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Bisphenol A-A Major Concern For Ovarian Function: Protective Role Of Black Tea Extract

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Abstract

Bisphenol A, an endocrine disruptor and also an environmental toxicant which is correlated with adverse estrogenic effects in both humans and other environmental species. This study depicts the investigation of the disintegration in ovarian function caused by BPA and the ameliorative effects of black tea extract (BTE). Eight-week-old female Wistar rats were treated with BPA (10µg and 100 µg/kg body weight/day) for 14 days by oral gavage, and then supplemented with BTE (1ml/100gm body weight/day) for 14 days orally. The oxidative and antioxidative stress parameters were measured in the ovaries. In this study, elevated ovarian level of nitric oxide and were observed in malondialdehyde BPA exposed animals as compared to the control animals. Furthermore, BPA exposure resulted in marked reduction in the activities of antioxidant enzymes, superoxide dismutase and catalase. All of these BPA induced effects were overturned to good dimension by the oral supplementation of black tea extract.

Keywords: Bisphenol A, black tea extract, stress, superoxide dismutase, catalase

Introduction

The primary goal of biomedical research is to identify the impacts of specific environmental exposures on human health. Among various environmental pollutants, Bisphenol A (BPA) stands out as an endocrine-disrupting chemical widely present in numerous consumer products. BPA has detrimental effects on both wildlife and humans (Fenichel *et al.*, 2013). With an annual production exceeding 8 billion pounds, BPA ranks as one of the most prevalent industrial chemicals globally (Rubin, 2011). Products incorporating BPA-containing plastics, such as food and beverage containers, toys, eyeglasses, computers, kitchen appliances, and medical equipment, contribute to its widespread usage. BPA serves extensively as a synthetic plasticizer in the production of polycarbonates, polystyrene, and certain epoxy resins (Rubin, 2011). Numerous studies have revealed that both human and wildlife populations are exposed to levels of BPA that can have detrimental effects on reproductive, developmental, and metabolic functions in various wildlife species and

laboratory animal experiments. Recognized as a typical endocrine-disrupting chemical, BPA exhibits hormone-like activities, leading to significant health concerns. It has been linked to conditions such as testicular cancer, endometriosis, precocious puberty, and breast cancer (Takeuchi *et al.*, 2004). Beyond reproductive issues, BPA is implicated in various diseases, including cardiovascular diseases, respiratory diseases, diabetes, kidney diseases, obesity, and reproductive disorders. Recent epidemiological studies have demonstrated an association between BPA exposure and a range of reproductive dysfunctions and endocrine abnormalities in women.

BPA exposure has been associated with inadequate development of the primordial germ cell population and a reduction in the primordial follicle pool in the fetal mouse ovary. This leads to a consequential decrease in the primordial follicle reserve and an increased incidence of premature ovarian insufficiency (POI) in adult female mice. POI, a prevalent heterogeneous endocrine disorder affecting 1% of women, is characterized by a loss of ovarian function before the age of 40 and is considered a significant cause of female infertility (Berger *et al.*, 2010). Numerous studies have reported that Bisphenol A could induce morphological and functional alterations in the female genital system, particularly in the ovaries. Exposure to BPA during the blastocyst implantation period can obstruct pregnancy. This study reviews various adverse effects of BPA and discusses a potential mechanism of its action (Frei and Higdon, 2003).

