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Response of Wistar Rats to Low Doses of Bisphenol A

Almoeiz Y. Hammad[1*], Shama I. Y. Adam[2] and Warda S. Abdelgadir[3]

[1]Al-manhal Academic of Science, Khartoum, Sudan.
[2]Department of Biochemistry and Molecular Biology, Faculty of Science and Technology, Al Neelain University, P.O. Box 12702, Khartoum, Sudan.
[3]National Food Research Centre, Ministry of Agriculture and Natural Resources, P.O. Box 213, Khartoum North, Sudan.

Authors' contributions

This work was carried out in collaboration among all authors. Author AYH designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SIYA and WSA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Bisphenol A is an industrial chemical widely used in the manufacture of polycarbonates (PCs), epoxy resins, and other polymeric materials. In this study the toxicity of different doses of oral bisphenol A to wistar rats was investigated. bisphenol A was given to rats at 2.5, 5, 10 and 20 µg/kg body weight /day for 12 weeks. All the treated rats had a significantly increased body weight, but none of the rats died during the 6 and 12 weeks' period. Haematological and biochemical parameters were measured after six and twelve weeks of the experimental period. Behavioral changes and dosing resistance were observed in all the tested groups. After 12 weeks, different types of anemia (microcytic hypochromic, microcytic normochromic and normocytic normochromic) were observed in the different treated groups. The toxicity on the liver, toxicity on kidney and spleen was correlated with changes in the concentrations of AST (aspartate aminotransferase), ALT

**Corresponding author: E-mail: Almoeiz7@gmail.com;*

(alanine aminotransferase) and ALP (alkaline phosphatase) and the concentrations of globulins, glucose, cholesterol and urea. The toxic effect of bisphenol A was evident on the Haematological and Serobiochemical parameters.

Keywords: BPA (bisphenol A); toxicity; haematological; serobiochemical.

1. INTRODUCTION

Bisphenols are organic synthetic compound that contain two hydroxyl phenyl as functional groups [1]. One of the well-known important type is bisphenol A (BPA) which is an industrial chemical widely used in the manufacture of polycarbonates (PCs), epoxy resins, and other polymeric materials. Although PC and epoxy resins are the major applications of BPA, other uses include unsaturated polyester resins, polyarylate resins, polysulfone resins, polyetherimide resins and flame retardants [2].

BPA is a widely used compound in daily life. Oral, inhalation and transdermal are the major routes for human exposure. While the primary sources of exposure to BPA for most people are through diet or food products, other sources include food packaging and dust, dental materials, healthcare equipment, thermal paper and baby bottles [3]. The most important source of dietary exposure to BPA is canned foods, but it may also be present in fresh foods such as meat, milk or eggs, when animals are bred or reared in polluted areas or watered with the contaminated water [4,5,6] reported that factors like pH, salt, oil and glucose concentration have been shown to influence BPA migration from the lacquer to the can contents. BPA was also detected in the food products stored in the cardboard boxes [7].

BPA is known to have toxic effects on various systems especially on reproductive system as it possesses estrogenic property [8,9,10,11]. It is implicated in carcinogenesis [12], endocrine abnormalities [13], neural and behavioral alterations [14], cardiac and hepatic abnormalities [15,16] denoting the long- term effects of BPA.

Due to the fast rhythm of daily life, too much fast food and soft drink are utilized using containers where BPA is one of the manufacturing materials. People put on weight and get diseased. Changes in sexual behavior, numerous cases of miscarriages, sterility in addition to increased incidence of cancer are continuously observed.

Acute exposure to BPA produces lethality with a very narrow range of lethal and survival dose for iv route. The lethality appears to be due to respiratory arrest and hypotension [17].

Long term toxicity experiment was designed to obtain information on the effect of various dietary doses (2.5, 5, 10 and 20 µg/kg per day) of bisphenol A on Wistar rats for 12 weeks. Emphasis was put on changes in growth, biochemical and hematological, characteristics of Wistar rats.

