CHAPTER-9

EXPERIMENT-V

9.0. EXPERIMENT-V

Study of mitigation of sodium arsenite aggravated female reproductive ailments by dietary NAC.

9.1. Objective

Objective of this experiment was oriented to delineate whether NAC could eliminate the arsenic driven ovarian steroidogenic disorders; ovarian and uterine oxidative stress and uterine apoptotic and necrotic event when it was supplied through diet in a post supplementation mode.

9.2. Animal selection and study design

Wistar strain female rats weighted about 75-100 gm have been allocated for this experiment. All the animals were then settled in polycarbonate cages in the animal house of the institution and acclimatized for 7-10 days. Five different groups were allocated to equally distribute the Wistar rats to continue the treatment for 16 days (initial 8 days arsenic and later on 8 days dietary NAC supplementation). The study design is given below:

Group 1: Control group of rats were provided with vehicle treatment,

Group 2: Orally As^{3+} (sodium arsenite) was given in this group (10mg/kg body weight) for initial 8 days then no further treatment of As^{3+} was considered,

Group 3: This group was given distilled water as vehicle orally for initial 8 days only and then NAC (250 mg/kg body weight) for next 8 days through the diet,

Group 4: This group was given As^{3+} orally as the same dosage of group 2 for initial 8 days and for the next 8 days above dose of NAC was given through the diet,

Group 5: The treatment procedure was same as group 4 only DMSA was given at 250 mg/kg body weight instead of NAC.

NAC was given through the diet whereas sodium arsenite was treated with oral gavage. The composition and analysis of nutrients of the diet was presented in Table 9.1. Water plus food consumption and body masses of animals were supervised regularly from day 1 upto 16 days of treatment schedule. Following the termination of treatment protocol, rats were permitted for sacrifice on day 17. They were anaesthetized with taking ketamine HCl. Uterus, ovary, liver and kidney and blood samples were acquired and then fridged at -20˚C. The animals were then euthanized by overdose of barbiturate (≥86 mg/kg body weight).

Table 9.1

Nutrients	Ingredients %	Energy (Kcal)	Carboydrate (gm)	Total protein (gm)	TSFA (gm)	TMUFA (gm)	TPUFA (gm)	Vit. A (Thiamine) (mg)	Vit. C (mg)	Vit.B_6 (mg)	Coline (mg)	Crude fibre (gm)
Weat, flour, atta	56.42	341.00	69.40	10.57	0.50	0.30	1.27	0.49	0.00	--	--	1.9
Bengal gram, Sattu	31.56	369.00	39.56	18.77	0.30	1.2	3.7	0.20	0.00	--	--	1.00
Whole Milk powder	10.52	496.00	38.00	25.80	26.7	--	--	0.31	4.00	--	--	--
Soyabean oil	1.00	900.00	--	--	13.1 (%)	28.9 (%)	57.2 (%)	--	--	--	--	--
Sodium chloride	0.5	--	--	--	--	--	--	--	--	--	--	--

Amino acid content (mg per gm N)

Nutrients	Ingredients %	Approx total N/100gm	Arginine	Lysine	Tryptophan	Methionine	Phenylalanine
Wheat, flour, atta	**56.42**	**1.89**	**290**	**170**	**070**	**090**	**280**
Bengal gram, Sattu	**31.56**	**2.74**	**570**	**440**	**050**	**080**	**360**
Whole Milk powder	**10.52**	**4.13**	**220**	**490**	**090**	**170**	**310**
Soyabean oil	**1.00**	--	--	--	--	--	--
Sodium chloride	**0.5**	--	--	--	--	--	--

Table 9.1: Diet composition and nutrient analysis of the rats' diet (per 100 gm) according to Gopalan et al., 1989 (Nutrient Value of Indian Foods).

9.3. Results

9.3.1. Food habits, body growth & organ weights

A significant deviation of body mass was prominent following arsenication when judged against control group (Table 9.2). After the supply of dietary NAC a noteworthy gain of body mass in arsenic amplified group was spotted. The repro-organs' weight was also reduced remarkably in arsenic ingested animals in respect of control but when dietary NAC was provided a notable increase of utero-ovarian weight was seen (Table 9.2). Although no considerable modification of food and water intake was observed among the groups.

