Chapter 5

Integrated Discussion

The food products like turmeric powder, ladoo and besan processed by the unorganized sectors and marketed in the rural areas in West Bengal mostly contained MY (MY) above the maximum permissible limits as provided in the Prevention of Food Adulteration Act of India (PFA, 2008). It was found that 58 of the 253 numbers of samples, i.e. 20.94% of total samples contain MY in which 36.21% of the positive samples contain the MY below the maximum permissible limit and 63.79% of the positive samples contain above the maximum permissible limit (i.e., 100 mg/kg food samples) as per Prevention of Food Adulteration Act of India (PFA, 2008). It was observed that all the samples contain in MY were prepared by the unorganized sectors. The food samples collected from the organized sectors were free from adulteration by MY. This might be due to indiscriminate use of MY in the food processing of unorganized sectors due to lack of governmental control and inadequate infrastructures.

The effect of MY on the whole body weight, and absolute and relative organ weights of the ovary and uterus; and effect of vitamin C on MY induced decrease in body weight and absolute and relative weights of ovary and uterus have been studied. I found a significant decrease in the gross body weight, and absolute and relative weights of the ovary and uterus in MY exposed rats compared to the control groups of rats. I found a significant counteraction in MY induced decrease in gross body weight, and decrease in absolute and relative weights of ovary and uterus when vitamin C was applied in combination with MY in both durations in comparison with the MY treated groups of rats. From the result it may be hypothesized that MY decreases the body weight, and absolute and

relative weights of ovary and uterus probably by inducing the oxidative stress induced necrosis or hypotrophy of the tissues, including the tissues of ovary and uterus; and the vitamin C counteracts the MY induced decrease in body weight and absolute and relative weights of the ovary and uterus probably by preventing the oxidative stress induced necrosis of the tissues.

An estrous cycle is a rhythmic reproductive cycle occurring in sexually mature female mammals which depends upon the periodic release of gonadotropin releasing hormone, gonadotropins and sex hormones that gives a fair index of ovarian function. The normal rats exhibited regular estrous cycle and normal duration of each phases of estrous cycle. In the study, I have examined the estrous cycle variables of MY exposed rats as a holistic indicator about the toxic effects of MY on female reproductive system function involving the component roles of hypothalamic-pituitary-gonadal axis and ovary. It was observed that MY significantly alters the estrous cycle physiology of rats. From the results, it may considered that the effects of MY on the set point secretion and function of some female reproductive hormones secreted from the anterior pituitary and ovary that control the estrous cycle physiology in female rats. I have observed a significant reduction in the level of LH, FSH and estradiol in MY treated rats. Estradiol is responsible for change in the reproductive tract and the regulation of gonadotropins. The stages of estrous cycle and their inter conversions are mainly governed by the hormones estrogen and progesterone. Any change in these hormones would lead to changes in the cyclicity of estrous cycle. Hence, the persistent of diestrus phases of estrous cycle in the MY treated rats could be correlated with decreased estradiol levels. The set point functions of female reproductive system are governed by hypothalamic-pituitary-gonadal axis. Hypothalamic neurons release gonadotropin releasing hormone (GnRH) which helps to release FSH and LH from the anterior pituitary. The FSH and LH act on the ovary and promote the release of estradiol, the hormone essential for the regulation of the estrous cycle physiology. The mechanism by means of which hypothalamus controls the ovary is called positive feedback mechanism of the hypothalamic-pituitary-gonadal axis. The level of estradiol in turn regulates the release of GnRH from hypothalamus and FSH and LH from anterior pituitary by negative feedback loop mechanism. I have observed a significant decreased in the level of LH, FSH and estradiol in MY treated female rats. Therefore a positive correlation between the level of FSH and LH; and estradiol was found. From the results, it is suggested that MY might decrease the synthesis and production of estradiol presumably by promoting negative feedback loop mechanism of hypothalamic-pituitary-gonadal axis. Thus, the changes in the cellular characteristics in vaginal smear in different phases of estrous cycle and increase in diestrus index might be due to MY induced inhibition of the secretion of LH

and FSH from the anterior pituitary and estradiol from the ovary as a result of the stimulation of negative feedback loop of hypothalamic-pituitary-gonadal axis. The gonadotropin hormones are needed for the early growth of the follicles. Normally follicles begin to develop at all times and as they grow, they produce large number of thecal and granulosa cells. In my present study, I found a significant reduction in the number of healthy follicles in stage I, II, III, V, VI and the total number of healthy follicles in both treatment durations in case of higher doses of chronic exposure. From the results, it is suggested that MY probably interferes the ovarian folliculogenesis by reducing the secretion of FSH and estradiol. MY might inhibit the positive loop of the hypothalamic-pituitary-gonadal axis and/ or inhibit the development of the follicles by deforming or deactivating the gonadotropin receptors.

