HISTOLOGICAL EFFECT OF BISPHENOL A ON THE RATS’ PAROTID SALIVARY GLANDS

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ABSTRACT

INTRODUCTION: Bisphenol A (BPA), an endocrine disruptor, is a chemical widely used in the manufacture of polycarbonate plastics and epoxy resins found in many consumer products and dental sealants. Concerns were raised against the excessive usage of such substances. Consumption of BPA has been associated with several diseases, including cardiovascular disease, diabetes mellitus and reproductive problems. Oxidative toxicity, on numerous tissues in the body, has also been stated after BPA exposure.

MATERIALS AND METHODS: Twenty adult male albino rats (180-200 gm in weight) were used in this study. They were randomly separated into 2 equal groups: Group I (control group), Group II (BPA group). In group I, rats received 1 ml corn oil as a vehicle. In group II, rats received 50 mg/kg body weight of BPA dissolved in corn oil and administered orally once daily for six weeks. The animals were euthanized at the end of the experimental. Right parotid glands were taken out and prepared for light microscopic analysis.

RESULTS: Control group showed normal histological features of parotid gland. Regarding the BPA group, there were changes in the parotid glands’ histological structure. The serous acini showed structural disorganization as well as loss of their cell boundaries. According to body weight changes, there was a considerable increase in body weight within the group receiving the BPA.

CONCLUSION: BPA oral administration for six weeks in adult male rats led to significant histological changes in the parotid salivary glands and thus oral homeostasis disruption.

KEY WORDS: Endocrine disruptors, Bisphenol A, BPA, Parotid salivary glands, Albino rats.

INTRODUCTION

Endocrine disruptors (EDs) are compounds that are either naturally found or chemically made that have been found in our environment and, as their name indicates, they disturb the hormonal balance (1,2). They may inhibit the secretion, transport, receptor binding, synthesis or even the elimination of endogenous hormones, changing the endocrine and homeostatic systems. Medications, dioxin compounds, polychlorinated biphenyls, various insecticides, components of plastics such as bisphenol A (BPA) are all considered examples to EDs (3,4).

BPA is a xenoestrogen that has been used in medical fields as an artificial estrogen, binding to estrogen receptors, since the 1930s (2). BPA production globally is calculated to be 6 million tons annually with a tendency to increase in the future (5). Owing to its cross-linking features, BPA has been employed widely in the production of polycarbonate plastics. These plastics can be used in making epoxy resins, electronics, medical equipment, baby bottles, water storage tanks, food plastic wraps, paper towels and dental sealants (6,7). The most common BPA derivative used is bis-Glycidyl dimethacrylate (bis-GMA) which is considered as a base for resins used in dentistry (8). People are regularly subjected to BPA through many sources. For example, during the heating process, BPA can seep from the inside of metal cans and microwave food dishes into the food as well as from plastic bottles into drinks due to recurrent use or...
interaction with acidic or basic substances (9) as well as from dental sealants or fillings into saliva. BPA has also been found in humans urine, blood, tissues and breast milk (10,11). Unfortunately, BPA consuming have been related to various diseases such as cardiovascular disease, polycystic ovarian disease, reproductive illnesses, diabetes mellitus (DM), obesity and cancers (2,12–17). Moreover, oxidative toxicity has been stated by several studies after exposure to BPA in rats and mice (18,19). Tissue damage of the brain, kidney, liver and other organs is caused by BPA through the creation of reactive oxygen species (ROS), thus causing oxidative stress (20,21). ROS have a significant role in tissue injury. They promote intracellular signal transmission. Excessive ROS attenuate the antioxidant capacity of cells through oxidative stress, which further leads to severe cellular damage (20).

Recent research postulates that BPA exposure may participate in the etiology and pathogenesis of oral cancers via variety of mechanisms that include epigenetic, genetic, immune, inflammatory, hormonal, and oxidative stress alterations, as well as oral microbiome alterations. The oral cavity area and oropharyngeal region are the earliest signs of BPA exposure following eating. In addition to the growing frequency of OC and OPC in recent decades, which has coincided with rising BPA manufacturing globally (22).

