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Original Research Article

Immunohistochemical characterization of pancreatic delta cell as potential target for dibutyl phthalate toxicity in Wistar rats

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Abstract

Purpose: *To investigate the endocrine disrupting potential of dibutyl phthalate (DBP) on δ-cells of the pancreas.*
Methods: *A total of 40 adult Wistar rats comprised of 20 males and 20 females were each grouped into control and study groups. Control groups had spontaneous access to food and water (control-spontaneous), and oral gavage normal saline (3.2 mL/kg; control-forced) every day for 8 weeks. Study group were exposed to DBP in their drinking water (0.8 µg/L; study-spontaneous), and oral gavage 0.8 µg DBP/kg for 8 weeks (study-forced). Blood glucose level was monitored weekly, and oral glucose tolerance test (OGTT) was conducted at the end of the experiment. The animals were sacrificed, pancreas excised, re-sectioned and processed for hematoxylin and eosin (H&E) staining, and immunohistochemical assay for identification of somatostatin (SST) protein.*
Results: *The H & E-stained pancreatic sections revealed a typical structural pattern of the endocrine and exocrine pancreas. The immunohistochemical assay using an anti-SST antibody indicated δ-cell immunoreactivity, mainly localized at the periphery of the islet population. The cell body of δ-cells exhibited characteristic neuron-like shaped filopodia-like extensions. The DBP-exposed animals demonstrated significant SST immunoreactivity compared to control ($p < 0.05$). The forced mode of DBP exposure showed a more significant effect on SST absorbance with no sex differences compared to drinking water exposure ($p < 0.05$).*
Conclusion: *This study shows that DBP toxicity induces alteration in the pancreatic δ-cells' SST immunoreactivity, which depends on exposure irrespective of sex.*

Keywords: *Immunohistochemistry, Pancreas, δ-cells, Environmental toxicity, Phthalate, Endocrine disrupting potential*

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INTRODUCTION

Owing to the rapid progress and advancement of technology in different fields such as medicine, industry, and agriculture, human activities have generated new chemicals of emerging concerns that leak into the surrounding environment. Many of those chemicals are potential endocrine disruptors, affecting the health of living organisms [1]. Endocrine-disrupting chemicals (EDCs), e.g. phthalates, are toxic compounds that negatively affect human health

and the environment. Reproductive diseases, cancers, cardiovascular risks, and autoimmune diseases are associated with EDC exposure. Due to widespread environmental distribution and the inevitable human exposure to EDCs throughout life, phthalate pollution is a global public health concern, and its interactive effects have gained significant attention [2]. Dibutyl phthalate (DBP) is a phthalate ester used as a plasticizer in the plastic and resin industries. It is a well-known EDC detected in the environment due to its leaks during manufacturing, transportation, disposal, and recycling [3].

Somatostatin (SST), a peptide hormone with diverse biological functions, is secreted by neurons and neuroendocrine cells, collectively known as SST-secreting cells. It is involved in different endogenous signaling pathways as an inhibitory regulator of cellular functions such as growth, proliferation, development, metabolism, and neuromodulation. Pancreatic delta (δ) cells secrete SST to inhibit insulin and glucagon release. Hypothalamic SST suppresses the secretion of growth hormone and thyroid-stimulating hormone by the pituitary gland. In the gastrointestinal tract (GIT), it acts as a negative regulator of gastrin, cholecystokinin, and gastric acid secretion [4].

The gastrointestinal δ cells are responsible for about 65 % of total circulatory somatostatin, and 5 % arises from the pancreatic delta cells. Meanwhile, the remaining 30 % originates from the central nervous system. Also, SST exerts its actions through five SST receptors (SSTR 1-5 with two isoforms, SSTR2a and SSTR2b) distributed in various organs such as nervous tissue, digestive tract, pituitary gland, and pancreas [5]. Although the neuroendocrine system is a potential target for EDCs [6], the impact of DBP on SST-secreting cells, particularly pancreatic δ cells, has not been fully investigated. Delta cells are easily identified as being SST immunoreactive, in addition to their characteristic neuron-like shape having long processes or filopodia-like cellular projections [7].

A previous study [7] reported that the toxic potential of graphene oxide on the pancreatic delta cells of Japanese medaka fish manifested as increased SST immunoreactivity and its direct release to the nearby and far islet cells. As an extension of an earlier study [8], this study focused on pancreatic δ cells, amongst other SST-secreting cells, because the pathogenesis of diabetes is related to cellular signaling and paracrine regulation between the three main pancreatic islet cell types (α, β, and δ) [9]. Therefore, DBP effects may be sex-specific or dependent on the mode of exposure. Therefore, the current study investigated the pancreatic δ cells of male and female Wistar rats as a potential target of DBP toxicity in different modes of administration.

EXPERIMENTAL

Animals

A total of 20 male and 20 female Wistar rats (6 - 8 weeks of age) weighing 220 - 235 g were randomized into 2 main groups, control and study groups. The animals were kept in the animal house of the College of Pharmacy, King Saud University, Saudi Arabia under standard conditions (ambient temperature of 24 ^{o}C, 55 % humidity, 12-h light/dark cycle exposure) with unrestricted access to food and water *ad libitum*. The rats were allowed to acclimatize for one week before DBP treatment commenced. To avoid any potential plasticizer contamination, water was provided in glass bottles and metal cages were used for housing the experimental animals. The study was approved by the Research Ethics Committee, Vision College, Riyadh, Saudi Arabia (approval no. KSU-SE-20-37) and adhered to the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health [10]. Within limits, the experimental procedures were refined to avoid or minimize the animals' discomfort, distress, and pain.