Black tea, the favored tea variety in the West, owes its popularity to its robust flavor and extended shelf life, containing 2% to 4% caffeine. Beyond being a non-sweetened, low-calorie beverage, it offers various health benefits attributed to potent polyphenolic compounds. These include epigallocatechin gallate, theaflavins, thrubigins, the amino acid L-theanine, and several other catechins or flavonoids with potential anti-inflammatory properties, reducing the risk of chronic conditions (Langley-Evans, 2000; Rao *et al.*, 2005). Flavonoids exhibit diverse biological activities, such as antibacterial, antifungal, antiviral, anticancer, and anti-inflammatory effects (Bahorun *et al.*, 2012). Black tea polyphenols have been found to possess anti-obesity effects, contributing to a reduced risk of major diseases like coronary heart disease, stroke, and cancer. Prolonged consumption of black tea has been associated with decreased serum cholesterol levels (Pan *et al.*, 2016; Fujita and Yamagami, 2008; Langley-Evans, 2000). Extensive research supports the notion that, next to water, black tea, derived from the *Camellia sinensis* plant, is one of the most consumed beverages. Regular consumption of black tea has been shown to enhance the body's antioxidant potential, thereby lowering the risk of chronic disorders and improving overall health. Studies indicate that drinking black tea 1–6 times per day significantly increases plasma antioxidant potential in humans while reducing oxidative biomolecular damage, including nucleic acids and lipids. Additionally, black tea polyphenols inhibit the activity of pro-oxidative enzymes like nitric oxide synthase and xanthine oxidase (Mukherjee *et al.*, 2006).

Materials and Methods

Preparation of aqueous Black Tea Extract (BTE)

A process was undertaken where 25 grams of black tea was introduced to 500 milliliters of boiling water and allowed to steep for 15 minutes. Subsequently, the infusion was cooled to room temperature and then filtered. In a second filtration step, the tea leaves underwent extraction with an additional 500 milliliters of boiling water. The resulting two filtrates were combined to yield a 2.5% tea water extract, which corresponds to 2.5 grams of tea leaf per 100 milliliters of water. The clear solution obtained closely resembles the tea brews commonly consumed by humans.

Experimental animals

This study utilized eight-week-old female adult albino rats (Wister strain) weighing between 130 to 150 grams. Prior to the initiation of the experiment, the rats underwent a seven-day acclimatization period in an experimental animal house. Throughout the study, the animals were housed in cages under standard laboratory conditions, maintaining a temperature of 25 ± 2°C, humidity at 55 ± 5%, and a 12-hour light/dark cycle schedule. They had free access to water supply and were provided with a standard diet consisting of 71% carbohydrate, 18% protein, 7% fat, and 4% salt mixture (Mukherjee *et al.*, 2006). The rats were allowed ad libitum access to food and water throughout the experimental period. To ensure hygiene, cages were regularly cleaned, and feces and spilled feed were removed daily. All experiments adhered to ethical guidelines established by the Institutional Animal Ethics Committee (IAEC) of Serampore College.

Experimental design

25 Healthy female adult albino rats were randomly selected and were divided into five groups (n = 5):

- **Group 1** – Control group (received normal diet)
- **Group 2** – Treated with BPA (10µg/kg body weight) for 14 days by oral gavage.
- **Group 3** –Treated with BPA (100µg/kg body weight) for 14 days by oral gavage.
- **Group 4** - Treated with BPA (10µg/kg body weight) and then supplemented with black tea extract (1ml/100gm body weight by oral gavage) for 14 days.
- **Group 5-** Treated with BPA (100µg/kg body weight) and then supplemented with black tea extract (1ml/100gm body weight by oral gavage) for 14 days.

Preparation of ovarian tissue extract

The experimental animals had their ovaries removed for the assessment of various oxidative stress markers. The preparation of ovarian tissue extracts was carried out using ice-cold Tris-HCl buffer (pH 7.4). To estimate the activity of superoxide dismutase (SOD) and catalase (CAT), the tissues were homogenized in ice-cold isotonic phosphate buffer saline (PBS) at two different pH levels (7.0 and 8.0) (Mukherjee *et al.,* 2006).

Estimation of nitric oxide production

Nitric oxide (NO) undergoes rapid decomposition in aerated solutions, resulting in the formation of stable nitrite/nitrate products. In this study, nitrite accumulation was determined using the Griess reaction (Raso *et al.,* 2001), serving as an indicator of NO production. A sodium nitrite standard curve was employed to calculate the quantity of nitrite present in the sample, measured in micromolar units. This approach provided an assessment of NO production in the study.

