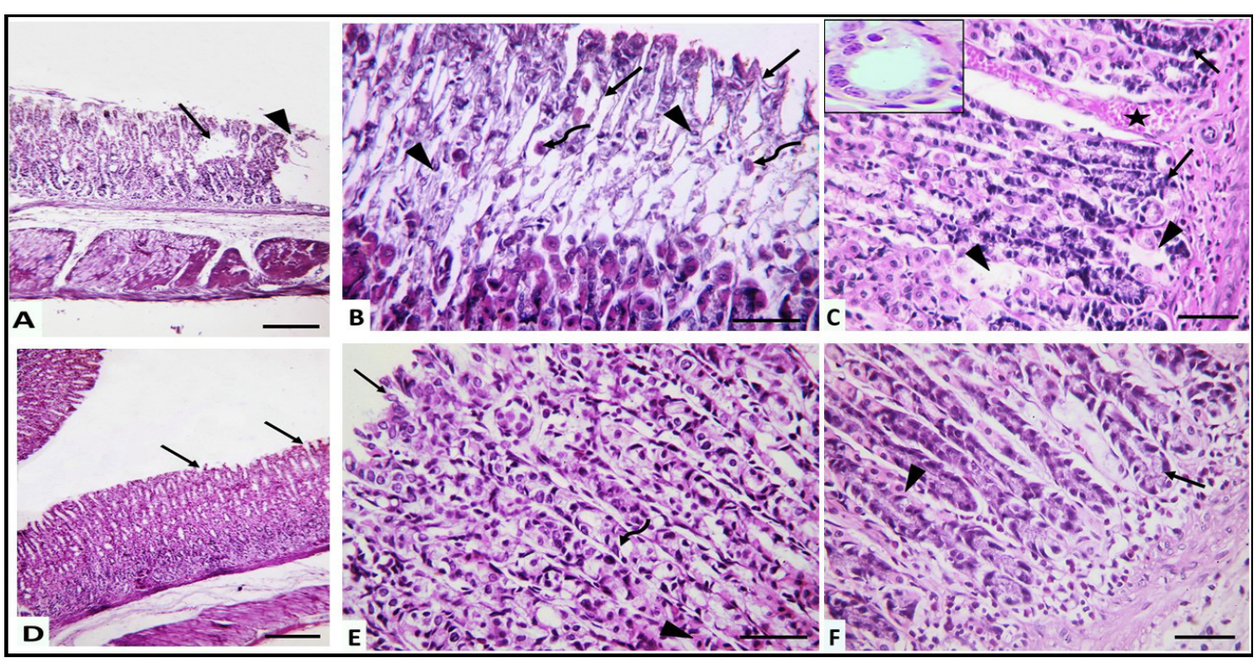

Fig. 2: photomicrographs of the fundus from group III (diabetic) and group IV (diabetic-BV) [A-C] Group III: [A] marked thinning of the mucosa, focal epithelial cell depletion (↑) and ulceration (▲) of the fundic glands. [B] the apical part of fundic glands is distorted with sloughed epithelial lining (↑). Some cells appear with vacuolated cytoplasm and pale stained nuclei (▲), sloughed parietal cells (curved arrow) are also seen. [C] the basal part of fundic glands is seen with focal sloughing of epithelial lining (▲). Many cells have deeply stained pyknotic nuclei (↑). Congested blood vessel (*) and inflammatory cells are seen in the lamina propria between widely spaced fundic glands. Inset: cubical chief cells appeared with pale cytoplasm and large nuclei. [D-F] Group IV: [D] the fundic glands are regularly arranged with short narrow pits (↑). [E] the surface is lined by closely packed surface mucous cells with oval vesicular nuclei (↑). Many mucous neck cells (curved arrow) are noticed. Some Parietal cells (▲) are also detected. [F] the basal part is lined by many Chief cells (↑) and few parietal cells (▲).HandE [A, D X 100, Scale bar: 200 µm] [B, C, E, F X 400, Scale bar: 50 µm] [Inset in C X 1000]

