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Original article

EFFECTS OF BISPHENOL A ON PANCREAS AND THYROID GLAND OF YOUNG AND ADULT FEMALE SPRAGUE DAWLEY RATS

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Summary

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Bisphenol A (BPA), a chemical involved in formation of plastic vessels, is one of the most widespread endocrine disrupting chemicals. The study was designed to investigate the effect of BPA on pancreas and thyroid gland of young and adult female Sprague Dawley rats. The rats were exposed to 330 mg/kg BPA orally every other day for 12 weeks; control rats were exposed orally to ethyl alcohol and corn oil. Samples were collected at 4, 8 and 12 weeks for hormonal, biochemical assays and histopathological examination. The insulin hormone in exposed young rats was decreased, but its level in adult ones was increased; the biochemical assay for blood sugar level showed a significant increase in young rats and decrease in adult ones. T_3 hormone was increased in treated young and adult rats; T4 hormone was increased in treated adults, while calcium level was decreased in treated adult rats. The histopathological findings of pancreas revealed vacuolation in its endocrine parts in young rats, while in adult ones there was intralobular fatty infiltration – a typical picture of diabetes. The thyroid gland in treated young female rats showed increased cellularity of parafollicular cells; moreover there was parafollicular haemorrhage, and in adult ones – desquamation in lining epithelium of follicular cells. In conclusion, exposure of young and adult female rats to BPA resulted in changes in the pancreatic and thyroid gland cells manifested by morphological, hormonal and biochemical parameters.

Key words: bisphenol A, female rats, glucose, pancreas, thyroid gland

INTRODUCTION

Bisphenol A (BPA) is a chemical involved in the production of some plastics, coating the inner surface of cans used for preserving food and many other products. It is an endocrine disruptor identified to induce serious health effects to humans.

As an endocrine disrupting agent, it has been reported that exposure to BPA resulted in endocrine and metabolic effects. An epidemiological study clarified that there was a linkage between BPA exposure and the risk of developing type 2 diabetes mellitus (T2DM) (Silver *et al.,* 2011). In the samples collected from children, Menale *et al.* (2017) demonstrated an association between hyperinsulinaemia and presence of BPA in urine samples. Low urinary BPA concentrations in children coupled with enhancement of the peak in insulin levels during an oral glucose tolerance test were reported, whereas higher urinary BPA concentrations correlated with lower peak insulin values (Carlsson *et al.,* 2018). Moreover, BPA was also associated with hyperinsulinaemia, insulin resistance, but also impaired insulin secretion and adverse glucose homeostasis (Tai & Chen, 2016)

Manukyan *et al.* (2019) stated that exposure to BPA 8 times, via drinking water during gestation and early development, at a dose lower than the current tolerable daily intake of BPA delivered by the European Food Safety Authority which is 4 μg/kg body weight per day resulted in increased secretion of insulin in rats up to one year after exposure. The effects of exposure to BPA on the endocrine part of the pancreas can promote the development of metabolic diseases like T2DM. Also, the hypersecretion of insulin, was proposed to be an early initiating event, which may lead to adiposity build-up and in turn development of metabolic disease, including T2DM (Astley *et al.,* 2018). Manukyan *et al.* (2019) reported that the effects of BPA exposure on pancreas may promote the development of metabolic disease including T2DM; an excess of ERα action mediated by BPA would provoke an increase in pancreatic β-cell content and secretion (Biddinger & Kahn, 2006). Estradiol levels above or below the physiological range may promote insulin resistance and type II diabetes (Ding *et al.,* 2009).

BPA can perturb the action of thyroid hormones (THs) in all body tissues through different mechanisms, in particular, antagonising the activity of the thyroid receptor (Sun *et al.,* 2009; Sheng *et al.,* 2012; Gentilcore *et al.,* 2013) as thyroxine (T4), triiodothyronine (T3), and thyroid-stimulating hormone (TSH), are essential for development, growth, metabolism, and play an important role in neurodevelopment. Therefore, alterations of thyroid hormone functions can interfere with these vital functions; the levels of THs can be easily measured in the blood samples (Kim & Park, 2019).

The BPA might alter thyroid homeostasis antagonising TH signalling pathways. However, it is not possible to exclude a direct action of BPA on thyrocytes. This point was assessed *in vivo* in zebrafish embryos exposed to low-dose BPA (Terrien *et al.,* 2011).

