5.0 EXPERIMENT

5.1 Experiment-I

Dose Dependent response of Sodium Arsenite on reproductive organs of female albino rat.

5.1.1 Aims and objectives:

To find out the effective doses of sodium arsenite ($NaASO_2$) to develop the arsenic toxicity in reproductive organs of female albino rats.

5.1.2 Experimental design:

First experiment in this thesis has been conducted by using 0.2 ppm and 0.4 ppm of sodium arsenite as these two doses are present at drinking water of the wide areas of our state West Bengal along with other contaminated states in India. The dose dependent experiment has been conducted here to search out the most effective dose of sodium arsenite for its toxic effect on female reproductive system.

5.1.3 Animal grouping treatment protocol:

Eighteen female albino Wistar strain rats (each group six) aged 60 days with weight of 120±10 g were selected in this experiment. They were nourished according to standard laboratory provision 12hrs light- 12hrs dark and 25±2 ^{0}C temperature with opened admittance to food (Standard Laboratory Diet) and water *ad libitum* and kept for 15 days to acclimatize before this first experimental study. The Principle of Laboratory Animal Care (CPCSEA guide line) and ethical authorization from Vidyasagar University was executed throughout the duration of experiment. All rats were synchronized for the maintenance of estrous cycle by single orally introduction

of estardiol at a dose of 3μg/100g.body wt. The treatment duration was extended for 28 days.

5.1.4 Treatment protocol:

For conducting first experiment 18 rats were arranged into three groups equally by recording the initial body weight. The pattern of group is as followed:

Group I: Control- 0.05ml distilled water /100g body weight/day as vehicle orally.

Group II: 0.2 ppm/100g body wt. /day sodium arsenite orally. .

Group III: 0.4 ppm/100g body wt./day sodium arsenite orally.

In this experiment, 28 days duration of treatment was preferred to investigate the chronic effect of $NaAsO_2$ on female reproductive system in rat. However, after 28 days duration just covering the 7 estrous cycles is considered as chronic type of treatment and so, this length of time was selected here.

This aforesaid concentration of $NaAsO_2$ was prepared in every week and stored it in cool and dark place. Vaginal smears were collected twice in a day at 8 O'clock in the morning and 5 O'clock in the evening from all animals. Feeding behavior of each and every animal was observed cautiously during the experimental schedule. On the 29^{th} day of this experiment final body weights of all the investigational rats were measured. After anesthesia by ketamine HCI, blood was collected from dorsal aorta of each rat using a 21-gauge needled syringe. Serum samples were separated after centrifuging the blood and stored at -20^0C in separate ampoules until all the samples had been used for the determination of serum level of gonadotrophins and estradiol.

After collection of blood all the animals were euthanized by sodium barbiturate. The ovary and uterus of the 18 animals of experimental groups were dissected out and wet weights of these organs were measured.

Both ovaries and the uterine horns were dissected out and its wet weights were noted by electronic balance. Right ovaries and right uterine horns from all animals were utilized for the biochemical and others assays whereas left ovaries along with left portion of the uterine horns of all rats were fixed in 10% formalin solution for histological study.

After fixation in 10% formalin solution, 5µ thick paraffin section of ovary and uterus were stained by haematoxylin and eosin for histometric analysis by using microscope.

5.1.5 Results:

5.1.5.1 Pattern of estrous cycle:

After 18±2 days of $NaAsO_2$ treatment at the dosage of 0.4 ppm, experimental rats exhibited consistent diestrus stage in their vaginal smear but there was no considerable variation after 0.2 ppm $NaAsO_2$ treatment when compared with control group of rats.

5.1.5.2 Body weight and organ weight:

After treatment of 0.2 ppm $NaAsO_2$ for 28 days, there was no significant change in the ovarian in addition to uterine-somatic index when compared with control. But after the treatment of 0.4 ppm $NaAsO_2$ for 28 days, a significant attenuation (decrease) was noted in ovarian along with uterine somatic indices when compared with the control group and 0.2 ppm sodium arsenite treated group (**Table -5.1.IA.I**).

5.1.5.3 Ovarian Δ^5, 3β-HSD activities and 17 β-HSD activities:

Sodium arsenite treatment throughout twenty eight days at the 0.4 ppm dose resulted a significant deterioration of ovarian Δ^5, 3 β-HSD and 17 β-HSD activities in compared with the control group. There was no significant attenuation (decrease) in the above enzymatic activities at 0.2 ppm $NaAsO_2$ treatment continued for same extent in compared with the control group. Besides, a significant diminution in these enzymes activity was observed at the 0.4 ppm $NaAsO_2$ dose in comparison with 0.2 ppm **(Table 5.1.IA.II).**

5.1.5.4 Serum levels of FSH, LH and estradiol:

$NaAsO_2$ treatment at the 0.4 ppm dose was resulted in significant drop in serum levels of LH, FSH and estradiol without showing any significant alteration of the above hormonal levels at 0.2 ppm when compared with that of control group of animals. (**Table-5.1.IA.II).**

5.1.5.5 Antioxidative enzymes:

A significant diminution of catalase, superoxide dismutase activity in uterine (**Fig.5.1.1**) and ovarian (**Fig**.**5.1.1**) tissue and uterine peroxidase enzyme activities (**Fig.5.1.2**) were observed following the dose of 0.4 ppm arsenic for 28 days where as no significant level of changes noted in 0.2 ppm dose of arsenic treated group when compared to vehicle treated group.

5.1.5.6 Lipid Peroxidation biomarkers:

The end product level of lipid peroxidation markers as conjugated diene (CD) and malondialdehyde (MDA) in uterine and ovarian tissue were measured. In arsenic treated group (0.4ppm) these concentration were increased significantly than the

other arsenic treated group (0.2ppm) in comparison with respective vehicled control group (**Fig. 5.1.3**).

5.1.5.7 Histometric study of ovary and uterus:

Arsenic treated group at the higher dose of $NaAsO_2$ (0.4 ppm) for 28 days exhibited increasing the number of follicular atresia and a significant reduction in uterine luminal diameter along with a significant reduction in the thickness of epithelial cell height, myometrium and endometrium without showing any significant in alteration or in reduction in these above histometric parameters at 0.2 ppm arsenic **(Fig. 5.1.4 and Fig. 5.1.5).**

Mode of treatment	**Initial body wt. (gm)**	**Final body wt. (gm)**	**Ovarian - somatic index (mg %)**	**Uterine - somatic index (mg %)**
Control (Vehicled)	124 ± 3.5^{a}	145 ± 2.9^{a}	95 ± 4.0^{a}	140 ± 5.6^{a}
$NaAsO_2$ (0.2 ppm)	125 ± 3.5^{a}	141 ± 2.5^{a}	93.3 ± 4.1^{a}	136 ± 4.5^{a}
$NaAsO_2$ (0.4 ppm)	125 ± 3.1^{a}	139 ± 2.1^{b}	73.7 ± 4.7^{b}	110 ± 3.5^{b}

Table.5.1.IA.I: Dose dependent changes in body weight, ovarian-somatic index and uterine-somatic index after arsenic treatment. Data represents here is mean ± SE, N=6, $p<0.001$. ANOVA followed by multiple student's t test. Same alphabet (superscript) in each column did not change from each other significantly.

Mode of treatment	Δ^5, 3 β-HSD (units/mg of tissue/hr)	17 β-HSD (units/mg of tissue/hr)	LH (mIU / ml of serum)	FSH (mIU / ml of serum)	Estradiol (pg / ml of serum)
Vehicle treated control	33.8 ±2.2 [a]	31.5 ±2.1 [a]	2.5± 1.8 [a]	6.3 ±1.0 [a]	141.2± 3.2 [a]
$NaASO_2$ (0.2 ppm)	29.3±2.5 [a]	28.9 ±3.1 [a]	2.1 ±9.7 [a]	5.8 ±0.9 [a]	136.5±3.8 [a]
$NaASO_2$ (0.4 ppm)	19.4 ±3.5 [b]	16.9 ±1.8 [b]	1.4 ±1.0 [b]	2.9 ±0.7 [b]	115.4±3.7 [b]

Table. 5.1.IA.II: Dose dependent Changes in ovarian steroidogenic enzyme activities, serum levels of gonadotrophins and estradiol after arsenic treatment. Data represents here is mean±SE, N=6, $p<0.001$. ANOVA followed by multiple student's t test. Same superscript in each vertical column did not differ from each other significantly.

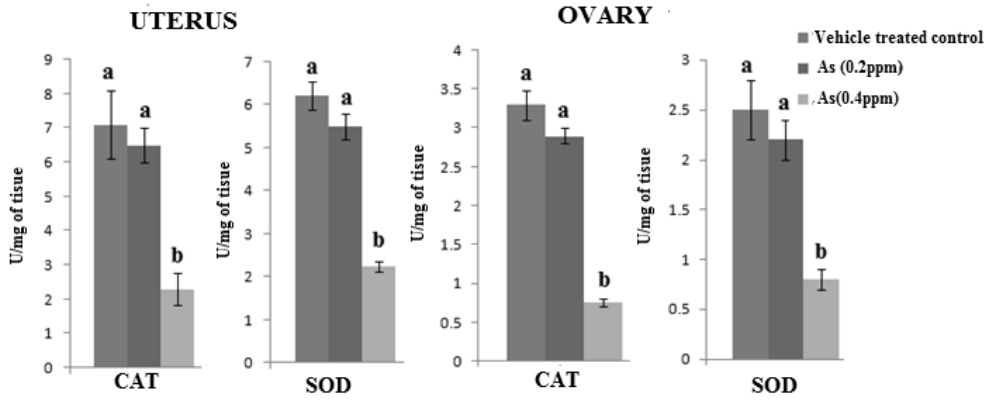


Figure 5.1.1: Dose dependent changes of catalase and superoxide dismutase activities in uterine and ovarian tissue after arsenic treatment. Data represents here is mean±SE, N=6, $p<0.001$. ANOVA followed by multiple student's t test. Values of bar diagrams with same superscript did not differ from each other significantly.

Figure 5.1.2: Dose dependent changes of peroxidase activities in uterine tissue after arsenic treatment in 8% native polyacrylamide gel electrophoresis.

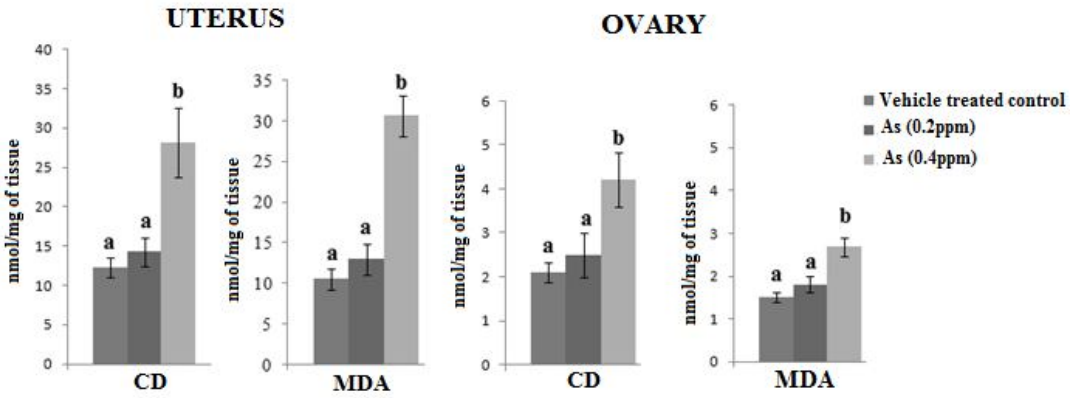


Figure 5.1.3: Dose dependent changes of conjugated diene and malondialdehyde activities in uterine and ovarian tissue after arsenic treatment. Data represents here is mean ±S E, N=6, $p<0.001$ and ANOVA followed by multiple student's t test. Values of bar diagrams with same superscript did not differ from each other significantly.

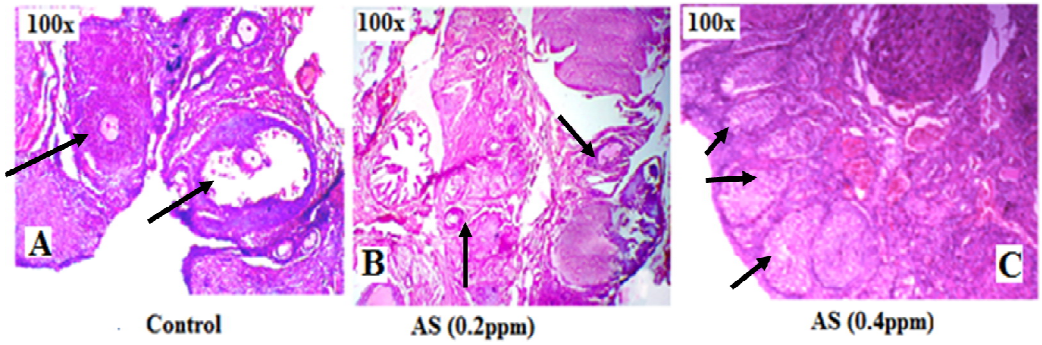


Figure 5.1.4: Histological sections show the ovaries of (A) vehical treated control focusing normal folicals , (B) after sodium arsenite treated(0.2ppm) rat. (C) after sodium arsenite treated(0.4ppm) rat showing follicular atresia.

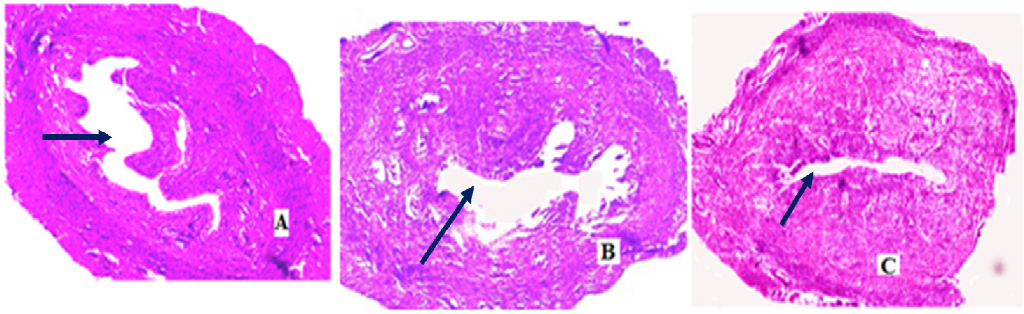


Figure 5.1.5: Histological sections show the uterus of (A) vehical treated control focusing normal internal lumem diameter, (B) No change internal lumem dameter after sodium arsenite treated(0.2 ppm) rat. (C) after sodium arsenite treated (0.4 ppm) rat showing reduce of internal lumem dameter.

5.1.6 Conclusion: From the above experimental studies it was explored that 0.4 ppm $NaAsO_2$ is the effective dose to generate arsenic toxicity in female albino rats' reproductive organs.

5.2 Experiment-II

Duration dependent response of Sodium arsenite on reproductive organs female albino rat.

5.2.1 Aims and Objective: In this experiment it is established that which duration is most effective to produce arsenic toxicity after selection of effective dose (0.4 ppm).

5.2.2 Experimental Design: Twenty four albino Wistar strain rats (each groups six) aged 60 days with weight of 120±10 g were selected in this experiment. They were nourished according to standard laboratory provision 12 hrs light and 12 hrs dark along with 25±2 ^{0}C temperature with opened admittance to food (Standard Laboratory Diet) and water *ad libitum* and kept for 15 days to acclimatize before this experimental study. The Principle of Laboratory Animal Care (CPCSEA guide line) and ethical authorization from Vidyasagar University was executed throughout the duration of experiment. All rats were synchronized for the maintenance of estrous cycle by single orally introduction of estardiol at a dose of 3µg/100g.body wt. The treatment duration was extended for 16 days and 28days.