The effect of MY on the activities of the antioxidant enzymes in brain tissue homogenate and structural alterations in hypothalamic tissues in MY exposed rat have been studied. It has been observed that the activities of antioxidant enzymes were significantly decreased and the amount of MDA production was significantly increased in MY treated rats compared to the control groups of rats. These results suggest that MY might produce oxidative stress in brain tissues including in hypothalamic regions. In our study, I have observed a significant cytoarchitectural changes in brain of MY exposed rats compared to the control groups of rats. This result suggests that MY might alter the normal cellular structures by causing oxidative stress. Thus, from the results it may be suggested that MY might depress the hypothalamic function probably by causing oxidative stress induced damages in hypothalamus.

The effect of MY in the production of oxidative stress induced structural degeneration, the activities of the antioxidant enzymes, MDA production and cytoarchitectural changes in ovary and uterine tissues of MY exposed rat have been studied. I found a significant decrease in the enzymatic activities of SOD, CAT, GPx, GR and GST with a concomitant increase in MDA production in uterine and ovarian tissue homogenate of MY exposed rats compared to the control groups of rats. These results suggest that MY might induce oxidative stress in uterine and ovarian tissues probably by inhibiting the enzymatic activities of antioxidant enzymes and promoting the peroxidation of lipids found in plasma membranes and other membranes of the organelles in uterine and ovarian tissues of rats. I also found significant degenerative structural changes in ovarian and uterine tissues in MY exposed rats. This result suggests that MY might induced the structural degenerations of the uterine and ovarian tissues probably by producing oxidative stress and inducing lipid peroxidation.

The protective role of vitamin C in the mitigation of MY induced oxidative stress in ovarian and uterine tissues of rats was examined. In present study, I found no significant changes in the activities of SOD, CAT, GPx, GR, GST and MDA production in ovarian and uterine tissues of rats received MY in combination with vitamin C compared with the control and MY treated rats. These results suggest that vitamin C might provides the protective role against the MY induced depression of the activities of antioxidant enzymes and damages of the structural membrane lipids by means of MY induced peroxidation. Besides, I did not find any significant structural alterations in the tissues of ovary and uterus in rats received MY in combination with vitamin C compared with the control groups of rats. These results suggest that vitamin C might provide mitigative role against the MY involved oxidative stress induced degeneration of the structure of the ovary and uterus.

The effect of MY on the contraction of uterine smooth muscle of rats was examined. I found a significant inhibition in the amplitude and frequency of uterus in a dose dependent manner in two sub-chronic treatment durations compared to the amplitude and frequency of the contraction of uterus in control group of rats. These results suggest that MY might inhibits the contraction of the uterine smooth muscle presumably by inhibiting the release of neurotransmitters that promotes contraction and/ or stimulating the release of transmitters that is/ or are involved in the inhibition of movements from the autonomic efferents. To examine the mechanism of action of MY in MY induced inhibition of the contraction of uterine smooth muscles, the effect of MY on the movement of the uterus *in vitro* has been studied in a single dose acute study. I found a significant inhibition of the contraction of uterus dose dependently in a single dose acute experiment. This result support our hypothesis that MY induces the inhibition of the contraction of the uterine smooth muscles probably by promoting the release of inhibitory neurotransmitters and/ or inhibiting the release of excitatory neurotransmitters from the autonomic efferents.

