Summary and Conclusions

6.1. Summary

Summary of the results are as follows-

a) It has been observed that, BPA (50mg/kgBW/day for 20 and 30 days) significantly inhibits the amplitude and frequency of duodenal movements in vitro of BPA treated rats in ante-mortem treatment experiment. From the results, it is hypothesized that BPA depresses the movement of duodenum presumably by inducing the release of inhibitory neurotransmitters (NTs) and/or by inhibiting the release of facilitatory NTs from the efferents of local Myenteric plexus innervating the duodenal smooth muscle.

b) In order to examine the effect of BPA on duodenal movement, if applied post-mortem as single dose acute exposures, the doses of BPA were applied in duodenal preparation in tissue organ bath. It has been observed that, BPA dose dependently depresses the amplitude and frequency of duodenal movement. This finding also suggests that BPA depresses the duodenal movements presumably by inducing the release of inhibitory neurotransmitters (NTs) and/or by inhibiting the release of facilitatory NTs from the efferents of local Myenteric plexus innervating the duodenum.

c) In order to examine the role of cholinergic and/or adrenergic efferents in the local Myenteric reflex in BPA induced inhibition of the duodenal movement, the doses of BPA were applied post-mortem after the application of cholinergic and adrenergic agonists and antagonists (i.e. ACh, adrenaline, atropine, propranolol and phentolamine) in duodenal preparations in tissue organ bath. It has been observed that, BPA induced inhibition of duodenal movement is not significantly counteracted by atropine (the cholinergic muscarinic receptor blocker) and propranolol (the β-adrenergic receptor...
blocker). But the BPA induced inhibition of the movement of duodenum partially counteracted by the phentolamine (the \( \alpha \)-adrenergic receptor blocker). Therefore, from the results it is suggested that the BPA induced inhibition of duodenal movements mediated partially by the activation of \( \alpha \)-adrenergic efferents of local Myenteric reflex arc innervating the duodenum.

d) In order to ascertain the role of non-adrenergic, non-cholinergic (NANC) influences in BPA induced inhibition of duodenal movement, the doses of BPA were applied post-mortem in NANC agonists and antagonists [i.e. sodium nitroprusside (SNP), methylene blue (MB) and \( N_\omega \)-Nitro-L-arginine methyl ester (L-NAME)] pre-incubated duodenal preparations in tissue organ bath. It has been observed that, SNP (the NO donor) potentiates the BPA-induced inhibition of movement of duodenum. Further, inhibition of the movement of duodenum by BPA was significantly counteracted by the MB (the guanylyl cyclase blocker) and L-NAME (the NOS blocker). Thus, it is suggested that BPA inhibits the duodenal movement presumably by inducing the release of nitric oxide (NO) from NANC efferent neurons of local Myenteric reflex arc innervating the duodenum through guanylyl cyclase mediated signal pathway.

e) In order to study the effect of BPA on gross body weight, absolute and relative weight of intestine, the body weight, absolute and relative weight of intestine were measured in BPA treated rats. No significant alterations in body weight, absolute and relative weight of intestine in BPA treated rats were observed. Therefore, it is suggested that BPA induced inhibition of duodenal movement may not be due to atrophy of the duodenum or atrophy of the other systemic organs in treated rats.

f) To study the role of ACh in BPA induced inhibition of duodenal motor function, the acetylcholinesterase (AChE) activity of duodenal tissue in BPA treated rats have been measured. It has been observed that the BPA significantly increases the activity of AChE in duodenal smooth muscle in BPA treated rats. From the results it is suggested that the BPA induced inhibition of motor activity of duodenal smooth muscle might be due to the partial facilitation of ACh decay at the local synapse on to the duodenal smooth muscle in Myenteric reflex arc.

g) In order to examine the role of nitric oxide synthase (NOS) and \( \text{Ca}^{2+} \) homeostasis in BPA induced inhibition of duodenal smooth muscle motor function, the expression of NOS and deposition of calcium salts in paraffin impregnated duodenal tissue sections of BPA treated rats have been studied histochemically. The expression of NOS in NADPH-diaphorase stained duodenal tissue section and deposition of calcium salts in muscularis and submucosal layers of Von Kossa's stained duodenal tissue section were
The results suggested that BPA may inhibit the duodenal movement presumably by promoting the synthesis of NO at the local synapse of duodenal smooth muscle by inducing the activity of NOS. BPA may also inhibit the duodenal movement partially by impairing the intracellular Ca\(^{2+}\) homeostasis by promoting the chelation of Ca\(^{2+}\) and deposition of chelated Ca\(^{2+}\) within the smooth muscle cells.

h) To study the effect of BPA on cytoarchitectural changes in duodenal smooth muscle layers in BPA treated rats, the paraffin impregnated duodenal tissue sections of BPA treated rats have been examined histologically. Remarkable structural alterations like inflammation of the muscle layers, lesions of the muscle layers due to degenerative changes, plaque formation; and other serosal and submucosal structural changes of the duodenal wall have been observed. Therefore, it can be said that the BPA induced inhibition of the duodenal movement is mediated partially by the structural degeneration of the muscle layers of duodenal wall. The structural degenerations of the smooth muscle and their innervating efferents of Myenteric (Auerbach’s) plexus located in the muscular layers may partially responsible for the inhibition of the duodenal movement in BPA treated rats.

i) In order to study the effect of BPA on oxidative stress in duodenal tissue, the activities of antioxidants enzymes- superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST); and malondialdehyde (MDA, as a marker of membrane lipid peroxidation) production of duodenal tissue homogenate have been measured. Results indicate that BPA significantly decreases the activity of SOD, CAT, GPx, GR; and increases the activity of GST and MDA production in duodenal tissue in BPA treated rats. Thus, it is suggested that BPA may cause the oxidative stress in duodenal smooth muscle cells by inducing the production of reactive oxygen species (ROS) in the cells due to the BPA induced inhibition of the antioxidant enzymes; and by inducing the peroxidation of membrane lipids of the duodenal smooth cells. This oxidative stress may also responsible for the BPA induced inhibition of duodenal movement.

j) To study the possible action of vitamin C in BPA induced oxidative stress in duodenal smooth muscle, the effect of BPA in association with vitamin C has been studied on the activities of antioxidant enzymes (SOD, CAT, GPx, GR and GST) and MDA production. It has been seen that the co-administration of BPA with vitamin C, produced no significant decrease in the activities of SOD, CAT, GPx, GR and increase in the activity of GST and production of membrane lipid peroxidation. Thus, it can be said that vitamin C efficiently counteracts the BPA induced oxidative stress in duodenal smooth muscle cells.
6.2. Conclusions

In conclusion,

a) BPA inhibits the motor function of the duodenal smooth muscle through \( \alpha \)-adrenergic and NO-mediated signal pathways.

b) At the cellular level, the BPA induced inhibition of the motor function of duodenal smooth muscle is mediated by-
   i) Increase in AChE activity,
   ii) Over expression of NOS,
   iii) Decrease in the availability of intramuscular free \( \text{Ca}^{2+} \),
   iv) Increase in membrane lipid peroxidation,
   v) Increase in oxidative stress as a result of the alterations of the antioxidant enzymes,
   vi) Oxidative stress induced cellular damage.

c) Vitamin C can potentially prevent the BPA induced inhibition of the motor function of intestinal smooth muscle presumably by reducing the oxidative stress.

Thus, the results obtained from this study in rat model may be extrapolated in human beings.