



Original Research Article

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GALLIC ACID AMELIORATED BPA INDUCED HEMOLYSIS IN HUMAN RED BLOOD CELLS: AN IN VITRO STUDY

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ABSTRACT: Bisphenol A (BPA) is a well known artificial environmental endocrine disrupting (ED) chemical that also provokes many deleterious impacts on human health. Accumulating evidence indicates that exposure to BPA may contribute to blood damage through production of reactive oxygen species. However, the information on impact of BPA on human blood is very scattered and incomplete. Up to now, very few studies have been realized to assess the impact of BPA on human Red Blood Corpuscular (RBC) at environmental exposure level and no study has been conducted to check attenuated impact of the most commonly available antioxidant like Gallic Acid (GA). Therefore, present investigation is an attempt to study hemolysis due to BPA on human red blood cells and its amelioration with GA in dose dependent manner.

KEYWORDS: BPA, ROS, RBC, GA, Hemolysis, Amelioration.

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1. INTRODUCTION

Bisphenol A (BPA) is the most discussed xenobiotics and endocrine disruptors due to its mimicking nature of estrogen and estradiol 17β . However, even after knowing its adverse effect production of BPA is still in demand throughout the world due to its ubiquitous nature, everyday use products like water bottles, baby bottles, food packaging containers, dental sealants etc. [1] [2] [3] [4] [5]. Extensive evidence indicates that BPA ultimately get exposed to humans through atmosphere, hydrosphere and lithosphere [6] [7] [8] and found in maternal blood, amniotic fluid [8] breast milk, follicular fluid, placental tissue [9] umbilical cord blood [10] [11] serum, saliva,

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urine [12] and breast milk [13]. It is also linked with reproductive health [14] decreased sperm count; endometriosis [15], metabolic disorders, diabetes and cancers [16]. However, all these deleterious effects are mainly due to generation of Reactive Oxygen Species (ROS) due to BPA exposure. ROS also produces during normal metabolic process of cells as well as exposure to environmental factors like UV, environmental pollutants, xenobiotics etc. ROS includes different types of chemically reactive form of oxygen and this free radical oxygen in turn damages cell membrane, proteins, lipids and DNA. It is believed that ROS is hallmark of all disease including cancer [16]. General population exposure is highest by eating food or drinking beverages that contains trace amount of BPA. Not all but mostly plastic coded with recycle codes 3 or 7 may be made with BPA [17]. Literature revealed that BPA is rapidly and efficiently absorbed in the gastrointestinal tract, following oral administration, initially being metabolized by the gut wall and liver, where it's main metabolite to quinone, semiquinone [18] and BPA-glucuronide [19]. Before, BPA eliminate from the blood via the kidneys and it remains in blood for minimum 5 h as the half-life of it is less than 6 h. [20]. However to protect against xenobiotics our body has its defense mechanism and also to compensate the damage in form of antioxidant supplements like Gallic acid which is mainly found from blueberries, walnuts, apples, tea, gall nuts, hazel, and other plants and herbs. GA is a phenolic plant secondary metabolite which has beneficial effects to human health, including anti-inflammatory, antimicrobial, antiallergic, antitumor activity [21]. It has gained lot of recognition due to its effective anti-oxidant activity and its protective mechanism to combat against liver fibrosis [22]. Human RBC is the most significant medium to carry out almost all necessary metabolic activity to keep human alive including transportation of oxygen, energy molecules, immunological response and hormonal balance. Therefore, Erythrocyte is used as a model to study cell membrane damage and oxidative stress [23] [24] as it has no nucleus as well as mitigation/ repair mechanism by antioxidant like Gallic acid. However, information on Gallic acid amelioration on BPA induced hemolysis is not available. Therefore, the present study is an attempt to assess the impact of BPA on human RBC and its attenuation by GA.

2. MATERIALS AND METHODS

2.1 Protocol Approval

Present study was approved by Bioethics committee of Gujarat Adani Institute of Medical Sciences (GAIMS), Bhuj, Kachchh with approval No. KSKV/DEES/MHT/SERB-LS-425/128/036 dated 26/11/15 for the purpose to undertake experiments on human blood and responsibility to dispose Bio-Medical waste as per the Gujarat Pollution Control Board regulation 2016.

2.2 Chemicals

Bisphenol A (99%, 2,2-bis(4-hydroxyphenyl)propane) (BPA), Gallic acid (3,4,5-trihydroxybenzoic acid), Phosphate buffer saline (PBS) were procured from Hi-media, Bombay.

2.3 Dose preparation

BPA stock solution (500 ppm) was prepared by dissolving in water with constant stirring and heating at 60-70°C condition for at least 24 hours. Final concentration of ethanol in stock solution was 0.2% which is not toxic to the cells [25] [26]. For working solution it was further diluted with PBS to obtain a final concentration ranges from (10-200 µg/ml) of BPA. The prepared stock and working solutions were kept in amber bottle at room temperature throughout the experiment as BPA is photo-sensitive compound. For Gallic acid the stock solution (200 ppm) was prepared in PBS and working solution was prepared ranges from (10-50 µg/ml) by further diluting it using PBS.

2.4 Blood sample collection

Blood samples were obtained with voluntary consent of well-nourished, healthy adult male student donors of KSKV Kachchh University, age 22-25 years residing in Bhuj, Kachchh. 5 ml venous blood sample was collected from 20 students with almost no addiction of packet food or exposure of plastic water bottles and pouches as they belong to far rural area of Kachchh. Blood was withdrawn by trained laboratory technician using disposable syringe in EDTA vials.