Animal grouping treatment protocol: For conducting this experiment 24 rats were arranged into four groups equally by recording the initial body weight. The pattern of group is as followed:

Group I: Control- 0.05 ml distilled water /100g body weight/day as vehicle orally.

Group II: 0.4 ppm/100g body wt. /day sodium arsenite orally for 16 days

Group III: Control- 0.05 ml distilled water /100g body weight/day as vehicle orally.

Group IV: 0.4 ppm/100g body wt. /day sodium arsenite orally for 28 days

The untreated control animals were provided with pure drinking water as vehicle treated control (**Group I and Group III**) rats without any arsenic contamination for 16 days and 28 days respectively.

Here 16 days duration of treatment was selected to search out the short term effect of sodium arsenite on female reproductive system. Sixteen days treatment just covers the 4 estrous cycles. Twenty-eight days duration of treatment was selected to search out the long term effect of sodium arsenite on female reproductive system in rat.

The rats (**Group II** & **Group IV)** were fed orally at a dose of 0.4 ppm of sodium arsenite /100g body weight /day for consecutive days of 16 and 28 days respectively. The aforesaid concentrations of $NaAsO_2$ were prepared in every week and kept in cool and dark place. Vaginal smears were collected regularly at the morning and evening as before from all experimental animals. After anesthesia by ketamine HCI, blood was collected from dorsal aorta of each rat using a 21-gauge needled syringe. Serum samples were separated after centrifuging the blood and stored at -20^0C in separate ampoules until all the samples had been used for the determination of serum level of gonadotrophins and estradiol.

After collection of blood all the animals were euthanized by sodium barbiturate. After collection of blood all the animals' ovaries and uterus of the 6 animals of each experimental group were dissected out and wet weights of these organs were measured by electronic balance. One ovary along with both uterine horns from each animal was used for biochemical assay activities whereas other one ovary of each rat was fixed in 10% formalin solution to study histometry.

5.2.3 Results:

5.2.3.1 Pattern of estrous cycle:

After 18±2 days of $NaAsO_2$ treatment at the dose of 0.4 ppm, experimental rats shown persistent diestrus of their vaginal smear but there was no significant variation after 0.4 ppm sodium arsenite treatment continued for 16 days when compared with the control group of rats.

5.2.3.2 Body weight and organ weight:

There was no significant alteration in the somatic growth at both durations after 16 days and 28 days of 0.4 ppm sodium arsenite treatment in compared with the control rats. .After treatment of higher dose of $NaAsO_2$ for 16 days, there was no significant change in the ovarian and uterine-somatic index when compared with the control. But 0.4 ppm $NaAsO_2$ for 28 days reflected a significant drop in these somatic indices when compared with the vehicled control group **(Table 5.2.IIA.I**).

5.2.3.3 Ovarian Δ^5, 3 β-HSD and 17 β-HSD activity:

$NaAsO_2$ treatment throughout twenty eight days at a higher dose (0.4 ppm) was exhibited in the significant inhibition of ovarian Δ^5, 3 **β-HSD** activities and 17 **β-HSD** activities in comparison with the respective control group without showing any significant diminution in these above enzymatic activities at 0.4 ppm sodium arsenite treatment continued for 16 days in comparison with that of control group (**Table 5.2.IIA.II**).

5.2.3.4 Serum levels of FSH, LH and estradiol:

There is FSH and LH level in serum is markedly reduced in the application of higher dose (0.4 ppm) for 28 days treatment where as this type of changes were not visible

in 0.4 ppm treated group for 16 days duration in contrast to the vehicled control group (**Table 5.2.IIA.II**)

5.2.3.5 Antioxidative enzymes:

Catalase, superoxide dismutase activity in ovarian and uterine tissue were markedly attenuated (decreased) in 0.4ppm dose group for 28 days whereas this type of change was not noted in 0.4ppm treated group for 16 days in contrast to the vehicled control group (**Fig. 5.2.1**).

5.2.3.6 Lipid Peroxidation biomarkers:

The end product level of lipid peroxidation as CD and MDA in uterine tissue was done. In the level of lipid peroxidation in arsenic treated group at a dose 0.4 ppm for 28 days were markedly elevated where as any no significant changes were found in the duration of 16 days treatment in respective to the vehicle treated control group (**Fig. 5.2.2**).

5.2.3.7 Histometric study of ovary:

There was no foliculogenesis hampered at a dose of 0.4 ppm for 16 days in respect to the vehicled control group. On the other hand significant follicular atresia was noted after arsenic treatment at a dose of 0.4 ppm for 28 days (**Fig. 5.2.3).**

Mode of treatment	**Initial body wt. (gm)**	**Final body wt. (gm)**	**Ovarian - somatic index (mg%)**	**Uterine - somatic index (mg%)**
Vehicle treated control (16 days)	123±3[a]	138±4[a]	97.2±4[a]	136.4±6.9[a]
Sodium Arsenite 0.4 ppm for 16 days	124±3[a]	136±3[a]	88.5±5[a]	121±7.2[a]
Vehicle treated control for 28 days	125±3[a]	148±2[a]	95.5±4[a]	145±7.1[a]
Sodium Arsenite 0.4 ppm for 28 days	126±2[a]	138±4[a]	69.2±5[b]	109.5±7.3[b]

Table-5.2.IIA.I: Duration dependent changes in body weight, ovarian-somatic index and uterine-somatic index after arsenic treatment. Data represents here is mean ± SE, N=6, $p<0.001$ and ANOVA followed by multiple student's t test. Same superscript in each vertical column did not differ from each other significantly.

Mode of treatment	Δ^5, 3 β-HSD (units/mg of tissue/hr)	17 β-HSD (units/mg of tissue/hr)	LH (mIU / ml of serum)	FSH (mIU / ml of serum)	Estradiol (pg / ml of serum)
Vehicle treated control (16 days)	37.6±2.1	27.3±3.2	3.5±0.2	7.1±0.63	133.7±5.5
0.4 ppm sodium arsenite (16 days)	35.5±1.9	24.2±2.2	3.3±0.4	6.1±0.62	129.77±4.8
Vehicle treated control (28 days)	35.2±1.6	31.3±3.1	3.4±0.7	7.2±0.79	135.7±5.7
0.4 ppm sodium arsenite (28 days)	19.8±1.8*	18.0±3.4*	1.9±0.2*	3.2±0.58*	109.4±6.7*

Table 5.2.IIA.II: Duration dependent changes in ovarian steroidogenic enzyme activities, serum levels of gonadotrophins and estradiol after arsenic treatment. Data represents here is mean ± SE, N=6. ANOVA followed by two tailed student's t test were used to find out statistical significance at $p<0.001$. An asterisk indicates an experimental value significantly different from the respective control value.

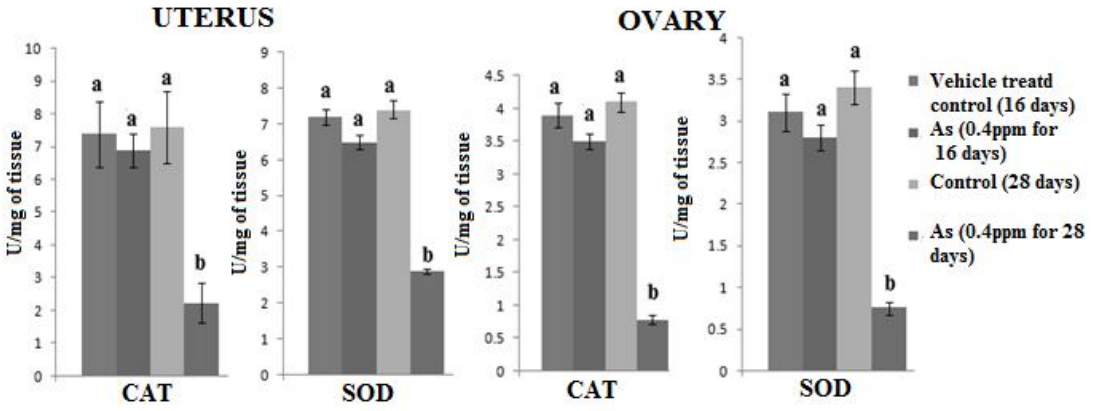


Figure 5.2.1: Duration dependent changes of catalase and superoxide dismutase activities in uterine and ovarian tissue after arsenic treatment. Data represents here is mean±SE, N=6, p<0.001 and ANOVA followed by multiple student's t test. Values of bar diagrams with same superscript did not differ from each other significantly.

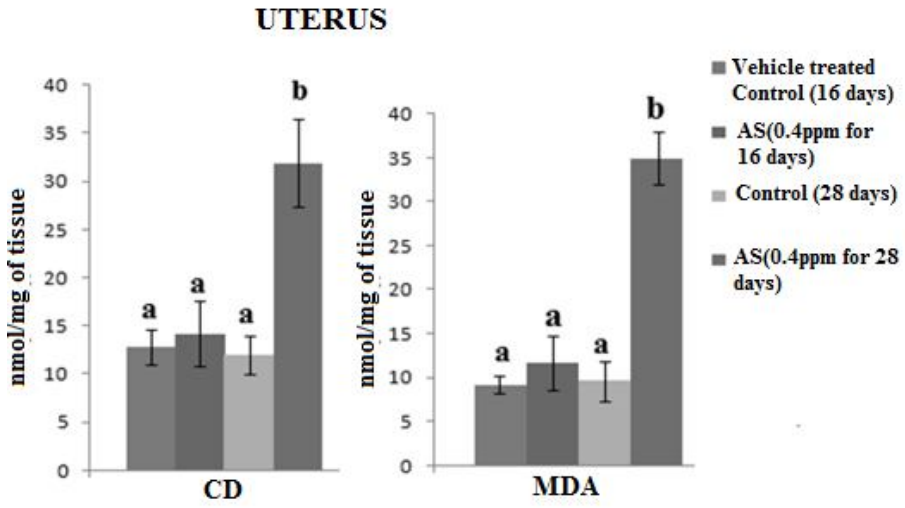


Figure 5.2.2: Duration dependent changes of conjugated diene and malondialdehyde activities in uterine tissue after arsenic treatment. Data represents here is mean ± SE, N=6, p<0.001 and ANOVA followed by multiple students t test. Values of bar diagrams with same superscript did not differ from each other significantly.

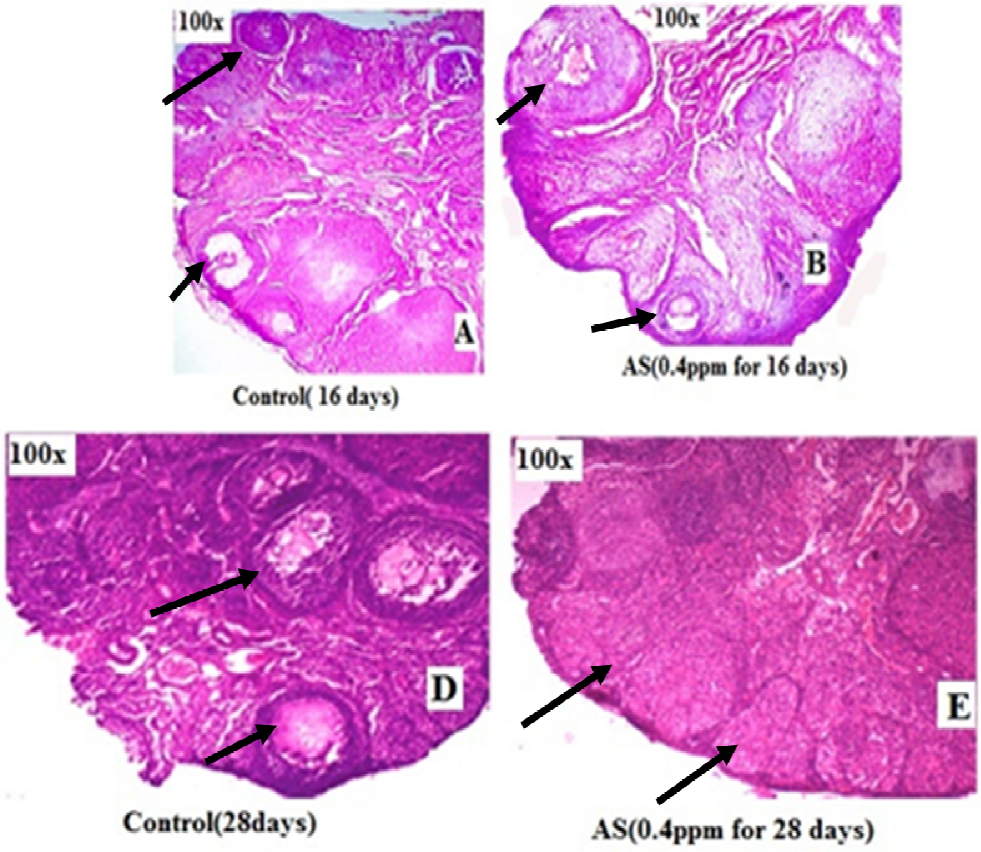


Figure 5.2.3: Histological sections show the ovaries of (A) vehical treated control focusing normal folicals , (B) after sodium arsenite treated (0.2ppm) rat for 16 days focusing normal folicals, (D) vehical treated control focusing normal folicals for 28 days, (E) after sodium arsenite treated (0.4ppm) rat for 28days showing follicular atresia.

5.2.4 Conclusion: According to the collective data it is revealed that 28 days is the critical period to generate reproductive toxicity on rodents by ingestion of 0.4 ppm dose of arsenic.

5.3 Experiment-III

Dose Selection of Vitamin-$B_{12}$and folic acid by ameliorative effect to reduce the arsenic mediated toxicity on female reproductive organs.

5.3.1: Aims and objectives: To find out the minimal effective doses of vit-B_{12} and /or folic acid to delineate the arsenic toxicity in reproductive organs of female albino rats.

5.3.2: Experimental design:

Forty eight female albino Wistar strain rats (each groups six) aged 60 days with weight of 135±5 g were selected in this experiment. They were nourished and acclimatized are followed as the before experiment. . The duration of the experiment was extended for 28days. All the rats are synchronized by orally introducing of estardiol at a dose of 3µg/100g.body wt.

For experimental purpose after recording the body weights all the female rats were divided equally and the experimental schedule is as followed:

Group I: Vehicle treated control

Group II: $NaAsO_2$ (0.4 ppm/100g body wt. /day).

Group III: $NaAsO_2$ (0.4 ppm/100g body wt. /day) + Vit-B_{12} (0.04µg/100g body wt. /day).

Group IV: $NaAsO_2$ (0.4 ppm/100g body wt. /day) + Folic acid (2µg /100g body wt. /day).

Group V: $NaAsO_2$ (0.4 ppm/100g body wt. /day) + Vit-B_{12} (0.04µg/100g body wt. /day) + Folic acid (2µg/100g body wt. /day).

Group VI: $NaAsO_2$ (0.4 ppm/100g body wt. /day) + Vit-B_{12} (0.07µg /100g body wt. /day).

Group VII: $NaAsO_2$ (0.4 ppm/100g body wt. /day) + Folic acid (4µg/100g body wt. /day).

Group VIII: $NaAsO_2$ (0.4 ppm/100g body wt. /day) +Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day).

The aforesaid concentrations of $NaAsO_2$ and two B vitamins were prepared in every week and kept in cool and dark place. Vaginal smears were collected regularly at the morning and evening as before from all investigational animals. After anesthesia by ketamine HCI, blood was collected from dorsal aorta of each rat using a 21-gauge needled syringe. Serum samples were separated after centrifuging the blood and stored at -20^0C in separate ampoules until all the samples had been used for biochemical assay.