Table 9.2

	Bodyweight (gm)		Organo-somatic indices (gm%)		Average food intake(g/100g body weight/24h)	Average water intake(ml/100g body weight/24h)
	Initial	Final	Uterus	Ovary in pairs		
Control	84.16±5.13	99.66±2.46	0.132±0.013	0.0484±0.002	4.41±0.35	9.31±0.52
As^{3+}	78.50±3.6	78.66±4.66**	0.100±0.018	0.0349±0.004*	5.08±0.33	10.23±1.11
NAC	84.66±4.31	98.00±3.45#	0.135±0.027	0.0446±0.002	5.13±0.31	10.34±0.92
As^{3+} + NAC	79.00±1.76	95.16±4.96#	0.121±0.019	0.0518±0.003#	4.61±0.31	9.54±0.55
As^{3+} + DMSA	82.33±2.66	93.50±3.44#	0.114±0.010	0.0463±0.002	5.31±0.31	8.93±0.87

Table 9.2: The significance of dietary post-supplementation of NAC on body growth, utero-ovarian weight and water and food consumption. 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 groups.

9.3.2. Status of estrous cycle

The evenness of estrous phase was not sustained after 3-4 days of arsenic ingestion while estrous cycle normalcy was renowned in control group provided only vehicle (Fig. 9.1). The typical pattern of estrous cycle was deviated by the episode of met or diestrous stage consistently following arsenic intake which was re-established towards usual mode by the dietary post-treatment with NAC (Fig. 9.1).

Figure 9.1

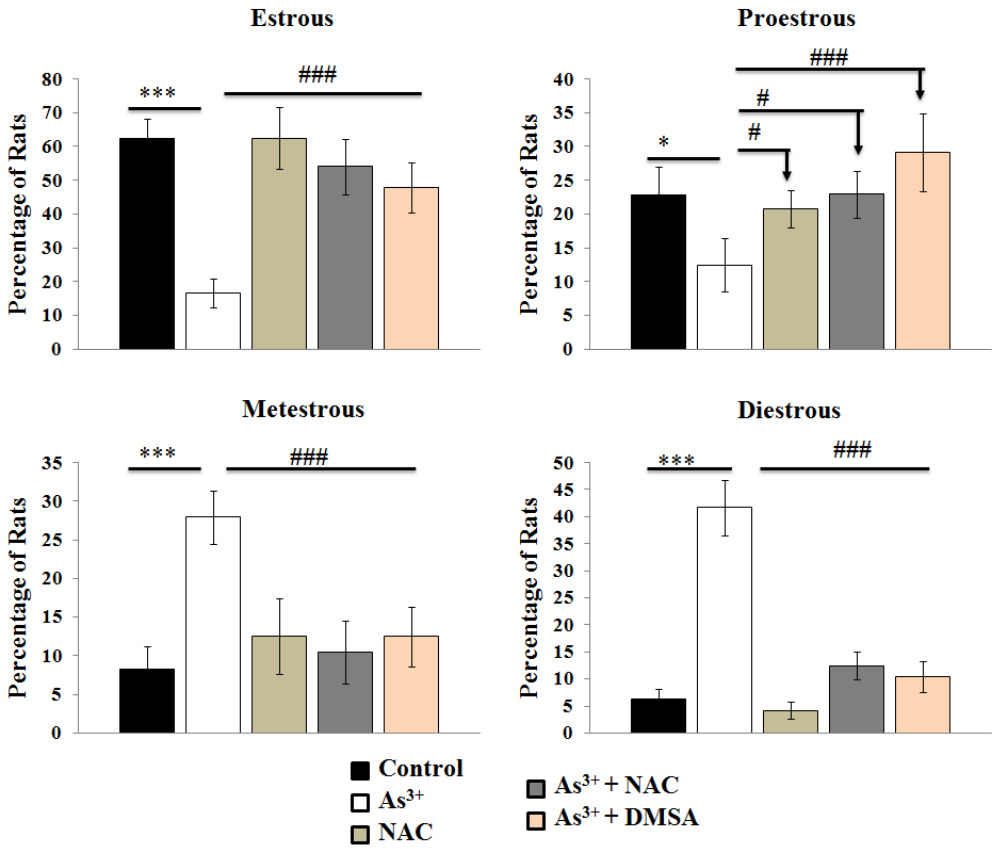

Figure 9.1: The curative effect of dietary NAC post-supplementation on the type of estrous phase sequence in arsenic fed animals. The results represent Mean ± SE

(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.

