Bisphenol A Inhibits The Motor Function Of Duodenal Smooth Muscle In Rat

Kaushik Sarkar, Panchali Tarafder, Partha Pratim Nath, Mukti Mondal, Goutam Paul

***Abstract*—The aim of the present study was to examine the effect of BPA (50mg/kgBW/day for 20 and 30 days exposure period) on the movement of duodenum in rat model. It was observed that BPA significantly depresses the amplitude and frequency of duodenal movements in test animals of both exposure groups. Besides, the activity of acetylcholinesterase (AChE) of duodenal tissue homogenate was also increased significantly in test animals. From the results it is suggested that BPA inhibits the movement of duodenum presumably by inducing the activity of AChE in smooth muscle membrane. We also found significant facilitation in nitric oxide synthase (NOS) expression and increase in deposition of calcium salts in Von Kossa's stained duodenal smooth muscle tissue section in test animals. From the results we may suggest that BPA may inhibit the duodenal movement by facilitating the synthesis of nitric oxide (NO) and decreasing the availability of free Ca^{2+} in duodenal smooth muscle cells. In order to study the BPA induced oxidative stress in duodenal smooth muscle, we have measured the oxidative stress indices in duodenal tissue homogenate. We found that BPA significantly decreases the activity of antioxidant enzymes. Thus, we may conclude that BPA inhibits the motor function of duodenal smooth muscle presumably by inducing oxidative stress, decreasing the availability of free Ca^{2+}, inducing the activity of AChE and promoting the synthesis of NO in duodenal smooth muscle cells.**

Keywords- BPA; NOS; AChE; Ca^{2+}; oxidative stress; duodenal smooth muscle.

I. INTRODUCTION

Bisphenol A (BPA) is an organic compound, rapidly used in the plastic industry. It is used commercially to produce polycarbonate plastics to make water and baby bottles, impact-resistant safety equipments, medical devices etc. and epoxy resins to make inner coating of the cans used to store food items and drinks (soft and heavy) [1-5]. It has been studied that humans are exposed to the BPA as a result of the consumption of BPA-tainted foods, water and drinks. Some dental sealants and composites may also play as important sources of BPA for human exposure [1,6,7]. The possible health hazards of BPA have been studied in some animal models as well as in humans.

It has been reported that BPA alters the function of coronary smooth muscle by activating Maxi-K (KCa.1.1) channels [8]. Besides, the studies of Pant J and associates have revealed that BPA causes depression of the atrial contractility in rat through NO-dependent guanylyl cyclase signaling pathway [9]. But the effect of BPA on the movement of intestine has not been reported before this study.

However, from the toxicological profile of BPA, it has been suggested that BPA induces oxidative stress. Previous studies demonstrated that BPA induces oxidative damages in the brain in male rats by generating reactive oxygen species (ROS) [10-12]. Chitra et al., 2003 have reported that BPA damages the epididymal cell in rats by causing oxidative damage [13]. Bindhumol et al., 2003 have shown that BPA reduces the activities of some antioxidant enzymes in liver mitochondria of rats [14]. But the effect of BPA on oxidative stress related variables in intestinal smooth muscle has not been studied till to date.

Therefore, the aim of the present study was to examine the effect of BPA on the motor function of intestine.

II. MATERIALS AND METHODS

A. Reagents and chemicals

All the common chemicals used for this study were of analytical grade. Bisphenol a (BPA ≥99%), acetylethiocholine iodide, reduced glutathione, DTNB, NADPH.Na_4, TCA, $K_2Cr_2O_7$, Tris, pyrogallol, NaCL, KCl, $MgCl_2$, $CaCl_2$, $NaHCO_3$, dextrose etc.were procured from SRL and EMerck, India.

B. Animal used for this study

Studies were carried out on 3-4 months old White Albino rats of the Sprague Dawley Strain weighing about 110-150 gm. Animals were maintained in Animal House as per recommendations of the Kalyani University Animal Ethics Committee. The animals were sacrificed by cervical dislocation on the 24th hr. after the completion of the last treatment.

