CHAPTER-6

EXPERIMENT-II

6.0. EXPERIMENT-II

Protective efficacy of NAC in the lessening of reproductive injury following sodium arsenite ingestion.

6.1. Objectives of this experiment

i) This study has been conducted using two unlike doses of NAC i.e. 50 mg/kg body weight and 100 mg/kg body weight with respect to judge sodium arsenite persuaded reproductive injury on female Wistar models.

ii) To recognize the most effective therapeutic co-administered dosage of NAC in resisting arsenite instigated female reproductive hazardous impact.

6.2. Selection of animal for experiment & treatment

About 100-110 grams of female Wistar strain rats were utilized for the continuation of this present experiment. All the experimental models were allocated in four distinguished groups and each group consisted of 6 evenly distributed animals (n=6). Animal experiment was followed for 8 days continually with subsequent treatment with oral gavage. Prior to start animal experimentation, the estrous cycle of animals in every group was synchronized with ethinyl estradiol at the dose of 0.1 µg/kg body weight. The study design of this experiment is given underneath:

Group 1: This was control group and only vehicle treatment was implemented,

Group 2: This was sodium arsenite treated group (dose: 10mg/kg body weight),

Group 3: This group was provided sodium arsenite with same dose as Group 2 accompanied with NAC (dose: 50 mg/kg body weight),

Group 4: The treatment protocol of this group was same as Group 3 only the dosage of NAC was higher (100 mg/kg body weight).

The dosage of NAC (100 mg/kg body weight) was decided based on the earlier investigation (Kannan and Flora, 2006). The other lower dosage of NAC i.e. 50 mg/kg body weight was selected here against arsenite to explore the dose dependent functional status of NAC and simultaneously to establish the high therapeutic index of NAC with minimum dose. Accessing the rats freely towards a standard pellet diet maintained under standard animal house condition. The rat's food habit and general growth were monitored during the entire treatment. The monitoring of cyclic stages of estrous was done for all animals in every group. Simultaneously, the organo-somatic indices were also determined as percentage of total body weight. Finally on day 9, the anaesthesia of rats was made using HCl ketamine (24 mg/kg body weight) and finally followed by euthanasia by barbiturate overdose (≥86 mg/kg body weight). Then the reproductive tissues ovaries, uterine horns, liver and blood samples were assembled as stated by the standard method of concern institution. Gathered samples were then placed in the fridge at -20˚C temperature using the sterile bags separately.

6.3. Results

6.3.1. Body growth & organ weights

All the animals from each group showed a normal sustained growth pattern and no remarkable transformation of body weight were noted (Table 6.1). Considering the water intake among the four groups, no such significant distinction was noticed. Arsenic ingestion revealed a significant exhaustion of wet weight of reproductive

organs (uterus and ovary) in contrast to control group (Table 6.1). Co-administration by NAC significantly discouraged the deteriorative action of As^{3+} on uterus and ovary. In this background, NAC at the dosage of 100 mg/kg body weight exhibited more prominent result.

Table 6.1

	Body Weight (g)		Organo-somatic indices (g)		Water intake (ml/100g body weight)
	Initial	Final	Ovary in pair	Uterus	
Control	108.2±6.74	123.2±7.09	0.0640±0.011	0.181±0.025	8.42±0.52
Arsenic	102.14±5.27	109.14±3.96	0.0412±0.002*	0.099±0.011*	8.28±0.57
As^{3+} +NAC 50	102±10.26	111.66±18.4	0.0507±0.007	0.121±0.017#	10.73±1.21
As^{3+} + NAC 100	111.5±5.35	116.33±4.07	0.0547±0.002#	0.141±0.017##	9.86±0.97

Table 6.1: Effect of different doses of NAC on body growth, water consumption and reproductive organs. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). *$p<0.05$ was considered for significance analysis when compared between control and As^{3+} group, whereas #$p<0.05$ and ##$p<0.01$ were considered for comparison between As^{3+} and rest of additional NAC oriented groups.

6.3.2. Study on the pattern of estrous cycle

During the experimental protocol of 8 days, the regular patterns of estrous cycles were monitored (Fig. 6.1). After 3-4 days of As^{3+} treatment, a continual phase of met or diestrous was significantly experienced in distinction with control group.

NAC treatment in arsenic incorporated rats reinstated the significant estrous phase towards normal condition. No considerable changes were signified between two separate dosages of NAC (Fig. 6.1).

