

# Bisphenol A Depresses the Duodenal and Cardiac Movement through Nitric Oxide Mediated Guanylyl cyclase Signal Pathway

Goutam Paul, Kaushik Sarkar and Panchali Tarafder

**Abstract**— Humans are exposed to Bisphenol A (BPA) contaminated packaged drinking water, foods and beverages. The effects of BPA on the function of intestinal smooth muscle and cardiac myocytes have been examined in this study. The record of the movements of isolated intestine and perfused heart of toad in response to single dose acute exposure of BPA have been recorded with isotonic transducer coupled to the RMS Polyrite-D. We found significant depression of the movement of duodenum and heart in a dose dependent manner. Further, it was observed that sodium nitroprusside (SNP), a nitric oxide (NO) donor, significantly potentiated the BPA induced inhibition of duodenal and cardiac movement. However, methylene blue (MB), a guanylyl cyclase (GC) inhibitor, and N<sup>ω</sup>-nitro-L-arginine methyl ester hydrochloride (L-NAME), a nitric oxide synthase (NOS) inhibitor, significantly counteracted the BPA induced inhibition of duodenal and cardiac movement. Thus, we may conclude that BPA depresses the duodenal and cardiac movement through NO-mediated GC signal pathway.

**Keywords**- BPA; nitric oxide; intestine; heart; guanylyl cyclase

## I. INTRODUCTION

Bisphenol A (BPA) is a man-made organic compound with two phenol functional groups. It is the building block of several plastics and plastic additives. The commercial use of the monomeric form of BPA in the production of polycarbonate plastics and epoxy resins [1-6] and as a non-polymer additive to other plastics [7,8] is increasing enormously. Polycarbonate plastics due to its clarity and toughness has been in use to make various types of products like water bottles, baby feeding bottles, soft and hard drink containers etc. Besides, BPA is widely used to manufacture other plastic products like- eyeglass lenses, medical equipments, CDs, DVDs, electrical equipments, sports safety equipments and many more household appliances. Epoxy resin is used for lining metal cans to maintain quality of canned food and beverages due to its chemical resistance. However, recent studies have revealed that BPA leaches from the protective internal epoxy resin coatings of canned foods and from consumer plastic products into the food contents [9,10]. The degree of leaching of BPA from polycarbonate bottles into its content has been found to depend more on the temperature of the liquid or bottle, than the age of the container. Repeated washing of the bottle with harsh detergent also enhances the leaching process [11].

Widespread and continuous human exposure to BPA occurs mainly through dietary intake [12], with additional exposure

through dental sealant, dermal exposure and inhalation of indoor and outdoor dusts [13-15]. It exerts multi-system toxicity in animal models. The mode of action of BPA appears to mimic that of the female hormone estrogen. Therefore, BPA can be said as a man made endocrine disrupting chemical.

Moreover, it has been reported that BPA alters the function of coronary smooth muscle by activating Maxi-K (KCa1.1) channels [16]. 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 [17]. Further the study of Liang Q et. al. reported that BPA progressively increase sarcoplasmic reticulum (SR) Ca<sup>2+</sup> release and Ca<sup>2+</sup> reuptake and inhibit the L-type Ca<sup>2+</sup> current (I<sub>cal</sub>) in cardiac myocytes [18]. But, there is lack of information regarding the effect of BPA on the movement of heart in animal models.

However, it has already been reported that the gastrointestinal mucosae are directly exposed to BPA. But, the effect of BPA on the gastrointestinal motility has not been studied till to date. The gastrointestinal motility causes the movements of ingested food stuffs from stomach to intestine for digestion in systemic order and absorption of digested food stuffs from intestine to the intestinal blood vessels and lymphatic ducts. The principal motor of the intestinal wall is the smooth muscle, arranged circularly and longitudinally, in the muscular layer of the intestinal wall. The motor functions of the smooth muscle are locally controlled by Myenteric (Auerbach's) plexus, and centrally by sympathetic and parasympathetic efferent fibres that innervates the Myenteric central neural pool. In that case, there may be a chance of occurrence of BPA induced toxicity in intestinal motor function.