Other studies stated that BPA exposure (40 mg/kg, 15 days, orally) in adult rats resulted in increased T4 levels (Da Silva *et al.,* 2019). Neonatal BPA exposure (2.5 to 6.2 mg/kg, 10 days, subcutaneously) led to the reduction in T4 levels and increased TSH levels in adulthood (Fernandez *et al.,* 2018). Also, the environmental exposure of BPA resulted in adverse health outcomes including bone loss. BPA disturbed bone health by decreasing the plasma calcium level and inhibiting calcitonin secretion (Thent *et al.,* 2018). Moreover, it affected gut permeability reducing Ca++ adsorption in pregnant mice (Braniste *et al.*, 2010; Otsuka *et al.,* 2012).

The aim of the current study was to evaluate the effect of BPA on pancreas and thyroid gland of young and adult female rats.

MATERIALS AND METHODS

Chemicals

Bisphenol A (BPA, purity 97%) was obtained from Sigma Aldrich, Co., St. Louis, USA, corn oil (purity 99%) – from Alpha Co, Egypt, ethyl alcohol (Analar) was obtained from Fisher Chemical Co., U.K. EIA kit for rat insulin: from CALBIOTECH, Spring Valley, USA and blood sugar kit: from the Egyptian Company for Biotechnology, Cairo, Egypt.

Animals

One hundred and twenty female Sprague Dawley rats (sixty rats aged one month and sixty rats aged three months) were obtained from the Laboratory Animal House, Faculty of Medicine, Assiut University, housed in polypropylene cages and fed a standard laboratory diet, with water *ad libitum*. All environmental facilities were controlled. The female rats were kept under observation for one week prior to the experiment for acclimatisation. All rats were handled according to the standard guidelines for care and use of experimental animals.

Experimental design

Sixty young female rats were used in the study, 30 rats per group. Bisphenol A – 330 mg/kg body weight equal to 1/10 of the LD_{50} according to Morrissey *et al.* (1987) was dissolved in 5% absolute ethyl alcohol and suspended in 95% corn oil according to Tunmise & Patrick (2015). The prepared BPA was given every other day for 12 weeks to treated young and adult female Sprague Dawley rats. The other thirty female rats were kept as control young and adult groups (15 rats each); they were given only ethyl alcohol and corn oil every other day for 12 weeks.

Blood sampling and necropsy

At the time of necropsy, diets were withheld from the experimental rats for 12 h. Blood samples were collected by retro-orbital bleeding into plain tubes without anticoagulant. Sera were separated and preserved at –20 ºC for biochemical analyses. Then, rats were euthanised by cervical dislocation following ethical animal care standards. Pancreas and thyroid gland samples were fixed in 10% formalin solution for histological examination. Samples were collected on 4, 8 and 12 experimental weeks.

Serum analyses

Insulin. Blood serum samples were separated according to Coles (1986) and used for determination of insulin hormone using rat insulin ELISA kit supplied by Calbiotech (Spring Valley, USA) according to the kit instructions. The plate was read by using Stat fax 2100 microplate reader (Awareness Technologies, USA).

Thyroxine (T4) and triiodothyronine (T3). Serum samples were analysed for T4 and T3 concentrations by using rat T4 enzyme immunoassay (EIA) kit and rat T3 EIA kit (Biocheck, Inc, Foster city, USA) respectively. The tests were based on competitive ELISA and performed according to the manufacturer instructions.

Serum calcium and glucose. Serum calcium and glucose levels were measured using commercial kits supplied by Spectrum Diagnostics (Cairo, Egypt) on Optizen 3220, UV Spectrophotometer (Sweden).

Histopathology

Fresh samples of pancreas and thyroid were fixed in 10% neutral buffered formalin. The tissues were dehydrated in a graded alcohol series and embedded in paraffin. The paraffin blocks were cut into 5 µm sections and stained with H & E (Bancroft *et al.*, 1996). All the microscopic findings for each group were presented in tables to demonstrate the type of lesion, severity, and percentages according to Radad *et al.* (2013) as followed: severe: +++, moderate:++, and slight: +.

Statistical analysis

The data were analysed using Statistical Package for the Social Sciences for Windows (SPSS, version 16, USA) by comparing treated and control rats using paired samples *t*-test (Levesque, 2007). All data were expressed as mean±SD.

