CHAPTER-7

EXPERIMENT-III

7.0. EXPERIMENT-III

Preventive efficiency of NAC in erasing of sodium arsenite dominated female reproductive mal-functions via *in vivo* approach.

7.1. Objectives of this experiment

This investigation has been perceived to justify the therapeutic efficiency of NAC in preventive manner (pre-treatment to be executed) to combat sodium arsenite directed female reproductive hypo-functions.

7.2. Selection of animal for experiment & treatment

Three (3) groups allocated with 6 animals in each. They were rared separately within a cage made with polycarbonate and free water and foods were provided. The investigation protocol was followed for 16 days successfully. The group separation and experimental plan were mentioned below:

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

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

Group 3: The animals of this group were treated orally with sodium arsenite of same dose like group 2 followed by 100 mg NAC /kg body weight.

Oral gavage has been introduced for execution of treatment plan on experimental models. NAC was treated for the initial 1st day to day 8 in group 3 whereas sodium arsenite was treated once daily for the next 8 days (9-16 days) to group 2 and group 3. Here, the dose of NAC was considered as per the outcome of experiment-II wherein NAC at a dose of 100 mg/kg body weight was more reproducible in erasing arsenite aided reproductive dysfunctions than the lower dose of 50 mg/kg body

weight. The changes of animal's body weight plus water consumption were supervised throughout the experimentation. The rhythmic status of estrous cycle of every animal was also monitored. Day after last day treatment, the entire animals of each group were anesthetized by ketamine HCl (24 mg/kg body weight) via intramuscular injection and then the ovarian samples and uterine horns and blood were collected and stored in cold chamber (-20˚C). Ultimately, considering the instructions of CPCSEA all the experimental animals were euthanized by overdosing of barbiturate (≥86 mg/kg body weight).

7.3. Results

7.3.1. General observation

No noteworthy divergence of body masses was noticed among the animals of entire groups (Table 7.1). Accordingly the average water consumption also revealed no alteration during the investigation. But the weights of reproductive organs manifested a significant deviation because of arsenication in Wistar rats (Table 7.1). Pre-treatment with NAC improved the reduced organs' weight towards the control.

Table 7.1

	Body weight (gm)		Organo-somatic indices (gm%)		Average water intake (ml/100 g of body weight/24h)
	Initial	Final	Uterus	Ovary in pairs	
Control	86.66±5.89	87.66±8.35	0.189±0.007	0.0342±0.001	8.62±0.49
As^{3+}	85.00±2.83	88.83±3.45	0.131±0.009**	0.0283±0.002***	9.42±0.51
As^{3+} + NAC	83.83±2.53	91.16±2.85	0.152±0.01##	0.055±0.003###	9.38±0.57

Table 7.1: Outcome of pre-treatment of NAC on whole body masses, organ's weight and water consumption in sodium arsenite challenged rats. 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 of 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.

7.3.2. Estrous cycle pattern

A regular inspection of estrous cycle status was followed while treatment was pursued. After the restricted days of arsenication, the experimental animals of this specified group sustained diestrous phase (Fig. 7.1). Pre-supplementation of NAC before the commencement of arsenic exhibited the occurrence of synchronization of estrous cycle in rats by replacing consistent diestrous significantly (Fig. 7.1).