Therefore, the aim of the present study has been designed to examine the effect of BPA on the motor function of intestine and the movement of heart in animal models. Motor function of intestine and heart are mainly controlled by cholinergic, adrenergic and non-adrenergic non-cholinergic (NANC) pathways. Here, we only focused on the NANC mechanism in the control of smooth muscle and cardiac muscle function.

## II. MATERIALS AND METHODS

### A. Reagents and chemicals

All the common chemicals used for this study were of analytical grade. Bisphenol a (BPA  $\geq 99\%$ ),  $N_{\omega}$ -nitro-L-arginine methyl ester hydrochloride (L-NAME) were purchased from sigma chemicals co. (USA). Dimethyl sulfoxide (DMSO), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ( $MgCl_2$ ), calcium chloride ( $CaCl_2$ ), sodium bicarbonate ( $NaHCO_3$ ), sodium dihydrogen phosphate ( $NaH_2PO_4$ ), sodium nitroprusside (SNP), methylene blue (MB) etc. were procured from EMerck, India.

### B. Animal used for this study

Studies were carried out on 14-16 weeks old White Albino rats of the Sprague Dawley Strain weighing about 110-150 gm (for study of motor function of intestine) and adult male common Indian Toads (*Duttaphrynus melanostictus*) (for study of motor function of heart). Animals were maintained in Animal House as per recommendations of the Kalyani University Animal Ethics Committee. In the experimental set up, toad's heart was perfused to study the effects of intended drugs (BPA) on the motor function of heart *in vivo* in anaesthetized toad. Because, the perfusion set up in rat is very difficult due to non-sustainability of heart movement in perfused state for a long period required for the experiment under anesthesia.

### C. Recording of intestinal 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 used for recording of intestinal motility according to our standard laboratory protocol. After removing the duodenal segments it was placed in Tyrode's solution consisting of 8.0 g/l NaCl, 0.2 g/l KCl, 0.2 g/l  $CaCl_2$ , 0.1 g/l  $MgCl_2$ , 1.0 g/l  $NaHCO_3$ , 0.05 g/l  $NaH_2PO_4$  and 1.0 g/l Glucose (pH 7.4). The luminal content of each segment was gently flushed out. 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  $37^\circ C \pm 0.5$ . Therefore, continuous recording of duodenal movement was achieved with isotonic transducer (IT-2245) coupled to RMS-Polyrite-D (RMS, Chandigarh, India).

### D. Perfusion method of toad heart

The acute study was performed on perfused heart of toad according to our standard laboratory protocol. After the anesthetization of toad by pithing, the heart was perfused with the toad Ringer's solution (NaCl- 0.65gm%, KCl- 0.014gm%,  $CaCl_2$ - 0.012gm%,  $NaHCO_3$ - 0.02gm%,  $NaH_2PO_4$ -0.001gm%, Dextrose-0.2gm%, pH 7.4). The apex of the ventricle was hooked at one end of the transducer. The recording was taken in the monitor fitted with the RMS polyrite-D machine.

### E. Experimental design

The experimental set up for this study is given below-

Set 1-A	Application of graded doses of BPA (10, 20, 40 $\mu$ M) on duodenal segments.
Set-1-B	Application of equivalent doses of DMSO (2, 4, 8 $\mu$ M) on duodenal segments.*
Set-1-C	Application of doses of BPA on duodenal segments pre-treated with SNP (3.3 $\mu$ M for 5mins), MB (200 $\mu$ M for 10mins) and L-NAME (180 $\mu$ M for 10mins).
Set-2-A	Application of graded doses of BPA (10, 20, 40 $\mu$ M) on perfused heart of toad.
Set-2-B	Application of equivalent doses of DMSO (2, 4, 8 $\mu$ M) on perfused heart of toad.*
Set-2-C	Application of doses of BPA on perfused heart of toad pre-applied with SNP (3.3 $\mu$ M for 5mins), MB (200 $\mu$ M for 10mins) and L-NAME (180 $\mu$ M for 10mins).

\* DMSO was used as solvent of BPA in this study.

