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Original Article

Plastic Toxin Bisphenol-A Depresses the Contractile Activity of Rat Ileum and Colon *in vitro*

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Abstract

Bisphenol A (BPA), a plastic toxin, is required in the production of various plastic items including water bottles, baby feeding bottles and other food and beverage containers. Since, the primary source of human exposure to BPA is the leachate from food and beverage containers, gastro intestinal tissues are particularly susceptible to BPA-induced changes. Therefore, the present study was undertaken to explore the possible effects of BPA on contractility in adult rat ileum and colon. In an organ bath preparation, isometric contractions were recorded from segments of dissected out colon and ileum, with the help of force transducer and digitized data acquisition system. The results indicated that BPA (1-100 µM) significantly ($p<0.05$) depresses contractile tension and frequency of ileum and colon in a dose dependent manner. Further, the exploration of possible mechanisms for BPA-induced decline in contractile responses revealed that the decrease in contractility was independent of estrogen receptors, nitric oxide and cholinergic system.

Introduction

The chemical, 4, 4'-Isopro-pylidenediphenol, commonly known as Bisphenol A (BPA), is produced in a high volume and used primarily in the production of polycarbonate plastics and epoxy resins (1). Polycarbonate plastics are mainly used to make various types of plastic products including water bottles, baby feeding bottles and various food and beverage containers. Epoxy resins are used for lining metal cansto avoid erosion and maintain quality of canned food and beverages. However, it is known that BPA leaches from these plastic products into the foods contents and thus, humans are exposed to the BPAduring the consumption of foods, water and drinks served in plastic containers (2). Some dental sealants and composites may also play as important sources of BPA for human exposure (3). Because of extensive use of BPA in plastic industry, there iswidespread and well documented human exposure of BPA. BPA has been detected in various body fluids and tissues in a large number of people across the world. For example, the national health and nutrition examination survey on U.S. population above 6 yrs. of age, reported detectable urinary BPA levelsin more than 90% of population (4). Human exposure to BPA in several Asian countries has also been

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documented (5). BPA has been known as endocrine disruptor and mimics estrogen in its mode of action (6). Owing to estrogenic activities, it produces various reproductive and behavioral toxicities (7). Also alteration in function of coronary smooth muscle (8) and depression of the atrial contractility in rat (9) has been reportedly induced by BPA.

Given that the main route of exposure to BPA is oral, intestinal tissues remains to be vulnerable to BPA-induced changes. Although, some studies have shown that BPA alters gut barrier and immune responses (10, 11), its effect on intestinal motility has not been adequately addressed.A recent study has reported that BPA inhibits duodenal movement via nitric oxide mediated pathway (12). However, its effect on ileum and colonic motility is not clearly understood.Therefore, the present study was undertaken to explore the possible effects of BPA on contractility of rat ileum and colon using*in vitro* experiments in organ bath preparations.

Methods

The present experiments were carried out on Charles Foster strain of rats after the approval of institutional ethical committee for animal experiments. The adult rats of either sex weighing 150-200 gm (4-6 months old) were procured from institutional animal house. Rats were housed in the departmental animal house in an environment of controlled temperature (25±0.5ºC), and light (12: 12 hr light dark) with *ad libitum* supply of rat feed and potable water.

Dissection of animal

Rats, fasted overnight, were sacrificed by cervical dislocation. The abdomen was opened quickly by midline incision. The ileum and proximal colon were dissected out and immediately placed in a petri dish containing 100% oxygenated fresh Krebs-Ringer solution and the intestinal contents, if any, were flushed out by this solution with the help of a syringe.

Mounting and recording of contractile response

The procedure for mounting and recording of contractile responses has been described earlier (13, 14). Briefly, after cleaning the tissues, the segments of 1-1.5 cm of intestine were placed in Krebs-Ringer solution filled organ bath (15 ml) maintained at 37°C±1°C and continuously bubbled with 100% O_2. One end of tissue segment was fastened to a glass tube support, and the other end was fixed to a force transducer (MLT 0210, AD instruments, Australia). Strips were mounted vertically for primarily recording of contractions of longitudinal muscle. The tissue segment was placed under optimum resting tension (0.5 gm.) and then left to equilibrate for 30 minutes, with replacement of Krebs-Ringer solution every 15 minutes. After stabilization, the initial recordings of spontaneous contractions were made for 30 minutes. Before, as well as after recording the contractile responses, calibration for the tension (0-10 g) was performed. After recording of contractions, the segment of tissue was removed from the organ bath and placed on blotting paper for lightly soaking the extra water from the tissue. The two ends of the strips were cut to remove the injured parts. The wet tissue was then weighed in a fine balance to express the contractile response per unit weight of tissue (g/g wet tissue).