C. Experimental design

Group 1	Animal received DMSO for 20 and 30 days-Vehicle control
Group 2	Animal received 50mgBPA/Kg/BW/day for 20 days – Treated 1
Group 3	Animal received 50mgBPA/Kg/BW/day for 30 days – Treated 2

D. Recording of intsetinal movement

For study of intestinal motility the rats were sacrificed by cervical dislocation after overnight fasting conditions; the abdomen was immediately opened and the duodenal segments (3cm each) were removed by transverse incision and were

DOI: 10.5176/2345-7201_1.2.21

used for recording of intestinal motility according to our standard laboratory protocol. After removing the duodenal segments it was placed in Tyrode's solution. Then the duodenal part was placed longitudinally in 40ml organ bath containing Tyrode's solution and continuously bubbled with 95 % O_2 and 5% CO_2, and temperature was maintained within a range of 37ºC ± 0.5. Therefore, continuous recording of duodenal movement was achieved with isotonic transducer (IT-2245) coupled to RMS-Polyrite-D (RMS, Chandigarh, India) [15].

E. Biochemical assays

The activities of AChE, superoxide dismutase (SOD), catalase (CAT) of duodenal tissue homogenate were assayed by the method of Ellman et al., 1961; Marklund and Marklund, 1974; and Sinha AK, 1972 respectively [16-18].

F. Histochemical study

Cytoplasmic calcium deposits in intestinal smooth muscle cells and extracellular spaces of muscle layer were detected by Von Kossa's staining techniques [19] and the expression of nitric oxide synthase (NOS) in duodenal smooth muscle layers were detected by the NAPH-diaphorase staining technique with slight modifications of the method used by Sandell, 1986 [20].

G. Statistical analysis

The data were expressed as mean± SEM of the value of each experimental group. Statistical comparisons were carried out by using Student's t-test. $p \leq 0.05$ was considered statistically significant.

III. Results and Discussion

A. Effect of BPA on duodenal movement in vitro of BPA treated rats

It has been observed that the mean amplitude of duodenal movement was decreased significantly in 20 days treatment duration and mean frequency was decreased significantly in 30 days treatment duration compared to vehicle control groups (Fig.1 and Table1).

Therefore, from the results, it is hypothesized that BPA depresses the duodenal motor function presumably by inhibiting the release of facilitatory neurotransmitters (NTs) (like ACh) or by inducing the inhibitory NTs (like norepinephrine, epinephrine, nitric oxide etc.) from the efferents of local Myenteric plexus innervating the duodenum. This finding supports our previous findings [15].

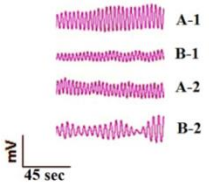

Figure 1: Representative records of the movement of isolated duodenum of vehicle control and BPA treated rats *in vitro*. A-1 and A-2: Records of the movement of duodenum of vehicle treated rats for 20 and 30 days durations. B-1 and B-2: Records of the movement of duodenum of BPA treated rats of 20 and 30 days durations.

Table 1: Showing the percent changes in amplitude and frequency of duodenal movements for 20 and 30 days exposure groups of rats. Value are represented as Mean±SEM (n=7), *p<0.05 *vs.* vehicle control.

Percent changes in amplitude and frequency of duodenal contraction in BPA treated rats				
Duration	Amplitude		Frequency	
	Vehicle control	BPA	Vehicle control	BPA
20 days	94.54±9.36	53.63±3.01*	99.99±5.98	99.99±6.04
30days	100.58±1.25	94.11±1.25	97.61±4.65	81.42±5.67*

B. Effect of BPA on AChE activity of duodenal tissue homogenate

BPA significantly increases the activity of AChE in both treatment groups (Table 2).

From the results it is suggested that the BPA induced inhibition of intestinal motor function might be due to the partial facilitation of ACh decay at the local synapse on to the duodenal smooth muscle in Myenteric plexus which support our previous findings [15].

Table 2: Showing the changes in activities of AChE, SOD, and CAT of duodenal tissue homogenate of 20 and 30 days exposure groups of rats. Value are represented as Mean±SEM (n=7), *p<0.05 *vs.* vehicle control.

AChE, SOD, and CAT activities of duodenal tissue homogenate of BPA treated rats				
	20 days		30 days	
	Vehicle control	BPA	Vehicle control	BPA
AChE (µmoles/min/mg protein)	41.90± 1.56	49.88± 1.13*	44.04± 1.33	58.36± 1.99*
SOD (Units/mg protein)	2.59± 0.33	1.57± 0.10*	2.83± 0.25	1.44± 0.31*
CAT (µmoles/min/mg protein)	61.90± 3.59	48.57± 2.23*	59.42± 2.05	44.18± 1.80*

C. Effect of BPA on the activities of SOD and CAT of duodenal tissue homogenate

The activities of SOD and CAT of duodenal tissue homogenate of BPA treated rats were decreased significantly compared to vehicle control group of rats (Table 2).