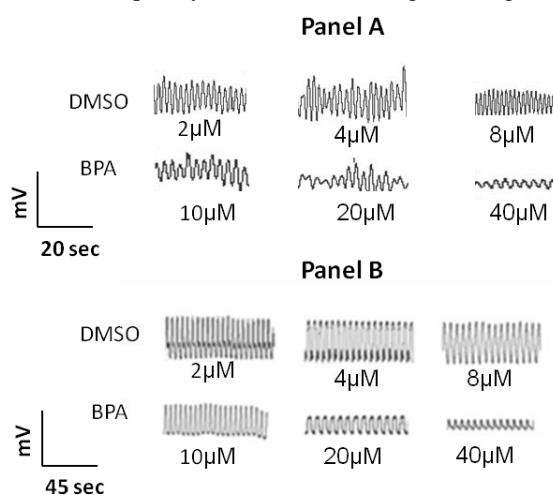
### F. Statistical analysis

The data were expressed as mean  $\pm$  SEM of the value of each experimental group. Force of contractions of duodenal and cardiac movements were measured in terms of amplitude and frequency. For analysis, the values of the treated preparations were expressed as percent change of the basal (or control) values. Statistical comparisons between the values obtained in control and in treated rats were carried out by using ANOVA followed by Student's t-test.  $p \leq 0.05$  was considered statistically significant.

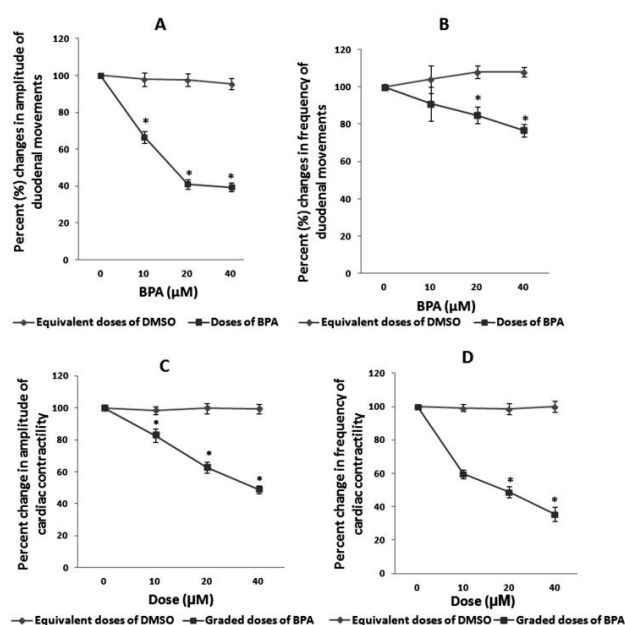
## III. RESULTS

### A. Effect of BPA on duodenal and cardiac movement of animals

After the application of graded doses of BPA (10-40 $\mu$ M) on duodenal segments (n=7) and perfused heart of toad (n=7), it has been observed that BPA dose dependently depresses the duodenal and cardiac movement significantly in terms of amplitude and frequency of contractions (Fig. 1 & Fig.2).



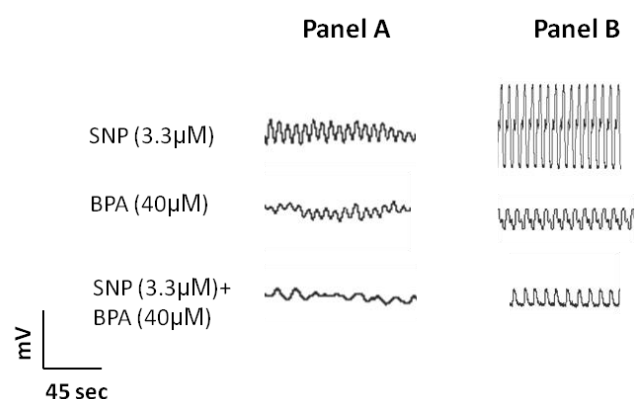
**Figure 1:** Representative records of movements of duodenum and heart after the application of graded doses of BPA. Panel A: Records of movement of isolated duodenum of rat after the application of DMSO and BPA. Panel B: Records of movement of perfused heart of toad after the application of DMSO and BPA.



