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Haematological and Biochemical Changes in Albino Rats in Response to Bisphenol A (BPA) Exposure

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ABSTRACT: The aim of this study was to investigate the haematological and biochemical alterations in albino rats induced by bisphenol A (BPA). Fifteen adult male albino rats were procured and separated into 3 different groups viz: group 1 (control), group 2 (50 mg/kg), group 3 (250 mg/kg); and were orally administered BPA at doses of 50 and 250 mg/kg/week. After 6 weeks of exposure, blood samples were collected for haematological and biochemical assay. The parameters analyzed included: red blood cell count, haemoglobin concentration, packed cell volume, white blood cell count, differential white blood cell count, kidney and liver function tests. The results obtained revealed that there was a sequence of significant decrease ($P<0.05$) from the control group to group 3 (250 mg//kg) of the BPA-treated groups in the following parameters; total RBC count 7.33-4.40, Hb concentration 16.63-11.13 and PCV count 43.67-34.67. A significant increase ($P<0.05$) was observed in all leucogram values of BPA-treated groups when compared to the control. The serum levels of AST (44.67-71.00), ALT (13.33-46.67), ALP (64.00-86.67), Na^+, K^+, urea and creatinine significantly increased ($P<0.05$) in the groups that received BPA-treatment. There was also a non-significant reduction ($P>0.05$) in total protein (72.67-61.00) and albumin (49.00-25.00) in BPA-treated groups when compared to the control. These findings demonstrate that exposure of albino rats to BPA resulted in haematological defects and alteration in several biochemical parameters that indicate renal and hepatic toxicity.

Keywords: Biochemical assay; Bisphenol A; Haematogical assay.

التغيرات الدموية والكيميائية الحيوية في الجرذان البيضاء استجابةً لتعرض Bisphenol A (BPA)

أنوانجو إفينيوج و أوبيميتا أوريكبونو

الملخص: كان الهدف من هذه الدراسة هو التحقيق في التغيرات الدموية والكيميائية الحيوية التي يسببها بيسفينول أ .(BPA)تم شراء خمسة عشر ذكورًا من الجرذان البيضاء البالغة وتم فصلها إلى 3مجموعات مختلفة :المجموعة) 1مجموعة التحكم (، المجموعة 50) 2مجم /كجم (، المجموعة 250) 3مجم / كجم (؛ وكانت تدار عن طريق الفم BPAبجرعات 50و 250ملغم /كغم /أسبوع .بعد 6أسابيع من التعرض ، تم جمع عينات الدم لفحص الدم والكيمياء الحيوية .تشمل المعلمات التي تم تحليلها ؛ خلايا الدم الحمراء ، تركيز الهيموجلوبين ، حجم الخلايا المكدسة ، خلايا الدم البيضاء ، تعداد خلايا الدم البيضاء التفاضلية ، فحص وظائف الكلى والكبد .أظهرت النتائج التي تم الحصول عليها أن هناك تسلسل انخفاض معنوي ($P<0.05$)من مجموعة التحكم إلى المجموعة 250) 3مجم /كجم (من المجموعات المعالجة بـ BPAفي المعلمات التالية ؛ إجمالي عدد كرات الدم الحمراء 7.33-4.40، تركيز الهيموغلوبين 16.63-11.13وعدد خلايا الدم الحمراء .43.67-34.67لوحظت زيادة معنوية ($P<0.05$)في جميع قيم leucogramللمجموعات المعالجة بـ BPAبالمقارنة مع المجموعة الضابطة .زادت مستويات مصل AST (44.67-71.00)، ALT (13.33-46.67)، ALP (64.00-86.67) ، Na +، K +، اليوريا والكرياتينين بشكل ملحوظ ($P<0.05$)في المجموعات التي تلقت علاج .BPAكان هناك أيضًا انخفاض غير معنوي ($P>0.05$)في البروتين الكلي (72.67-61.00)والألبومين (49.00-25.00)في المجموعات المعالجة بـ BPAمقارنةً بمجموعة التحكم .توضح هذه النتائج أن التعرض لـ BPAأدى إلى عيوب دموية وتغيير في العديد من المعلمات البيوكيميائية التي تشير إلى سمية كلوية وكبدية.