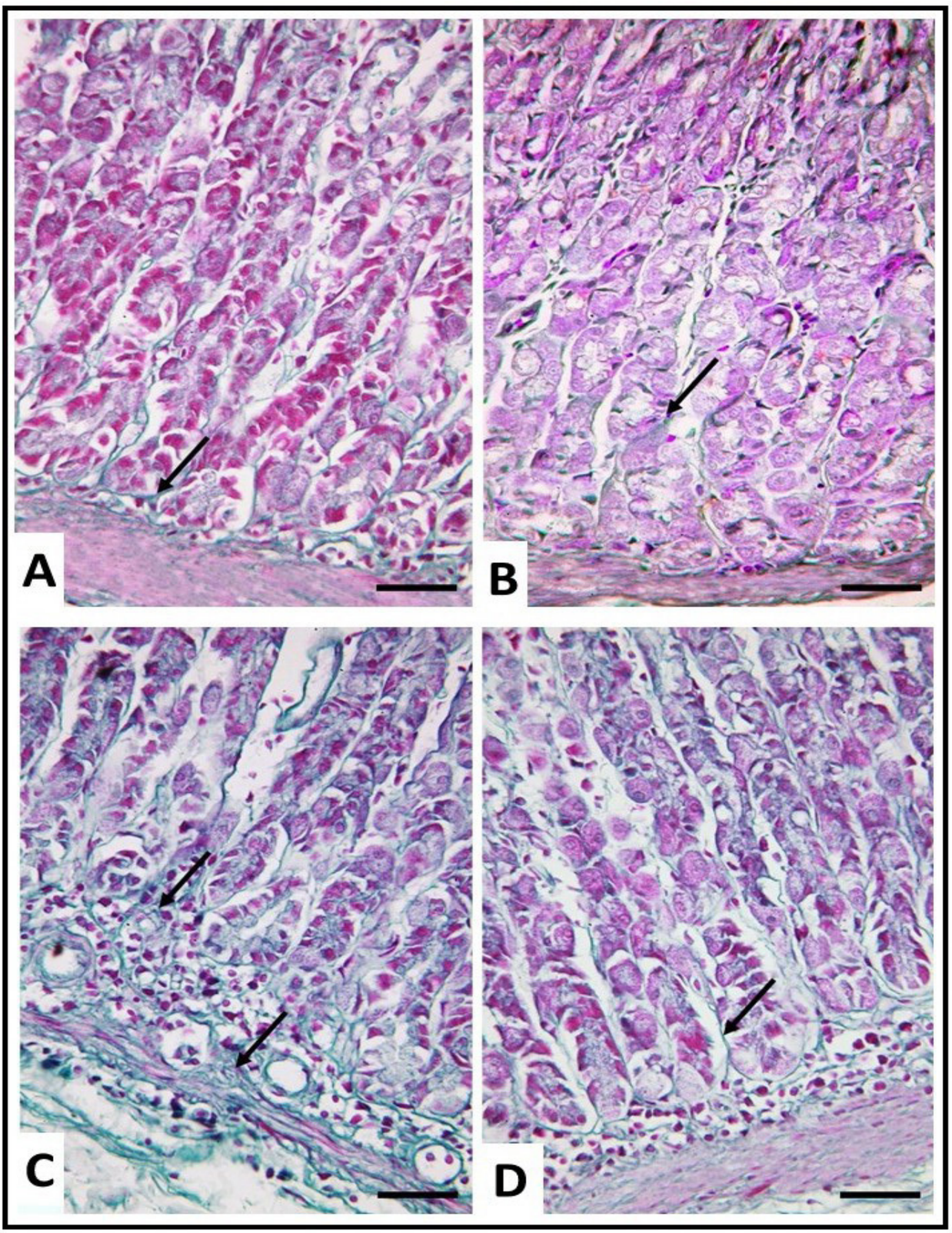

Fig. 3: photomicrographs of the basal part of fundic mucosa [A] Group I (control) and [B] Group II (bee venom): few collagen fibers (↑) in the lamina propria of the mucosa are noticed. [C] Group III (diabetic): an apparent increase in the collagen fibers (↑) in the lamina propria of fundic glands. [D] Group IV (diabetic-BV treated): few collagen fibers (↑) in the lamina propria. [Masson's trichrome stain X400, scale bar: 50µm]