Design

The animals were divided into 2 main groups: control (water and oral gavage normal saline groups) and study groups (DBP-exposed group). Each main group included 20 rats distributed equally into male and female subgroups. Study group was further subdivided, based on the mode of DBP exposure, into spontaneously (drinking water) or forcedly (gastric gavage). Control group was exposed to parallel experimental conditions. So, the study included 8 groups with 5 animals each.

- Control-spontaneous (male): The male rats had spontaneous free access to drinking water for 8 weeks.
- Control-spontaneous (female): The female rats had spontaneous free access to drinking water for 8 weeks.
- DBP-spontaneous (male): The male rats were spontaneously exposed to dibutyl phthalate (DBP; Sigma-Aldrich, Saint Louis,

MO, USA) in their drinking water (0.8 µg/L) for 8 weeks.

- DBP-spontaneous (female): The female rats were spontaneously exposed to DBP in their drinking water (0.8 µg/L) for 8 weeks.
- Control-forced (male): The male rats were forcedly given normal saline (3.2 mL/kg) orally via gavage, every day for 8 weeks.
- Control-forced (female): The female rats were forcedly given normal saline (3.2 mL/kg) orally via gavage, every day for 8 weeks.
- DBP-forced (male): The animals were forcedly exposed to DBP orally via gavage (0.8 µg DBP/ kg body weight of animal), once daily for 8 weeks.
- DBP-forced (female): The animals were forcedly exposed to DBP orally via gavage (0.8 µg/kg), once daily for 8 weeks.

The dose of DBP and the route of exposure were selected based on relevance to humans and to simulate real-world exposure [11]. The DBP was dissolved in tween 80 (1:1250 v/v) and further diluted to 1:1000 in drinking water (final concentration is 0.8 µg/L).

Glucose homeostasis profile and serum somatostatin

Oral glucose tolerance (OGTT), blood glucose and serum insulin levels were measured. A glucometer (Accu-Check; Roche Diagnostics, Benzberg, Germany) was used to monitor the blood glucose level every week. Animals with blood glucose levels > 250 mg/dL were considered diabetic [12]. At the end of the experiment, tail vein blood samples were obtained, centrifuged (3000*g* for 10 min), and sera were frozen (-80 ^{o}C) for insulin levels assay using ELISA kit (Novus Biologicals, USA, Cat no. NBP2-62854). Serum concentration of SST in animals was determined using the rat-specific SST ELISA kit (MyBioSource, California, USA, Cat no. MBS260025). The OGTT was carried out by fasting the animals for 12 h, after which they were given a glucose solution (2.5 g/kg) through gastric gavage [13]. Blood samples were withdrawn at different times (0, 15, 30, 60, 90, and 120 min) post-glucose loading and glucose levels were checked. After 24 h, the rats were anesthetized and sacrificed by cervical dislocation. The abdomen was opened, pancreas was excised, sectioned and fixed in 10 % formol saline for histological and immunohistochemical study.

Histological assays

Following a 48-hour fixation in 10 % formol saline at room temperature, the pancreatic specimens were subjected to the paraffin micro-technique. The samples were dehydrated by immersion in ethyl alcohol (70, 90 and 100 %) for 15 min for each of the changes, cleared in xylene for 20 min for each of the changes, followed by paraffin infiltration for 30 min at 60 °C, and embedded in paraffin blocks. Paraffin blocks were sectioned using a rotatory microtome and five-micrometer cut-sections were prepared. One per ten serial sections were selected, mounted on glass slide, deparaffinized, rehydrated through immersion in ethanol (100, 95, 70 and 50 %) for 5 min each and manually stained with hematoxylin and eosin [14].

Immunohistochemical assays

The immunohistochemical assay [15] was carried out for the identification of the SST-secreting pancreatic δ cells. Mouse monoclonal anti-somatostatin primary antibody (Santa Cruz Biotechnology Inc., Texas, USA, Cat no. sc-74556) was used as the primary antibody. Sections were exposed to heat-mediated antigen retrieval by boiling in citrate buffer (pH 6, 95 °C). Then, the sections were incubated with the diluted primary antibody (1:200) at 4 ^{o}C overnight. ImmunoCruz ABC secondary kits (sc-516216) were used for completion of the reaction. Thereafter, 3,3-diaminobenzidine (DAB) chromogen and hematoxylin counterstaining were used. A brownish cytoplasmic cellular reaction was considered positive. The SST positive control slide was prepared with the human pancreas. Negative control slide was prepared by replacing primary antibody with phosphate-buffered saline (PBS). Slide examination and image photographing were carried out in the College of Science, King Saud University, Riyadh, Saudi Arabia using a light microscope (Nikon Eclipse 80i). The built-in Nikon DXM1200C digital camera combined with Nikon's NIS-elements Ar imaging software (Nikon Corporation, Japan) was used for image capturing.

Morphometric evaluation

The image J software (Fiji image j, NIH, USA) was used for quantitative measurement. The measured parameters were the percentage area of SST immunoreactivity, absorbance of SST immunopositivity, and number of delta cells to

the total islet cells (percentage delta cell). Distorted pancreatic islets were excluded from evaluation and only round or nearly rounded islets were considered. Ten islets per section/group were randomly captured for measurements.