The decrease in activities of SOD and CAT signifies the generation of peroxy radicals O_2 in duodenal smooth muscle cells which leads to oxidative stress. SOD is an important defense enzyme which catalyzes the dismutation of superoxide radicals to H_2O_2 thereby reducing the activity of superoxide anions [21]. These superoxide anions also react with the NO to form peroxynitrite which leads to oxidative damages into the cells [22]. H_2O_2 is successively metabolized into water and non-reactive oxygen species by the activation of CAT (Mates et al., 1999). CAT decomposes the H_2O_2 into harmless products such as water and molecular oxygen [23]. H_2O_2 also produces the toxicity into the cells due to its formation of

reactive hydroxyl radical. From these results, the inhibition of CAT activity may leads to the depression of conversion of H_2O_2 to H_2O and molecular O_2, thus in turn produces the oxidative stress into duodenal smooth muscle cells.

D. Calcium salts deposits in duodenal smooth muscle layers in BPA treated rats

It has been observed that the deposition of calcium salts in submucosal and muscularis layers of Von Kossa's stained duodenal transverse sections were increased in duration dependent manner compared to vehicle control group of rats (Fig. 2).

From the results, it is suggested that the BPA induced deposition of calcium salts in the smooth muscle layers may be due to the facilitation of Ca^{2+} in the matrix of the smooth muscle with some Ca^{2+} binding protein like calmodulin, calsequestrin etc. As a result of the formation of Ca^{2+} chelate, the availability of the free Ca^{2+} to initiate excitation-contraction coupling is inhibited and thus, the muscle undergoes to relaxation instead of contraction which supports our earlier reports [15].

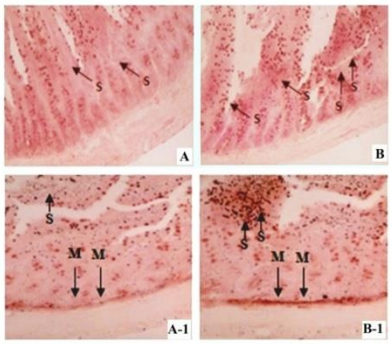

Figure 2: Representative photomicrographs of Von Kossa's stained duodenal tissue section (5µm) for 20 and 30 days exposure groups. A and A-1: Control sections (vehicle control) for 20 and 30 days durations. B and B-1: BPA treated sections for both 20 and 30 days durations. Arrow sign indicates the sites of calcium salts deposition. Submucosal and muscularis smooth muscle layers are indicated by alphabet 'S' and 'M'. Images were obtained by digital SLR Olympus Camera (E-620) fitted with Olympus light microscope (CH20i) (100X magnification).

E. NOS expression on duodenal smooth muscle layers of BPA treated rats

It has been observed that the expression of NOS enzyme in muscularis layers of NADPH-diaphorase stained duodenal transverse sections were increased in an exposure duration dependent manner (Fig. 3).

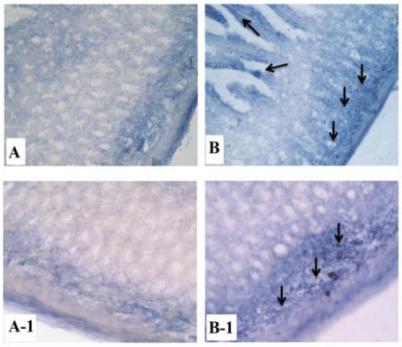

Figure 3: Representative photomicrographs of NADPH diaphorase-stained duodenal tissue sections (15µm) of rat for 20 and 30 days exposure groups. A and A-1: Control sections (vehicle control) for 20 and 30 days durations. B and B-1: BPA treated sections for both 20 and 30 days durations. Arrow sign indicates the sites of NOS expression in muscle layer of duodenal smooth muscle. Images were obtained by digital SLR Olympus Camera (E-620) fitted with Olympus light microscope (CH20i) (100X magnification).

From the results it is suggested that BPA may facilitate the synthesis of nitric oxide (NO) in Myenteric neurons presumably by inducing the synthesis of NOS which further leads to the inhibition of duodenal movement.

IV. CONCLUSION

It is concluded that the motor activity of duodenal smooth muscle is depressed by BPA presumably through the excitation of NO, released from Myenteric efferents on to the smooth muscle; shortening of the activity of the ACh as a result of the facilitation of the activity of AChE at ACh efferent-smooth muscle junction; reduction of the availability of free Ca^{2+} ; and promotion of the oxidative stress in smooth muscle cells.