After collection of blood all the animals were euthanized by sodium barbiturate. The ovary and uterus of the 48 experimental animals were dissected out and wet weights of these organs were measured by electronic balance. One ovary along with one sided uterine horns from each animal were used for biochemical assay activities whereas other one ovary and another one sided uterine horns of each rat were fixed in 10% formalin solution for histometric study.

5.3.3: Results:

5.3.3.1 Estimation of Δ5,3β-HSD and 17β-HSD:

$NaAsO_2$ treatment at the dose of 0.4 ppm resulted a conspicuous reduction in ovarian Δ5,3β-HSD and 17 β-HSD activity compared with the vehicled control

group. Co-administration of Vit-B_{12} and folic acid at a dose of 0.04µg /100g body wt. /day and 2µg /100g body wt. /day respectively did not show any significant restoration of the aforesaid enzyme activities. On the other hand co-administration of Vit-B_{12} and folic acid at a comparatively higher dose [Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day)] could mitigate arsenic induced ovarian enzymes inhibition activities significantly alone or in combination.

The effect was more pronounced when Vit-B_{12} and folic acid at higher dose were combined **(Fig.5.3.1**).

5.3.3.2 Estimation of LH, FSH, Estradiol in serum:

$NaAsO_2$ treatment at the dose of 0.4 ppm resulted in remarkable reduction in serum levels of LH, FSH and estradiol in compared with the vehicled control group. Co-administration of Vit-B_{12} and folic acid at a lower dose [Vit-B_{12} -0.04µg /100g body wt. /day + folic acid -2µg /100g body wt. /day] did not show any promising restoration of the ovarian steroidogenesis. In contrast, co-administration of Vit- B_{12} and folic acid at a comparatively higher dose could mitigate arsenic induced sterodogenic inhibition significantly alone or in combination.

The effect is more pronounced when Vit-B_{12} and folic acid at higher dose are combined (**Fig. 5.3.2**).

5.3.3.3 Status of oxidative stress bio-sensors:

5.3.3.3.1 Antioxidative enzymes (Catalase, Superoxide dismutase, Peroxidase) activity:

Allover ovarian and uterine SOD, CAT and uterine POD activity in arsenic intoxicated group was protected significantly following comparatively higher dose

of Vit-B_{12} and folic acid alone or in a combined manner. But combination of Vit-B_{12} and folic acid ameliorated the said effects of arsenic are more pronounced (**Fig. 5.3.3, Fig. 5.3.4) .**

5.3.3.3.2 End products of Lipid peroxidation levels monitoring:

There was significant augmentation in the ovarian and uterine MDA and CD level in $NaAsO_2$ induced group compared to the control group. However co-administration with low dose of Vit-B_{12} and folic acid alone or combine manner in arsenic intoxicated rats were unable to correct the alteration of MDA and CD levels in these sex organs of arsenic exposed rats. But comparatively higher doses of Vit-B_{12} and folic acid alone or in combine manner antagonize the sodium arsenite induced reduction of MDA and CD levels significantly. Furthermore, the combined level was showing more intense recovery of these end products of lipid peroxidation in arsenic treated rats (**Fig. 5.3.6**).

5.3.3.4 Total Serum Lactate Dehydrogenase Status: Serum LDH is markedly elevated in 0.4ppm dose group in contrast to the vehicled control group. Co-administration of Vit-B_{12} and folic acid at a dose of 0.04µg /100g body wt. /day and 2µg /100g body wt. /day respectively did not show any considerable restoration of the aforesaid enzyme level. But co-administration of Vit-B_{12} and folic acid at a comparatively higher dose [Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day)] could mitigate arsenic induced alteration significantly alone or in combination.

The effect was more well-defined when Vit-B_{12} and folic acid at higher dose were combined (**Fig. 5.3.5**)

5.3.3.5: Histoarchitectural assessment female reproductive organs:

A strong recovery in ovarian folliculogenesis after co-administration of higher doses of Vit-B_{12} and folic acid alone or in combine manner antagonize the sodium arsenite induced follicular atresia in comparatively co-administration with lower dose of Vit-B_{12} and folic acid alone or combine manner in arsenic intoxicated rats was unable to correct the aforesaid matter. The tortuosity of the uterine secretory glands is destroying in arsenite induced group compare to the vehicled control group. Similarly lower dose of Vit-B_{12} and folic acid alone or combine manner in arsenic intoxicated rats were unable to restoration the tortuosity of the uterine secretory glands whereas the higher doses of Vit-B_{12} and folic acid alone or in combination partial protection were noted. (**Fig. 5.3.7, Fig. 5.3.8, Fig. 5.3.9**)

5.3.3.6: DNA fragmentation study: The reproductive tissues injury at the dose of 0.4 ppm after 28 days duration is significantly protected by the co-administration of higher dose of Vit-B_{12} and folic acid in combination which evident in DNA Ladder assay and Comet assay. Results of agarose gel electrophoresis of uterine tissues of different investigational groups showed that prolongation of DNA Ladder in respect to the control group where the higher doses of two B Vitamins co-administration either alone or in combination restrained this DNA brekase. Among them in combined group of these B vitamins in higher dose is more pronounced (**Fig. 5.3.10 & Fig. 5.3.11**).

Mode of treatment	**Initial body wt. (g)**	**Final body wt. (g)**	**Ovarian-somatic index (mg %)**	**Uterine-somatic index (mg %)**
Vehicle treated control	137±3.0	157±2.4	95±1.2	139.3±7.0
Sodium arsenite (0.4ppm / 100g body wt. /day).	139±3.8	148±2.5	58±4.4**	109±4.5**
Sodium arsenite (0.4 ppm /100g body wt. /day) + Vit-B_{12} (0.04µg /100g body wt. /day).	138±3.5	149±2.2	60±5.1**	112±5.0**
Sodium arsenite (0.4 ppm /100g body wt. /day) + Folic acid (2µg /100g body wt. /day).	137±3.2	148±2.0	62±4.5**	111.5±5.5**
Sodium arsenite (0.4 ppm /100g body wt. /day)+ Vit-B_{12} (0.04µg /100g body wt. /day) + Folic acid (2µg/100g body wt./day).	139±3.5	150±3.0	63±3.9**	115.2±6.3**
Sodium arsenite (0.4 ppm /100g body wt. /day) + Vit-B_{12} (0.07µg /100g body wt. /day).	138±2.9	150±3	76±4.0*	120.5±6.8*
Sodium arsenite (0.4ppm /100g body wt. /day) + Folic acid (4µg/100g body wt. /day).	139±2.8	151±2.8	74±5.1*	122.3±6.8*
Sodium arsenite (0.4ppm /100g body wt. /day) +Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg / 100g\ body wt. /day).	138±3.0	154±2.5	86±3.0	134.5±5.5

Table 5.3.IIIA.1: Dose dependent changes in general body growth, ovarian-somatic index and uterine-somatic index of rat is shown in response to sodium arsenite ingestion and its protection of the ovary and uterine weight by vitamin B_{12} and folate though As^{3+} has no significant impact on the general growth status of the animal. Data represents here is mean ± SE, N=6. Data of As^{3}+ -ingested or vitamins co-administered groups are compared to the corresponding vehicle treated group (Student's t test) *$p<0.05$; **$p<0.01$.

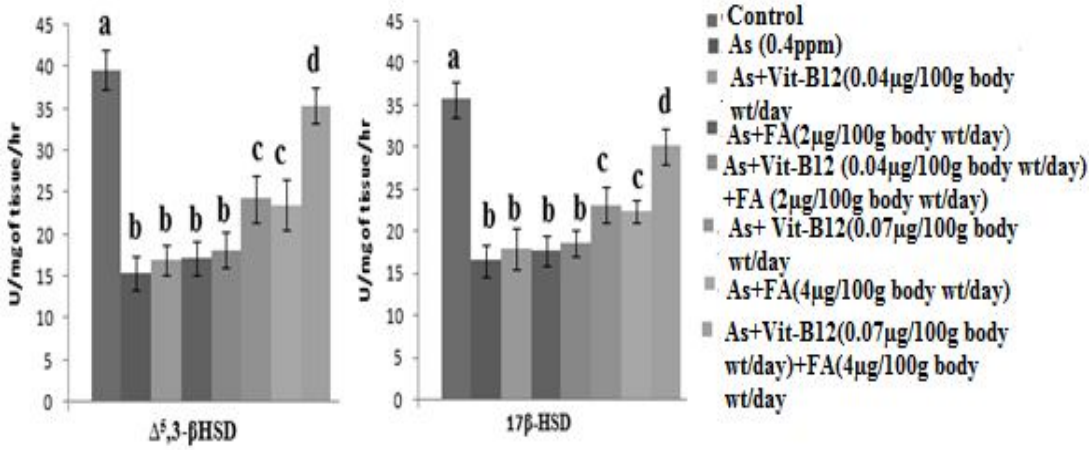


Figure 5.3.1: Correction of ovarian Δ^5, 3β-HSD and 17β-HSD in arsenic induced toxicity with co-administration of Vit-B_{12} (0.07µg) and/ or folic acid (0.4µg). Each bar represents mean ± SE, N=6. ANOVA followed by two tailed student's t test were used to find out statistical significance at $p<0.001$. Bar with different superscripts (a, b, c, and d) differ from each other.

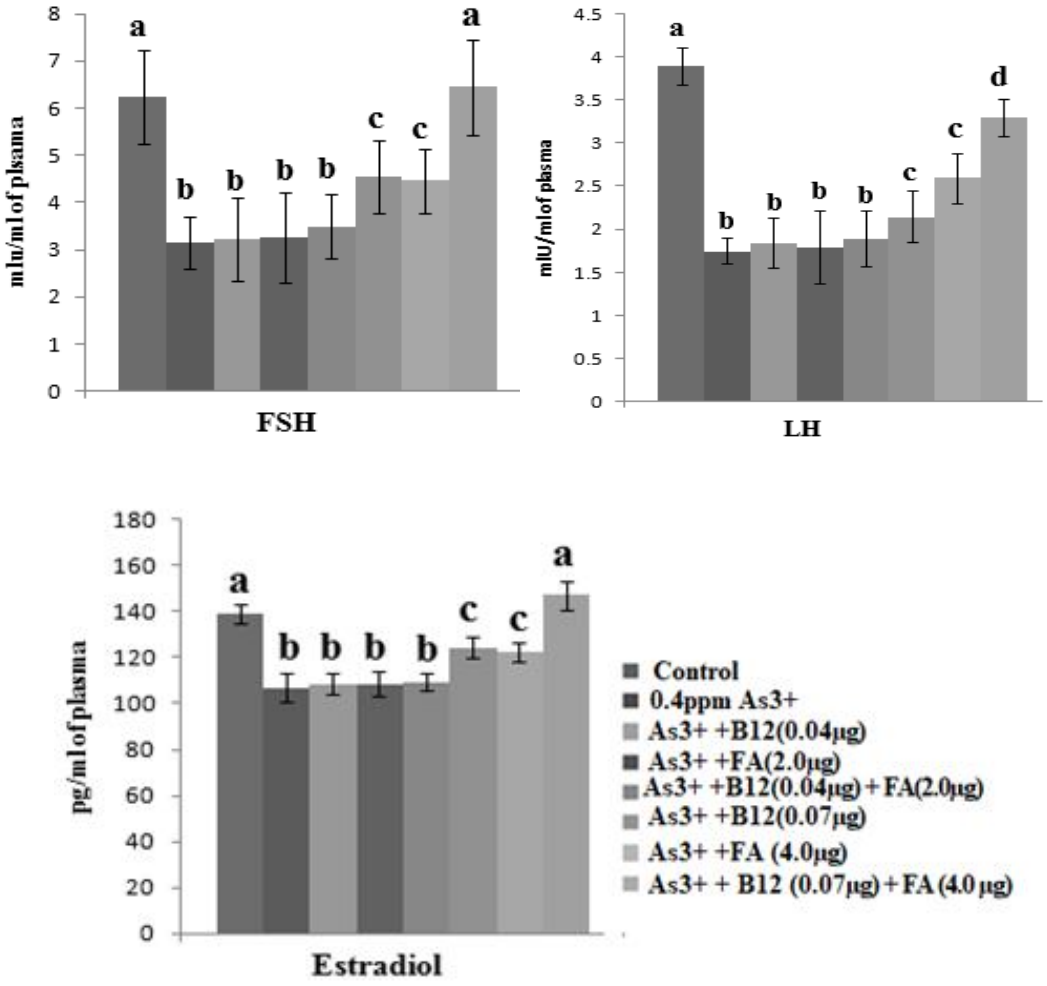


Figure 5.3.2: Resettlement of serum FSH, LH and Estradiol in arsenic induced toxicity with co-administration of Vit -B_{12} and/ or folic acid. Each bar represents mean ± SE, N=6. ANOVA followed by two tailed student's t test were used to find out statistical significance at $p<0.001$. Bar with different superscripts (a, b, c, and d) differ from each other.

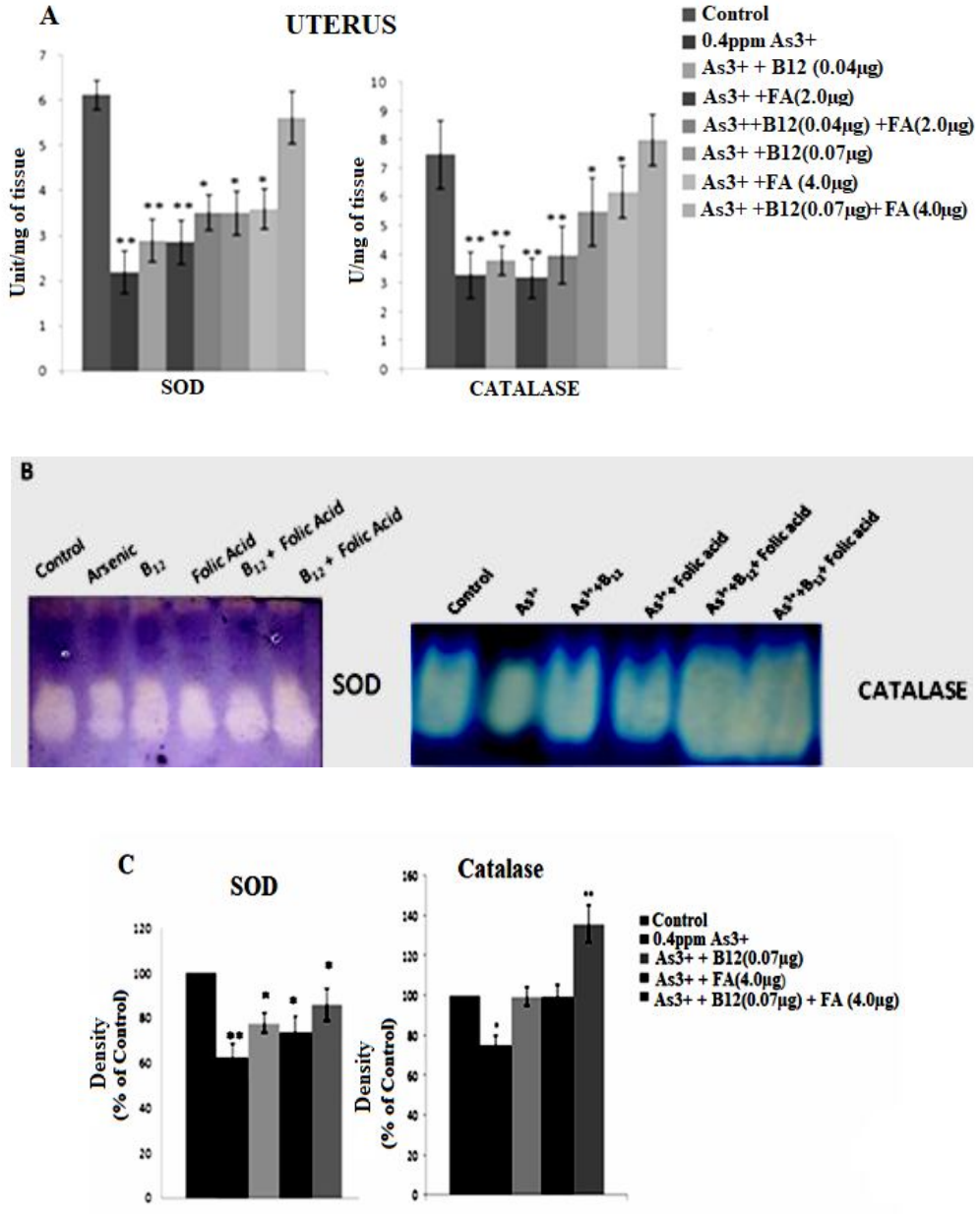


Figure 5.3.3: SOD and catalase activity shows differential pattern in uterine tissue of the female albino rats. [A] represents SOD activity measured in cell free extracts. Since the higher dose of vitamin B_{12} (0.07 µg) and folic acid (4.0 µg) alone or in combination in cell free extract depicted more pronounced protection of SOD and catalase activities. Hence, in case of [B], tissue extracts from rats (co-treated with these above comparatively higher doses of these two B vitamins) containing protein in each lane was electrophoresed on 12% and 8.0 % native gel followed by substrate specific development of SOD and catalase bands respectively. [C] Represents densitometric analysis with respect to control. Each bar represents mean ± SE, N=6. ANOVA followed by two tailed student t test were used to find out statistical significance at *$p<0.05$; **$p<0.01$.