Figure 7.1

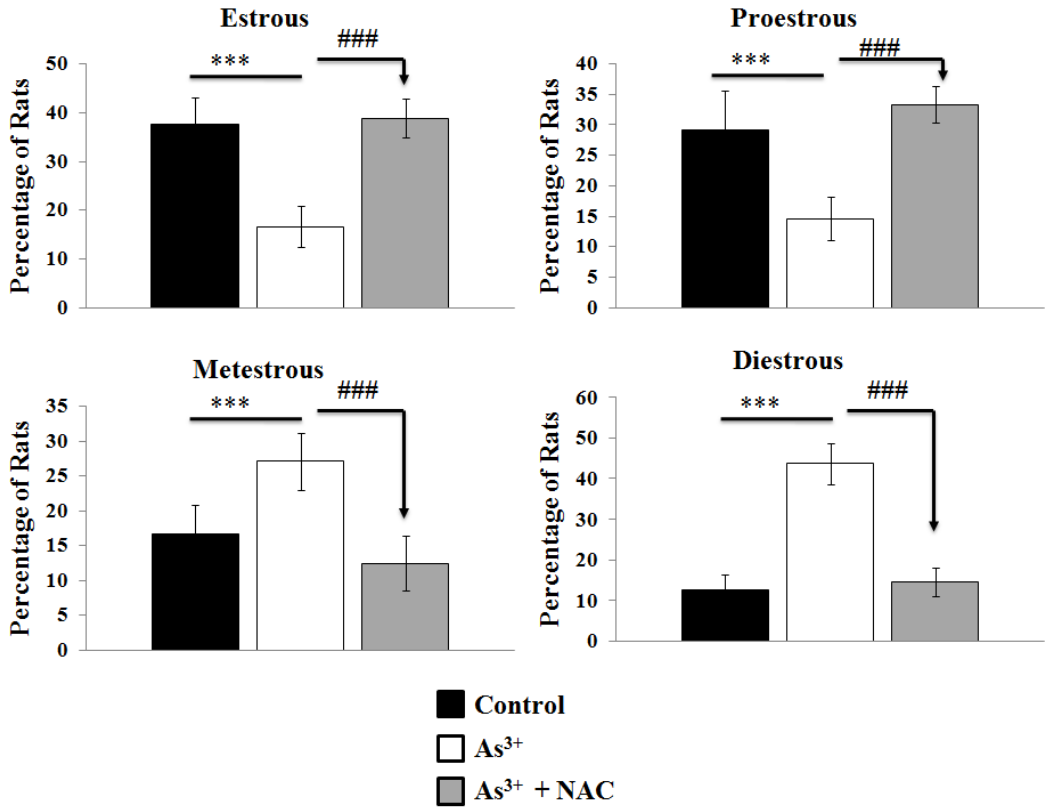

Figure 7.1: Outcome of pre-treatment of NAC on estrous cycle status of arsenic fed rat. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***p<0.001 was considered for significance of analysis when compared between control and As^{3+} group; whereas ###p<0.001 was considered for comparison between As^{3+} and rest of additional groups.

7.3.3. Status of MDA, CD and NPSH

A significant and abrupt increase of redox state of lipid peroxidation e.g. MDA-CD was visible in ovarian-uterine tissues following the introduction of arsenic (Fig. 7.2 A & B). Sodium arsenite further diminished the soluble thiols in reproductive tissues (Fig. 7.2 C & D). Pre-amplification with NAC counteracted the initiation of lipid

peroxidation driven by arsenic treatment and decreased the yield of MDA-CD and exhibited in a recovery of thiol status (Fig. 7.2 A, B, C & D).

