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THE AMELIORATIVE EFFECT OF BLACK TEA EXTRACT AND QUERCETIN ON BISPHENOL A – INDUCED CYTOTOXICITY

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Abstract: The purpose of our study was, to explore the possible ameliorating effects of black tea extract and quercetin, against bisphenol A-induced cytotoxicity. For this, human red blood corpuscles (RBC) were taken as the model. Blood samples collected in EDTA vials from healthy adults were used for preparation of RBC suspension. This suspension was treated with bisphenol A (0-150 μg/mL) with and without black tea extract or quercetin (0-200 μg/mL). The results showed that addition of bisphenol A causes concentration-dependent increase in rate of hemolysis. Addition of black tea extract or quercetin alone to RBC suspension did not cause any significant reduction. However, concurrent addition of bisphenol A (0-150 μg/mL) and black tea extract or quercetin caused concentration-dependent amelioration in bisphenol A-induced cytotoxicity.

Keywords: Black tea extract; cytotoxicity; hemolysis; bisphenol A; quercetin

Bisphenol A (4, 4'-isopropylidene-2-diphenol, BPA) is a small estrogenic monomer used in the production of polycarbonate and epoxy resins (1, 2). This compound is found in diverse range of plastic products such as baby bottles, microwave ovens, and canteen as well as, the packaging materials of many foods and beverages. Polycarbonate is routinely subjected to heat treatment and bisphenol A has been detected in the thermal degradation products formed during this thermal treatment. It has also been well documented that polymerization reaction may not be fully completed and a significant proportion of unreacted reactants existed in plastic materials (3, 4). The European Union (5) noted that the highest potential for human exposure to bisphenol A is through products that directly contact food.

Studies (6-8) have revealed that concentration of bisphenol A found in human blood samples is above daily tolerable intake (0.01 mg/kg body mass per day) thus, it may produce adverse effects. However, effect of bisphenol A on RBC is not clear.

In a rapidly developing country like India, tea is one of the most common and widely consumed beverages. It contains large number of antioxidant polyphenols; typically 93% of total tea phenolic compounds are flavonoids. Quercetin is one of the most active compound which gives major contribution for the antioxidant property of black tea (9).

The present investigation is an attempt to evaluate the effect of bisphenol A on RBC *in vitro* condition.The ameliorative effects of black tea extract and quercetin on bisphenol A-induced cytotoxicity on (RBC) were also studied (10).

EXPERIMENTAL

Bisphenol A and quercetin were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai, India.

Extract preparation

Black tea extract was prepared as per WHO protocol CG-06 (11). 15 g of the powdered tea (Lipton yellow label) was suspended in 100 mL of deionized water and heated up to 90^{o}C for 30 min. After cooling, the mixture was filtered twice through Whatman No. 1 filter paper. The filtrate was collected and evaporated to dryness in a hot air oven at 90^{o}C. Dried extract was used in the experiment.

Preparation of RBC suspension

Intravenous blood samples from healthy adult volunteers (25-30 years) collected in EDTA vials were diluted with normal saline (0.9% NaCl) and centrifuged at 1000 g for 10 min. This procedure was repeated twice and finally RBC pellet was collected and diluted with saline to have a cell density of 2×10^{4} cells/mL (12).

The experiment was conducted in two phases. Phase I – To study the effect of bisphenol A-induced hemolytic effect the following sets of tubes were prepared:

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(a) Control tubes containing 2 mL of RBC suspension. (b) Bisphenol A treated tubes containing 2 mL of RBC suspension and 0 -150 µg/mL of bisphenol A.

Total volumes of tubes were made up to 4 mL with additional saline.

Phase II – To assess the efficacy of black tea extract and quercetin in amelioration of bisphenol A-induced cytotoxicity (hemolysis), the following sets of tubes were prepared: (a) Control tubes containing 2 mL of RBC suspension. (b) Bisphenol A treated tubes containing 2 mL of RBC suspension and 150 µg/mL of bisphenol A. (c) Antidote control containing 2 mL of RBC suspension and 200 µg/mL of black tea extract or quercetin. (d) Bisphenol A and antidote treated tubes containing 150 µg/mL of bisphenol A and 0-200 µg/mL of black tea extract or quercetin. The volume of each tube was made up to 4 mL with additional saline in order to have the required concentration of bisphenol A and black tea extract or quercetin which were also prepared in normal saline.