RESULTS

Hormonal and biochemical assays

The concentrations of random insulin in the serum of young female rats were significantly (P≤0.01) decreased while in adult ones, they significantly (P ≤ 0.01) increased (Table 1).

There was a significant (P≤0.01) increase in the blood levels of T3 hormone in treated female young rats, while in treated adult females, T3 levels showed significant (P≤0.01) increase by the 4th and 8th weeks but no significant changes at week 12 of exposure (Table 2).

The levels of T4 hormone showed no change in exposed young female rats in comparison with the control groups. In

Table 1. Effect of BPA on random insulin level (µIU/mL) in young and adult female rats. Data are presented as mean ± SD (n=5)

Time after exposure (weeks)	Groups			
	Young female rats		Adult female rats	
	Control	330 mg/kg BPA	Control	330 mg/kg BPA
4	1.65±1.02	1.35±0.81	2.12±0.90	8.80±0.0.49**
8	4.46±0.62	2.10±0.85**	2.44±0.86	8.70±0.21**
12	4.20±1.03	1.03 ±0.99**	2.95±1.03	71.14±29.20**

* P≤ 0.05; ** P ≤ 0.01 vs control group.

Table 2. Effect of BPA on T3 level (nmol/L) in young and adult female rats. Data are presented as mean ± SD (n=5)

Time after exposure (weeks)	Groups			
	Young female rats		Adult female rats	
	Control	330 mg/kg BPA	Control	330 mg/kg BPA
4	1.73±0.30	3.64±0.93**	2.47±0.93	3.97±0.33*
8	2.50±0.09	6.39±1.62**	2.96±0.06	3.67±0.27**
12	4.28±0.89	6.23±0.75**	2.65±0.41	2.84±0.16

* P≤ 0.05; ** P ≤ 0.01 vs control group.

Table 3. Effect of BPA on T4 level (nmol/L) in young and adult female rats. Data are presented as mean ± SD (n=5)

Time after exposure (weeks)	Groups			
	Young female rats		Adult female rats	
	Control	330 mg/kg BPA	Control	330 mg/kg BPA
4	10.55±0.98	10.87±0.59	6.78±1.99	11.66±0.61**
8	8.19±1.29	9.16±0.76	7.18±0.46	8.44±0.73*
12	10.55±0.98	10.87±0.59	6.78±1.99	11.66±0.61**

* P≤ 0.05; ** P ≤ 0.01 vs control group.

Table 4. Effect of BPA on serum glucose level (mmol/L) in young and adult female rats. Data are presented as mean ± SD (n=5)

Time after exposure (weeks)	Groups			
	Young female rats		Adult female rats	
	Control	330 mg/kg BPA	Control	330 mg/kg BPA
4	2.33±0.25	2.54±0.41	2.40±0.20	1.04±0.12**
8	1.96±0.15	2.51±0.17**	2.34±0.20	1.33±0.14**
12	1.98±0.20	2.64±0.46**	2.35±0.23	2.64±0.31

* P≤ 0.05; ** P ≤ 0.01 vs control group.

Table 5. Effect of BPA on calcium level (mmol/L) in young and adult female rats. Data are presented as mean ± SD (n=5)

Time after exposure (weeks)	Groups			
	Young female rats		Adult female rats	
	Control	330 mg/kg BPA	Control	330 mg/kg BPA
4	2.16±0.24	2.07±0.77	2.60±0.21	2.67±0.04
8	2.51±0.15	2.50±0.07	2.50±0.01	1.39±0.35**
12	2.02±0.37	1.99±0.06	2.44±0.11	0.90±0.02**

* P≤ 0.05; ** P ≤ 0.01 vs control group.

adult rats, however, T4 hormone concentrations showed a significant increase at week 4 (P≤0.01) and 8 (P≤0.05) (Table 3).

The serum glucose levels were decreased (P≤0.01) in young female rats while in adults they were increased (P≤0.01) at weeks 4 and 8 (Table 4). The calcium level were significantly (P≤0.01) reduced in treated adult female rats at weeks 8 and 12, yet in young female rats no changes were detected in comparison with control ones (Table 5).