Figure 7.2

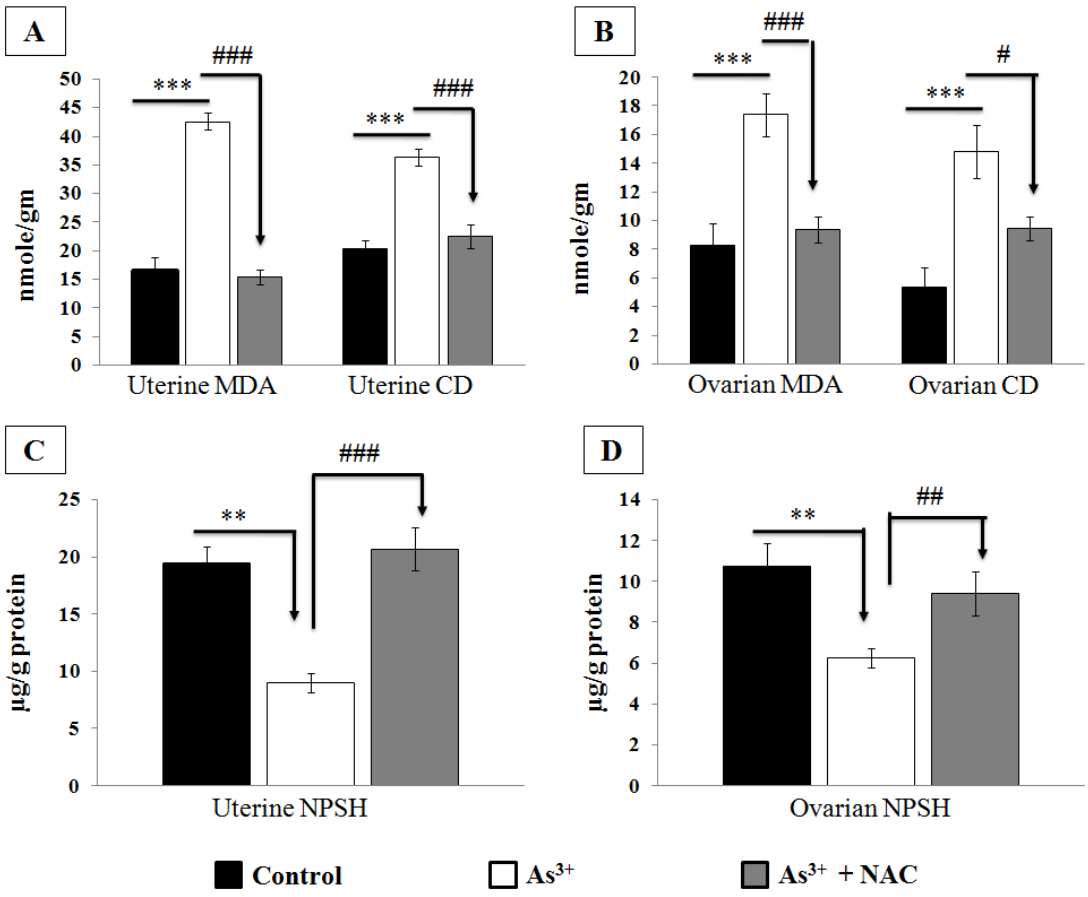

Figure 7.2: The preventive significance of NAC (pre-treatment) in antagonism of arsenic driven lipid peroxidation and soluble thiol production. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). **p<0.01 and ***p<0.001 were 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.

7.3.4. Status of antioxidant enzymes and serum LDH

Arsenic incorporation significantly prohibited the functionality of SOD, catalase and GPx as visualized from both spectrophotometric as well as native gel exploration (Fig. 7.3 A, B, C & D). Pre-administration of NAC secured the defensive act of these enzymes against oxidative stress and setback the deteriorative activity of arsenic (Fig. 7.3 A, B, C & D).

The status of LDH was noted to be prevailing after arsenication in the experimental model animals (Fig. 7.3 E & F). This dominancy and effectivity of LDH remained under control when NAC was pre-treated in this group (Fig. 7.3 E & F).

