Protective Effect of Turmeric against Bisphenol-A Induced Genotoxicity in Rats

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Summary In this study, the protective role of turmeric on genotoxic effects of Bisphenol-A exposure in Wistar rats by in vivo experiment were investigated. Bisphenol-A is a known endocrine disruptor and suspected carcinogen, that comes diet through plastics for food packaging and food processing. In this study, rats were divided into three groups of twelve animals each and were administered with Bisphenol-A by oral gavage with levels of 0, 50 and 100 μg. Half of the animals in each group were fed with feed which contained 3% turmeric (wt/wt), for a period of 4 wk, while the rest of the rats received the same diet treatment without the addition of turmeric. At the end of the experiment, all rats were terminated and the internal organs such as liver, kidney, femurs were collected and analyzed. Mean and SD values were compared by one-way ANOVA and Kruskal-Wallis-Wilcoxon test, the formation of micronuclei was compared using Mann-Whitney U-test. Significant decrease in serum malondialdehyde and urinary 8-hydroxy-2′-deoxyguanosine levels were observed in Bisphenol-A+turmeric groups as compared to Bisphenol-A groups. Bisphenol-A groups exhibited significantly higher mean levels of DNA damage in liver and kidney as compared to the untreated control group. Bisphenol-A group showed significant increase in the formation of micronuclei which was approximately threefold higher as compared to the control group. A significant decrease in DNA migration was observed in Bisphenol-A+turmeric fed groups in liver and kidney. Turmeric feeding significantly inhibited the micronuclei formation induced by Bisphenol-A. The study results indicate that turmeric can protect against Bisphenol-A induced genotoxicity in rats.

Key Words comet assay, Rodent bone marrow micronucleus test, 8-hydroxy-2′-deoxyguanosine, MDA

Bisphenols are large groups of aromatic chemicals which are made up of two phenyl groups attached via a bridging carbon or other chemical groups. Bisphenols are widely used to produce polycarbonate, epoxy resins, and thermal paper. Therefore, bisphenols are found in several consumer products, such as baby feeding bottles, plastic bottles, toys, food cans, medical equipment, thermal paper tickets, food cartons, etc. (1). The most extensively used bisphenol is bisphenol-A (2,2-bis-[4-hydroxyphenyl] propane; BPA). BPA is an organic, synthetic compound with the chemical formula of (CH3)2C(C6H4OH)2. BPA as one of the highest volume chemicals, produced at about 4.5 million tons per year worldwide. The widespread use of BPA-containing materials, in food processing, and packaging chains led to its contamination in samples, produced at about 4.5 million tons per year worldwide. The widespread use of BPA-containing materials, in food processing, and packaging chains led to its contamination in samples, ranging from dietary products, human fluids, to environmental media. Several studies on BPA found it to exhibit toxicological properties such as genotoxicity, oxidative stress, endocrine disruption, mutagenicity and carcinogenicity in both in vitro and in vivo models (2).

BPA is a model xenoestrogen, despite possessing only modest estrogenic activity compared with 17β-estradiol. Over the last decade, BPA has been shown to produce a range of adverse effects in laboratory animals, with major concerns in reproductive system (3). The estrogenic activity of BPA exerted through binding to estrogen receptors is likely involved in the various occurrence of its adverse effects, and several studies in animal models have shown that such effects were observed after being exposed in low concentration level (4). In humans, the half-life of BPA in oral administration is about 6 h. However, it requires approximately 24 h for almost complete excretion through urine (5). In vitro studies found that BPA and its analogues were capable of inducing genotoxicity in human peripheral blood cells and macrophages (6, 7), indicating that the genotoxic effect of BPA was exhibited via oxidative stress. It was also reported that BPA caused the increase in lipid peroxidation in blood and tissues and altered the expression of peroxisome proliferator-activated receptors (PPARs), which could be one of the possible mechanisms in causing genetic toxicity (8).

