CHAPTER 6

Summary and Conclusions

6.1 Summary

1. The percentage level of occurrence of metanil yellow (MY) in some food items produced by the organized and unorganized sectors of different districts in West Bengal, India was identified and determined. It was found that 20.94% of total samples contains MY in which 63.79% of the positive samples contain above the maximum permissible limit as per Prevention of Food Adulteration Act of India (PFA, 2008). From the results, it may be suggested that the unorganized sectors prefers to use MY as food additive to create the brightness of the food ignoring the possible toxic effects of MY. This might be due to lack of governmental control and inadequate infrastructures.

2. The effect of MY on body weight, and absolute and relative weights of ovary and uterus; and study of the role of vitamin C in the mitigation of MY induced reduction of body weight, and absolute and relative weights of ovary and uterus in treated rats. I found a significant decrease in gross body weight, and absolute and relative weights of ovary and uterus of MY treated rats was examined. I also found a significant counteraction in MY induced decrease in gross body weight, and decrease in absolute and relative weights of ovary and uterus when MY was applied in combination with vitamin C. From the results, it may be suggested that the decrease in body weight; and absolute and relative weights of ovary and uterus might be due to atrophy of the ovary and uterus or atrophy of the whole tissue of the organs. MY induces the necrotic degeneration of

the tissues of uterus and ovary probably by inducing oxidative stress in tissues. Vitamin C can provide protective action against the MY induced tissue degeneration of the ovary and uterus probably by preventing the oxidative stress induced necrosis of the tissues.

3. The effect of MY on estrous cycle rhythmicity, ovarian folliculogenesis and female reproductive hormone profile in MY treated rats was examined. It was observed that MY significantly alters the estrous cycle rhythmicity, impairs the ovarian folliculogenesis and decrease the serum female reproductive hormonal levels in MY treated rats. From the results it may be hypothesized that MY impairs the estrous cycle physiology and folliculogenesis probably by suppressing the production of FSH and LH from the anterior pituitary and estradiol from the ovary as a result of the stimulation of negative feedback loop of hypothalamic-pituitary-gonadal axis.

4. The effect of MY on the activities of antioxidant enzymes in brain tissue homogenate and structural alterations in hypothalamic tissues in MY exposed rat have been studied. It was observed that MY significantly reduces the activities of the antioxidant enzymes (SOD, CAT, GPx, GR and GST) and increases the MDA production in brain tissue homogenate of MY exposed rats. It was also observed that MY significantly produces the structural degenerations in the brain tissues of MY exposed rats. From the results it may be suggested that MY might depresses the hypothalamic function probably by causing oxidative stress induced damage of the cytoarchitectural structures of the hypothalamic neurons.

5. 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 tissues of MY exposed rat have been studied. I found a significant decrease in the activities of the antioxidant enzymes and increase in MDA production in ovarian tissue homogenate of MY exposed rats. Further, MY produced a significant structural alterations in ovary tissue of exposed rats. These results suggest that MY inhibits the ovarian functions presumably by inducing the oxidative stress induced structural degeneration in ovary tissues as a result of the inhibition of the enzymatic activities of antioxidant enzymes and stimulation of the peroxidation of lipids of plasma membranes and other membranes of the organelles.

6. The effect of MY in the production of oxidative stress induced structural degeneration, the oxidative stress linked variable and cytoarchitectural

changes in uterine tissues of MY exposed rat have been studied. It was observed that MY significantly decreases the activities of antioxidant enzymes with concomitant increase in the MDA production in uterine tissue homogenate and produces degenerative structural changes in the wall structure of uterus. From the results it can be suggested that MY induces the structural degenerations of the uterine tissue probably by producing oxidative stress and lipid peroxidation of uterine muscle fibres and other tissue cells.

7. The protective role of vitamin C in the mitigation of MY induced oxidative stress in ovary tissue of rats was examined. I found no significant changes in the activities of the antioxidant enzymes, MDA production and no significant structural alterations in the tissues of ovary in rats received MY in combination with vitamin C compared to control rats. These results suggest that vitamin C can provide the protective action against the MY induced oxidative stress in ovarian tissue probably by augmenting the MY induced depression of the antioxidant enzymes, production of MDA by means of lipid peroxidation and stress induced damages of ovarian tissues.

8. The protective role of vitamin C in MY induced oxidative stress in uterus, the oxidative stress linked variables in uterine tissue homogenate and structural changes in uterine tissues of rats received MY in combination with vitamin C have been studied. I found no significant changes in the oxidative stress linked variables in uterine tissue homogenate and no significant cytoarchitectural degeneration in uterine tissues of rats received MY in combination with vitamin C compared to control rats. From the results it may be suggested that vitamin C can provide protective action against MY induced oxidative stress probably by antagonizing the MY induced depression of the antioxidant enzymes, production of MDA and stress induced damages of uterine tissues.

9. The movement of isolated uterine segment has been recorded in vitro, to examine the effect of MY on the movement of uterus. I found a significant inhibition in the amplitude and frequency of the movement of uterus in subchronic and single dose acute experiments. These results suggest that MY induces the inhibition of the contraction of uterine smooth muscles presumably by promoting the release of inhibitory neurotransmitters and/ or inhibiting the release of excitatory neurotransmitters from the autonomic efferents.

10. In order to find out the role of cholinergic and adrenergic efferents in MY induced inhibition of the contraction of the uterine smooth muscles, dose dependent effect of MY in combination with cholinergic agonists and antagonists, and nor adrenergic agonists and antagonists have been studied on the movement of uterus in a single dose acute study. I found no significant alterations in the movement of uterus when MY was applied in uterine preparations pre-incubated with propranolol. Significant counteraction of MY induced movements of uterus was observed when MY was applied in phentolamine pre-incubated uterine tissue preparations. I also 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. These results suggest that MY promotes the inhibition of the contraction of uterine smooth muscles probably by α-adrenergic signaling pathway; and partially by cholinergic pathways.