Isometric contractions were amplified by bridge amplifier and digitized via an analog/ digital interface (Power Lab 4/ST system) to acquire onto a personal computer. The recordings were displayed and analyzed with the help of software Chart-5 for windows (AD Instruments, Sydney, Australia).

Drugs and solutions

The physiological solution (Krebs-Ringer solution) was prepared with following compositions (in mmol): NaCl, 119; KCl, 4.7; $CaCl_2.2H_2O$, 2.5; KH_2PO_4, 1.2; $MgSO_4.7H_2O$, 1.2; $NaHCO_3$, 5; and glucose, 11, with pH adjusted to 7.4. BPA was obtained from HIMEDIA laboratories Pvt. Ltd, Mumbai. It was dissolved in 50% ethanol to have stock solution (10 mM). For dose response curve, 1 µM 10 µM, 30 µM and 100 µM concentrations were used. In experiments where tissue was pretreated with various antagonists, 100 µMconcentration of BPA was used.

Antagonists- L-NAME (N – nitro – L – arginine methyl ester, a Nitric Oxide synthase inhibitor), Tamoxifen

(an estrogen receptor blocker), Atropine (a muscarinic receptor blocker), Hexamethonium (a ganglion blocker) were procured from Sigma Chemicals Inc. (St Louis MO, USA). The stock solution (10 mM) of these chemicals was prepared in distilled water. The stock solution was refrigerated and required dilutions were made in Krebs – Ringer solution just before the experimentations. The concentration used in experiments was 10 µM for Tamoxifen and 100 µM for all other antagonists.

Experimental protocol

There were three sets of experiments. Initially tissue was allowed to stabilize for 30 min and control recording was taken. In first set after stabilization, tissue was exposed to cumulative concentration of BPA (1, 10, 30, 100 µm), to assess the cumulative dose response of BPA. For each concentration, tissue was exposed to BPA for 10-15 minutes, followed by exposure to next higher concentration, without wash. In second set the tissue was exposed to equi-volumes of ethanol present in respective BPA concentrations. In third set, the gut tissue was exposed to one of four antagonists in different subsets for 15 min & subsequently it was exposed to BPA at concentration of 100 µm for 15 min. At the end of each experiment the tissue segment was removed, blotted and weighed.

Parameters studied and statistical analysis

Parameters studied were contractile tension and frequency**.** The tissue strips were subjected to initial tension of 0.50 gm. The maximum height of contractions were converted to tension (gram) with help of chart-5 software and then the tension so developed was expressed as tension per unit mass (g/g wet tissues) using the tissue weight determined at the end of the experiments. Frequency of contractions was calculated frequency per minute.

The values were then pooled to calculate Mean±SEM. The statistical significance of differences between mean values was determined by using paired *t*-test and unpaired *t*-test as applicable. One or two-way ANOVA were applied for multiple comparison depending on the requirement. A *p*-value of < 0.05 was considered statistically significant.

Results

Characteristics of contractions in normal recordings in ileum and colon of rat

The contractions observed in the ileum were phasic type (short lasting) and in colon were tonic type (long lasting). The Mean±SEM values of spontaneous contractions and frequency of contractions in control samples of ileum and colon were observed as in Table I.

TABLE I : Mean±SEM values of spontaneous contractions expressed as g/g wet tissue and frequency of contractions/min in ileum (n=6) and colon (n=6) of rats in untreated samples.

Parameters	*Untreated samples*	
	Ileum	*Colon*
Contractile tension (g/g wet tissue)	10.13±1.78	11.31±2.48
Frequency (contractions/min)	18.17±0.98	1.33±0.23

Effect of different concentrations (1, 10, 30 and 100 µM as cumulative doses) of BPA on spontaneous contraction in ileum tissue.

