Trop J Nat Prod Res, December 2018; 2(12):502-505 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of Bisphenol A and Di-(2-ethylhexyl) phthalate on Haematological and Renal Function Parameters

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ARTICLE INFO

Article history:
Received 11 October 2018
Revised 19 December 2018
Accepted 26 December 2018
Published online 03 January 2019

ABSTRACT

The ubiquitous nature of plastics has caused increased concerns about the effects of exposure of humans to plastic polymers particularly the endocrine-disrupting chemicals (EDCs) like Di-(2-ethylhexyl) phthalate (DEHP) and Bisphenol A (BPA) used in plastic production. The aim of this study was to investigate the effects of BPA and DEHP on haematological and renal function parameters. A total of 60 adult Wistar rats were divided into four groups (I-IV) of 15 animals each. Group I was fed with rodent feed only (control), while groups II - IV were administered 5 mg/kg/day of BPA, 0.5 mg/kg/day of DEHP and a mixture of 0.5 mg/kg/day of BPA and 0.5 mg/kg/day of DEHP, respectively. The animals were monitored for 42 days. The result shows that there were significant ($p < 0.05$) increase in pack cell volume, haemoglobin, red blood cell, reticulocyte, total white blood cell, lymphocyte, platelet, sodium ion and creatinine concentrations in group II, while the concentrations of urea, potassium and bicarbonate were reduced. In group III, there were increase in the concentrations of pack cell volume, mean cell haemoglobin, and mean cell volume, lymphocyte, platelets, sodium ion, and urea while creatinine concentration was reduced. In group IV, there were increase in the concentration of mean cell haemoglobin concentration, reticulocyte, total white cell count, neutrophil, lymphocyte, platelet, sodium ion and a decrease in creatinine and potassium ion concentration. The present study has shown a possible haematinic effect of BPA and DEHP. They also cause significant alterations in kidney function test parameters.

Keyword: Bisphenol A, Di(2-ethylhexyl) phthalate, Renal, haematological, plastic polymers.

Introduction

The universal usage of plastics has raised concerns about the continuous exposure to plastic polymers and their health implications is of worrisome concern especially the use of endocrine-disrupting chemicals (EDCs) including Di-(2-ethylhexyl) phthalate (DEHP) and Bisphenol A (BPA) used in the production of plastics. Widespread and continuous exposure to DEHP and BPA occurs through dietary intake, inhalation, dermal and intravenous exposure via consumer products and medical devices. BPA is a molecule which is used as a monomer in polymerization reaction to yield polycarbonate plastics. Polycarbonates are used in numerous consumer products, comprising food and water containers, baby bottles, covering of food and beverage metal cans, medical tubing, epoxy resins and dental fillings.[1,2] Small amounts of BPA can leach from the polymers to food or water especially when heated.[3] Research conducted in the USA, Europe and Japan, have documented widespread human exposure to BPA, with detected levels ranging from 0.3 to 5 ng/mL in serum as well as breast milk.[2] Being lipophilic, BPA also accumulates in human fat.[4] BPA is one of the highest-volume chemicals produced worldwide.[5] Heat and contact with either acidic or basic compounds accelerate hydrolysis of the ester bond linking BPA molecules in polycarbonate and resins. Specifically, heating of cans to sterilize food, the presence of acidic or basic food or beverages in cans or polycarbonate plastic, and repeated washing of polycarbonate products have all been shown to result in an increase in the rate of leaching of BPA.[6-8] The most commonly used phthalate is DEHP with a production of 1 to 4 million tons per year, which makes it one of the most common environmental contaminants worldwide.[9-11] Phthalates do not form strong molecular linkages with the polymer so they can diffuse throughout the matrix and leach into the environment.[12,13] As a result, the general population is widely and continuously exposed to these compounds through ingestion, inhalation, or skin absorption. No acclaimed and organized center where these plastic hazards are characterized, in Enugu, Nigeria thereby predisposing members of the populace to danger. The need to ensure that the plastic products we use does not contain dangerous chemical substances that poses as threat to our external and internal body tissues and organs, led to our investigating the effects of these chemical substances (BPA and DEHP) on Renal and haematological parameters in Wistar rats.

Materials and Methods

Animals

A total of 60 adult, male Wistar rats of age 2-3 months; with average weight of 200-250 g were purchased from the animal house, University of Nigeria, Enugu Campus. The animals were caged in a well-ventilated room and maintained under standard condition (12 h light/dark cycle). The animals were allowed to adapt to the room for 7 days. All the rats had access to standard commercially prepared pelleted rodent feed and pyrogen-free water.