Histopathological findings

The endocrine part of the pancreas was affected by the BPA exposure and the

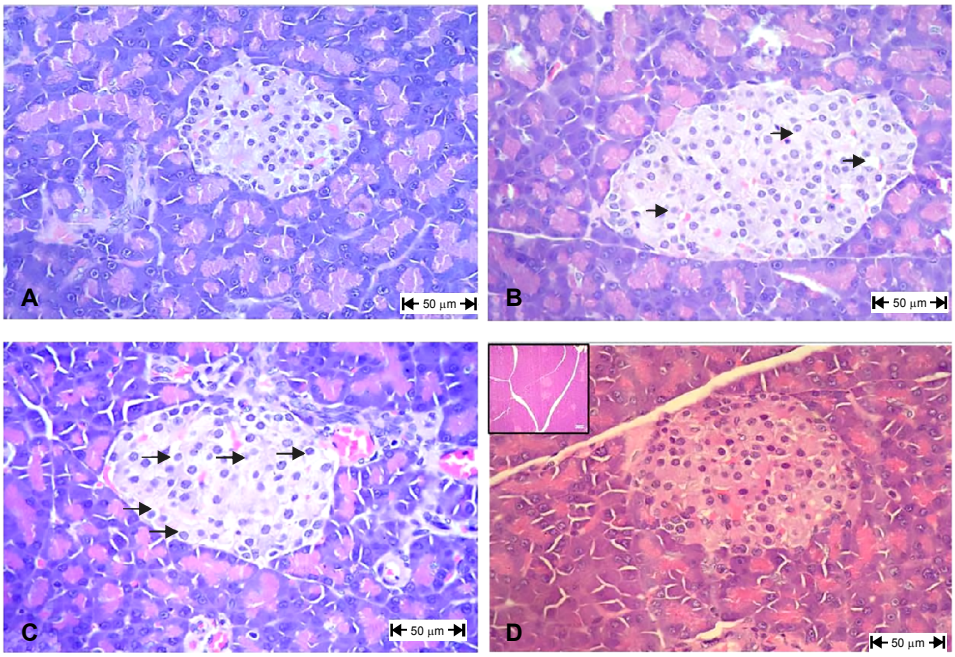

Fig. 1. Histopathology of the pancreas in female young rats. **A.** Pancreas of control rats showing normal apperance of exocrine and endocrine parts; **B.** Pancreas of treated rats collected after 4 weeks showing slight (+) vacuolation of B cells (arrows); **C.** Pancreas of treated rats collected after 8 weeks showing moderate (++) vacuolation and pale cytoplasm of B cells (arrows); **D.** Pancreas of treated rats collected after 12 weeks and showing disorganisation of cellular element of the islets. H&E, bar=50 µm.

findings in young and adult exposed rats were quite different. In young female rats there was an increase in the vacuolation in the endocrine part parallel to increasing the period of exposure; slight vacuolation was found 4 weeks post exposure (Fig. 1B) in comparison with control ones (Fig. 1A), moderate vacuolation occurred after 6 weeks (Fig. 1C) and at the end of experiment, the pancreas showed disorganisation in its endocrine part (Fig. 1D).

In adult female rats there was hyperaemia of blood vessels in the pancreas (Fig. 2B), after the 8th week of exposure there was a moderate vacuolation (Fig. 2C), with severe vaculation (Fig. 2F) at the end of experiment. Moreover, interlobular fatty infiltration (Fig. 2D & 2E) was found out in comparison with control group (Fig. 2A).

The thyroid follicles were affected by BPA exposure both in young and adult female rats. Treated young females exposed for 4 weeks had normal thyroid follicles (Fig. 3B); however rats exposed for 8 weeks showed increasing cellularity of parafollicular cells (Fig. 3C); in rats exposed for 12 weeks there was necrosis of parafollicular cells and extravasated red blood cells (Fig. 3D, 3 E) compared with normal control rats which showed normal appearance of thyroid follicles with acidophilic colloid (Fig. 3A). Treated adult female rats exposed for 8 weeks showed normal thyroid follicles (Fig. 4B), but those exposed for 12 weeks lining epithe-