Figure 7.3

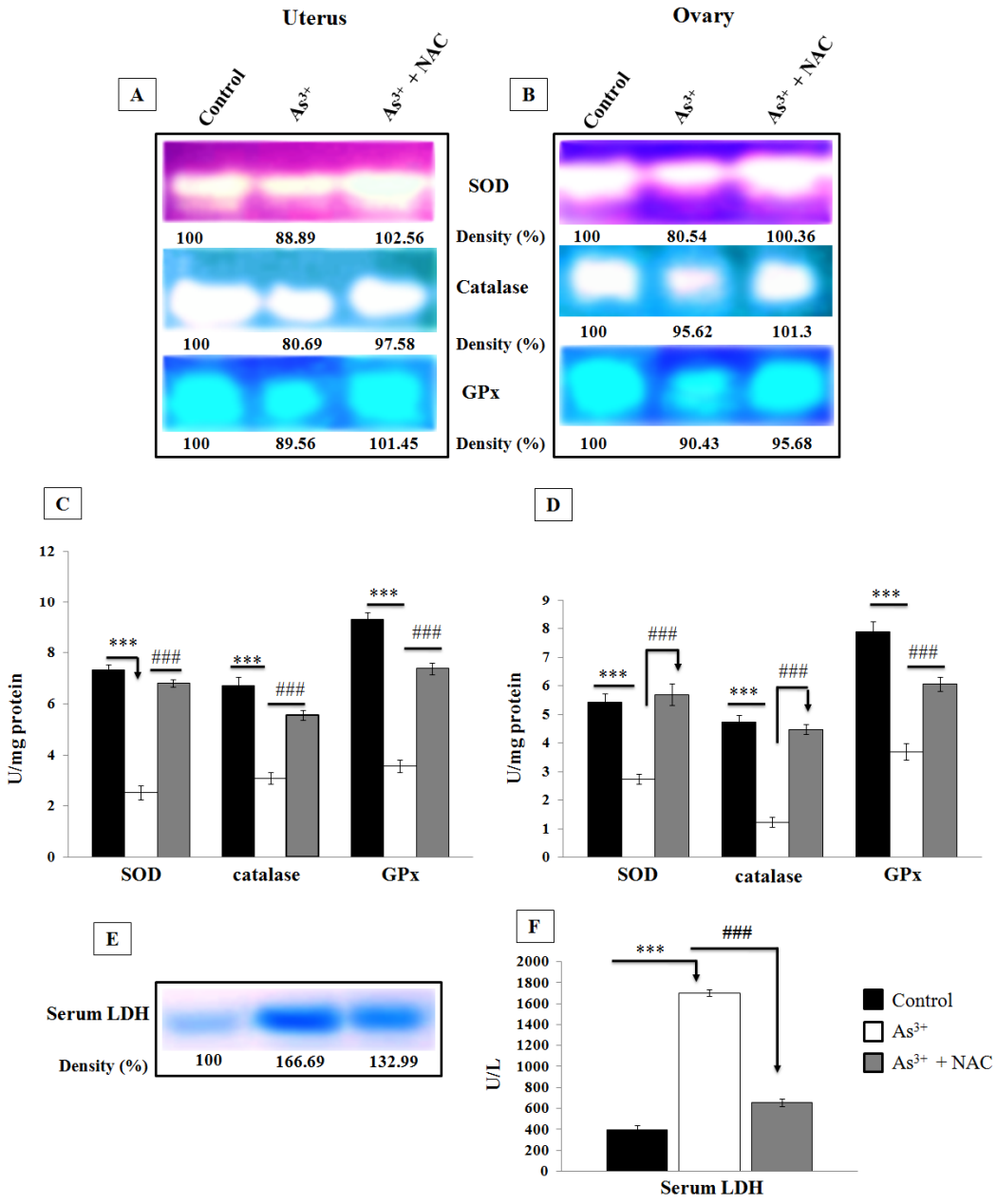

Figure 7.3: The outcome of pre-administration of NAC on antioxidant enzymes and LDH status in arsenic fed animals. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***p<0.001 was considered for significance analysis when compared

between control and As^{3+} group, whereas ###p<0.001 was considered for comparison between As^{3+} and rest of additional groups.

7.3.5. Status of DNA fragmentation

A significant number of cellular DNA degradation was appeared in uterus in terms of appearance of comet cells in arsenic introduced group (Fig. 7.4 B). The length of forming comet was prominent too following arsenication (Table 7.2). NAC administration prior to the supply of arsenic (pre-treatment) discouraged the cell disintegration (Fig. 7.4 B) and made an obstacle for comet formation (Table 7.2) to a noticeable level in arsenicated animals.