Data analysis

All data were collected, organized, and tabulated in an Excel file before importation into GraphPad Prism software (San Diego, California, USA) for analysis. Kolmogorov- Smirnov test was used for the normality check. Measurement data were presented in mean ± standard deviation (SD; n = 5) and compared using one-way analysis of variance (ANOVA) and Tukey's test. $P < 0.05$.

RESULTS

Effect of oral DBP exposure on glucose homeostasis

The animals had normal blood glucose levels throughout the eight-week experiment indicating a non-diabetic state. Furthermore, study group showed significantly improved glucose homeostasis compared to control groups ($p < 0.05$; Figure 1).

Oral glucose tolerance test

There was significant difference in glucose tolerance test following the different modes of DBP exposure ($p < 0.05$). Gavage administration of DBP resulted in significantly lower glucose levels irrespective of sex at 90 and 120 min ($p < 0.05$; Figure 2 A and B). With the same mode of exposure (Figure 2 C and D), there was no significant difference between the OGTT of the control male / female rats (Figure 2 D) as well as DBP-exposed male/female rats (Figure 2 C) in the different time points ($p > 0.05$). With the same sex (Figure 2 E and F), the OGTT of the forced mode of DBP was significantly different compared to the forced control at 60, 90, and 120 min ($p < 0.05$). There was no significant difference in serum insulin levels of the male and female rats following DBP exposure (by oral gavage or in drinking water) when compared to the corresponding control group (Table 1).

Effect of oral DBP exposure on serum somatostatin levels

Exposure to DBP in both groups (by oral gavage or in drinking water) resulted in non-significant changes in serum SST levels compared to their corresponding control groups.

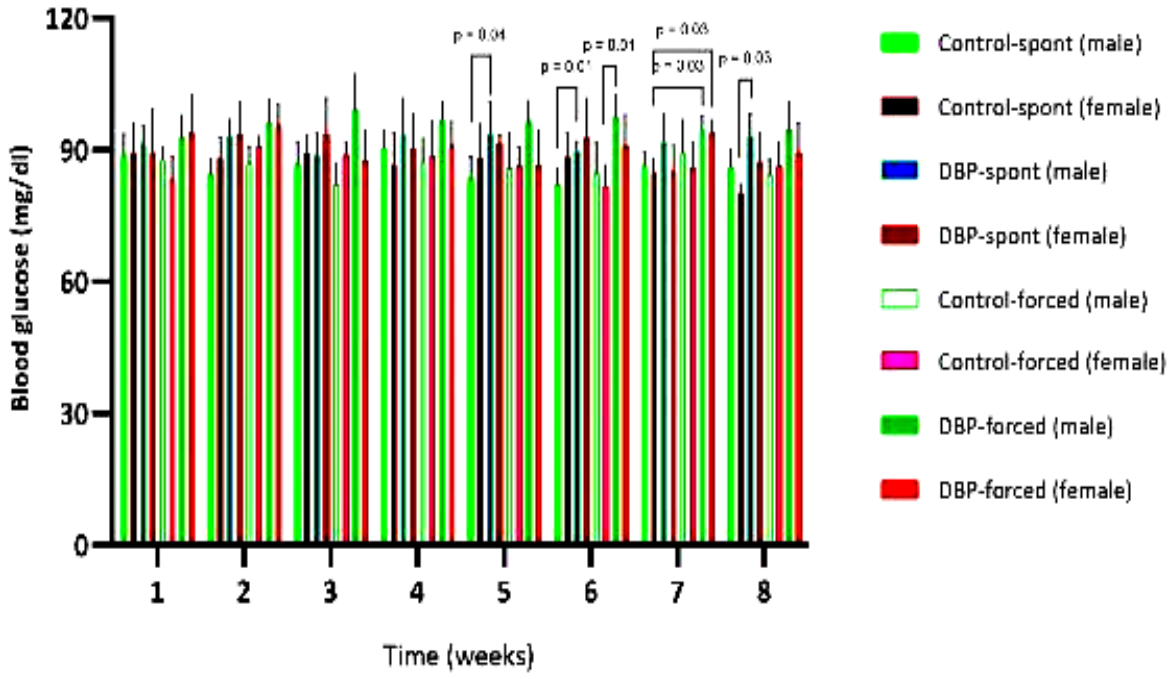

Figure 1: Effect of oral DBP exposure on the mean blood glucose levels of experimental animals. The values are expressed as mean ± SD (n = 5)