Oral homeostasis is dependent on several elements, including healthy salivary glands that secrete enough amounts of saliva in both quality and quantitative forms. These parameters change depending on hormonal state and the neuroendocrine system, which controls salivary secretions (23). Furthermore, the existence of estrogen receptors $\beta$ (Er$\beta$) inside the salivary glands, as well as the neuroendocrine system regulation of salivary gland secretions, allows us to identify the salivary glands as potential targets for BPA harmful effects (24).

Few investigations on the impacts of estrogen on male salivary glands have been conducted, however experimental experiments have indicated that synthetic estrogen can promote secretory hypertrophy similar to that caused by sex hormones (25).

There is now a debate over the toxicity of BPA. Despite the fact that the Food and Drug Administration has categorized BPA as a safe chemical, new information has shown additional studies on health risks of BPA exposure, particularly in areas where plastic consumption has grown (26). Moreover, there have been relatively few studies that have investigated the effects of BPA exposure on salivary glands, especially the parotid glands.

Accordingly, the purpose of this work is to detect the consequences of BPA administration on parotid glands histological structure. Null hypothesis of this study proposes that there will be no significant difference between the control group and the study group.

MATERIALS AND METHODS

Study sample
In this experiment twenty adult male albino rats with approximate weight of 180–200 gm (approximately 2-3 months) were used. The rats were excluded if underweight or overweight, or if any of the rat with any illness or wounds. Animals were taken from the Medical Research Institute's animal house at Alexandria University. (Total sample size=20 rats) based on previous literature reviews (27,28).

The study was conducted after the research ethics committee approval in Faculty of Dentistry, Alexandria University. The approval number by the ethical committee is 0215 and IORG 0008839

Grouping (Randomization technique)
Rats were randomly assigned by (using computer generated random numbers) to one of the two equal groups (10 rats each).

- **Group I (control group):** 10 rats were kept under normal environmental and nutritional conditions. They received oral administration of corn oil (vehicle) 1 ml daily throughout the experimental period.

- **Group II (BPA group):** 10 rats kept under the same conditions as group I. They received 50 mg/kg body weight of BPA dissolved in corn oil orally once daily for 6 weeks (29,30).

Bisphenol A (manufactured by Loba Chemie, India), was packed in the form of white powder that dissolves in corn oil.

Throughout the experiment, animals were clinically examined every week for general health, activity, and body weight. The animals in each group were euthanized, by overdose of diethyl ether, after six weeks, and the right parotid salivary glands were dissected out, cleaned from adhering fat, and prepared for histological examination. The histological procedures were done in the laboratory of the Oral Biology Department at Alexandria University.

Histological procedures (31)
Specimens were labelled and preserved in a 10% solution of neutral buffered formalin. The specimens were then washed, dehydrated in increasing alcohol concentrations, cleared with xylene, infiltrated, and embedded in paraffin blocks. Five micrometers thickness sections were cut by the micromote rotary device. The sections of parotid glands were stained with Hematoxylin and Eosin stains (H&E) then
observed under a light microscope to examine their histological structure.

**Statistical Analysis**
The body weight was statistically calculated by IBM SPSS software version 20.0. (Armonk, NY: IBM Corp). For continuous data, they were tested for normality by the Shapiro-Wilk test. Distributed data were expressed as mean and standard deviation. For normally distributed quantitative variables Student t-test was used to compare two groups. While ANOVA with repeated measures was used to compare between more than two periods and followed by Post hoc test (Bonferroni adjusted) for pairwise comparisons. Significance of the obtained results was judged at the 5% level (32).

**RESULTS**
1- Body weight changes results and general health observations:
The rats showed good general health. They also maintained their general health and activity. No rats died throughout the whole experimental period. The body weight changes of rats (gm/week) of the two studied groups which are group I (control), group II (BPA group) are shown in table (1) and Figure (1).