Figure 6.1

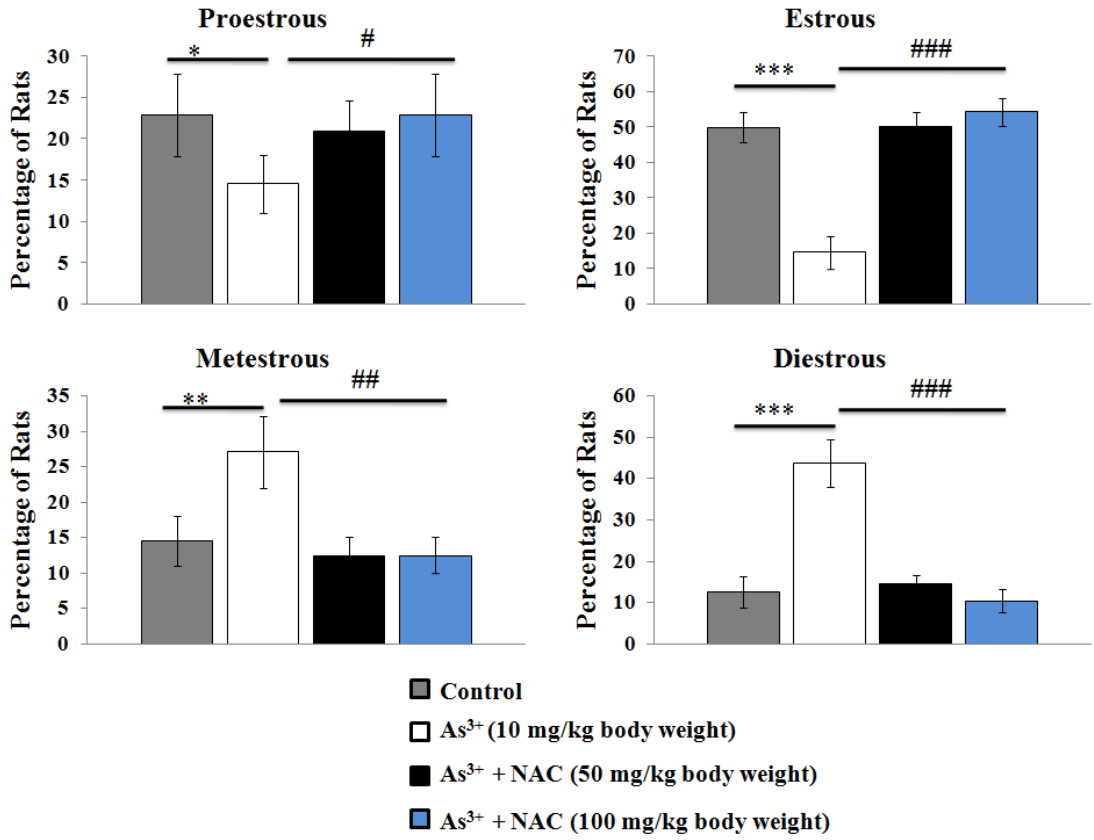

Figure 6.1: Effect of two diverse dosages of NAC on the pattern of estrous cycle in opposition to arsenication. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***p<0.001 was considered for significance analysis when compared between control and As^{3+} group, whereas ###p<0.001 considered for comparison between As^{3+} and rest of additional groups e.g. NAC co-administered groups.

6.3.3. Monitoring of lipid peroxidation and NPSH in uterus

Arsenic ingestion caused an enhancement of lipid peroxidation which further resulting in significant rise in the construction of downstream products like MDA & CD in uterus compared with vehicle treated control (Fig. 6.2 A). Moreover, co-introduction of separate diverse doses of NAC corrected and declined the peroxidation of lipid. However, NAC lowered the yield of these downstream products in the reproductive organs. Indeed, arsenic depleted the amount of soluble thiol in the uterus and increased the predisposition of oxidative stress. NAC supplementation eventually recovered this thiol level significantly in uterus and diminished the susceptibility of cellular oxidative stress (Fig. 6.2 B). Any noteworthy discrepancy was not observed between the separate two dosage of NAC in lipid peroxidation as well as NPSH level.