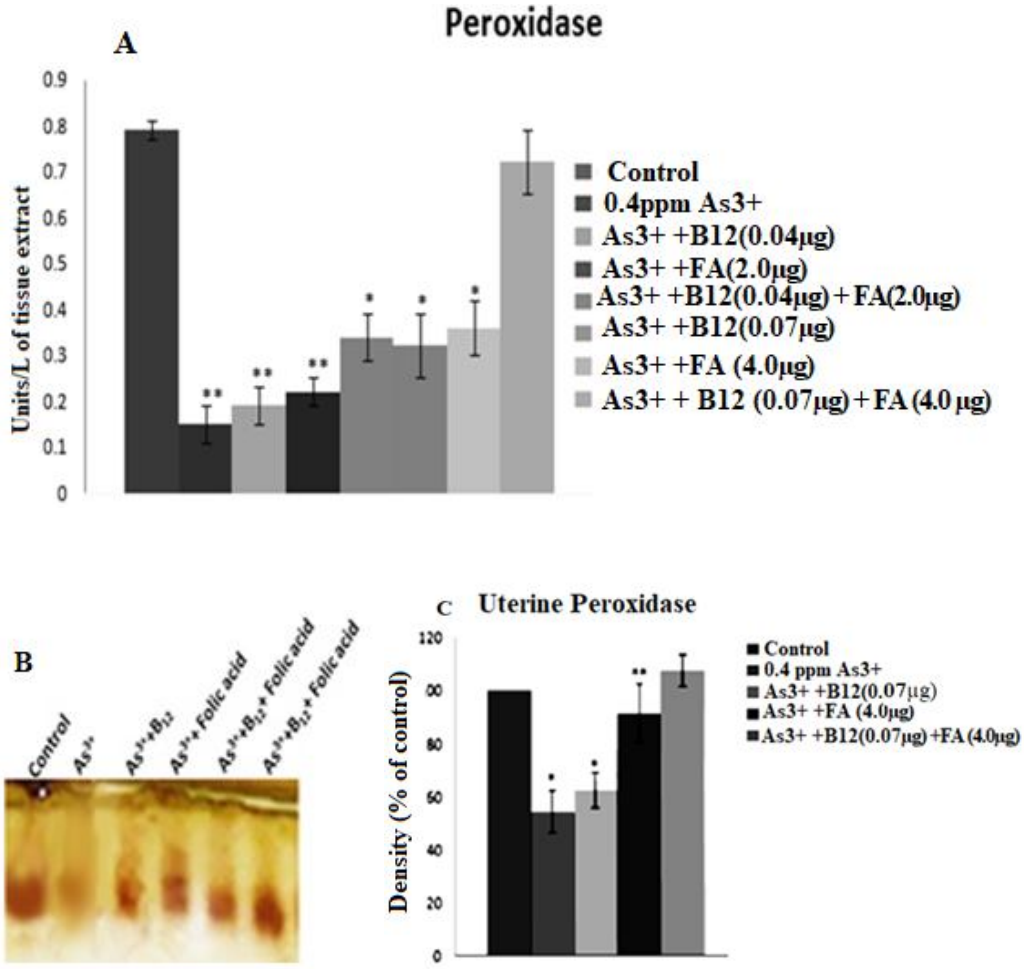


Figure 5.3.4: Total peroxidase activities in cell free extract [A] protected better with comparatively higher dose of Vitamin B_{12} (0.07 µg) and folic acid (4.0 µg). Electrozymogram showing total peroxidase profile [B] using tissue extracted protein and comparatively these two above higher doses of B vitamins considered in the zymogram. The electrophoresis was carried out using conventional 8% polyacrylamide gel. The gels were stained for peroxidase activity using benzidine, glacial acetic acid and H_2O_2. In panel [C] relative densitometric values of peroxidase band from experimental group, as % of the control lane are shown. Each bar represents mean ± SE, N=6. .ANOVA followed by two tailed student t test were used to find out statistical significance at *p<0.01; **p<0.001.

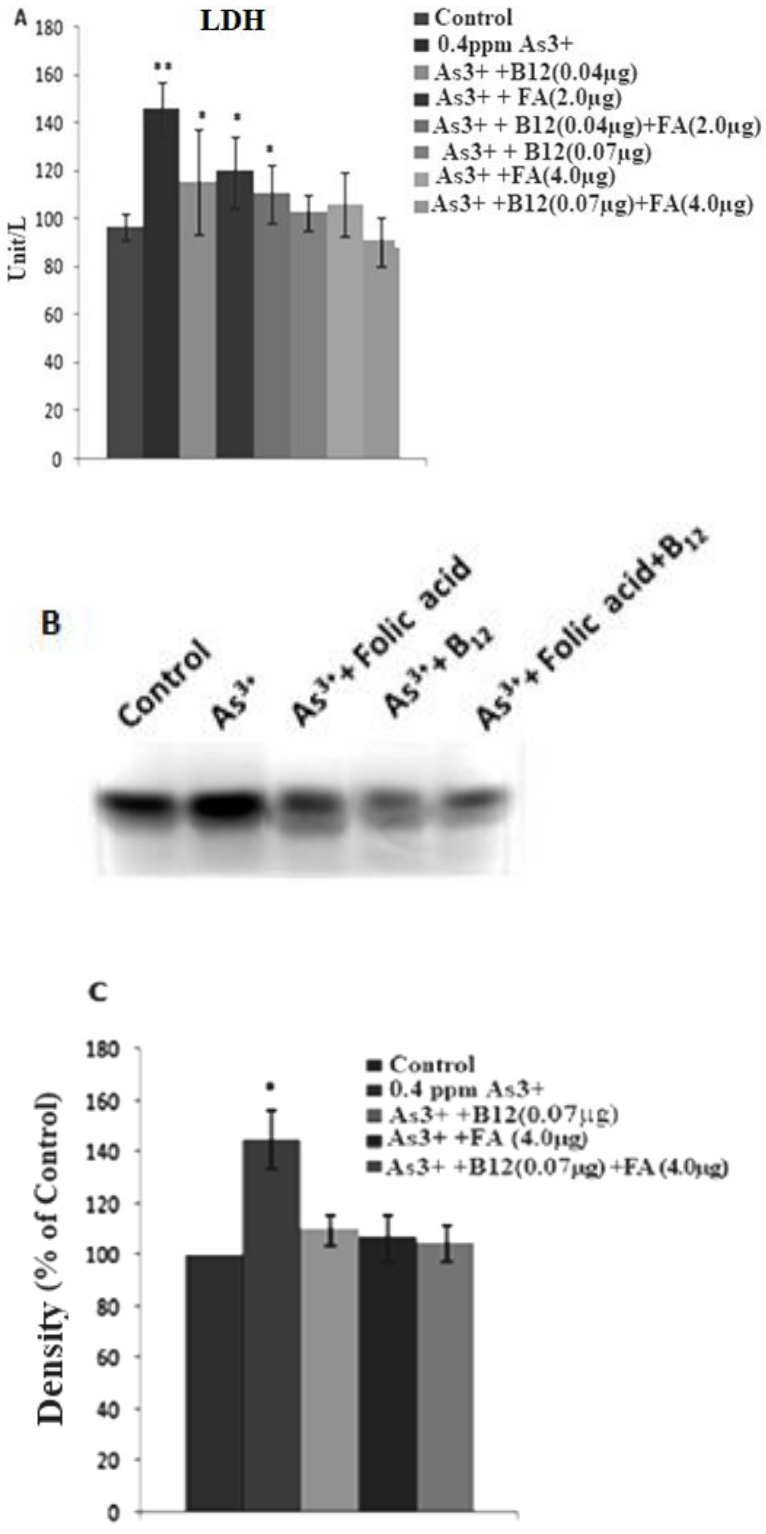


Figure 5.3.5: Total serum LDH activity illustrated for the extent of cellular damage. [A] is showing enzymatic activity in cell free assay system following dose dependent co-administration of B vitamins in arsenic intoxicated rats. Vitamin B_{12} (0.07 µg) and folic acid (4.0 µg) had comparatively higher effectiveness in the restoration of arsenic induced tissue damage. Hence, in panel [B] these above two doses of

vitamins were considered and serum protein in each lane was electrophoresed on agarose gel followed by substrate specific development of LDH bands. Each bar represents mean ± SE, N=6. ANOVA followed by two tailed student t test were used to realize statistical significance at *p<0.05; **p<0.01.

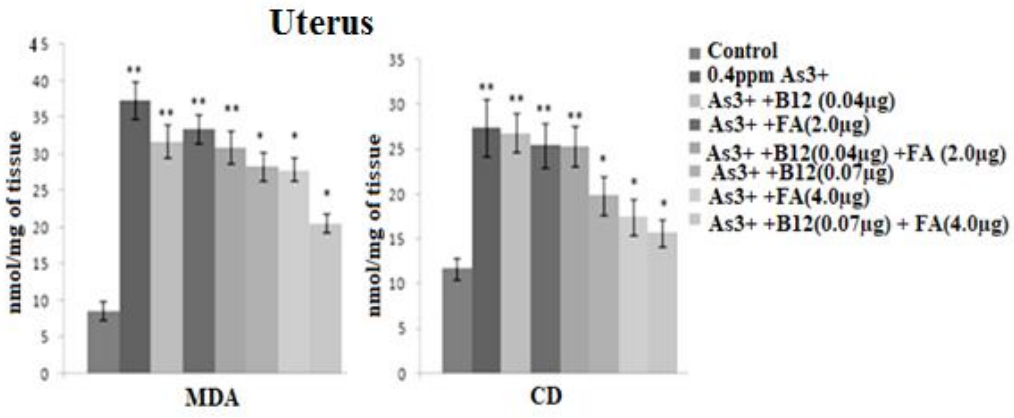


Figure 5.3.6: Protective effect (dose dependent) on malondialdehyde (MDA) and conjugated dine (CD) in uterine tissue by co-administration of Vitamin B_{12} and folic acid in As3+ intoxicated rats. Comparatively higher dose of vitamin B_{12} (0.07 µg) and folic acid (4.0 µg) alone or in combination were more effective on the above protection. Each bar represents mean ± SE, N=6. ANOVA followed by two-tailed student 't' test were used *p<0.05; **p<0.01.

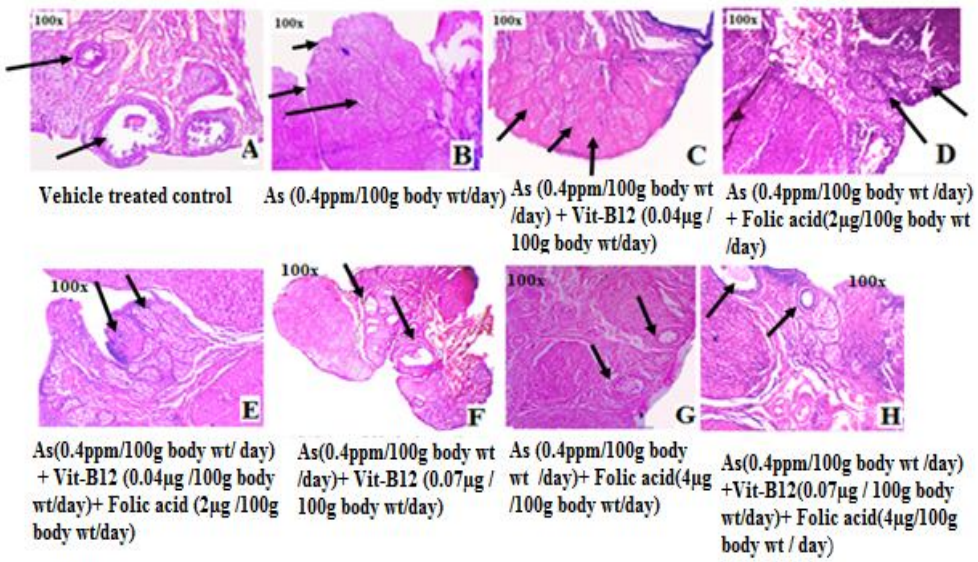
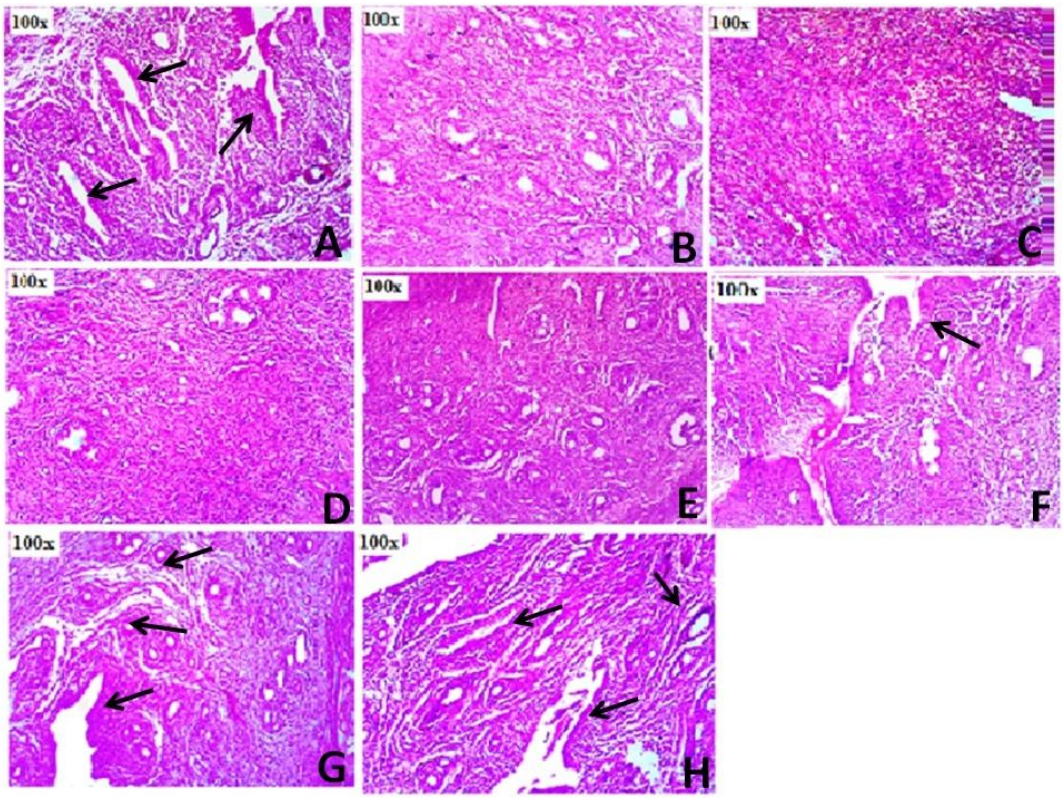


Figure 5.3.7: Figure shown (histology of ovary) dose dependent effect of Vitamin-B_{12} and folic acid by amelioration to reduce the arsenic mediated follicular atresia in ovary of female rat.