![Figure 1](image)

**Table (1):** Comparison between the two studied groups according to body weight in each week

<table>
<thead>
<tr>
<th>Body weight</th>
<th>Group I (n = 8)</th>
<th>Group II (n = 8)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>186.9 ± 25.1</td>
<td>190.0 ± 6.0</td>
<td>0.343</td>
<td>0.741</td>
</tr>
<tr>
<td>2 week</td>
<td>195.0 ± 25.5</td>
<td>203.1 ± 7.5</td>
<td>0.864</td>
<td>0.412</td>
</tr>
<tr>
<td>3 week</td>
<td>205.6 ± 27.2</td>
<td>211.3 ± 14.8</td>
<td>0.514</td>
<td>0.615</td>
</tr>
<tr>
<td>4 week</td>
<td>209.4 ± 26.8</td>
<td>213.1 ± 14.1</td>
<td>0.350</td>
<td>0.731</td>
</tr>
<tr>
<td>5 week</td>
<td>213.1 ± 25.6</td>
<td>223.8 ± 12.5</td>
<td>1.055</td>
<td>0.309</td>
</tr>
<tr>
<td>6 week</td>
<td>219.6 ± 28.6</td>
<td>243.8 ± 13.0</td>
<td>2.173*</td>
<td>0.047*</td>
</tr>
<tr>
<td>F (p)</td>
<td>2.187 (0.129)</td>
<td>31.170 (&lt;0.001)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data was expressed using Mean ± SD.
SD: Standard deviation
 t: Student t-test
p: p value for comparing between the studied groups

F: F test (ANOVA) with repeated measures, Sig. bet. periods was done using Post Hoc Test (Bonferroni)
*: Statistically significant at p ≤ 0.05
#: Significant with 1 week

The readings represent the mean body weights in grams for each group in successive six weeks. No statistically significant differences in mean weights between the two groups from week 1 till week 5. At week 6, group II was significantly higher than group I. In Group II, there was a significant difference in body weight within the same group as shown in the statistics. There was a significant difference between week 1 and week 5 as well as between week 1 and week 6. (A significant increase in weight occurred in group II in Weeks 5 and 6)

2- Histological results
**Group I (control group)**
Results obtained from the histological sections prepared from the control specimens revealed normal parotid gland architecture. Serous acini with well-defined rounded boundaries, large spherical basally located basophilic nuclei and narrow lumen. Intralobular ducts consisted of intercalated and secretory striated ducts. The intercalated ducts appeared small with cuboidal cells, centrally located nuclei and narrow lumens. Regarding the striated ducts they appeared larger and with wider lumen than the intercalated ducts. They are lined by columnar cells with basal striations. **(Figure 2)**

![Figure 2](image)

**Figure 2:** Photomicrograph of the control group showing: Normal architecture of the serous acini, intercalated ducts, and striated ducts. Serous acini are rounded with narrow lumen and spherical basally located central nuclei (blue arrows). Intercalated ducts are smaller, with narrow lumen and lined with cuboidal cells (yellow arrows). Note the normal secretory striated ducts with wider lumens than the intercalated ducts. They are lined with columnar cells (red arrows). (H & E stain x400)
Group II (BPA 50 mg/Kg)
Group II showed structural disorganization of the parotid salivary gland tissues. Extreme vacuolization was evident in the acinar cells’ cytoplasm. (Figure 3) The acinar cells showed generalized loss of architecture, with vacuolated cytoplasm and most of their nuclei appeared pyknotic. Moreover, some of the secretory striated ducts exhibited slight widening in the lumen in addition to significant loss of the basal striations. (Figure 4) Intercalated ducts showed as well widening in their lumens. Cytoplasmic vacuolations not only found in acinar cells’ cytoplasm but also the striated ducts cells’ cytoplasm. (Figure 5)

**DISCUSSION**
Bisphenol A is an endocrine disruptor that affects numerous hormonal systems and is manufactured in enormous amounts per year. BPA is found in a wide range of consumer and industrial items, including food containers, beverage bottles, can linings, dental sealants, and medical equipment, and is therefore widely distributed in the environment (33). The BPA polymer is hydrolyzed when a BPA product is exposed to heat or basic or acidic substances, and the BPA monomers can then leak out into the environment and to humans (34). Unfortunately, exposure to BPA have been related to many systemic diseases in addition to the oxidative toxicity that has been reported in several tissues and organs through the formation of reactive oxygen species, thus inducing oxidative stress (19–21).