The role of the adrenergic and/ or cholinergic influences in MY induced inhibition of the contraction of uterus *in vitro* of rats was examined. I found a significant inhibition of the movements of uterus in response to the application of MY when MY was applied after the application of atropine. From the observations, it may be hypothesized that MY blocks the contraction of the uterine smooth muscles partially by inhibiting the release of ACh from the cholinergic nerve terminals and/ or blocking ACh mediated transmission from cholinergic efferents to smooth muscles. Besides, it was observed that adrenaline potentiates the MY induced inhibition of the movements of uterus. This result suggests that MY might partially inhibits the contraction of the

uterine smooth muscles probably by augmenting the activity of nor-adrenergic efferents innervating the uterine smooth muscles. So, to examine the pathway (s) involved in MY induced adrenergic signaling, the effect of MY on the movement of the uterus has been recorded after the incubation of uterus with propranolol, a β-adrenergic antagonist/ phentolamine, an α-adrenergic antagonist. I found no significant alterations in the movements of uterus when MY was applied in uterine preparation pre-incubated with propranolol but a significant counteraction of the MY induced movement was observed after the application of MY in phentolamine pre-incubated uterine tissue preparations. From the results, it can be suggested that MY induces the inhibition of the contraction of uterine smooth muscles predominantly by α-adrenergic signaling pathway in smooth muscle cells.

The involvement of non cholinergic non adrenergic (NANC) efferents, if any, in MY induced inhibition of the contraction of uterine smooth muscles has been studied. I did not find any significant alteration in MY induced inhibition of the contraction of uterus in SNP pre-incubated uterine preparations. So, the result suggests that NO produced in the cell from the extrinsic source could not affect the MY induced relaxation of uterine smooth muscles. Further, it was observed that L-NAME significantly counters the MY induced relaxation of uterine smooth muscles. L-NAME potentially blocks the synthesis of NO by inhibiting the enzymatic activity of NO synthase. So the result suggests that intrinsically NO produced might be partially involved in MY induced relaxation of uterine smooth muscles. NO induces the relaxation of uterine smooth muscles through soluble guanylyl cyclase (sGC) signaling pathway. To find out the action of MY in sGC signaling pathway, the effect of MY on the movement of the uterus in MB pre-incubated uterus was observed. I found a significant recovery of the MY induced relaxation of uterine smooth muscles in presence of MB. MB is a chemical that can block the action of soluble guanylyl cyclase, the enzyme responsible for the production of cyclic GMP from GTP. So, the result suggests that MY probably counters the relaxation n action of NO by inhibiting the enzymatic action of sGC. Considering the entire results, it can be suggested that NANC efferents, that secretes NO might be partially involved in MY induced inhibition of the contraction of the uterine smooth muscles.

Nitric oxide synthase is the key enzyme for the synthesis of NO from L-arginine in smooth muscle cells. If the enzymatic activity of NOS is increased, the level of NO at myoneural junctions to promote relaxation of uterine cell is increased and vice versa. In the present study, the expression of NOS in uterine smooth muscle cells was increased in MY exposed uterine tissue dose dependently in both exposure durations. From the result, it can be ascertained that MY promotes the

relaxation of the smooth muscles by inducing the expression of NOS in uterine smooth muscle cells.

I can determine the amount of extracellular free Ca^{++} ions by examining the deposition of Ca^{++} salts at the intracellular spaces of the smooth muscles in Von Kossa's stained histochemically prepared tissue sections of MY exposed and control rats. In the present study, a significant increase in the deposition of Ca^{++} salts at the intercellular spaces of the uterine muscle layers of MY exposed rats was observed. From the results, it can be suggested that MY inhibits the contraction of the uterine smooth muscles probably by decreasing the intercellular Ca^{++} concentration required for actin-myosin cross bridge interactions. The neurotransmitters nor epinephrine, nitric oxide and acetylcholine might be involved in MY induced inhibition of the diffusion of extracellular Ca^{++} into the cells and/ or efflux of intracellularly stored Ca^{++} ion into the cytosol of the smooth muscle cells.

Considering the results presented in all chapters of the thesis, I can suggests that MY depresses the female reproductive functions probably by impairing the functions of ovary and uterus. MY probably interferes the function of ovary by inhibiting the function of hypothalamus-pituitary-gonadal axis, decreasing the release of female reproductive hormones, altering the rhythmicity of estrous cycle, inhibiting the folliculogenesis and promoting the oxidative stress induced tissue degenerations of the ovary. MY impairs the functions of the uterus probably by promoting the inhibition of the contraction of uterine smooth muscles through changing the activity of cholinergic, α-adrenergic and nitrergic efferents to the uterus and inducing the oxidative stress induced cytoarchitectural changes of uterus. I have tried to examine the protective functions of vitamin C against MY induced suppression of ovarian and uterine functions. Vitamin C showed significant protective action against the MY induced depression of ovarian and uterine functions.