V. ACKNOWLEDEGEMENT

Financial grant from University Grants Commission (UGC) [F.No.-36-260/2008 (SR) dated 27.03.2009] and PRG of Professor Goutam Paul (2014-2015) are gratefully acknowledged to carry out this study.

VI. REFERENCES

[1] J.E. Biles, T.P. McNeal, T.H. Begley, and H.C. Hollifield, "Determination of bisphenol-A in reusable polycarbonate food contact plastics and migration to food simulating liquids", J. Agric. Food. Chem. Vol. 45, pp. 3541-3544,1997.

[2] B.E. Erickson, "Bisphenol A under scrutiny. Chemical and Engineering News", American Chemical Society, vol. 86, no. 22, pp. 36-39, 2008.

[3] M. Biedermann, K. Grob, M. Bronz, R. Curcio, M. Huber, and F. Lopez-Fabal, "Bisphenol A-diglycidyl ester (BADGE) in edible-oil-containing canned foods: determination by LC-LC-fluorescence detection", MittGeb. Lebensmittelunters. Hyg., vol. 87, pp. 547-558, 1998.

[4] National Toxicology Program (NTP), "NTP Technical Report on the carcinogenesis bioassay of bisphenol A (CAS No. 80-05-7) in F344 rats and B6C3F1 mice (feed study)." NTP-80-35. NIH Publ. No. 82-1771. 1982.

[5] H. Inoue, G. Yuki, H. Yokota, and S. Kato, "Bisphenol A glucuronidation and absorption in rat intestine", Drug. Metab. Dispos., vol. 31, pp.140–144, 2003.

[6] N. Olea, R. Pulgar, P, Perez, F. Olea-Serrano F, A. Rivas, A. Novillo-Fertrell, V. Pedraza, A.M. Soto, and C. Sannenschein, "Estrogenicity of resin-based composites and sealants used in dentistry", Environ. Health. Perspect., vol. 104, pp. 298-305, 1996.

[7] R.M. Facciolo, R. Alo, M. Madeo, M. Canonaco, and F. Dessi-Fulgheri, "Early cerebral activities of the environmental estrogen bisphenol A appear to act via the somatostatin receptor subtype sst2", Environ. Health. Perspect., vol. 110, no. 3, pp. 397-402, 2002.

[8] S. Asano, J.D. Tune, and G.M. Dick, "Bisphenol A activates Maxi-K (K(Ca)1.1) channels in coronary smooth muscle", Brit. J. Pharmacol., vol. 160, pp. 160–170, 2010.

[9] J. Pant, P. Ranjan, and S.B. Deshpande, "Bisphenol A decreases atrial contractilityinvolving NO-dependent G-cyclase signaling pathway", J. Appl. Toxicol., vol. 31, no. 7, pp. 698-702, 2011.

[10] H. Kabuto, S. Hasuike, N. Minagawa, and T. Shishibori, "Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues", Environ. Res., vol. 93, no. 1, pp. 31-35, 2003.

[11] M. Aydogan, A. Korkmaz, N. Barlas, and D. Kolankaya, "The effect of vitamin C on bisphenol A, nonylphenol and octylphenol induced brain damages of male rats", Toxicol., vol. 249, no. 1, pp.35-39, 2008.

[12] Ke C, Liu X, Zuo H, Zhao J, Yang X, Yuan J (2013). The oxidative damage of bisphenol A on the organs of the mice. Health 5: 1190-1194.

[13] K.C. Chitra, C. Latchoumycandane, and P.P. Mathur, "Induction of oxidative stress by bisphenol A in the epididymal sperm of rats", Toxicol., vol. 185, pp. 119-127, 2013.

[14] V. Bindhumol, K.C. Chitra, and P.P. Mathur, "Bisphenol A induces reactive oxygen species generation in the liver of male rats", Toxicol., vol. 188, no. 2-3, pp.117-124, 2003.

[15] K. Sarkar, P. Tarafder, P.P. Nath, and G.Paul, "Bisphenol A Inhibits Duodenal Movement in Rat by Increasing Acetylcholinesterase Activity and Decreasing Availability of Free Ca^{2+} in Smooth Muscle Cells", Int. J. Pharm. Bio. Sci., vol. 4, no. 2, pp. (B) 679-688, 2013.