1.1 Objective

This study was conducted to assess the chronic toxic effects of Bisphenol A on wistar rats

2. MATERIALS AND METHODS

Fifty Wistar rats were obtained from the Faculty of Pharmacy of the University of Khartoum, reared within the premises of the animal house under 12 hours' photoperiod with standard feed and drinking water provided *ad libitum* before the commencement of experimental feeding. Room temperature was maintained at 25 ± 2°C at adequate house ventilation. Then the animals were randomly allotted into five groups 1, 2, 3, 4 and 5 each of ten rats. Group 1 was designated as the control group.

Extra Pure Bisphenol A powder (Sangon, China) was thoroughly dissolved in distilled water and rats received the test chemical by oral gavage doses at 2.5, 5, 10 and 20 µg/kg per day. Whereas Group 1 was fed the basal diet and served as control. Experiment was continued for 12 weeks. 50% of rats from each group (five rats) was humanely slaughtered at the end of week 6. The remaining 50% rats was slaughtered by the end of the experimental period (12 weeks).

2.1 Data Collection

2.1.1 Body weight changes

The initial weight of rats was measured on the first day of the experiment, after sex week and at the end of the experimental period (12 weeks) and weight gain was calculated.

2.1.2 Hematological parameters

By the end of 6 weeks (half of experiment period), 50% of each group was slaughtered and the blood samples was collected in dry test tubes containing EDTA (Ethylene diamine tetra acetic acid) and examined according to sysmex CBC analyzer protocol-sysmex corporation kobe, Japan for Hemoglobin Concentration (Hb), Red Blood Cells (RBC), total White Blood Cell (WBC) Counts and Packed Cell Volume (PCV). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

2.1.3 Serobiochemical parameters

Blood samples was collected and allowed to clot and sera was separated by centrifugation at 3000 r.p.m for 5 min and stored at -20°C and analyzed by UV Spectrophotometer method [18] to determine the total proteins, albumin, globulins, urea, cholesterol and the serum enzymes, AST, ALT and ALP.

2.2 Statistical Analysis

Mean values in body weight and serum data were compared using student's t-test [19].

3. RESULTS

3.1 Clinical Observations

The control Group 1 remained clinically normal throughout the experimental period. All other tested groups showed aggressiveness and dosing-resistance.

3.2 Body Weight

The effects of receiving 2.5, 5, 10 and 20 µg/kg /day of extra pure bisphenol A on growth of rats are shown in Table 1. By the end of week six of the experimental period, the rats receiving 5, 10 and 20 µg/kg /day BPA (group 3, 4 and 5 respectively) had significant increase (P<0.05-0.01) in body weight gain, while that of group 2 received 2.5 µg/kg /day BPA did not change when compared to the control group 1.

At the end of the experimental period (12 weeks), all the test groups showed significant increase (P<0.01) in body weight gain, with group 4 showing the highest increase when compared to the control and the other test groups.

3.3 Haematological Findings

These data are presented in Table 2. After the end of the first six weeks, the value of HB was higher (P<0.05) in group 2 and that of RBCs was lower (P<0.05) in the same group. The values of MCV increased significantly (P<0.05-0.01) in groups 3 and 4 and depressed (P<0.05) in group 5. PCV values were higher (P<0.05) in groups 2 and lower (P<0.05-0.01) in groups, 3, 4 and 5. The value of MCHC was significantly lower (P<0.05) in group 5 than the control group 1 while the values of MCH and WBCs were not different among the treatment groups.

At the end of the experimental period (12 weeks), the values of RBCs and MCV were lower (P<0.05) in groups 2 and 3. The PCV values increased significantly (P<0.05-0.01) in all the test groups with group 5 recorded the highest value. The values of MCH and MCHC were lower (P<0.05) in group 2 but did not changed in groups 3, 4 and 5. The counts of WBCs were lower (P<0.05) in groups 2, 3 and 4 than the control group 1.

