CHAPTER-8

EXPERIMENT-IV

8.0. EXPERIMENT-IV

Curative mode of correction of arsenic challenged reproductive deformities by direct oral application of NAC.

8.1. Objectives of this experiment

This experimentation was undertaken to explore the remedial significance of NAC along with a positive control DMSA separately or jointly for the abolition of arsenic driven reproductive toxicity on female experimental model when NAC was applied in the system directly via gavage.

8.2. Selection of animal for experiment & treatment

A total number of 30 animals weighted about 70-95 gm were allocated into 5 groups of having 6 animals per group. The treatment was persisted regularly for 16 days. The schedule and experimental plan is as followed herewith:

Group 1: This was control group wherein only distilled water was given as vehicle,

Group 2: This group was ingested only with sodium arsenite orally (dose: 10 mg/kg body weight) for the initial 8 days followed by no further treatment from 9^{th} day onwards,

Group 3: This group was administered with sodium arsenite for 8 days where the dose and duration was same as group 2. DMSA (dose: 100 mg/kg body weight) was given from day 9 onwards upto day 16,

Group 4: The treatment mode was same as previous group of 3, only NAC (dose: 100 mg/kg body weight) was given for the last 8 days instead of DMSA,

Group 5: This group of rats were provided with above dose of DMSA and NAC from day 9 to day 16 after the completion of sodium arsenite treatment for initial 8 days.

In many investigations DMSA was implemented to mitigate arsenic allied discrepancies (El-Saad et al., 2016; Mittal et al., 2018; Maehashi and Murata, 1986) hence, in this experiment DMSA was applied as positive control with respect to arsenication. In previous investigations DMSA was given at the dose of 50 mg/kg body weight (El-Saad et al., 2016; Mittal et al., 2018) and 100 mg/kg body weight (Maehashi and Murata, 1986) intraperitoneally against arsenic poisoning. Here DMSA was given 100 mg/kg body weight as per the same dose of NAC against arsenite propagated reproductive mis-functionality via oral route. The treatment of sodium arsenite, DMSA and NAC were performed by using oral gavage. A regular monitoring of water intake, body weight alteration and rhythmicity of estrous phases were considered. At the time of sacrifice, the recording of last body weight was performed and then using ketamine HCl (24 mg/kg body weight) the animals were anesthetized. The ovarian-uterine tissues and blood samples were acquired from anesthetized animals and finally as per direction of CPCSEA all experimental animals were euthanized with overdosing of ketamine (≥86 mg/kg body weight).

8.3. Results

8.3.1. General observation

The mean body growth of animals among groups were not changed significantly (Table 8.1). However, the mean weight of ovarian-uterine tissues revealed a noteworthy reduction with respect to control (Table 8.1). This altered weight of

reproductive organs was regained after the supplementation of NAC. Although the average water intake in animals among the groups remain unchanged (Table 8.1).

Table 8.1

	Body weight (gm)		Organo-somatic indices (gm%)		Average water intake(ml/100g body weight/24h)
	Initial	Final	Uterus	Ovary in pairs	
Control	80.5±4.55	88.00±3.22	0.155±0.023	0.048±0.004	10.53±0.59
As^{3+}	79.00±4.33	86.83±4.78	0.079±0.007***	0.023±0.002**	11.46±0.84
As^{3+} + DMSA	81.83±4.42	89.16±4.11	0.181±0.023##	0.06±0.002###	10.47±0.97
As^{3+} + NAC	84.00±2.3	88.66±2.21	0.198±0.029###	0.058±0.002###	9.57±0.79
As^{3+} + DMSA + NAC	85.66±3.42	90.00±2.85	0.202±0.026###	0.0568±0.005###	8.64±0.69

Table 8.1: Represents the differences of body mass alteration, weight changes of reproductive organs and mean water consumption among the group. 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 the additional groups.

8.3.2. Estrous cycle pattern

The type of estrous cyclicity was screened on regular basis during the conduction of experimental treatment (Fig. 8.1). The animals of control group maximally encouraged either estrous or proestrous phase throughout the experimentation. After 3-4 days of arsenication almost all the experimental animals from this group lost their usual estrous cyclicity and sustained with diestrous or metestrous (Fig. 8.1).

NAC post-administration in arsenicated animals changed the irregularity of estrous cycle pattern and re-established their status towards normalcy (Fig. 8.1).

