6.0 DISCUSSION

In this present study, **Experiment-I** projected the results of the ovarian and uterine somatic indices and these levels were decreased only at high dose without any significant alteration in these indices at low dose of sodium arsenite treated group (**Table 5.1.IA.I**). It also reflected that there was a significant diminution in the steroidogenic and gametogenic activities in ovary at a dose of 0.4 ppm sodium arsenite**,** though there was no significant alteration in these parameters at the dose of 0.2 ppm. On application of comparatively high dose of arsenic a marked reduction of ovarian, uterine weight and prolongation of diestrus phase was prominent may be due high concentration of arsenic in plasma and massive deposition of arsenic in reproductive tissues that may ultimately promote the alteration of normal cellular mechanism and could attribute to necrotic and/ or apoptotic manifestation.

Serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (**Table 5.1.IA.II**) were decreased at this higher dose (0.4 ppm) of sodium arsenite ($NaAsO_2$) without any significant modulation at lower dose (0.2 ppm). Relevant parameters like luminal diameter, thickness of myometrium, endometrium and epithelial cell height of uterine histometry (**Fig 5.1.5**) were decreased along with consistent diestrus at this higher dose of sodium arsenite without any significant change in these parameters at the lower dose. The result of 1st experiment revealed that 0.4 ppm of $NaAsO_2$ which is noted in the drinking water at wide areas of West Bengal also exerts a intense deleterious effect on pituitary ovarian axis and so, this dose has been selected in the present study and it has an applied value in community health and environment toxicology because this dose is available in the drinking water at wide areas of West Bengal.

In this experiment (**Experiment-I**) the diminution in serum FSH**,** LH [**Table 5.1.IA.II**] at the dose of 0.4 ppm of sodium arsenite is consistent with our previous report [**Chattopadhyay et al., 1999**] where serum LH and FSH both were decreased significantly after 28 days of $NaAsO_2$ treatment at the dose of 0.4 ppm. It clearly emphasized that sodium arsenite induced reduction in serum estradiol level is the reflection of parallel changes in serum FSH and LH as both these gonadotrophins are regulator of estradiol synthesis [**Fukuda et al., 1979**] according two cell two steroid hypothesis [**Chattopadhayay et al., 2000**]. Moreover, this low level of estradiol at the high dose of sodium arsenite treated rat was again supported by our previous report where 28 days is the critical period for diminution in estradiol level in plasma [**Chattopadhyay et al., 1999**]. Low estradiol in response to low level of plasma FSH, LH proliferation and differentiation of reproductive cells were prevented, resulted in the occurrence of low weight of ovary and uterus along with a higher number of regressing follicles [**Fig. 5.1.4**] [**Chattopadhyay et al., 2003**].

$\Delta^5$3β-HSD and 17β-HSD are the key regulatory enzymes in ovarian steroidogenesis. Due to imposing of arsenication and decreasing of scavenger enzymes in reproductive cells by overproduction of free radicals resulted abundant cell degeneration, which leads to poor activities of Δ^5**,** 3β-HSD and 17β-HSD. Deterioration of both ovarian Δ^5**,** 3β-HSD and 17β-HSD activities (**Table 5.1.IA.II**) following sodium arsenite treatment at higher dose (0.4 ppm) may be due to the low gonadotrophins signaling [**Nimrod et al., 1980**] as these are the regulators for the maintaining the expression of relevant genes of these enzymes [**Carr, 1998**]. Lessened levels of plasma LH and FSH in arsenic treated rats may be due to hyper secretion of adrinocorticotrophic hormone (ACTH) and glucocorticoids [**Ghosh et al., 1999**]. An elevation in plasma glucocorticoids reduces the sensitivity of

gonadotroph cells to gonadotrophins releasing hormone (GnRH) resulted in the inhibition of gonadotrophin secretion resulted in the retardation of follicular growth and enhancement of follicular atresia. Moreover, diminution in the levels of dopamine (DA), norepinephrine (NE) as well as high level of 5-hydroxytryptamine (5-HT) in midbrain and diencephalon may be another causative factor as these are the regulators for gonadotrophin secretion [**Chattopadhyay et al., 2003**]. From other research works it has been revealed that these brain monoamines are altered when sodium arsenite was given to the animals [**Chattopadhyay and Ghosh, 2010**].

