ABSTRACT: A sub-acute toxicity study of Bisphenol A (BPA) was conducted on Wister rat to investigate its potency and impact on mammals. The doses chosen were 50, 200 and 600 mg/kg body weight for group III, IV and V respectively and group II served as vehicle control and group I was negative control. BPA caused significant ($P < 0.01$) decrease in humoral immune response to sheep red blood cell as antigen (SRBC) in group V and significant ($P < 0.01$) depression of cell mediated immunity (CMI) were observed in BPA treated rats as measured by dinitrofluorobenzene (DNFB) skin contact sensitization test.

Key words: Bisphenol A, Humoral immune response, CMI.

INTRODUCTION

Bisphenol A (BPA; 4, 4'-dihydroxy-2, 2-diphenyl propane) is an organic compound with two phenol functional groups. It is produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics are used in certain food and drink packaging, e.g., water and infant bottles, compact discs, impact-resistant safety equipment, medical devices, can be blended with other materials to create molded parts for use in mobile phone housings, household items, and automobiles. Epoxy resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Some polymers used in dental sealants or composites contain BPA-derived materials. Bisphenol A is known to leach from dental composites as well as food containers such as cans and polycarbonate plastic water bottles. Detectable levels of BPA have been found in the general population. The consumption as well as production of plastic material is increasing by the time and simultaneously exposure of BPA to animals, wildlife species and humans is also increasing. Concern is mounting regarding the human and animal health and environmental effects of BPA due to its toxic effects. Immunosuppression leads to change in span of life, increased susceptibility to infectious diseases and decreased immune response to foreign antigen (Mondal et al., 2009).
Therefore, it is necessary to understand the effects of BPA in the immune system. In view of the reason, attempt has been made to assess quantitative risk in rat model system. The present study was designed to elucidate the effect on different organs along with immunological effects induced by BPA in Wistar rat.

**MATERIALS AND METHODS**

**Animals:** The present study was conducted on 6 weeks old healthy Wistar rats. The rats were procured and housed in cages at Animal House, College of Veterinary Science and Animal Husbandry (Indira Gandhi Krishi Vishwavidyalaya), Anjora, Durg, Chhattisgarh, India. Animals were acclimatized to experimental room for 7 days before start of the experiment. Experimental protocol was approved by institutional animal ethics committee (IAEC, CVS&AH) before starting the experiment.

**Husbandry:** The animals were housed in polypropylene cages under control temperature and hygienic conditions in the animal house using sterilized husk as bedding materials. Light and dark cycle of 12 hr was maintained throughout the experimental period. Animals were provided standard feed (Nutri Lab, rodent feed, Vetcare Pvt. Ltd, Bangalore) and allowed water *ad libitum* (water purification was done by reverse osmosis followed by ultraviolet [UV] treatment).

**Chemicals:** Bisphenol A was procured from the Department of Veterinary Pharmacology and Toxicology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, India. Dinitrofluorobenzene was obtained from Sigma-Aldrich (cat#42080fluka), USA.

**Formulation:** Bisphenol-A was formulated using propylene glycol as a vehicle. Bisphenol-A solution was administered orally, dose volume 10 ml/kg. Body weights were recorded before administration of BPA. The daily oral administration was continued for 28 days.

Dinitrofluorobenzene was formulated in acetone: olive oil (4:1) solution in different concentration as described by Tamang *et al.* (1988).

**Experimental Design**

Total 90 rats were divided into five groups having 18 rats each. Rats of group I was kept as negative control and was given only distilled water orally. Rats of group II served as vehicle control and were given propylene glycol. Rats of group III, IV and V were administered BPA at the dose rate of 50, 200 and 600 mg/kg of body weight respectively for 28 days.

**Assessment of humoral immune response**

Humoral immune response was assessed by micro Haemagglutination (HA) test as described by Hudson and Hay (1989) with slight modification (Mondal *et al.*, 2009). The venous blood from the sheep was collected aseptically from the jugular vein of the animal in equal volume of Alsever’s solution. It was kept in 2°-8°C for a week long period for stabilization. Sheep red blood cells were obtained by centrifugation and washing with phosphate buffer saline thrice at 2000 rpm for 10 minutes. Finally a suspension of 5 per cent SRBC was adjusted with PBS.

For immunization, 1 ml of 5% SRBC was injected intraperitoneally in six rats of each group on day 16 of the experiment. Blood from SRBC injected rats was collected on 28th day without anticoagulant and serum was separated. Serum was kept in water bath at 56°C for 30 minutes to inactivate the complement fraction.
of the sample. The haemagglutinins produced in response to SRBC was determined by micro HA test. The test was performed in micro titre plates. For this 1% suspension of SRBC in NSS was used as working suspension. The plate was thoroughly cleaned and each test was carried out in duplicate according to the protocol given in appendix. The reciprocal of the highest dilution of serum causing complete haemagglutination was taken as HA titre of the serum sample and expressed in log2 values.

Assessment of cell mediated immune response

Dinitroflurobenzene (DNFB) contact skin sensitivity test:

Cell mediated immune response based on delayed type hypersensitivity reaction was measured by Dinitroflurobenzene (Sigma Chemical Co. Ltd., U.S.A.) test as described by Phanuphak et al. (1974) with some modification by Tamang et al. (1988) and Mondal et al. (2009). DNFB test for monitoring cell mediated immunity was done on day 25 after primary sensitization at day 21.