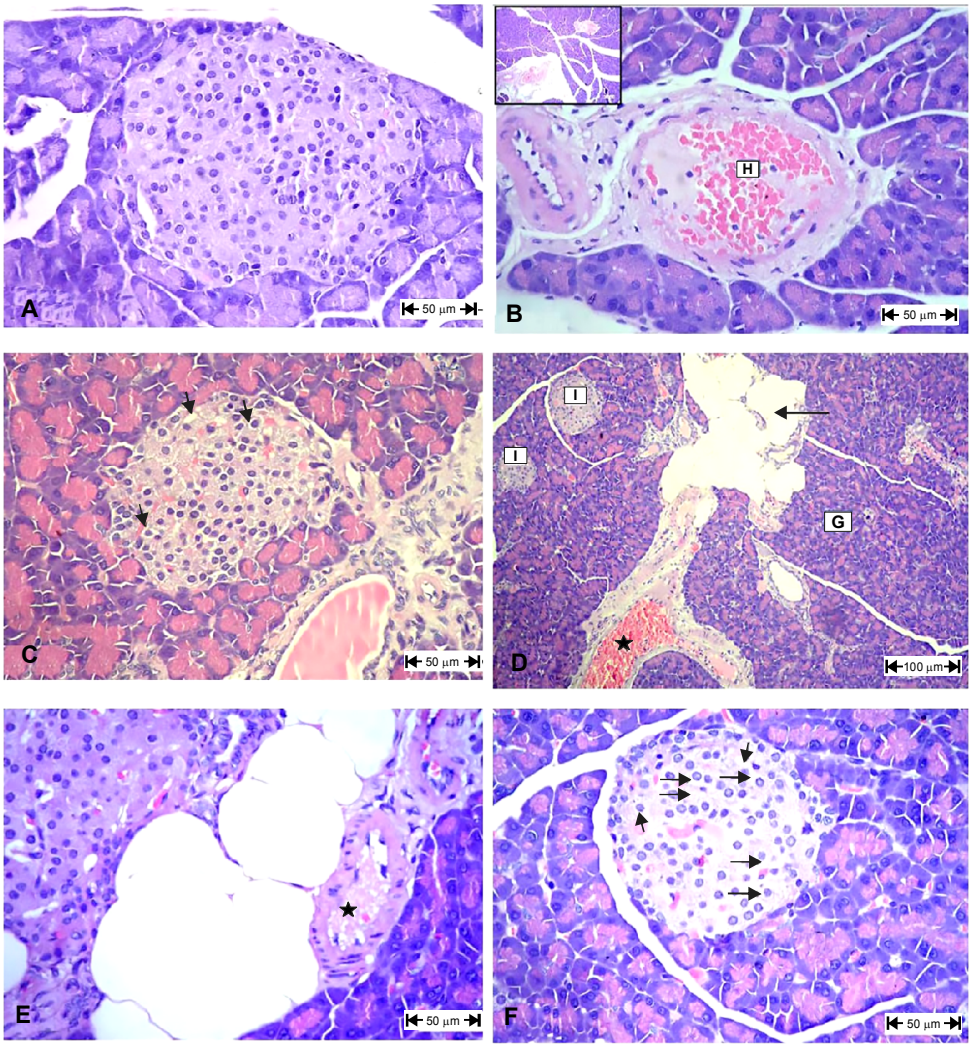

Fig. 2. Histopathology of the pancreas in female adult rats. **A.** Pancreas of control rats showing normal exocrine and endocrine parts; **B.** Pancreas of treated rats collected after 4 weeks showing hyperamia (H) of blood vessels; **C.** Panceas of treated rats collected after 8 weeks showing moderate vacuolation (++) of beta cells; **D.** Pancreas of treated rats collected after 12 weeks showing hyperaemia of blood vessels in islets of Langerhans (I), interlobular fatty infiltration (arrow) and exocrine gland (G); **(E).** Higher magnification of **D.** showing infilteration of fat as well as fat droplet in the lumen of blood vessels (star); **F.** Pancreas of treated rats collected after 12 weeks showing severe vacuolation (+++) of beta cells (arrows). H&E, bar = 50 µm.