Figure 8.1

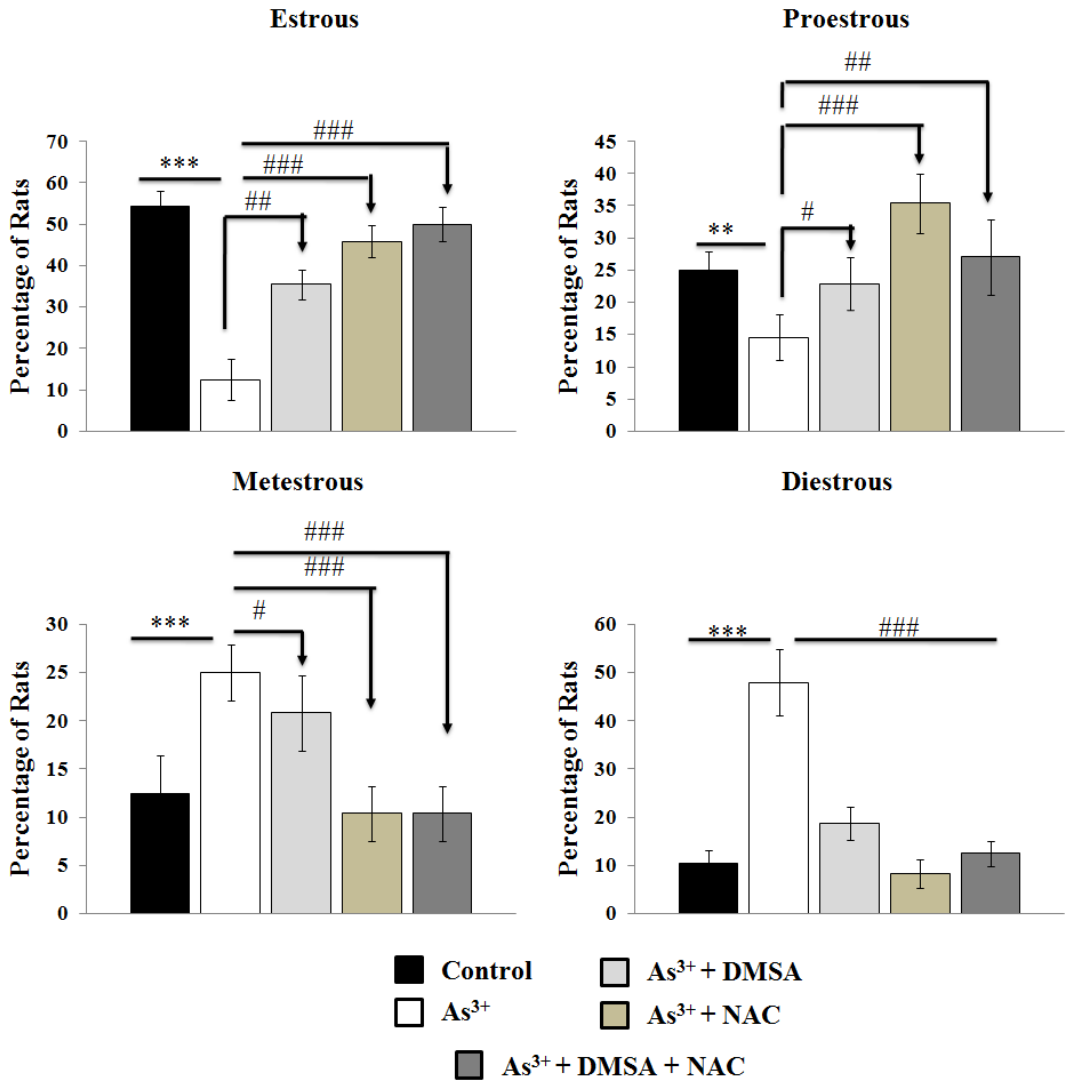

Figure 8.1: Represents the curative approach of NAC on arsenic directed changes of estrous cycle pattern. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***p<0.001 was considered for significance of analysis when compared between control and As^{3+} group, ###p<0.001 was considered for comparison between As^{3+} and rest of additional groups.

8.3.3. Effect of NAC on MDA-CD and NPSH with reference to DMSA

MDA along with CD level were abruptly enhanced in ovarian-uterine tissues following arsenication (Fig. 8.2 A, B, C & D). Therefore, a robust diminution of thiol status in these sexual organs was noted as proven from the decreased level of NPSH (Fig. 8.2 E & F). Administration of NAC plus DMSA alone or jointly overcome this condition significantly and diminished the MDA-CD level towards control and accordingly restored the usual status of cellular NPSH in these organs (Fig. 8.2 A, B, C, D, E & F). In this perspective, NAC combined with DMSA acts more robustly than DMSA and NAC alone.