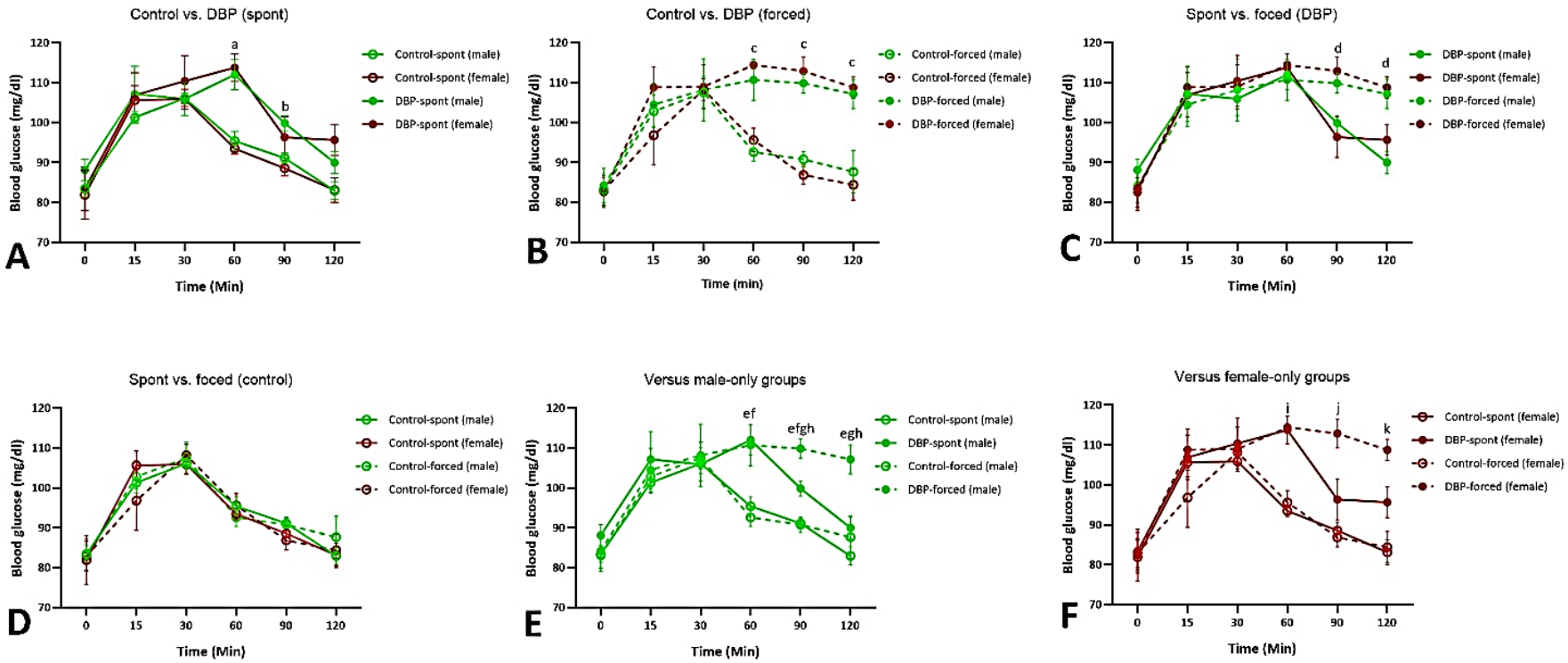

Figure 2: Effect of DBP exposure on OGTT at the end of the experiment. Results are expressed as mean ± SD (n = 5). A shows significant difference ($p < 0.05$) between the spontaneously exposed (control vs DBP) male and female rats. B shows significant difference between the forcibly exposed (control vs DBP) male and female rats. C: shows significant difference between the DBP exposed (spontaneous vs forced) male and female rats. D: shows significant difference between the control (spontaneous vs forced) male and female rats. E: Shows significant difference between male rats (control vs DBP and spontaneous vs forced). F: shows significant difference between the female rats (control vs DBP and spontaneous vs forced). Green lines indicate male animals, red lines indicate female animals, empty dots refer to the control group, filled dots refer to the DBP-exposed group, continuous lines refer to spontaneous mode of exposure, and dashed lines refer to the forced mode of exposure. $^{a}P < 0.05$ DBP female vs control male and DBP male vs control female, $^{b}p < 0.05$ between DBP male vs control female, $^{c}p < 0.05$ between DBP female vs control male & DBP male vs control female, $^{d}p < 0.05$ between DBP forced female vs DBP spontaneous male and DBP forced male vs DBP spontaneous female, $^{e}p < 0.05$ between DBP forced vs control spontaneous, $^{f}p < 0.05$ between DBP spontaneous vs control spontaneous and DBP spontaneous vs control forced and DBP forced vs control forced, $^{g}p < 0.05$ difference between DBP forced vs DBP spontaneous, $^{h}p < 0.05$ between DBP forced vs control forced, $^{i}p < 0.05$ between DBP spontaneous and forced DBP vs control spontaneous and DBP spontaneous, DBP forced vs Control forced, $^{j}p < 0.05$ between DBP forced vs control spontaneous and control forced and DBP forced vs DBP spontaneous, and $^{k}p < 0.05$ between control spontaneous vs DBP spontaneous and DBP forced

Table 1: Effect of different modes of DBP exposure on serum levels of insulin and SST in male and female rats

Parameter	Control-spont (male)	Control-spont (female)	DBP-spont (male)	DBP-spont (female)	Control-forced (male)	Control-forced (female)	DBP-forced (male)	DBP-forced (female)
Serum insulin (U/mL)	5.81±0.24	5.92±0.48	5.70±0.19	5.87±0.44	5.80±0.60	6.06±0.17	5.91±0.30	5.81±0.60
Serum SST (pg/mL)	260.5±7.89	265.6±7.50	258.8±9.5	263.8±4.66	258.7±8.63	260.2.8±11.98	259±9.18	256±9.12

Data presented in mean ± SD (n = 3). $P < 0.05$. DBP: Di Butyl phthalate, SST: somatostatin, Spont: spontaneously exposed