3.4 Serobiochemical Changes

After the end of the first six weeks of the experimental period, there was a significant decrease (P<0.01) in AST and an increase (P<0.05) in ALT activities in groups 2, 3 and 4. ALP activity was significantly depressed (P<0.01-0.001) in all the treatment groups. The concentration of the total protein was lower (P<0.05) in groups 2, 3 and 4 while those of globulins was lower (P<0.05) in groups 2 and 3. Albumin concentration did not change in any of the test groups when compared to the controls (group 1). Significantly lower (P<0.05) concentration of glucose was recorded in group 2 and 3, while those of groups 4 and 5 did not change. The concentration of cholesterol was lower (P<0.05) in group 3 and higher (P<0.01) in group 4 while urea concentration was higher (P<0.05) in groups 2 and 3 and lower (P<0.05) in group 4 than the control group 1.

At the end of the experimental period (12 weeks), the activities of AST and ALP were lower (P<0.05-0.01) in all the treatment groups than the control rats. Significant increase (P<0.05-0.01) in ALT activity was observed in groups 2,3 and 4 and that of group 5 did not change. The total protein concentration was lower (P<0.05) in group 5 and that of globulin was lower (P<0.05) in groups 3 and 5. The concentration of albumin did not change in any of the test rats. Glucose concentration was significantly higher (P<0.05-0.01) in groups 3, 4 and 5. Cholesterol and urea concentrations were higher (P<0.05-0.01) in all the treatment groups than the control rats of group 1.

Table 1. Average (mean±S.D) values of rats changes in body weight gain (g) during treatment (6 weeks) and (12 weeks) with orally BPA

Groups	Initial weight (g)	Final weight (g)	Weight gain (g)
6 weeks			
1. Control	104.2±0.37	125.4±0.39	21.2±0.2
2. 2.5 µg/kg /day BPA	106.2±1.01	129.2±0.98	23.0±0.03^{NS}
3. 5 µg/kg /day BPA	104.0±1.12	131.1±1.01	27.1±0.11**
4. 10 µg/kg /day BPA	107.4±1.43	133.1±0.89	25.7±0.54**
5. 20 µg/kg /day BPA	105.4±1.20	132.4±1.50	27.0±0.3**
12 weeks			
1. Control	125.4±0.39	151.3±0.98	25.9±0.59
2. 2.5 µg/kg /day BPA	129.2±0.98	164.2±1.02	35.0±0.04**
3. 5 µg/kg /day BPA	131.1±1.01	163.2±0.54	32.1±0.47**
4. 10 µg/kg /day BPA	133.1±0.89	171.8±0.12	38.7±0.77**
5. 20 µg/kg /day BPA	132.4±1.50	167.9±0.67	35.5±0.83**

*Values are means ±SE, NS=not significant, * Denotes mean values significant at (P<0.05), **Significant=(P<0.01)*