Figure 8.2

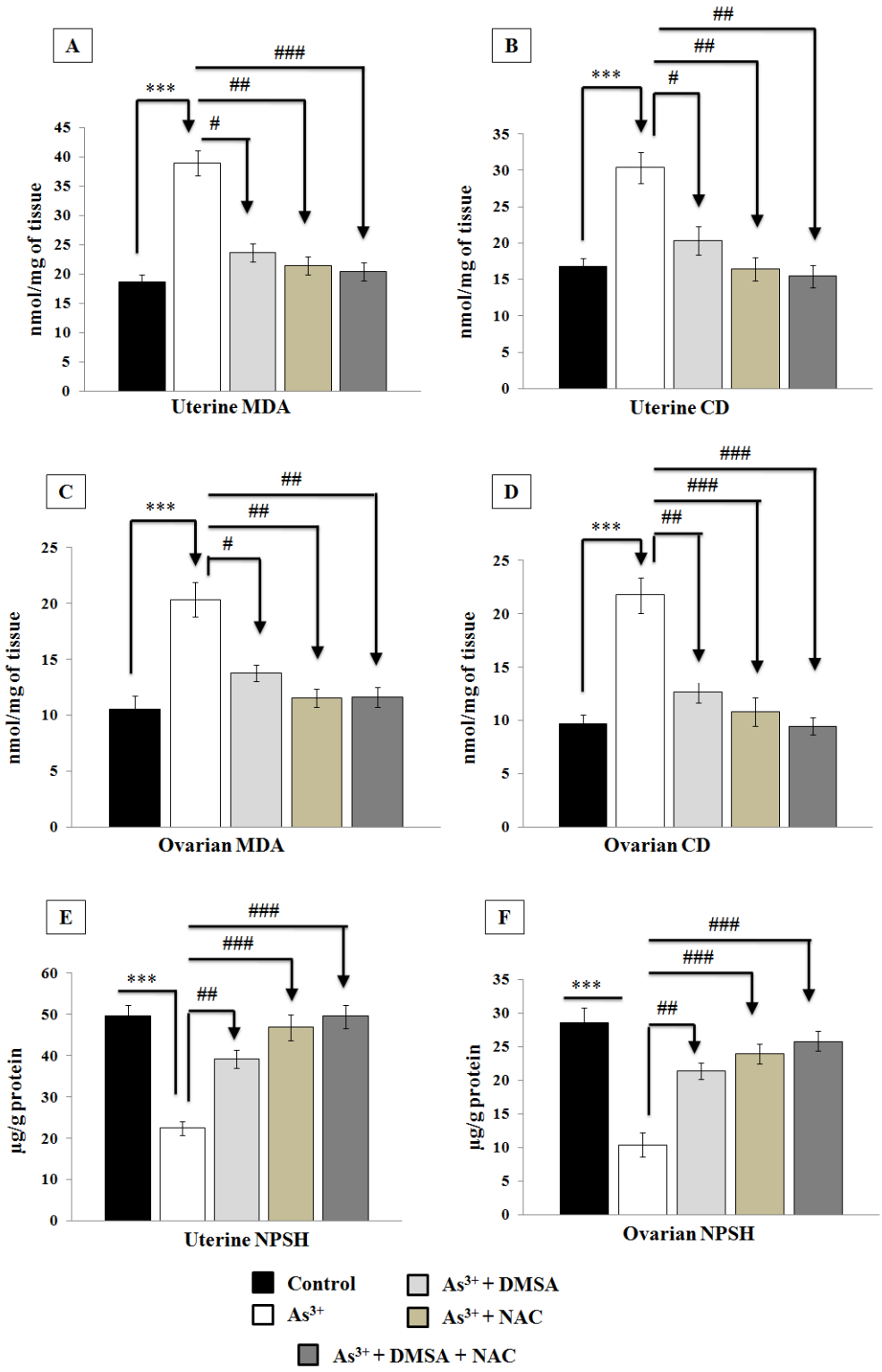

Figure 8.2: Presents the curative mode of significance of NAC, DMSA and NAC plus DMSA combination in the safeguarding of arsenic driven MDA, CD and NPSH

status on ovarian-uterine tissues. 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, #p<0.05, ##p<0.01 and ###p<0.001 were considered for comparison between As^{3+} and rest of additional groups.

8.3.4. Effect of NAC on antioxidant enzymes with reference to DMSA

Arsenic associated augmented lipid peroxidation further leading to suppressed activity of SOD, catalase and GPx in ovarian-uterine tissues (Fig. 8.3 C & D). Likewise parallel trends of outcome were revealed from native gel running. A lessened level of protein expression was observed for these enzymes owing to arsenication (Fig. 8.3 A & B). Post-supply of DMSA plus NAC separately or jointly in arsenicated animals caused notable counteraction and re-stimulation of the functions of these enzymes towards normalcy and significantly abolished the negative effect of arsenication (Fig. 8.3 A, B, C & D).

