National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Effects of chronic ingestion of Bisphenol A on gut contractility in rats

Devarshi Dixit, Sanjeev K Singh, Anil Kumar Tiwari, Maloy B Mandal

Department of Physiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence to: Maloy B Mandal, E-mail: maloy_mandal@yahoo.com

Received: May 14, 2017; **Accepted:** Jun 06, 2017

ABSTRACT

Background: Bisphenol A (BPA) is a plastic toxin widely used in manufacturing of plastic containers. It is known to produce a variety of toxic effects on body functions including reproduction, metabolism, and development. Despite the fact that the gastrointestinal tract is chronically exposed to food containing BPA, its effect on the gut motility is poorly understood. **Aims and Objectives:** The present study attempted to assess the small and large gut contractility *in vitro* in male rats fed with BPA-mixed food for 28 days. **Materials and Methods:** This study was carried out in 8 adult male albino rats of 150-200 g weight. One group (4 rats) was subjected to daily oral administration of BPA (50 µg/kg/day) for 28 days. Another group of 4 rats was Sham-fed and served as control. With the help of organ bath preparations and computerized data acquisition system, *in vitro* isometric contraction (spontaneous and agonist-induced) was recorded from small gut (distal ileum) and large gut (mid colon) segments. **Results:** Recordings of spontaneous contractions revealed significantly ($P < 0.05$) decreased contractile tension in small gut and a decrease in both tension and frequency in large gut of the BPA-fed group of rats. **Conclusion:** Chronic exposure of BPA depresses spontaneous and agonist-induced contractility of small and large gut of rats.

KEY WORDS: Bisphenol A; Contractility; Ileum; Colon; Acetylcholine; Serotonin; Histamine

INTRODUCTION

Bisphenol A (BPA) is extensively used in the production of polycarbonate plastics and epoxy resins. A variety of food and beverage containers including water bottle, baby feeding bottles, and food containers are made up of polycarbonate plastics.[1,2] Metal cans used for packing different types of foodstuff are lined with epoxy resins. Thus, there is widespread consumption of BPA-contaminated food and liquid in human beings. The National Health and Nutrition Examination Survey reported the detectable BPA levels in urine of more than 90% of US population.[3] BPA has been known to be an endocrine disruptor and having estrogen-like effects, resulting into reproductive and developmental impairments in laboratory animals.[4] Since main route of exposure to BPA is oral, therefore, the BPA-induced changes in the intestine cannot be ruled out. Some studies regarding the effects of BPA on gut barrier and immune responses are available.[5,6] A study suggested the inhibitory effects of BPA on duodenal movement. However, its effects on other part of small intestine and that on large intestine are not well documented. 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.

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.0518906062017	

MATERIALS AND METHODS

Animals and Groups

The present experiments were carried out on Charles-Foster strain of rats after the approval of Institutional Ethical Committee for animal experiments (Ref No. Dean/2015/CAEC/1426 dated 03-10 2015). The adult rats of either sex

weighing 150-200 g (4-6 months old) were procured from institute animal house. Rats were housed in the departmental animal room in an environment of controlled temperature (25 ± 0.5°C) and light (12:12 h light dark) with *ad libitum* supply of rat feed and potable water.

The present investigation was carried out on eight rats. They were divided into two groups. In one group of rats (n = 4) *in vitro* recording of contractility of gut was assessed after chronic exposure of BPA, that is, rats fed with BPA (50 µg/kg/day) for 28 days. The feed was prepared by adding BPA dissolved in olive oil in rat food pellets. *In vitro* contractility of gut was also assessed in the other group of rats (n=4) fed with food pellets containing only olive oil (without BPA) for same duration, that is, 28 days. This group served as control or sham-fed group.

Dissection of Animals

After 28 days feeding of BPA, overnight fasted rats were sacrificed by cervical dislocation. Abdomen was opened by midline incision and segments of distal ileum and proximal colon were dissected out quickly and cleaned by flushing out the intestinal content by fresh Krebs-Ringer solution. Thereafter, it was quickly placed in a Petri dish containing freshly prepared Krebs-Ringer solution bubbled with 100% oxygen. The segments (1-2 samples from each rat) of distal ileum and proximal colon were prepared to assess the contractility responses.

