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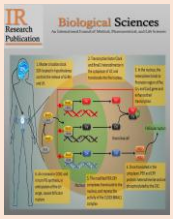

Curcumin Attenuates Cytotoxic Effect of Bisphenol A in the Liver of Adult Wister Rats

Sunday Okon Elijah, Moses Ibrahim Auza, Abigail Abraham, Charles O. Ayara

Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine and Allied health Sciences, Bingham University, Karu, Nasarawa State

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ABSTRACT

This study investigates the effect of curcumin on bisphenol A induced cytotoxicity in the liver of adult Wister rats. Twenty adult Wistar rats (weight = 190±10g) were placed into four groups (N=4). Group 1 (control) received vital feed, water and sunflower oil only throughout the research, group 2 (BPA) received vital feed, water and 65mg/kg of bisphenol A, group 3 (BPA and Curcumin) received vital feed, water and 65ml/kg of bisphenol A and 100mg/kg of Curcumin, and group 4 (Curcumin) received vital feed, water and 100mg/kg of curcumin for a total 28 days. Rats exposed to BPA for 28 days demonstrated liver damages as evidence by increased concentration of Alkaline phosphatase (ALP) in blood, increase in Malondialdehyde (MDA) concentration and decrease in Glutathione (GSH) level. In regard to histological analysis, curcumin had positive effects on the liver except for group 2 (BPA) which showed degenerative changes due to the administration of bisphenol A only. This study reveals that curcumin has ameliorative and cytoprotective effect on bisphenol A cytotoxocity on the liver.

Introduction

Bisphenol A [BPA, 2,2-bis (four-hydroxyphenyl) propane], is a popular artificial organic molecule utilised in polycarbonate plastics and epoxy resins as an intermediate combination product. It is one of the largest manufacturing chemicals within the global synthetics industry in volume [1]. BPA is used for manufacturing epoxy resins, which are found in plastic food containers, piping, wire insulations, and healthcare consumables. Due to the increased popularity of the production and usage of this product, BPA has been released into our ecosystem and food chain in high quantity. BPA contamination is primarily from the leaching out of plastic products. It is also an additive in the manufacture of polyvinyl chloride plastics, which have wide applications in healthcare consumables, piping, wire insulation and construction material [2]. The sources of BPA ingestion may vary according to environmental, social, and age factors, including baby and beverage bottles, as well as the repeated use of containers, food cans, and even medical equipment such as polycarbonate hemodialysis equipment [3]. Ingested BPA is eliminated by the liver, hence, can induced hepatoxicity and injury via various mechanisms [4] since it is the primary organ engaged in the detoxification of a variety of medications and xenobiotics. Preclinical studies revealed that administration of BPA in animals was correlated with the alteration of blood lipid profile and interfered with the oxidant/antioxidant mechanism in the liver which eventually lead to liver

*Address for correspondence
Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine and Allied health Sciences, Bingham University, Karu, Nasarawa State.
Email: auza.moses@binghamuni.edu.ng
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damage [5]. vom Saal et al. observed that the majority of studies they reviewed were showing effects due to BPA at concentrations significantly below the stated safety threshold and that there was a discernible effect of funding source on the results of these studies. More than 90% of government-funded studies were showing BPA to have effects at a low dose, while industry-funded studies were showing no effect [6].

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), also called diferuloylmethane, is the main natural polyphenol found in the rhizome of Curcuma longa (turmeric) and in others Curcuma spp. [7]. It has been shown to target multiple signaling molecules while also demonstrating activity at the cellular level, which has helped to support its multiple health benefits [8]. It has also been reported to benefit inflammatory conditions [9], metabolic syndrome, pain [10], and help in the management of inflammatory and degenerative eye conditions [11]. While there appear to be countless therapeutic benefits to curcumin supplementation, most of these benefits are due to its antioxidant and anti-inflammatory effects [12]. Despite its reported benefits via inflammatory and antioxidant mechanisms, one of the major problems with ingesting curcumin by itself is its poor bioavailability [13], which appears to be primarily due to poor absorption, rapid metabolism, and rapid elimination.