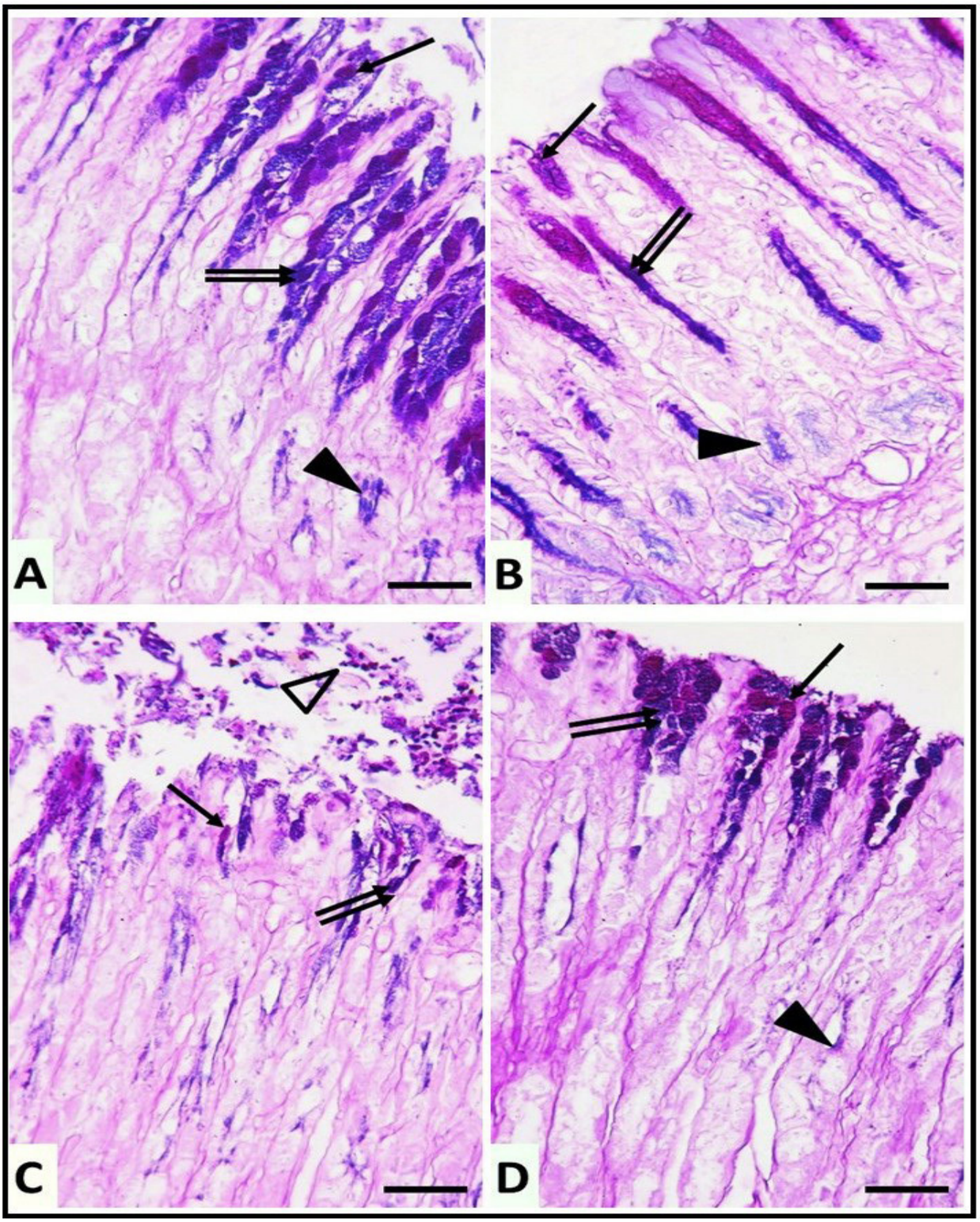

Fig. 4: photomicrographs of fundic mucosa [A] Group I (control) and [B] Group II (Bee venom): showing surface mucous secreting cells with periodic acid–Schiff (PAS)-positive reaction (↑), whereas the mucous neck cells show strong Alcian blue-positive reaction (↑↑). A faint Alcian blue-positive reaction is noticed over scattered basal cells (▲). [C] Group III (diabetic): marked reduction in both PAS (↑) and Alcian blue (↑↑) positive reaction is noticed in most cells of the fundic gland. Notice sloughed out fundic glands (Δ). [D] Group IV (diabetic- BV treated): some surface mucous secreting cells have PAS-positive reaction (↑), whereas some mucous neck cells show strong Alcian blue-positive reaction (↑↑). Notice weak Alcian blue-positive reaction over scattered cells (▲). [Combined Alcian blue–PAS X 400, scale bar: 50µm]