Estimation of lipid peroxidation

The activity of lipid peroxidase was evaluated by monitoring the generation of malondialdehyde (MDA), serving as an indicator of lipid peroxidation levels. The quantitative assessment of lipid peroxidation was conducted through the thiobarbituric acid (TBA) test (Wills, 1987). The amount of MDA formed was quantified using TBA and employed as an index for lipid peroxidation. The outcomes were presented as nanomoles of MDA per milligram of protein, utilizing the molar extinction coefficient (€ = 1.56 x 10^5 cm^2/mM).

Estimation of superoxide dismutase activity

The activity of superoxide dismutase (SOD) was determined using the nitroblue tetrazolium (NBT) method, which relies on the inhibition of NBT reduction by SOD (Sun *et al.,* 1988). The relative absorbance was subsequently converted into units of SOD activity per milligram of protein. One unit of SOD activity was defined as the amount of SOD that caused a 50% reduction in the background rate of NBT reduction.

Estimation of catalase activity

Catalase activity was assessed using the method outlined by Aebi (Aebi, 1984), which involves monitoring the decomposition of hydrogen peroxide (H_2O_2) at 240 nm. The change in absorbance per unit time was utilized as a measure of catalase (CAT) activity. The results were expressed as units (U) per milligram of protein.

Estimation of reduced glutathione concentration

The level of glutathione (GSH) was determined using 5,5'-dithiobis-2-nitrobenzoic acid. The absorbance of the reduced chromogen was monitored spectrophotometrically at 412 nm. GSH levels were calculated based on a standard curve and expressed as millimoles per milligram of protein (mM/mg protein) following the method described by Ellman (1959) (Ellman, 1959).

Estimation of protein

The quantification of protein in the ovarian tissue extract was carried out using the method described by Lowry et al. (1951) (Lowry *et al.,* 1951), with bovine serum albumin (BSA) serving as the standard.

Statistical analysis

The data were presented as mean ± standard error (SE). The Kruskal–Wallis nonparametric analysis of variance test was conducted to assess whether the scores of different groups exhibited significant differences.

For intergroup comparisons, the Mann–Whitney U multiple comparison test was employed to identify correlations between the study variables. Statistical analysis was performed using StatsDirect 2.7.2, and differences were deemed significant if $p < 0.05$.

Results

To investigate the role of oxidative stress damage in the mechanism behind the toxicological effects induced by BPA in mice ovarian tissue, various parameters, including nitric oxide production, lipid peroxidation, glutathione content, and the activity of catalase and superoxide dismutase, were analyzed in ovarian tissue homogenates.The results indicated a significant increase in MDA and NO levels, which are indicative of lipid peroxidation and an inflammatory response, in the BPA-treated groups. Supplementation of black tea extract in BPA treated groups caused a marked decrease in both MDA level and NO production. Furthermore, chronic BPA administration resulted in a significant decrease in the tissue glutathione (GSH) levels of the ovaries compared to the control group. Nevertheless, the decrease in tissue glutathione (GSH) levels induced by BPA in the ovarian tissue was reversed by supplementation with black tea extract. Furthermore, following BPA intoxication, a significant decline in the activity of catalase (CAT) was observed in the BPA-treated group compared to the control group. The catalase (CAT) activity was restored to normal levels in the black tea extract treated groups, compared to the BPA-treated group. Moreover, the superoxide dismutase (SOD) activity was significantly compromised ($p < 0.01$), experiencing a decrease in the BPA-injected group. However, supplementation with black tea extract in the other BPA-treated groups led to a substantial recovery.