There was concentration dependent decrease in the maximum height of contractions after application of each concentration of BPA (Fig. 1a). There was significant ($p<0.05$, one way ANOVA), decrement in the response/tone at higher dose of BPA (30 and 100 µM); (Fig. 1b). There was a significant decrease in frequency when the concentration was increased to 30 and 100 µM of BPA (Fig. 1c). The frequency became zero, indicating no spontaneous contraction at 100 µM bath concentration (Fig. 1c).

Effect of different concentrations (1, 10, 30 and 100 µM as cumulative doses) of BPA on spontaneous contraction in colon tissue.

The effect of BPA on colon tissue was similar to ileum (Fig. 2a). On comparison of responses induced by different concentrations (1-100 mM) of BPA in colon and ileum, it was observed that there was continuous decrease in contractile tension with increasing concentration of BPA in colon (Fig. 2b), while in ileum response remains almost same up to

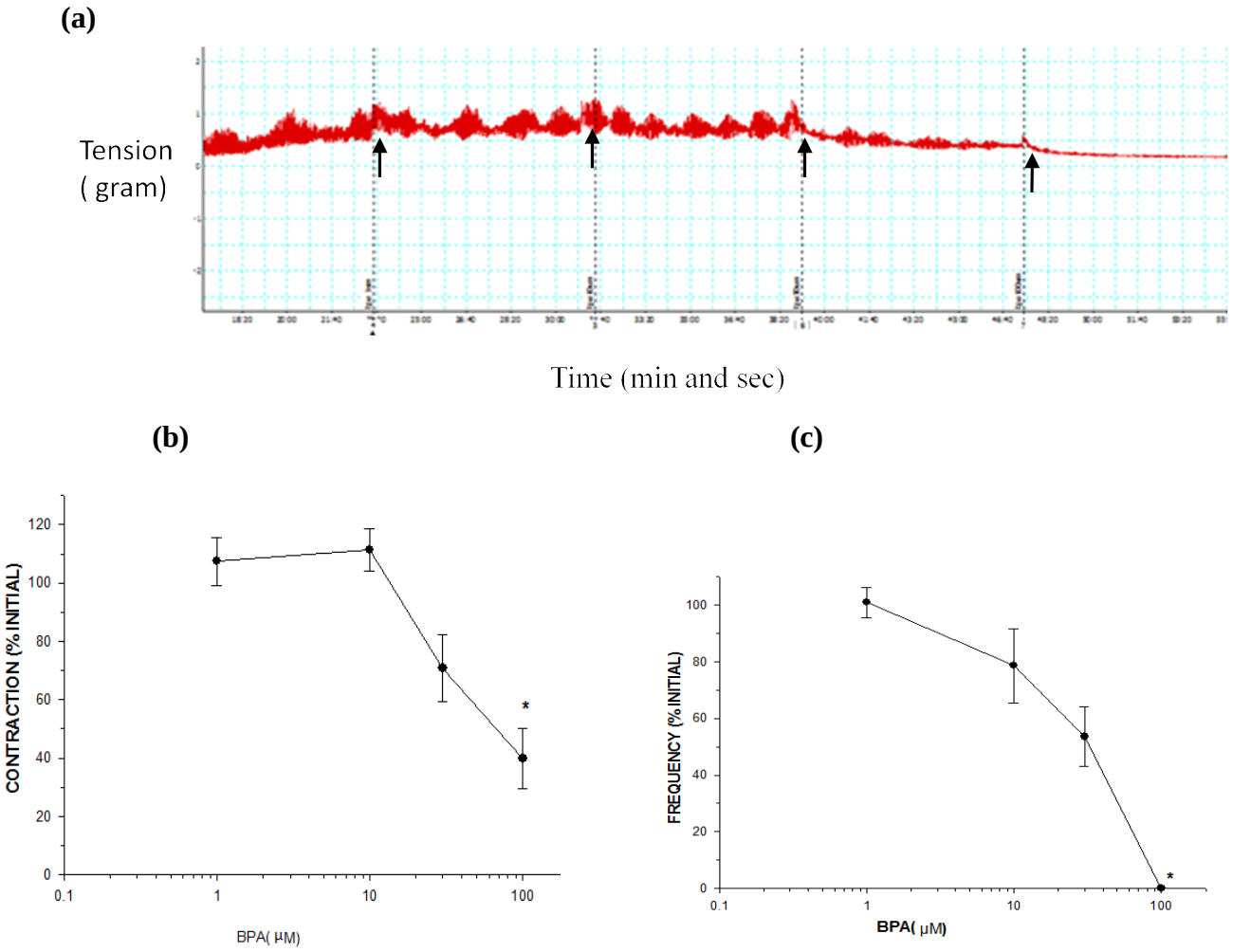

Fig. 1 : Above (a) showing original representative recording of dose response of ileum on application of different concentrations (1, 10, 30 and 100 mM) of BPA. Arrows indicate the point of application of BPA, vertical & horizontal calibrations represent the tension (gram) and time (min. and sec.) respectively. Below graphs showing the effect of BPA on contractile tension (b) and frequency (c) of contraction per min (Mean±SEM, n=6). The asterisk indicate significantly (p-value <0.05, one way ANOVA) different from previous concentrations.

10 mM concentration and then it decreased with 30 and 100 mM concentration. However, the frequency of contractions declined progressively and reached to zero at highest concentration (100 mM) of BPA in both (Fig. 1c, 2c).