9.3.3. Effects of NAC on redox status in uterus and ovary

The efficacy of antioxidant enzymes i.e. SOD, catalase and GPx were hindered following arsenication in utero-ovarian tissues (Fig. 9.2 C & D) and it was documented to be reduced ~2.6, 2.9 and 2.5 folds in uterine tissue respectively and~ 4.4, 1.6 and 2.6 folds in ovarian tissue correspondingly. For further validation zymographic study enumerated that the band compactness of those enzymes were reduced with an appearance of lighter band impression in reproductive tissues following the intake of arsenic among rats (Fig. 9.2 C & D). Post-supplementation of NAC with diet improved the above enzymes expression towards the usual mode. Therefore, a greater amplification with improved band width was notable after the supplementation of NAC in arsenite consumed animals (Fig. 9.2 A, B, C & D).

Arsenic intensified redox discrepancy in female sex glands was determined by MDA-CD assessment (Fig. 9.2 E & F). An advanced level of lipid peroxidation was found because of arsenic with respect to control (Fig. 9.2 E & F). Arsenic also weakened the endogenous GSH status which was clearly evidenced following the diminution of NPSH level and detected~2.1 times and~1.8 times drop in its level in uterus and ovary (Fig. 9.2 G). Dietary NAC post-supplementation significantly revived the formation frequency of MDA-CD and correspondingly elevated NPSH in these sex organs.