For this test, 6 rats were randomly selected from each group. Dorsal aspects of ears of the selected rats were cleaned for the application of DNFB. All the selected rats of each group were sensitized with one drop of 2% DNFB in 4:1 acetone olive solution to the right ear. The left ear was kept as respective control, on which only the vehicle i.e. the acetone olive solution (4:1) was applied. Four days after primary sensitization, the sensitized rats were challenged with 1% DNFB in 4:1 acetone olive oil solution on day 25. Ear thickness was measured with disk micrometer (model# 223-101, Mitotoyo, New York) at 0, 6, 12, 24 and 48 hours after post challenge.

RESULTS AND DISCUSSION

Humoral immune response

The status of the humoral immunity of the Wister rats exposed to subacute BPA toxicity was assessed by micro HA against sheep red blood cell (SRBC). The log2 of the micro HA titres of the rats of all groups against SRBC are shown in Table 1. The HA titre of the rats of all the BPA treated groups were decreased. However, significant decrease in HA titer was observed in the rats of group IV (P ≤ 0.05) and V (P ≤ 0.01) as compared to rats of the control groups (group I and II). The level of serum antibodies against the antigen is the conventional index of humoral immunity, which could be measured accurately by HA titre (Gatne et al., 2006). It was evident that the compound might have adverse effect on

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA Titre (log2 values)</td>
<td>Group I</td>
</tr>
<tr>
<td>8.17±0.47cd</td>
<td>8.5±0.43d</td>
</tr>
</tbody>
</table>

Superscripts are read row wise for comparison of mean. Different superscripts differ significantly (*P=0.05) and (**P= 0.01).
antibody response against SRBC when exposed up to 28 days at the dose level employed in the present study. A progressive decrease in HA titre with increasing dose of BPA indicates adverse effect of BPA on humoral immunity. The decline in HA titre to SRBC in the present study could be due to effect of BPA in the inhibition of lymphocyte mitogenesis (Sakazaki et al., 2002).

Segura et al. (1999) investigated in vitro effect of BPA on viability, and substrate adherence capacity of macrophages and observed that the resin component of BPA can alter macrophage adhesion. Taking into account that, adhesion is the first step in the phagocytic process of macrophages and in antigen presentation, BPA could inhibit macrophage function and modulate immune and inflammatory responses in dental pulp and periapical tissues.

### Table 2. DNFB response (Mean increase in ear thickness in mm) of Wister rats exposed to subacute bisphenol A toxicity (Left side served as vehicle control and right side treated with DNFB).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ear Side</th>
<th>Before Sensitization</th>
<th>After challenge with DNFB at different time interval (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Gr I</td>
<td>Left</td>
<td>0.305 ± 0.004a</td>
<td>0.333 ± 0.01a</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>0.303 ± 0.004a</td>
<td>0.41 ± 0.01b</td>
</tr>
<tr>
<td>Gr II</td>
<td>Left</td>
<td>0.308 ± 0.012a</td>
<td>0.33 ± 0.013a</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>0.305 ± 0.007a</td>
<td>0.403 ± 0.007b</td>
</tr>
<tr>
<td>Gr III</td>
<td>Left</td>
<td>0.285 ± 0.008a</td>
<td>0.33 ± 0.01a</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>0.301 ± 0.011a</td>
<td>0.49 ± 0.014a</td>
</tr>
<tr>
<td>Gr IV</td>
<td>Left</td>
<td>0.29 ± 0.01a</td>
<td>0.31 ± 0.011a</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>0.3 ± 0.008a</td>
<td>0.435 ± 0.014a</td>
</tr>
<tr>
<td>Gr V</td>
<td>Left</td>
<td>0.31 ± 0.018a</td>
<td>0.316 ± 0.009a</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>0.338 ± 0.010b</td>
<td>0.351 ± 0.011***</td>
</tr>
</tbody>
</table>

Superscript may read column wise for mean comparison. Similar superscript showing means do not differ significantly. (P ≤ 0.05) and (**P ≤ 0.01).

Cell mediated immune response by DNFB contact skin sensitization test

Application of the challenge dose of DNFB caused erythema, oedema, vesiculation and scabbing. These changes were more pronounced in the rats of control group than in BPA treated rats. The mean increment in ear thickness of rats at different hours post challenge is shown in Table 2. A significant (P ≤ 0.01) increase in ear thickness of control rats compared with the BPA treated rats at 6 to 48 h post challenge was observed. This indicated the severe depression of cellular immunity due to BPA treatment.

Youn et al. (2002) also evaluated the immune
response to BPA @ of 0.015, 1.5 and 30 mg/ml for 4 weeks in mice and observed that BPA induced prolactin production in the spleen. Exposure of BPA increased the activity of splenocyte proliferation. Interestingly, the production of a strong Th-1 type cytokine (IFN-gamma) was induced, while Th-2 type (IL-4) was suppressed by BPA treatment. Based on those findings, the authors speculated that stimulation of prolactin production by estrogenic effects of BPA can affect cytokine production, thus leading to imbalanced cellular immune response.

Lee et al. (2003) also examined the effects of BPA and nonylphenol on production of interleukin-4 (IL-4), a pro-inflammatory cytokine closely associated with allergic immune responses. Both BPA and nonylphenol significantly enhanced IL-4 production in CD4+ T cells and elevated immunoglobulin E levels in a concentration-dependent manner.

**CONCLUSION**

It may be concluded that (a) sublethal dose of Bisphenol-A suppressed both CMI and humoral immunity in wistar rats of both sexes. (b) Bisphenol-A imparts immense harm to immune system which is the most sensitive of all body system due to their continuous growth and differentiation. (c) Indiscriminate usage of plastic and their leaching to food chain may affect immunity of animal and human which may ultimately cause high susceptibility to various secondary infections.

**ACKNOWLEDGEMENT**

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