الكلمات المفتاحية: فحص الكيمياء الحيوية .ثنائي الفينول أ؛ فحص الدم.

1. Introduction

Bisphenol A (BPA) is a synthetic organic compound that is widely used throughout the world. It is primarily known as an important ingredient in the production of polycarbonate (PC) plastics and epoxy resins. It serves as an intermediary to the manufacture of various products. BPA is widely used because it is lightweight and for its rigidity, lucidity and resistance to temperature. Polycarbonates are used in plastic containers especially in the food industry and for domestic products such as for plastic bottles, and for lenses, sports safety equipment and components of medical devices. Epoxy resins are used as protective coatings in metal cans in order to protect the integrity and safety of our food supply by preventing corrosion and contamination of canned foods and beverages with metals and bacteria, hence extending their shelf life. Thus besides many other applications, both PC and epoxy resins are widely used as food contact materials (FCMs) which means that they are used for the manufacture of items that have direct contact with food [1]. These items include: infant feeding bottles, tableware, food containers, water bottles, ovenware, water pipes and so on [2]. Food contact materials, however, comprise only about 3% of produced PC plastics and 10% of epoxy resins [3]. The general human population can be exposed to BPA through the oral, inhalatory and dermal routes [4, 8], but the most significant mode of exposure of humans to BPA is through food, being present in canned food and drinking water as a result of the leaching of this chemical from the plastic lining of cans of drinks and food. The leaching of BPA from plastic is increased when it is cleaned with a strong detergent, when it contains acidic liquids or when it is exposed to high temperatures. It is also known to leak into food from the protective inner epoxy resin coating of canned food, and this usually occurs when the cans are being heated during sterilization or food preparation [9]. Moreover, the high production volume of BPA is ubiquitous and this leads to contamination of the environment, especially the soil and groundwater [10].

BPA is absorbed from the gastrointestinal tract into the blood and redistributed to other tissues [11]. It is highly conjugated in the liver to form bisphenol A glucuronide, a major metabolite, which is excreted in urine [12] and this has been detected in the urine and serum samples of individuals in the USA [13]. These studies have shown that humans are being exposed to BPA. BPA has been demonstrated in both in vivo and in vitro experiments to act as an endocrine disrupting chemical [14]. This has raised great concern with regards to human health. The harmful effects of BPA are mainly linked to its estrogenic activity [15]. The exposure of different laboratory animals to BPA elucidates the multiple effects on the male and female reproductive systems. Different doses of BPA caused inhibition in spermatogenesis and seminiferous tubules in male chicks [16]. BPA is also known to cause a decrease in sperm count and motility and also to affect sperm morphology of adult male rats [17,18]. BPA has been associated with declined semen quality and increased sperm DNA damage; this also supports the toxicity of BPA on germ cells [19].
This study therefore investigated the effect of oral administration of BPA in adult male rats by examining alterations in their haematological and biochemical parameters.

2. Materials and methods

2.1 Test material

Bisphenol A [2, 2-bis (4-hydroxyphenyl propane)] (purity 99.9%, CAS no. 80-05-7) was procured from Geochem Ventures Limited, Choba, Rivers State. BPA solution was prepared by dissolving this chemical in olive oil on a weekly basis according to the dose to be orally administered to each of the groups.

2.2 Test animals

A total of fifteen (15) adult male albino rats, weighing 180-230 grams were used to carry out these studies. The rats were bred in the animal house with a 12-hour light and 12-hour dark photoperiod, in the Department of Animal and Environmental Biology, University of Port Harcourt. Before the administration of this chemical, the test animals were allowed to acclimatize to the laboratory environment for a period of fourteen days. They were kept in a wooden cage and given a standard feed and water throughout the study.

2.3 Experimental design

The rats were divided into three groups with each group containing five animals which were approximately equal to their average body weight. The doses were selected according to Jayashree *et al*. [20]. The oral LD_{50} of BPA in rats was 3250 mg/kg body weight as described by MSDS [21].

The experimental groups were designated as follows: Group I: Control (vehicle-treated, n = 5); Group II (received 50 mg/kg body-weight/week BPA, n = 5); Group III (received 250mg/kg body-weight/week BPA, n = 5). BPA was administered weekly via the oral route for 6 weeks. All experimental procedures and animal use were approved by the University of Port Harcourt Local Committee on Research Ethics.

