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NIGELLA SATIVA (KALONJI) SEED OIL AMELIORATES THE PANCREATOXIC EFFECTS OF BIFENTHRIN IN MICE

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ABSTRACT: The aim of the present study was to examine, in mice, the pancreatoxic effects of bifenthrin (Bn) exposure and the rehabilitative capacity of *Nigella sativa* (NgS) seed oil. The six groups (N=5) of male mice and their treatment regimes were: (i) the negative control (NC) group (0.1 mL corn oil for 14 days); (ii) the positive control (PC) group (0.1 mL corn oil on days 1–6 and 10% v/v NgS oil in corn oil on days 8–14); (iii) and (iv) the Bn2.5 and Bn5 groups (2.5 and 5 mg/kg Bn in 0.1 mL corn oil, respectively, on days 1–7 and 0.1 mL pure corn oil on days 8–14); (v) and (vi) the Bn2.5NgS and Bn5NgS groups (the respective Bn treatments on days 1–7 and 10% NgS oil on days 8–14). The treatments were given intra-gastrically by gavage. Food and water were provided *ad libitum.* The pancreas from each animal was recovered for histological examination on day 15. The Bn exposure was found to cause pancreatitis with prominent acinar-to-ductal metaplasia (ADM) with a simultaneous hypotrophy and necrotic lesions of the acini. The mean count of exocrine cells per acinus was significantly lower in the Bn2.5 (9.4±0.38) and Bn5 (8.8±0.47) groups than in the NC (13.1±0.62), PC (14.84±0.79), Bn2.5NgS (11.24±0.68), and Bn5NgS (12.76±0.72) groups. The islets of Langerhans showed hypertrophy in the Bn2.5 group and secondary atrophy in the Bn5 group. However, endocrine cell hyperplasia and a consequential hypertrophy of the islets was seen in both the Bn2.5NgS and Bn5NgS groups. Additionally, pancreatic islet metaplasia was also observed in Bn5NgS group. The micrometry estimations likewise showed a significantly (p=0.05) higher mean cross-sectional area (CSA) of the pancreatic islets in the PC (16521±1744 μm^2), Bn2.5NgS (16930±1700 μm^2), and Bn2.5 (13703±1572 μm^2) groups than in the Bn5 (6382±1405 μm^2), NC (9464.75±955 μm^2), and Bn5NgS (11451±1099 μm^2) groups. The mean CSA of the endocrine cells of islets in the Bn2.5 (30.1±1.39μ^2) group remained significantly higher than in all the other groups (NC: 23.1±0.83 μm^2; PC: 21.6±0.85 μm^2; Bn5: 24.4±1.03 μm^2; Bn2.5NgS: 25.2±0.95 μm^2, and Bn5NgS: 22.1±0.71 μm^2). The mean number of cells in the islets/unit area (289 μm^2) in the Bn2.5 and Bn5 (141.37±6.3 and 132.03±6.5, respectively) groups remained significantly lower than in the NC (150.89±6.7), PC (174.43±6.6), Bn2.5NgS (172.97±6.4), and Bn5NgS (150.61±6.5) groups. These findings suggest that Bn exposure at levels of 2.5 mg/kg and above for seven or more days can result in exocrine and endocrine pancreatic pathologies and that NgS seed oil helps to ameliorate the tissue damage and restore the structural and functional attributes of the pancreas in mice.

Keywords: Bifenthrin; *Nigella sativa* (Kalonji) seed oil; Pancreatic histopathology.

INTRODUCTION

The Type-I pyrethroid insecticide bifenthrin (Bn) has been used worldwide to control insect pests.[1,2] Like most of the other insecticides, the hazardous outcomes of Bn exposure on the vital body organs and the endocrine glands are well documented.[3-7] However little is known about its pancreatoxic potential. The pancreas is a secretory organ consisting primarily of the acinar tissue and the islets of

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Langerhans. The acinar tissue produces pancreatic juice containing the digestive enzymes (trypsinogen, chymotrypsinogen, lipase, phospholipase A2, lysophospholipase, cholesterol esterase, and amylase) while the islets mainly comprise the α and β cells that secrete the hormones glucagon and insulin, respectively, for the regulation of the plasma glucose level.[8-10] Delta cells (δ) in the islets also secrete somatostatin which decreases the release of insulin and glucagon. Pancreatic toxicity may result in acute pancreatitis and diabetes mellitus with digestive incapability and an inability to maintain the blood glucose level.[11]