Effect of Ethanol (Vehicle) on spontaneous contractions in ileum and colon

There was no statistically significant(p-value >0.05, one way ANOVA) change in contractions/tone as well as frequency of contractionswith different concentration of ethanol used to dissolve respective concentrations of BPA (Table II).

BPA (100 µM) response after pre-treatment of various antagonists

Pre-treatment of all antagonists viz. estrogen receptor antagonist tamoxifen, nitric oxide synthatase inhibitor L-NAME, ganglion blocker hexamethonium and atropine failed to block BPA (100 µM) induced depression in contractile responses in ileum (Fig. 2b) as well as colon (Fig. 3b), because there was no significant (p>0.05) difference between the response of BPA (100 µM)with or without pretreatment of antagonists (Fig. 3a, 3b). The frequency of contraction were not recordable in all the pre-treated groups, as the recording was flattened, therefore the

TABLE 2 : Mean±SEM values of contractile tension (g/g wet tissue) and frequency of contractions (per min) expressed as percentage of initial (i.e. control as 100%) and after application of different concentrations of ethanol (v/v%) from six experiments in rat ileum and colon. Please note that there was no significant($p>0.05$) change in contractility and frequency of contraction induced by various concentrations of ethanol.

	Ethanol (v/v %)									
	Control		*0.005*		*0.05*		*0.15*		*0.50*	
	Ileum	*Colon*	*Ileum*	*Colon*	*Ileum*	*Colon*	*Ileum*	*Colon*	*Ileum*	*Colon*
Contractions	100.00± 0.00	100.00± 0.00	118.79± 7.71	115.34± 7.37	119.63± 10.15	116.24± 10.05	118.36± 6.58	109.75± 6.49	114.66± 8.13	114.05± 7.40
Frequency/min	100±0	100±0	88±2	96±8	94±5	98±10	86±6	96±14	87±3	89±16