Figure 8.3

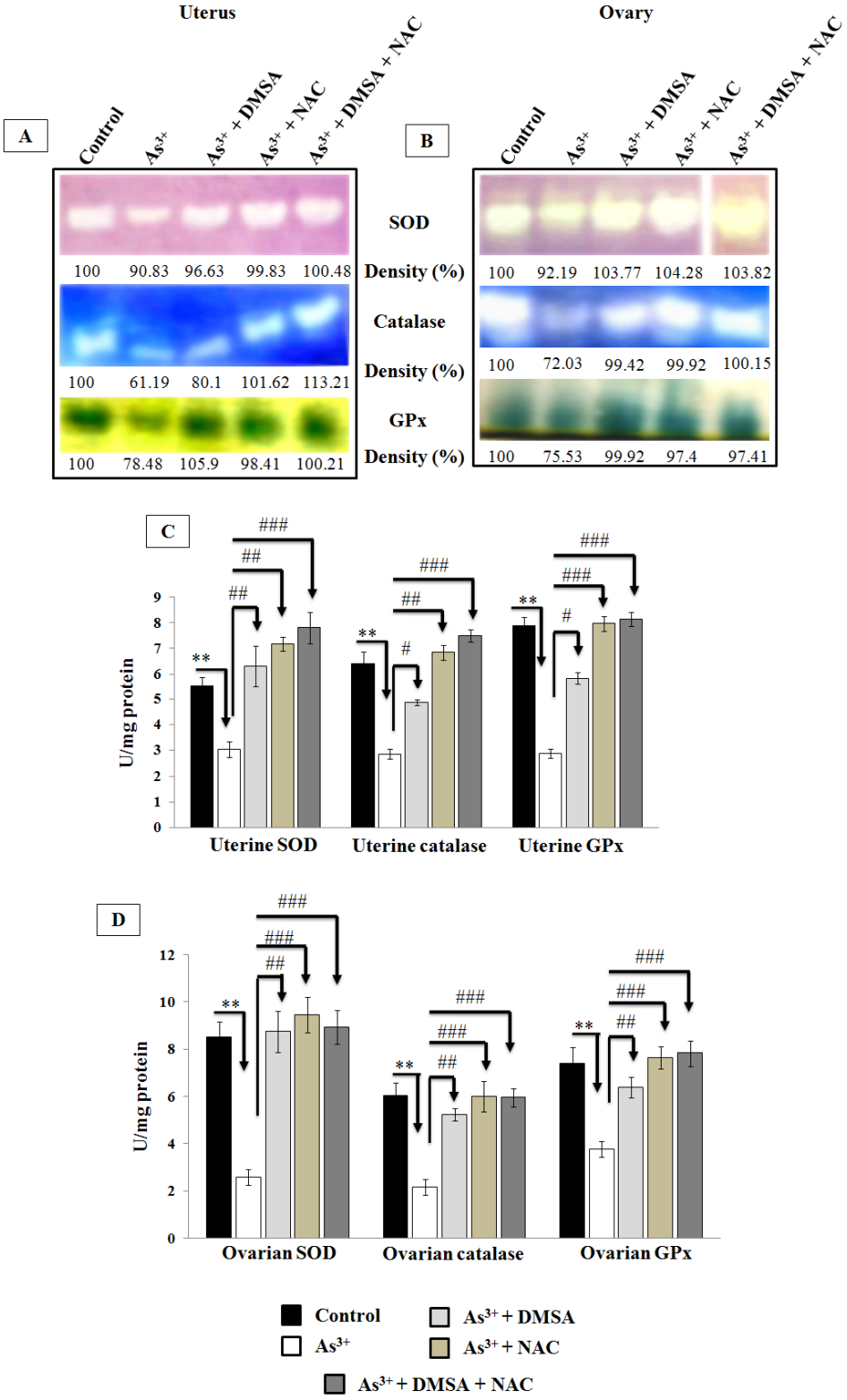

Figure 8.3: Curative mode of effect of DMSA, NAC separately and conjointly on arsenic inhibited functionality of antioxidant enzymes within arsenicated

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<0.01 and ***p<0.001 were considered for significance of analysis when compared between control and As^{3+} group, #p<0.05, ##p<0.01 and ###p<0.001 were considered for comparison between As^{3+} and rest of additional groups.

8.3.5. Effect of NAC on serum LDH with reference to DMSA

The functionality as well as expression of LDH was enormously increased after arsenication in rats with respect to control (Fig. 8.4 A & B). The elevated band girth on agarose gel affirmed that arsenic caused over-stimulation of LDH. Supply of DMSA and NAC separately or combinely down-regulated the over-expression of LDH and headed towards its usual status (Fig. 8.4 A & B). DMSA plus NAC combination showed more viable action.