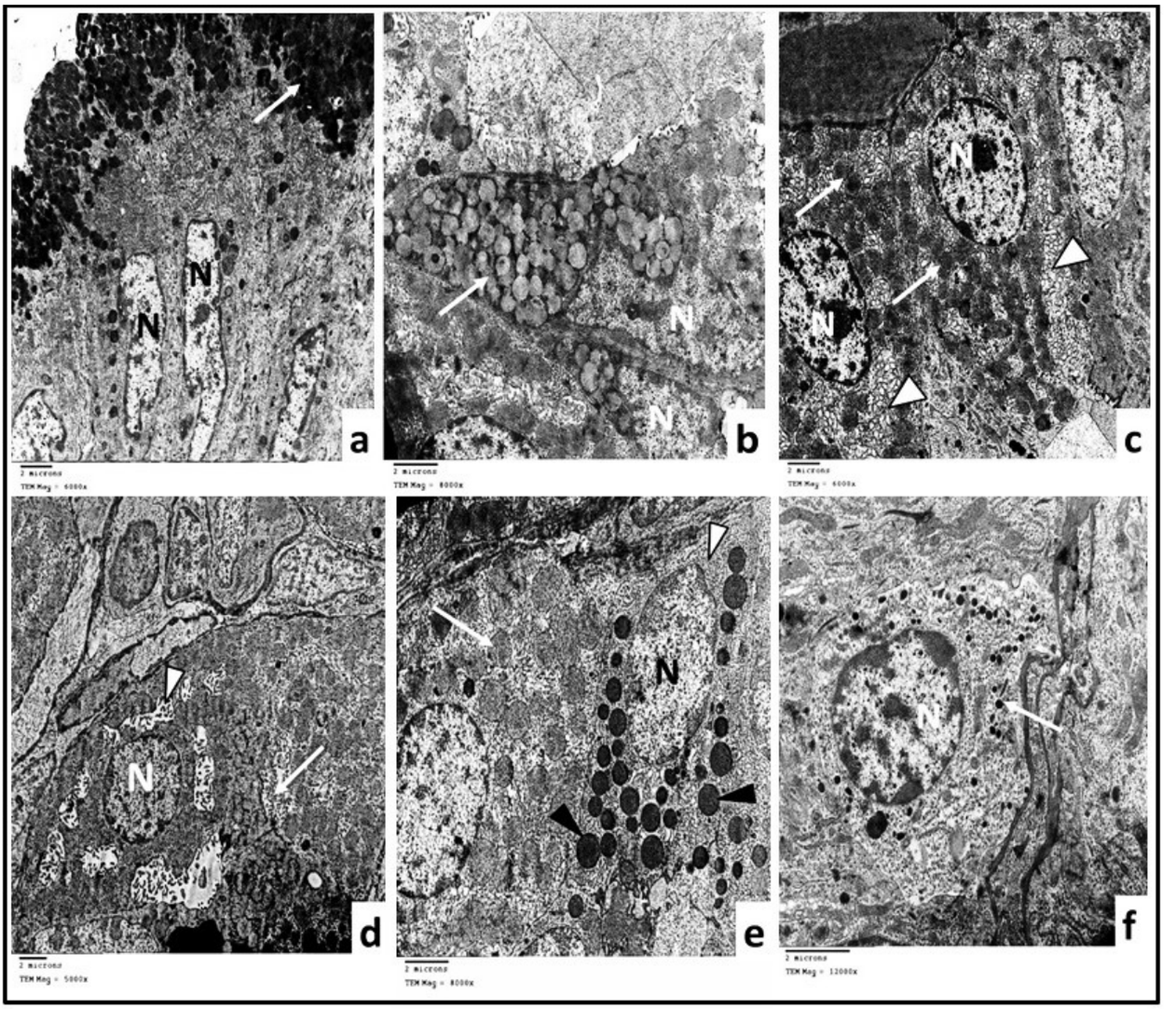

Fig. 5: electron micrographs of different cells lining fundic glands from Group I (control) showing: (a) Surface mucous secreting cells with numerous apical electron dense mucous granules (↑) and basal rod shaped euchromatic nuclei (N). (b) Mucous neck cell with numerous apical electron lucent mucous granules (↑) and basal euchromatic nucleus (N). (c) Parietal cells with rounded euchromatic nuclei and prominent nucleoli (N). The cytoplasm shows numerous mitochondria (↑) and intracellular canaliculi (Δ). (d) Parietal cell with rounded euchromatic nucleus (N) and intracellular canaliculi (↑) with many microvilli (Δ). (e) Chief cell with some apical electron dense granules (▲), basal oval shaped euchromatic nucleus (N) and basal rough endoplasmic reticulum (Δ). Notice part of parietal cells with their characteristic tubulovesicular system and numerous mitochondria (↑). (f) Enteroendocrine cell with a euchromatic nucleus (N) and numerous small electron-dense basal secretory granules (↑). TEM [(a, c) X 6000 (b, e) X 8000 (d) X 5000 and (f) X 12000]