Mounting and Recording of Contractile Response

After cleaning the tissues, 1.5-2.0 cm segment of intestine was vertically placed in Krebs-Ringer solution filled organ bath (12 ml) maintained at 37 ± 1°C and continuously bubbled with 100% O_2. One end of the gut segment was fastened to a glass tube support and other end was fixed to a force transducer (MLT 0210, AD Instruments, Sydney, Australia) with an initial tension of 0.5 g and then left to equilibrate for 30 min with replacement of Krebs solution every 15 min. Spontaneously generated isometric contractions were amplified by bridge amplifier and digitized through an analog/digital interface (Power Lab 4/ST system) to acquire onto a personal computer. The contraction recordings were displayed and analyzed with the help of software Chart-5 for Windows (AD Instruments, Sydney, Australia). Calibration for the tension (0-5 g) was performed before and after recording the contractile responses. After stabilization, the initial recording was made for 30 min without any external chemical interventions.

In addition to the spontaneous contractions, various agonist (10 µM; acetylcholine [ACh], histamine, and serotonin) and antagonist (10 µM; atropine [Atrp], pheniramine, and ondansetron) were used to assess the mechanisms of contractile responses in BPA-fed rats.

After the recordings of contractile responses, 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 wet tissue was then weighed to express the contractile response per unit weight of tissue (g/g wet tissue). The details of recording procedure have been described earlier.[7,8]

Drugs and Solutions

BPA was obtained from HiMedia laboratories Pvt. Ltd., Mumbai, India. ACh, Atrp sulfate, histamine, serotonin, and creatinine sulfate monohydrate were procured from Sigma Chemical Co., St. Louis, Mo, USA. Pheniramine maleate was procured from Aventis Pharma Limited (Bangalore, India). Ondansetron hydrochloride was obtained from Cipla Limited, Mumbai, India. Krebs solution was prepared containing (in mM) NaCl = 137, KCl = 3.7, $CaCl_2$ = 1.02, $MgCl_2$ = 0.05, NaH_2PO_4 = 0.32, $NaHCO_3$ = 11.9, and glucose = 5. All the chemicals used were of analytical grade. Krebs-Ringer solution was prepared containing (in mM/L) 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 7.4 pH of the solution.

Analysis of Data

Amplitude and frequency of spontaneous gut contractions were measured. Amplitude was converted to gram tension using calibration. The tension developed per gm of tissue was also calculated. The data for each group were expressed as mean ± standard error mean. Student's t-test was used for comparison of mean values. A $P < 0.05$ was considered as statistically significant.

RESULTS

In this study, chronic exposure of BPA was evaluated in the rats after 28 days of BPA feeding (50 µg/kg/day). The effects of BPA on contractility (tension and frequency) of spontaneously contracting small gut and large gut segments were evaluated and various agonists/antagonists were used to assess the mechanisms of contractility.

Effects on Contractility of Ileum: Control versus BPA-fed Group

The spontaneous average contractile response in ileum control sample (n=15) was 10.54±1.22 g/g wet tissue (Figure 1a and e) and frequency was 12.13 ± 0.43 peaks/min (Figure 1a and f). In chronically BPA-fed ileum sample, spontaneous average contractile response was 6.71 ± 1.56 g/g wet tissue (Figure 1b and e) and frequency was 11.04 ± 1.29 min (Figure 1b and f). The contractile tension in chronically BPA-fed group was significantly lower ($P < 0.05$) as compared to

control (sham-fed) group. There was no significant change in frequency ($P > 0.05$) in BPA-fed group as compared to control group.

Effects on Contractility of Colon: Control versus BPA-fed Group

The spontaneous average contractile response in colon control sample ($n = 15$) was 6.29 ± 0.66 g/g wet tissue (Figure 1c and e) and frequency was 2.19 ± 0.53 min (Figure 1c and f). In chronically BPA-fed colon sample ($n = 12$), the spontaneous average contractile response was 3.16 ± 0.62 g/g wet tissue (Figure 1d and e) and frequency was 0.63 ± 0.13 min (Figure 1d and f). The contractile tension in chronically BPA-fed group was significantly decreased ($P < 0.05$) as compared to the control (sham-fed) group. There was statistically significant ($P < 0.05$, unpaired *t*-test) decrease in frequency and BPA-fed group as compared to sham-fed group (Figure 1d and f).