Figure 9.2

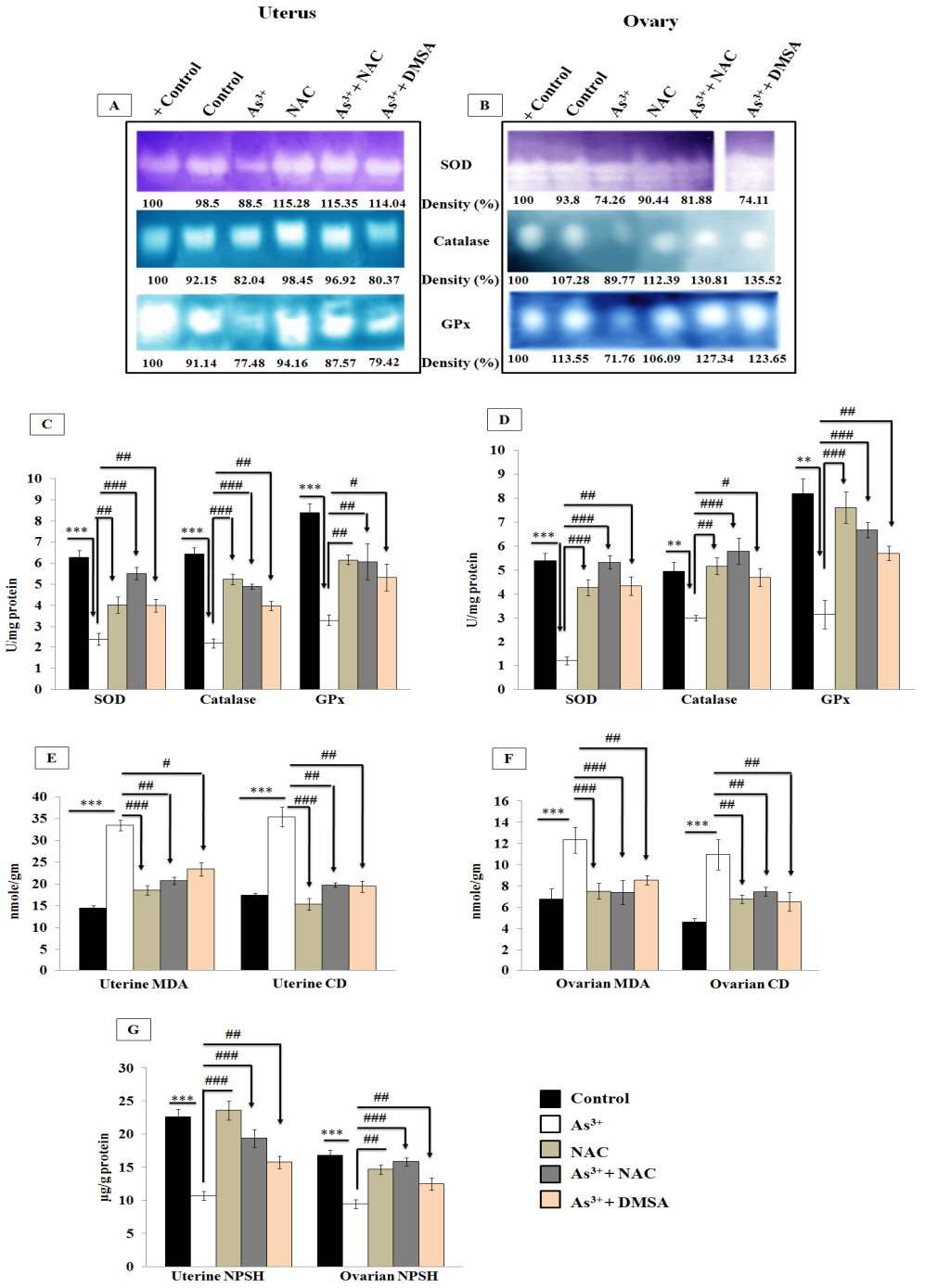

Figure 9.2: The curative mode of action of dietary NAC post-supplementation on redox status in female sex glands following arsenic consumption. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). **p<0.01 and ***p<0.001 were

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.

9.3.4. Effect of NAC on the status of serum LDH

Arsenic stimulated the functionality of LDH in serum and probably leading to tissue necrosis. A~ 2.1 times elevation was noticed in arsenic exposed animals when compared with control (Fig. 9.3 B). The appearance of higher amplified band was also seen in that group on electrzymogram (Fig. 9.3 A). Dietary NAC post-ingestion revealed a noteworthy counteraction against LDH functionality (Fig. 9.3 B) and concurrently down-regulated its expression by the manifestation of much weaker band (Fig. 9.3 A).

Figure 9.3

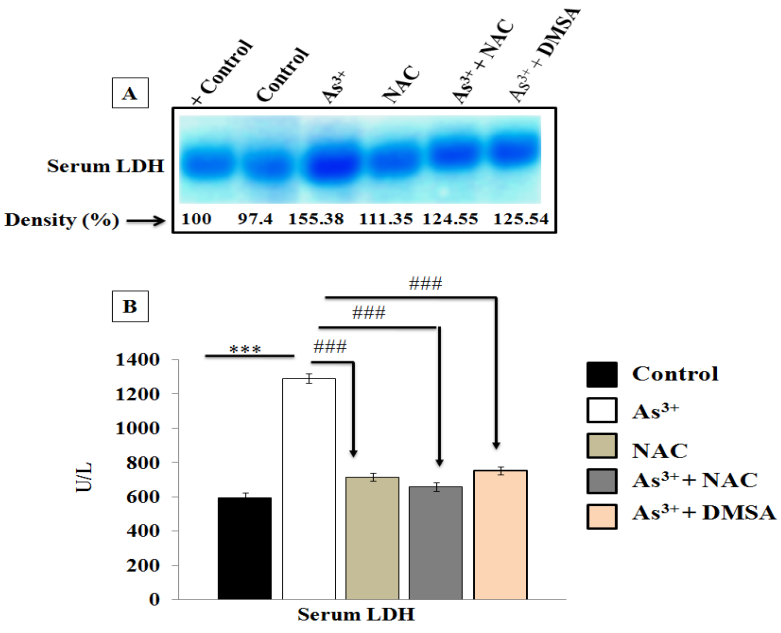

Figure 9.3: The effect of dietary NAC post-supplementation to oppose arsenic driven LDH activity. The results represent Mean ± SE (Standard Error), n=6. Data

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.

9.3.5. Effect of NAC on hepato-renal functionality

Arsenic consumption elevated the functional aspects of SGOT and SGPT in serum and thus enhanced the liver toxicity when compared with control animals (Fig. 9.4 A & B). Alongside the creatinine level in serum was raised that suggesting the kidney malfunction (Fig. 9.4 C). Dietary NAC post-administration significantly returned these toxic markers' level to their normal status and helped to re-establish the usual hepato-renal profile.