Nigella sativa, a medicinal plant of the family Ranunculaceae, has been recommended for the treatment of many diseases in Tibb-e-Nabwi (Islamic Prophetic medicine).[12,13] The seed oil of NgS contains many important constituents such as thymoquinone, thymol, thymohydroquinone, p-cymene, polyphenols, tocopherols, α-spinasterol, β-sitosterol, cholesterol, citronellyl acetate, campesterol, citronellol, carvone, limonene, nigellone, stigmasterol, linolenic, linoleic arachidonic, eicosadienoic acid, myristic, palmitic, oleic, palmitoleic acids, and stearic acid.[14,15] Evidence indicates that NgS has ameliorative and regenerative potential for treating the toxic effects on the body organs and organ systems of various noxious environmental chemicals such as insecticides.[16,17] The beneficial effects of NgS on endocrine and homeostatic activities include the regulation of metabolic activities and the blood glucose concentration.[18-19] We reported previously on the curative and regenerative potential of NgS oil treatments on the Bn-induced histopathologies of the liver and the adrenal gland.[1,2] In the present study we investigated the pancreatic histopathology of Bn exposure and the mitigating capacities of NgS-oil on Bn-induced pancreatoxic effects.

MATERIALS AND METHODS

Animals: Thirty adult male Swiss Webster mice were distributed into 6 groups (n=5). As reported earlier, the animals were kept under standard conditions (i.e., humidity, temperature, and light-dark cycles).[2] Free access to food and water were ensured throughout the study period.

Preparation of the Bn solutions: The technical grade insecticide (Bn) was diluted in corn oil to produce the required treatment solutions (i.e., 2.5 mg/kg and 5 mg/kg).[2]

Preparation of the NgS oil solution: The NgS seed oil (extracted and marketed by Marhaba Laboratories limited, Lahore Pakistan) was purchased from the local market, to produce a 10% (v/v) NgS/corn oil mixture for the treatments of the animals in the PC, Bn2.5NgS, and Bn5NgS groups.

Animal treatment groups:

(i) the negative control (NC) group: 0.1 mL corn oil for 14 days;

(ii) the positive control (PC) group: 0.1 mL corn oil on days 1–6 and 10% v/v NgS oil in corn oil on days 8–14;

(iii) the Bn2.5 group: 2.5 mg/kg Bn in 0.1 mL corn oil, on days 1–7 and 0.1mL pure corn oil on days 8–14;

(iv) the Bn5 group: 5mg/kg Bn in 0.1 mL corn oil on days 1–7 and 0.1 mL pure corn oil on days 8–14;

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(v) the Bn2.5NgS group: 2.5 mg/kg Bn in 0.1 mL corn oil, on days 1–7 and 10% NgS oil on days 8–14.;

(vi) the Bn5NgS group:5mg/kg Bn in 0.1 mL corn oil on days 1–7 and 10% NgS oil on days 8–14.

All treatments were intra-gastric and provided by appropriate gavages on a 24 hourly basis.

Excision of pancreas and the histological preparations: The animals were euthanized by cervical dislocation on day 15 of the study to exteriorize the entire pancreas. The organs were readily fixed in acidified formyl ethanol for 48 hours and finally processed for dehydration in different grades of ethanol, clearing in xylene, wax embedding in molten histological wax (melting point: 58–60ºC), and serial sectioning (3 µm thick) on a rotary microtome (ERMA TOKYO 422). The serial sections were mounted on the albumenized histological slides in a water bath at 45ºC and were finally stained with hematoxylin and eosin.

Histological observations and digital micrometry: Sections of pancreas were carefully observed and snapshots (at 100× and 400×) were obtained in a digital (7.2 megapixels) camera (Sony DSC-W35) on a trinocular research microscope (Labomed CXR_2). The digital photographs of the pancreatic sections of all the groups were used for the micrometric estimations. The selected photographs were further processed (color and contrast adjustments, digital cropping, and on-snap labeling to highlight various signs of histopathology) in CorelDRAW11® for the presentation of the photographic data.

Micrometric measurements were obtained for the mean number of exocrine cells per acinus, the mean CSA of the islets of Langerhans, the mean number of endocrine cells per unit area (289 μm^2) in the islets, and the mean CSA of endocrine cells in islets, for which digital images (100× and 400×, respectively) of three randomly selected sections of pancreas from each animal were used. The measurements were obtained from these sections projected in CorelDRAW11® using a calibrated digital scale (obtained from the 100× and 400× photographs of the stage micrometer).