Present data documented an elevation of atretic follicles and diminution of healthy follicles (**Fig. 5.1.4**) that may result in the impairment of plasma FSH, LH and estradiol signaling. Nonetheless, overproduction of malondialdehyde (MDA) and conjugated diene (CD) (**Fig. 5.1.3**) along with the reduction of the superoxide dismutase (SOD), catalase (CAT) [**Fig. 5.1.1**] and peroxidase activity [**Fig. 5.1.2**] indicated the possible loss of cellular integrity that finally resulted in ovarian follicular and uterine cell degeneration in response to reactive oxygen species (ROS) generation following arsenic intoxication [**Chatterjee & Chatterji, 2010; Chattopadhyay et al., 2010**].

According to **Experiment-II** represented duration dependent arsenicosis that is responsible for the alteration of the pituitary-gonadal axis activity. After 28 days arsenic treated rats exhibited a marked suppression of uterine and ovarian somatic indices (**Table-5.2.IIA.I**) along with reduction of plasma FSH, LH and estradiol (**Table-5.2.IIA.II**) level rather than 16 days arsenic treated group which revealed that arsenic mediated reproductive injury may be fatal according to duration prolongation.

Diminution in plasma/serum level of estradiol in arsenic treated rat was again supported by consistent diestrus after 18±2 days and it may be due to low plasma levels of estradiol. This consistent diestrus in sodium arsenite treated rat is in agreement with our previous report [**Chattopadhyay et al., 1999**].

Limited number of healthy follicles and elevation of regressive follicles after sodium arsenite treatment also supported the inhibition in plasma gonadotrophins and ovarian steroids as these hormones are modulators for the growth of the follicles and its maintenance [**Carr, 1998**].

Retarded ovarian and uterine growth at higher dose of sodium arsenite for 28 days is consistent with our previous report. This diminution may be due to low plasma levels of gonadotrophins and estradiol since ovarian weight is under the influence of gonadotrophins whereas uterine weight is regulated by estradiol.

Inhibition in cell proliferation at endometrium and myometrium along with low epithelial height at luminal border of uterus again supported the low levels of ovarian steroid as uterine cell proliferation is under the influence of ovarian steroid especially estradiol [**Dash et al., 2018**]. As there was no significant alteration in the body weight of arsenic treated rats with respect to control**,** so these adverse effects of arsenic on pituitary ovarian axis should not be explained by its generalized toxicity rather than some specific toxic effect on target organ.

Data exhibited an elevation of atretic follicles and diminished number of healthy follicles that may result in the impairment of plasma FSH, LH and estradiol in 28 days with respect to 16 days treatment protocol **(Fig. 5.2.3)**. Nonetheless, overproduction of malondialdehyde (MDA) and conjugated diene (CD) along with the reduction of the superoxide dismutase (SOD), catalase (CAT) activities indicated

the possible loss of cellular integrity in response to reactive oxygen species (ROS) generation following arsenic feeding (**Fig. 5.2.1 &Fig. 5.2.2**).

In remediation against arsenical injury on female reproductive health, we selected two B vitamins (Vit-B_{12} and Folic acid) of different doses in the **Experiment III**. From the tables, bar diagrams, plates it is revealed that co-administration of vitamin B_{12} and B_9(folic acid) in combination at a dose 0.07µg /100g body wt. /day and 4µg/100g body wt. /day respectively are more effective to mitigate the arsenic mediated reproductive ailments.