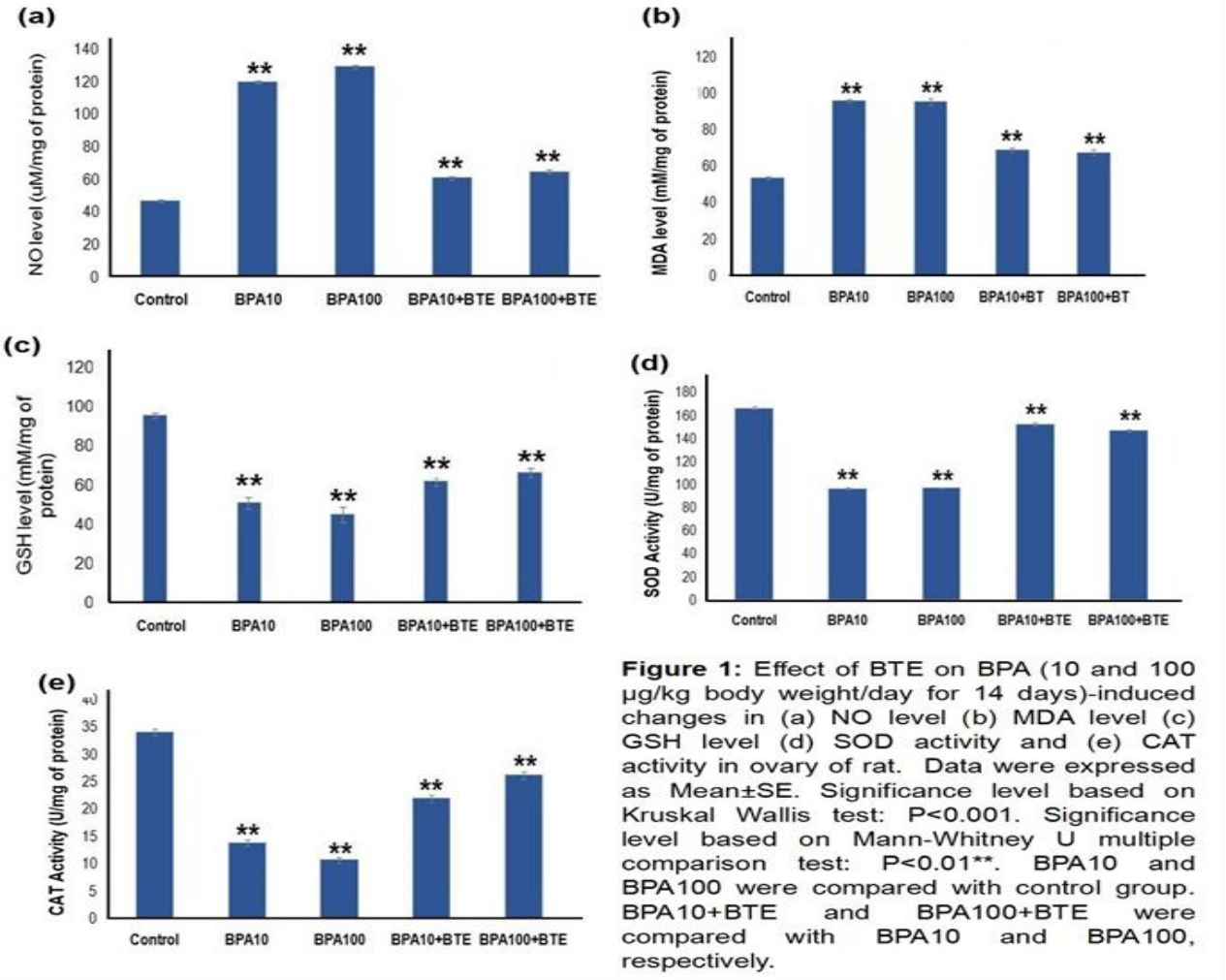

Figure 1: Effect of BTE on BPA (10 and 100 µg/kg body weight/day for 14 days)-induced changes in (a) NO level (b) MDA level (c) GSH level (d) SOD activity and (e) CAT activity in ovary of rat. Data were expressed as Mean±SE. Significance level based on Kruskal Wallis test: P<0.001. Significance level based on Mann-Whitney U multiple comparison test: P<0.01**. BPA10 and BPA100 were compared with control group. BPA10+BTE and BPA100+BTE were compared with BPA10 and BPA100, respectively.