Effects of ACh with and without Atropine on Ileum in Sham-fed and BPA-fed Groups

In the ileum control group ($n = 5$), ACh (10 μM) only produced 205.32 ± 21.91% of initial tension and with pre-treatment of Atrp produced 145.09 ± 35.87% of initial

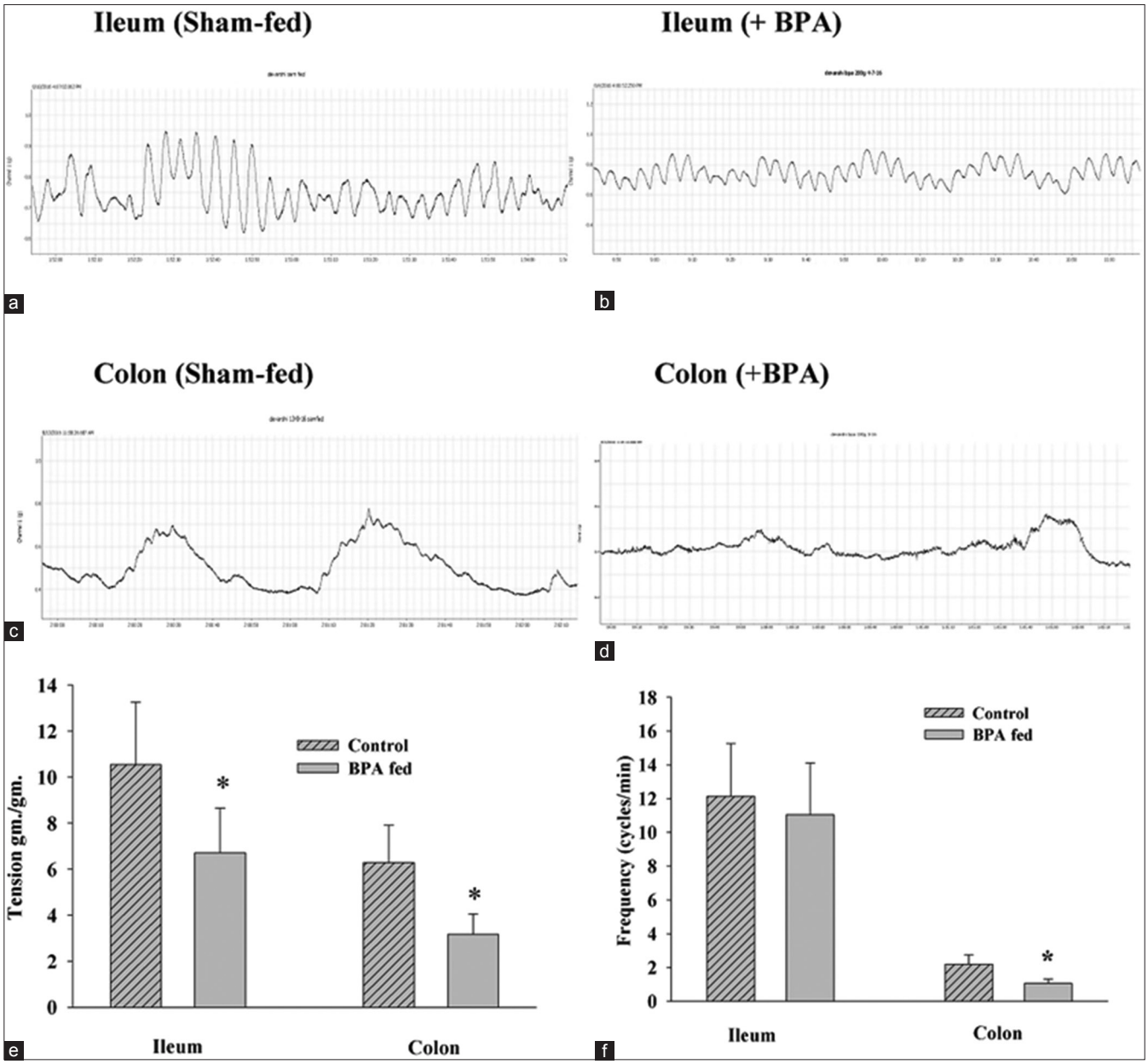

Figure 1: (a) Original tracings showing spontaneous contractions of ileum in Sham-fed group, (b) original tracings showing spontaneous contractions of ileum in bisphenol A (BPA)-fed group, (c) original tracings showing spontaneous contractions of colon in Sham-fed group, (d) original tracings showing spontaneous contractions of colon in BPA-fed group, (e) histograms showing contractile tension in g/g wet tissue in ileum and colon tissues, (f) histograms showing contractile frequency in ileum and colon tissue. An asterisk "*" indicates $P < 0.05$ for unpaired *t*-test

tension. The blockade response was statistically significant ($P < 0.05$; unpaired *t*-test; Figure 2a).

In BPA-fed group ($n = 5$) in the ileum tissue, ACh (10 μM) only produced 138.96 ± 11.08% of initial tension and with pre-treatment of Atrp produced 93.50 ± 5.76% of initial tension. The responses in the ACh only and Atrp pre-treated group are significantly different than each other ($P < 0.05$; unpaired *t*-test; Figure 2a).

In the ileum tissue, when the ACh-induced contractile response of control and BPA-fed groups were compared; it was observed that ACh-induced tension in BPA-fed group (39% of initial) was significantly lower ($P < 0.05$, unpaired *t*-test) than the ACh-induced tension in sham-fed group (105% of initial; Figure 2a).

