8.0 SUMMARY

The critical value of arsenic as a important water pollutant via drinking water at the wide spectrum of India, especially in our state West Bengal, were studied by corresponding some important parameters for the assessment of female reproduction. To assessment the ovarian steroidogenic activities the ovarian Δ^5-3βHSD, 17β-HSD activities along with plasma level of estradiol and gonadotrophins were measured. To evaluate the gametogenic functions of ovaries, qualitative study of folliculogenesis was performed by showing the number of healthy follicles and regressing follicles in ovarian histopathological section. Uterine histometry was examined by considering some important relevant parameters like uterine luminal diameter, uterine tissue arrangement architecture. As estrus cycle is the primary indicator of ovarian steroidogenic activity and duration of diestrus is the most important indicator of the cyclical rhythm of estrus cycle, therefore the qualitative nature of diestrus was considered in different experimental schedules. Arsenic also promotes oxidative stress in several organs, accompanied with female reproductive organs; status of oxidative stress was evaluated by measuring the ovarian and uterine SOD, CAT, peroxidase activities which are act as important scavenger enzymes. To evaluate the toxicity in cellular level we performed histological assessment in ovarian and uterine tissue and also DNA fragmentation study by conducting DNA ladder assay and comet assay.

In this thesis the effect of sodium arsenite has been studied under various endocrine manipulations which are as follows:

In Experiment I, there are two arsenical doses such as 0.2 ppm and 0.4 ppm are taken to evaluate the effective dose. From those studies it is concluded that 0.4 ppm of sodium

arsenite is the effective dose to produce arsenic toxicity in female albino rat's reproductive organs. This critical dose is impairment to degeneration of ovarian tissue which enhance the follicular atresia and disorganization of uterine histoarchitecture by inhibition of steroidogenic enzymes activities of Δ^5, 3β-HSD and 17β-HSD resulted low level of steroids hormone (FSH, LH and estradiol) secretion rather than 0.2 ppm of sodium arsenite addicted rats. There significant diminution is noted in SOD, CAT and peroxidase (POD) level and elevation of MDA, CD in 0.4 ppm in respect of 0.2 ppm. That is why 0.4 ppm dose of sodium arsenite is selected as affective dose to run in the whole research work which is abundantly found in drinking water at different regions of our state West Bengal as India.

To examine the effective duration of the selective dose (0.4 ppm.) of sodium arsenite was administered for 16 days and 28 days in the Experiment II. A deleterious effect of arsenicosis on reproductive organs (ovary and Uterus) are found in 28 days duration in comparison to 16 days treatment. In 28 days protocol showed a significant reduction of ovarian and uterine mass along with retardation of steroidogenesis and disorganization of ovarian tissue rather than 16 days duration. An evaluation of duration dependent response our data exhibited a significant augmentation of MDA and CD in uterine tissue along with reduction of scavenging enzymes in ovarian tissue and uterine tissue in 28 days treatment. Whereas in 16 days duration there was no promising outcome seen in comparison with 28 days treatment protocol. That is why 28 days is selected as a critical period at the dose 0.4 ppm of sodium arsenite to progress the research work.

The Experiment III is conducted to evaluate the effective dose of Vitamin- B_{12} and folic acid either singly or combined along with sodium arsenite at a dose 0.4 ppm for

28 days as therapeutic agent .For that purpose there are two doses chosen of Vitamin B_{12} as 0.04µg/100g body wt./day and 0.07µg/100g body wt./day. And also two doses chosen of Folic acid as 2µg/100g body wt. /day and 4µg/100g body wt. /day. Overall experimental data expressed that combination of Vit- B_{12} and folic acid at the doses 0.07µg/100g body wt. /day and 4µg/100g body wt. /day respectively are most effective to mitigate the arsenic mediated injury. Therefore the Vit- B_{12} and folic acid at the doses 0.07µg/100g body wt. /day and 4µg/100g body wt. /day respectively was selected as effective dose to combat sodium arsenite (0.4 ppm) causes hazards for 28 days duration.

Experiment IV is an evaluation of any generation of toxicity in organs (ovary, uterus, liver, kidney) of body by the administration of effective doses of Vit- B_{12} (0.07µg/100g. body wt. /day) and folic acid (4µg/100g body wt. /day) either singly or in combination. There is no such injury is noticed either biochemically or genetically form our data either singly or in combination of the selective doses of B vitamins. But in combination of Vit-B_{12} (0.07 µg/100g body wt. /day) and folic acid (4µg/100g body wt. /day) is shown more healthy impact in respect to the control rather than their single administration.

Whether the adverse effect of sodium arsenite ovarian and uterine function is reversible or irreversible, the experiment was performed by treating the animals with sodium arsenite at a dose of 0.4 ppm. For 28 days followed by withdrawal of this treatment in a duration dependent manner for 16 days and 28 days. To search out whether the effective dose of Vit-B_{12} and folic acid has reversible or irreversible, the withdrawal experiment was conducted administration of 0.4 ppm sodium arsenite with co-administration of Vit- B_{12} (0.07µg/100g. body wt. /day) and folic acid

(4µg/100g body wt. /day) for 28 days followed by cessation of all doses for 16 days followed by 28 days. In Experiment V in withdrawal programme it is proved the toxicity generated by arsenic imposition is reversible where as supplementation of B vitamins along with sodium arsenite is irreversible.

To find out the efficacy of the effective dose of Vit- B_{12} (0.07µg/100g. body wt. /day) and folic acid (4µg/100g body wt. /day) along with sodium arsenite treated rat's metabolic organ as liver was performed. Collective data revealed that arsenic mediate hepatic injury is retrieved by the co-administration of Vit-B_{12} and folic acid at selective doses bimolecular level as well as tissue level.

To explore the possibility of direct effect of sodium arsenite on ovarian steroidogenesis, *in vitro* study was performed. In Experiment VII is demonstrated that major arsenic associated problems are occurred whether in direct action on ovarian steroidogenesis and their recovery by direct effect on co- administration of two vitamin B supplementation. As my way is mainly concerned about the toxic effect of sodium arsenite at the selective dose availability in concern about in the drinking water of our country on ovarian activities in albino rats, so in this *in vitro* experiment we selected ovarian steroidogenic enzyme activities. To conduct this experiment ovaries are kept in 0.4 ppm arsenic mediated KRB solution for 2 hrs along with co-administered with supplemented vitamins B either singly or both combined as required doses. The results of this experiment indicated that there was no significant alteration in ovarian steroidogenesis in each group in contrast to control, so it may be concluded that arsenic mediated ovarian steroidogenic inhibition is not responsible on its direct effect on ovarian steroidogenic enzymes.