Figure 6.2

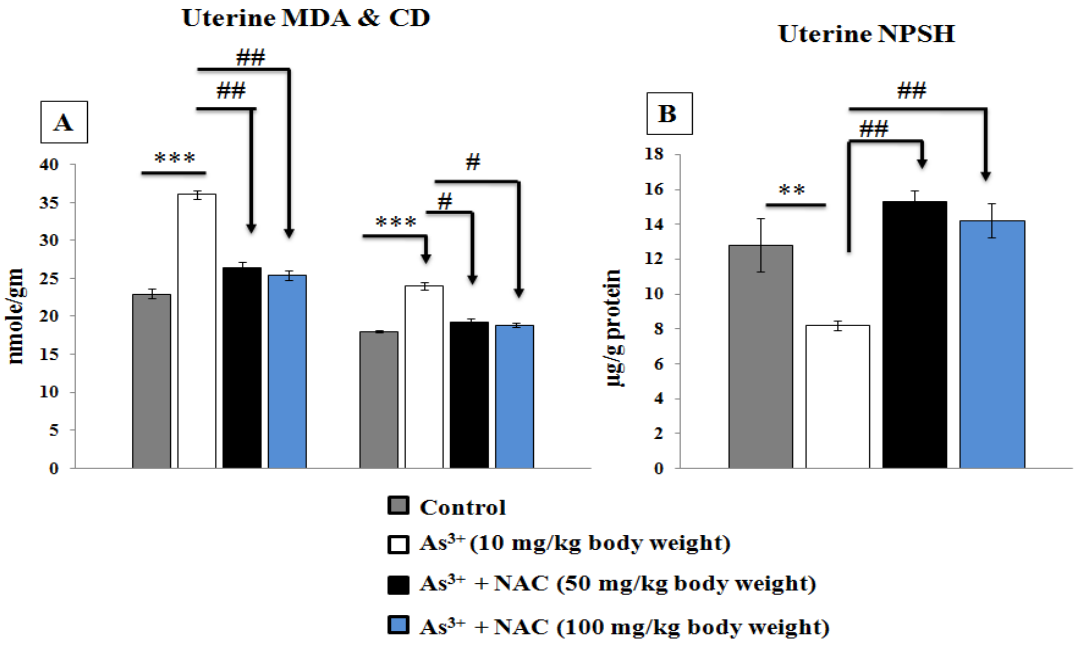

Figure 6.2: Effect of two dosages of NAC on arsenic influenced lipid peroxidation plus NPSH in uterus. The results represent Mean ± SE (Standard Error), n=6. Data

were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). **p<0.01 and ***p<0.001 were considered for significance analysis when compared between control and As^{3+} group, whereas #p<0.05 and ##p<0.01 were considered for comparison between As^{3+} and rest of additional groups.

6.3.4. Effect of NAC on uterine antioxidant status

The spectrophotometric evaluation documented the diminuted activity of uterine antioxidant enzymes catalase, SOD and GPx in arsenicated group compared with vehicle treated control (Fig. 6.3 A) and this reduction was 4.0, 3.2 and 2.6 fold correspondingly. Around 1.1, 1.3 and 1.55 fold deviation was prominent between the two distinct dosages of NAC (NAC 50 and NAC 100 mg/kg body weight) correspondingly in case of SOD, catalase and GPx activity.

Native gel study further confirmed the similar outcome where arsenic treatment executed lower expression of these enzymes profile as evidenced from lower intensity of bands with 15%, 10% and 11% loss of band strength of catalase, SOD and GPx respectively (Fig. 6.3 B). Co-introduction of NAC in arsenicated animals significantly antagonized the hazardous effect of arsenic in functioning of these enzymes in this sex organ (Fig. 6.3 A). Simultaneously the band impression was also returned to the high intensity due to giving NAC in arsenic challenged group (Fig. 6.3 B). Nevertheless, NAC at a dosage of 100 mg/kg body weight exhibited better expressional result for catalase (Fig. 6.3 B).