Figure 9.4

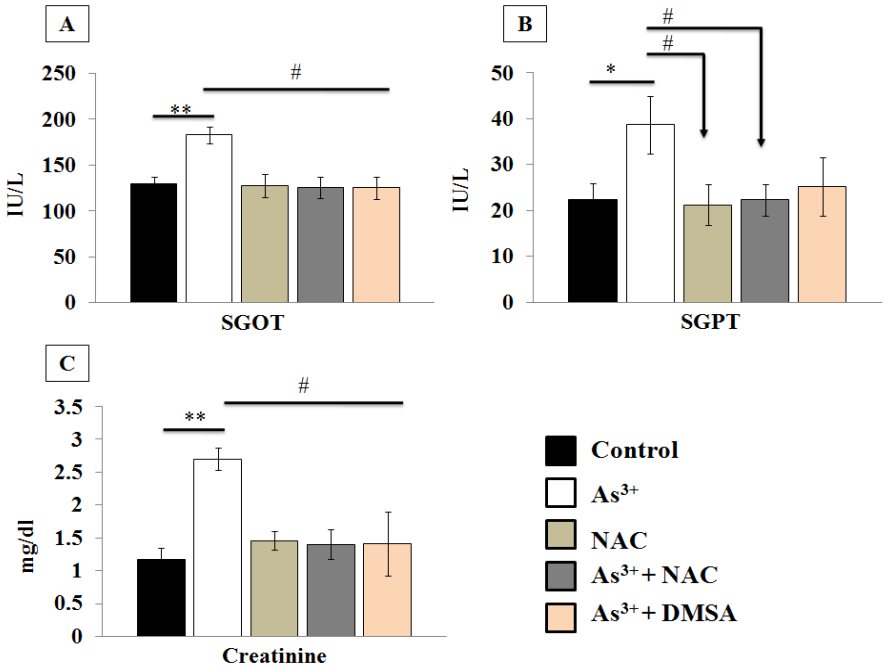

Figure 9.4: Showing the curative effect of dietary NAC post-supplementation against arsenicated hepato-renal profile. The results represent Mean ± SE (Standard

Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). *$p<0.05$ and **$p<0.01$ were considered for significance analysis when compared between control and As^{3+} group, whereas #$p<0.05$ was considered for comparison between As^{3+} and rest of additional groups.

9.3.6. Effect of NAC on DNA fragmentation via comet assay

Intake of arsenic directed DNA disintegration in uterus that caused cellular damage which has been emerged by distinct cell DNA assessment with enhanced tail width (Fig. 9.5; Table 9.3). Dietary NAC post-administration defended the DNA distortion significantly and declined the comet formation with decreased length of comet tail (Fig. 9.5; Table 9.3).