Data analysis and statistical application: The micrometric data were analyzed through ANOVA, ANCOVA, Tukey's Multiple Range Test, and the Least Significant Multiple Comparison employing SPSS20.

RESULTS

Histological findings: The histological slides in the NC group showed the acini and the exocrine duct system (containing the intercalated, intra-lobular, and interlobular ducts) of the pancreas had a normal appearance with an even distribution. The islets of Langerhans appeared in close association with the blood vessels and were imbedded randomly throughout the acinar tissue (Figures 1A and 2A). The acinar tissue formed aggregations of exocrine cells (the acini). The acinar cells contained prominent peripheral nuclei and central eosinophilic cytoplasts (consisting mainly on the pancreatic zymogenic proteins) (Figure 2A). The islets were mostly spherical in shape and contained aggregations of small endocrine cells (Figures 1A and 2A).

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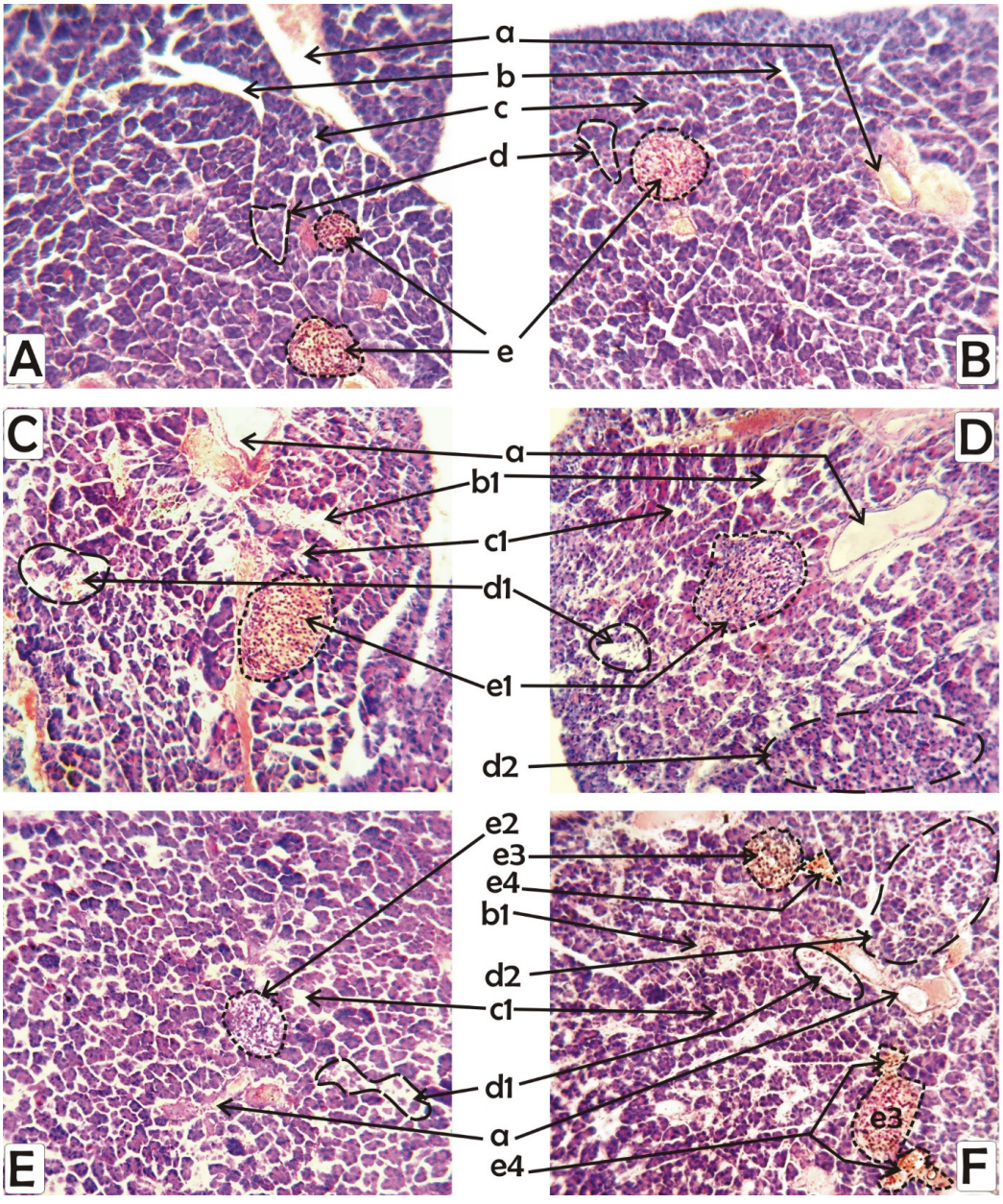