Figure 7.4

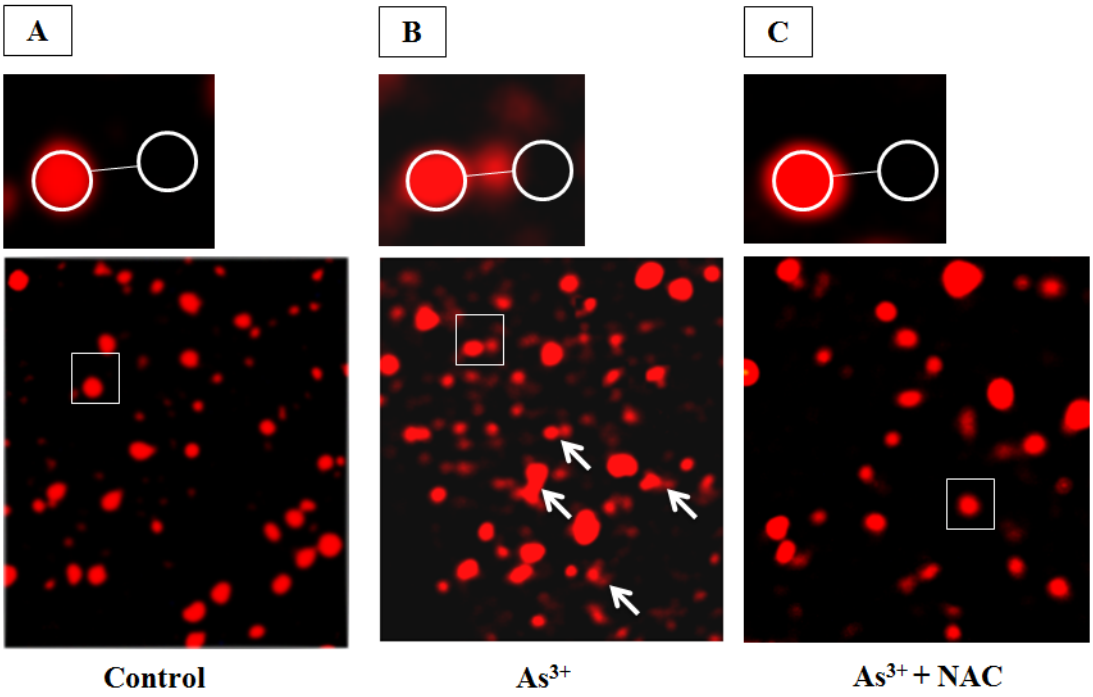

Figure 7.4: The adequacy of pre-treatment of NAC in the correction of arsenic challenged cellular DNA distortion via preventable mode. The arrow mark specified the comet cell appearance.

7.3.6. Status of sex hormones, steroidogenic enzymes and ER-α

An interrupted and appreciably reduced amount of sex hormones i.e. LH-FSH along with estradiol respectively were apparent because of arsenic administration with respect to control (Fig. 7.5 A, B & C). Alongside arsenic treatment also manifested the exhaustion of 17β-HSD and Δ^5, 3β-HSD status (Fig. 7.5 D & E). An increasing and re-influencing level of these hormones and enzymes with adequate efficacy were found when arsenic administered group was pre-supplemented with NAC (Fig. 7.5 A, B, C, D & E).

Arsenic accumulation further lessened the signaling of ER-α within uterus that might be denoted for estradiol level exhaustion (Fig. 7.5 F). A concomitant increase of ER-α signaling was noticed following pre-application of NAC into arsenicated group (Fig. 7.5 F).

Figure 7.5

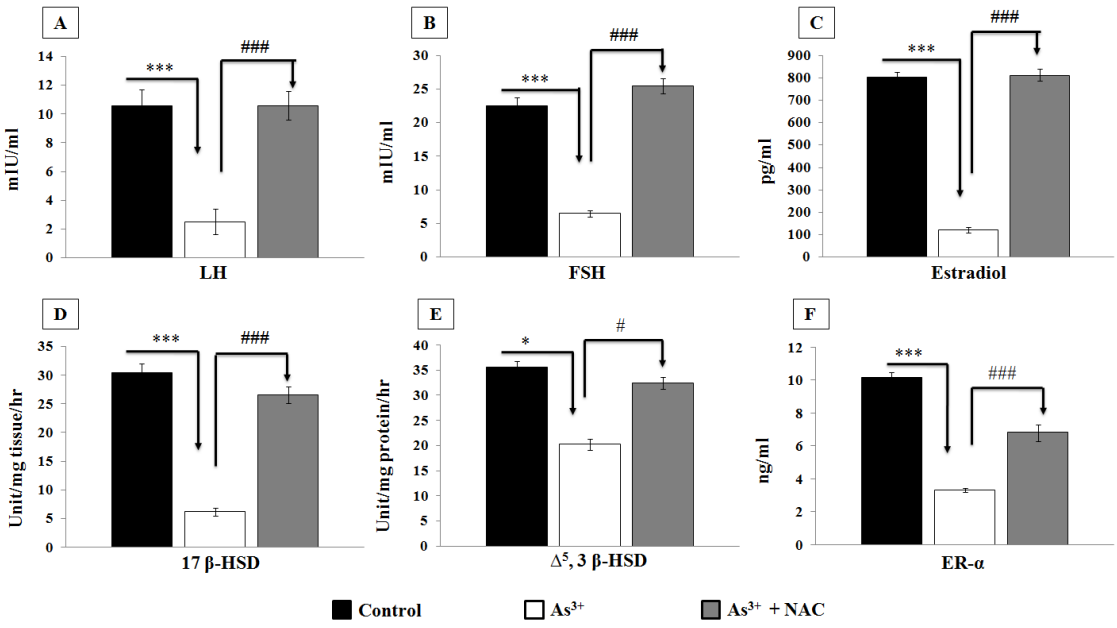

Figure 7.5: The preventive effect of NAC (pre-treatment) in the correction of arsenic exhausted sex hormones, steroidogenesis and ER-α level in uterus. The results

