Acute toxicity of Bisphenol A in rats
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Bisphenol A (BPA), an estrogenic compound, is used in manufacturing plastics and is known to produce toxic effects on various systems in man and animals. Since the use of plastics in day-to-day life is increasing, exposure to BPA will also increase. Therefore, this study was undertaken to determine the median lethal dose (LD$_{50}$) of BPA via intraperitoneal and intravenous route in adult rats (by Dixon’s up and down method) and also to know the acute systemic changes (in blood pressure, respiration and ECG) produced by lethal dose of BPA. Adult female albino rats of Charles Foster strain were used in the study. LD$_{50}$ of BPA was 841 and 35.26 mg/kg body weight for ip and iv route, respectively. Injection of lethal dose of BPA (40 mg/kg body weight) produced acute toxicity manifesting as immediate respiratory arrest and hypotension after the injection of BPA followed by bradycardia. The animals died within 7.3 ± 0.7 min. Volume of ethanol (vehicle; 0.1 mL) present in the lethal dose of BPA was not lethal and had no effect on respiration, blood pressure and heart rate. The results provide evidence that the acute exposure to BPA produces lethality with a very narrow range of lethal and survival dose for iv route. Further, the lethality appears to be due to respiratory arrest and hypotension.

Keywords: BPA, Hypotension, Lethal dose, Respiratory arrest, Toxic chemical from plastics

Bisphenol A (BPA), a chemical produced extensively worldwide, is used in the manufacturing of polycarbonate plastics. Plastics are used in our daily life in the form of infant feeding bottles, beverage bottles, food packaging containers, dental fillings, eyeglass lens, etc. BPA is known to have toxic effects on various systems especially on reproductive system as it possesses estrogenic property$^1-^4$. Besides the reproductive effects, BPA is implicated in endocrine abnormalities$^5$, carcinogenesis$^6$, neural and behavioural alterations$^7$, cardiac and hepatic abnormalities$^8,^9$, denoting the long term effects of BPA. Recently, National Health and Nutrition Examination Survey (NHANES) reported that urinary BPA levels in more than 90% of US population was around 0.27–10.6 ng/mL$^{10-12}$. Further, it is reported that the daily human consumption of BPA is around 1 µg/kg body weight$^{13}$ and is expected to increase with increasing usage of plastics. Recently, concentration as low as 0.1 µM of BPA, produced drastic changes in force of atrial contractions in vitro$^{14}$. However, there are no acute in vivo toxicity reports available till date. Hence, this study has been undertaken to determine the LD$_{50}$ of BPA via parenteral route and acute systemic changes associated with lethal dose of BPA in rats.

Materials and Methods

Animals—Adult female albino rats of Charles Foster strain weighing 150–200 g were used. The female rats were chosen in order to eliminate the estrogenic effect of BPA on male animals. The animals were housed in a temperature (25 ± 0.5 °C), humidity (50% of RH) and light (12:12 h light: dark) controlled room. Food (Raj Scientific Corporation, Varanasi) and water were provided ad libitum. The animal experiments were performed as per the guidelines given by the Ethical Clearance Committee of the Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Drugs and solutions—BPA was obtained from HiMedia Laboratory Pvt. Ltd (Mumbai, India) and was dissolved in 100% ethanol. In all the experiments, the volume of injection was kept 0.1 ml at any concentration of BPA. The control animals received 0.1 ml ethanol (100%).

The experiments were performed in two groups. In Group I, LD$_{50}$ of BPA via intraperitoneal (n = 7) and intravenous route (n = 5) was determined. In group II (n = 8), the systemic effects produced by lethal dose of BPA (iv) were examined.

Determination of LD$_{50}$ via intraperitoneal route—Dixon’s up and down method for estimation of median lethal dose (LD$_{50}$) was used to minimize the number of animals$^{15}$. In Dixon’s method, the LD$_{50}$
determination begins with a dose of the test chemical. The survival or death of the animal at this concentration decides for the next dose either to be increased or decreased accordingly. In this method survival is represented as “o” and death as “x” (Table 1) and the score is made accordingly. In the present study, female rats weighing 150 ± 20 g were injected with increasing doses of BPA (ip) to get lethality and then decreasing the doses to obtain a survival (Table 1). The animals were observed for 48 h for survival. The process was terminated upon finding two live animals at a dose and one death at immediately higher dose. Median lethal dose (LD₅₀) was calculated as given in Table 1.

**Determination of LD₅₀ by intravenous route**—For determining LD₅₀ by iv route, the trachea, jugular vein and femoral artery were cannulated in urethane anesthetised rats as described earlier. The tracheal cannulation was done to keep the respiratory tract patent. Jugular vein was cannulated for injection of BPA and femoral artery cannulation was done for recording blood pressure via pressure transducer. The respiratory movements were recorded by a force transducer on a chart recorder. The electrocardiographic potentials were recorded by standard limb lead 2 configuration, using needle electrodes connected to Bio-amplifier. The respiratory rate and heart rate were computed manually and mean arterial pressure (MAP) was calculated from the built-in software (Lab Chart 7, AD Instruments, Australia). In case of LD₅₀ determination the observation period was 120 min for survival as described earlier for scorpion venom. Dixon’s up and down method was performed for iv route similar to intraperitoneal route.

**Statistical evaluation**—The values are presented as mean ± SE. Dixon’s up and down method was used for the LD₅₀ determination as described earlier. Comparison of the changes in blood pressure, respiratory rate and heart rate in BPA and ethanol treated groups was performed using two-way ANOVA. A P < 0.05 was considered significant.