Figure 1. Histological sections (100×) of mouse pancreas: A: (NC), B: (PC), C: (Bn2.5), D: (Bn2.5NgS), E: (Bn5), F: (Bn5NgS); a: interlobular duct; b: intralobular duct; b1: intralobular duct, enlarged; c: intercalated duct; c1: intercalated duct, enlarged; d: single acinus; d1: acinus focal degenerations; d2: acinar tissue regeneration (probable proliferation of the acini); e: islet of Langerhans; e1: islet of Langerhans, hypertrophy; e2: islet of Langerhans, probable secondary atrophy; e3: islets of Langerhans, hyperplasia / proliferation; e4: islet metaplasia

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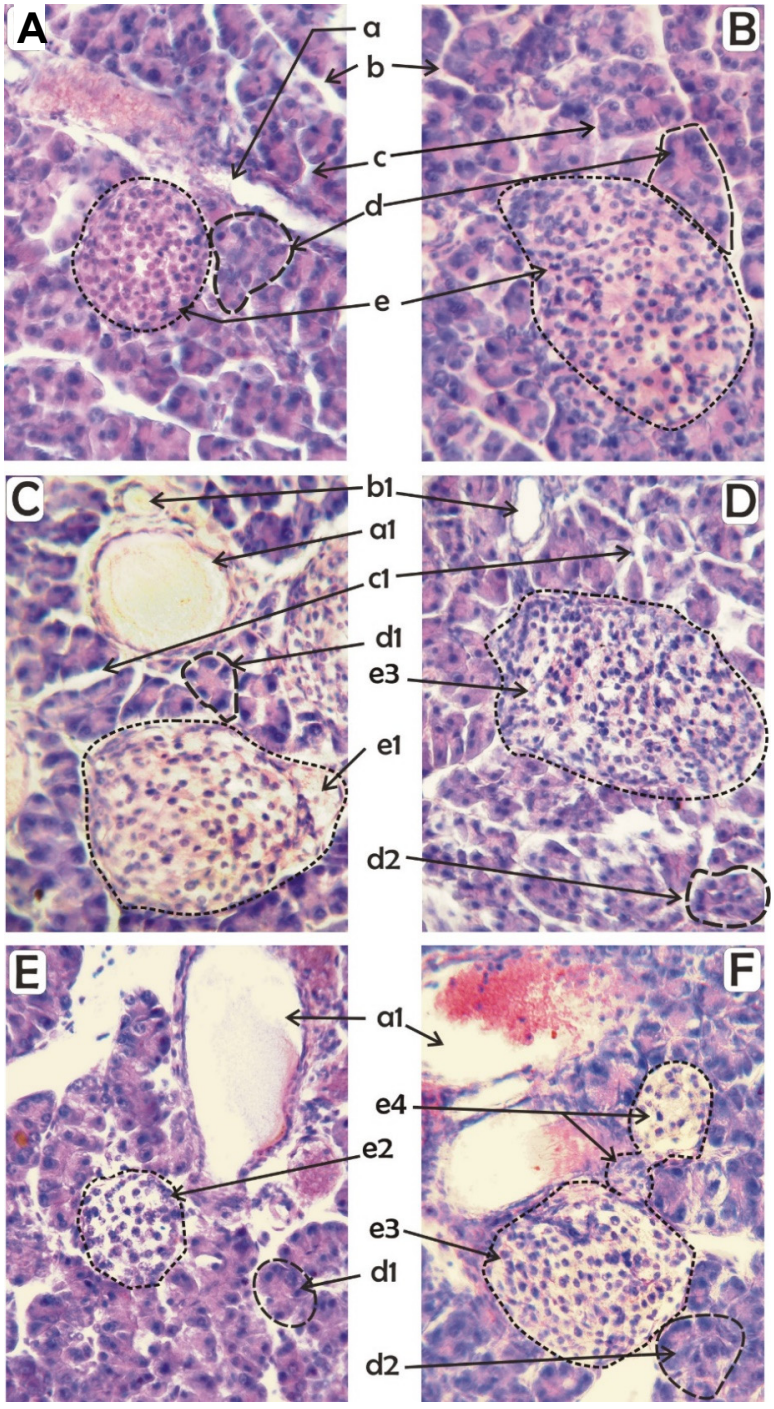