From the **Table 5.3.IIIA.I** it is evident that sodium arsenic mediated poor occurrence of ovarian and uterine somatic indices are restored when compared with control; this suggested the prohibition of tissue degradation of reproductive organs. Similarly elevation of serum FSH, LH and estradiol (**Fig. 5.3.2**) in supplemented group with respect to arsenic treated group revealed that steroidogenesis driving prime regulatory enzymes Δ^5,3β-HSD and 17-βHSD maintain their homeostasis and thereby properly projected towards a healthy assignment of pituitary- gonadal axis. Therefore, these two B vitamins played a crucial role to maintain the homeostasis in arsenic intoxicated rat either by the replacing the degeneration of follicular cells or by altering hormonal signaling pathway. As a result, diminution in the number of atretic follicles and elevation of the number of healthy follicles were observed (**Fig 5.3.7)**. Due to normal elevation of plasma estradiol in supplemented group there is a noteworthy protection and proliferation of lining epithelial tissue, endometrial tissues, myometrial tissues and also luminal diameter of uterine horns. The elevation in the tortuosity of uterine glands promotes the normal estrous cyclicity as an upshot of normal estradiol (E_2) regulation which indicating the restoration of steroidogenic enzyme activity in reproductive organ

(**Fig. 5.3.8**). [**Gray et al., 2001**]. However, the above combination of selective doses of these two B vitamins plays a critical role in maintaining tissue histoarchitecture.

The present results in the **Experiment-III** explored that trivalent form of arsenic decreases the H_2O_2 scavenging activity, which increase reactive oxygen species occurring with H_2O_2, and thus generates several lipid peroxides and conjugated diene as the end products. A significant increase in the uterine MDA (323%) and CD (129%) level in the arsenite-induced group compared to the control group (**Fig. 5.3.6**) supports this assumption. Adherence with SOD inhibition exhibited that uterine tissue may encounter by abundant generation of superoxide anion (O_2) due to arsenic ingestion because of scarcity of conversion of superoxide anion radical into H_2O_2 in uterine tissue. Therefore, this drastic enzymatic exhaustion shown electrozymographically supported that free radicals are produced in uterus during arsenic metabolism. (**Fig. 5.3.3 & Fig.5.3.4**).

In the present study, uterine SOD (200%), catalase (180%) and peroxidase (316%) activities were diminished drastically (**Fig. 5.3.3A, Fig. 5.3.4A**) in arsenic-exposed compared to control group when assessed spectrophotometrically. Apparent from electrozymogram shown in **Fig.5.3.3B, Fig.5.3.3C and Fig.5.3.4C** revealed that electrophoretic intensity of these three enzymatic bands were reduced in arsenic ingested group significantly in comparison to vehicle treated control. Sluggish antioxidative enzymatic activities were concluded into cell cycle arrest at 2/M phase, necrosis and apoptosis in the male reproductive organ [**Fang, 2013**].

Our present histological data expresses that a severe follicular degeneration is occurred in arsenic imposed rats which elevated the number of atretic follicles [**Fig. 5.3.7**]. It has been reported that arsenic inhibits the estradiol production as resulted

from the impairment of pituitary-gonadal axis that resulted in suppressed release of FSH and LH. Arsenic also acts as ovotoxic substance that provides oxidative stress during the promotion of cellular toxicity as well as necrotic cell death. It has been also revealed that a severe uterine disorder is occurred during arsenic imposition. Estradiol is main enzyme that controls the proliferation and differentiation of endometrial tissue and this regulation was lost after arsenic ingestion for 28 days. Not only that arsenic may impair the binding of estrogen receptor by competing and inhibiting estradiol to the estrogen receptor. Thus, lowering estradiol leads to reduction of luminal diameter in uterus with thinning luminal epithelium, endometrium and myometrium. The histoarchitecture of uterus also exhibited that the number of endometrial glands are reduced along its tortuosity due to arsenication in rats (**Fig. 5.3.8**). This incidence hints the loss of secretory property and prolongation of diestrous phase. Electron microscopic (SEM) configuration of uterine tissue also suggested the occurrence of distorted nature of uterine tissue by sodium arsenite **(Fig. 5.3.9)** but this state was partially improved when arsenicated rats were given vitamin B_{12} and B_9 orally **(Fig. 5.3.9)**.