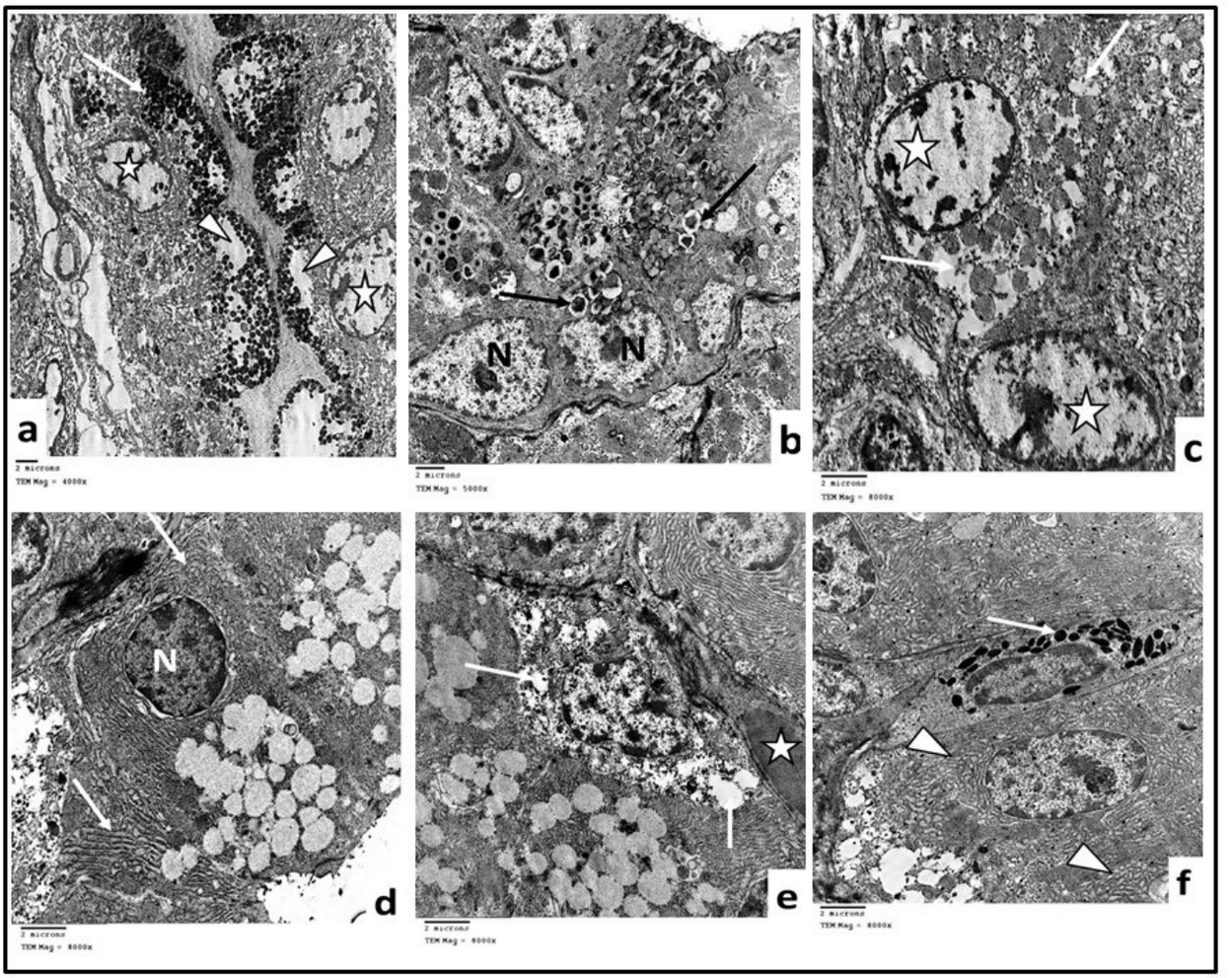

Fig. 6: electron micrographs of different cells lining fundic glands of Group III (diabetic) showing: (a) Surface mucous secreting cells with some apical electron dense granules (↑). Notice focal cytoplasmic vacuolation that separates the apical granules (Δ) and the nuclei are karyolitic (*). (b) Mucous neck cells with numerous apical electron lucent mucous granules with central electron dense core (↑). Notice their nuclei are basal and euchromatic with prominent nucleoli (N). (c) Parietal cells with rounded karyolitic nucleus (*) and cytoplasmic vacuolations (↑). (d) Chief cell with some apical variable size electron lucent granules, basal oval shaped euchromatic nucleus (N) and prominent dilated basal rough endoplasmic reticulum (↑). (e) Enteroendocrine cell with a euchromatic nucleus and cytoplasmic vacuolations (↑). Notice nearby blood capillary (*). (f) Eosinophil cell with characteriśtic oval granules with a cryśtalline body core (↑). Notice cells with prominent dilated rough endoplasmic reticulum (Δ). TEM [(a) X 4000 (b) X 5000 (c, d, e, f) X 8000]