At the end of the experiment, the test animals were sedated by placing them in a sealed jar containing cotton wool soaked with chloroform anaesthesia. Blood samples were obtained by cardiac prick and 1 ml of blood was placed in an ethylenediaminetetraacetic acid (EDTA) anticoagulant tube for measurements of haematological parameters. The blood was centrifuged at 6000 rpm for ten minutes to separate the serum which was used for the measurement of biochemical parameters.

2.4 Haematological assays

Red blood cell (RBC) and leucocyte counts were ascertained using an improved Neubauer haemocytometer. Packed cell volume (PCV) was estimated by the micro haematocrit technique. Haemoglobin (Hb) concentration was assessed with Drabkin's method [22]. The differential leucocyte count was performed on Giemsa stained blood smears [23].

2.5 Biochemical analysis of serum

2.5.1 Kidney function

The levels of urea and serum creatinine were ascertained using the principles of Tabacco, and Fabiny and Eringhausen respectively [24, 25]. The method for electrolyte [calcium ion (Ca^{2+}), chloride ion (Cl^{-}), potassium ion (K^{+}), and sodium ion (Na^{+})] analysis was performed by direct measurement [26]. The activity of the specific ion in the sample at the electrode was joined with an electrical potential which was measured by a voltmeter. Voltage is theoretically proportional to ionic activity. The voltage was finally converted to an electrical signal and displayed as a value on the screen. The bicarbonate (HCO_3^{-}) analysis was carried out using standard laboratory procedures [27].

2.5.2 Liver function

Serum samples were assayed for aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) using standard diagnostic kits with a clinical spectrophotometer. Serum total protein (TP) was ascertained using the method of Weichselbaun, while albumin (ALB) was ascertained using the method of Dumas and Biggs [28, 29].

2.6 Statistical analysis

The results were presented as mean ± standard deviation and subjected to analysis by using one-way analysis of variance (ANOVA). A post hoc test was used to determine the significant difference among means of different groups. The SPSS (Statistical Package for Social Sciences) software (version 20) was used for the analysis of data and the level of significance was set at $P \leq 0.05$.

3. Results

3.1 Effects of BPA on haematological parameters of albino rats

Table 1 shows the effect of BPA on some haematological parameters. There was a significant decrease ($P<0.05$) in RBC count, Hb concentration and PCV in rats after oral administration of both doses (50 and 250 mg/kg) of BPA when compared to the control. The results also revealed that there was a significant increase ($P<0.05$) in white blood cell (WBC) count and in the percentage of neutrophils, lymphocytes, monocytes and eosinophil in the groups that received BPA-treatment when compared to the control.

Table 1. Effect of oral administration of BPA on some haematological parameters of albino rats.

Parameters	Control	50 mg/kg	250 mg/kg
RBC ($x10^9$/L)	7.33 ± 0.41^{a}	6.23 ± 0.25^{b}	4.40 ± 0.46^{c}
Hb (g/dL)	16.63 ± 0.31^{a}	13.33 ± 0.35^{b}	11.13 ± 0.83^{c}
PCV (%)	43.67 ± 1.53^{a}	40.00 ± 1.00^{b}	34.67 ± 1.53^{c}
WBC ($x10^9$/L)	8.07 ± 0.31^{a}	9.50 ± 0.20^{b}	10.03 ± 0.15^{b}
Neutrophils (%)	23.00 ± 2.00^{a}	28.67 ± 1.53^{a}	36.00 ± 3.46^{b}
Lymphocyte (%)	45.33 ± 4.51^{a}	53.33 ± 1.53^{b}	59.00 ± 1.73^{b}
Monocyte (%)	5.00 ± 0.00^{a}	7.00 ± 0.00^{b}	7.67 ± 0.58^{c}
Eosinophil (%)	2.67 ± 0.58^{a}	4.00 ± 0.00^{b}	5.33 ± 0.58^{c}

Data are presented as mean ± SD; Values with different superscript characters ($^{a-c}$) indicate a significant difference ($P \leq 0.05$); RBC - Red Blood Cell; Hb - Haemoglobin concentration; PCV - Packed Cell Volume; WBC - White Blood Cell; (n = 5)

3.2 Effect of BPA on renal function of albino rats

Figure 1 shows a summary of the effect of BPA on kidney parameters. The mean values of serum urea, creatinine, Na^+, K^+ and Ca^{2+} increased significantly ($P \leq 0.05$) in the groups that received BPA-treatment when compared to the control. The table also reveals that there was no significant difference ($P > 0.05$) in the level of serum HCO^-.