2.5 RBC suspension and *in vitro* hemolysis

According to method of [27] Blood samples were diluted with PBS and centrifuged at $1000 \times g$ for 10 min. The RBC pellets were washed twice and finally suspended in PBS to have a cell density of 2×10^4 cells/ml.

Following sets of tubes were prepared.

1. Control tubes: These tubes contained 2.0 ml of RBC suspension and 2.0 ml of PBS.
2. BPA treated tubes: BPA (10-200 ppm) were mixed with 2.0 ml of RBC suspension. The total volume was made up to 4.0 ml with PBS.
3. GA treated tubes with different doses of BPA: GA (50 ppm) alone and in combination with highest dose of BPA 200 ppm was mixed with 2.0 ml of RBC suspension. The total volume was made up to 4.0 ml with PBS.
4. 100% hemolysis/ Positive control: 2 ml of distilled water was added to 2 ml of RBC suspension. All tubes were incubated at 37°C for 4 h with intermittent shaking. Thereafter, tubes were centrifuged at $1000 \times g$ for 10 min and the absorbance of the supernatant was read spectrophotometrically at 540 nm. Percent hemolysis was calculated by using the formula:

$$\% \text{ Hemolysis} = \frac{\text{Abs of Individual tubes}}{\text{Abs with 100\% hemolysis}} \times 100$$

$$\% \text{ Reduction in Hemolysis} = \frac{A-B}{A} \times 100$$

A: BPA induced hemolysis

B: Hemolysis caused by addition of BPA and different dose of GA

Data analysis

Each set of experiment was repeated at least three times. The results were expressed as the mean \pm SEM. The data were statistically analyzed using Graph Pad Prism 6.0 software & statistical comparisons were performed using the paired Student's *t*-test values of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered significant.

3. RESULTS AND DISCUSSION

Table 1: Hemolysis due to BPA on RBC suspensions

Sr. No.	Concentration of BPA ($\mu\text{g/ml}$)	Hemolysis (%)
1	Control	5.90 ± 0.04
2	100 % Hemolysis	100 ± 0.36
3	10	$16.04 \pm 0.22^*$
4	40	$27.46 \pm 0.35^{**}$
5	80	$35.23 \pm 0.31^{**}$
6	120	$49.76 \pm 0.25^{**}$
7	160	$72.13 \pm 0.32^{**}$
8	200	$88.29 \pm 0.34^{**}$

**Significant at level of $p < 0.001$, *Significant at level of $p < 0.05$

Table 2: Percentage hemolysis induced by BPA and Percent reduction in hemolysis due to GA

Sr. No.	BPA ($\mu\text{g/ml}$)	GA ($\mu\text{g/ml}$)	% Hemolysis	% Reduction
1	0	0	5.90 ± 0.04	-
2	0	50	11.12 ± 0.05	-
3	200	0	88.29 ± 0.34	-
4	200	10	76.32 ± 0.31^{abgh}	13.54
5	200	20	67.54 ± 0.28^{abcegh}	23.49
6	200	30	61.10 ± 0.20^{abch}	30.78
7	200	40	48.34 ± 0.21^{abcde}	45.24
8	200	50	34.67 ± 0.29^{abcdef}	60.72

^aCompared with control; ^bCompared with GA control; ^cCompared with BPA 200; ^dCompared with BPA 200+GA 10; ^eCompared with BPA 200+GA 20; ^fCompared with BPA 200+GA 30; ^gCompared with BPA 200+GA 40; ^hCompared with BPA 200+GA 50.

In present *in vitro* experiment, low dose (10 ppm) of BPA show significant hemolysis as compared to control (16.04 ± 0.22) with $p < 0.05$. Impact of BPA is dose dependent as the concentration increases red blood cells damage increases gradually from 10-200 $\mu\text{g/ml}$ as shown in table 1. Maximum hemolysis (88.29 ± 0.34) was found at concentration of 200ppm BPA which is highly significant as compare to control with $p < 0.001$. It might be due to production of Reactive Oxygen

Species that damages cellular membrane and increases the membrane permeability of RBCs that eventually burst out and causes hemolysis. Addition of GA alone does not show any significant damage in RBC [28]. Concurrent addition of GA (10-50 ppm) with 200 ppm of BPA, shows significant reduction in hemolysis as the dose increases as shown in Table 2. 60.72% amelioration was achieved with 50 ppm of concurrent addition of GA with 200 ppm of BPA after 4h incubation as compare to 200 ppm BPA alone, the result is highly significant with $p < 0.001$. Literature review revealed that the ROS production creates oxidative stress in RBCs [16] [28] with increase level of lipid peroxidation, decreases level of GSH, decrease activity of SOD, CAT and GSH-Px which results into increase in hemolysis [28]. Ameliorative effect of GA was mainly due to its electron donating ability and thus it satisfy the electron scavenging need of oxidative stress molecules produced due to BPA toxicity [29]. The literature clearly revealed that BPA exposure in any mode i.e. via ingestion of food, inhalation of air or direct skin contact it might disrupts RBCs as shown in present study. High concentration of BPA could also overcome the defense mechanism of RBC by reducing activity of antioxidative stress related enzymes viz. SOD, CAT and GSH-Px. In accordance to earlier finding our study also revealed that incubation of BPA alone with RBC caused dose dependent hemolysis. However, concurrent addition of GA showed amelioration. The protective mechanism of GA was mainly due to its antioxidant property.

4. CONCLUSION

Incubation of BPA (10 – 200 ppm) with RBC suspension for 4h at 37 °C caused dose dependent significant increase in hemolysis as compare to control. However, concurrent addition of GA (10 – 50 ppm) with 200 ppm in RBC suspension for 4h at 37 °C showed significant ameliorative effect.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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