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

7.3.7. Status of B vitamins and inflammatory markers

Arsenic abrogated and diminished the serum content of vitamin B_{12} plus folic acid in the circulation as compared between arsenic and control group (Fig. 7.6 A & B). Arsenic mediated 1.41 and 1.22 folds degradation of above stated vitamins was observed respectively (Fig. 7.6 A & B) contrasted with control group. NAC pre-administration augmented their functional status by 1.35 and 1.18 folds correspondingly with respect to arsenic. A noteworthy higher signaling of TNF-α, NF-κB and IL-6 was prominent after application of arsenic (Fig. 7.6 C & D) and this was around 2.4, 2.63 and 2.13 folds increase respectively. When the animals of this group were pre-supplied with NAC, a significant suppression of these pro-inflammatory markers' level was observed and this reversal was around 2.16, 1.63 and 1.97 folds respectively of above cited inflammatory markers.

Figure 7.6

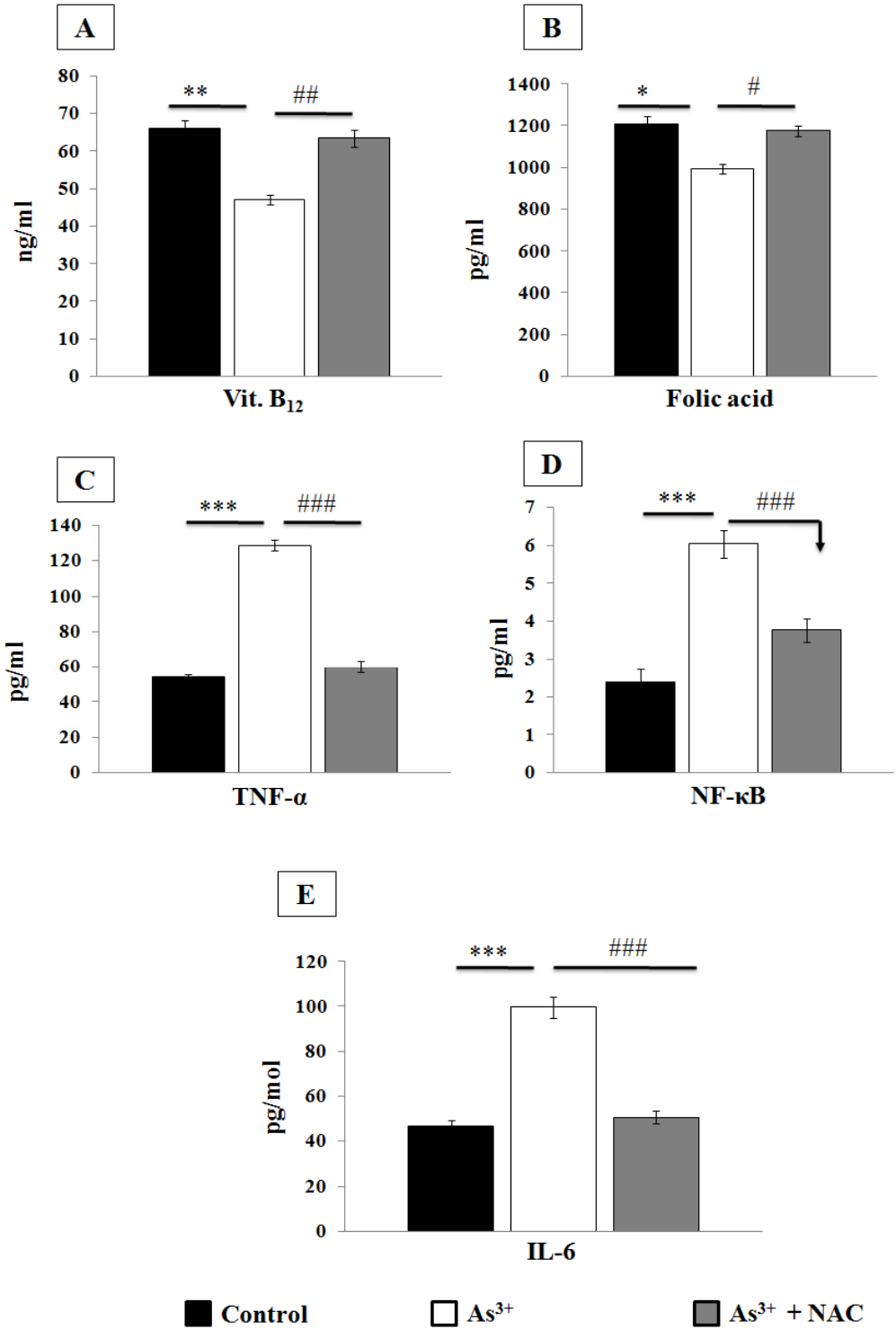

Figure 7.6: The significance of pre-treatment of NAC regarding arsenic aggravated diminution of B vitamins and elevation of measured inflammatory markers. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-

way-ANOVA following Dunnett's test (post-hoc). *p<0.05, **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.

7.3.8. Status of utero-ovarian morphology