Oxidative stress has been implicated in many chronic diseases, and its pathological processes are closely related to those of inflammation, in that one can be easily induced by another. In fact, it is known that inflammatory cells liberate a number of reactive species at the site of inflammation leading to oxidative stress, which demonstrates the relationship between oxidative stress and inflammation (Biswas, 2016). Tumor necrosis factor α (TNF-α) is a major mediator of inflammation in most diseases, and this effect is regulated by the activation of a transcription factor, nuclear factor (NF)-κB. Whereas TNF-α is said to be the most potent NF-κB activator, the expression of TNF-α is also regulated by NF-κB. In addition to TNF-α, NF-κB is also activated by most inflammatory cytokines; gram-negative bacteria; various disease-causing viruses; environmental pollutants; chemical, physical, mechanical, and psychological stress; high glucose; fatty acids; ultraviolet radiation; cigarette smoke; and other disease-causing factors. Therefore, agents that downregulate NF-κB and NF-κB–regulated gene products have potential efficacy against several of these diseases [14]. Curcumin has been shown to block NF-κB activation increased by several different inflammatory stimuli. Curcumin has also been shown to suppress inflammation through many different mechanisms, thereby supporting its mechanism of action as a potential anti-inflammatory agent [15]. Curcumin is purported to exert its anti-inflammatory effects by inhibiting cyclooxygenase-2 (COX-2), lipoxygenase (LOX), and inducible nitric oxide synthase (iNOS), which are significant enzymes involved in inflammatory pathways. Dysregulated upregulation of COX-2 or iNOS, or both, has been implicated in the development of specific human cancers and inflammatory conditions. Given the intimate connection between inflammation and tumor progression, curcumin's strong anti-inflammatory properties are believed to contribute to its chemopreventive effects against carcinogenesis [16].

Bisphenol A (BPA), an environmental chemical, has been widely used in the manufacture of polycarbonate plastics and epoxy resins for many years and have been used for various ways in the packaging of food or beverage containers and the coating of food cans, people of different ages are inevitably exposed on a daily basis. BPA is said to mimic estrogen compound, resulting in an array of health complications including prostate and breast cancer [17,18]. The adverse effects of BPA are largely related to its estrogenic activity [19,20]. However, BPA has other effects such as inflammatory cytokine dysregulation [21,22], and mitochondrial mediated apoptosis in the hepatic tissue [23]. Thus, this study investigates the effect of curcumin on bisphenol A induced hepatotoxicity on the liver of adalt wister rats.

Materials and Methods

Ethical approval for Study

All protocols on animal handling strictly followed the guidelines of the Institutional Animal Care and Use Committee (IACUC) as approved by the BHU Ethics Review Committee, Bingham University, Karu, Nasarawa State, Nigeria. efficacy as a therapeutic agent for the management of diabetes mellitus.

Study Material Procurements

Animals

Adult Wistar rats (weight = 190±10g) were acquired from the animal house, Bingham University animal holdings, Karu, Nasarawa State. Wistar rats were kept in standard polypropylene cages, allowed to acclimatize to their new environment for 2 weeks, under standard laboratory conditions at Bingham University animal holdings facility where they had liberal access to rat chow and water ad libitum.

Bisphenol A and Curcumin procurement

Bisphenol A (400mg) manufactured by Laboratory Reagents and Fine Chemicals, with batch number L387192111, Curcumin (5mg) manufactured by Molychem, with batch number MCR-12517-03 and Sunflower oil manufactured in Casa De Campo were purchased from life gates stores, New karu, Karu LGA of Nasarawa State, Nigeria, in May 2023.

Animal grouping and treatments

Twenty adult Wistar rats (weight = 190±10g) were placed into four groups (N=5). Group-I received 1ml of sunflower oil and Group-II received a single dose of Bisphenol A (65mg/kg) daily treatment for 28 days. Group-III received 65mg/kg body weight of Bisphenol A in the morning and 100mg/kg body weight Curcumin in the evening for 28 days, Group-IV received 100mg/kg body weight of curcumin daily for 28 days. All administration was done orally and thereafter, histomorphology and biochemical assessments were carried out.

Table 1. Experimental design

Group	Treatment	Feeding
Group I (control)	Food + distilled water + sunflower oil only	28 days
Group II (bisphenol A)	Distilled water + food + Bisphenol A dissolved in sunflower oil at a dose of 65mg/kg body weight orally	28 days
Group III (Bisphenol A+ Curcumin)	Distilled water + food + Bisphenol A dissolved in sunflower oil at a dose of 65mg/kg body weight and Curcumin dissolved in sunflower oil at a dose of 100mg/kg body weight orally	28 days
Group IV (Curcumin)	Distilled water + food + Curcumin dissolved in sunflower oil at a dose of 100mg/kg body weight orally	28 days

Body Weight assessment

The animals were weighed before the experiment began and after the experiment was concluded using a weighing scale (Atom electronic compact scale), to determine if the treatment affected the body weight of the adult Wistar rats.