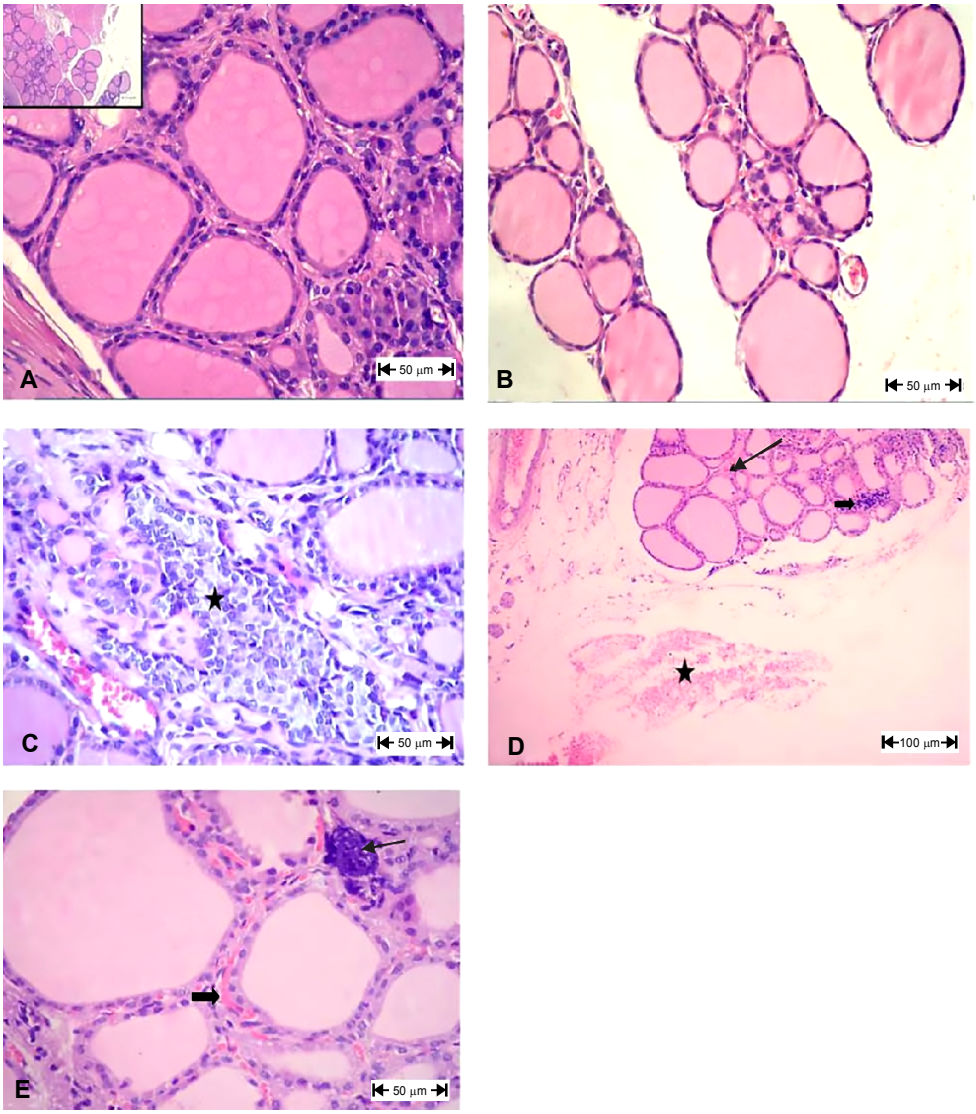

Fig. 3. Histopathology of the thyroid gland in female young rats. **A.** thyroid gland of control rats showing normal appearance of thyroid follicles with acidophilic colloid; **B.** Thyroid gland of treated rats collected after 4 weeks showing normal thyroid follicles; **C.** Thyroid gland of treated rats collected after 8 weeks with increased cellularity of parafollicular cells (star); **D.** Thyroid gland of treated rats collected after 12 weeks showing necrosis of parafollicular cells and extravasated red blood cells(thin arrow), increased cellularity (star) and necrosis of parafollicular cells (thick arrow); **E.** High thyroid gland power of treated rats collected after 12 weeks showing necrosis of parafollicular cells (thin arrow) and extravasated RBCs (thick arrow). H&E, bar=50 µm.

lium of thyroid follicles was desquamated (Fig. 4C), compared with normal adult controls which showed normal appearance of thyroid follicles with acidophilic colloid (Fig. 4A).

DISCUSSION

BPA is a monomer of plastic materials that are widely used in daily life. BPA is detectable in the environment and present in drinking water, canned goods, and even milk bottles. The current study estimated the effect of BPA on pancreas, thyroid gland and their relation with blood glucose level both in young and adult female rats.

The decreased insulin concentrations in young female rats exposed to BPA after weaning was in line with an *in vitro* study by Lin *et al.* (2013), affirming that decreased insulin concentration in young female rats might be due to mitochondrial damage in β cells of pancreas. The mitochondrial damage of β cells occurred at weaning age and increased with age resulting in progressive loss of pancreatic island function (Szendroedi *et al.,* 2011).