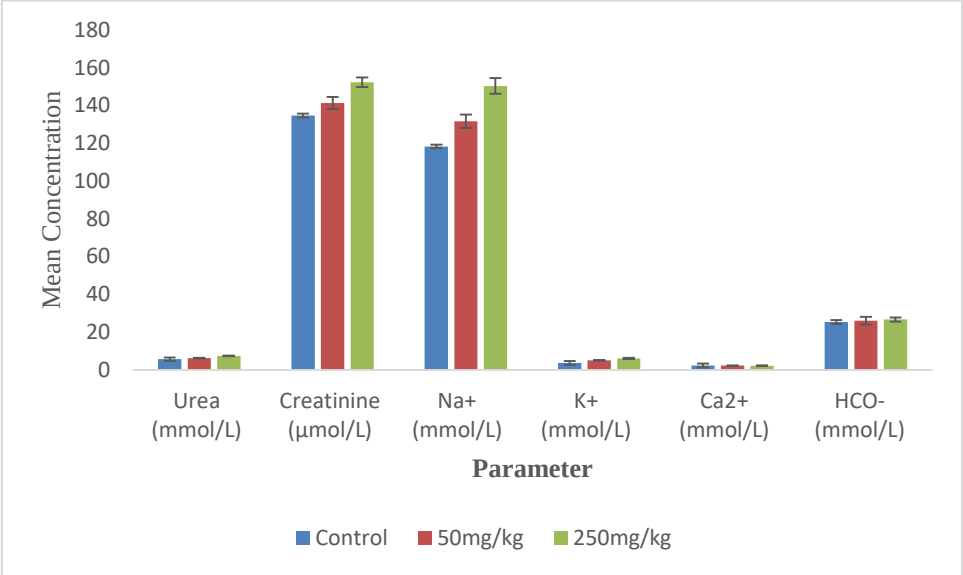

Figure 1. Effect of oral administration of BPA on renal function of albino rats.
Data are presented as mean ± SD. Values with different superscript characters ($^{a\text{-}c}$) indicate a significant difference ($P \leq 0.05$). Na^+ = Sodium ion; K^+ = Potassium ion; Ca^{2+} = Calcium ion; HCO_3^- = Bicarbonate ion; (n = 5)

3.3 Effect of BPA on liver function of albino rats

Figure 2 shows the effects of BPA on liver parameters. The mean values of AST, ALT and ALP increased significantly ($P < 0.05$) in rats after oral administration of both doses (50 and 250 mg/kg of BPA) when compared to the control. The mean values for total protein and albumin showed no significant difference ($P > 0.05$) in the group that received BPA treatment when compared to the control.