**Results**

**LD₅₀ of BPA by intraperitoneal route**—Median lethal dose (LD₅₀) of BPA was determined as mentioned in Table 1. Increasing doses of BPA were injected intraperitoneally in different rats and observed for survival up to 48 h. The animals became sluggish and lethargic after injection of BPA (300-750 mg/kg body weight) but, recovered slowly and survived for the entire period of observation (o, o, o). However, at 1000 mg/kg body weight dose, the animal died within 24 h (x). Hence, previous lower dose of BPA (750 mg/kg body weight) was repeated. This dose also killed the rat within 24 h (x), hence the same dose was repeated in another rat, which survived (o). Finally, 1000 mg/kg body weight of BPA was injected again in a separate rat and which died within 24 h (x). Therefore, the final score according to Dixon’s up and down method was oooxxox and with this score the LD₅₀ was 841 mg/kg body weight (Table 1).

**LD₅₀ determination by intravenous route**—Initially 30 mg/kg body weight BPA was injected intravenously and observed for survival up to 120 min and the animal survived for the period of observation (o). Next higher dose of BPA (35 mg/kg body weight) was injected in a separate rat and at this dose also animal survived for 120 min (o). Then the next higher dose of BPA (40 mg/kg body weight) was injected and at this dose the animal died within 10 min of injection of BPA (x). Then the dose was lowered to 35 mg/kg body weight and the animal survived (o). Finally, 40 mg/kg body weight dose of BPA was repeated in another animal which killed the animal (x). Therefore, the final score was oooxox and the LD₅₀ by intravenous route was 35.26 mg/kg body weight (Table 2).

### Table 1—Effect of different doses of BPA (intraperitoneal) on survival of rats for 48 h. (*x* indicates death; ‘o’ indicates survival)

<table>
<thead>
<tr>
<th>Real Dose(mg/kg body weight)</th>
<th>Log Dose</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>2.478</td>
<td>o</td>
</tr>
<tr>
<td>600</td>
<td>2.778</td>
<td>o</td>
</tr>
<tr>
<td>750</td>
<td>2.875</td>
<td>o x o</td>
</tr>
<tr>
<td>1000</td>
<td>3.000</td>
<td>x x</td>
</tr>
</tbody>
</table>

Therefore, final score is oooxxox and k = -0.144 (value obtained from Dixon’s table). 

**Antilog (1.547) = 35.26 mg/kg body weight.**

### Table 2—Effect of different doses of BPA (intravenous) on survival of rats for 120 min. (*x* indicates death; ‘o’ indicates survival)

<table>
<thead>
<tr>
<th>Real Dose(mg/kg body weight)</th>
<th>Log Dose</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>1.477</td>
<td>o</td>
</tr>
<tr>
<td>35</td>
<td>1.544</td>
<td>o o</td>
</tr>
<tr>
<td>40</td>
<td>1.602</td>
<td>x x</td>
</tr>
</tbody>
</table>

Therefore, final score is ooxx and k = -0.439 (from Dixon’s Table). 

**Antilog (1.547) = 35.26 mg/kg body weight.**
Acute systemic changes induced by lethal dose of BPA—To know the acute systemic changes associated with lethality, the lethal dose (40 mg/kg body weight) was injected in 5 different animals. Recordings of ECG, respiration and blood pressure of one experiment are presented in Fig. 1. In these animals the initial value of heart rate was 288 ± 19.3 beats per min, respiratory rate was 75 ± 5.1 breaths per min and MAP was 89.1 ± 2 mm Hg. After BPA injection (40 mg/kg body weight iv) there was respiratory arrest and hypotension within 10 min (Fig. 2) and the animals died. On the other hand the heart rate decreased progressively for a longer period. The mean survival time of these animals (n = 5) was 7.3 ± 0.7 min. The above changes were consistently observed in all the animals exposed to BPA and were significantly different from ethanol control group (P < 0.05, two-way ANOVA).

In another set of experiments (n=3), equi-volumes of ethanol (0.1 ml solvent present in 40 mg/kg BPA) was injected to the animals. The initial value of heart rate was 276 ± 19.1 beats per min, respiratory rate was 100 ± 14.4 breaths per min and MAP was 84.2 ± 1.3 mm Hg. These animals survived for 120 min without any changes in respiration, blood pressure and heart rate (Figs 1 and 2).

Discussion

BPA, one of the important components for manufacturing plastics has been proven to be present in body fluids. With the increasing use of plastic wares the daily exposure to BPA is increasing, hence...
In a study elsewhere the urinary levels of BPA were correlated with hypertension\(^9\). The hypertension is attributed to obesity, metabolic syndrome and estrogenic effects of BPA. However in this study, acute exposure to BPA produced hypotension while acute respiratory arrest was prominent. It is likely that such hypotensive effects especially at renal artery level can trigger the renin-angiotensin regulatory mechanisms to produce hypertension in addition to those described earlier\(^9\).

In the present experiments, compensatory reflex changes were expected due to chemoreceptor /baroreceptor activation by apnoea or hypotension. However, the results did not show hyperventilatory or hypertensive or tachycardiac responses reflecting the reflex alterations. Lack of reflex responses may be due to pre-existing medullary depression by BPA. However, the presence of ECG activity indicates that the heart did not stop simultaneously with apnoea or hypotension. This suggests that cardiac depression is not responsible for the lethality produced by BPA within such a short time.

The present observations indicate that BPA poses toxic effects with very low safety margin in iv route. Further, BPA depresses the medullary centres to produce apnoea, hypotension and lethality.

**References**