Figure 2. Histological sections (400×) of mouse pancreas: A: (NC), B: (PC), C: (Bn2.5), D: (Bn2.5NgS), E: (Bn5), F: (Bn5NgS); a: interlobular duct; a1: interlobular duct, enlarged; b: intra-lobular duct; b1: intra-lobular duct, enlarged; c: intercalated duct; c1: intercalated duct, enlarged; d: single acinus; d1: acinar tissue atrophy; d2: acinar tissue regeneration; e: islet of Langerhans; e1: islet of Langerhans, hypertrophied due to extra-cellular edema; e2: islet of Langerhans, secondary atrophy; e3: islet of Langerhans, hyperplasia; e4: islet metaplasia.

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The histological sections of the PC group were not much different from the NC group. However, the acinar cells seemed to be slightly enlarged due to an increase in the eosinophilic cytoplasts and the islets were more globular and showed hypertrophy as well as hyperplasia (Figures 1B and 2B).

The Bn2.5 and Bn5 groups sections showed enlarged intercalated, intra-lobular, and interlobular ducts with a concomitant hypotrophy and hypoplasia (decrease in the number and size of the acinar cells per acinus) of the acini indicating the so-called acinar to ductal metaplasia (ADM). Some acini also gave an impression of disassociation of the adjacent exocrine cells with focal acinar cell degeneration (Figures 1C, 1E, 2C, and 2E). The islets showed enlarged individual endocrine cells. Very subtle apoptotic signs in the form of nuclear distortions and dissolutions were also seen in various endocrine cells of the islets (Figures 2C and 2E). However, in general, the islets in the Bn2.5 groups showed prominent signs of hypertrophy (as indicated by the accumulation of tissue fluid [extra-cellular edema]) (Figure 2C). In contrast, in the Bn5 group secondary atrophy of the islets was observed (Figure 2E).

The histological slides in the Bn2.5NgS and Bn5NgS groups, on the other hand, showed signs of exocrine and endocrine tissue regeneration in terms of proliferation in the acinar tissue cells with a probable simultaneous splitting of the acini and the enlargement of islets due to a probable hyperplasia in the islets along with the emergence of new islets possibly by means of migration of nascent endocrine cells in the vicinity of the pre-existing islets (Figures 1D, 1F, 2D, and 2F).

Micrometric findings: The micrometric findings are shown in Table 1.

Table 1. Micrometric results of endocrine pancreas (CSA=cross-sectional area)

Micrometric parameters	Groups. Values are means±SEM					
	NC	PC	Bn2.5	Bn5	Bn2.5NgS	Bn5NgS
*Mean number of exocrine cells per acinus†	13.1 ±0.62^{ab}	14.84 ±0.79^{b}	9.4 ±0.38^{cd}	8.8 ±0.47^{d}	11.24 ±0.68^{ac}	12.76 ±0.72^{ab}
*Mean CSA of the islets of Langerhans (μm^2)†	9464 ±955^{ab}	16521 ±1744^{c}	13703 ±1572^{bc}	6382 ±1405^{a}	16930 ±1700^{c}	11451 ±1099^{b}
§Mean number of endocrine cells in the islets per unit area (289 μm^2)†	150.89 ±6.7^{a}	174.43 ±6.6^{c}	141.37 ±6.3^{ab}	132.03 ±6.5^{b}	172.97 ±6.4^{c}	150.61 ±6.5^{a}
*Mean CSA of endocrine cells in the islets (μm^2)†	23.1 ±0.83^{a}	21.6 ±0.85^{a}	30.1 ±1.39^{b}	24.4 ±1.03^{a}	25.2 ±0.95^{a}	22.1 ±0.71^{a}

*: analyzed by ANOVA), §: analyzed by ANCOVA taking the cross-sectional area (CSA) of the islet as the covariate), †: $p \leq 0.0001$.
The mean values in a row not sharing a common superscript differ significantly ($p \leq 0.05$) with each other.