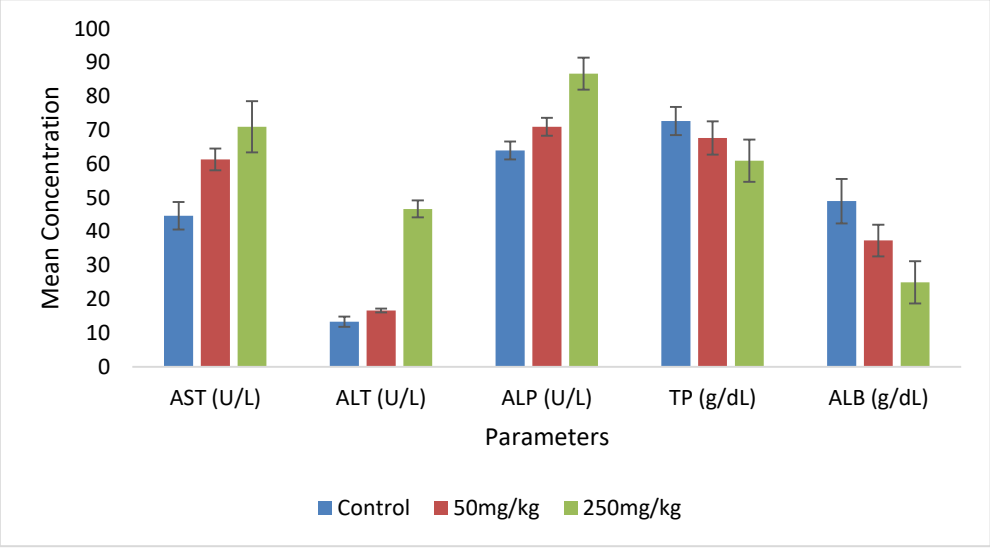

Figure 2. Effect of oral administration of BPA on liver function of albino rats.
AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alkaline phosphatase; TP = Total protein; ALB = Albumin; (n = 5).

4. Discussion

The current research revealed that the oral exposure of adult male rats to BPA resulted in a significant decrease in the RBCs count, Hb concentration and PCV compared to the values for the control. The data obtained were close to the results of Ulutas, and of Yamasaki and Okuda [30, 31]. They both determined the impact of BPA on rats at a dose of 125 mg/kg and 100 mg/kg respectively and later discovered that this chemical had instigated a significant decrease in total erythrocyte count, Hb concentration and PCV. The decrease in RBCs may signify a disruption in erythropoiesis or may be due to damaged erythrocytes [32]. These defects may also lead to a reduction in the lifespan of RBCs. The decrease in haemoglobin content of blood may be due to the decrease in RBC count, destruction of synthesized RBC or destruction of RBC membranes [32]. Erythrocyte deformation might cause low oxygen levels and dysfunction of cellular respiration or radical-induced stress which might cause haemolysis; therefore altering the total erythrocyte count [33]-[34]. The decrease in the concentration of haemoglobin may result in stress which can cause deleterious effects in the experimental animals [35]. PCV is a major haematological parameter that changes with environmental stress [36]. The reduction in PCV value is result of a decrease in the total RBC count. There was also a significant increase in leucogram values of BPA-treated groups in comparison with the control. This may signify that this compound has the capacity to induce some non-specific immune response in the test animal [36]. This would help in resisting potential pathogens in the test animal. The elevated leucogram values of BPA-treated groups might initiate a defense mechanism against this chemical. The elevated values of WBC may be to enhance wound healing and inflammatory disease [37].

The results also revealed that there was a significant increase in serum urea, creatinine and some electrolytes (Na^+, K^+) in BPA-treated groups in comparison with the control group. These alterations in renal function are close to the results obtained from previous research [38,39]. Sangai and Verma stated that the nephrotoxic effect of BPA is as a result of kidney damage which leads to the accumulation of toxic metabolites in the body [40]. There was an insignificant increase in serum Ca^{2+} whereas a non-significant reduction was also noticed in the level of HCO_3^- in BPA-treated groups in comparison with the control groups. The above results confirmed that electrolyte imbalance is characterized as renal problems [41]. The increases in urea, creatinine, Na^+ and K^+ indicate renal impairment which resulted in the accumulation of unwanted materials. The current research revealed that rats exposed to BPA displayed a significant increase in AST, ALT and ALP levels in comparison with the control group. Serum AST, ALT and ALP have been proven to be released into the blood when the liver is damaged [42]-[44]. Adedapo and Aiyelotan had previously reported similar higher levels of serum hepatic enzymes [37]. According to Korkmaz *et al*., exposure to BPA might result in a rise in oxidative stress in the liver which explains the higher levels of AST, ALT and ALP [14]. There was an insignificant reduction in the serum levels of total protein and albumin in the groups that received BPA-treatment. The reduced level of total protein in BPA-treated groups may suggest impairment of liver function which might result in a disease condition called hypoproteinemia.

5. Conclusion

It can be concluded that oral administration of BPA for 6 weeks in adult male rats resulted in haematological defects and alteration of several biochemical parameters that indicate renal and hepatic toxicity.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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Ethical Statement

This study was conducted in accordance with the ethical standards of the European and German Animal Welfare legislation, declaration principles set out by Helsinki and the National Institutes of Health guidelines for care and use of animals in research. All protocols were approved by the local ethics committee of the University of Port Harcourt, Nigeria (regulation CEE 86/609).

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