Figure 8.4

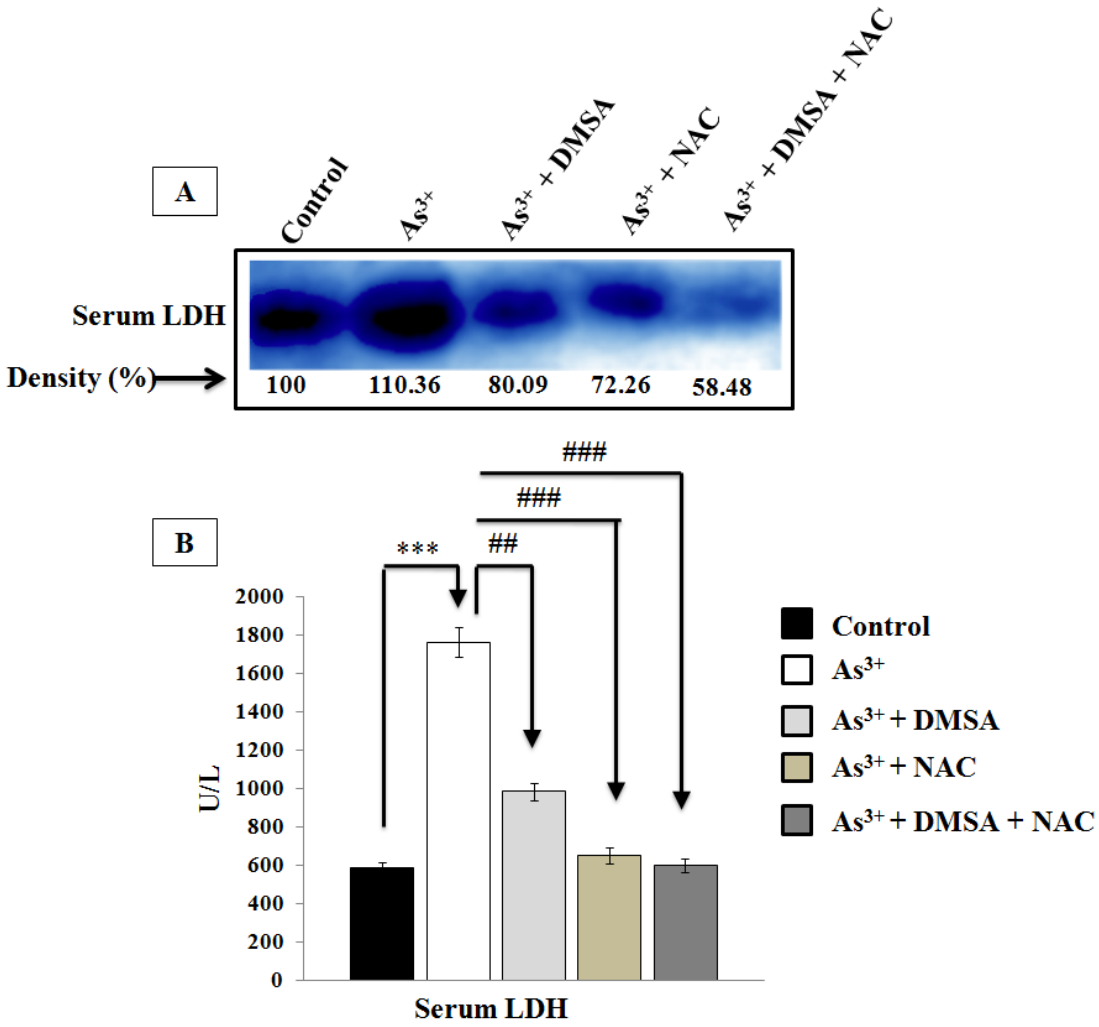

Figure 8.4: Curative action of DMSA plus NAC with respect to LDH status following arsenication. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***p<0.001 was considered for significance analysis when compared between control and As^{3+} group, ##p<0.01 and ###p<0.001 were considered for comparison between As^{3+} and rest of additional groups.

8.3.6. Effect of NAC on DNA comet with reference to DMSA

Arsenic lead to an advanced cellular DNA disintegration which was recognized from the appearance of comet (Fig. 8.5). The wideness of formed comet along with its number was surprisingly elevated in consequence of arsenication (Table 8.2). NAC post-administration alone and or DMSA notably diminished the amount of comet formation and decreased their tail length as well (Fig. 8.5 & Table 8.2).