Drastic as well as significant follicular erosion was dominant after arsenic administration in rats (Fig. 7.7 B; Table 7.2). Simultaneously, the lesion of uterine layers with decreasing status of secretory cells was also prominent with arsenic application (Fig. 7.7 A; Table 7.2). Significant and better reversal of these degenerative circumstances towards normal follicular development and uterine growth were notable when NAC was applied before arsenic treatment (pre-treatment of NAC).

Figure 7.7

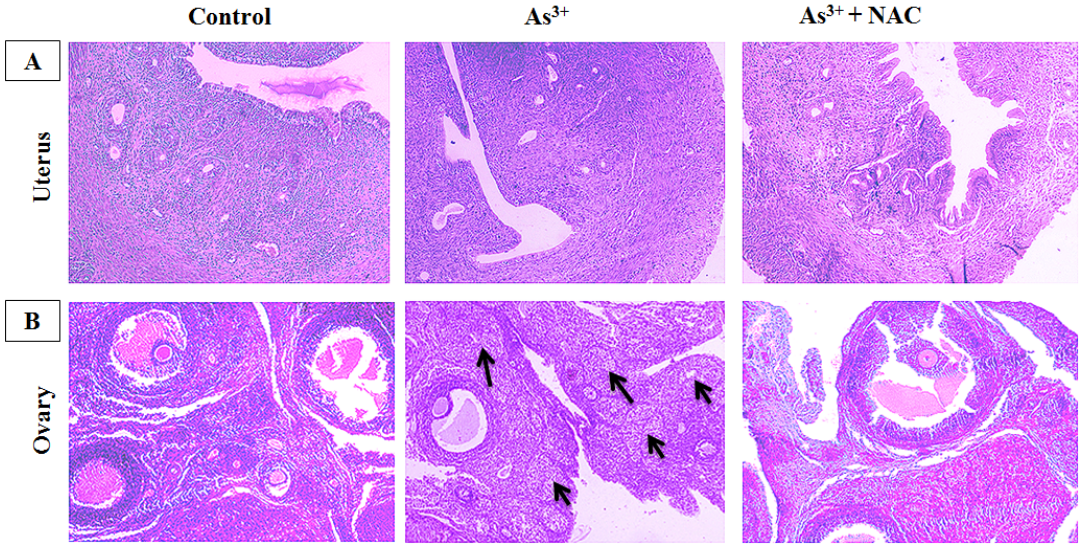

Figure 7.7: Preventive outcome of pre-treatment of NAC in opposing the deteriorative action of arsenic in sex organs. The black coloured arrows specified atretic follicles.

Table 7.2

	Control	As^{3+}	As^{3+}+ NAC
Comet in number	0.98±0.38	7.48±0.76***	2.23±0.33##
Comet tail length (µm)	20.21±2.21	38.48±1.12**	26.75±1.49##
SAF	7.38±0.69	3.1±0.32**	5.46±0.43#
MAF	4.37±0.31	1.52±0.43**	4.62±0.37##
LAF	3.62±0.83	1.64±0.26*	3.86±0.87#
GF	2.31±0.38	0.43±0.17**	2.12±0.28##
AF	2.11±0.53	15.38±1.68***	2.33±0.31###
Endometrium (µm)	311.23±6.78	121.44±5.67***	254.12±5.38###
Myometrium (µm)	169.22±3.12	59.56±3.77***	118.58±4.38###

Table 7.2: Showing the preventive effect of pre-treatment of NAC on comet formation, follicular quantity in ovary and breadth of uterine 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, **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.