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The results revealed a highly significant ($p \leq 0.0001$) difference among the groups for all four micrometric parameters. The mean number of exocrine cells per acinus and the mean number of endocrine cells per unit area (289 μ^2) in the islets of Langerhans were significantly ($p \leq 0.05$) lower in the Bn5 groups than in the rest of the groups except for the Bn2.5 group. The mean CSA of the endocrine cells of the islets in the Bn2.5 group remained significantly higher than rest of the five groups. However, the mean CSA of the islets of Langerhans in the Bn5 group was significantly lower than the rest of the groups except the NC group (Table 1).

DISCUSSION

The endocrine toxicity of pesticides (leading to diabetes) is a current hot issue in research.[20] Among the insecticides, organophosphates are almost established endocrine disruptors.[21,22] Insecticide-related neurotoxicity and disruptions of the hypothalamic-pituitary-thyroid and hypothalamic-pituitary-gonadal axes are also well established.[23-25] Among the various groups of the insecticides, pyrethroids are now being preferred globally over organophosphates because of a general perception that they are less toxic to non-target animals and humans and because of their non-accumulative nature. However, unfortunately, the fluoridated pyrethroid insecticide Bn has been found to cause pathological damage in the mouse liver and adrenals thus indicating its hepatic and endocrine toxicological potential.[1,2]

Pancreas is an extremely important 'two-in-one" organ that produces vital exocrine (enzymatic) and the endocrine (insulin and glucagon) secretions. The general physiology of the exocrine pancreas involves the release of digestive enzymes by the acinar cells into the intercalated ducts for onward transport to the intestine (duodenum) through the intra-lobular, interlobular, and finally the main pancreatic ducts. The intercalated duct cells simultaneously secrete bicarbonate rich mucoid secretions primarily for pH adjustments in the small intestine. The loss of integrity of the duct system may lead to the enzymatic secretions entering into the interstitial spaces and causing pancreatic tissue damage or pancreatitis.[26] Unfortunately the pancreas has mostly been overlooked by toxicologists and histo-pathologists. Nevertheless, there are indications that toxic insults to this organ may lead to pancreatitis, primarily shown by partial damage to and replacement of the enzyme producing acinar cells by the duct cells causing the so called "acinar-to-ductal metaplasia" (ADM).[27,28] Because of the dearth of information on the pancreatic pathologies induced by pesticide exposure, the results of the present study are not directly comparable to any of the existing information in the reported literature. Nevertheless, the characteristic changes observed in the exocrine pancreas histology (i.e., a general enlargement of the intercalated, intra-lobular, and interlobular ducts with a simultaneous shrinkage of the acini which are indicative of ADM) seen in the the Bn2.5 and Bn5 groups in the present study should be considered a logical consequence of the pancreatoxic effects of Bn exposure.[29] The micrometric results further consolidated the histopathological preview.

Pyrethroid exposure has been found to cause hyperglycemia which may be considered to be a logical consequence of β-cell destruction in the endocrine pancreas.[11,30] The hypertrophy of the islets seen in the Bn2.5 group and the secondary atrophy seen in Bn5 groups is likely to be the consequence of tissue fluid accumulation in the Bn2.5 group and the severe damage and destruction of the

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endocrine cells of the islets in the Bn5 group. Again, the micrometric findings were in line with the histological observations.

The histological sections in the Bn2.4NgS and Bn5NgS groups showed clear repair and rehabilitative activity in the exocrine tissues (the proliferation of the acinar cells with a simultaneous splitting activity in the acini) and endocrine tissues (hypertrophy and hyperplasia of the islets with a simultaneous migration of the endocrine cells for the emergence of new islets) of the pancreas. These rehabilitative activities are likely to be a consequence of the stress alleviating and regenerative potentials of the ingredients of NgS oil (such as thymoquinone).[31,32]

CONCLUSIONS

The results of the present study indicate that Bn is highly toxic for both the endocrine and the exocrine pancreas. Exposure with Bn at 2.5 mg/kg/day and 5mg/kg/day for 7 or more days in laboratory mice can lead to various histopathological and micrometric alterations in the pancreas. However, such destructive changes can be ameliorated with NgS oil therapy, indicating that NgS oil has appreciable potential rehabilitative activities.

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