In Atrp pre-treated groups, ACh-induced contractions were attenuated more in the BPA-fed group (100 %) than the sham-fed group (60%). While comparing the responses of both the groups, it was not found significant ($P > 0.05$, unpaired *t*-test; Figure 2a).

Effects of ACh with and Without Atropine on Colon in Sham-fed and BPA-fed Groups

In the colon control group ($n = 5$), ACh (10 μM) only produced 221.0 ± 54.9% of initial tension and with pre-treatment of Atrp produced 99.08 ± 4.00% of initial tension. The blockade response was statistically significant ($P < 0.05$; unpaired *t*-test; Figure 2b).

In BPA-fed group ($n = 5$) in the colon tissue, ACh (10 μM) only produced 192.8 ± 31.5% of initial tension and with pre-treatment of Atrp produced 112.3 ± 16.15% of initial tension. The Atrp blocked the ACh-induced responses significantly ($P < 0.05$; unpaired *t*-test; Figure 2b). There is no significant difference between the control and BPA-fed group responses (Figure 2b).

Effects of Serotonin with and without Ondansetron on Ileum in Sham-fed and BPA-fed Groups

In the ileum control group ($n = 5$), serotonin (10 μM) only produced 147.6 ± 16.6% of initial tension and with pre-treatment of ondansetron produced 135.5 ± 12.9% of initial tension. The blockade response was not significant ($P > 0.05$; unpaired *t*-test; Figure 3a).

In BPA-fed group ($n = 5$) in the ileum tissue, serotonin (10 μM) only produced 102.9 ± 2.6% of initial tension and with pre-treatment of Atrp produced 109.7 ± 4.4% of initial tension. The responses in the serotonin only and ondansetron pre-treated group are not different than each other ($P > 0.05$; unpaired *t*-test; Figure 3a).