Animal Sacrifice and Tissue Processing

After weighing the animals on the final day of experimentation, the animals for histology were euthanized using chloroform vapor and then subjected to trans-cardial perfusion in which a flush of 50 ml of 0.1 M PBS (pH 7.4) was followed by 50 ml of 10% buffered formalin. Rats anterior abdominal wall was incised using surgical blade, scissor and scalpel. The liver was dissected out with an incision made from the pylorus to the duodenojejunal flexure. The liver of two animals per group was then rinsed in 0.1 M PBS (pH 7.4) three times, for 5 mins each, and then post-fixed in 10% buffered formalin solution for 24 hours after which they were taken for histological (H&E) tissue processing. Three animals per group, processed for biochemical study were not subjected to trans-cardial perfusion. The liver was excised, rinsed in 0.1 M PBS (pH 7.4) for 5 mins each, and then placed in PBS in which they were stored at 4°C. after which sections of the liver were homogenized for biochemical assay.

Data analysis

Results obtained were analysed using GraphPad Prism® software (Version 8.1) and tested for analysis of variance (ANOVA) with Tukey's multiple comparisons test. Significance was set at 95% confidence interval ($p<0.05$).

Results

Body weights of animals treated with both bisphenol A and curcumin (group III) and curcumin only (group IV) gained less weight compared to the other groups. There was a relative increase in body weight of the control group (group I), while the group treated with bisphenol A only (group II) showed high increase in body weight.

Discussion

The group administered bisphenol A showed significant increase in the alkaline phosphotase concentration, while the group administered curcumin had the lowest concentration in alkaline phosphatase. The group administered both bisphenol A and curcumin showed ALP levels which were lower compared to group 2 but higher compared to the control and curcumin group. This is in accordance with the findings of Elbakry et al., [24] which stated that intoxication with BPA induces liver damage which can be evident through impaired liver functions accompanied by excessive leakage of ALP. Alkaline phosphatase is a membrane bound glycoprotein that catalyses the hydrolisis of phosphate monoesters and helps breakdown protein in the body with the liver as its main source [25]. This result is also in line with the study Abarikwu et al., [26] which states that

curcumin decreases the level of ALP to support the hepatoprotective effects of curcumin because of the increased activity of marker enzymes such as glutathione (GSH).

This study examines the effect BPA on the liver. Additionally, it aimed to assess whether curcumin could effectively protect against any negative effects caused by BPA on the liver tissues. This research showed that the liver of the animals treated with bisphenol A only, showed degenerative changes later cause DNA alteration in cell [30]. The group treated with curcumin had the highest level of glutathione and lowest level of malondialdehyde, while group 2 had the highest malondialdehyde concentration and the lowest glutathione concentration. The group treated with bisphenol A and curcumin showed improvement in the malondialdehyde level and glutathione level. This is in line with the study by Yuanyuan et al, [27] that is an antioxidant and has capacity for free radical scavenging. This also agrees with the study by Meli et al., [31] that bisphenol A contributes to organ

Table 2. Descriptive statistics of the body weight of the animals.

VARIABLES (N=5)	MEAN±S.D	STD. ERROR
GRP I-CI	161.50±13.50	6.04
GRP I-CF	170.54±10.22	4.57
GRP II-BPAI	203.12±19.20	8.59
GRP II-BPAF	250.26±35.45	15.86
GRP III-BPA&CURI	194.58±4.70	2.10
GRP III-BPA&CURF	195.40±6.24	2.79
GRP IV-CURI	180.50±14.70	6.57
GRP IV-CURF	184.96±14.60	6.53

CI: Initial weight of control group; CF: Final weight of control group; BPAI: Initial weight of bisphenol A group; BPAF: Final weight of bisphenol A group; BPA &CURI: Initial weight of bisphenol A and curcumin group; BPA&CURF: Final weight of bisphenol A and curcumin group; CURI: Initial weight of curcumin group; CURF: Final weight of curcumin group; STD: Standard error. Body weight of Wistar rats in grams expressed as mean ± standard deviation.

Table 3. Descriptive statistics of weight of the liver

VARIABLES (N=5)	MEAN±S.D	STD. ERROR
GRP1-CONTROL	2.24+0.23	0.10
GRP2-BPA	2.80+0.20	0.09
GRP3-BPA&CURCUMIN	2.44+0.24	0.12
GRP4-CURCUMIN	2.32+0.24	0.16

The liver weight in grams obtained are expressed in mean ± standard deviation. STD: Standard error.