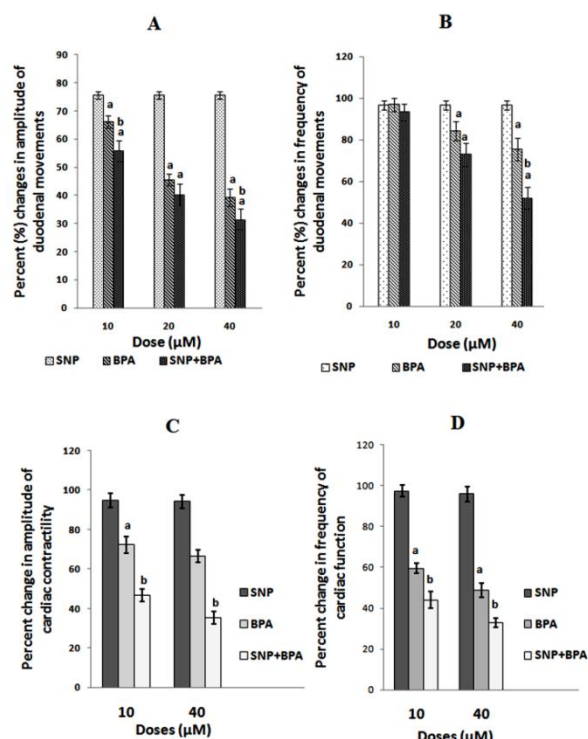
**Figure 2:** Showing the percent changes in amplitude and frequency of movement of duodenum and heart after application of graded doses of BPA. A & C: Percent changes in amplitude of movement of duodenum and heart after application of graded doses of BPA. B & D: Percent changes in frequency of movement of duodenum and heart after application of graded doses of BPA. Data were expressed as mean±SEM, n=7; \*p<0.05 vs. vehicle control (DMSO).

#### B. Effect of BPA on duodenal segments and perfused heart of toad pre-applied with SNP

From the results it was observed that SNP (n=5) significantly potentiated the BPA induced inhibition of duodenal and cardiac movement compared to BPA alone. Whereas, application of SNP alone initially depresses the movement slightly but the movement returns to the resting level after 2 minutes (Fig. 3 & Fig.4).



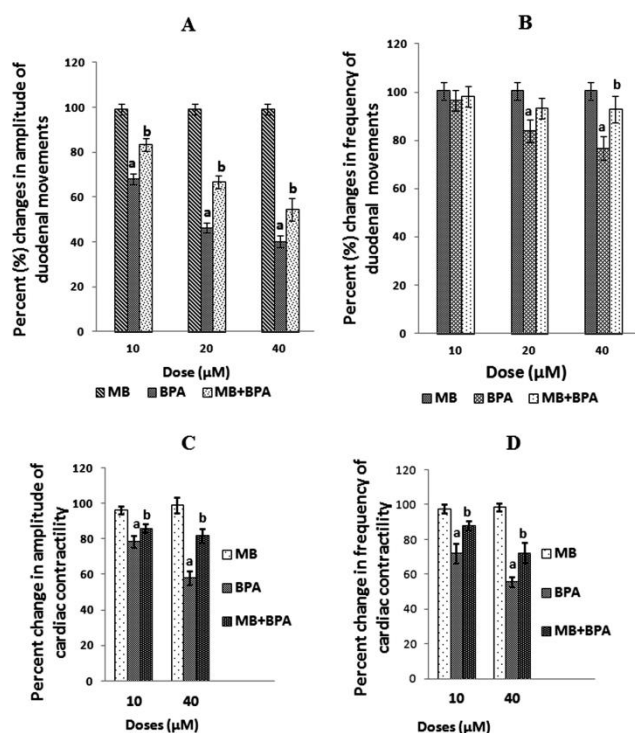
**Figure 3:** Representative records of the effects of BPA on movements of duodenum and heart after the application of SNP. Panel A: Records of the movement of duodenum. Panel B: Records of the movement of heart.