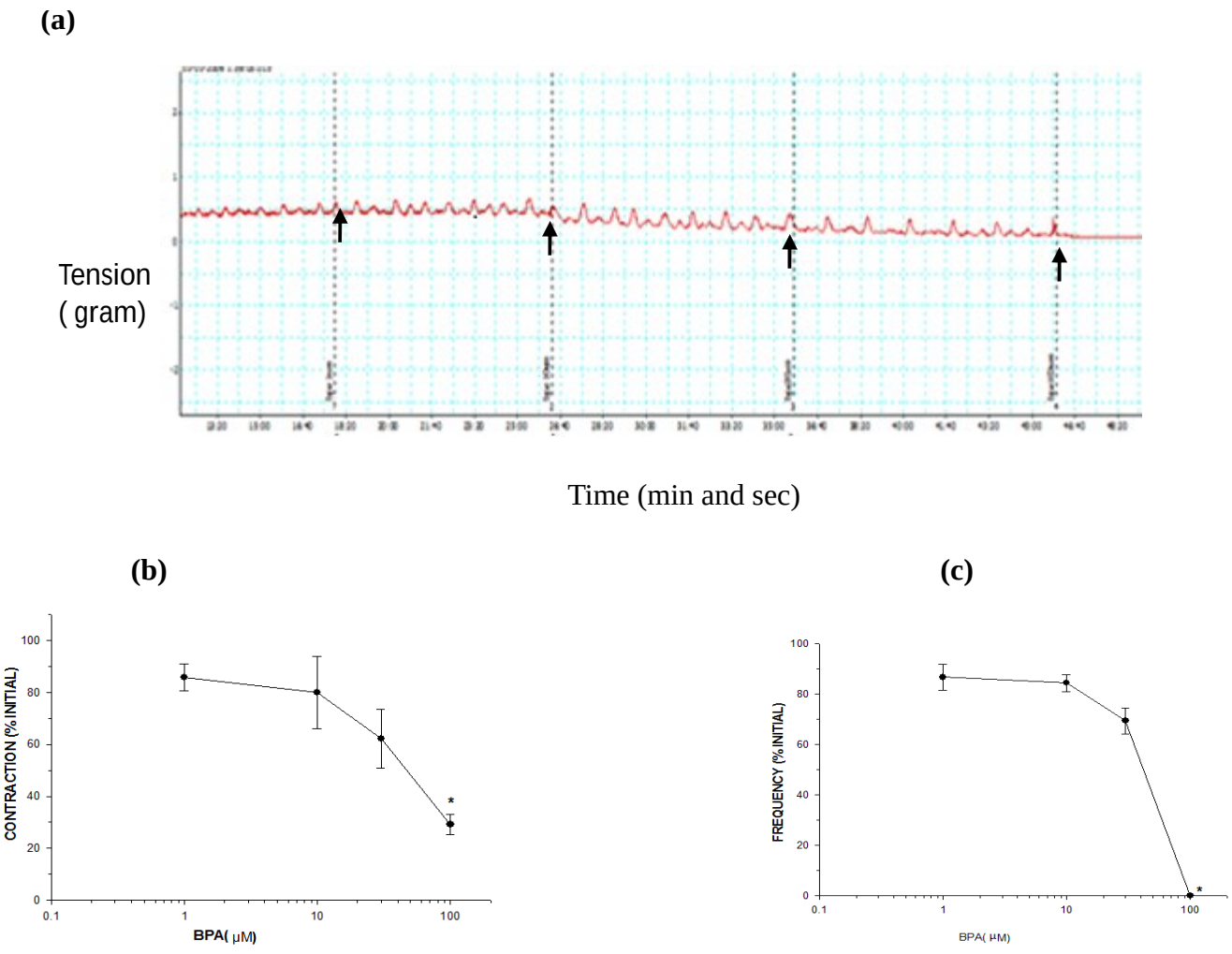

Fig. 2 : Representative recording (a), contractile tension (b), frequency (c) from colon issue (n=6), after application of different concentrations (1, 10, 30 and 100 mM) of BPA. Rest as described in Fig. 1.

frequency was considered zero after the addition of BPA 100 µM.

Discussion

The present study clearly demonstrated that BPA decreased contractile tension as well as frequency of spontaneously occurring contractions in segments of both small and large intestine (ileum and colon) of rats. It was apparent that the inhibition was by per se action of BPA and not the vehicle (i.e. ethanol), because the vehicle control experiments clearly