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Citation: Omorodion TN, Achukwu UP, Belonwu NJK. Effect of Bisphenol A and Di-(2-ethylhexyl) phthalate on Haematological and Renal Function Parameters. Trop J Nat Prod Res. 2018; 2(12):502-505. doi.org/10.26538/tjnpr/v2i12.2

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Ethical consideration
The study ethical approval was granted by College of Medicine Ethical Committee (COMREC), University of Nigeria with approval number: 076/08/2018. The study was also conducted in compliance with policies outlined in the Guide for the Care and Use of Laboratory Animal (NRC, 2011).

Acute Toxicity Study
Acute toxicity study was carried out using standard Lorke's method.[14] The median lethal dose (LD_{50}) was calculated using the formula:

$LD_{50} = \sqrt{}$ (Highest nonlethal dose) x (Lowest lethal dose).

The LD_{50} and the possible dose animals and humans could be exposed to at a time or accidentally formed the basis for the experimental doses administered.

Haematological and Renal function studies
The animals were divided into four groups (I-IV) of 15 animals per group. All the rats had access to standard commercially prepared pelleted rodent feed and pyrogen-free water. From the results of the acute toxicity testing, experimental Groups II, III and IV were administered with BPA and DEHP daily mixed with rodent pellet orally in the morning and evening. The group I animals received normal rodent pellet and water and served as control group, group II received 5 mg/kg/day of BPA mixed with rodent pellet orally, group III received 0.5 mg/kg daily dose of DEHP mixed with rodent pellet while group IV received orally mixture of 0.5 mg/kg of BPA and 0.5 mg/kg DEHP + pelleted rodent feed. The rats were fed regularly for 42 days and on completion of 42 days, the animals were weighed, sacrificed by cranial dislocation. The blood samples were obtained from caudal vein for full blood count and kidney function test. Standard operating procedure was used in the analysis of complete blood count and Renal function test.[15,16]

Statistical analysis
The obtained data were subjected to statistical analysis using SPSS (version 25). The test groups' values were compared with the values of the control group using one-way analysis of variance (ANOVA) and Dunnett's test. P-Values < 0.05 were considered statistically significant.

Results and Discussion

The present study shows significant increase ($p < 0.05$) in the concentrations of pack cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), reticulocyte, total white blood cell count (TWBC), lymphocyte, platelets (Table 1) and significant increase in the concentration of sodium ion (Na^+), creatinine and a significant reduction in the concentrations of urea, potassium ion (K^+), and bicarbonate (Table 2) in group II (treated with bisphenol A) in comparison with the control group I.
The study of the effect of Bisphenol A on haematological parameters reveals significant increase in pack cell volume, haemoglobin, and red blood cell which could be the present of haematinic effect in the substance Bisphenol A. The significant increase in haemoglobin concentration could have been due that Bisephenol A increases the oxygen carrying capacity of the red blood cell in the Wistar rat. The significant increase in the white blood cell count and lymphocyte count of the group fed with BPA could have been due to increased immune response as a result of the presence of Bisphenol A in the blood. The increase in the platelet count may probably have resulted from the rat generating enough platelet due to the toxic substance (BPA). The increase in creatinine and sodium concentration could be due to the inhibitory effect of the substance on the glomeruli filtratration function of the kidney. Creatinine clearance is part of the measures in ensuring the glomeruli is functioning properly, if the serum creatinine ($p < 0.05$) is increased that means the glomeruli could be affected in comparison with the control . The study reveals significant ($p < 0.05$) increase in the concentration of red blood cell, mean cell haemoglobin concentration, reticulocyte count, total white blood cell count, lymphocyte and platelets (table 1). Urea and sodium ion (Na+) concentrations were also significantly increased ($p < 0.05$), while the concentration of creatinine was significantly ($p > 0.05$) decreased (table 2) in the group treated with di (2-ethylhexyl) phthalate in comparison with the control group I. The increase ($p < 0.05$) in the total white blood cell count and lymphocyte count could have been a result of the body's immunological response to the foreign substance (Diethyhexyl phathalate). The significant increase ($p < 0.05$) in the urea and sodium ion concentrations in the albino rats could have resulted from the inhibitory effect of DEHP on the selective reabsorption and glomeruli filtration function of the kidney. This increase could be attributed to impairment of the kidney function.[17-20]

Table 1: Complete blood count parameters in BPA, DEHP, BPA + DEHP Treated Rats and control.