In the present study in relation to comet formation (**Fig. 5.3.11**) as well as uterine DNA laddering and smearing (**Fig. 5.3.10**) exhibited single cell DNA damage occurring in excess free radicals by arsenic ingestion. This phenomenon may initiate the induction of apoptotic and necrotic changes in uterine tissue [**Ciaccio et al., 1998**]. Occurrence of DNA damage by arsenic induced ROS could affect cell survival of the intestinal epithelial tissue by encouraging DNA-fragmentation in WBC population. Arsenic (III) may enhance uterine DNA hypomethylation at the cost of methyl donor S-adenosyl methionine (SAM) during arsenic metabolism. Resulting possible tissue necrosis and apoptosis along with extreme DNA damage

were validated by the arsenicals and its methylated derivative and this might be further influenced by the SOD activity [**Kligerman et al., 2003; Salnikow and Zhitkovich, 2008**].

Lactate dehydrogenase (LDH) is an important enzyme that converts pyruvic acid into lactic acid under anaerobic and hypoxic state. It is generally found in intracellular space but comes out in extra cellular fluid by rupturing cell membrane and might play a crucial role during malignancies development and progression. Due to prognostic value, LDH is designated to be a necrotic toxicity (cancer) biomarker and is not generally elevated in humans without cancer [**Zhang et al., 2015**]. Necrotic tissue lesions as found in the present architecture of uterine tissue section (**Fig.5.3.8**) in accordance to our result where elevated serum LDH level [**Gimeno et al., 1979**] following arsenic ingestion was documented (**Fig.5.3.5**). Fractal dimension assessment of histological section demonstrated the fibrotic status of the tissue and in the present study found an increase in fractal dimension in the stained tissue of arsenic treated group (**Fig.5.3.8**). This may point out that occurrence of uterine fibrosis after arsenic ingestion due to massive infiltration and deposition of collagen in uterine tissue [**Zouein et al., 2014**]. In this investigation elevated LDH level (58%) in arsenic exposed rats (**Fig. 5.3.5**) may act a crucial role in the fibrotic changes of the organ by stimulating collagen deposition [**Judge et al., 2015**]. Deficiency of the B vitamins may increase serum LDH level [**McCarthy et al., 1966**]. This is the reason why supplementation of adequate levels of the B vitamins is critical in the protection of uterine necrosis.

Advanced peroxidase activity in uterine cells proved that the estrogen acts a vital role in the restoration uterine peroxidase activity as well as uterine development and

proliferation [**DeSombre and Lyttle, 1979**]. Down regulation of plasma estradiol signaling may causes the inhibitory effect on DNA synthesis in uterine epithelium cells (**Fig.5.3.11**) and reduced reproductive organ mass (**Table 5.3.IIIA.I**) in arsenic treated group. In response to the production of free radicals the existence of a non-harmonized estrous cycle (4.1 ± 0.2 cycles per animal) and persistence diestrus were observed following arsenic exposure when compared with control group (7.2 ± 0.2 cycles per animal) in the present study. Data from the previous studies implied that sodium arsenite plays a crucial inhibitory role on key ovarian steroidogenic enzyme (Δ^5, 3-βHSD and 17-βHSD) activities which interrupts ovarian estradiol synthesis [**Chattopadhyay and Ghosh, 2010; Chattopadhyay et al., 2003**].