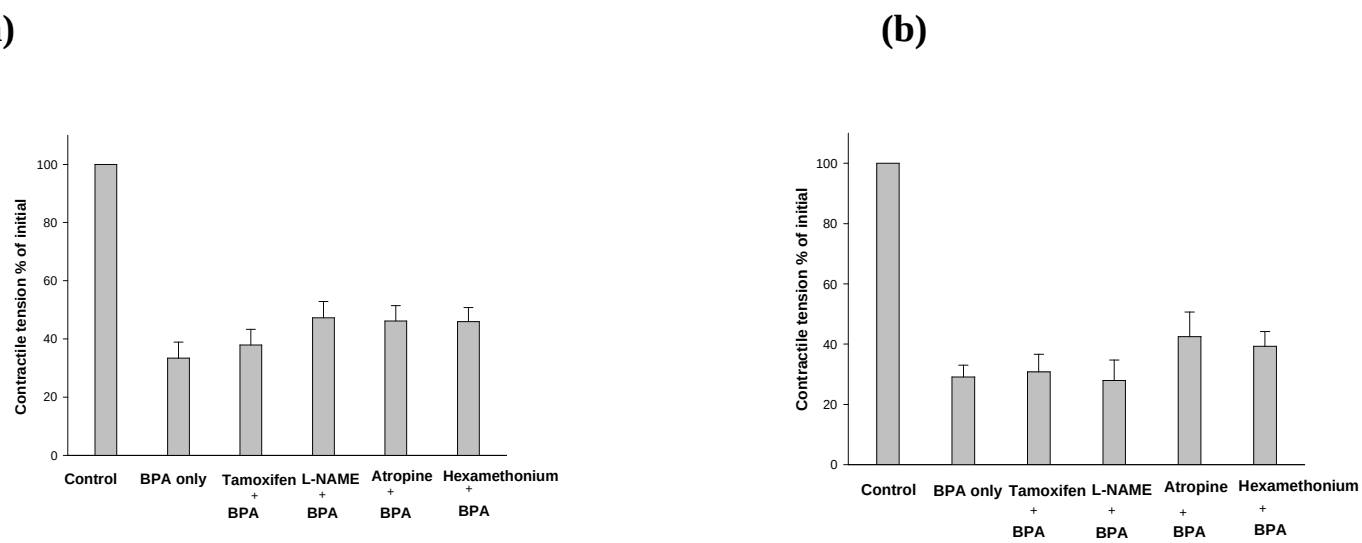

Fig. 3 : Bar diagrams showing (Mean±SEM, n=6) the contractile tension in ileum (a) and colon (b) after addition of BPA only (100 µM) and addition of BPA after treatment with L-NAME(100 µM), Tamoxifen (10 µM), Atropine (100 µM) and Hexamethonium (100 µM), expressed as percentage of initial.

showed that the amount of ethanol used for dissolving various concentration of BPA, in the present study, did not alter the smooth muscle contractile activity significantly.

BPA level has been detected in human urinary samples in several studies (4, 5). BPA concentrations used in our experiments are higher than these human urinary BPA levels. The various doses (1-100 µM) were used to assess the dose response of BPA and its toxicity, as used in studies carried out in other tissues in *in-vitro* experiments (9, 12).

It was observed that the depression of contractile functions was characterized by reduction of both contractile tension and frequency. The observation, therefore, signified that BPA might affect contractile machineries for reduced tension generated in intestinal smooth muscle and also interstitial cells of Cajal for observed change in the frequency of contractions.In the present study, an attempt has been made to assess the mechanisms of BPA induced inhibition of intestinal smooth muscle contractions. BPA is known to have estrogen like action (15) and estrogen has been found to impair contractile activity of gut muscle (16). Therefore, the decreased contractile functions of gut observed in the present experiments may be attributed to the estrogen like activity of BPA. Estrogen is known to act via two types of estrogen receptors (ER), namely ERá and ERâ. The ERâ is known to be expressed in intestine (17). However, in our study the estrogen receptor antagonist tamoxifen failed to block the BPA induced inhibitory response, thus indicating the ER-independent mechanisms for the action of BPA. It was postulated that the BPA might mediate its action via neural elements in enteric nervous system. However, the experiments using ganglion blocker hexamethonium discarded this proposition too. Further, BPA has been found to depress the atrial activity through nitric oxide (NO) mechanisms (9). In order to evaluate the involvement of NO mechanisms, some experiments with nitric oxide synthase inhibitor, L-NAME were also carried out in this study. It was observed that prior application of L-NAME failed to protect BPA-induced contractile impairment, thus, suggesting non-involvement of NO mechanisms in mediating the BPA induced attenuation of contractile responses.However a recent study reported that BPA inhibits the movement of the duodenum through NO mediated mechanisms (12). Further, any cholinergic involvement also was excluded by experiments having pre-treatment with atropine.

Thus, it appeared that the inhibitory response of BPA was brought about by its ER-independent action on intestinal smooth muscle. Similar ER-independent action of estrogen through activation of potassium

channels or inhibition of calcium channels has been proposed earlier (18). However, it is not possible to confirm if similar mechanisms are responsible for inhibitory action of BPA in the present investigation.