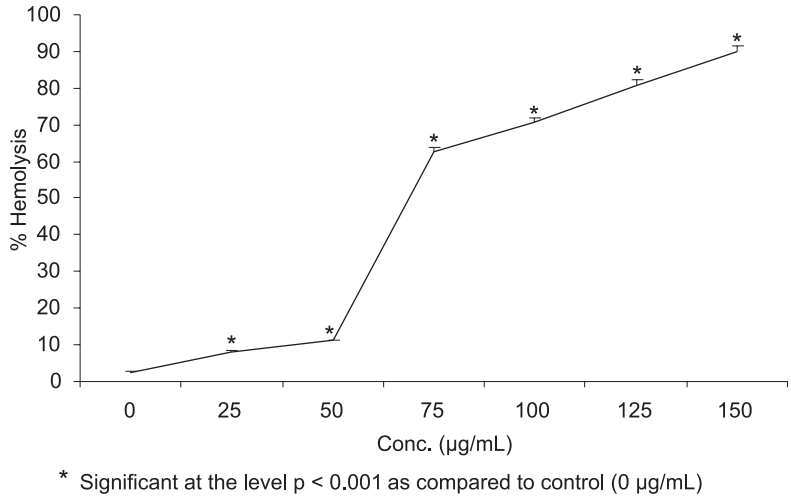

Figure 1. Bisphenol A-induced hemolysis in human RBC –*in vitro*

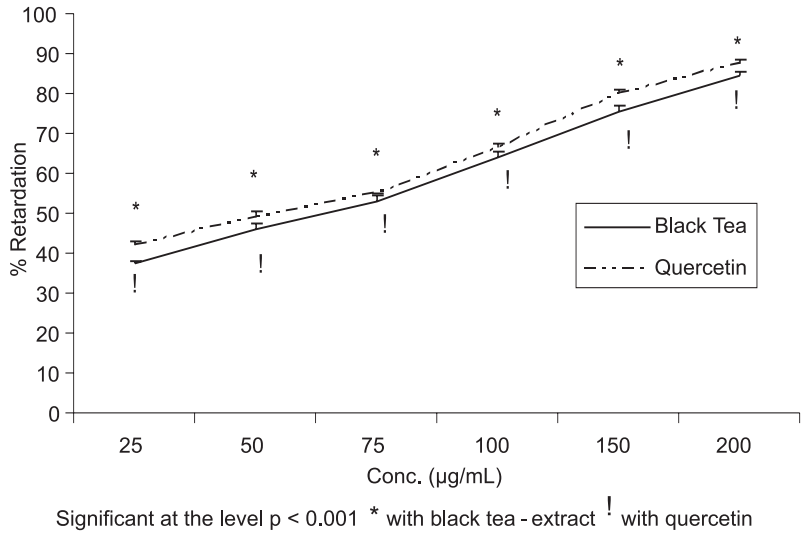

Figure 2. Retardation in Bisphenol A-induced hemolysis by black tea and quercetin

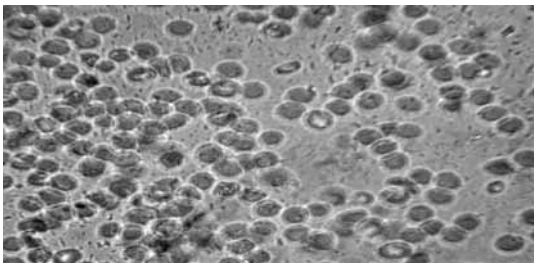

Plate 1. Normal RBC.

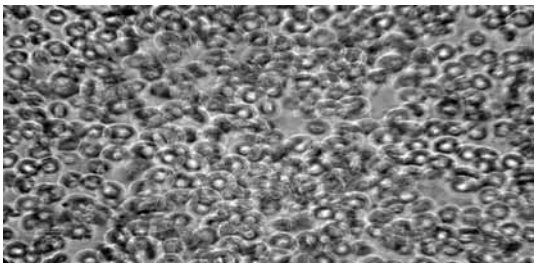

Plate 3. Antidote treated RBC's showing normal shape and size.

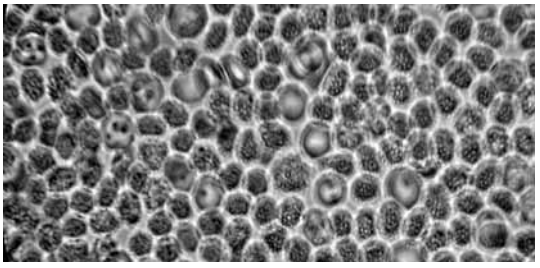

Plate 2. Bisphenol A treated RBC's.
Note: Swollen RBC's as well as blebbing of plasma membrane.