Uterine injury was primarily due to the remarkable loss of secretory cells of uterine tissue along with the distortion of endometrial layer. Co-treatment of Vit-B_{12} and folic acid [Vit-B12 (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day)] significantly secured such arsenic induced uterine-hazards (**Fig. 5.3.11**). These protective mechanisms might have ended by synergistic action of vit-B_{12} and folate thereby protect arsenic induced apoptotic and nacrotic damages as evidenced from the improvement of uterine histopathological status (**Fig.5.3.8 & Fig.5.3.9**) and subsequent restoration of DNA (**Fig. 5.3.10**). In addition, the uterus in these animals was significantly protected by oxidative stress, as shown by the restrained generation of free radical products MDA and CD possibly by conserving SOD and CAT function. This might have been resulted in a lesser accumulation of tissue arsenic. Though tissue arsenic levels were not measured in the present investigation, several studies suggest that folic acid and vitamin B_{12} [Vit-B_{12} (0.07µg/100g body wt. /day)+ Folic acid (4µg /100g body wt. /day)] could reduce tissue content of arsenic by

enhancing its urinary excretion (**Mukherjee et al., 2006; Flora et al., 2007; Gamble et al., 2007**). Further studies are required to obtain a concrete result. However, animals in the vitamin-supplemented group showed a re-established morphology as compared to those in arsenic-exposed group. Earlier investigation confirmed that 7 estrous cycle duration with low dose (0.4 ppm) of arsenic treatment significantly increases arsenic concentration in uterine tissues (**Chattopadhyay & Ghosh, 2010**). A higher tissue arsenic concentration is obviously allied with the impaired antioxidant status and tissue damages. This most likely reflects the determination that inorganic arsenic may be incorporated with reduced plasma level of vitamin B_{12} and folate, which enhances the reducing of the arsenic detoxification, resulted in a delay in the biliary excretion of arsenic (**Kile and Ronnenberg., 2008**). Hence, exogenous vitamin B_{12} and folate may enhance the arsenic excretion through bile in the methylated form (As -III).

Methylation of arsenic is the process in the excretion, removal and detoxification of arsenic from the system. A derivative of vitamin B_{12} named as methyl cobalamin (CH_3B_{12}) along with reduced glutathione (GSH) are established as the coenzymes and involved in the methylation reaction of inorganic arsenic accompanied with SAM [**Nakamura, 2011**]. It has already been established that arsenic is detoxified via methylation reaction in association with SAM (S-adenosylmethionine) and the enzyme AS_3MT (methyl transferase). Hence methylation of As (III) is occurred via one-carbon metabolism to yield methylarsonic acid (MMAs) and SAH (S-adenosyl homocysteine) followed by homocysteine generation and adenosine by the hydrolysis of S-adenosyl homocysteine [**Hall and Gamble, 2012**]. Methionine synthase association with N5 further remethylates this homocystein presence of methyl tetra hydrofolate as a co-substrate thereby eventual regeneration of SAM

during removal of arsenic. Hence, the involvement of methionine synthase is required to folate metabolism to maintaining the folic acid pool in the system. Folic acid also maintains the homeostasis of endogenous methionine level by converting homocysteine consistently. In this methylation process, the toxic derivative of arsenic (III) is oxidized and converted into less harmful arsenic forms (V). Their intracellular mechanism is regulated by vit-B_{12} and folate (**Kligerman et al., 2003**). This study approaches a plausible effective drainage of free radicals, which might be helpful in protection as in tissue or DNA level.

Excretion of arsenic from the body or organ is necessary to inhibition of uterine stress and regularization the normal histoarchitecture. Moreover, S-adenosyl homocysteine is catalyzed by methionine synthase in continuous association with exogenous folic acid and vit-B_{12} during the regularization of normal endogenous methionine level. Therefore, supplementation of previously mentioned vitamins at physiological concentration acts as a therapeutic agent favouring detoxification and elimination of arsenic via urine.

Consequently, poor folate in circulation enhanced tissue retention of arsenic and generates the cellular toxicity of inorganic arsenic due to the inhibition of biotransformation and excretion of either inorganic arsenic or its derivatives. Therefore, it is explored that impairment of folate and homocysteine are required during arsenic methylation and detoxification cum removal process (**Spiegelstein et al., 2003**).

