Effect of bisphenol-A- on some immunological parameters of female rats *(Rattus Norvegicus)*

تأثير البسفينول –أ-على بعض المعايير المناعية لأناث الجرذ

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Abstract

Bisphenol-A-(BPA) is one of endocrine disruptor substances. The present study was conducted to investigate effect of BPA on some immunological parameters such as interleukin-1β (IL-1β),interleukin-6(IL-6),interleukin-2(IL-2), tumor necrotic factor-α(TNF-α),complememt$_3$ (C_3) and Immunoglobulin G (IgG).Twenty four female rats were used and divided into three groups (8 animals for each group) randomly. Animals of first group was negative control group and second group was positive control group received normal saline and corn oil orally respectively, while female rat of third group were received BPA suspended with corn oil (250mg/kg B.W/day) orally as treatment group for 30 days . Results of the present study revealed significant increase ($P\leq0.05$) in serum level of (IL-1β, IL-6, IL-2, TNF-α, C_3 and IgG) in group administrated BPA in compared with all other groups.
Keywords: bisphenol-A-, IL-6, TNF-α, rat

الخلاصة

البسفينول-أ- هو أحد المواد المعرقلة لعمل الغدد الصم .الدراسة الحالية توصلت لكشف تأثير البسفينول-أ-على بعض المعايير المناعية مثل انترلوكين-1-، انترلوكين-2-، انترلوكين-3-، انترلوكين-4-، المتمم-3- والامينوكلوبيولين-ج- . أربع وعشرون أنثى جرذ أستخدمت وقسمت الى ثلاث مجاميع (8 حيوانات لكل مجموعة) عشوائيا. حيوانات المجموعة الأولى هي مجموعة السيطرة السالبة والثانية هي مجموعة السيطرة الموجبة تناولت المحلول الملحي وزيت الذرة فمويا على التوالي، بينما أناث الجرذان للمجموعة الثالثة تناولت البسفينول-أ-المعلق بزيت الذرة (250 ملغم/كغم من وزن الجسم) فمويا وعملت كمجموعة معاملة . نتائج الدراسة الحالية عكست ارتفاع معنوي ($P\leq0.05$) في مستويات المصل للانترلوكين-1-، انترلوكين-2-، انترلوكين-3-، انترلوكين-4-، المتمم-3-والامينوكلوبيولين-ج-في المجموعة المعطية البسفينول-أ-بالمقارنة مع كل المجاميع الأخرى.

Introduction

Bisphenol A (BPA) is one of the manufacturing compounds, that interfered in production different plastic compounds and polycarbonate and become universally used in the production of paper, food and beverage containers, consumer goods, and in many other industrial applications (1). Recently researches showed that BPA has ability to leach out of some products, include tableware, plastic lining of cans used for food, white dental fillings sealants and polycarbonate babies' bottles. The leaching was occured by exposure of the plastic to high temperatures (2). About 93% of urine samples in the US population contain on BPA (3). (1) BPA found in the fluid portion of many classes of vegetables such as green beans, mushrooms, mixed vegetables, peas, corn and artichokes, which take from Cans with epoxy resin linings. (4) Were reported that pro inflammatory cytokines at high concentrations lead to disruption the homeostasis of oxidants/anti-oxidants and DNA repair enzymes, these pro inflammatory cytokines increased in BPA-associated inflammatory processes. (5) Were reported that Kupfer Cells considered as the cause of the inflammatory response, because they lead to release proinflammatory cytokines, include IL-6 and interleukin (IL)-1beta when activated. Exposure to BPA lead to increase in concentration of IgG (6)

Materials and Methods

Animals of the Study

The present study was conduct at College of Veterinary Medicine – University of Kerbala. Twenty four mature *female Ratus Norvegicus* rats were purchased from care center and medicinal researches in Baghdad, Iraq. They were 14 to 16 weeks old with an average body weight (200-250gm).

The animals were clinically healthy, kept under hygienic conditions, metal cages and glassy bottles were used to avoid exposure to BPA from old polycarbonate cages. Water and feed were giving *ad –libitum* throughout the experimental period.

Female albino rats (24females) divided into three main groups (8animals) of each group as following:

1-Negative control group: Eight female rats that received only normal saline orally as vehicle (0.5ml/kg BW).

2-Posative control group: Eight female rats that received only corn oil orally as vehicle (0.5ml/kg BW).