Several studies showed that chemoprevention can
The extent of DNA damage in the tissues was quantified by olive tail moment (OTM) and tail length (TL). Olive tail moment was calculated by multiplying %DNA tail with tail moment length i.e. measurement from the

MATERIALS AND METHODS

Animals. Male Wistar NIN (WNIN) rats were obtained from the National Centre for Laboratory Animal Science (NCLAS) and housed in the animal facility, where the temperature was maintained at 24–25°C with 12-h dark/light cycle. This study was approved (Study approval no. P43F/IAEC/NIN/20/11/Wistar Rats M54) by the Institutional Animal Ethics Committee (IAEC) under the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Turmeric powder was obtained from a commercially available standard grade (AGMARK).

Study design. Age (9–10 wk) and weight (150–180 g) matched inbred male WNIN rats (n = 36) were divided into three groups of twelve animals each and were administered with BPA (Sigma-Aldrich, St. Louis, MO, USA) by oral gavage at 0, 50 and 100 μg/kg body weight/d. Half of the animals in each group were fed with standard diet containing 3% turmeric (wt/wt), for a period of 4 wk while the rest of the group received the same diet treatment without the addition of turmeric. The standard diet consisted of wheat flour 15%, roasted Bengal gram flour 58%, groundnut flour 10%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture 4% and vitamin mixture 0.2%. The animals had free access to food and water. At the end of the experiment, all animals were euthanized by carbon dioxide (CO₂) asphyxiation method and the internal organs, such as liver, kidney and femurs were collected for further analysis.

Urine waste was collected 24 h prior to euthanization and analyzed for the presence of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of oxidative stress and carcinogenesis. Blood was collected from the orbital plexus prior euthanization and used for the comet assay, whereas serum was separated and used for lipid peroxidation estimation. Tissue samples were also processed for comet assay. Femurs were collected for rodent bone marrow micronucleus test to determine the effect of turmeric on BPA exposure.

Lipid peroxidation. Blood serum was separated and used for lipid peroxidation estimation using Aldetect (MA-Specific) Lipid peroxidation Assay kit (AK 171, Biomol International Inc., USA). The concentration of malondialdehyde (MDA) in an unknown sample was determined from the absorbance of the unknown sample at 586 nm with the MDA standard curve as per kits protocol.

Detection of 8-hydroxy-2′-deoxyguanosine (8-OHdG). 8-OHdG, one of the by-products of oxidative DNA damage, is physiologically found and enhanced by chemical carcinogenesis. 8-OHdG is formed during the repair of DNA damage in vivo by exonucleases and excreted in urine without further metabolism. Urine was centrifuged at 3,000 rpm for 10 min. About 1.0 mL of supernatant was used for 8-OHdG estimation. 8-OHdG levels in unknown urine samples were measured using Oxiselect™ oxidative DNA damage ELISA kit (Cell Biolabs, Inc., USA) by comparing its absorbance at 450 nm with the known amount of 8-OHdG using a standard curve. The urinary 8-OHdG was expressed as the total amount excreted in 24 h.

Comet assay. The use of comet assay is a relatively simple and rapid method to examine DNA damage and repair as an important biomarker for the study of the effects in nutrition and cancer (11). Single-cell suspension was made using a cell dissociation technique and the resulting cell suspension/whole blood (40 μL) was mixed with 100 μL of 0.5% low melting point agarose (LMPA) and placed on frosted microscope slides that have already been pre-layered with 1% normal melting point agarose (NMPA). After solidification, slides were covered with a third layer of 0.5% LMPA (200 μL). Later, the slides were immersed into a lysing solution (1% sodium saccharine, 2.5 M NaCl, 100 mM sodium EDTA, 10 mM Tris-HCl (pH-10), 1% Triton X-100) for 1 h at 4°C.

The slides were kept in alkaline electrophoresis and DNA unwinding was allowed for 20 min at room temperature and then the slides were subjected further to electrophoresis for 20 min at 25 V and 300 mA. The slides were rinsed thrice with 0.4 M Tris (pH 7.4), then dipped in 70% ethanol followed by 100% ethanol for a minute (1.2). Then the slides were allowed to dry, stained with ethidium bromide and analyzed using a fluorescence microscope (BX51T1, Olympus, Japan) with a monochrome CCD camera with grabber and Komet 5.5 (USA) image analysis software.