The present work studied the effect of BPA when it was orally administrated for 6 weeks on the parotid glands of male albino rats. This was evaluated by using histological examination.

The study was designed with a BPA dosage of 50 mg/Kg/day (29,30). This dose is considered the lowest-observed-adverse-effect level (LOAEL); which is defined as the lowest dose that showed harmful effects and studied for risk assessment purposes (29). Moreover, this dose has shown harmful effects when used in the study of both Mohamed et al (2019) (30) and Morsy et al (2019) (27) who studied the outcomes of BPA on the thyroid glands in rats. Regarding the use of corn oil as a vehicle substance for the BPA supplement in our study, It was shown...
that one of the techniques of administration of BPA included oral gavage through feeding the chemical in oil (29). Moreover, there is some evidence that mode of administration impacts the metabolism of BPA where a greater involvement from intake of BPA in comparison to inhalation or absorption through the skin (35,36).

Concerning the statistical analysis of the rats’ body weights, showed that there was a statistically significant increase in body weights within the group receiving the BPA. This increase in weight is in accordance with the study of Vom Saal et al (2012) (37) who studied the relation between environmental chemicals, especially the BPA and its contribution to the epidemic obesity. The study reported that exposure to BPA during prenatal, perinatal and in adulthood has been found to increase body weight. However, in another study BPA didn’t influence the weight of rats (24). Other study stated a decrease in weight of rats receiving BPA when compared with those not receiving (38).

In the present study, the group exposed to BPA showed loss of parotid gland architecture. The histological changes included the acinar and ductal cells of the gland. These light microscopic results coincides with the study of Mireille et al (2013) (24) who examined the potential effects of BPA on oral homeostasis. The study reported histological changes in the submandibular glands’ acinar structure upon BPA exposure along with other disturbances in oral homeostatic parameters. These results were interpreted as sensitivity of salivary glands to estrogen activity due to the presence of estrogen receptors (ERs), especially ER subtype beta. Thus, sensitivity of these tissues to xenoestrogens as BPA (24).

Regarding the presence of ERs within tissues of the oral cavity, it was documented the wide expression of ER beta subtype mainly in keratinocytes and salivary gland acinar and ductal cells. However, it was reported the absence of ER alpha subtype in buccal, gingival epithelium or in salivary glands (39).

Dinesh et al (2012) (40) documented the oxidative stress effect of BPA through multiple in vivo tests at several doses (10 µg, 5 mg and 50 mg/kg bw) of BPA when evaluated with the control group. BPA caused a significant increase in the lipid peroxidation levels thus causing liver membrane damage. In addition to a decrease in the reduced glutathione (GSH) activity in the liver with rise in BPA concentrations shows increase in consumption of glutathione for catching these BPA generated ROS. The BPA oxidative stress action may interpret the cytoplasmic vacuolations seen in both acinar and ductal cells in our study.

The oxidative reactions and the ROS caused by BPA were also responsible for liver toxicity in the study of Kazemi et al (2016) (38) who investigated the effects of different dosages of BPA on rats’ gene expression of the hepatic oxidative stress. The researchers utilized three dosages of the BPA (5, 25, and 125 µg/kg) in corn oil which were given as gavage for 35 days. These deleterious effects of BPA were mostly related to its oxidative stress generating action.

Moreover, the study of Ibrahim et al (27) demonstrated that exposure of BPA for 8 weeks in adult male rats at an oral dose of 50 mg/Kg daily led to significant histological impairments in the thyroid gland. The results showed vacuolated follicular cells which agree with our histological observations in parotid gland cells in rats taking the same dose of BPA.

In conclusion these findings clearly show that long-term exposure to BPA might have negative impacts on human health, implying the need to reconsider the rules for risk assessments.

CONCLUSIONS
The findings of this study showed significant histological changes in the structure of parotid glands. Thus, it may as well lead to disturbance in oral homeostasis. It is recommended to pay more attention to this substance and its usage in different types of plasticizers, as well as other domestic products. The usage of BPA in different plasticizers and other industries should be restricted.

Conflict of interest
We declare that we have no conflicts of interests.

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REFERENCES


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