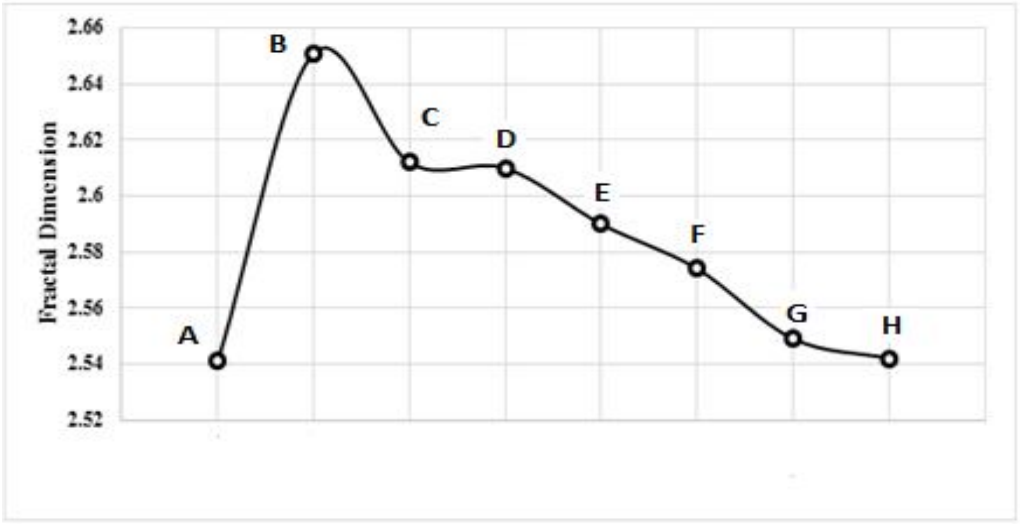


Figure 5.3.8 Uterine tissue was implanted in paraffin, serially sectioned laterally at 5 µM, stained with eosin and haematoxylin (Harris) and observed under a microscope (magnification 340) to study the uterine histoarchitecture (A-H). Secretary nature of uterine bed was lost following arsenic intoxication (panel-B) and gradual and or significant reversal of secretary tubules was observed following the treatment with these B vitamins (panel E-H). Arrow denotes secretary vesicles. Panel distribution: control rat (panel A) or treated with As3+-0.4 ppm (panel B) or As3+-0.4 ppm+ vitamin B_{12}-0.04µg (panel C) or As3+-0.4 ppm plus Folic acid-2.0 µg (panel D) or

As3+-0.4 ppm+ vitamin B_{12}-0.04µg + Folic acid-2µg (panel E) or As3+-0.4 ppm+ vitamin B_{12}-0.07µg (panel F) or As3+-0.4 ppm + Folic acid-4.0 µg (panel G) or As3+-0.4 ppm+ vitamin B_{12}-0.07 µg + Folic acid-4.0 µg (panel H).Graphical representation denotes fractal dimension of uterine bed was increased in As^{3+} ingested rats. Subsequent co-administration of vitamin $B_{12}$and folic acid gradually and dose dependently decreased the fractal dimension from intoxicated state to convalescent state. NB: There is no unit for Fractal dimension, this is ratio.

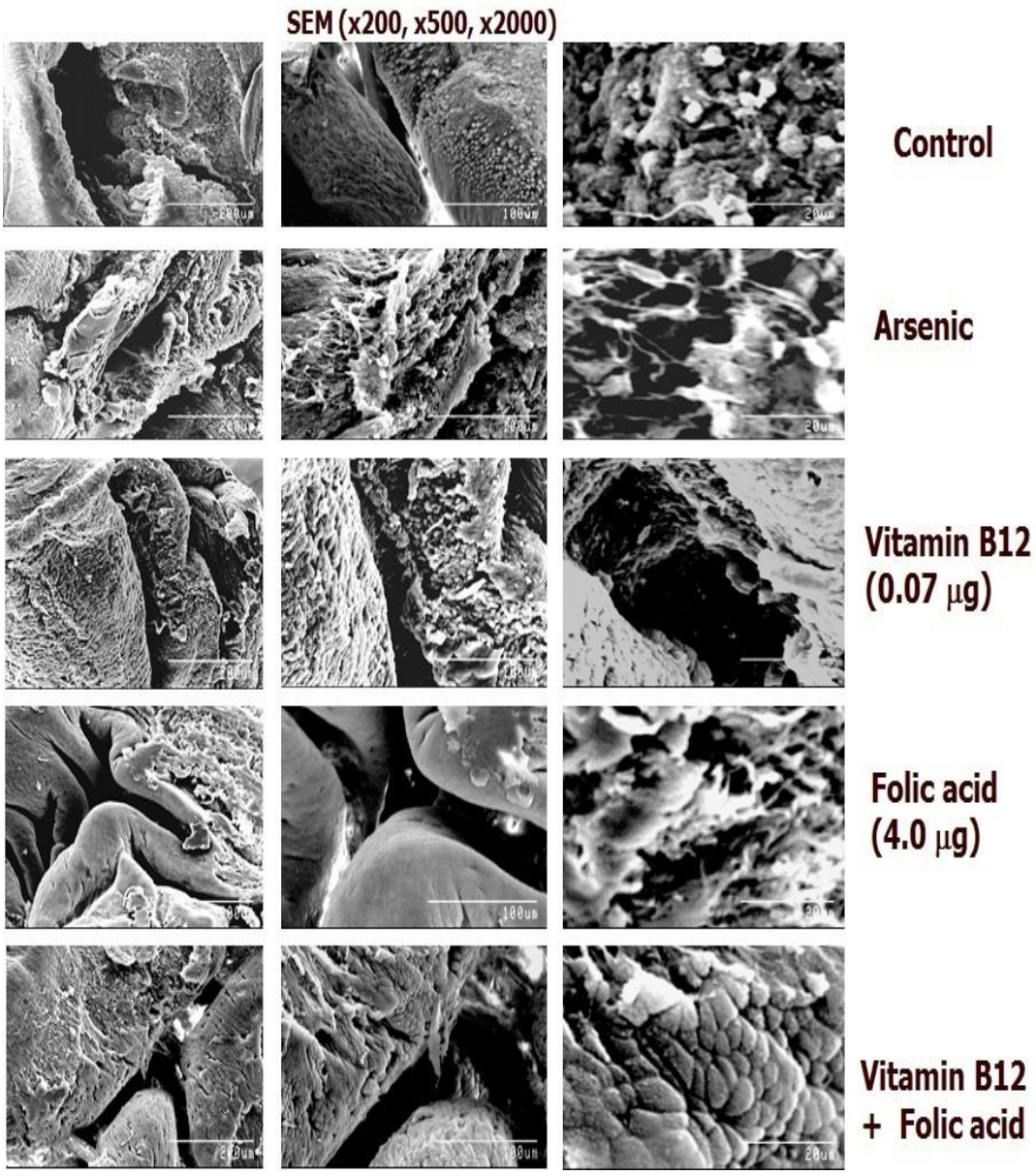


Figure 5.3.9: Figure shown the protective effect in uterine tissue architecture by co-administration of Vit-B_{12} and /or folic acid in arsenic induced female rat.

DNA fragmentation study : a) Ladder assay

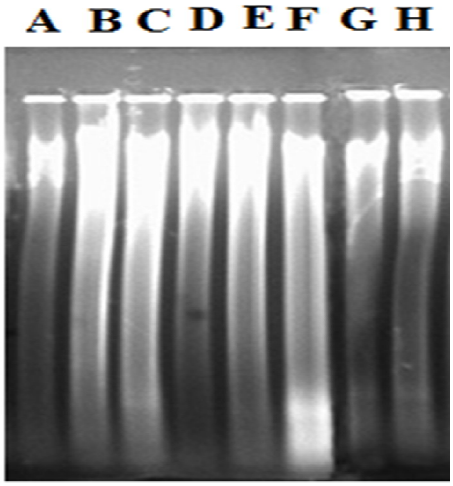


Figure 5.3.10 DNA fragmentation in uterus of female rat treated with arsenic.

A: Vehicle treated control **B:** As (0.4 ppm/100g body wt./day). **C:** AS (0.4 ppm/100g body wt./day) + Vit-B_{12} (0.04µg /100g body wt./day). **D:** AS (0.4 ppm/100g body wt./day) + Folic acid (2µg /100g body wt./day). **E:** AS (0.4 ppm/100g body wt./day) + Vit-B_{12} (0.04µg /100g body wt./day) + Folic acid (2µg/100g body wt./day). **F:** AS (0.4-ppm/100g body wt./day) + Vit-B_{12} (0.07µg /100g body wt./day). **G:** AS (0.4 ppm/100g body wt. /day) + Folic acid (4µg/100g body wt. /day). **H:** AS (0.4 ppm/100g body wt./day) +Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt./day).

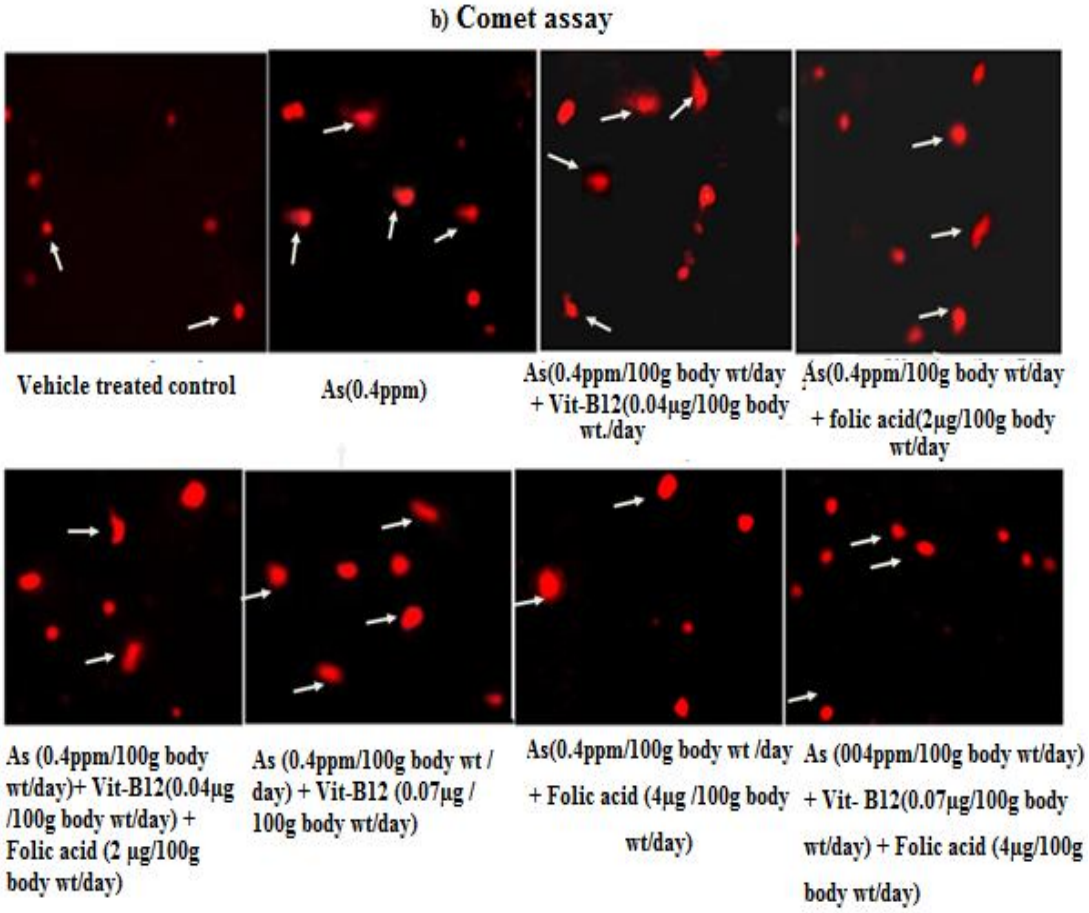


Figure 5.3.11 Protection of DNA breakage in the comet formation is shown in arsenic induced uterine cell by the co-administration of Vit- B_{12} and/ or folic acid in different doses.

5.3.4 Conclusion: From the above cumulative data explored that the two vitamins at the higher dose [Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day)] might have potent against arsenic mediated reproductive health injury.

5.4 Experiment-IV

Toxicity assessment of Vit-B_{12} and folic acid on female albino rat:

5.4.1 Aims and Objectives: This experiment has been performed to search out the any toxicity was produced in female albino rat after administration of selective dose either alone or in combination of Vit-B_{12} and folic acid.

5.4.2: Experimental Design:

Twenty four female albino Wistar strain rats (each groups six) aged 60 days with weight of 120±10 g were selected in this experiment. They were nourished and acclimatized were followed as the previous experiments. The duration of the experiment was extended for 28days. All the rats were synchronized by orally introducing of estardiol at a dose of 3µg/100g.body wt.

Group I: Vehicle treated control

Group II: Vit-B_{12} (0.07µg /100g body wt. /day)

Group III: Folic acid (4µg/100g body wt. /day).

Group IV: Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day).

The untreated control animals were given pure drinking water as vehicled control (Group I) rats without any arsenical combination for 28 days.

The rats (**Group II ,Group III & Group IV)** were fed orally at a dose of 0.07µg /100g body weight /day, 4µg/100g body wt. /day, 0.07µg/100g body wt. /day + 4µg /100g body wt. /day for twenty eight days only Vit-B_{12}, only folic acid, Vit-B_{12} + folic acid respectively. The above concentrations of these drugs were prepared in

each week and kept in cool and dark place. Vaginal smears were collected twice daily as earlier experimental schedule. On the 29th day of experiment final body weights of all the investigational rats were noted. After anesthesia by ketamine HCI, blood was collected from dorsal aorta of each rat using a 21-gauge needled syringe. Serum samples were separated after centrifuging the blood and stored at –20^{0}C in separate ampoules until all the samples had been used for biochemical assay.

After collection of blood all the animals were euthanized by sodium barbiturate. The ovary and uterus of the 24 experimental animals were dissected out and wet weights of these organs were measured by electronic balance. Liver, Kidney, ovary and uterus from each animal was used for the biochemical assay whereas another some portion of liver, kidney ovary and uterus of each rat were fixed in 10% formalin solution for histometric analysis.

5.4.3 Result:

5.4.3.1 Body weight and oregano-somatic indices (female reproductive) and toxicity assessment:

In comparison of vehicled control group no significant alteration were noted in either singly or combined administration of Vit-B_{12} and folic acid therapeutic agents (**Table 5.4.IVA.I**).

5.4.3.2 Estimation of LH, FSH, Estradiol in serum:

The serum LH, FSH and Estradiol level in alone or combined administration of Vit-B_{12} and folic acid groups, there were no any changes noted in contrast to vehicled control group (**Table 5.4.IVA.II**).

5.4.3.3 Ovarian Δ^5, 3 β-HSD and 17 β-HSD activities:

The activities of Δ^5, 3 β-HSD and 17 β-HSD in ovarian tissue of aforesaid administered drugs either alone or in combination there were no dissimilarity observed in comparison to corresponding vehicled control group (**Table 5.4.IVA.II)**

5.4.3.4 Serum Total Protein /SGPT/SGOT/ALP/Urea/Creatnine status:

Vit-B_{12} and folic acid alone or in combination did not show any toxic effect on liver and kidney in comparison with control group as evident from the assessment of the parameters like serum total protein, SGPT, SGOT, alkaline phosphatase, creatinine and urea (**Fig. 5.4.1**).