Figure 8.5

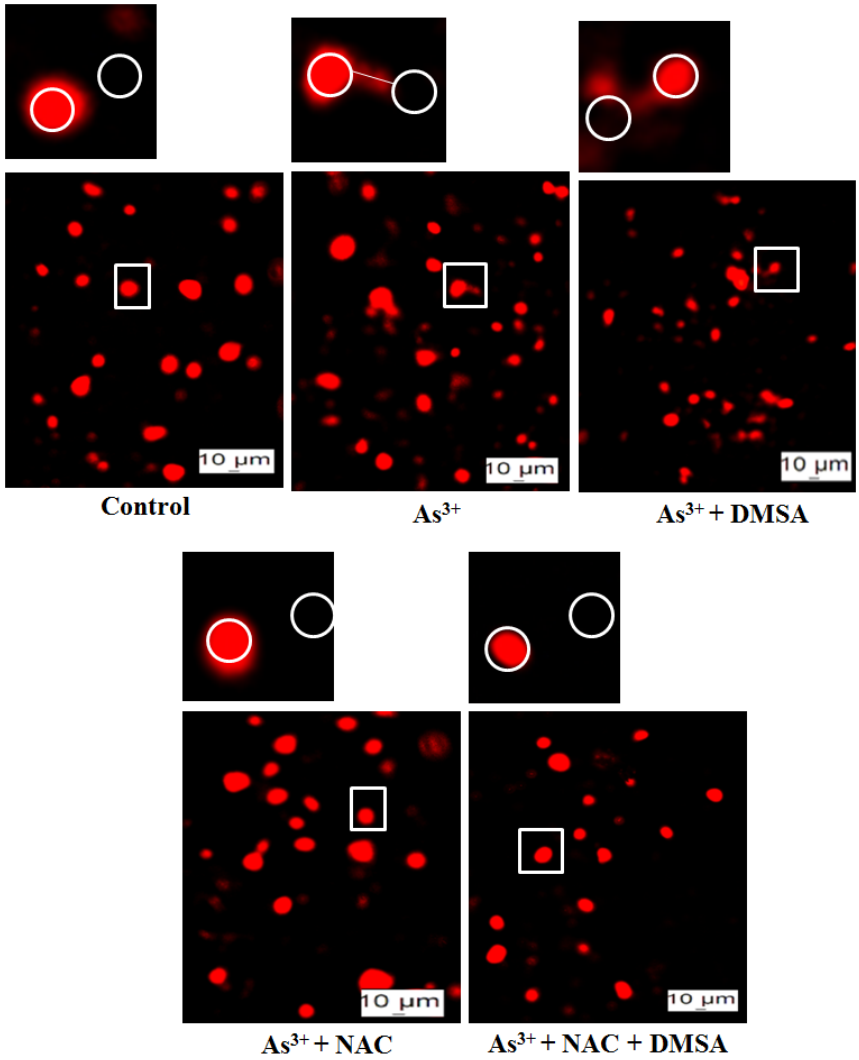

Figure 8.5: Represents the curative action of DMSA, NAC and their additive effect on uterine comet formation on account of arsenication.

Table 8.2

	Control	As^{3+}	As^{3+} + DMSA	As^{3+} + NAC	As^{3+} + DMSA + NAC
Comet in number	0.76±0.07	7.45±0.33***	5.33±0.54	1.43±0.13###	1.57±0.32###
Comet tail length (µm)	2.27±0.29	11.22±1.35***	7.36±0.83	3.05±0.17###	2.27±0.62###
SPAF	5.63±0.52	1.83±0.41***	3.52±0.28##	4.37±0.31###	4.71±0.27###
LPAF	4.61±0.32	1.42±0.17***	3.12±0.23##	3.93±0.31##	4.00±0.15###
SAF	4.85±0.17	2.32±0.16**	3.62±0.17#	4.13±0.25##	4.43±0.2###
MAF	3.51±0.4	1.12±0.11**	2.71±0.19#	3.1±0.22##	3.34±0.13##
LAF	2.51±0.21	0.54±0.03***	1.43±0.1#	2.00±0.15##	2.38±0.16###
GF	2.17±0.11	0.23±0.06***	1.21±0.12##	1.79±0.13###	2.43±0.15###
AF	2.29±0.33	18.72±1.12***	5.44±0.76###	4.76±0.49###	2.84±0.34###
Endometrium (µm)	298.94±5.76	110.54±3.65***	202.63±3.82###	234.74±4.29###	256.83±4.76###
Myometrium (µm)	176.74±3.84	86.96±2.93***	109.75±2.54##	142.28±2.39###	155.95±2.87###

Table 8.2: Remedial effect of DMSA, NAC separately and their additive effect on comet number, tail length and ovarian follicular quantity and breadth of uterine layers with respect 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, ###p<0.001 was considered for comparison between As^{3+} and rest of additional groups.

8.3.7. Effect of NAC on ovarian steroidogenesis, sex hormones and hormone receptor with reference to DMSA

An interruption of steroidogenesis was noted owing to arsenic treatment. Arsenic influenced abolition of 17β-HSD and Δ^5, 3β-HSD functions that dismissed the usual mode of steroidogenesis (Fig. 8.6 D & E). Accordingly, there was a concomitant disruption of LH-FSH and estradiol signaling following arsenic intake (Fig. 8.6 A, B

& C). The changing status of these important sex hormones perhaps because of the destruction and diminution of hormone receptors which were liable for estradiol production. A noteworthy diminished level of expression of ER-α was noticeable after arsenication (Fig. 8.6 E). The impaired status of these hormones and enzymes were significantly restored following the post-application of NAC alone and combined with DMSA. Here in most instances DMSA alone was persisted with mild recovery which was not significant against arsenication. However, NAC alone and combined with DMSA showed more distinguishable positive outcome regarding ovarian steroidogenesis.