Figure 6.3

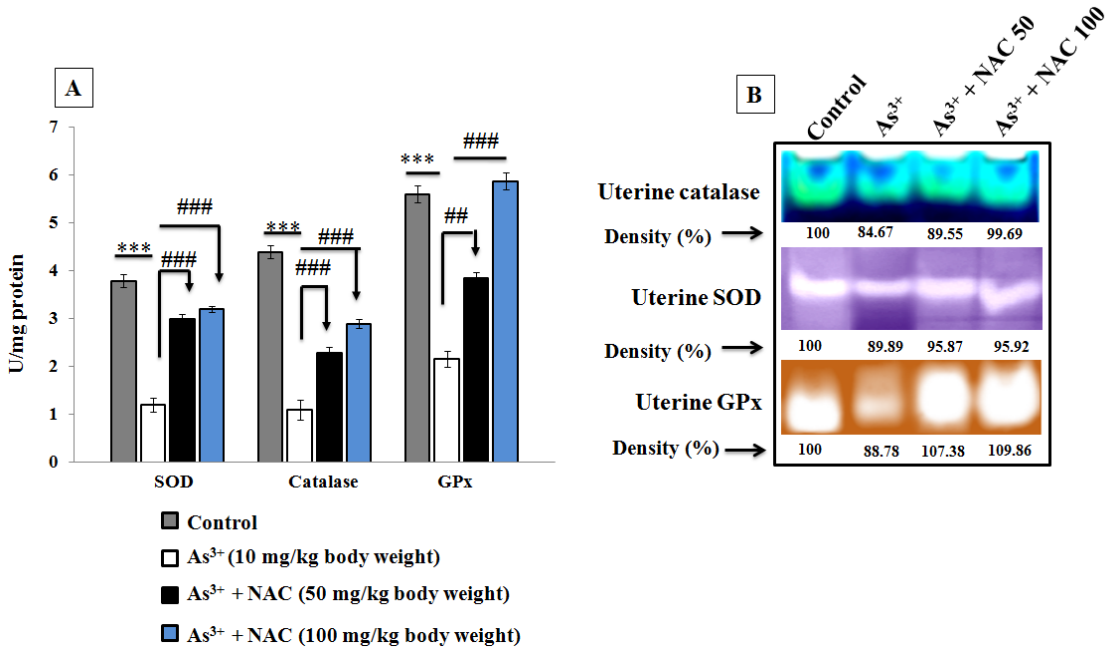

Figure 6.3: Effect of NAC on uterine antioxidant status via spectrophotometric study (A). Native gel study documented the demonstration of intracellular antioxidant enzymes (B). The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***p<0.001 was considered for significance analysis when compared between control and As^{3+} group, whereas ##p<0.01 and ###p<0.001 were considered for comparison between As^{3+} and rest of additional groups.

6.3.5. Status of serum LDH

About 3.3 fold increased level of LDH activity was perceived in sodium arsenite fed group (Fig. 6.4 A). A parallel nature of result was noted on electrozymogram where LDH band showed a strong expression in arsenicated models in opposition to control (Fig. 6.4 B). A noteworthy opposed action against arsenicated degradation of LDH was found following the supplementation of NAC. The band strength was also

weakening when NAC with both doses were applied on arsenic ingested animals (Fig. 6.4 B).