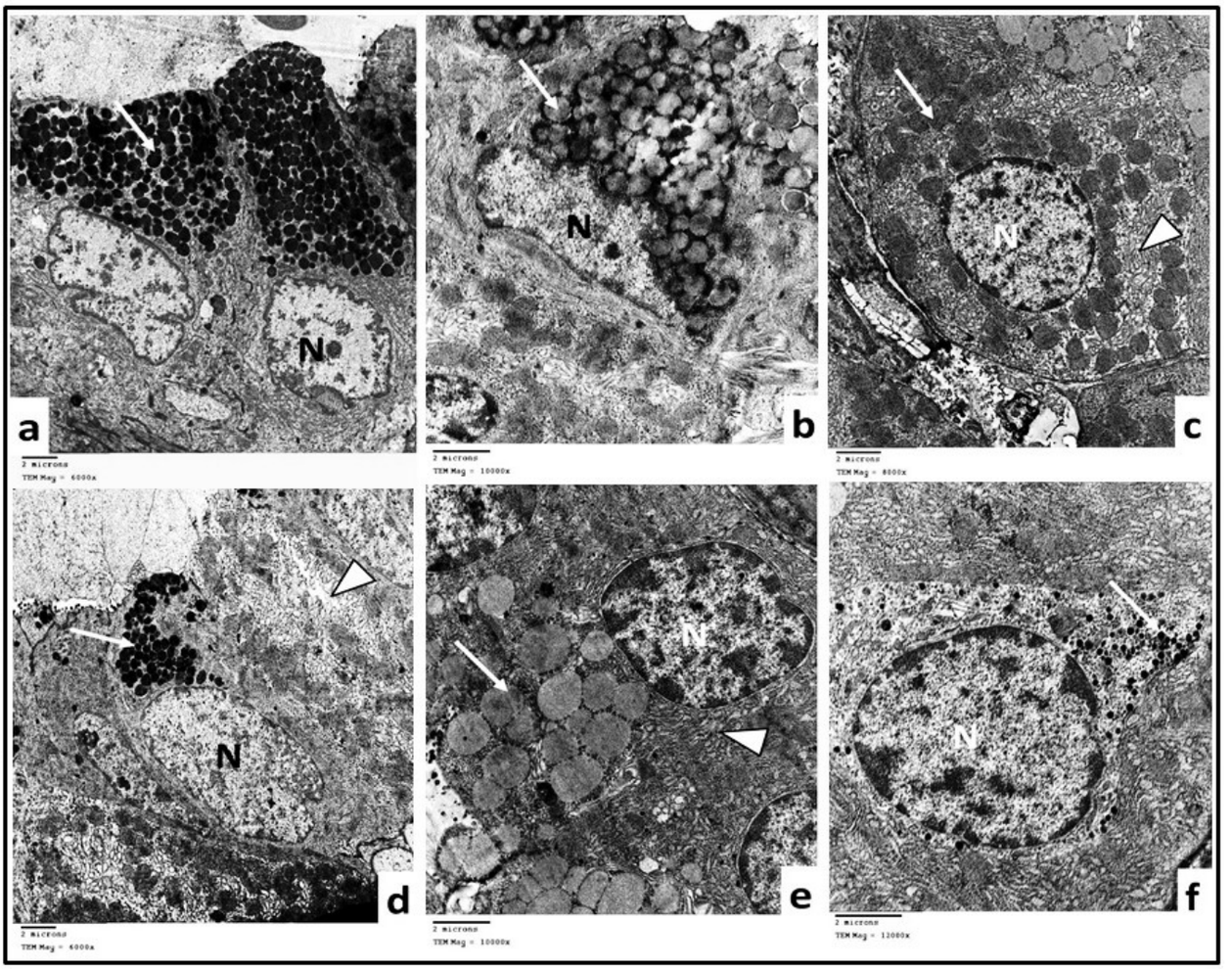

Fig. 7: electron micrographs of different cells lining fundic glands of Group IV (diabetic- BV treated) showing: (a) Surface mucous secreting cells with numerous apical electron dense mucous granules (↑). Notice the irregular euchromatic nuclei with prominent nucleoli (N). (b) Mucous neck cell with numerous apical electron lucent mucous granules (↑) and basal rod shaped euchromatic nucleus (N). (c) Parietal cell with rounded euchromatic nucleus (N). The cytoplasm shows numerous mitochondria (↑) and characteristic tubulovesicular system (Δ). (d) A cell contains apical variable size electron dense granules (↑) and basal oval shaped euchromatic nucleus (N) was detected between parietal cells (Δ). (e) Chief cell with some apical variable size electron lucent granules (↑), basal oval shaped euchromatic nucleus (N) and prominent basal rough endoplasmic reticulum (Δ). (f) Enteroendocrine cell with a euchromatic nucleus (N) and numerous small electron-dense secretory granules (↑). TEM [(a, d) X 6000 (b, e) X 10000 (c) X 8000 and (f) X 12000]

Table 1: showing mean ± SD of blood glucose levels (mg/dl), thickness of the gastric fundic mucosa, area percentage of collagen fibers and different types of mucous secreting cells in different groups

Groups	Blood glucose levels (mg/dl)	Thickness of gastric fundic mucosa (µm)	Area % of Collagen fibers	Area % of mucous secreting cells
Control group I	108±1.58	644.17±11.59	3.37±0.31	18.14±0.2
Group II	105.6±1.51	648.98±9.68	2.59±0.25	17.73±0.27
Group III	338.8±4.43▲	348.22±6.43Δ	16.14±0.96▲	2.94±0.39Δ
Group IV	127.4±5.72■	509.55±9.50○	5.78±0.68■	10.29±0.41○

▲Significant increase compared with all other groups.
Δ Significant decrease compared with all other groups.
■ Significant decrease compared with group III.
○ Significant increase compared with group III.

DISCUSSION

Diabetes is a chronic disease that affects approximately 170 million people worldwide, numbers that are expected to double in the next few years[20]. Diabetes and its complications affect many organs, especially the gastrointestinal tract[21]. Despite the wide range of pharmacological drugs that have been used as an antidiabetic agent, there is a continuous search for new alternatives, due to their side effects. This study investigated the role of bee venom (BV) on the structure of fundic mucosa in STZ induced diabetes.

Diabetic complications are caused by prolonged hyperglycemia which causes excess production of reactive oxygen species (ROS). ROS increases the level of lipid peroxidation which is the cause of long-term complications of diabetes[5]. Diabetic complications are also caused by defects in the body`s antioxidant defense systems, reduced angiogenesis, more rapid apoptosis, damages to DNA, cellular membranes and organelles. Hyperglycemia can add to the oxidative stress when the production of ROS exceeds the antioxidant capacity[22]. Hyperglycemia also leads to increased protein glycation. Glycation is a non-enzymatic reaction between sugars and a free amino group of proteins resulting in structural and functional alteration in proteins with formation of advanced glycation end-products (AGEs). Protein glycation and AGEs are accompanied by increased free radical activity that leads to the biomolecular damage in diabetes. It is important to identify and develop AGE inhibitor that can suppress AGE formation[14]. Meanwhile, formerly other researchers[23] suggested that early lesion of gastric mucosa might be associated with the direct action of streptozotocin, the severity of which may be further intensified by diabetic state.

The isthmus of the fundic gland harbored active multipotent stem cell that could replenish all mature lineages within the gland daily[24]. It is suggested that all mature gastrointestinal epithelial cell lineages arise from a common stem cell, and this is known as the "Unitarian Theory". The stem cell niche of gastric fundic epithelium is nearer the lumen than in the base of the glands so, it is likely to be more exposed to surface irritants and requires bidirectional migration of its daughter cells[25]. It was added that fundic gland respond to injury via superficial response and glandular response. Superficial response is a rapid adaptation which involves changes in the rapidly dividing surface epithelium lining the stomach. It includes: epithelial restoration by cell migration, increase mucous production, increase blood flow and isthmus cell proliferation. While, glandular response involves slower adaptations by deeper cells in the gastric unit (parietal cells and chief cells). It is proposed that stomach, exhibits marked cellular plasticity which involves reprogramming of mature cells to serve as auxiliary stem cells that replace lost cells[24].