Figure 8.6

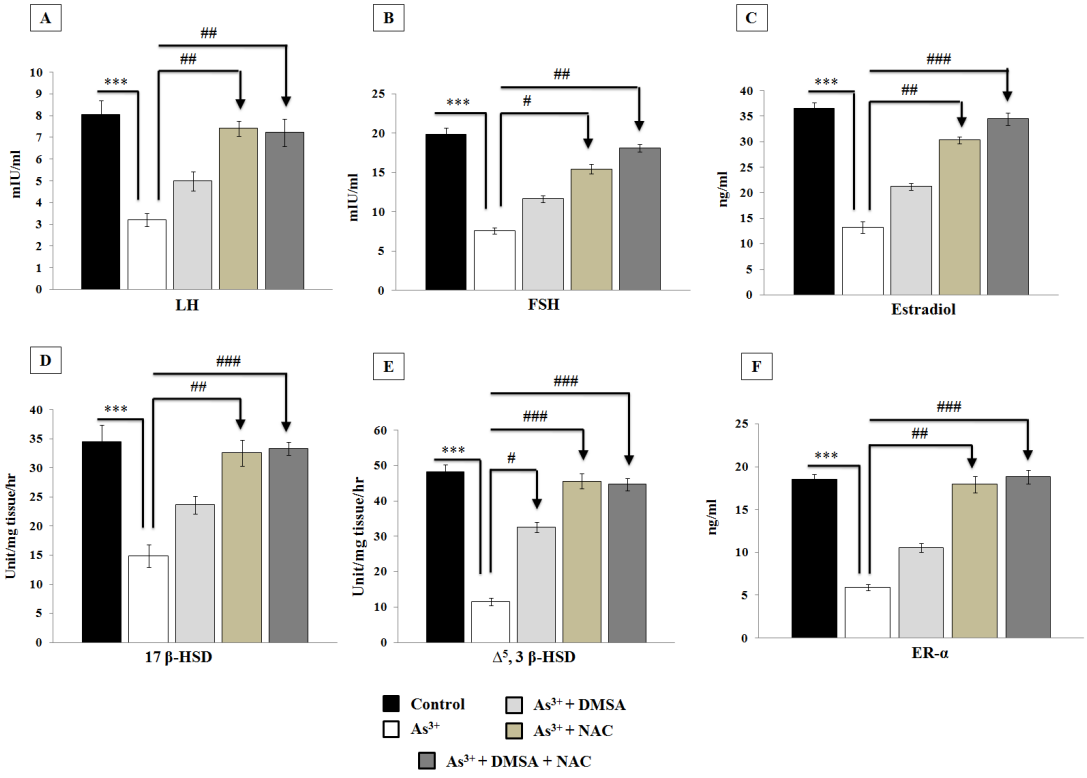

Figure 8.6: Presents the curative action of DMSA, NAC and its combined form in the down-streaming of arsenic mediated deleterious effect on sex hormones, steroidogenesis in ovary and ER-α receptor. The results represent Mean ± SE

(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, #$p<0.05$, ##$p<0.01$ and ###$p<0.001$ were considered for comparison between As^{3+} and rest of additional groups.

8.3.8. Effect of NAC on serum status of vitamin B_{12}, folic acid, homocysteine and hepatic MT-I with reference to DMSA

Persistent arsenic treatment responded for significant decreased in serum status of vitamin B_{12} and folic acid (Fig. 8.7 A & B) while increasing the propensity of hepatic storage of MT-I and homocysteine in the circulation (Fig. 8.7 C & D) with respect to control. The advanced level of MT-I in liver indicated probable extent of arsenic oriented metalloid existence inside the body. NAC delivery alone or additively with DMSA in arsenicated animals served to re-establish these B vitamins' level and consequently lessened the degree of MT-I in hepatic cell and homocysteine in circulation (Fig. 8.7 A, B, C & D), though DMSA caused insignificant but mild degree of lessening of homocysteine level in the circulation.