Figure 6.4

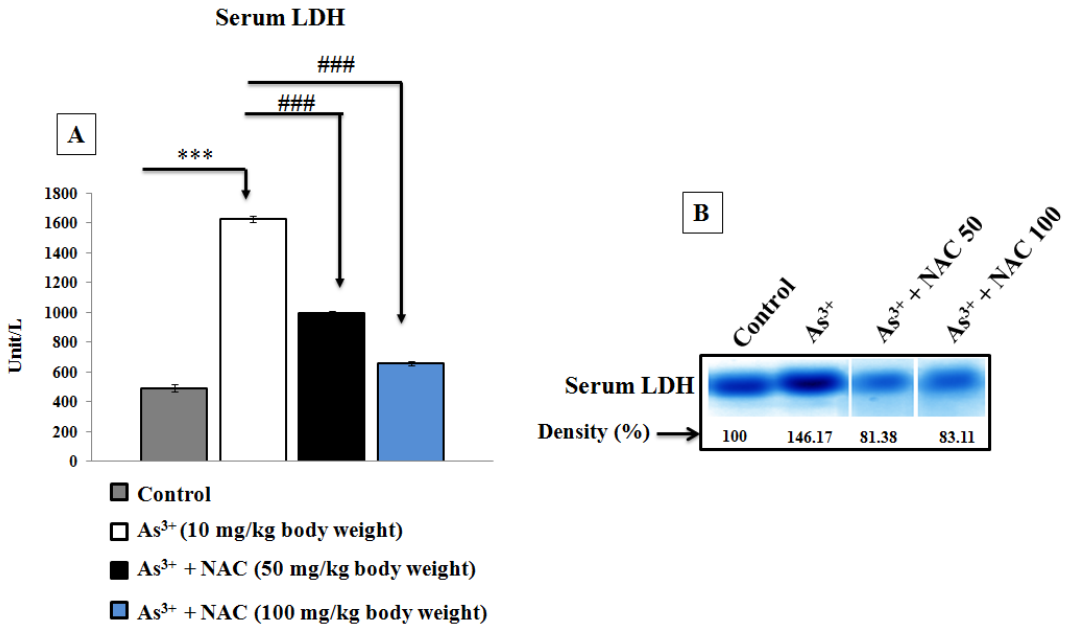

Figure 6.4: Functioning of NAC on LDH studied through spectrophotometric analysis (A). Study on agarose gel exhibited the propagation of LDH with respect to the co-application of As^{3+} and NAC (B). The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***$p<0.001$ was considered for significance analysis when compared between control and As^{3+} group, whereas ###$p<0.001$ was considered for comparison between As^{3+} and rest of additional groups.

6.3.6. Study of DNA fragmentation and comet assay

A higher strength of DNA lesion and fragile type of band was focused in arsenic aided group in respect of unexposed rats (Fig. 6.5 A1). This was further affirmed by the quantity of band compactness of uterine DNA (Fig. 6.5 A2). Variable dosages of NAC co-supply in arsenic aided group decreased the DNA lesion and damage by

forming the high intensity bands significantly (Fig. 6.5). Considering the two variable dose of NAC, a higher relative band density with lower migration was viewed when NAC was given at 100 mg/kg body weight in arsenite fed group (Fig. 6.5 A2). From this context this is affirmed that NAC of higher dose is comparatively more suited for erasing arsenite aided DNA lessening than that of lower dose (50 mg/kg body weight).

DNA degradation by arsenic was additionally confirmed by comet assay (Fig. 6.5 B) on uterine tissue. Interruption in DNA structure and enlarged number of broken DNA with high length of degraded tail was seen from bulk of cells in arsenic supplied animals (Table 6.2). NAC ingestion in different doses partially yet significantly erased arsenic persuaded DNA lesion and also lessened the creation of comet along with diminishing tail length (Table 6.2).