In diabetic group of the current study, focal sloughing of gastric mucosal epithelium was noticed, with intact surface epithelium above the sloughed areas. This might be due to the rapid superficial response induced by cellular migration. It was suggested that focal erosions of the stomach are repaired by increased proliferation of neighboring stem cells[25]. Also, focal mucosal ulcerations were noticed in scattered areas of fundic glands in diabetic group of the present study. Disruption of surface epithelium was confirmed by loss of mucous granules in PAS/Alcian blue stained sections with a significant decrease in mucous producing cells. The observed decrease in mucous producing cells with consequent loss of mucosal barrier might be the cause of the observed gastric mucosal ulceration in diabetic group. Some authors reported that the defense mechanisms of the gastric mucosa are disordered in diabetes. This increases its sensitivity to ulcerogenic factors and inhibits healing of gastric mucosal lesions[18]. Other authors attributed the cause of mucosal ulceration to back diffusion of hydrogen ions in the stomach, reduction in gastric blood flow, formation of free radicals and loss of mucosal glycoprotein. Free radicals cause degradation of the epithelial basement components, alteration of the cell metabolism and DNA damage[4]. It was reported that diabetes is associated with delayed or impaired repair of injured tissue, because diabetes is associated with hypoxia, defective T cell immunity and defects in phagocytosis[22].

Moreover, a significant decrease in thickness of fundic mucosa was noticed in rats of diabetic group of the current work. Some authors[26] postulated that endocrine hormones secreted by the gastrointestinal tract influence most gastrointestinal functions. Increased plasma concentration of somatostatin, reduced plasma gastrin concentration and smaller antral G-cell population was noticed in diabetic rats. They also reported that somatostatin is a recognized inhibitor of gastrin release. Since gastrin has been implicated in the control of gastric growth, so reduced secretion of this hormone is an explanation of parietal cell hypoplasia, gastric atrophy and reduced stomach weight in diabetic rats. It was added that diabetes is also associated with neuropathy that may reduce vagal input to the stomach leading to the observed gastric atrophy. In the current study, ultrastructural changes (cytoplasmic vacuolations) were noticed in enteroendocrine cells of diabetic group. This was explained by some researchers[27] who stated that mucosal enteroendocrine cells are chemosensory cells that monitor luminal environmental changes and mucosal abnormalities. They added that the stomach has a large population of ‘closed’ type enteroendocrine cells that are localized to the lower half of gastric glands with parietal cells.

In the current study, eosinophilia and an apparent increase number of inflammatory cells were noticed in lamina propria of diabetic group. It was reported that diabetes is always associated with hypoxia which amplify the inflammatory response through augmentation of inflammatory mediators (e.g. IL-6) and increasing the levels of oxygen radicals[22]. The diabetic group of the present work also showed sloughing of apoptotic parietal

cells with ultrastructural changes as karolytic nuclei and vacuolations. It was proposed that chronic inflammation of the stomach leads to loss or atrophy of parietal cells. It was also suggesting that parietal cells might influence stem cell proliferation, following certain types of injury[25]. So, the observed affection of parietal cells in diabetic group, might be the cause of disturbed healing of gastric mucosa in the current study.

In addition, some investigators[28] revealed that chief cells arise from mucous neck cells that migrate into the basal part of the gland with gradual change of mucous secretion to digestive enzyme secretion as they enter the base of the gland. A transition zone is noticed between the end of the neck zone and the beginning of the base that harbored cells containing both mucus and zymogens granules. Immature zymogen cells contain abundant large granules than mature cells. Differentiation of zymogen cells normally occurs in many phases: neck cell; transitional zone cell; and mature zymogen cell. In the current study, mucus neck cells were frequently seen with numerous apical electron lucent granules with central electron dense core. These cells might represent the transitional mucous neck cells.

In the current study, dilated endoplasmic reticulum was frequently noticed in chief cells of diabetic group. This was explained by some investigators[29,30] who postulated that hyperglycemia induces oxidative stress by producing stress of the endoplasmic reticulum in the form of dilation of the endoplasmic reticulum. They also added that if endoplasmic reticulum dysfunction is severe or prolonged it can lead to endoplasmic reticulum stress–induced apoptosis and cell death

In the current study, dilated congested blood vessels and significant increase collagen fibers content were noticed in lamina propria of diabetic group. It was suggested that increased collagen content in diabetic rats can be attributed to increase hydroxyproline content which is a major component of collagen fibrils. Increase hydroxyproline is associated with insulin resistance[31]. Moreover, other scientists[32] reported that diabetes is associated with vascular dysfunction which lead to reduced blood flow and is correlated with insulin resistance.

In a trial of ameliorating the structural changes that had occurred to gastric fundic mucosa subjected to streptozotocin induced diabetes mellitus, Bee Venom (BV) was used as a possible agent in the current study in group IV. Recently, BV has proven its effectiveness in traditional medicine for treating a variety of disease conditions. In addition to its anti-inflammatory activity, BV also exhibits antioxidant, antimicrobial, and analgesic effects[10]. Moreover, some investigators[4] reported that agents that have gastro-protective properties are classified into two groups; those that decrease or counter acid/pepsin secretion and those that provide cyto-protection by their effects on mucosal defensive factors. These defensive factors include mucin secretion, cellular mucus, antioxidants, bicarbonate secretion, mucosal blood flow and cell turnover. In the present study, proliferation of mucous neck cells with a significant increase in mucous producing cells was noticed in PAS/Alcian blue stained sections in diabetic-BV treated group compared to diabetic untreated group. This might suggest that BV has cyto-protective effects on fundic mucosa.