5.4.3.5 Antioxidative enzymes (catalase, superoxide dismutase) activity and end product of lipid peroxidation (MDA and CD) monitoring:

Indicator of antioxidant system e.g. SOD, CAT and the end product of lipid peroxidation e.g. MDA and CD were not altered following treatment of selected dose of Vit-B_{12} and folic acid alone or in combination when compared with vehicled controls rats (**Fig.5.4.2, Fig 5.4.3, Fig. 5.4.4**).

5.4.3.6: Histoarchitectural assessment of Kidney and Liver:

Vit-B_{12} and folic acid alone or in combination did not show any disparity of histoarchitecture on liver and kidney in comparison with the control group (**Fig 5.4.5, Fig. 5.4.6**).

Mode of treatment	**Initial body wt. (g)**	**Final body wt. (g)**	**Ovarian-somatic index (mg %)**	**Uterine-somatic index (mg %)**
Vehicle treated control	130±3.2[a]	150±3.5[a]	95.5±2.5[a]	140±3.5[a]
Vit-B_{12} (0.07µg /100g body wt. /day)	132±3.1[a]	152±3.2[a]	96.3±3.0[a]	140.5±3.8[a]
Folic acid (4µg/100g body wt. /day).	135±2.5[a]	153±3.2[a]	95.8±3.2[a]	141±3.5[a]
Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day).	133±3.0[a]	155±3.4[a]	97.2±2.3[a]	143±3.4[a]

Table 5.4.IVA.I: Drug dependent changes in body weight, ovarian-somatic index and uterine-somatic index after drug administration. Data represents here is mean ± SE, N=6 and two tailed student's t test were used to find out statistical significance at $p<0.001$. Same superscript in each vertical column did not differ from each other significantly.

Mode of treatment	Δ^5, 3 β-HSD (units/mg of tissue/hr)	17 β-HSD (units/mg of tissue/hr)	LH (mlU / ml of serum)	FSH (mlU/ml of serum)	Estradiol (pg/ml of serum)
Vehicle treated control	36.4±2.1[a]	33.2±2.5[a]	4.0±0.3[a]	7.2±0.52[a]	136±4.5[a]
Vit-B_{12} (0.07µg /100g body wt. /day)	36.9±2.3[a]	33.9±2.1[a]	4.3±0.25[a]	7.5±0.6[a]	136.8±4.8[a]
Folic acid (4µg/100g body wt. /day).	36.7±2.4[a]	33.6±2.3[a]	4.2±0.22[a]	7.6±0.35[a]	136.7±5.0[a]
Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day).	37±2.0[a]	34.5±2.2[a]	4.8±0.2[a]	7.9±0.5[a]	138.5±4.0[a]

Table 5.4.IVA.II: Drug dependent changes in ovarian steroidogenic enzyme activities, serum levels of gonadotrophins and estradiol after drug administration. Data represents here is mean ± SE, N=6. ANOVA followed by two tailed student's t test were used to find out statistical significance at $p<0.001$. Same superscript in each vertical column did not differ from each other significantly.

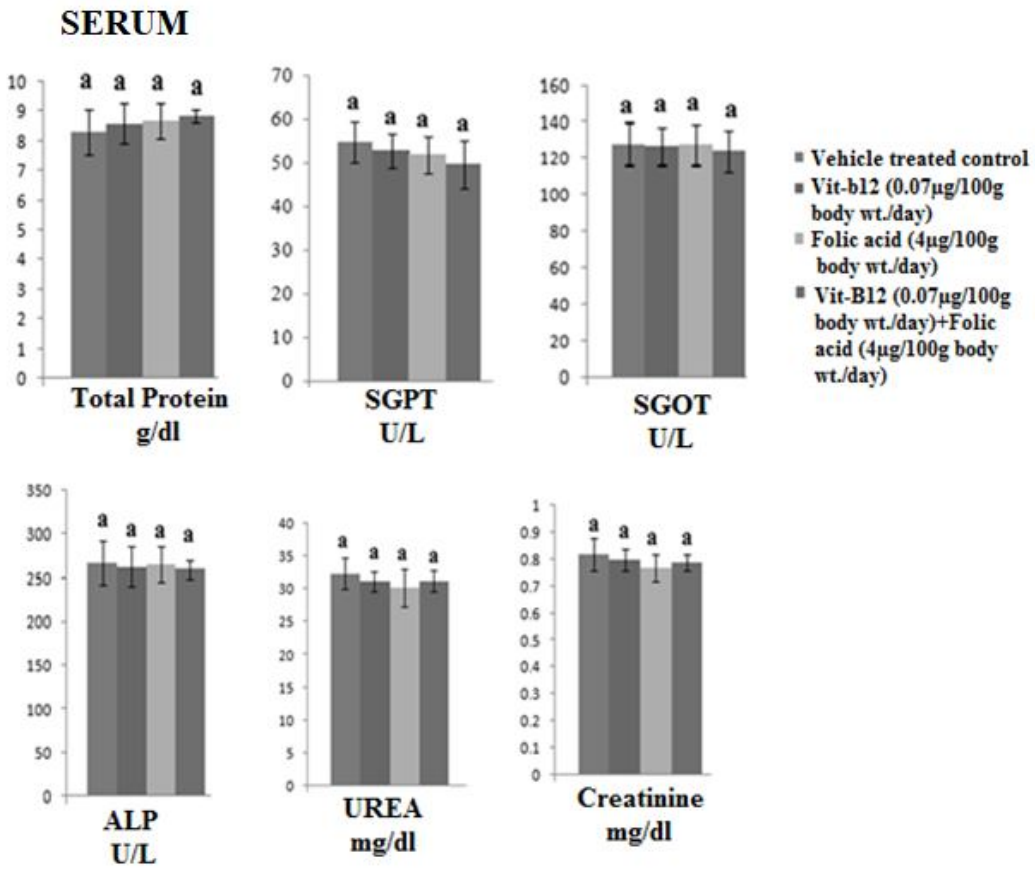


Figure 5.4.1: Effect of Vit-B_{12} and /or folic acid on Serum Total protein (TP), Serum Glutamate pyruvate transamynase (SGPT), and serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP), serum Urea and Creatinine. Each bar represents mean ± SE, N=6. ANOVA followed by two tailed student's t test were used to find out statistical significance at $p<0.001$. Bar with same superscripts did not differ from each other.

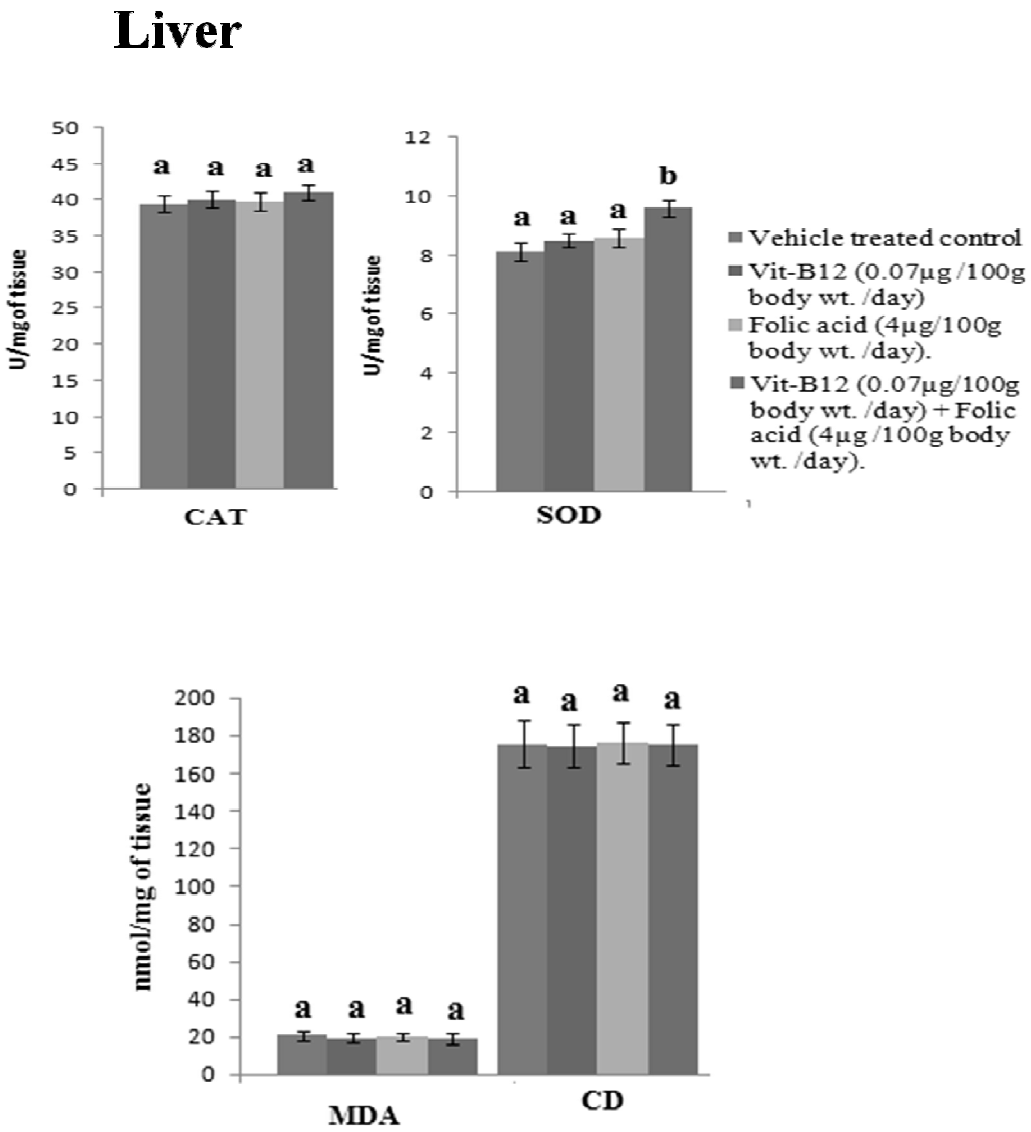


Figure 5.4.2: Effect of Vit-B_{12} and /or folic acid on CAT (catalase), SOD (superoxidedismutasae), MDA (malondialdehyde) and CD (conjugated diene) in hepatic tissue. Each bar represents here is mean ± SE, N=6, $p<0.001$. ANOVA followed by multiple student's t test. Bar with same superscripts did not differ from each other.

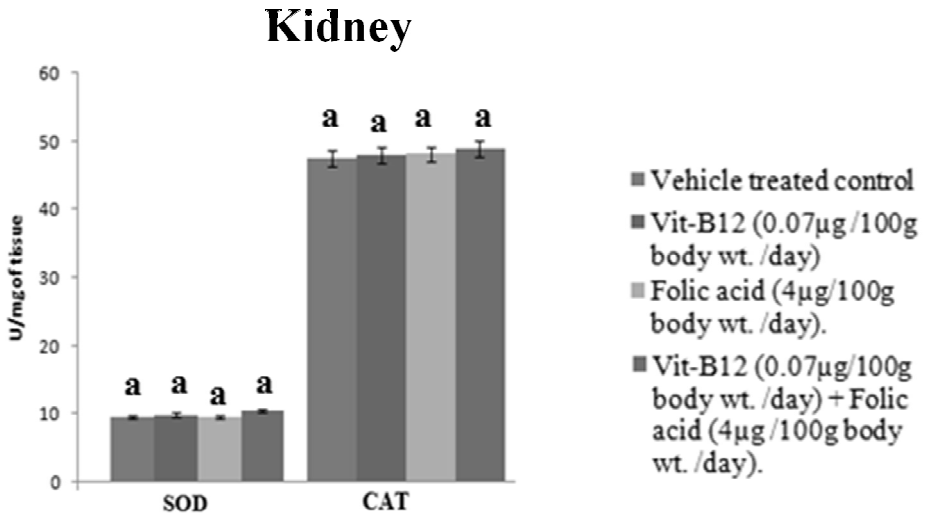


Figure 5.4.3: Effect of Vit-B_{12} and /or folic acid on Catalase (CAT), Superoxidedismutasae (SOD) in kidney tissue. Each bar represents here is mean ± SE, N=6, $p<0.001$ and ANOVA followed by multiple student's t test. Bar with same superscripts did not differ from each other.

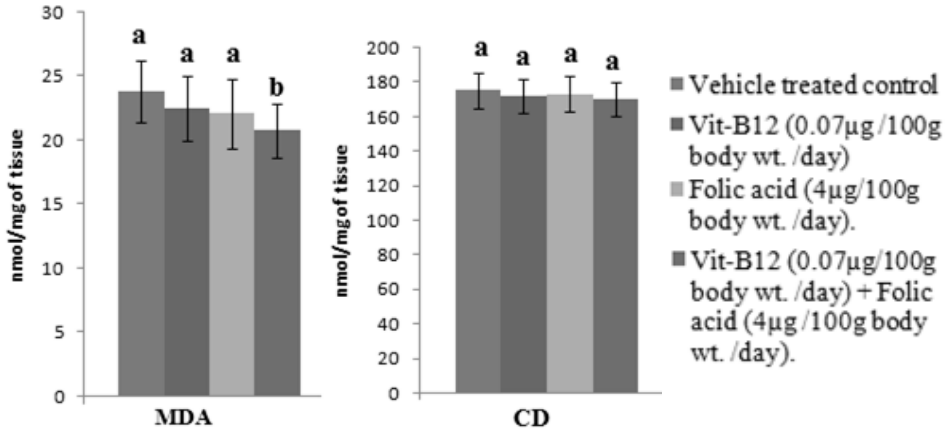


Figure 5.4.4: Effect of Vit-B_{12} and /or folic acid on MDA (malondialdehyde) and CD (conjugated diene) in kidney tissue. Each bar represents here is mean ± SE, N=6, $p<0.001$. ANOVA followed by multiple student's t test. Bar with same super scripts did not differ from each other.

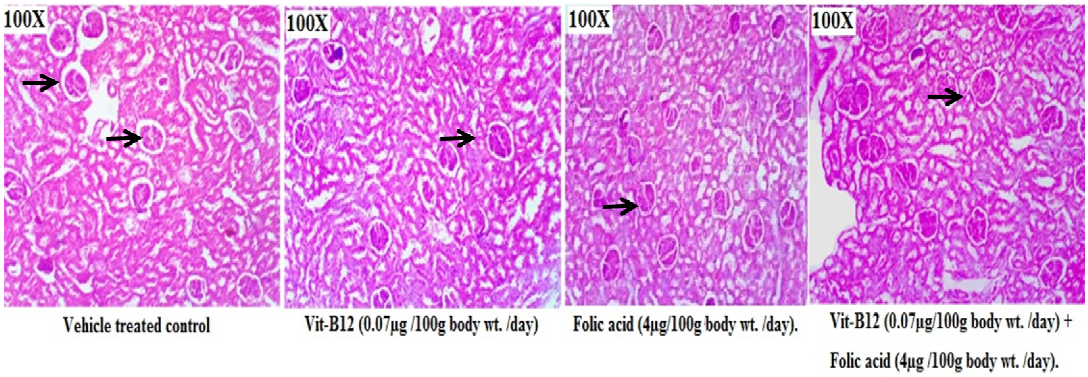


Figure 5.4.5: Figure shows that there is no dissimilarity of kidney histoarchitectural by the administration of Vit-B_{12} and/or folic acid in comparison with vehicle treated control of female albino rat.