Table 2. Haematological changes in rats received BPA at different concentrations

Groups	HBg/dL^{-1}	RBC(x10^6/mm)	MCV(m^3)	PCV(%)	MCH(Pg)	MCHCg/dL	WBC(x10^3/mm)
6 Weeks							
1. Control	12.80±0.37	7.68±0.39	54.00±1.45	59.40±0.44	15.20±0.66	33.00±1.79	5.68±0.24
2. 2.5 µg/kg /day BPA	14.09±0.21*	5.98±0.39*	52.60±1.97^{NS}	66.40±0.43**	15.20±0.58^{NS}	33.54±1.41^{NS}	5.26±0.72^{NS}
3. 5 µg/kg /day BPA	11.60±0.68^{NS}	7.46±0.86^{NS}	57.80±0.58*	56.01±0.26*	15.20±0.32^{NS}	32.84±1.01^{NS}	4.94±0.48^{NS}
4. 10 µg/kg /day BPA	13.20±0.86^{NS}	7.62±0.67^{NS}	61.40±0.81**	57.20±0.31*	15.20±0.51^{NS}	33.34±0.96^{NS}	4.40±0.25^{NS}
5. 20 µg/kg /day BPA	11.80±0.86^{NS}	8.04±0.26^{NS}	50.20±0.86**	52.46±0.53**	14.80±0.86^{NS}	28.22±0.95*	4.56±0.23^{NS}
12 Weeks							
Groups	HBg/dL^{-1}	RBC x10^6/mm	MCV(m^3)	PCV(%)	MCH(Pg)	MCHCg/dL	WBCx10^3/mm
1. Control	12.20±0.86	7.25±0.69	54.40±0.87	59.87±0.46	15.60±0.51	32.60±1.23	6.28±0.41
2. 2.5 µg/kg /day BPA	13.20±0.74^{NS}	4.94±1.02*	50.40±1.03*	69.49±0.31**	13.40±0.81*	30.52±1.09*	4.52±0.26*
3. 5 µg/kg /day BPA	13.00±1.63^{NS}	5.40±1.16*	51.40±1.86*	66.0±0.49*	15.00±0.32^{NS}	31.34±1.09^{NS}	4.96±0.34*
4. 10 µg/kg /day BPA	13.60±0.68^{NS}	7.50±0.33^{NS}	54.40±0.86^{NS}	68.4±0.87*	16.60±0.93^{NS}	32.00±1.49^{NS}	4.36±0.13*
5. 20 µg/kg /day BPA	15.41±0.31*	8.68±0.49^{NS}	55.80±2.76^{NS}	71.62±0.34**	16.80±1.53^{NS}	32.48±2.44^{NS}	5.42±0.62^{NS}

*Values are means ±SE, NS=not significant, * Denotes mean values significant at (P<0.05), **Significant= (P<0.01)*

Table 3. Serobiochemical changes in rats received BPA at different concentrations

6 weeks									
Groups	**ASTU/L**	**ALTU/L**	**ALPU/L**	**Total protein g/dL**	**Albumin g/dL**	**Globulins g/dL**	**Glucose mg/dL**	**Cholesterol mg/dL**	**Urea mg/dL**
1. Control	52.8±0.2	12.8±0.5	61.20±0.60	5.04±0.35	2.72±0.43	2.32±0.28	55.96±1.03	35.94±1.67	19.06±0.95
2. 2.5 µg/kg /day BPA	43.6±0.2**	17.6±0.2*	38.6±0.12***	3.86±0.34*	2.42±0.10 NS	1.84±0.24*	49.46±0.27*	34.50±1.31 NS	21.80±0.27*
3. 5 µg/kg /day BPA	46.2±0.4**	15.8±0.6*	55.2±0.1**	4.06±0.04*	2.36±0.14 NS	1.70±0.11*	50.68±1.40*	33.22±1.32*	23.22±0.97*
4. 10 µg/kg /day BPA	41.6±0.7**	17.4±0.8*	47.01±0.45***	4.14±0.40*	2.08±0.09 NS	2.02±0.41 NS	55.78±9.51 NS	40.40±1.54**	16.20±0.66*
5. 20 µg/kg /day BPA	54.2±0.9 NS	13.2±0.7 NS	54.0±0.82**	5.17±0.48 NS	2.46±0.12 NS	2.71±0.37 NS	55.16±1.44 NS	34.12±2.45 NS	19.38±1.57 NS
12 weeks									
Groups	**AST U/L**	**ALT U/L**	**ALP U/L**	**Total protein g/dL**	**Albumin g/dL**	**Globulins g/dL**	**Glucose mg/dL**	**Cholesterol mg/dL**	**Urea mg/dL**
1. Control	53.18±0.7	11.56±0.3	61.67±0.0	4.76±0.44	2.50±0.23	2.26±0.38	54.95±0.09	35.30±0.16	17.24±1.36
2. 2.5 µg/kg /day BPA	48.6±0.21*	14.80±0.8*	57.44±0.32**	4.17±0.21 NS	2.15±0.18 NS	2.03±0.04 NS	52.05±0.90	37.43±0.02 *	21.62±0.95*
3. 5 µg/kg /day BPA	42.0±0.83**	15.60±0.04*	55.30±0.6**	4.52±0.22 NS	2.78±0.12 NS	1.74±0.28*	61.77±0.51**	39.70±0.04 *	20.40±0.96*
4. 10 µg/kg /day BPA	44.6±0.32**	17.40±0.3**	53.20±0.5**	4.26±0.15 NS	2.24±0.24 NS	2.02±0.16 NS	58.39±0.94*	44.98±0.78**	25.92±0.78**
5. 20 µg/kg /day BPA	46.0±0.31*	12.80±0.7 NS	53.89±0.3**	3.8±0.16*	2.08±0.08 NS	1.72±0.20*	57.49±0.79*	37.99±0.19 *	24.42±2.62**