(vacuolation) and wasn't as intact compared to the control and curcumin group. BPA is capable of acting directly on the liver causing mitochondrial mediated apoptosis in the hepatic tissue as reported by Xia et al, [23]. Curcumin has hepatoprotective effects on CCL4 induced liver damage and is associated with its capacity for free radial scavenging and anti-oxidation as reported by Yuanyuan et al, [27] agreeing with this research.

The group administered bisphenol A (group 2) and both bisphenol A and curcumin showed presence of amyloidosis which can lead to organ dysfunction and cell death [28], though the protein aggregates in group 2 were larger compared to group three. The control group and the group administered curcumin showed no presence of amyloid build up. Therefore, curcumin prevents the buildup of amyloid protein agreeing with the study by Dai et al., [29].

Malondialdehyde is one of the free radicals which is formed from the reaction of free radical and lipid and may alter the structure of cell membrane and toxicity and alters oxidative balance by increasing oxidative stress related markers and decreases capacity of antioxidant defense.

In general, biochemical analysis indicated highest amount of glutathione concentration in the curcumin group compared to the control and higher in the BPA + Curcumin group than the BPA group. This shows that curcumin alleviate the rate of liver damage, helps in liver detoxification and boost the immune system. Lipid peroxidase level is highest in the BPA group and lowest in the curcumin group. It was also observed that the lipid peroxidase level was lower in the BPA+curcumin group than in the BPA only group indicating a high lipid accumulation in the liver of the BPA group animals. The same was recorded for the alkaline phosphatase analysis.

Conclusion

Curcumin has ameliorative effect on the BPA induced cytotoxicity in the liver of adult Wistar rats. Bisphenol A causes injury and oxidative damage to

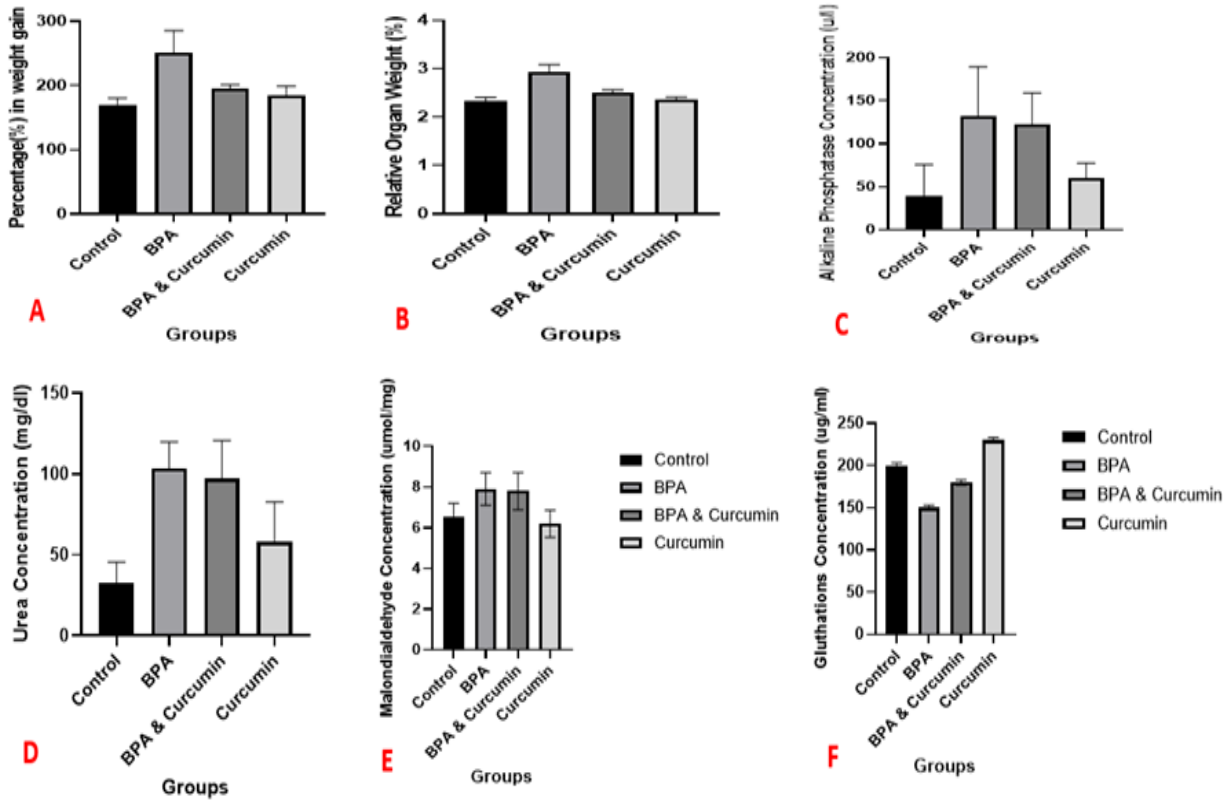

Figure 1. Diagrammatic representation of information concerning weight changes (A & B), blood parameters (C & D) and biochemical parameters (E & F). A: Effect on weight gain percentage, B: Effect on relative organ weight, C: Effect of alkaline phosphatase in blood serum, D: Effect of urea concentrations, E: Effect on malondialdehyde concentration, and F: Effect on glutathione level.