Figure 8.7

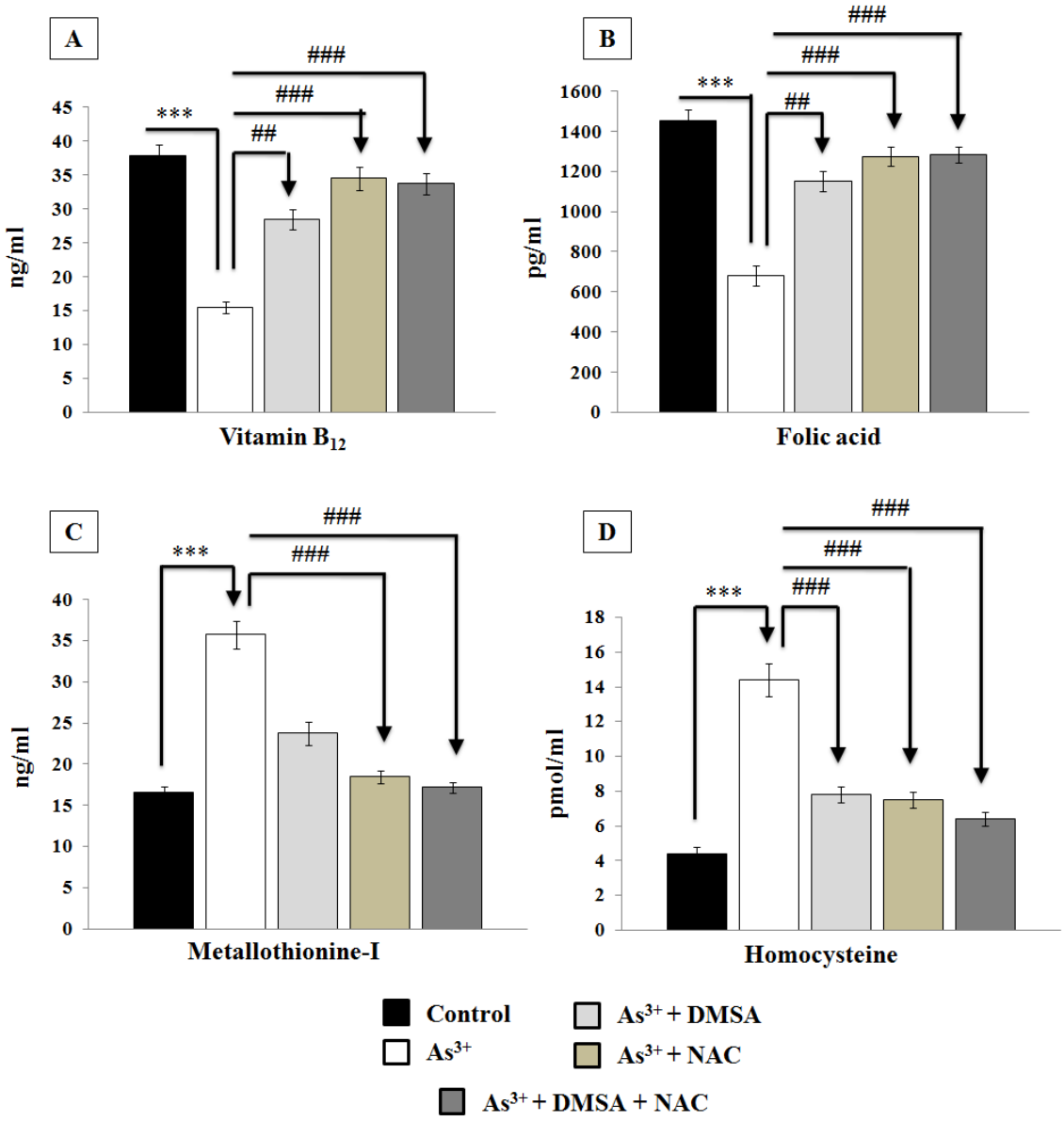

Figure 8.7: The remedial action of DMSA and NAC alone or in combined form in the up-gradation of vitamin B_{12} and folic acid and the suppression of MT-I and homocysteine level. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***p<0.001 was considered for significance of analysis when compared between control and As^{3+} group, ##p<0.01 and ###p<0.001 were considered for comparison between As^{3+} and rest of additional groups.

8.3.9. Effect of NAC on uterine and ovarian histology with reference to DMSA

Sodium arsenite treatment adversely affected the reproductive organs thus uterine and ovarian deformities were noted. Secretory glands in uterus were enormously decreased and breadth of uterine layers was too lessened after arsenication with respect to control (Fig. 8.8A; Table 8.2). In ovary, arsenite treatment proficiently enhanced the creation of follicular atresia whereas minimized the antral follicular generation (Fig. 8.8B; Table 8.2). DMSA and NAC post-administration significantly counteracted such deterioration in sex-organs and corrected the cellular architecture of ovary and uterus. More corrective outcome was noted in NAC supplemented group. The summative effect of DMSA-NAC was more reproducible than their alone effect (Fig. 8.8 A & B; Table 8.2).

Figure 8.8

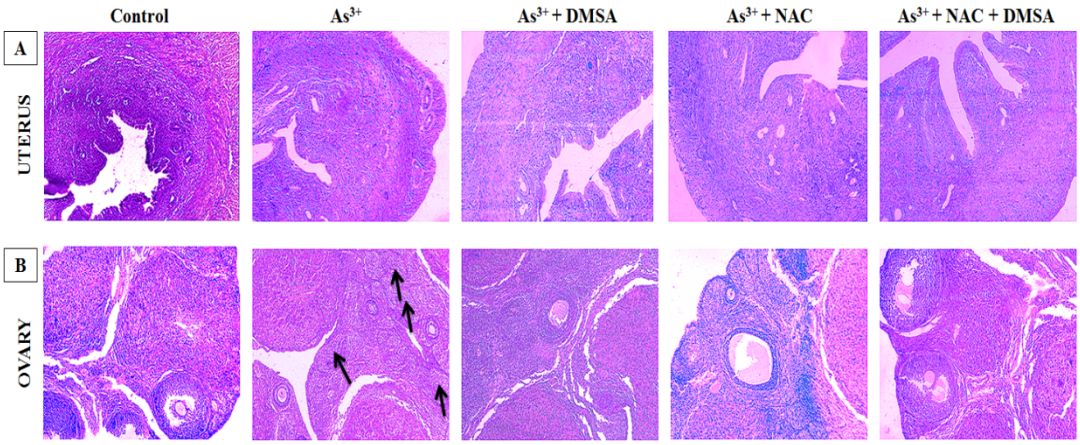

Figure 8.8: The curative action of DMSA and NAC separately or their additive form in the lessening of sodium arsenite propagated uterine-ovarian histo-architectural deformities. The arrow sign marked as atretic follicle produced by arsenite.