Discussion

The extensive use of BPA in plastic food or beverage containers has been linked to severe damage to the female reproductive system, particularly the ovaries, and disruptions in hormonal balance. These are significant concerns that warrant public awareness in today's world. BPA is also prevalent in toys, baby food containers, feeding bottles, and other items, making its impact on children substantial. People of all ages are unavoidably exposed to BPA in their daily lives. The objective of this study was to investigate the protective effects of black tea extract (BTE) against female reproductive disorders caused by BPA exposure. Black tea, derived from *Camellia sinensis*, is known for its antioxidant effects in both laboratory and living systems, capable of scavenging free radicals such as reactive oxygen and nitrogen species (Frei and Higdon, 2003). These antioxidant properties are often attributed to the polyphenol content of tea. Although both green tea and black tea are recognized as effective chemopreventors against reactive oxygen and nitrogen species, a prior study by Sarkar and Bhaduri in 2001 revealed that the theaflavin content of black tea is more proficient at scavenging ROS than green tea (Sarkar and Bhaduri, 2001). The current investigation revealed that exposure to BPA leads to ovarian toxicity, evident in reduced activities of antioxidant enzymes (SOD and CAT) and increased levels of NO and MDA in ovarian tissue. Notably, there was a significant rise in NO production in the ovaries of BPA-exposed rats, indicating heightened generation of reactive oxygen species (ROS). These ROS play a cohesive role in initiating and exacerbating oxidative stress, leading to lipid peroxidation. Elevated MDA levels in the ovaries of BPA-treated rats underscored increased lipid peroxidation, resulting in oxidative damage and impaired membrane function. Metabolic processing of BPA-generated ROS may surpass the capacity of the intracellular antioxidant system.

Glutathione (GSH), a crucial and widely available antioxidant, provides extensive cellular protection against oxidative damage. The study observed a reduced GSH level in BPA-treated rats, suggesting a failure of the primary antioxidant system to counteract free radicals. This decrease in GSH levels contributes to lipid peroxidation in the ovaries, aligning with previous findings reporting a decline in GSH concentration in BPA-exposed rat ovaries. Cells employ various defense mechanisms against oxidative damage, including enzymatic scavengers like SOD and CAT, which protect against the detrimental effects of ROS. Catalase, a common enzyme found in organisms exposed to oxygen, catalyzes the decomposition of H_2O_2 to water and oxygen, mitigating oxidative damage induced by various toxicants. The study observed significant decreases in CAT and SOD activities in response to BPA exposure, implying reduced CAT activity leads to less H_2O_2 conversion, causing greater oxidative damage.

Supplementation with Black Tea Extract (BTE) demonstrated enhanced antioxidant activity in the ovaries, increasing CAT and SOD intensity. The antioxidant effects of BTE, attributed to its flavonoids and phenolic acids, allow scavenging of free superoxide radicals, protecting biological systems from the harmful effects of oxidative processes on macromolecules such as carbohydrates, proteins, lipids, and DNA. The hypothesis suggests that flavonoids and phenolic compounds in BTE scavenge more ROS, thereby protecting antioxidant enzymes like SOD, GSH, and catalase from induced decrements.

Our study strongly indicates that exposure to BPA resulted in significant oxidative stress and damage to ovarian tissuewith more pronounced effects observed with higher BPA doses. The mechanisms responsible for these positive effects of BTE against BPA-induced damage may involve the reduction of lipid peroxidation and NO levels, along with an increase in the activities of antioxidant enzymes such as SOD and catalase, and improvement in female reproductive hormone levels. This collectively works to protect the normal ovarian morphology, the regular steroidogenic process, and the overall functioning of the ovary.

Conclusion

In conclusion, the present study suggests that Black Tea Extract (BTE) holds promise as a therapeutic agent with potent antioxidant activity, likely attributed to the presence of polyphenols, theaflavin, gallate, digallate, and thearubigens. BTE demonstrates efficacy in ameliorating the adverse effects of BPA on the rat ovary. Therefore, considering its potential benefits, BTE may be regarded as a valuable health supplement to help reduce the risk of permanent female reproductive infertility caused by prolonged BPA exposure, particularly during the prepubertal period of life.

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