3- Treatment group: Eight female albino rats, orally administer BPA 250mg/kg BW. /day (1/20 LD50) suspended in corn oil via gavage as high dose (7).

female rats' of each group were sacrificed at the end of the experiment after 30 days; the rats before sacrifice were first anaesthetized by placing them in a closed jar containing cotton sucked with chloroform anesthesia.

Blood samples were collected by heart puncture the blood was put in plane tube to be centrifuged (6000) rpm for 10 minutes to obtain the serum which is then transferred to epndrofe tubes, for the immunological estimation all tubes were stored at (-20c) until analyzed.

Immunological parameters

Interleukin (IL-1β), Interleukin (IL-2), Interleukin (IL-6) and Tumor Necrotic Factor-α (TNF-α) Boster's mouse IL-1β, IL-2, IL-6 and TNF-α ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology according to manufacture instruction.

C3 and IgG radial immunodiffusion plates for the accurate quantitative determination of proteins in the serum which was described by (8).

Statistical analysis:

The data were presented as mean ±SE and subjected to analysis of variance by using one way ANOVA Post hoc test was used LSD to specify the significant difference among means the software package IBM SPSS Program version 20 was used for the analysis of data (9).

Results

Effect of BPA on serum concentrations of C_3 and IgG in Mature Female Rats

A significant increase ($p \leq 0.05$) was noticed in serum C_3 and IgG concentrations of female rats treated with BPA 250 mg/kg B.W compared with control groups.

Table (1) the Effect of BPA on serum concentrations C_3 and IgG in Mature Female Rats (Means ± SE)

Parameters / Groups	C_3 mg/dl	IgG mg/dl
Normal saline group (Negative control group) (0.5ml/kg/B.W)	C 133.91±9.28	C 1214.12±95.96
Corn oil group (Positive control group) (0.5ml/kg/B.W)	C 131.88±9.96	C 1202.37±110.61
Bisphenol-A- group (Treatment group) (250 mg/kg/B.W)	A 229.62±4.25	A 3143.00±185.73

N=8

Different letters represent a significant difference at ($p \leq 0.05$).

Effect of BPA on serum concentration of IL-1β, IL-2, IL-6 and TNF-α in Mature Female Rats

The effect of exposure to (250 mg / kg B.W.) of BPA demonstrated a significant increase ($p \leq 0.05$) in serum of IL-1β, IL-2, IL-6 and TNF-α concentrations of female rats compared with control groups.

Table (2) the Effect of BPA on serum concentration of IL-1β, IL-2, IL-6 and TNF-α in Mature Female Rats (Means ± SE)

Parameters / Groups	IL-1β pg/ml	IL-2 pg/ml	IL-6 pg/ml	TNF-α pg/ml
Normal saline group (Negative control group) (0.5ml/kg/B.W)	C 16.37±1.28	C 37.00±1.73	C 36.37±0.94	C 38.50±1.41
Corn oil group (Positive control group) (0.5ml/kg/B.W)	C 17.25±1.68	C 38.25±2.05	C 37.50±1.01	C 38.75±2.13
Bisphenol-A- group (Treatment group) (250 mg/kg/B.W)	A 226.25±3.01	A 231.62±2.87	A 267.00±1.73	A 268.87±2.78

N=8

Different letters represent a significant difference at ($p \leq 0.05$).

Discussion

The present results revealed significant increase ($P \leq 0.05$) in C_3 and IgG concentration in group treated with (250 mg/kg B.W) of BPA compared to control groups table (1) and these results were matched with previous studies (10) (11) (12). Administration of BPA orally in mice lead to shift of the Th2 cytokine profile to Th1. BPA moderately increased IgG a representative of Th1 type antibody, was also augmented (11). (10) Were reported that BPA lead to significant increased ($P \leq 0.05$) in immunoglobin IgG antibodies in mice that were immunized with hen egg lysozyme. The effect of exposure to (250 mg / kg B.W.) of BPA demonstrated a significant increase ($P \leq 0.05$) in serum of IL-1β, IL-2, IL-6 and TNF-α concentrations of female rats compared with control groups and these results were matched with previous studies (13) (14) (10) (15). (16) were reported

that the pathway by which BPA rises IL-6 release remains to be determined. There is no evidence for gender differences in circulating IL-6 or TNF_α concentrations in humans. BPA caused in increase of innate immune response by increasing production of cytokines such as IL-1β and tumor necrosis factor (TNF-α) in macrophages, in the other hand, BPA stimulated both T and B cells in adaptive responses by using immune cells isolated from BALB/c mice (17).

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