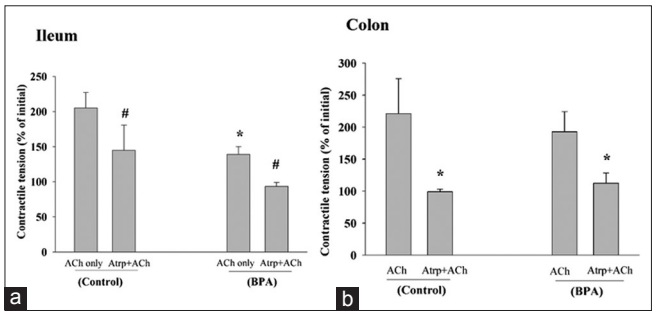

Figure 2: (a) Histograms showing the contractile tension (% of initial) of ileum developed after acetylcholine (ACh) with and without atropine (Atrp) pre-treatment in sham-fed (control) and chronically bisphenol A (BPA)-fed (50 μg/kg/day for 28 days) group of rats. Note that there is a significant ($P < 0.05$ unpaired t-test) decrease in ACh-induced contractile tension in chronically BPA-fed groups as compared to sham-fed group of rats (shown by asterisk). In both the groups of rats, Atrp caused blockade of ACh-induced contractions significantly ($P < 0.05$, unpaired t-test) as indicated by #, (b) histograms showing the contractile tension (% of initial) of colon developed after ACh with and without Atrp pre-treatment in sham-fed (control) and chronically BPA-fed (50 μg/kg/day for 28 days) group of rats. Note that there is no significant ($P > 0.05$, unpaired t-test) decrease in Ach-induced contractile tension in chronically BPA-fed groups as compared to sham-fed group. In both the groups, Atrp caused blockade of Ach-induced contractions significantly ($P < 0.05$, unpaired t-test) as shown by asterisk

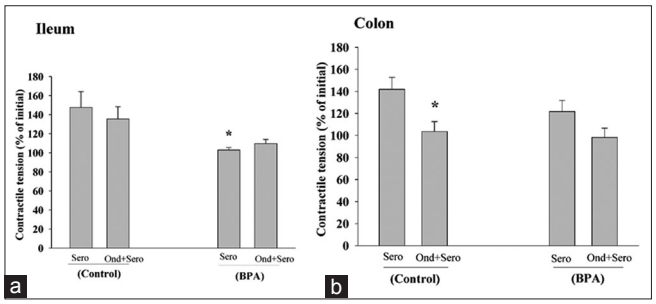

Figure 3: (a) Histograms showing the contractile tension (% of initial) of ileum developed after serotonin with and without ondansetron pre-treatment in sham-fed (control) and chronically bisphenol A (BPA)-fed (50 μg/kg/day for 28 days) group of rats. Note that there is a significantly decrease in serotonin-induced contractile tension in chronically BPA-fed groups as compared to sham-fed group of rats ($P < 0.05$ unpaired *t*-test) as shown by asterisk. Ondansetron failed to block the serotonin-induced contractions, either in control or in BPA-fed rats, (b) histograms showing the contractile tension (% of initial tension) of colon developed after serotonin with and without ondansetron pre-treatment in sham-fed (control) and chronically BPA-fed (50 μg/kg/day for 28 days) groups. Note that there is a significant blockade ($P > 0.05$, unpaired t-test, indicated by asterisk) by ondansetron in control group but no significant blockade in BPA-fed group

In the ileum tissue, when the serotonin-induced contractile response of control and BPA-fed groups were compared; it was observed that serotonin-induced tension in BPA-fed group (103% of initial) was significantly lower ($P < 0.05$, unpaired *t*-test) than the serotonin-induced tension in sham-fed group (147% of initial; Figure 3a).

There is no significant difference between the control and BPA-fed group responses (Figure 3a).

Effects of Serotonin with and without Ondansetron on Colon in Sham-fed and BPA-fed Groups

In the colon control group (n = 5), serotonin (10 μM) only produced 141.9 ± 10.9% of initial tension and with pre-treatment of ondansetron produced 103.6 ± 8.9% of initial tension. The ondansetron blocked the serotonin-induced response significantly ($P < 0.05$; unpaired t-test; Figure 3b).

In BPA-fed group (n = 5) in the colon tissue, serotonin (10 μM) only produced 121.8 ± 9.9% of initial tension and with pre-treatment of ondansetron produced 98.14 ± 8.52% of initial tension. The responses in the serotonin only and ondansetron pre-treated group are not different than each other ($P > 0.05$; unpaired t-test; Figure 3b).

There is no significant difference between the control and BPA-fed group responses (Figure 3b).

Effects of Histamine with and without Pheniramine on Ileum in Sham-fed and BPA-fed Groups

In the ileum control group (n = 5), histamine (10 μM) only produced 102.2 ± 7.12% of initial tension and with pre-treatment of pheniramine produced 89.8 ± 4.7% of initial tension. There is no significant effect of histamine in control group without pheniramine pre-treatment group. Pheniramine application reduced the tone below initial tension which is statistically significant ($P < 0.05$, unpaired t-test; Figure 4a).