The extent of DNA damage in the tissues was quantified by olive tail moment (OTM) and tail length (TL). Olive tail moment was calculated by multiplying %DNA tail with tail moment length i.e. measurement from the
center of head to the center of tail. Quantification of the comet images in blood was done by measuring the width of the head and length of the tail of the comet images using a visual scoring system with ocular micrometer. About 50 cells per slide were counted twice.

**Rodent bone marrow micronucleus tests.** The micronucleus is sensitive to many aneuploidy inducing agents and it is the most commonly used assay for identification in genotoxic effects (13). The femur bones were opened at the ends and the bone marrow was gently flushed out using fetal calf serum (FCS) and made a fine colloid with a syringe and centrifuged at 800 rpm for 5 min. The supernatant was decanted and the sediment was overlayered with one or two drops of FCS. The cell suspension was smeared on glass slide and air-dried. Slides were stained with May-Gruenwald and Giemsa stain respectively. About 2,000 polychromatic erythrocytes (PCEs) per animal were scored to determine the frequency of MNPCEs. The normochromatic erythrocytes (NCE) were also scored and the frequency of PCE among the first 200 NCE was counted using Leica microscope to calculate PCE/NCE ratio. The percentage of reduction in the frequency of MNPCE's was also calculated (12).

**Statistical analysis.** Statistical analyses were performed using Statistical Package for Social Science (SPSS) software package for Windows version 15.0. Mean and SD values were calculated for all variables and groups. Mean values were compared by one-way ANOVA and with post-hoc test of least significant differences (LSD) among the groups. Paired 't' test was used for comparison of mean differences of dependent samples for each group. Non-parametric tests of Kruskal-Wallis-Wilcoxon test would be performed whenever the assumptions of parametric tests were violated. For the analysis of micronuclei, 2,000 PCEs were scored to calculate the MN frequencies and 200 NCE were examined to determine the ratio of PCE to NCE. The differences in the incidence of MNPCE per group and of PCE per 2,000 erythrocytes (PCE+NCE) were compared between BPA control and BPA+Turmeric fed groups using Mann-Whitney U-test (two-tailed).

**RESULTS**

Reduction in MDA levels was observed in turmeric fed groups compared to control. Significant decrease in MDA levels were observed in BPA (50 µg and 100 µg)+turmeric groups compared to groups fed with 50 µg and 100 µg of BPA respectively (Fig. 1).

Urinary 8-OHdG levels in the samples without BPA were similar in turmeric fed groups and control but were higher in 50 µg and 100 µg BPA group. Reduction in urinary 8-OHdG levels occurred in both 50 µg and 100 µg of BPA+Turmeric groups compared to 50 µg and 100 µg of BPA groups respectively, indicates that feeding of turmeric inhibited the formation of urinary 8-OHdG (Fig. 2).

The difference of comet ratios (y/x i.e., width of the head/length of tail) was observed between normal and 50 µg and 100 µg BPA treated control groups in blood. There were significant differences of comet ratios in 50 µg and 100 µg of BPA+Turmeric groups compared to 50 µg and 100 µg of BPA groups respectively. There was a decrease in the extent of DNA damage in the turmeric fed groups compared to BPA treated groups (Table 1).

BPA (50 µg and 100 µg) treated positive control groups exhibited significantly higher mean levels of
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DNA damage in OTM & TL parameters in all organs, namely liver and kidney compared to the normal (without BPA) control group (Tables 2, 3). A significant decrease in DNA migration in response to BPA+turmeric fed groups was observed in liver and kidney. The decrease in TL was observed at 3% turmeric treated with 50 µg BPA compared to the BP A groups respectively which shows an inhibitory action on DNA damage (Tables 2, 3). These results show that turmeric protects the extent of DNA damage in the turmeric fed groups compared to BP A treated groups.

BP A (50 µg and 100 µg) treated groups showed a significant increase in the formation of micronuclei (MN) which was approximately threefold higher compared to the normal (without BPA) control group.

The frequencies of micronucleated polychromatic erythrocytes (MNPCE) in the normal control group, 50 µg and 100 µg BPA treated were 3.9±0.92, 9.1±2.41 and 12.3±3.14 respectively. Turmeric feeding significantly inhibited MN formation in 50 µg and 100 µg BPA+Turmeric groups. PCE/NCE ratio was also determined to see whether BPA had a cytotoxic effect on the bone marrow cells. The results showed a decrease in PCE/NCE ratio in BP A group, which was an indication of cytotoxicity. However, the PCE/NCE ratios observed in 50 µg BPA+turmeric group were found to be similar to the 3% turmeric group (Table 4).