Table 2: Effect of different modes of DBP exposure on serum levels of insulin and SST in male and female rats

Group	Serum insulin (U/mL)	Serum SST (pg/mL)
Control-spont (male)	5.81±0.24	260.5±7.89
Control-spont (female)	5.92±0.48	265.6±7.50
DBP-spont (male)	5.70±0.19	258.8±9.5
DBP-spont (female)	5.87±0.44	263.8±4.66
Control-forced (male)	5.80±0.60	258.7±8.63
Control-forced (female)	6.06±0.17	260.2±11.98
DBP-forced (male)	5.91±0.30	259±9.18
DBP-forced (female)	5.81± 0.60	256±9.12

Data presented in mean ± SD (n = 3). $P < 0.05$. DBP: Di Butyl phthalate, SST: somatostatin, Spont: spontaneously exposed

Effect of oral DBP exposure on histological structure of pancreatic islet cells

The H & E staining of the pancreatic sections showed the islet cells as pale-stained patches scattered among deeply stained pancreatic acini. The islet cells appeared as masses of cells with basophilic nuclei and eosinophilic cytoplasm. Blood capillaries were seen intervening with the islet cells. The islet cell population consisted mainly of central beta cells and peripheral non-beta cells. No histological changes were observed in the cellular subtypes of the different groups (Figure 3).

Effect of oral DBP exposure on somatostatin immunoreactivity

One of the types of pancreatic islet cells is the delta cell. Delta cells are easily detectable using anti-SST immunohistochemistry. This study revealed different staining intensities and area distribution of delta cells in the DBP-exposed animals compared to control group. Also, SST immunoreactivity in DBP exposed rats (either males or females) appeared to be strongly positive in forcedly exposed rats compared to spontaneously exposed rats (Figure 4).

The effect of oral DBP exposure on islet measurements

All the assessed parameters showed no significant difference ($p > 0.05$) among the studied groups. Also, there was a significant increase ($p < 0.05$) in mean % area of SST immunoreactivity (Figure 5 A) and the absorbance of SST sensitivity (Figure 5 B) in the DBP-exposed males and females (spontaneously and forcibly exposed) compared to control male and female rats. However, the SST absorbance was more pronounced in the forced DBP exposure compared to spontaneous exposure. There was no significant difference ($p > 0.05$) in the islet measurements between the sexes ($p < 0.05$; Table 3).

DISCUSSION

The pancreas is a mixed gland comprising of islet cell population (the endocrine portion), glandular element, secretory acini and ducts (the exocrine part). Each islet cell cluster is composed of five specific cell types: α, β, δ, γ, and ε, concerned with the release of glucagon, insulin, somatostatin, pancreatic polypeptide, and ghrelin, respectively [16]. While evaluating DBP as a putative endocrine disruptor, this study investigated the immunohistochemical characteristics of pancreatic delta cells in Wistar rats (male vs female) exposed (spontaneously through drinking water, and forcibly through oral gavage) to environmentally relevant doses of DBP. The reason for the rapid increase in incidences of diabetes worldwide remains unclear. The World Health Organization (WHO) reported a rise in the number of diabetics (from 108 million to 422 million) between 1980 and 2014 [17]. Epidemiological studies revealed an association between EDC exposure and development of type 2 diabetes (T2DM) [18]. To verify the endocrine-disrupting effects of DBP on islet cells, the hypothesis was based on whether the effect of DBP on pancreatic delta cells of Wistar rats is dependent on sex or mode of exposure.

Table 3: Effect of oral DBP exposure on pancreatic islet cell morphometric parameters

Groups	Islet cell perimeter (um)	Number of cells per islet (n)	Percent of delta cells per islets (%)
Control-spont male	675.10±53.82	209.3±25.82	6.39±1.61
Control-spont female	670.30±88.30	212.60±21.92	5.75±1.40
DBP-spont male	646.50±95.25	217.90±37.33	6.76±1.20
DBP-spont female	636.80±79.81	218.30±33.89	6.18±1.66
Control-forced male	603.10±126.60	221.50±36.80	5.91±1.62
Control-forced female	634.90±125.30	207.30±33.99	6.15±1.56
DBP-forced male	583.20±123.40	208±36.54	6.05±1.62
DBP-forced female	647.40±107.10	219.70±45.96	5.72±1.66

Values are presented in mean ± SD (n = 5). $P > 0.05$ among the groups for each parameter