In BPA-fed group (n = 5) in the ileum tissue, histamine (10 μM) only produced 103.2 ± 9.4% of initial tension and with pre-treatment of pheniramine produced 109.8 ± 16.4% of initial tension. The responses in the serotonin only and ondansetron pre-treated group are not different than each other ($P > 0.05$; unpaired t-test; Figure 4a).

There is no significant difference between the control and BPA-fed group responses (Figure 4a).

Effects of Histamine with and without Pheniramine on Colon in Sham-fed and BPA-fed Groups

In the colon control group (n = 5), histamine (10 μM) only produced 67.5 ± 13.2% of initial tension and with pre-treatment of pheniramine produced 76.1 ± 19.16% of initial tension. The pheniramine did not block the histamine-induced responses (Figure 4b).

In BPA-fed group (n = 5) in the colontissue, histamine (10 μM) only produced 36.6 ± 7.9% of initial tension and

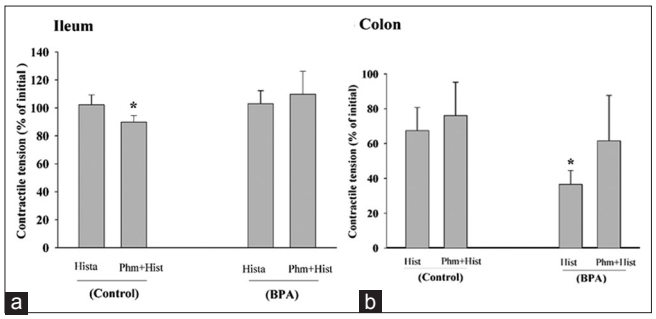

Figure 4: (a) Histograms showing the contractile tension (% of initial) of ileum developed after histamine with and without pheniramine pre-treatment in sham-fed (control) and chronically bisphenol A (BPA)-fed (50 μg/kg/day for 28 days) group of rats. Note that there is a decrease in histamine-induced contractile tension and pheniramine pre-treated histamine-induced contractile response in chronically BPA-fed groups as compared to sham-fed group of rats ($P < 0.05$ for unpaired t-test) as shown by asterisk. In the BPA-fed groups of rats, pheniramine did not cause blockade of histamine-induced contractions, (b) histograms showing the contractile tension (% of initial tension) of colon developed after histamine with and without pheniramine pre-treatment in sham-fed (control, n = 5) and chronically BPA-fed (50 μg/kg/day for 28 days, n = 3) group. Note that there is significant ($P < 0.05$, unpaired t-test, marked by asterisk) decrease in histamine-induced contractile tension in chronically BPA-fed group as compare to sham-fed groups. Pheniramine failed to block the effect of histamine

with pre-treatment of pheniramine produced 61.59 ± 26.1% of initial tension. There was increase in tension by histamine after pheniramine application ($P < 0.05$, unpaired t-test; Figure 4b).

There is no significant difference between the control and BPA-fed group responses (Figure 4b).

DISCUSSIONS

The present study investigated the contractile activity of ileum (small gut) and colon (large gut) in the chronically BPA-exposed rats by feeding the BPA for 28 days. The observations revealed that, in the BPA-fed group, the tension and frequency of ileum and colon both were decreased in comparison to their respective control groups. This observation indicated that chronic exposure of BPA affects the intestinal smooth muscle contractile mechanisms.

So far, there is no study available describing the chronic effect of BPA on ileum and colon. However, it is known that estrogen may depress the motility in the gastrointestinal (GI) tract as discussed elsewhere.[9] Therefore, the effect of BPA observed in the present investigation may also be mediated through estrogen receptor since BPA mimics the action of estrogen.[4] Further, a study demonstrated that the sensitivity is increased for colorectal distention in BPA-treated rats.[5]

The effect of BPA on frequency of contraction in small gut and large gut was not uniform. The frequency was significantly

decreased in colon only, the ileum remained unaffected in this regard. This is an interesting observation because it depicts that small and large gut may be differentially affected by BPA and large gut appears to be more vulnerable to BPA-induced adverse effects. The frequency of spontaneous contractions is a primary function of interstitial cell of Cajal.[10] Injection of lethal dose of BPA (40 mg/kg body weight) produced acute toxicity manifesting as immediate respiratory arrest and hypotension followed by bradycardia.[11,12] Tamoxifen was seen to block the BPA-induced depression of compound action potential of frog sciatic nerve.[13] Considering these observations, we hypothesized that BPA-induced decrease in force or amplitude of contractions in GI muscles may be mediated through estrogen receptor.