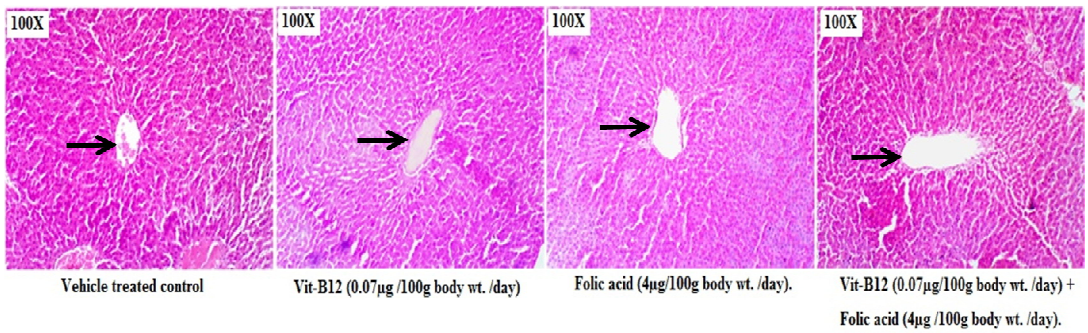


Figure 5.4.6: Figure shows that there is no disparity of hepatic histoarchitecture by the administration of Vit-B_{12} and/or folic acid in comparison with vehicle treated control of female albino rat.

5.4.3.7: DNA fragmentation study:

DNA fragmentation study
a) Ladder assay

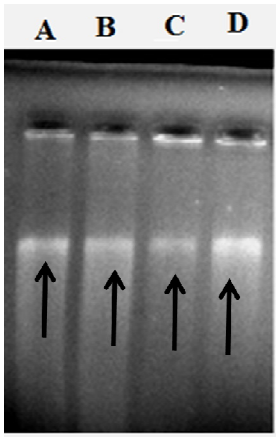
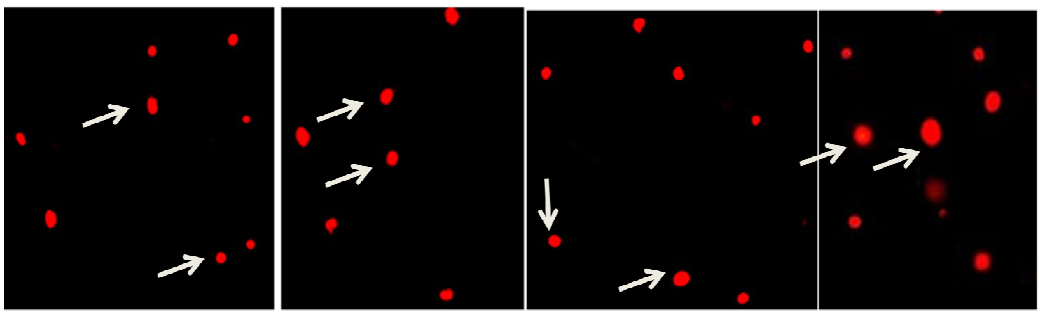


Figure 5.4.7: DNA fragmentation of uterus in Vit-B_{12} and/or folic acid induced female rat.

A:*Vehicle treated control* **B:***Vit-B12 (0.07µg /100g body wt. /day)* **C:** *Folic acid (4µg/100g body wt. /day).* **D:***Vit-B12 (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day).*

b) Comet Assay



Vehicle treated control | Vit-B12 (0.07µg /100g body wt. /day) | Folic acid (4µg/100g body wt. /day). | Vit-B12 (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day).

Figure 5.4.8: There is no Comet formation by DNA fragmentation in uterus by administration of Vit-B_{12} and/or folic acid induced female rat.

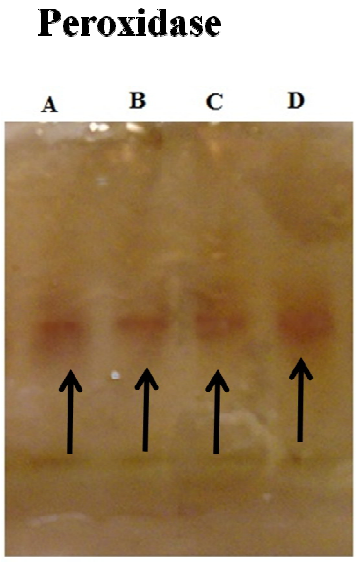


Figure: 5.4.9 Restoration of Peroxidase activity in Vit-B_{12} and/or folic acid in compare with vehicle treated control. But peroxidase activity is more pronounced in Vit-B_{12} and folic acid combination group.

5.4.4 Conclusion: Results from this experiment showed that there is no harmful effect on either reproductive organ or hepato-renal system by the application of selected higher doses of these two B vitamins either alone or in combination.

5.5 Experiment-V

Testing the efficacy of vitamin B_{12} and folic acid in metabolic organ

5.5.1 Animal Selection and Treatment:

Eighteen female albino rats weighing 110 ± 10 g were acclimatized for 15 days at 12-hour light-dark cycle, 25ºC ± 2ºC temperature, 50%–70% humidity in the institutional animal resource facility. They were nourished and acclimatized were followed as the previous experiments. All the rats were synchronized by orally introducing of estardiol at a dose of 3µg/100g.body wt. For experimental purpose after recording the body weights all the female rats are divided equally and the experimental schedule is as follows:

Group I: Vehicle treated control

Group II: Sodium arsenite (0.4 ppm/100g body wt. /day).

Group III: Sodium arsenite (0.4 ppm/100g body wt. /day) +Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day).

5.5.2 General Observations:

Results General observations Food and water consumption was unaffected in all groups of animals. General somatic indices are not differing in arsenic treated and vitamin B_{12} with folic acid co-administrated rats groups in comparison with the control group (**Table 5.5.VA.I**). But the hepato-somatic in the present data expressed that the percentage of body mass (hepato-somatic index), increased significantly after arsenic exposure. Co-administration of vitamin B_{12} with folic acid prevented the increase.

The liver-function markers such as ALT (SGPT) and AST (SGOT) activity (**Fig.5.5.1**) are markedly increased in sodium arsenite treated group. Co-treatment of vitamin B_{12} with folic acid to arsenic-treated rats significantly restricted the increase of these enzymatic activities in liver.

5.5.3 Lipid profile:

Administration of arsenic in female rats, with the present dose (0.4 ppm) and duration caused a significant diminution of total protein (TP) (**Fig.5.5.1**) along with augmentation ($P < 0.001$) of total cholesterol (CHL), triglyceride (TG), and low density lipoprotein (LDL) in serum (**Fig. 5.5.2**). Co-treatment of two B vitamins to arsenic-treated rats significantly elevated TP (**Fig 5.5.1**) and reduced CHL, TG, LDL as compared to arsenic treated group (**Fig. 5.5.2**).

5.5.4 Status of oxidative stress markers in liver:

A marked elevation in the levels of MDA and CD were noticed after sodium arsenite treatment (**Fig. 5.5.3**). However, a concomitant administration of present micronutrients to arsenic treated animals impeded this elevation. Moreover, a notable diminution ($P < 0.001$) in hepatic SOD and CAT activities and NPSH levels in arsenic treated groups only was significantly restored and protected by the vitamin supplementation ($P < 0.001$; **Fig 5.5.3**).

5.5.5 Liver histology and DNA fragmentation:

According to dose and duration of arsenic ingestion with the present dose data expressed in hepatocytic disarrangement with lobular degeneration. And a partial, but significant protection was noticed in co-administration of B vitamins along with in arsenic animals which was shown in the histoarchitecture picture **(Fig. 5.5.4).**

Agarose-gel electrophoresis of liver DNA of different experimental groups exhibited a prolonged DNA ladder in arsenic only treated group, which was prevented partially in the vitamin-supplemented, arsenic-exposed group (**Fig.5.5.5**).

Mode of treatment	**Initial body wt. (gm)**	**Final body wt. (gm)**	**Hepato-somatic index (g %)**
Vehicle treated control	150 ± 3^{a}	166 ± 5^{a}	2.87 ± 0.12^{a}
Sodium Arsenite (0.4 ppm)	152 ± 4^{a}	160 ± 2^{a}	3.79 ± 0.09^{b}
Sodium Arsenate (0.4 ppm) + Vit-B_{12} (0.07µg/100g body wt.)+ Folic acid (4µg /100g body wt..)	149 ± 5^{a}	160 ± 5^{a}	3.02 ± 0.27^{a}

Table-5.5.VA.I. Effect of vitamin B_{12} and folic acid on general somatic growth along with hepato-somatic indices in arsenic-exposed rats. Mean ± SE; N = 6. As compared with control, $P < 0.01$ (ANOVA followed by multiple comparison two-tailed t test). Same superscript did not differ from each other significantly.

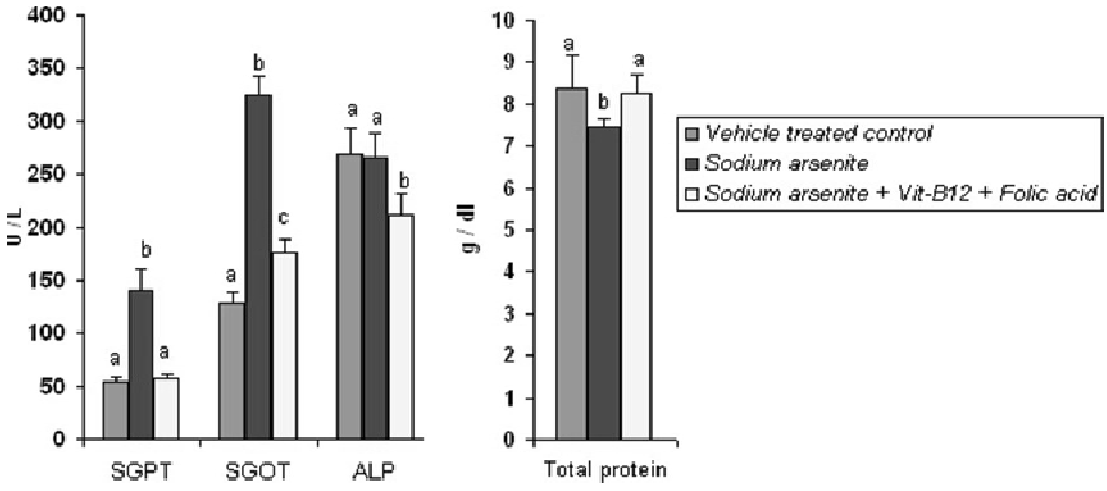


Figure 5.5.1 Effect of vitamin B_{12} and folic acid on arsenic-induced hepatic function markers (mean + SE; N = 6). As compared with control, $P < 0.001$ (ANOVA followed by multiple comparison two-tailed t test). Same superscript did not differ from each other significantly.

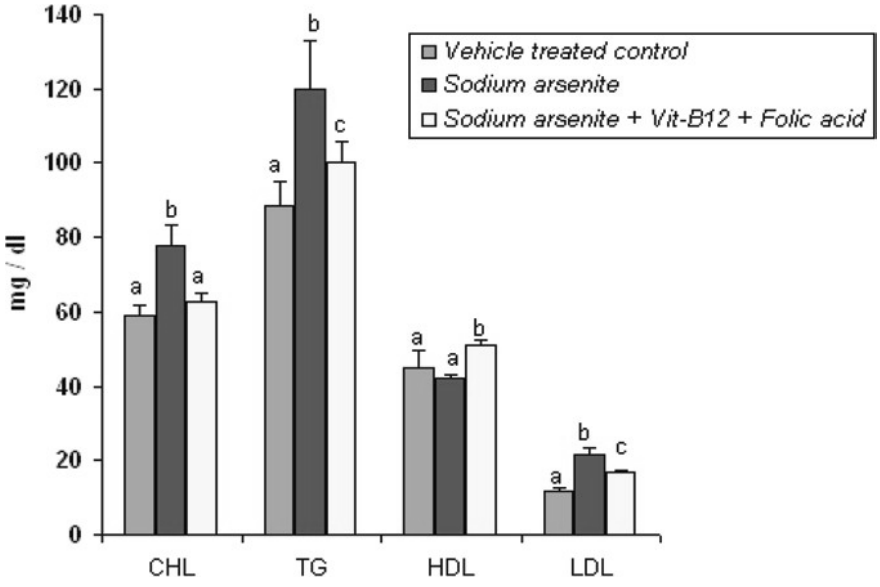


Figure 5.5.2 Effect of vitamin B_{12} and folic acid on lipid profile of arsenic-treated rats (mean + SE; N = 6). As compared with control, P < 0.001 (ANOVA followed by multiple comparison two-tailed t test). Same superscript did not differ from each other significantly.

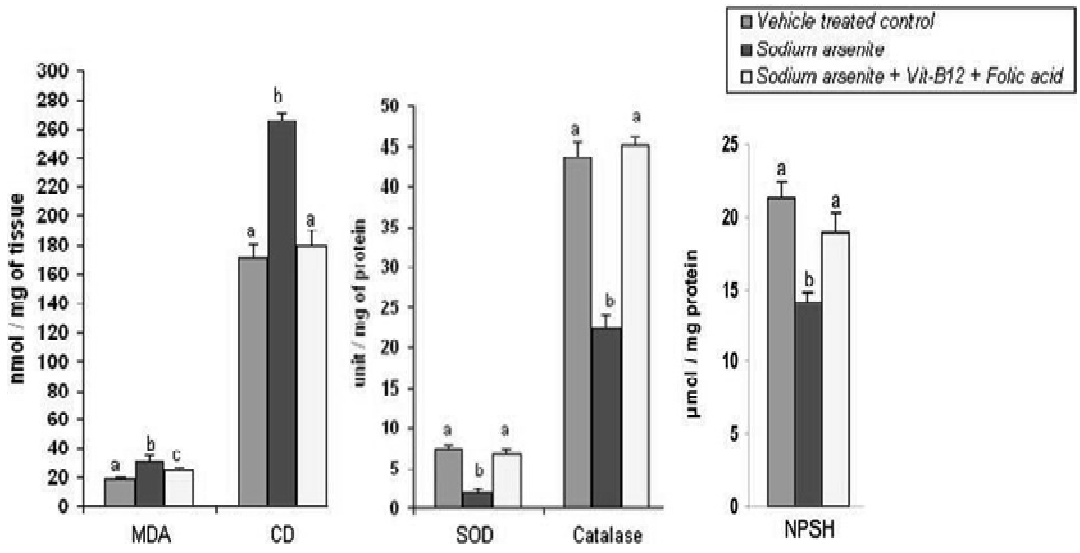


Figure 5.5.3: Effect of vitamin B_{12} and folic acid on arsenic-induced oxidative stress in liver of rats (mean + SE; $N = 6$). As compared with control, $P < 0.001$ (ANOVA followed by multiple comparison two-tailed t test). Same superscript did not differ from each other significantly.

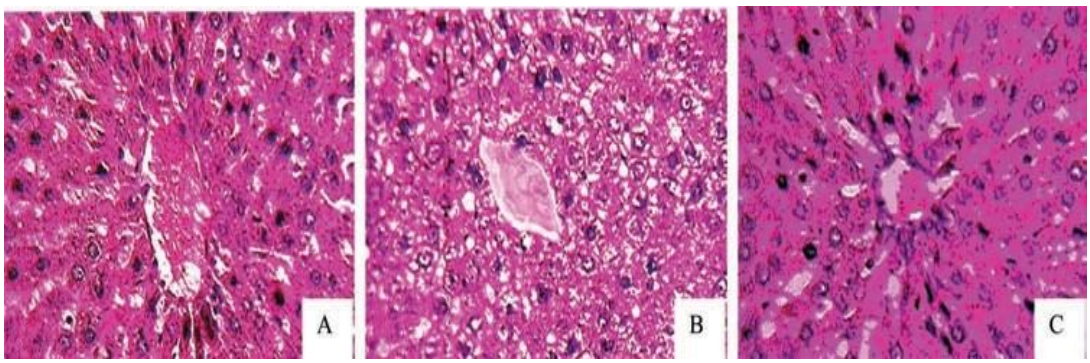


Figure 5.5.4: Hepatic histoarchitecture (magnification 400×) of female rat treated with arsenic. Control rat (plate A) or treated with sodium arsenite (plate B) or sodium arsenite + vitamin B12 + folic acid (plate C).