It may be concluded that, BPA diminish the contractile functions of small and large intestine in rats. The inhibitory mechanisms seem to be operating without involvement of estrogen receptors, nitric oxide and intrinsic neural plexuses. The exact mechanisms for the reduced contractile activity could not be ascertained from the present experiments. Further, the BPA-induced impaired contractile function of intestine is likely to have clinical implications like constipation and other gastrointestinal motility disorders and warrants further critical evaluation.

Referances

1. Vom Saal FS, Meyers JP. Bisphenol A and risk of metabolic disorders. *JAMA* 2008; 300(11): 1353–1355.
2. Erickson BE.. Bisphenol A under scrutiny. Chemical and Engineering News. *Am Chem* Soc 2008; 86(22): 36–39.
3. Fielding M, Fawell JK, Franklin GS. Exposure to endocrine disruptors via materials in contact with drinking water. 1999 Report No: DETR/DWI 4746. WRc plc, Marlow, Bucks
4. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. *Environ Health Perspect* 2008; 116: 39–44.
5. Zhang Z, Alomirah H, Cho HS, Li YF, Liao C, Minh TB, Mohd MA, Nakata H, Ren N, Kannan K. Urinary Bisphenol A Concentrations and Their Implications for Human Exposure in Several Asian Countries. *Environ Sci Technol* 2011; 15; 45(16); 7044–7050.
6. Matsumoto H, Adachi S, Suzuki Y. Bisphenol A in ambient air particulates responsible for the proliferation of MCF-7 human breast cancer cells and its concentration changes over 6 months. *Arch Environ Contam Toxicol* 2005; 48: 459–466.
7. Patisaul HB, Todd KL, Mickens JA, Adewale HB. Impact of neonatal exposure to the ERá agonist PPT, bisphenol-A or phytoestrogens on hypothalamic kisspeptin fiber density in male and female rats. *Neurotoxicology* 2009; 30: 350–357.
8. Asano S, Tune JD, Dick GM. Bisphenol A activates Maxi-K (K(Ca)1.1) channels in coronary smooth muscle. *Br J Pharmacol* 2010; 160: 160–170.
9. Pant J, Ranjan P, Deshpande SB. Bisphenol A decreases atrial contractility involving NO-dependent G-cyclase signaling pathway. *J Appl Toxicol* 2011; 31(7): 698–702.
10. Braniste V, Jouault A, Gaultier E at al. Impact of oral bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats. *PNAS* 2010; 107: 448–453.
11. Sawai C, Anderson K, Walser-Kuntz D. Effect of bisphenol A on murine immune function: Modificattion of interferon-γ, IgG2a, and disease symptoms in NZB × NZW F1 mice. *Environ Health Perspect* 2003; 111(16): 1883–1887.
12. Sarkar K, Tarafder P, Paul G. Bisphenol A inhibits duodenal movement ex vivo of rat through nitric oxide-mediated soluble guanylyl cyclase and α-adrenergic signaling pathways. *J of Applied Toxicology* 2015 April 16; 36: 131–139.
13. Tyagi P, Mandal MB, Mandal S, Patne SCU, Gangopadhyay AN. Pouch colon associated with anorectal malformations fails to show spontaneous contractions but respond to acetylcholine and histamine in vitro. *J Paeditr Surg* 2009; 44: 2156–2162.
14. Singh S, Mandal MB. *In vitro* study of acetylcholine and histamine induced contractions in colon and rectum of adult and neonate rats. *Indian J Physiol Pharmacol* 2013; 57(2): 104–113.
15. Vom Saal FS, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nagel SC, *et al.* A physiologicallybased approach to the study of bisphenol A and other estrogenic chemicals on the size ofreproductive organs, daily sperm production, and behavior. *Toxicol Ind Health* 1998; 14(12): 239–260.
16. Heinemann A, Pieber D, Holzer P. Inhibition by female sex steroids of peristalsis in the guinea pig small intestine. *Digestion* 2002; 65(4): 213–219.
17. Gustafsson JA. Estrogen receptor beta—a new dimension in estrogen mechanism of action. *J Endocrinol* 1999 Dec; 163(3): 379–383.
18. Díaz M, Ramírez CM, Marin R, Marrero-Alonso J, Gómez T, Alonso R. Eur Acute relaxation of mouse duodenum [correction of duodenun] by estrogens. Evidence for an estrogen receptor-independent modulation of muscle excitability. *J Pharmacol* 2004 Oct 6; 501(1-3): 161–178.