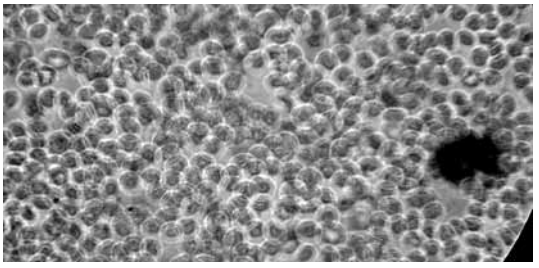

Plate 4. Antidote and bisphenol A treated RBC's showing normal shape and size.

The tubes were mixed gently and incubated at 37°C for 4 h with intermittent shaking. Thereafter, the tubes were centrifuged at 1000 g for 10 min and color density of supernatant was measured spectrophotometrically at 540 nm.

To achieve 100% hemolysis, 2 mL of distilled water was added to 2 mL of RBC suspension.

CALCULATIONS

Percent hemolysis was calculated by the formula:

$$\% \text{ hemolysis} = \frac{\text{Absorbance of individual tubes}}{\text{Absorbance with 100\% hemolysis}} \times 100$$

Percent retardation by different concentration of antioxidants was calculated as:

$$\% \text{ retardation} = \frac{A-B}{A} \times 100$$

where, A= bisphenol A induced hemolysis, B = hemolysis caused by concurrent addition of bisphenol A and antioxidants

DATA ANALYSIS

Data were analyzed statistically using Student's t-test.

RESULTS

In control tubes, RBC remained settled at the bottom of the tubes with almost clear ambient solution. With the addition of 50 µg/mL of bisphenol A there was appearance of tinge red color in the medium; most of the cells remained settled in the bottom of the tube. Morphological observations revealed concentration-dependent swelling of RBC (Fig. 1). Further increase in concentration of bisphenol A caused a concentration-dependent increase in the rate of hemolysis. A maximum of hemolysis occurred on addition of 150 µg/mL of bisphenol A in incubation medium (Plate 2)

Addition of 200 µg/mL of black tea extract or quercetin to RBC suspension did not cause any significant increase in the rate of hemolysis. However, concurrent addition of bisphenol A and various concentration of black tea extract or quercetin in RBC suspension significantly reduced bisphenol A-induced hemolysis (Plate 4). Maximal retardation in hemolysis was observed with 200 µg/mL concentration of black tea extract or quercetin (Fig. 2 Plate 3).The results also revealed that quercetin is comparatively more protective than black tea extract.

DISCUSSION

The result shown in Fig. 1 clearly indicates that lower concentration of bisphenol A causes swelling of cells. It might be due to destabilization of RBC membrane leading to influx of water into the cells. Further increase in concentration causes increasing breakdown of cells resulting in a sigmoidal curve. The exact mechanism of action of bisphenol A on RBC is not clear. Moreover, involvement of lipid peroxidation and oxidative damage induced by bisphenol A cannot be neglected.

Hiroshi et al. (14) revealed that exposure to bisphenol A brought about morphologic changes in the cells, such as membrane blebs, cell rounding, cytoskeletal collapse, and chromatin condensation or fragmentation of cultured Sertoli cells.

The data shown in Fig. 2 clearly indicate that addition of black tea extract or quercetin in incubation medium containing 150 µg/mL of bisphenol A significantly reduced the rate of hemolysis. The effect was concentration-dependent. Ameliorative effect of black tea extract on RBC suspension is mainly due to the presence of polyphenols in black tea extract. Various amounts of polyphenols like quercetin, myricetin, kaempferol, catechin gallate and gallocatechin gallate are found exclusively in tea (15). Polyphenols are well known for their ability to reduce membrane lipid peroxidation and to increase the production of malondialdehyde, which can prevent oxidative damage caused by bisphenol A (16).

The results revealed that quercetin is comparatively more potent ameliorating agent than that of black tea extract as it exhibits the highest antiradical property toward hydroxyl radical, peroxyl, and superoxide anion compared with other flavonoids (19, 20). Quercetin is rapidly and avidly taken up by human RBC via a passive diffusion mechanism, driven by flavonoid binding to hemoglobin and resulting in almost quantitative accumulation of the flavonoid (17). Also, it may be useful in diminishing oxidative damage to RBC (18).

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