In the present study, in small gut segment of control rats, ACh-induced contraction was increased two-fold from its initial value. On the other hand, in BPA-fed rats, the same ACh-induced contractile response was significantly less (66%) in comparison to the control group. Normally, ACh mediates its contractile actions through M_3 receptors and phospholipid (IP3/DAG) second messenger systems.[14,15] However, the present data could not ascertain the involvement of BPA affecting the intracellular cholinergic signaling mechanisms.

Atrp application in the present experiments inhibited the ACh-evoked response up to 60%. Atrp is known to only block the muscarinic receptors. However, it is interesting that there was 100% blockade by Atrp for ACh-induced reduced response in BPA-fed rats. It is possible that the alteration of ACh receptor might have taken place in neuronal plexus which are nicotinic type. Thus, it appears that, BPA might have reduced the ACh-mediated receptor (nicotinic type only) activity in neuronal plexus sparing the smooth muscle muscarinic receptor, so that Atrp could produce 100% inhibition of ACh-induced contraction in BPA-fed rats.

When the action of ACh was examined in large gut, it was evident that both sham-fed and BPA-fed rats responded similarly to ACh application and like small gut Atrp blocked the ACh-induced response in the large gut. Thus, it may be inferred that BPA treatment for 28 days did not affect the cholinergic mechanisms in large gut.

Serotonin is an important regulator of contractility in healthy gut. It is an amine and about 90% of body serotonin is present in enterochromaffin cells of gut mucosa.[16] It acts through serotonin subfamily $5HT_1$, $5HT_2$, $5HT_3$, and $5HT_{4-7}$ receptors. Among these receptors, $5HT_3$ mediate the contractile response in intestinal smooth muscle in rats.[17] In control rats, the increased response could not be reversed by pre-treatment with selective $5HT_3$ receptor antagonist.[18] In BPA-fed rats, the reasons for ineffectiveness of serotonin on small gut may be attributed to the BPA-induced changes on serotonin receptor activity.

In colon, serotonin increased the contractile tension in control rats only and was completely inhibited by ondansetron pre-treatment but serotonin had no significant effects on contractility in BPA-fed rats. Thus, the actions of BPA seemed to be same in both small gut and large gut segment (i.e., BPA might have changed the serotonin receptor activity). Further, the large gut contraction appeared to be by largely mediated through $5HT_3$ receptor but same might not be true for the small gut.

In the present investigation, small gut contractions were not influenced by the histamine application either in control or BPA-fed rats. While in large gut, there was significant reduction in both control and BPA-fed groups and the effect was more pronounced in BPA-fed rats. Thus, the actions of BPA on large gut appeared to potentiate the effect of histamine. This relaxing effect of histamine on colonic smooth muscle of rat could be reversed by antihistamine pheniramine. This indicated that histamine-mediated it relaxing effect through H_1 receptor in the present experiment. Histamine acts through H_1 receptor and causes smooth muscle contraction.[19] The potentiating effect of histamine action by BPA might play a crucial role in BPA-induced inflammatory process.[20] A recent report[21] indicated that BPA inhibits movements of rat duodenum through NO-mediated mechanisms. However, whether the similar NO mechanisms are involved in ileum and colon needs further investigation.

It is further emphasized that in view of extensive use of plastic containers for packaging of food and beverages in the modern era; the study is important and relevant. The present study, for the first time, revealed the effects of chronic exposure of plastic toxin BPA on GI motility that might lead to various motility disorders after long-term use of plastic food containers.

CONCLUSION

Chronic exposure of BPA depresses spontaneous and agonist-induced contractility of small and large gut of rats, possibly without involving cholinergic mechanisms. The findings may have implications in altered gut motility conditions including chronic constipation in people consuming foodstuff from plastic containers.