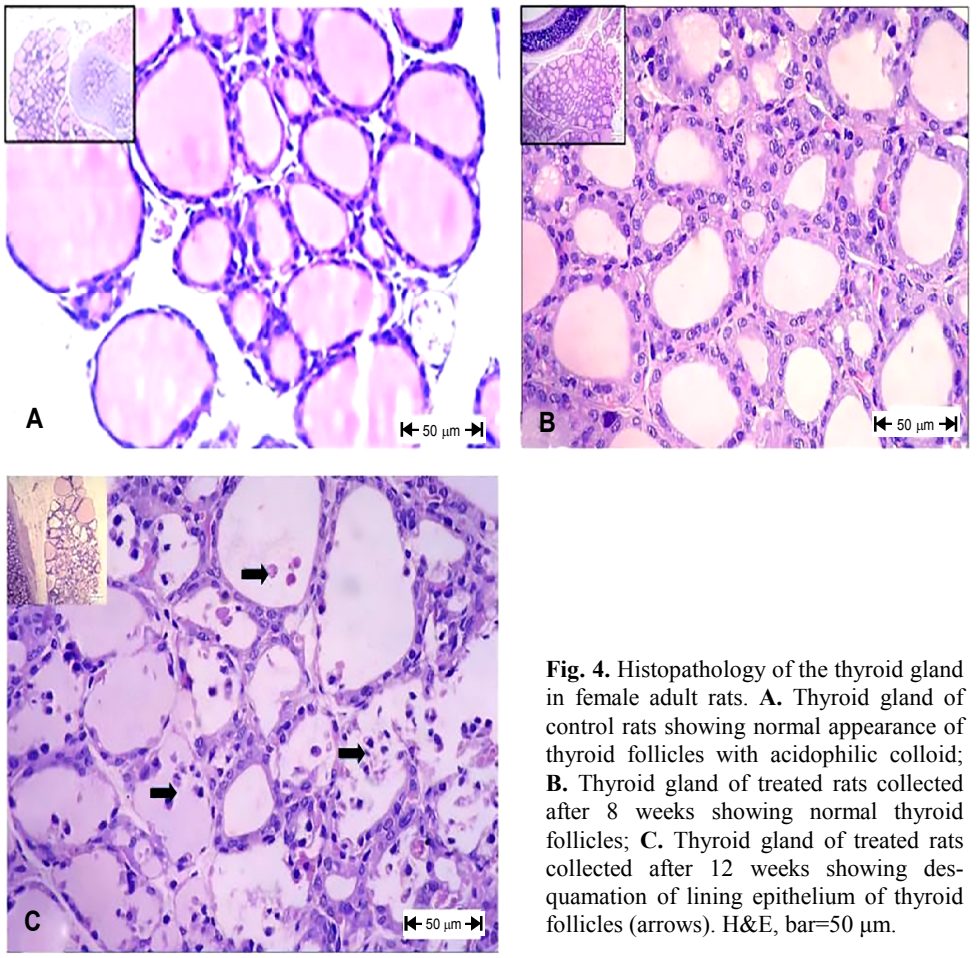

Fig. 4. Histopathology of the thyroid gland in female adult rats. **A.** Thyroid gland of control rats showing normal appearance of thyroid follicles with acidophilic colloid; **B.** Thyroid gland of treated rats collected after 8 weeks showing normal thyroid follicles; **C.** Thyroid gland of treated rats collected after 12 weeks showing desquamation of lining epithelium of thyroid follicles (arrows). H&E, bar=50 µm.

The increasing insulin concentration in adult female rats exposed to BPA was in accordance with Alonso-Magdalena *et al.* (2006), who reported that prolonged exposure of adult mice to a low oral dose of BPA (10 µg/kg/day) resulted in stimulation of insulin secretion. Increased insulin concentration resulted in lower blood glucose levels. Fritsche *et al.* (2008) stated that insulin, in order to control blood glucose levels, promoted glucose utilisation in adipocytes and muscles and inhibited hepatic glucose production. Moreover, the intralobular fatty infiltration, shown in the pancreatic samples of adult female rats, was associated with type 2 diabetes mellitus (Ou *et al.,* 2013). Animal feeding studies have shown that long-term consumption of BPA may lead to adiposity build-up and in turn development of metabolic disease including glucose intolerance, insulin resistance, and ultimately T2DM (Punthakee *et al.,* 2007; Corkey, 2012; Moon *et al.,* 2015; Astley *et al.,* 2018). The pathogenesis of type 2 diabetes is complex. In most instances, it develops due to a progressive reduction in the response of the pancreas to produce sufficient insulin to compensate for insulin resistance (Kahn, 2003; Leahy, 2005).