These results indicate that 3% turmeric does not possess genotoxic or cytotoxic effect but it reduces BPA-induced chromosome damage and genotoxicity in the rodent bone marrow micronucleus assay.

**DISCUSSION**

In this study, we investigated the protective role of turmeric, on in vivo genotoxic effects of two different
doses (50 and 100 µg/kg/bw) of BPA on various genotoxicity parameters in WNIN rats. The rats were divided into three groups and administered with BPA by oral gavage. Half of the animals in each group were fed with diets containing 3% turmeric (wt/wt), for one month while the rest of the animals received the same diet without turmeric. The BPA exposure induced DNA damage in blood, liver, and kidney by increasing serum MDA and urinary 8-OHdG levels and formation of micronuclei in bone marrow cells. Turmeric feeding protected against BPA-induced genotoxicity significantly as compared to control animals.

To assess genotoxicity, various biomarkers are available and have been extensively studied. There are evidences from epidemiological, in vitro and in vivo studies that plant-based diet can reduce the risk of cancer and other chronic diseases (14). The molecular mechanism of nutrients which protect against carcinogenesis and the biomarkers which are involved in molecular effect of dietary factors both in vivo and in vitro studies are crucial.

In this study, rats were fed with turmeric (3%) through diet and a significant decrease in malondialdehyde (MDA) levels was observed in turmeric fed groups treated with BPA. This may be due to the active compound curcumin present in turmeric that may have protection role against lipoperoxidation of subcellular membranes. An earlier study showed that turmeric itself had no genotoxic effect, but instead had a protective role against benzopyrene toxicity by modulating lipid peroxidation (12). In the previous study, increased MDA levels were observed due to the exposure of BPA, which indicated an increase in generation of ROS, leading to high lipid peroxidation activity, thereby enhancing membrane disruption and DNA damage (8). The increase in the degree of MDA level due to increase in concentration of BPA. It suggests that there was of excessive generation of free radicals (8). A group of researchers studied the protective activity of nanomicelle curcumin in bisphenol A-induced cardiotoxicity in rats. The results revealed that nanomicelle in curcumin could ameliorate BPA-induced toxic effects. Reduction in MDA level was observed in 50 mg/kg nanomicelle curcumin group compared to BPA groups (15).

Urinary 8-OHdG is a sensitive marker for in vivo oxidative DNA damage and oxidative stress. It is formed in promutagenic DNA lesion induced by the reaction of hydroxyl radicals with guanosine at C8 site in DNA. Upon DNA repair, 8-OHdG is excreted in the urine. One of the products of DNA oxidation, 8-OHdG has been extensively studied and its levels in target tissues were correlated with oxidative stress and the incidence of cancers (12). Numerous studies had proven that 8-OHdG was not only a biomarker of DNA damage and cellular oxidative stress, but also be the possible reason for many diseases, namely cancer, diabetes, etc. (16).

To evaluate the potential oxidative stress markers, the effect of turmeric on BPA induced oxidative damage at different levels was studied. In all experimental groups treated with BPA resulted in increasing 8-OHdG levels compared to normal control. Reduction in urinary 8-OHdG levels were seen in both 50 µg and 100 µg BPA+curcumin groups compared to 50 µg and 100 µg of BPA treated groups respectively. Feeding of turmeric inhibited the formation of urinary 8-OHdG. Our previous study showed the reduction in urinary 8-OHdG level by feeding turmeric in induced benzopyrene toxicity in rats (12). The results obtained in this study shows the suppressive role of turmeric against genotoxic effect of BPA. In an animal study, supplementation with 1% curcumin for one month eliminated the formation 8-OHdG in the kidneys of Ferric nitrito triacetate treatment group, and these protective effects might be mediated by antioxidant properties in curcumin (17). In another study, researchers showed that curcumin could inhibit diabetes-induced retinal 8-OHdG levels, so it suggests that curcumin could inhibit the accumulation of oxidized DNA in retina and eventually inhibited diabetic retinopathy (18).