Figure 6.5

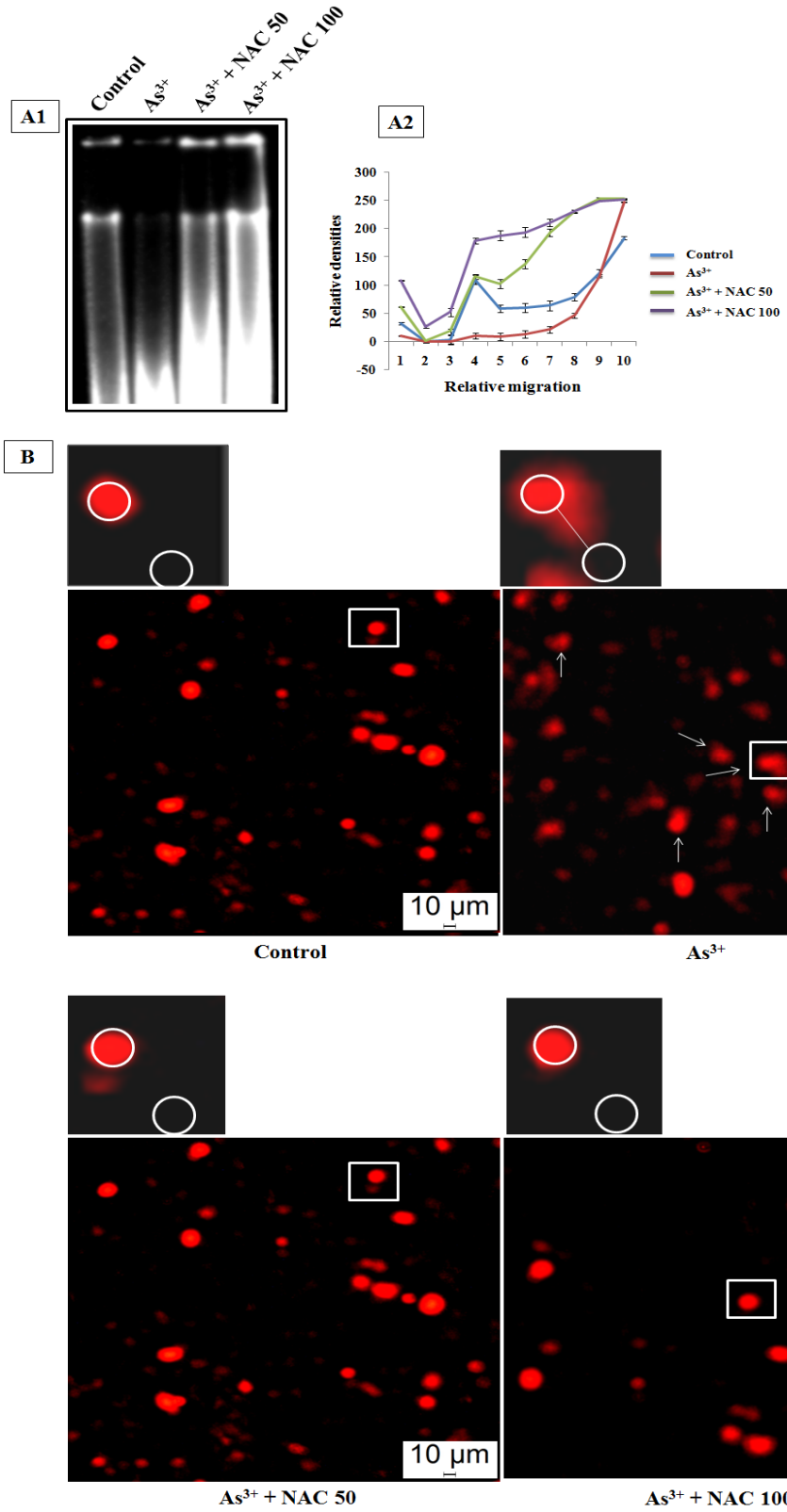

Figure 6.5: Effect of changed dosage of NAC in arsenicated uterine DNA lesion (A1) estimated on agarose gel. The evaluation of band density was made using

Image J software (A2). Arsenic persuaded formation of comet was indicated by arrows (B). NAC allocation significantly opposed the arsenic influenced comet formation.

6.3.7. Ovarian steroidogenesis and the level of gonadotropins & estradiol in serum

A noteworthy interruption of ovarian steroidogenesis was observed following arsenic application (Fig. 6.6 A & B). The status of ovarian 17 β-HSD (16.7 fold) and Δ^5, 3β-HSD (1.9 fold) was remarkably diminuted because of arsenic action when contrasted with control rats. A significant recovery of steroidogenesis was noticeable after the management of NAC in variable doses in arsenicated animals (Fig. 6.6 A & B). Taking into account of two dosages, around 5.6 and 11.2 fold recoveries were accounted for ovarian 17 β-HSD activity for 50 mg/kg body weight and 100 mg/kg body weight of NAC respectively with respect to arsenite given animals (Fig. 6.6 A). Considering the functional status of ovarian Δ^5, 3β-HSD, approximately 1.6 and 1.89 fold improvement was observed in above cited groups respectively in contrast with arsenite driven group (Fig. 6.6 B). Besides, the surge of gonadotropin hormones like LH (2.9 fold) plus FSH (2.59 fold) and estradiol (3 fold) were observed significantly when arsenic treatment administered with respect to control group (Fig. 6.6 C, D & E). Co-supplementation of NAC significantly interrupted the negative action of arsenic therefore, re-established the gonadotropin hormones signaling towards normalcy (Fig. 6.6 C, D & E). When NAC was given at a dose of 50 mg/kg body weight, it was showing 2.2, 2.0 and 2.0 fold enhancement of gonadotropins (LH and FSH) and estradiol signaling respectively whereas 2.7, 2.4 and 2.7 fold up-gradation was noted in above stated hormones when NAC was given

at 100 mg/kg body weight in arsenite given animals (Fig. 6.6 C, D & E). Hence, all instances perceived that NAC at a dose of 100 mg/kg body weight was more reproducible than that of 50 mg/kg body weight in opposing sodium arsenite propagated uterine ailments.