*Values are means ±SE, NS=not significant, * Denotes mean values significant at (P<0.05), **Significant= (P<0.01)*

4. DISCUSSON

The results of the present study indicated that administration of BPA at 2.5, 5, 10 and 20 µg/kg /day is toxic but not lethal as evidenced by a significant increase in body, alter the function of some organs, such as the liver, haematological and serobiochemical alterations.

The mean body weight gains of Wistar rat given BPA at 2.5, 5, 10 and 20 µg/kg /day were significantly increased at the end of the experimental period (12 weeks) when compared to the control rats. Studies on rodents have also revealed that prenatal and postnatal exposure to BPA increases adipose tissue mass and hence promote the increment of body weight [20]. Increased body weight was observed in offspring of Sprague-Dawley female rats soon after birth and continued into adulthood when the female rats were exposed to approximately 0.1 mg BPA/kg body weight (BW)/day (low dose) or 1.2 mg BPA /kg/BW/day (high dose) in drinking water from day 6 of pregnancy through the period of lactation [21]. In the last few years, evidence of a relationship between BPA and obesity in humans has been emphasized but is confined to certain populations. Epidemiological studies in the USA [22,23], China [24], Korea [25,26] and Canada have reported positive associations between BPA and adiposity measures in adults. *In vitro* studies have shown that BPA was able to enhance terminal differentiation of 3T3-L1 cells into adipocyte leading to excess fat accumulation [27]. In that study, the action of BPA was mediated by the mechanism that involved the PI 3-kinase and Akt kinase pathway.

The anemia in group 2 received 2.5 µg/kg /day was microcytic hypochromic, a conclusion indicated by low values of MCV and MCHC, and that in groups 3 was microcytic normochromic while that of groups 4 and 5 was normocytic normochromic with normal values of MCV and MCHC. anemia could be referring to the effect of BPA on bone marrow.

In This study, the depressed activity of AST and ALP and elevated activity of ALT and high cholesterol concentration indicate liver damage. The development of nephrotoxicity in all the test groups was indicated by the high serum urea concentration. In this study there was an increase of serum glucose concentration, the increase in glucose levels was evident for concentrations of BPA ≥5 µg/kg/day. It was demonstrated by [28] that doses well below the current lowest observed adverse effect level considered by the US-EPA, disrupt pancreatic β-cell function producing insulin resistance in male mice. Therefore, this altered blood glucose homeostasis by BPA exposure may enhance the risk of developing type II diabetes.

BPA produces down-regulation of glucose transporters in adipocytes [29], an action that may induce insulin resistance. Moreover, BPA combined with insulin favors the conversion of fibroblasts to adipocytes [30], enhancing the risk of obesity, a metabolic disorder that has been related to endocrine disruptors exposure in the last years [31,32]. Hence, the direct effect of BPA on peripheral tissue might also be of importance to develop insulin resistance.

5. CONCLUSION

This study concluded that BPA is a toxic substance at all levels. The toxic effect was evident on the Haematological and Serobiochemical parameters also the vital organs was directly affected.

ETHICAL APPROVAL

Animal ethic Committee approval has been collected and preserved by the author.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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