11. The involvement of non cholinergic non adrenergic (NANC) efferents, if any, in MY induced inhibition of the contraction of uterine smooth muscles have been studied. A significant reversal of MY induced inhibition of the contraction of uterine smooth muscles was observed when MY was applied after the application of L-NAME (blocker of NO synthase) and MB (blocker of soluble guanylyl cyclase) in uterine tissue preparation. From the results, it may be suggested that NANC pathway might be partially involved in MY induced inhibition of the contraction of uterine smooth muscles.

12. To ascertain the involvement of nitric oxide synthase (NOS) in MY induced relaxation of uterine smooth muscles, the expression of NOS in uterine tissue sections of MY exposed rat have been studied. I observed a significant increase in the expression of NOS in uterine smooth muscle cells of MY exposed rats. Form the result it can be ascertained that MY promotes the relaxation of uterine smooth muscle cells probably by inducing the expression of NOS in uterine smooth muscle cells.

13. The effect of MY on the availability of free Ca^{++} in smooth muscle layers of uterine wall structure of MY exposed rats was examined. It was observed that the deposition of the Ca^{++} salts increases significantly in uterine tissue sections of MY exposed rats. From the result, it can be suggested that MY inhibits the contraction of uterine smooth muscles probably by reducing the availability of free Ca^{++} ions in smooth muscle cell and thus, increasing the extracellular Ca^{++} in bound form.

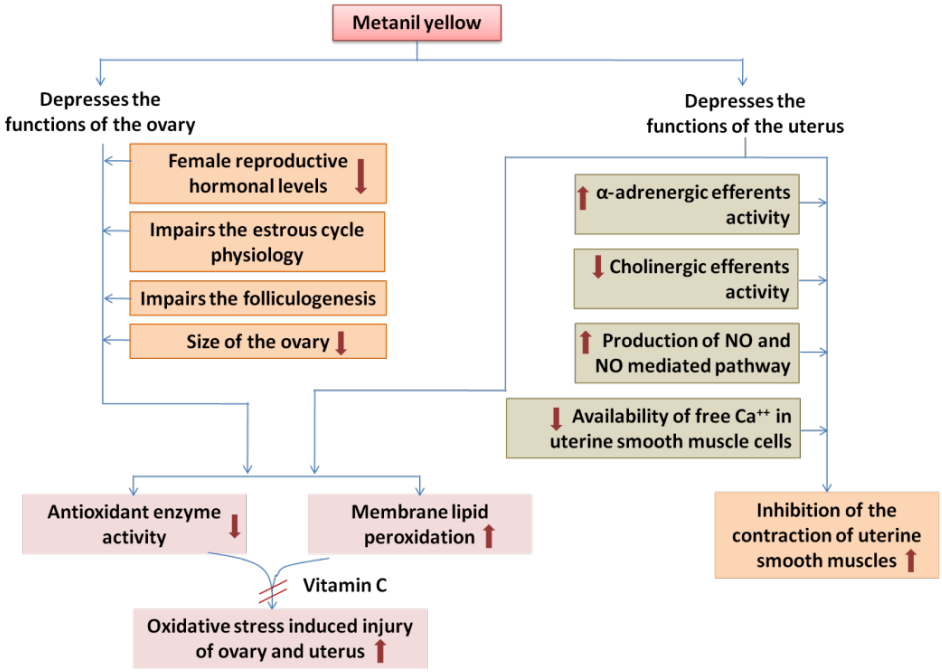

Figure 6.1.1: The probable mechanism of action of MY on uterine and ovarian functions and the mitigative role of vitamin C in MY induced oxidative stress in ovary and uterus of rats.

6.2 Conclusion

In conclusion,

1. The unorganized sectors practice to use MY indiscriminately due to lack of adequate infrastructures and governmental control to adulterate food items for financial gain.
2. Metanil yellow depresses the function of ovary and uterus in female rat.
3. Metanil yellow inhibits the ovarian functions by impairing the estrous cycle physiology, decreasing the female reproductive hormonal (FSH, LH and estradiol) levels, decreasing the size of the ovary, impairing the folliculogenesis, increasing the membrane lipid peroxidation of ovarian tissue cells and oxidative stress induced cellular damages.
4. Metanil yellow depresses the uterine function by promoting the inhibition of the contraction of uterine smooth muscle cells.
    a. Metanil yellow promotes the inhibition of the uterine smooth muscles predominantly by promoting the activity of α-adrenergic efferents innervating the uterine smooth muscles and partially by inhibiting the stimulatory cholinergic efferents and stimulating the activity of NANC or nitrergic efferents innervating the uterine smooth muscles.

b. Metanil yellow probably stimulates the NANC efferents by stimulating NO synthase in NANC neurons and activating soluble guanylyl cyclase in uterine smooth muscle cells.
c. In addition to the promotion of the production of the NO and stimulation of the secretion of nor epinephrine from α-adrenergic pathways, metanil yellow probably inhibits the smooth muscle contraction by decreasing the availability if intracellular free Ca^{++} in smooth muscle cells.

5. Metanil yellow depresses the uterine functions probably by inducing the oxidative stress induced damages of the uterine smooth muscle cells, uterine epithelium, uterine glands and blood vessels.
6. Vitamin C provides the protective functions against metanil yellow induced depression of the function of ovary and uterus.

Thus, metanil yellow inhibits the function of female reproductive system probably by depressing the functions of ovary and uterus. The results in rat models may be extrapolated in human beings.