REFERENCES

1. vom Saal FS, Akingbemi BT, Belcher SM, Birnbaum LS, Crain DA, Eriksen M, et al. Chapel H bisphenol A expert panel consensus statement: Integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. Reprod Toxicol. 2007;24(2):131-8.
2. vom Saal FS, Myers JP. Bisphenol-A and risk of metabolic disorders. JAMA. 2008;300(11):1353-5.
3. 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(1):39-44.
4. Pflieger-Bruss S, Schuppe HC, Schill WB. The male reproductive system and its susceptibility to endocrine disrupting chemicals. Andrologia. 2004;36(6):337-45.
5. Braniste V, Jouault A, Gaultier E, Polizzi A, Buisson-Brenac C, Leveque M, et al. Impact of oral bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats. Proc Natl Acad Sci U S A. 2010;107(1):448-53.
6. Sawai C, Anderson K, Walser-Kuntz D. Effect of bisphenol-A on murine immune function: Modulation of interferon-gamma, IgG2a, and disease symptoms in NZB X NZW F1 mice. Environ Health Perspect. 2003;111(16):1883-7.
7. Tyagi P, Mandal MB, Mandal S, Patne SC, Gangopadhyay AN. Pouch colon associated with anorectal malformations fails to show spontaneous contractions but responds to acetylcholine and histamine *in vitro*. J Pediatr Surg. 2009;44(11):2156-62.
8. 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):104-13.
9. Heinemann A, Pieber D, Holzer P. Inhibition by female sex steroids of peristalsis in the guinea pig small intestine. Digestion. 2002;65(4):213-9.
10. Huizinga JD, Berezin I, Chorneyko K, Thuneberg L, Sircar K, Hewlett BR, et al. Interstitial cells of Cajal: Pacemaker cells? Am J Pathol. 1998;153(6):2008-11.
11. Pant J, Deshpande SB. Acute toxicity of bisphenol-A in rats. Indian J Exp Biol. 2012;50(6):425-9.
12. 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.
13. Pandey AK, Deshpande SB. Bisphenol A depresses compound action potential of frog sciatic nerve *in vitro* involving Ca(2)-dependent mechanisms. Neurosci Lett. 2012;517(2):128-32.
14. Ehlert FJ, Sawyer GW, Esqueda EE. Contractile role of M2 and M3 muscarinic receptors in gastrointestinal smooth muscle. Life Sci. 1999;64(6-7):387-94.
15. Ehlert FJ. Contractile role of M2 and M3 muscarinic receptors in gastrointestinal, airway and urinary bladder smooth muscle. Life Sci. 2003;74(2-3):355-66.
16. Gershon MD. Review article: Serotonin receptors and transporters-roles in normal and abnormal gastrointestinal motility. Aliment Pharmacol Ther. 2004;20 Suppl 7:3-14.
17. Chetty N, Irving HR, Coupar IM. Activation of 5-HT3 receptors in the rat and mouse intestinal tract: A comparative study. Br J Pharmacol. 2006;148(7):1012-21.
18. Kordasti S, Sjövall H, Lundgren O, Svensson L. Serotonin and vasoactive intestinal peptide antagonists attenuate rotavirus diarrhoea. Gut. 2004;53(7):952-7.
19. Breunig E, Michel K, Zeller F, Seidl S, Weyhern CW, Schemann M. Histamine excites neurones in the human submucous plexus through activation of H1, H2, H3 and H4 receptors. J Physiol. 2007;583:731-42.
20. Liao SL, Tsai MH, Lai SH, Yao TC, Hua MC, Yeh KW, et al. Prenatal exposure to bisphenol-A is associated with Toll-like receptor-induced cytokine suppression in neonates. Pediatr Res. 2016;79(3):438-44.
21. Sarkar K, Tarafder P, Paul G. Bisphenol-A inhibits duodenal movement *ex vivo* of rat through nitric oxide-mediated soluble guanylyl cyclase and a-adrenergic signaling pathways. J Appl Toxicol. 2016;36:131-9.

How to cite this article: Dixit D, Singh SK, Tiwari AK, Mandal MB. Effects of chronic ingestion of Bisphenol A on gut contractility in rats. Natl J Physiol Pharm Pharmacol 2017;7(10):1109-1115.

Source of Support: Nil, **Conflict of Interest:** None declared.