Administration of BV also lead to restoration of the ultrastructure of cells of fundic gland. It was suggested that in tissue injury, honeybee product stimulates rapid development and differentiation of stem cells into needed cells[33]. It was also reported that BV and its components are effective in proliferation, survival and differentiation of stem cells[34]. In diabetic-BV treated group of the current study, an almost normal structure of parietal cells was noticed. It was stated that increase parietal cell count in BV treated rats suggests a potential proliferative effect of BV on parietal cells[35].

In the present work, focal reduction of inflammatory cells was noticed in BV treated group compared to diabetic untreated group. It was postulated that BV exerts anti-inflammatory effects. It is as effective, if not, more effective than known antibacterial drugs involving no side effects in animal models[12]. Administration of BV stimulates the function of immune system and induces the release of cortisol which is known as natural anti-inflammatory agent[35].

In the current study, a significant decreased in blood glucose level was noticed following BV treatment. This could be attributed to mellitin and phospholipase-A which constitutes about 52% of the venom peptides. They diminish inflammation of islets of Langerhans and stimulate them to secret insulin[7]. BV also inhibits proinflammatory cytokines and free radicals which induce apoptosis of beta cells[36]. Moreover, other scientists[14] suggested that BV has a significant antiglycation effect so it can prevent glycation-induced alteration in diabetes. Bee venom has been used as a traditional medicine to treat chronic inflammatory conditions. Bee venom therapy is done by various methods, as Apitherapy (using live honeybee stings), apipuncture (BV acupuncture) and by direct injection.

CONCLUSION AND RECOMMENDATION

Bee venom had prevented structural changes in the fundic mucosa in a rat model of STZ induced diabetes. So, it might potentially provide a possible solution for gastric structural changes induced by diabetes mellitus.

However, there is still a wide diversity of questions remain open. Therefore, it is recommended that long term investigations are still highly desired to understand the new molecular targets for treatment of diabetes mellitus.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربى

الدور المحتمل لسم النحل على الغشاء المخاطي لقاع المعدة في مرض السكري المحدث بواسطة الستربتوزوتوسين في الجرذان. دراسة هستولوجية

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المقدمة: يمثل مرض السكري أحد الامراض المزمنة الأكثر انتشارا كما يمثل تحديا رئيسيا في مجال الصحة العامة في جميع أنحاء العالم ويؤدي هذا المرض إلى عواقب وخيمه في العديد من الأنسجة بما في ذلك الجهاز الهضمي. ويصنف سم النحل ضمن الطب التقليدي المستخدم لعلاج الأمراض المختلفة.

الهدف من البحث: دراسة اثار مرض السكري علي التغيرات النسيجية في الغشاء المخاطي لقاع المعدة، وتقييم الدور العلاجي المحتمل لسم النحل علي هذه التغييرات في نموذج مرض السكري المحدث بواسطة الستربتوزوتوسين في الجرذان.

المواد وطرق البحث: قسم ثلاثون من ذكور جرذان الويستر المهق البالغين إلى أربع مجموعات. المجموعة الأولى (الضابطة)، المجموعة الثانية (مجموعة سم النحل) تلقت حقنتين أسبوعيا من سم النحل (٠.٥ مج⁄كم) داخل الصفاق لمدة أربع أسابيع، المجموعة الثالثة (مجموعة مرض السكري) تلقت حقنة واحدة من ستربتوزوتوسين (٤٥ مج⁄كم) داخل الصفاق. المجموعة الرابعة (مجموعة مرض السكري- وسم النحل) بعد أسبوع من التأكد من الإصابة بمرض السكري تم إعطاء الجرذان سم النحل لمدة أربع أسابيع كما في المجموعة الثانية. بعد انتهاء مدة التجربة تم جمع عينات من قاع المعدة من جميع الجرذان، وتم تجهيز العينات لدراستها بالمجهر الضوئى والإلكترونى النافذ. كما أجريت أيضا دراسات قياسية وإحصائية.

النتائج: أظهرت الجرذان المصابة بمرض السكري انسلاخا في النسيج الظهاري لقاع المعدة، وانخفاضا ذو دلالة إحصائية في الخلايا المفرزة للمخاط، وزيادة ذات دلالة إحصائية في ألياف الكولاجين بالإضافة إلى زيادة خلايا الالتهاب في الصفيحة المخصوصة. كما شوهدت خلايا غدد المعدة بأنوية منحلة و توسع في الشبكة الإندوبلازمية الخشنة. وأظهر إعطاء سم النحل تحسنا في البنية النسيجية الهيكلية الناجمة عن مرض السكري.

الاستنتاجات: أن استخدام سم النحل له امكانية في علاج التغيرات الناجمة عن مرض السكري المحدث في الغشاء المخاطي لقاع المعدة فى الجرذان. مما يمكن أن يوفر حلا محتملا للتغيرات المعدية المرتبطة بمرض السكري.