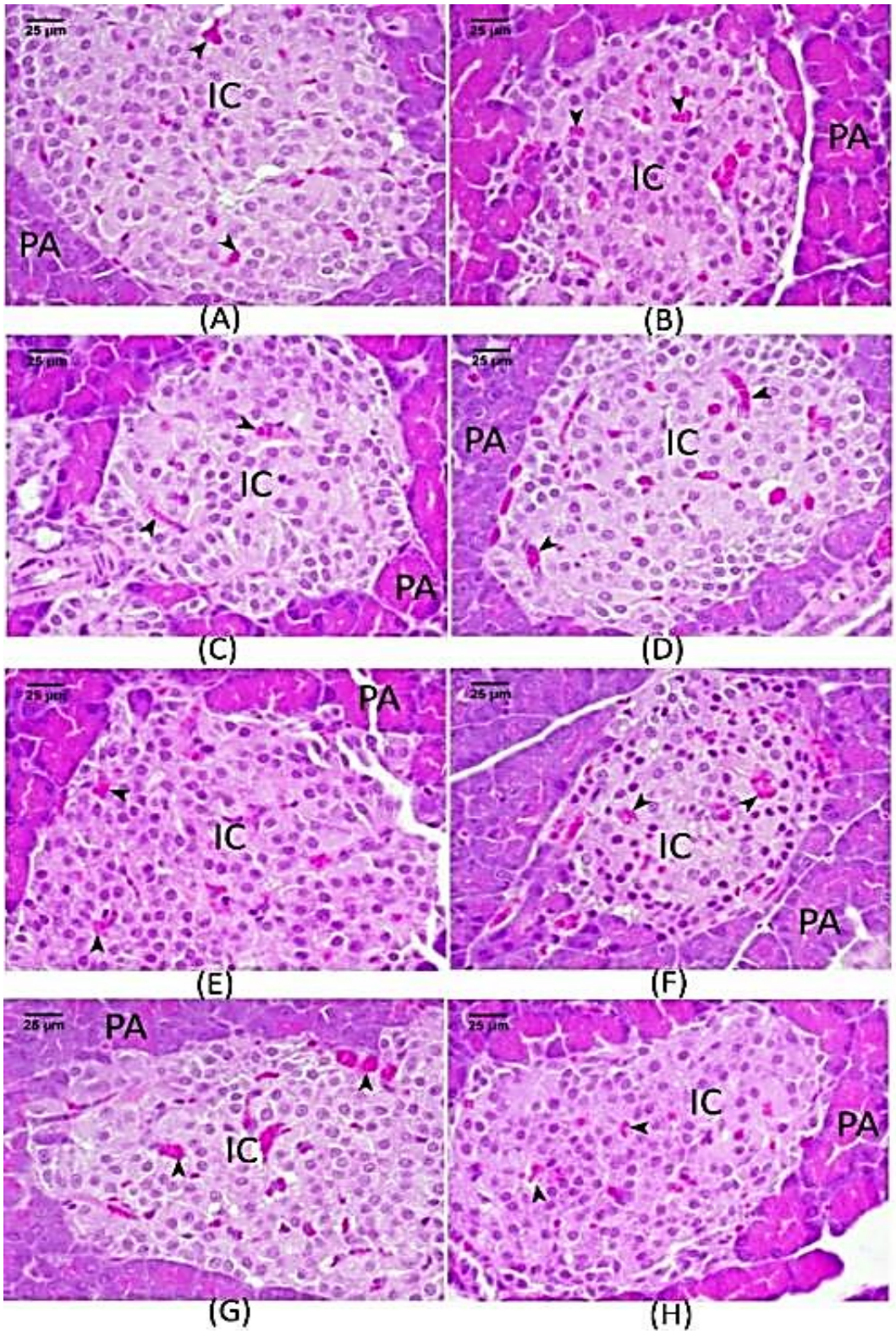

Figure 3: Effect of oral DBP exposure (spontaneous versus forced) on pancreatic islet cells of male and female rats (light photomicrographs, H & E staining, x400). Islet cell (IC), and pancreatic secretory acini (PA), and blood capillaries (arrowheads) were shown. The figure displays representative images for each experimental group; the control spontaneously exposed male (a), control spontaneously exposed female (b), DBP spontaneously exposed male (c), DBP spontaneously exposed female (d), control forcedly exposed male (e), control forcedly exposed female (f), DBP forcedly exposed male (g), and DBP forcedly exposed female (h). Scale bar: A-H, 25 µm