Comet assay is a sensitive and rapid method to study DNA damage and repair (11). In this study, we observed a decrease of DNA damage in turmeric fed groups compared to BPA treated groups in blood samples. We also observed a decrease in DNA migration in response to BPA+curcumin fed groups in liver and kidney with respect to OTM and TL. The protective role of turmeric against lipid peroxidation and oxidative DNA damage in rats could be related with the prevention of carcinogenesis.

In a study, inhibitory effects of turmeric on the genotoxic effect of a powerful mutagen, urethane, was observed (19). Curcumin is a hydrophobic polyphenol and it is capable of scavenging free radicals. In other study, curcumin was found to be highly effective in reducing genotoxicity and oxidative stress induced by arsenic (20). Our study proved that spices like ginger, garlic, and turmeric have antioxidant activity. The turmeric showed to have 43 to 91% inhibition in different concentrations and IC50 was 183.38 µg/mL when compared with vitamin C as standard. It is known that the phenolic properties in curcumin are responsible for its antioxidant properties (21). Recently, several studies had reported BPA exposure induced DNA damage in zebra fish, which was correlated with the increase in ROS production (22).

There was not enough evidence to show the exact molecular mechanism of BPA on the production of ROS. However, many scientific reports stated that BPA generates ROS during biotransformation and certain ROS, such as quinones, could react with DNA and could induce DNA damage (23). In a study, it was shown that BPA-induced oxidative stress could be one of the possible mechanisms for the genotoxic activity of BPA (8). In another study, it is reported that the trend of cytotoxicity in macrophages was similar to BPA-exhibited apoptosis and genotoxicity. These results indicate that the cytotoxicity was induced by BPA via apoptosis and genotoxicity in macrophages (24). Rodent bone marrow micronucleus assay is a commonly used genotoxic assay to detect both clastogenic and aneugenic poten-
cies of genotoxic agents or radiation (25). Several epidemiological studies have suggested that the formation of micronuclei might serve as an effective biomarker to estimate cancer risk.

In this study, the feeding of turmeric reduced the number of micronuclei in the groups that were given turmeric + BPA in comparison to groups given BPA alone. There was also a dose-dependent increase in micronuclei numbers PCE groups 50 μg and 100 μg of BPA compared to normal untreated control group, which indicates that BPA produces chromosomal damage in erythrocytes. Turmeric itself does not possess genotoxic or cytotoxic effect, but it protects by inhibiting chromosome damage and genotoxicity of BPA in rodent bone marrow micronucleus assay.

Such chromosomal damage is associated with the onset and/or progression of tumors with adverse reproductive and developmental outcomes. Curcumin showed to have protective effects against chromosomal damage. The bone marrow micronucleus test significantly reduced the frequency of micronucleated PCE induced by the full exposure to gamma radiation (26).

In our earlier study, the overall mean percentage reduction of micronuclei was nearly 80% in the benzopyrene+turmeric fed groups, indicating that turmeric exerted protective effect against benzopyrenotoxicity (12).

Furthermore, this study was the first to demonstrate that BPA-induced genotoxicity could be attenuated by turmeric feeding. The active compounds in turmeric provide mechanistic and pharmacologic profile that might be useful to characterize the chemopreventive potential (27). The research on biomarkers which are responsible for lifestyle related diseases will give a scope in understanding the onset and progression of disease. By using these biomarkers, researchers may have the lead in developing new health check that will contribute in preventive medicines (28).

In summary, the results of this study shows that BPA (50 and 100 μg) exposure may generate ROS, which results in elevated levels of MDA in blood and 8-OHdG in urine. BPA induced DNA damage in blood, liver and kidney and MN formation in bone marrow cells, thereby causing oxidative stress and genotoxicity. Turmeric feeding significantly decreased the serum MDA and urinary 8-OHdG levels. Significant decrease in DNA migration as a response to 50 μg BPA+turmeric fed groups was observed in liver and kidney. Turmeric feeding significantly inhibited the micronuclei formation. This study results indicate that turmeric has protective roles against BPA-induced genotoxicity in rats.

**Disclosure of state of COI**

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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