**Figure 4:** Showing the effect of BPA on isolated duodenum and perfused heart of toad when applied after the application of SNP. A & C: Percent changes in amplitude of movement of duodenum and heart after the application of graded doses of BPA pre-applied with SNP. B & D: Percent changes in frequency of movement of duodenum and heart after the application of graded doses of BPA pre-applied with SNP. Data were expressed as mean±SEM, n=7; \*p<0.05 vs. SNP; <sup>b</sup>p<0.05 vs. BPA.

#### C. Effect of BPA on duodenal segments and perfused heart of toad pre-applied with MB

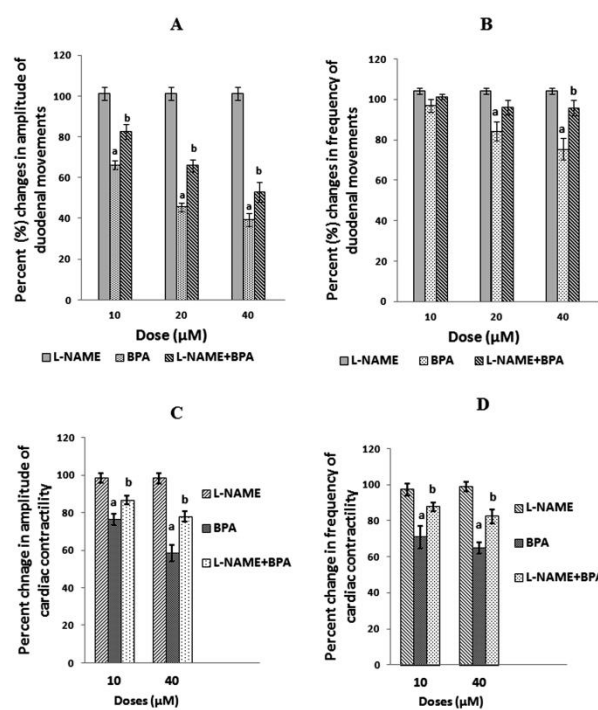
It has been observed that the application of the same dose of SNP in MB (the guanylyl cyclase inhibitor) pre-applied duodenal and cardiac preparations did not produce any significant inhibition of the movement. But the application of BPA in MB pre-incubated preparations (200 μM for 10 minutes) produced inhibition of the movement immediately after the application, but the movement of duodenum and heart were reversed significantly towards the resting level after 15 minutes of the application of BPA (Fig. 5 & Fig.7). Further, it was observed that the MB alone initially increases the duodenal and cardiac movement but the movement returned to the resting level after 1 minute.



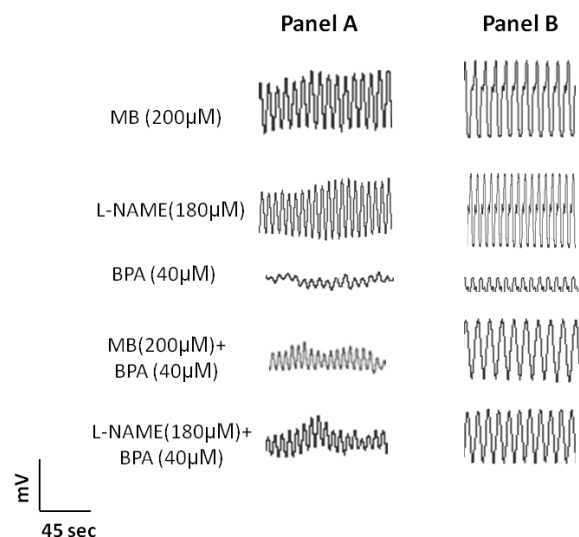
**Figure 5:** Showing the effect of BPA on isolated duodenum and perfused heart of toad when applied after the application of MB. A & C: Percent changes in amplitude of movement of duodenum and heart after the application of graded doses of BPA pre-applied with MB. B & D: Percent changes in frequency of movement of duodenum and heart after the application of graded doses of BPA pre-applied with MB. Data were expressed as mean $\pm$ SEM, n=7; \*p<0.05 vs. MB; ^p<0.05 vs. BPA.