Figure 6.6

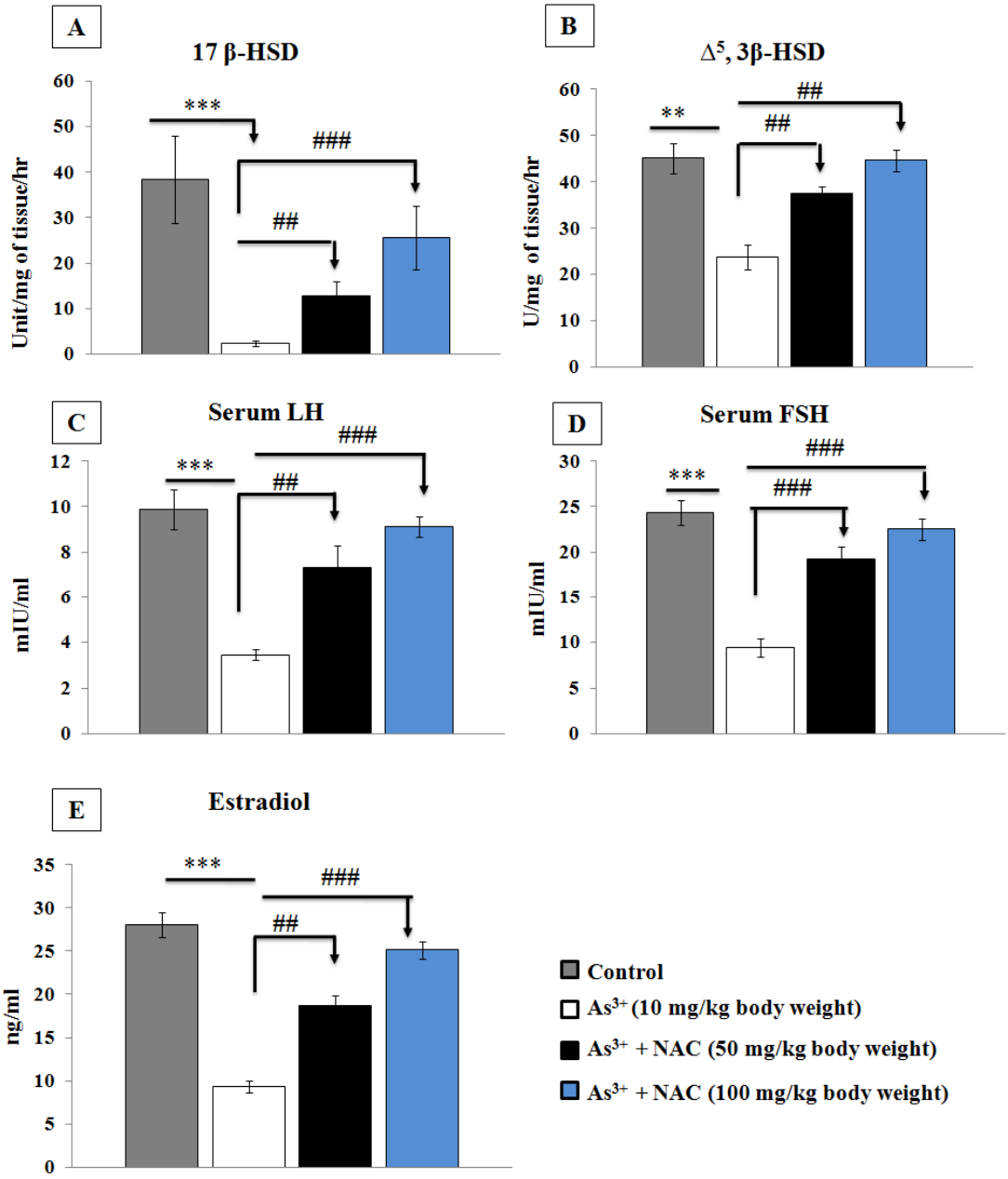

Figure 6.6: Affectivity of NAC (various doses) in the opposition of arsenic dominated disruption of ovarian steroidogenesis (A & B), and serum status of

gonadotropins and estradiol (C, D & E). The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). **p<0.01 and ***p<0.001 were considered for significance analysis when compared between control and As^{3+} group, whereas #p<0.05, ##p<0.01 and ###p<0.001 were considered for comparison between As^{3+} and rest of additional groups.

6.3.8. Histopathology of uterus and ovary

Arsenic treatment showed the deteriorative cum degraded effect on secretory glands. The uterine tissue layers i.e. endometrium and myometruim were also distorted in opposition with unexposed rats (Fig. 6.7 A). Co-supplementation of separate two dosages of NAC extensively re-established the regular width of uterine layers and also restored the secretory glands into the normal condition (Fig. 6.7 A; Table 6.2). A considerable loss of ovarian preantral follicle along with graafian follicle; elevated numbers of atretic and regressive follicle were manifested by the intake of arsenic compared to arsenic untreated group (Fig. 6.2 B; Table 6.2). Application of individual dosage of NAC significantly counteracted and diminished follicular atresia by replacing these with considerable numbers of growing and matured follicle in the way of maintaining the normal folliculogenesis (Fig. 6.2 B; Table 6.2). Here no noteworthy recovery of SAF, MAF, LAF and GF were visualized in NAC co-treated group (50 mg/kg body weight) wherein NAC at a dose of 100 mg/kg body weight co-treated group revealed a significant recovery of above stated ovarian follicles (Table 6.2). Thus NAC at a dosage of 100 mg/kg body weight was more advantageous in the betterment of sodium arsenite allied reproductive health than the lower dose of NAC (50 mg/kg body weight).