Parameter	Value			
	Group I (control)	**Group II**	**Group III**	**Group IV**
PCV (l/l)	0.58 ± 0.00	0.63 ± 0.00*	0.58 ± 0.005	0.58 ± 0.00
Hb (g/dL)	19.22 ± 0.13	20.71 ± 0.04*	18.41 ± 0.96	17.72 ± 0.12
RBC (x 10^{12}/L)	8.20 ± 0.12	9.18 ± 0.13*	8.85 ± 0.1*	7.85 ± 0.27
MCH (pg)	21.08 ± 0.26	20.50 ± 0.29	19.82 ± 0.88	20.33 ± 0.14
MCV (Fl)	71.00 ± 0.37	68.00 ± 11.0.50	63.45 ± 0.56	66.42 ± 0.67
MCHC (g/L)	297.92 ± 0.47	293.67 ± 0.80	314.83 ± 0.91*	304.08 ± 0.92*
Rect. (%)	0.38 ± 0.27	0.82 ± 0.31*	0.58 ± 0.11*	0.88 ± 0.04*
TWBC (x 10^9/L	9.11 ± 0.07	12.96 ± 0.09*	14.47 ± 0.08*	15.91 ± 0.22*
Eosinophil (%)	Nil	Nil	Nil	Nil
Basophil (%)	Nil	Nil	Nil	Nil
Monocyte (%)	3.00 ± 0.37	3.17 ± 0.21	2.17 ± 0.24	1.17 ± 0.24
Lymphocyte (%)	63.08 ± 0.62	69.25 ± 1.15	78.58 ± 1.64*	32.08 ± 0.78*
Neutrophil (%)	33.91 ± 0.62	28.42 ± 1.22	19.25 ± 1.57*	66.67 ± 0.82*
Platelet (%)	374.92 ± 2.38	1113.91 ± 48.27*	864.00 ± 18.68*	1129.08 ± 34.73*

Values are expressed as mean ± standard deviation.
The sign (*) denote significant difference compared to the control ($p \leq 0.05$).
PCV - Pack Cell Volume, Hb - Haemoglobin, RBC - Red Blood Cell, MCH - Mean Corpuscular Haemoglobin, MCV - Mean Corpuscular Volume, MCHC - Mean Corpuscular Haemoglobin Concentration, TWBC - Total White Blood Cell Count.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Renal Function Test results in Treated Rats and control.

Parameters	Value			
	Group I (control)	Group II	Group III	Group IV
Urea	32.33 ± 0.45	20.33 ± 0.14*	46.92 ± 0.29*	92.33 ± 0.47*
Na^+	117.08 ± 0.34	142.08 ± 0.57*	123.05 ± 0.67*	138.83 ± 0.35*
K^+	16.10 ± 0.21	8.86 ± 0.29*	16.20 ± 0.05	8.917 ± 0.08*
HCO_3^-	20.42 ± 0.15	10.42 ± 0.19*	19.08 ± 0.15	20.00 ± 0.30
Cl^-	110.75 ± 0.25	107.25 ± 0.35	92.92 ± 0.38	108.75 ± 0.24
Cr	0.50 ± 0.02	0.58 ± 0.01*	0.43 ± 0.01*	0.28 ± 0.03*

Values are expressed as mean ± standard deviation.
The sign (*) denote significant difference compared to the control ($p \leq 0.05$).
Keys: Na^+ - Sodium ion, K^+ - Potassium ion, Cl^- - Chloride ion, HCO_3^- - Bicarbonate ion, Cr – Creatinine.

Table 3: Weight variations in Treated Rats versus control

Weight (g)			
Group I (control)	Group II	Group III	Group IV
199.50 ± 1.88	181.00 ± 5.51*	180.83 ± 2.92*	160.58 ± 1.78*

Values are expressed as mean ± standard deviation.
The sign (*) denote significant difference compared to the control ($p \leq 0.05$).

The concentration of mean cell haemoglobin, Reticulocyte count, total white blood cell count, Neutrophils, Lymphocyte and platelets count were significantly increased in the group treated with combination of BPA and DEHP (Group IV) (Table 1), also there was significant increase ($p < 0.05$) in the concentration of urea, Na^+ and significant reduction ($P < 0.05$) in the concentration of potassium ion (K^+), and creatinine (Table 2). The increase in total white blood cell count and neutrophils may have been due to increased immune response of the rats as a result of the combine effect of Bisphenol A and Di-(2-ethylhexyl) phthalate.
Body weights were significantly decreased in the treatment groups II – IV in comparison with the control group I (Table 3). The p-values shows that there are significant differences from the control ($p < 0.005$).
This suggests that the combined effects of BPA and DEHP could impair the functioning of the kidney. Significant reduction in the sizes of the rat may have resulted from growth or development reduction effect of BPA and DEHP. It could also be due to the inhibitory properties of DEHP and BPA on growth hormones as revealed by Wittassek, Angerer. [21, 22]

Conclusion

The present study reveals that bisphenol A and di(2-ethylhexyl) phthalate causes emaciation, affect the normal haematological parameters (have shown to have haematinic effect), and can cause a significant alterations in renal function parameters which could affect their normal functioning.

Conflict of interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors thank Mr Okey, Dr (mrs) Azubuike, Nkemdilim Ebo, Mr. Victor for their assistance and support throughout the duration of the work.

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