#### D. Effect of BPA on duodenal segments and perfused heart of toad pre-applied with L-NAME

In order to confirm the involvement of NO pathway in BPA induced inhibition of duodenal and cardiac movements, the doses of BPA were applied after the application of L-NAME (the NOS inhibitor) on duodenal and cardiac preparations. It has been observed that the application of L-NAME (180  $\mu$ M) does not produce any significant changes in duodenal and cardiac movements. Further, the application of BPA in L-NAME pre-applied preparations (180 $\mu$ M for 10 minutes), the duodenal and cardiac movement were reversed significantly towards the resting level after 20minutes of the application of BPA (Fig.6 & Fig.7) compared to the movement obtained after the application of BPA alone.



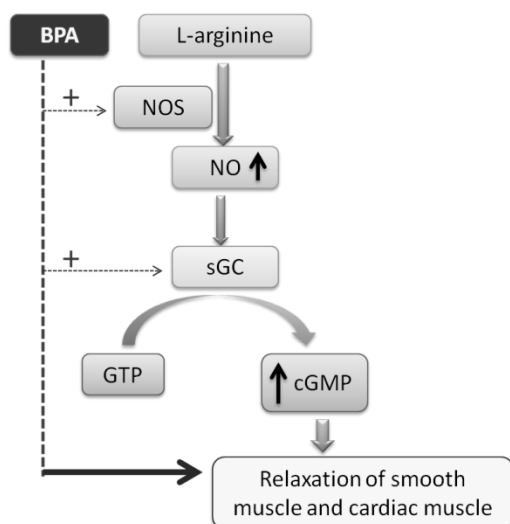
**Figure 6:** Showing the effect of BPA on isolated duodenum and perfused heart of toad when applied after the application of L-NAME. A & C: Percent changes in amplitude of movement of duodenum and heart after the application of graded doses of BPA pre-applied with L-NAME. B & D: Percent changes in frequency of movement of duodenum and heart after the application of graded doses of BPA pre-applied with L-NAME. Data were expressed as mean $\pm$ SEM, n=7; \*p<0.05 vs. L-NAME; ^p<0.05 vs. BPA.



**Figure 7:** Representative records of the effects of BPA on movements of duodenum and heart after the application of MB and L-NAME. Panel A: Records of the movement of duodenum. Panel B: Records of the movement of heart.

#### IV. DISCUSSION

From this study it has been observed that BPA dose dependently depresses the duodenal and cardiac movement. Results obtained from the study implies that NO is involved in the BPA induced inhibition of duodenal and cardiac movement. It has been studied that the neuronally generated NO diffuses to smooth and cardiac muscle cells which contain soluble guanylyl cyclase (sGC). sGC is an important NO-sensitive mediator in the signal transduction of smooth and cardiac muscle relaxation. The activated sGC catalyzes the conversion of GTP to cyclic GMP (cGMP), which in turn activates the cGMP-dependent protein kinase G (PKG). PKG subsequently stimulates the extrusion of intracellular  $\text{Ca}^{2+}$  by phosphorylating a number of important target proteins including ion channels, ion pumps, receptors and enzymes; thereby decreases the sensitivity of contractile apparatus to  $\text{Ca}^{2+}$  which leads to the muscle relaxation [19-25]. In this study, it has been observed that MB, the sGC inhibitor significantly counteracts the action of BPA induced inhibition of duodenal and cardiac movements. Further, from the results it was observed that L-NAME, the NOS inhibitor, also blocks the action of BPA-induced inhibition of duodenal and cardiac movements. From the results it is suggested that the BPA inhibits the duodenal and cardiac movements by inducing the release of NO from NANC neurons innervating the duodenal smooth muscle and cardiac myocytes; and the NO in turn produces the relaxation of muscle through guanylyl cyclase mediated signal pathway (Fig.8).



**Figure 8:** Probable mechanism of action of BPA in smooth and cardiac muscle relaxation.

#### V. CONCLUSION

From the results it is concluded that BPA inhibits the duodenal and cardiac movement presumably by inducing the relaxation of smooth muscle and cardiac muscle through nitric oxide-mediated guanylyl cyclase signal pathway.

#### VI. ACKNOWLEDGEMENT

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