Figure 9.5

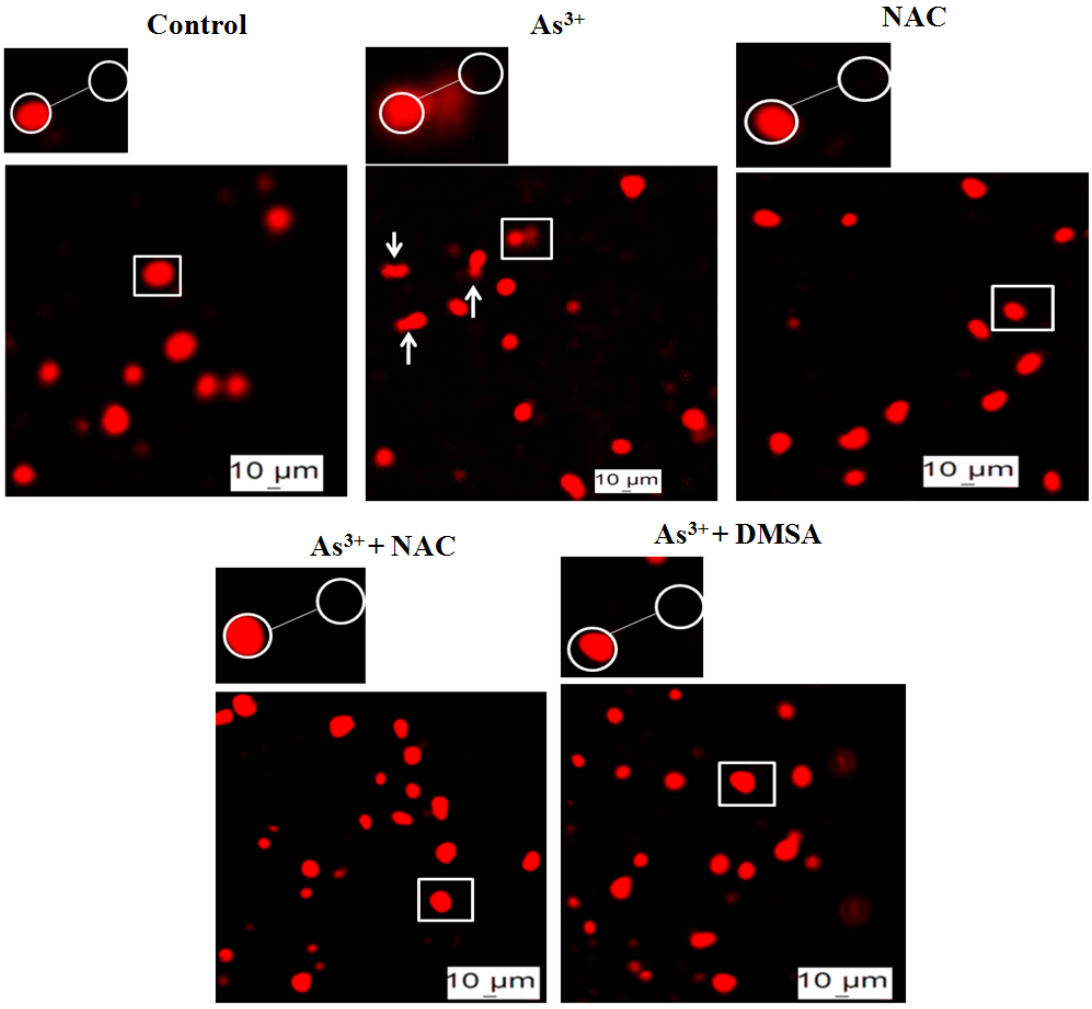

Figure 9.5: The curative effect of dietary NAC post-supplementation on comet assay in uterus. The arrow specifies the comet cell appearance.

Table 9.3

	Control	As^{3+}	NAC	As^{3+} + NAC	As^{3+} + DMSA
Comet in number	1.16±0.47	5.66±0.61***	1.33±0.33###	1.16±0.3###	3.33±0.42##
Comet tail length (µm)	25.33±1.15	41.12±1.01***	21.26±0.56###	23.16±1.00###	32.25±0.81###
SAF	6.33±0.49	2.5±0.42***	5.83±0.3###	3.66±0.33	3.33±0.49
MAF	3.16±0.3	1.16±0.3**	3.33±0.2##	3.16±0.3##	2.33±0.55#
LAF	2.00±0.25	1.00±0.36	1.5±0.42	1.33±0.33	1.33±0.49
GF	1.66±0.42	0.83±0.3	2.66±0.33##	1.33±0.33	0.93±0.3
AF	1.5±0.42	12.5±1.33***	1.5±0.42###	1.16±0.3###	7.00±0.57#
Endometrium (µm)	283.40±4.87	101.28±4.57***	224.14±10.87###	229.81±5.48###	199.29±9.07##
Myometrium (µm)	114.26±2.32	66.6±2.35***	109.33±3.69###	108.17±3.34###	100.15±0.94###

Table 9.3: The curative effect of dietary NAC post-supplementation on quantity of comet cell, width of comet tail, ovarian follicles and diameter of different layers of 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, ##p<0.01 and ###p<0.001 were considered for comparison between As^{3+} and rest of additional groups.

9.3.7. Effect of NAC on the level of vitamin B_{12} and folic acid

The circulatory status of vitamin B_{12} plus folic acid reflected noteworthy exhaustion following arsenic administration as contrary to control animals (Fig. 9.6A & B). Post-supply of NAC after arsenic ingestion via diet could significantly replete their normal status in the circulation and from this outcome we might speculate that arsenic might be removed by using NAC through the involvement of SAM pathway (Fig. 9.6A & B).