Figure 5.5.5: DNA fragmentation in liver of female rat treated with arsenic. Control rat (lanes 1, 2, and 3) or treated with sodium arsenite (lanes 4, 5, and 6) or sodium arsenite + vitamin B_{12} + folic acid (lanes 7 and 8).

5.5.6 Conclusion: All collective data elucidate that these two B vitamins at the selective doses can be used as a hepatoprotectant against arsenicosis.

5.6 Experiment-VI

Duration dependent withdrawal effect of selective formulation of co-administration of Vit-B_{12} and/or folic acid in arsenic induced toxicity on sex organs of female albino rat.

5.6.1 Aims and Objectives:

To search out whether the effective dose of vit-B_{12} and folic acid has reversible or irreversible effect on the recovery of arsenic induced toxicity phenomenon on female reproductive organs (uterus and ovary), this experiment has been designed. Withdrawal treatment was conducted after 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 aforesaid doses for 16 days or 28 days to find weather the role of this selective doses drugs to protect the arsenic mediated toxicity is permanent or temporary on arsenic induced albino female rat.

5.6.2 Experimental Design: Thirty six female albino Wistar strain rats (each group six) aged 60 days with weight of 125±5 g were selected in this experiment. They were nourished and acclimatized were followed as the previous experiments. The duration of the experiment was extended for 28days. All the rats were synchronized by orally introducing of estardiol at a dose of 3µg/100g.body wt.

This experimental schedule was as followed:

Group I: Control for 16 days withdrawal

Group II: 0.4 ppm/100g body wt. /day sodium arsenite orally for 28 days followed by 16 days of Withdrawal treatment.

Group III: 0.4 ppm/100g body wt. /day sodium arsenite +Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day) orally for 28 days followed by 16 days of withdrawal treatment.

Group IV: Control for 28 days withdrawal

Group V: 0.4 ppm/100g body wt. /day sodium arsenite orally for 28 days followed by 28 days of Withdrawal treatment.

Group VI: 0.4 ppm/100g body wt. /day sodium arsenite +Vit-B_{12} (0.07µg/100g body wt. /day) + Folic acid (4µg /100g body wt. /day) orally for 28 days followed by 28 days of withdrawal treatment.

The untreated control animals were provided with pure drinking water as vehicle treated control (**Group I &**. **Group IV**) rats without any arsenic contamination.

The above concentrations of these drugs were prepared in each week and kept in dark and cool place. Vaginal smears were collected twice daily at 8 a.m. and 5 p.m. from all animals. On the 45^{th} day and 57^{th} day of this experiment the rats final body weights of all the investigational rats were noted. After anesthesia by ketamine HCI, blood was collected from dorsal aorta of each rat using a 21-gauge needled syringe. Serum samples were separated after centrifuging the blood and stored at -20^0C in separate ampoules until all the samples had been used for biochemical assay.

After collection of blood all the animals were sacrificed. Now the ovary and uterus of the 6 animals of each group were dissected out and wet weights of these organs were measured by electronic balance. Ovary and uterus from each animal were used for biochemical assay.

5.6.3 Results:

5.6.3.1 Estimation of LH, FSH, And Estradiol in serum: Ovarian Δ^5, 3 β-HSD and 17 β-HSD activity:

Mode of treatment	Δ^5, 3 β-HSD (units/mg of tissue/hr)	17 β-HSD (units/mg of tissue/hr)	LH (mlU / ml of Serum)	FSH (mlU / ml of Serum)	Estradiol (pg / ml of Serum)
Control for 16 day withdrawal	38.7±2.5[a]	34.5 ±2.3[a]	4.0±0.5[a]	7.5±0.71[a]	135.5±5.7[a]
Sodium arsenite treatment at the dose of 0.4-ppm / 100 g body weight / day for 28 days followed by 16 days withdrawal treatment	22.6±2.1[b]	20.9 ±2.9[b]	2.9±0.4[b]	5.9±0.62[b]	119.77±4.8[b]
Sodium arsenite (0.4-ppm/100g body wt. /day) +Vit-B_{12} (0.07µg/100g body wt. /day)+ Folic acid (4µg /100g body wt. /day)for 28 days followed by 16 days withdrawal treatment	37.8±3.1[a]	32.9 ±2.1[a]	3.6±0.5[a]	7.9±0.69[a]	136.7±5.2[a]
Control for 28 days withdrawal	39.4±2.7[a]	35.6±2.4[a]	4.2±0.3[a]	7.6±0.61[a]	136.2±4.9[a]
Sodium arsenite treatment at the dose of 0.4-ppm / 100 g body weight / day for 28 days followed by 28 days withdrawal treatment.	37.5±1.9[a]	33.2±2.8[a]	3.7±0.2[a]	6.8±0.53[a]	132.4±3.2[a]
Sodium arsenite (0.4-ppm/100g body wt. /day) +Vit-B_{12} (0.07µg/100g body wt. /day)+ Folic acid (4µg /100g body wt. /day) for 28 days followed by 28 days withdrawal treatment.	40.5±2.1[a]	34.9±2.5[a]	4.1±0.02[a]	7.7±0.63[a]	136.5±5.1[a]

Table 5.6.VIA I: Withdrawal effect of selective formulation of co-administration ofVit-B_{12} and/or folic acid in arsenic induced female rat's ovarian steroidogenic enzyme activities, serum levels of gonadotrophins and estradiol. Data represents here is mean ± SE, N=6 and two tailed student's t test were used to find out statistical significance at $p<0.001$. Same superscript in each vertical column did not differ from each other significantly.

5.6.3.2 Antioxidative enzymes (Catalase, Superoxide dismutase, peroxidase) activity monitoring:

Mode of treatment	Catalase Unit /mg of tissue		SOD Unit/mg of tissue		Peroxidase Unit/mg of tissue	
	Ovary	Uterus	Ovary	Uterus	Ovary	Uterus
Control for 16 days withdrawal	**4.4±0.13[a]**	**7.9±1.1[a]**	**3.2±0.42[a]**	**6.2±0.36[a]**	**0.18±0.005[a]**	**0.2±0.003[a]**
Sodium arsenite treatment at the dose of 0.4-ppm / 100 g body weight / day for 28 days followed by 16 days withdrawal treatment	**1.2±0.08[b]**	**2.2±0.58[b]**	**2.1±0.33[b]**	**2.9±0.56[b]**	**0.09±0.006[b]**	**0.1±0.002[b]**
Sodium arsenite (0.4-ppm/100g body wt. /day) +Vit-B_{12} (0.07µg/100g body wt. /day)+ Folic acid (4µg /100g body wt. /day)for 28 days followed by 16 days withdrawal treatment	**3.8±0.2[a]**	**7.1±0.91[a]**	**3.4±0.4[a]**	**5.9±0.34[a]**	**0.19±0.004[a]**	**0.25±0.004[a]**
Control for 28 days withdrawal	**4.7±0.16[a]**	**7.7±1.2[a]**	**3.3±0.55[a]**	**6.5±0.29[a]**	**0.19±0.003[a]**	**0.24±0.004[a]**
Sodium arsenite treatment at the dose of 0.4-ppm / 100 g body weight / day for 28 days followed by 28 days withdrawal treatment.	**4.1±0.1[a]**	**6.9±1.1[a]**	**2.9±0.33[a]**	**5.5±0.24[a]**	**0.17±0.004[a]**	**0.17±0.003[a]**
Sodium arsenite (0.4-ppm/100g body wt. /day) +Vit-B_{12} (0.07µg/100g body wt. /day)+ Folic acid (4µg /100g body wt. /day) for 28 days followed by 28 days withdrawal treatment.	**4.4±0.12[a]**	**7.9±1.3[a]**	**3.4±0.65[a]**	**6.3±0.35[a]**	**0.2±0.002[a]**	**0.26±0.002[a]**

Table 5.6.VIA.II: Withdrawal effect of selective formulation of co-administration ofVit-B_{12} and/or folic acid in arsenic induced female rat's Catalase, Superoxide dismutase, peroxidase activity in ovary and uterine tissue. Data represents here is mean±SE, N=6 and two tailed student's t' test were used to find out statistical significance at $p<0.001$. Same superscript in each vertical column did not differ from each other significantly.

5.6.3.3 Comet Assay:

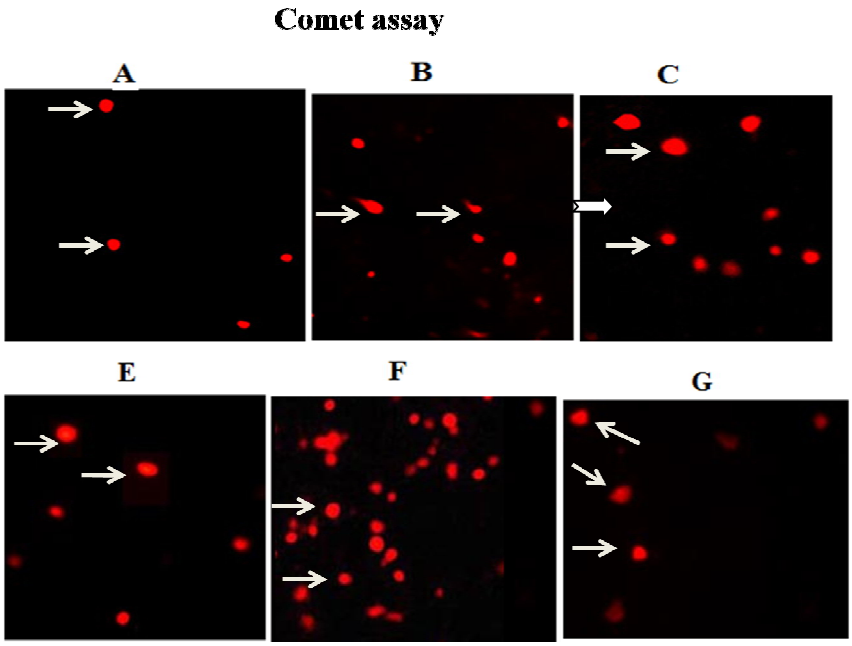


Figure 5.6.1: Withdrawal effect of selective formulation of co-administration ofVit-B_{12} and/or folic acid in arsenic induced female rat's protection of DNA breakage in the comet formation are shown in arsenic induced uterine cell by the co-administration of Vit- B_{12} and/ or folic acid in different doses.

A: Control for 16 days withdrawal**; B:** Sodium arsenite treatment at the dose 0.4 ppm/100g body weight/day for 28 days followed by 16 days of withdrawal treatment; **C:** Sodium arsenite (0.4 ppm/100g body wt./day) + Vit. B_{12} (0.07µg/100g body wt./day) + Folic acid (4µg/100g body wt./day) for 28 days followed by 16 days of withdrawal treatment.

E. Control for 28 days withdrawal; **F:** As treatment at the dose of 0.4 ppm/100g body wt./day for 28 days followed by 28 days of withdrawal treatment; **G:** As (0.4 ppm/100g body wt./day) + Vit.-B_{12} (0.07µg/100g body wt./day) + Folic acid (4µg/100g body wt./day) for 28 days followed by 28 days of withdrawal treatment.

5.7 Experiment-VII

***in vitro* studies on folic acid and Vit-B_{12} of sodium arsenite treatment on ovarian Δ^5, 3β-HSD and 17β-HSD activity in mature albino rat.**

5.7.1 Experimental Design:

For *in vitro* experiments 15 mature female albino rats of Wistar strain were taken with 120±10 g body weight bred in our colony. They were acclimatized by keeping for 7 days in temperature (25±2^{0}C) and lighting controlled (12h L: 12h D) animal house for acclimation. They were supplied with food and water *ad libitum*. Animals were divided into five equal groups.

In vitro studies were carried out in glass tubes containing 9 ml of Kreb-Ringer bicarbonate buffer (KRB) solution having the following composition:

i)	NaCl	8.00 g
ii)	KCl	0.20 g
iii)	$CaCl_2$	0.20 g
iv)	$MgCl_2$, 6H2O	0.10 g
v)	$NaHCO_3$	1.00 g
vi)	Na_2HPO_4	0.05 g
vii)	Glucose	1.00 g

Volume was made up to 1000 ml with redistill water containing pH 7.6. In the present study incubation was continued for 2 hrs at constant 37 ^{0}C and in a gas phase of 95% O_2 – 5% CO_2 (**Brady, 1951**).

Animals were sacrificed and ovaries were dissected out. Both ovaries were cut into small sizes that fluid can be entering easily in the tissue parts. Ovaries were placed directly in each glass tubes. One ovary from each animal was kept in control group and other ovary of the same animal was kept in investigational group. Animals in each group were arranged in the following manner to perform this experiment.

Group I: Incubation of ovaries *in vitro* tubes (one ovary per tube) containing 9 ml of KRB solution along with 1ml of drinking water without arsenic content. In this way total 3 tubes were taken and named as control group.

Group II: Incubation of ovaries *in vitro* tubes (one ovary per tube) containing 9 ml KRB solution along with 0.4ppm sodium arsenite dissolved in 1ml of drinking water. In this way a total 3 tubes were taken and named as treated group.

Group III: Incubation of ovaries *in vitro* tubes (one ovary per tube) containing 9 ml KRB solution along with 0.4 ppm sodium arsenite dissolved in 1ml of drinking water + Vit-B_{12} (0.07µg/100g body wt.). In this way a total 3 tubes were taken and named as treated group.

Group IV: Incubation of ovaries *in vitro* tubes (one ovary per tube) containing 9 ml KRB solution along with 0.4 ppm sodium arsenite dissolved in 1 ml of drinking water + Folic acid (4µg /100g body wt.). In this way a total 3 tubes were taken.

Group V: Incubation of ovaries *in vitro* tubes (one ovary per tube) containing 9ml KRB solution along with 0.4 ppm sodium arsenite dissolved in 1ml of drinking water +Vit-B_{12} (0.07µg/100g body wt.)+ Folic acid (4µg /100g body wt.). In this way 3 tubes were taken.

Towards the end of incubation phase ovaries were transferred individually to the tubes containing 0.1 M sodium phosphate buffer (pH 7.6) for washing. Then these ovaries were used to perform the assay of Δ^5, 3β-HSD and 17β-HSD activity.

5.7.2 Results:

Experimental results revealed that sodium arsenite at a concentration of 0.4 ppm did not able to alter the activities of Δ^5, 3β-HSD and 17β-HSD at a significant level **(Table-5.7.VII.A.I)**

Mode of treatment	**Δ^5, 3β-HSD (units/mg of tissue/hr)**	**17β-HSD (units/mg of tissue /hr)**
Vehicle treated control	35.5±3.1 [a]	33.8±3.2 [a]
Sodium arsenite 0.4 ppm	34.6±3.3 [a]	32.8±2.9 [a]
Sodium arsenite 0.4 ppm+Vit B_{12} (0.07µg/100g body wt.) + Folic acid (4µg /100g body wt.)	35.9±3.5[a]	33.5±3.1[a]

Table 5.7.VII.A.I: *In vitro* studies of sodium arsenite treatment on ovarian Δ^5, 3β-HSD and 17β-HSD activity in mature albino rat. Data represents here is mean ± SE, N=3. ANOVA followed by two tailed student's t test were used to find out statistical significance at $p<0.001$. Same superscript in each vertical column did not differ from each other significantly.