In spite of that, the free radicals generated degradation is restricted in co-treatment of exogenous Vit-B_{12} and folate [Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day)] in comparison to only the arsenic treated group. These vitamins

are also intended to the defense of the uterine tissues and restoration of genetic materials by ensuing the hindrance of necrosis and probable carcinogenesis (**Mukherjee et al., 2006**). Uterine histoarchitecture also shown a renovation of glandular tortuosity and wide lumen with expressed that secretory phase

However, renovation of uterine and ovarian weight in arsenic affected rats by the vitamin B_{12} and folate co-treatment (**Table 5.3.IIIA.I**) may be postulated due to the safeguarding of ovarian steroidogenesis and plasma gonadotrophin levels. Earlier study explored that vitamin B_{12} and folate may play a crucial role in restoring peroxidase activities in arsenic treated rats [**Stokstand, 1980**], and these results in this regard strengthen this interaction between these two vitamins and peroxidase. In group of vitamin B_{12} and folate co-administration either alone (Vit-B_{12} -6.0± 0.4 cycles/animal; folate- 6.3 ± 0.2 cycles / animal) or in combination, the frequency of estrous cycles was no dissimilarity (7.0 ± 0.2 cycles / animal) was seen in compared with the control level in relation to the probable upshot of the protection of E_2 signaling [**Bennett, 2001; Chattopadhyay and Ghosh, 2010**]. Cervical dysplasia , the abnormalities of the cell lining of the cervix and uterus is the possible outcome of B_{12} deficiency. Folate deficiency alleviated the evel of circulating estradiol, testosterone and LH [**Lepkovsky et al., 1951; Wallock-Montelius et al., 2007**].

Nonetheless, arsenic mediated elucidation of free radicals resulted over production of MDA, CD and reduction of catalase and superoxide dismutase enzymes that can affect the cell viability of ovary. In this investigation, the arsenic mediated oxidative stress injuries are prevented in selective dose of Vit-B_{12} and folate co-administration group by increasing the catalase and superoxide dismutase activity and diminution of ovarian MDA and CD level (**Fig.5.3.3, Fig.5.3.6**). The development of ovary is

noted by the elevation of healthy follicles and regression of atretic follicles suggested that they are also protecting apoptosis and necrosis at cellular level with a revival of hormonal signaling system (**Fig.5.3.7**).

To search out the toxicity of selective doses of Vitamin B_{12} and folic acid [Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day)] whether could generate toxicity on reproductive, metabolic and excretory organs; the toxicity assessment study was designed in the **Experiment IV**. In this experiment revealed that the selective doses of these B vitamins has no harmful injury to the hepato-renal system as well as reproductive health also. The ovarian and uterine somatic indices were within normal range that means these vitamins have no toxicity on reproductive organ either alone or in combination [**Table 5.4.IVA.I**]. This incidence also reflected on the balance of ovarian steroidogenesis that proved there was no drug dependent injury [**Table 5.4.IVA.II**]. Not only the reproductive organs but also in other organs such as liver and kidney these B vitamins also maintained the good hygiene without alteration of serum total protein, SGPT, SGOT, ALP, urea creatinine (**Fig.5.4.1**). Vitamin B_{12} and folic acid act as good co-factors in the regulation of ROS generation by maintaining the homeostasis of SOD, CAT and peroxidase activity in liver and kidneys (**Fig.5.4.2, Fig.5.4.3, and Fig.5.4.4**). The data from histoarchitecture (**Fig.5.4.5 & Fig.5.4.6**) also revealed that no necrotic changes in tissue along with no DNA breakage were found *in vivo* with the application of these B vitamins either alone or in combination (**Fig.5.4.7 & Fig.5.4.8**).