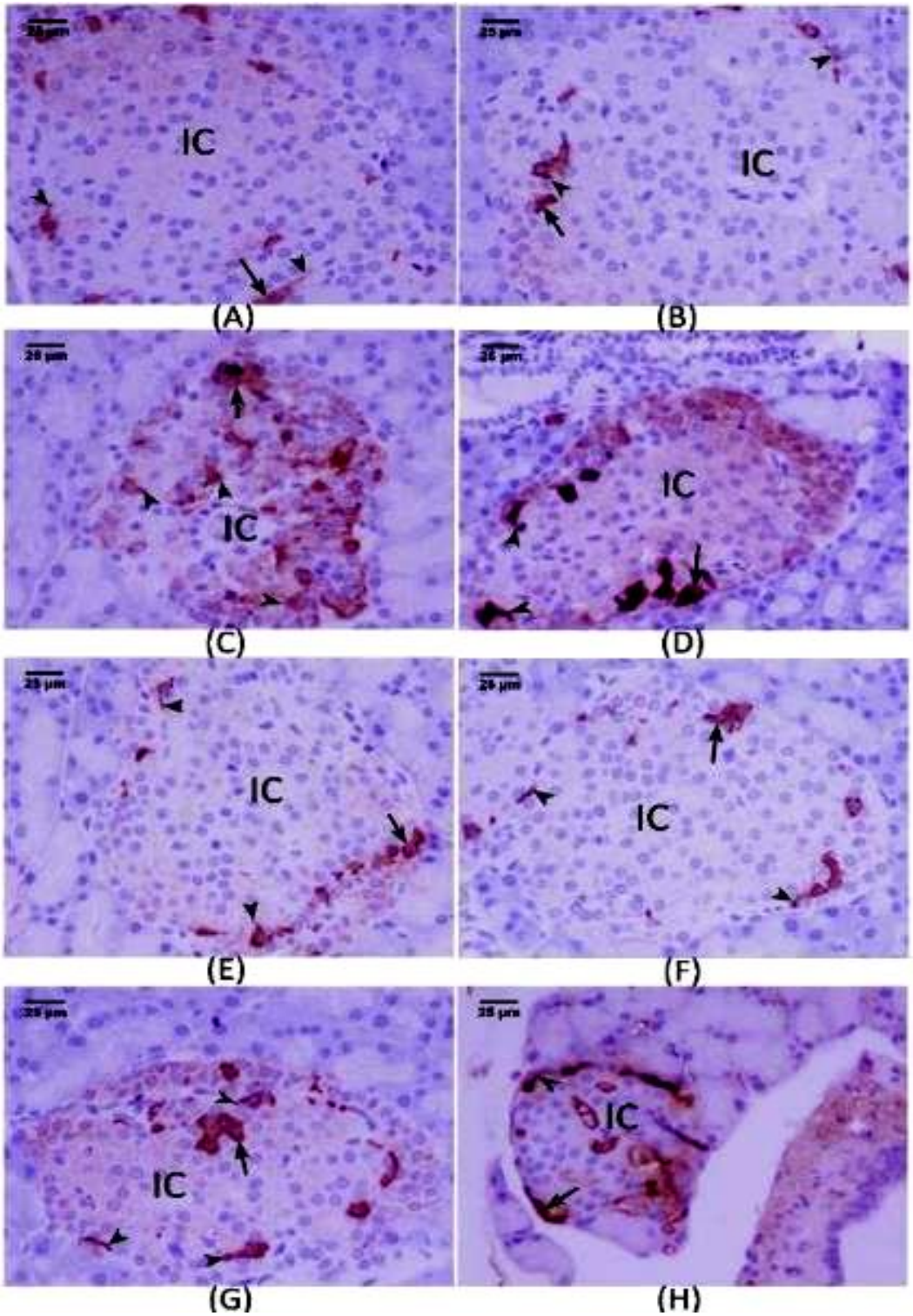

Figure 4: Effect of oral DBP exposure on anti-SST immunostained pancreatic sections. Scale bar: A-H, 25 µm. Immunostained-δ cells (arrows) appeared as neurons with filopodia-like cellular projections (arrowheads). They are located at the peripheral margins of islet cells (IC) surrounding central negative non-delta cells. The control-spontaneous male group (A), control-spontaneous female (B), DBP-spontaneous male (C), DBP-spontaneous female (D), control-forced male (E), control-forced female (F), DBP-forced male (G), and DBP-forced female (H) groups were presented. All groups showed the pancreatic δ cells with positive anti-SST immunoreactivity staining the cell bodies (arrows) and their cellular projections or processes (arrowheads). However, the DBP-exposed groups (C, D, G, and H) had a larger area of dense SST immunopositivity, irrespective of sex and exposure mode

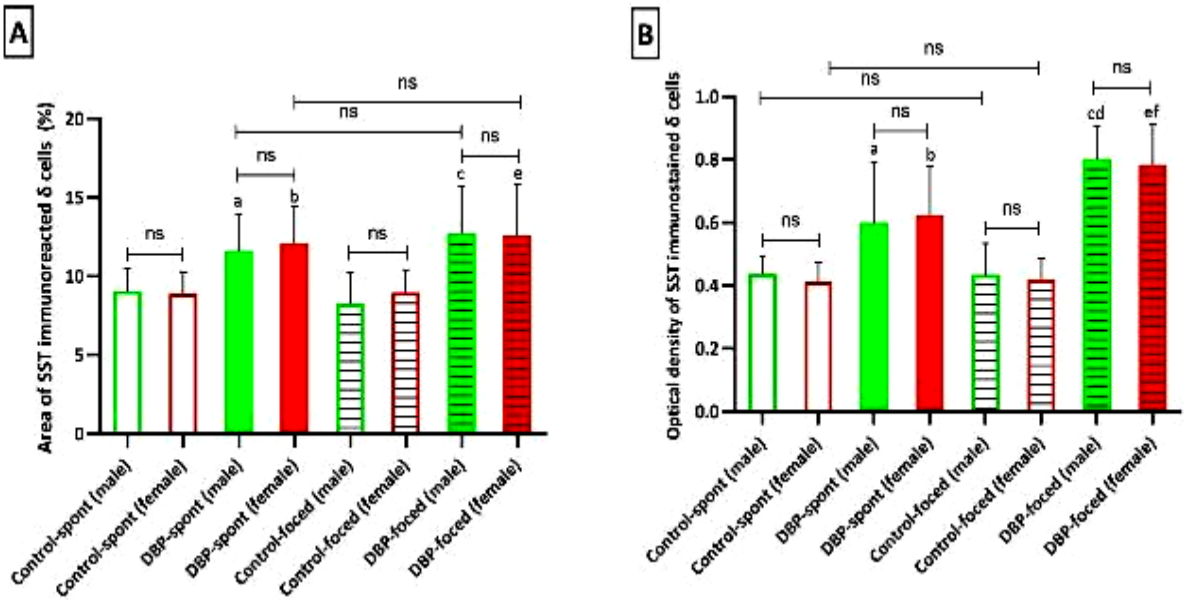

Figure 5: Effect of DBP exposure on morphometric evaluation of SST immunoreactivity of pancreatic delta cells. Results are expressed as mean ± SD (n = 5). Green bars signified male groups, red bars signified female groups, empty columns signified control, filled columns signified DBP exposure, and transverse lines within column signified forced mode of exposure. [a]*P* < 0.05 vs control spont male group, [b]*P* < 0.05 vs control spont female group, [c]*P* < 0.05 vs control forced male group, [d]*P* < 0.05 vs DBP spont male group, [e]*P* < 0.05 vs control forced female group, [f]*P* < 0.05 vs DBP spont female group, ns = not significant