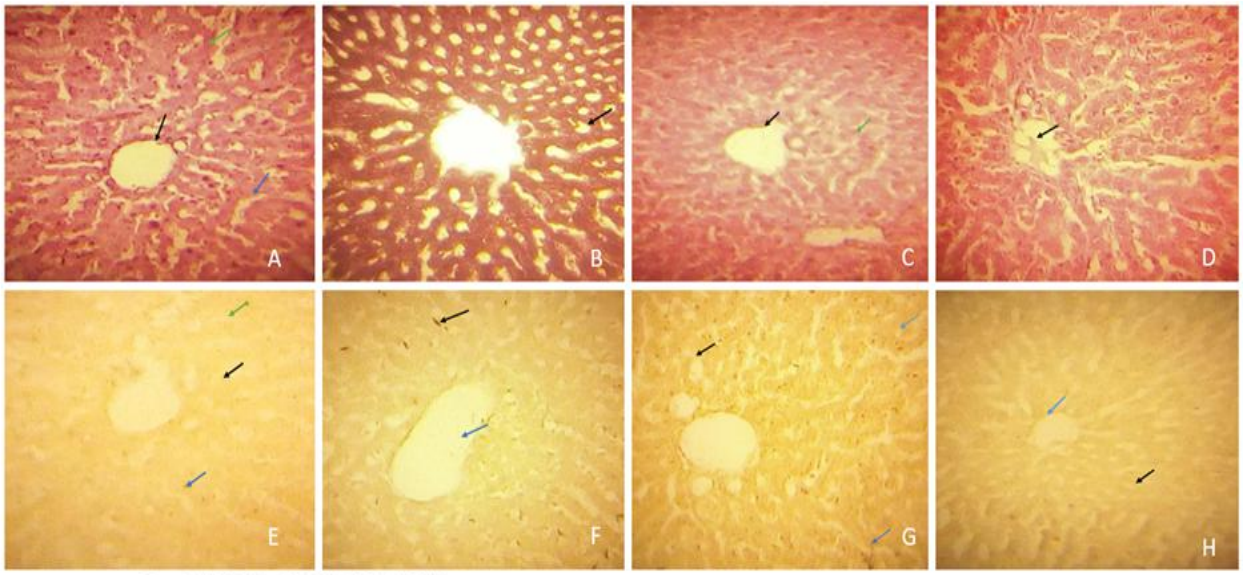

Figure 2. Histology and special stain analysis. A: Liver section of normal control animals showed typical central vein (black arrow), sinusoid (blue arrow), and radiating chords of hepatocytes (green arrow) (H&E X400). B: Liver section of animals administered with BPA showed degenerative changes (vacuolation) (black arrow) (H&E X400). C: Liver section of animals administered with BPA and curcumin showed radiating chords of hepatocytes (green arrow) and central vein (black arrow) (H&E X400). D: Liver section of animals administered with curcumin showed normal radiating chords of hepatocytes draining into central vein (black arrow) (H&E X400). E: Liver section of normal control animals showed typical central vein (black arrow), sinusoid (blue arrow), and radiating chords of hepatocytes (green). No degenerative changes (Congo red X400). F: Liver section of animals administered with BPA showed radiating chords of hepatocytes and central vein (blue arrow) (Congo red X400). G: Liver section of animals administered with BPA and curcumin showed vacuolization (black arrow), mild amyloid plaque (blue arrow) (congo red X400). H: Liver section of animals administered with curcumin showed radiating chords of hepatocytes (black) and central vein (blue arrow). No amyloid plaque (congo red X400).

curcumin by its anti-oxidative capabilities.

Contribution of authors

SOE., conceptualized and outlined the idea of the study, data collection, manuscript proof reading and supervision, MIA carried out the animal experimentation, streamlined research design and analyzed the data, Abigail Abraham drafted the manuscript and COA drafted proof read manuscript. All authors read and approved the final manuscript.

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Conflict of Interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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