Figure 6.7

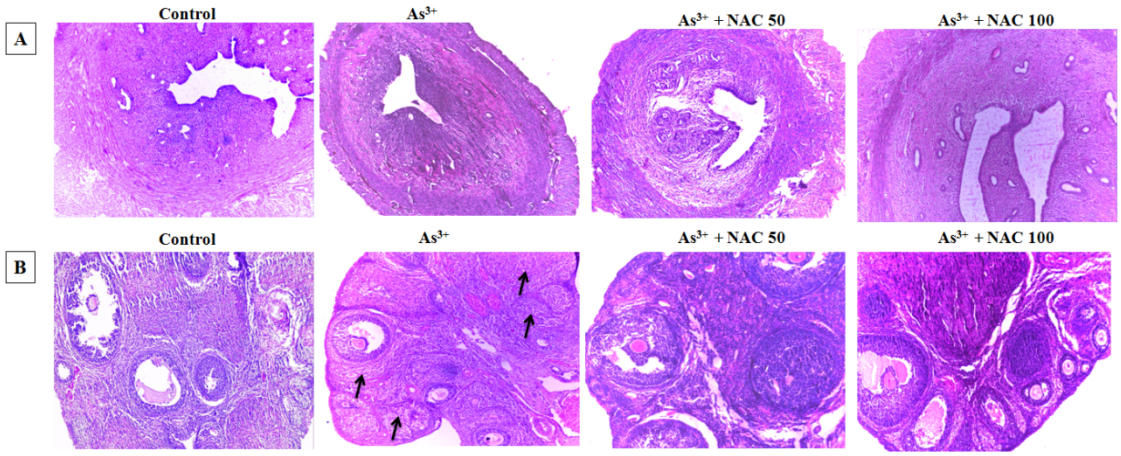

Figure 6.7: Effect of diverse dosages of NAC on arsenic intoxicated architecture of uterus and ovary (A & B). The indication of arrow mark denoted appearance of atretic follicle (B).

Table 6.2

	Control	**As^{3+}**	**As^{3+} NAC 50**	**As^{3+} NAC 100**
Comet in number	0.83 ± 0.3	5.83 ± 0.6***	2.5 ± 0.4##	1.66 ± 0.4###
Comet tail length (µm)	22.33 ± 1.11	39.37 ± 2.33***	23.51 ± 0.97###	21.7 ± 1.05###
SPAF	9.66 ± 1.05	2.16 ± 0.6***	5.83 ± 0.98##	7.66 ± 0.84###
LPAF	8.5 ± 0.8	1.5 ± 0.22***	4.83 ± 0.54##	7.66 ± 1.28###
SAF	6.16 ± 0.79	1.33 ± 0.21***	2.66 ± 0.4	2.83±0.4#
MAF	3.00 ± 0.25	1.33 ± 0.33*	2.00 ± 0.63	2.33 ± 0.42#
LAF	1.33 ± 0.2	1.00 ± 0.36	1.83 ± 0.3	2.16 ± 0.3##
GF	2.16 ± 0.3	1.00 ± 0.25*	1.5 ± 0.22	2.16 ± 0.3##
AF	1.33 ± 0.6	15.33 ± 2.29***	1.66 ± 0.4###	2.66 ± 0.8###
Endometrium (µm)	290.3 ± 8.88	115.31 ± 6.29***	185.82 ± 5.08###	196.39 ± 2.49###
Myometrium (µm)	108.6 ± 1.66	64.47 ± 2.87***	90.49 ± 3.65##	91.72 ± 2.88###

Table 6.2: Effect of NAC with both the dosages with respect to arsenic provoked comet formation i.e. dominated by lengthy tail formation, diminished quantity of

ovarian antral follicles with advanced level of follicular atresia and lessened uterine tissue layers. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). *$p<0.05$ and ***$p<0.001$ were considered for significance analysis when compared between control and As^{3+} group, whereas #$p<0.05$, ##$p<0.01$ and ###$p<0.001$ were considered for comparison between As^{3+} and rest of additional groups.