[16] G.L. Ellman, K.D. Courtney, V. Andres Jr, and R.M. Feather-Stone, "A new and rapid colorimetric determination of acetylcholinesterase activity", Biochem. Pharmacol., vol. 7, pp. 88–95, 1961.

[17] S. Marklund, and G. Marklund, "Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase", Euro. J. Biochem., vol. 47, no.3, pp. 469–474, 1974.

[18] A.K. Sinha, "Colorimetric assay of catalase", Anal. Biochem., vol. 47, no. 2, pp. 389-394, 1972.

[19] J.V. Kossa, "Ueber die im Organismus kuenstlich erzeugbaren Verkalkung. Ziegler's", Beitr. Path. Anat., vol. 29, pp.163-202, 1901.

[20] J.H. Sandell, "NADPH diaphorase histochemistry in the macaque striate cortex", J. Comp. Neurol., vol. 251, pp.388–397, 1986.

[21] J.M. Mates, C. Perez-Gomez, and I.N. De Castro, "Antioxidant enzymes and human diseases", Clin. Biochem., vol. 32, pp.595–603, 1999.

[22] A.C. Maritim, R.A. Sanders, and J.B. Watkins, "Diabetes, oxidative stress, and antioxidants: a review", J. Biochem. Mol. Toxicol., vol. 17, pp. 24-38, 2003.

[23] F. Lledı´as, P. Rangel, W. Hansberg, "Oxidation of catalase by singlet oxygen", J. Biol. Chem., vol. 273, no. 17, pp. 10630–10637, 1998.

AUTHORS' PROFILE

Professor (Dr) Goutam Paul is an excellent teacher, a distniguished scientist, and acclaimed author. Currently, he is the Senior Professor and Head of the Dept. of Physiology; and Dean of Science at the University of Kalyani, West Bengal, India. He teaches Physiology in UG, PG and PhD curriculum since 1988. He is the Prinicipal Investigator of various research projects in the Department of Physiology, Kalyani University in the filed of molecular neuro-toxicology and environmental physiology. He has already published more than 100 research articles, 08 books in the field of Enviornmental Science and Physiology, 05 book articles and 09 GenBank submissions till to date. He has supervised 08 PhD theses, 02 MPhil theses and many post graduate dissertations.

Dr. Kaushik Sarkar is an active reseracher. Currently, he is the Postdoctoral Research Fellow of Toxicology Unit of the Department of Physiology under the mentorship of Professor (Dr) Goutam Paul, Professor, Department of Physiology University of Kalyani, India. He did his PhD in the field of intestinal neurotoxicology as a Project Fellow of University Grants Commission, Govt. of India funded major research project. He has already published 12 research papers in peer reviwed journals, 12 conference proceedings and abstracts; and 02 GenBank submissions till to date. He has participated and presented papers in two international conferences. He has participated and presented the research paper orally in ARP-2014, GSTF held at Singapore, July, 2014.

Dr. Panchali Tarafder is an active reseracher. Currently, she is the Senior Research Fellow of Toxicology Unit of the Department of Physiology under the mentorship of Professor (Dr) Goutam Paul, Professor, Department of Physiology University of Kalyani, India. She did her PhD in the field of cardiovascular physiology as a SRF in DST-PURSE programme, Govt. of India. She has already published more than 08 research papers in peer reviwed journals, 12 conference proceedings and abstracts; and 02 GenBank submissions till to date. She has participated and presented research papers in various national and international conferences. She has participated and presented the research paper orally in ARP-2014, GSTF held at Singapore, July, 2014.

Mr. Partha Pratim Nath is an active reseracher. Currently, he is working as DST-INSPIRE Senior Research Fellow of Toxicology Unit of the Department of Physiology under the mentorship of Professor (Dr) Goutam Paul, Professor, Department of Physiology University of Kalyani, India. He is pursuing his PhD in the field of reproductive toxicology. He has published 05 research papers in peer reviwed journals, 03 conference proceedings and abstrcats. He has participated and presented the research paper orally in ARP-2014, GSTF held at Singapore, July, 2014.

Mrs. Mukti Mondal is an active reseracher. She is currently doing her PhD thesis as a DST-INSPIRE Junior Research Fellow of Toxicology Unit of the Department of Physiology under the mentorship of Professor (Dr) Goutam Paul, Professor, Department of Physiology University of Kalyani, India. She is pursuiing her PhD in the field of reproductive toxicology. She has already published 02 research papers in peer reviwed journals, 02 conference proceedings and abstrcats till to date.