The present histopathological examination of pancreas revealed that there was a pancreatic change in young rats exposed to BPA; vacuolation of beta-cells of the islets of Langerhans was observed. There was a gradation in the intensity of vacuolation varying with duration of administration. On the other hand, the pancreas of adult rats showed more pronounced vacuolation than that of young rats, and disorganisation of the beta-cells was prominent. These results may explain the alteration in insulin hormone release. Patel *et al.* (2014) reported that higher exposure to BPA may enhance cardiovascular diseases, obesity and diabetes morbidity in humans. Pancreatic beta cells responded to low BPA doses by increasing the insulin production and secretion, resulting in insulin resistance in adult animals (Alonso-Magdalena *et al.,* 2006). In the present study, by the 12 post-administration week, fatty infiltration in the vicinity of the islet of Langerhans was prominent. This result may suggest that diabetes occurred in these rats. The association between fatty infiltration and diabetes has been reported. Hou *et al.* (2013) mentioned that both pancreatic fat replacement with acinar cell death and pancreatic fat infiltration due to obesity contributed to pancreatic steatosis.

The increase in thyroid hormones in the blood of both young and adult female rats was in accordance with data of Wang *et al.* (2012) who reported that the concentrations of T3 and T4 hormones were elevated in humans as a result of occupational exposure to BPA. Similar result were obtained by Moriyama *et al.* (2002), Zoeller *et al.* (2005) and Sun *et al.* (2009), reporting that BPA increased serum T4 in rat pups on post natal day (PND) 15 and that the expression of a thyroid responsive gene in brain was increased when their dams were exposed to BPA via diet during gestation and lactation. Also, Xu *et al.* (2007) demonstrated a transient elevation of thyroid hormone levels on PND 7 in offspring of rat dams exposed to BPA in their drinking water from 11th day of gestation through PND 21. These findings might be due to the inhibition effect of BPA on thyroid peroxidase activity, interfering with the activity of transthyretin, increasing the metabolism via deiodinases (Zoeller *et al.,* 2007), or might be a result of the antagonistic action between BPA and T3 at the transcriptional level. *In vitro* studies demonstrated that BPA is a TR

antagonist (Kitamura *et al.,* 2002; 2005; Moriyama *et al.,* 2002).

The decreased calcium concentration in treated adult rats in the current study was analogous to data of Suzuki *et al.* (2003), who found that goldfish exposed to BPA resulted in lower calcium and calcitonin concentrations after 8 days exposure. The histopathological findings of thyroid gland was in line with those of Jassim (2015) who examined histopathologically the thyroid glands in adult male rats treated with BPA at 50, 100 and 200 mg/kg BW daily dissolved in corn oil for 30 days and observed vacuolated colloid and thickening of parafollicular cells, and some follicles with flattened thyrocytes. The elevation of thyroid hormones concentration might be due to the disturbance of the normal thyroid gland morphology.

The present histopathological findings of thyroid gland in young female rats revealed an increase in cellularity of parafollicular cells after 4 weeks and interfollicular haemorrhage and necrotic parfollicular cells after 8 weeks. In adult female rats, desquamation of lining thyroid follicular epithelium was observed at 12th week post exposure.

Marmugi *et al.* (2014) stated that exposure for several months to BPA disrupted expression of key genes related to lipid metabolism, mainly in cholesterol biosynthesis, associated with hypercholesterolaemia, hyperglycaemia, and glucose intolerance in male adult mice. BPA exposure during 8 months in adult mice resulted in metabolic disorders consisting in increased adipose tissue mass, (Marmugi *et al.,* 2014).

In the present study, by the 12 week post-administration, fatty infiltration in the vicinity of the islets of Langerhans was prominent. In addition, fatty pancreas has been suggested to have a role in type 2 diabetes mellitus. In rats, chronic exposure to a high-fat diet induces both interlobular and intralobular fat accumulation, inflammatory cell infiltration, and fibrosis in the pancreas, and thus damage to the normal pancreatic architecture and islets.

In conclusion, exposure of young and adult female rats to BPA resulted in changes in the pancreatic and thyroid gland cells manifested by morphological, hormonal, and biochemical parameters.

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