In **Experiment V**, from the collected data suggested that arsenic acts as a hepato-toxicant which enhances the hepatic injury. The elevation of the activities of ALT

and AST revealed that a massive liver tissues degradation caused by necrosis and apoptosis [**Chattopadhyay et al., 2003; McVicker et al., 2009**] (**Fig.5.5.1**). One study reported Liver cirrhosis occurs in smelter workers after chronic ingestion to arsenicals. Moreover, arsenic is a causative agent to infiltration of inflammatory cells in the liver periportal areas [**Liu et al., 2002].** The cumulative data on arsenic induced rats a significant decrease (10%) in liver protein postulated the suppression of protein synthesis (**Fig.5.5.1**). Arsenic has been shown to influence in regulation to the level of sulfhydryl-containing proteins [**Palaniappan and Vijayasundaram, 2008; Santra et al., 2000**]. In these experiments the arsenic mediated hyperlipidemic state indicates disorientation of hepatic function (**Fig.5.5.2**). Some cross sectional study reported that fatty infiltration in the liver and fibrotic changes have been occurred in arsenic-exposed animals [**Liu and Waalkes, 2008**]. This may be linked to the present elevation in hepato-somatic index by arsenic ingested group (**Table 5.5.VA.I**). Arsenic induced hyperlipidemic state and its peroxidation is responsible to generate oxidative stress in tissues [**Yang et al., 2007; Shila et al., 2005**]. The level of NPSH, which is a direct determinant of GSH pool, reduced after arsenic consumption in the present study [**Mieyal et al, 2008; Forman et al, 2009**] (**Fig.5.5.3**). Arsenic influences oxidative stress in cultured lung epithelial cells and isolated brain tissues [**Li et al, 2002; Shila et al., 2005**]. In our study stated that elevation of free radical products MDA, CD in sodium arsenite treated rats possibly by impairing CAT and SOD activities (**Fig.5.5.3**). Here, a larger hepatic DNA-smearing in arsenic exposed rat, which may be the yielding of ROS in hepatic tissues (**Fig.5.5.5**). Our histological slides exhibited a drastic damage in hepatic tissues in arsenicated rats (**Fig.5.5.4**). Metabolism of arsenical compounds is allied to generate oxidative stress [**Tabacova et al., 1992; Yamanaka et al., 1990**], which

ultimately influences DNA strand breakage and necrosis [**Kato et al., 1994**]. Arsenic is a genotoxic agent, also evident in human lymphocytes and whole white blood cells [**Avani and Rao, 2007; Maiti et al., 2012**] linking with the altered gene-expression–related premalignant skin lesions [**Kibriya et al., 2007; Argos et al., 2006**]. Co-administration of vitamin B_{12} and folic acid along with arsenic treated rats profoundly restricted disorientation of hepatic tissue and its DNA breakage. In addition, the livers in these animals were significantly defended from oxidative stress, as noticed by the retardation of free radical generation, such as MDA and CD, may be by restoration of CAT and SOD function and NPSH level. The diminution of free radical generation may have been the upshot of a lesser accumulation of tissue arsenic. Several cross sectional study expressed that folic acid and vitamin B_{12} can minimize tissue arsenic by enhancing its urinary excretion [**Mukherjee et al., 2006; Flora et al., 2007; Gamble et al., 2007**]. In the present observation, hepatic DNA was markedly protected via oxidative stress by the co-administration of Vit-B_{12} and folic acid (**Fig. 5.5.5)**. Some reports revealed that folic acid supplementation in rats resulted in increased arsenic methylation by folate-dependent, one-carbon metabolism in association with S-adenosyl methionine (SAM) [**Gamble et al., 2006**]. On the other hand, vitamin B_{12} also ameliorates methylation of inorganic arsenic in coalition with GSH [**Nakamura et al, 2009**]. Methylation of inorganic arsenic is impaired to nullify the imposition arsenic in tissue and promotes its excretion through urine [**Gamble et al., 2006; Nakamura et al., 2009; Nakamura, 2011**]. These protective mechanisms might have terminated arsenic induced apoptotic stimuli and protected multiple tissues from the necrotic changes.