Due to the paucity of information in literature, male and female animals were exposed to DBP spontaneously in their drinking water or administered via oral gavage. Histological assessment revealed no potential structural changes in the pancreatic sections of DBP-exposed groups compared to the corresponding control groups. Histological findings were supported by quantitative evaluation of pancreatic islet measurements, which revealed no change in islet perimeter or cell number per islet. These findings were consistent with previous studies [8] that reported no morphological changes in the exocrine or endocrine pancreas during DBP exposure. Evaluation of glucose homeostasis revealed normal serum insulin levels during the 8 weeks in all the studied groups. Although within the normal range, there were significant changes in the serum glucose level during the second 4 weeks of the study. On the other hand, OGTT revealed a significant increase in blood glucose levels of the forced DBP-exposed animals during time points of the test. In contrast, there was a significant difference in glucose level of the spontaneously-exposed animals at 60 min alone. This observation might explain the glucose tolerance impairment in rats forcibly exposed to DBP irrespective of sex.

Though the H & E staining procedure revealed no damage in pancreatic delta cells, this study reported a potential endocrine-disrupting impact of DBP in delta cells of the Wistar rats from immunohistochemical findings. The anti-SST immunohistochemical assays characterize the somatostatin-secreting δ-cells in the pancreatic islet. The current study revealed that the immunoreactive δ-cells are distributed in the periphery of pancreatic islets in both control and DBP-exposed rats. Similarly, an earlier study [19] reported peripheral distribution of δ-cells within the islet in rodents, with sparse δ-cells found in the islet center, and distribution was reported in humans. Also, it was observed that DBP enhances somatostatin expression within δ-cells cytoplasm in both male and female rats that were either spontaneously or forcibly exposed to it. Furthermore, SST immunohistochemistry was evaluated by measuring the percentage area and absorbance of SST immunoreactivity. The findings of this study revealed higher SST absorbance in the DBP forcibly exposed rats compared to spontaneous administration. However, the exposure mode did not affect SST % area, but there was a significant difference between DBP-exposed rats compared to control.

Immunohistochemistry-based counting of delta cells showed no significant difference between treatment groups, even though there was a significant difference in percentage area. This discrepancy may be due to possible δ cell swelling or distention by SST granules and or arborization of δ cell processes for effective intra-islet cellular communication. Significant changes

in OGTT and absorbance of SST expression in forced DBP-exposed groups may be explained by the fact that forced exposure via oral gavage ensured accurate dosage. Also, daily water consumption by the animals in spontaneously exposed groups might vary, thus affecting daily dosage. On the other hand, serum SST level was within normal range in the DBP-exposed animals compared to control group irrespective of sex or the mode of exposure. This observation might be due to the very short half-life of SST (about 1 min in the circulation) and the fact that pancreatic delta cells account for a small portion (5 %) of total SST sources in the body and the primary portion (65 %) is released from the gastrointestinal delta cells [20]. So, evaluating the effect of DBP on other SST-secreting cells is necessary. The impact of DBP on pancreatic δ-cells is still unclear. However, the δ-cells are vital regulators for insulin and glucagon synthesis [21]. For quantitative evaluation, the islet cells were categorized into two based on SST immunoreactivity: neuron-shaped SST immunoreactive δ-cells (DCs) and non-delta cells (NDCs, oval or rounded SST non-reactive cells). The percentage of DCs and NDCs was calculated showing a non-significant difference among the experimental groups.

Limitations of the study

This study lacks an *in vivo* component such as delta cell isolation and cell culture studies to further investigate other SST-secreting cells distributed in the body.

CONCLUSION

Pancreatic δ-cells in adult Wistar rats release somatostatin, which is enhanced by DBP exposure in both sexes. Furthermore, oral gavage showed more SST absorbance, and the percentage area of SST immunoreactivity was increased due to DBP exposure in both sexes, irrespective of exposure mode. However, exposure of Wistar rats to DBP, either by spontaneous or oral gavage resulted in specific cellular effects on pancreatic islets. These effects must be broadly investigated using other methods and different islet cell types.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

The study was approved by the Research Ethics Committee, Vision College, Riyadh, Saudi Arabia (approval no. KSU-SE-20-37).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ezat Mersal conceptualized the research idea, followed up on the experimentation, performed the biochemical analysis, and wrote the initial draft of the manuscript. Ahmed Morsi photographed the histology slides, wrote the image legends and assay results, performed the statistical analysis, and participated in writing the initial draft. Shaimaa Alakabawy, Riham Elfawal, and Bushra Assery followed up on the experiment, analyzed the data, type-edited the manuscript, and participated in the discussion. Omar Hasan, Nour Eddin Alzayed, and Ibrahim Koujan arranged the logistics, conducted the experiment, and tabulated the research records. All authors approved the final version of this manuscript.

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