Whether the selected doses of vit-B_{12} and folic acid [Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day) have any reversible or irreversible effect

on the recovery of arsenic induced toxicity phenomenon on female reproductive organs (uterus and ovary), to search it out, the **Experiment-VI** was also conducted by duration dependent withdrawal treatment.

The tables and plates in **Experiment-VI** indicated that ovarian as well as uterine somatic indices**,** ovarian Δ^5,3β-HSD**,** 17β-HSD activities, plasma levels of gonadotrophins along with estradiol (**Table 5.6.VIA.I**) and pattern of estrous cycle did not shown any reversible changes for 28 days of sodium arsenite ingestion followed by 16 days withdrawal treatment. In contrast, 28 days of sodium arsenite withdrawal after 28 days of sodium arsenite ingestion established significant reversible changes of the aforesaid parameters. In both 16 days and 28 days withdrawal experiment after 28 days co-administration of two B vitamins against sodium arsenite the above mentioned parameters remain unchanged significantly in compared with the vehicled control. The persistence of inhibitory ovarian enzyme activities after 16 days withdrawal treatment exhibited low level of FSH, LH and estradiol (**Table 5.6.VI.I**) that retardation of folliculogenesis was continued. The prolongation of diestrus phase proved that impairment of estrous cyclicity. In **Table 5.6.VI.II,** 16 days withdrawal treatment after 28days arsenic ingestion a remarkable persistence of low level scavenging enzymes (CAT, SOD and Peroxidase that leads to cellular necrosis and DNA breakage by the generation of ROS (**Fig.5.6.1**).

On the other hand 28 days sodium arsenite withdrawal the rejuvenation of steroidogenic activity leads to normalization of the plasma level of gonadotrophins and estradiol that explored the recovery of ovarian dysfunction (**Table 5.6.VI.I)**. The reversible changes of scavenging enzymes lead to the improvement of arsenic

mediated tissue injury by ROS resulted in the restoration of DNA breakage and normal regularization of cellular proliferation and differentiation (**Fig.5.6.1**).

But the above parameters were remained same following Vit-B_{12} and folic acid at selective doses either 16 days or 28 days withdrawal treatment.

From these results, it may be suggested that due to the intake of arsenic contaminated water**,** the developed ovarian toxicity may be reversed and settled to normal level due to cessation of the use of arsenic contaminated water as drinking water. A remarkable irreversible protective measure also exhibited in co-administration vitamins supplemented group also. Therefore**,** such adverse effects of sodium arsenite are not permanent and remediation of arsenic toxicity in co-administration of Vit-B_{12} and folic acid at the selective doses is irreversible.

To search out the possibilities of direct effect of arsenic in presence of vit-B_{12} and folic acid on ovarian steroidogenesis *in vitro* study has been conducted in **Experiment-VII**. Table of this experiment indicated that there was no inhibitory effect found in ovarian Δ^5, 3Δ-HSD and 17β-HSD activities for 2 hrs instant study. To conduct this experiment, ovarian tissues were incubated in a media containing 0.4 ppm sodium arsenite/ ml of drinking water and co-administration of vit-B_{12} and folic acid [Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day)] for 2 hrs duration. In this *in vitro* study, we highlighted the steroidogenesis only but not the folliculogenesis, because, from our previous experiments it has been established that arsenic mediated inhibition of folliculogenesis mainly via the modulation of ovarian steroidogenesis [**Chattopadhyay & Ghosh, 2010**]. At this dose, as there was no toxic effect on ovarian steroidogenesis in arsenic group and no alteration has been shown by the co-administration of vitamin supplemented group; this incidence

indicates that there was no possibility of direct action of vitamin B_{12} and folic acid on arsenic treated ovarian steroidogenesis. Therefore, it may be concluded that adverse effect developed by sodium arsenite *in vivo* condition may be due to the indirect effect of sodium arsenite via the modulation of hypothalamico-hypophyseal system and this abrupt injury is protected *in vivo* by modulation the ROS generation via methylation.