Figure 9.6

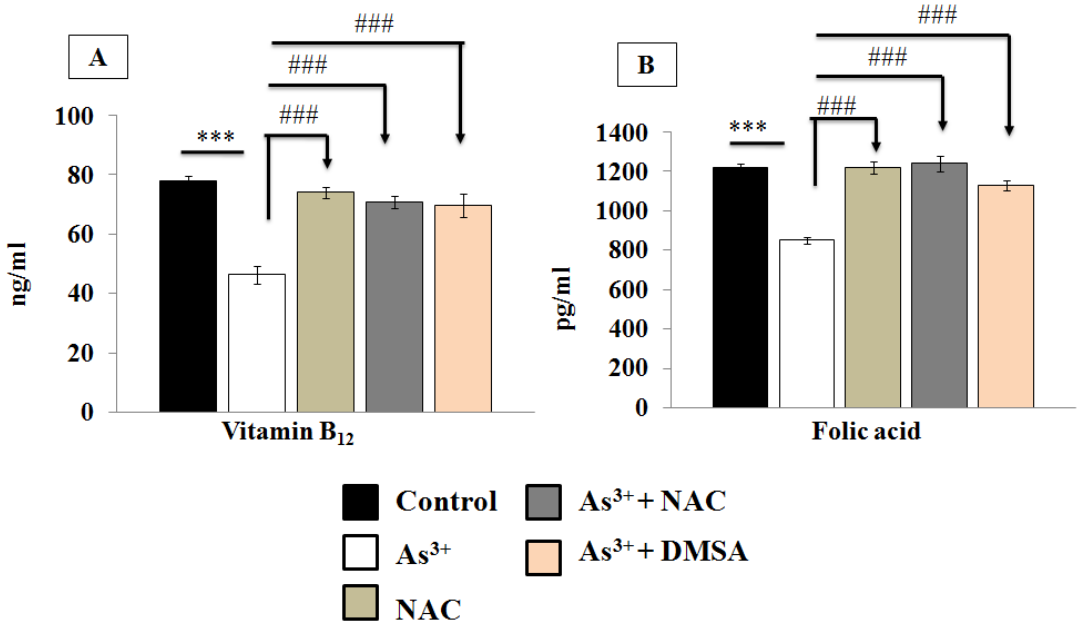

Figure 9.6: The curative effect of dietary NAC post-supplementation in maintaining the status of vitamin B_{12} plus folic acid in the circulation of arsenicated animals. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***$p<0.001$ 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.

9.3.8. Effect of NAC on utero-ovarian histology

Arsenic ingestion reflected reduction of secretory glands (Fig. 9.7) found in uterus with distortion of uterine layers i.e. endo and myometrium (Table 9.3). In ovary arsenic lessened the graafian follicle production together with decreasing the quantity of antral follicle (Fig. 9.7; Table 9.3). The increased amount of atretic follicles was visible due to arsenication (Fig. 9.7). Post-supplementation of dietary

NAC could restock the secretory glands and recovered the usual breadth of uterine layers. The decreased quantities of regressive follicles with increased number of growing follicle were reappeared after post application of NAC (Fig. 9.7; Table 9.3).

Figure 9.7

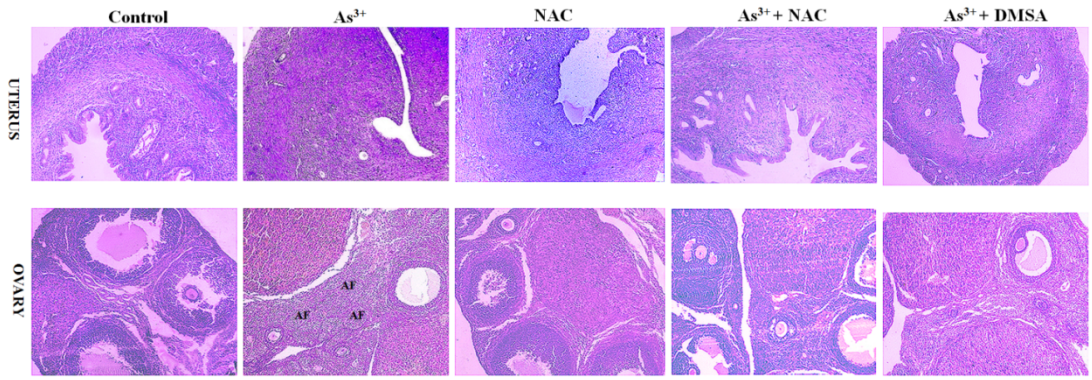

Figure 9.7: Curative effect of dietary NAC post-supplementation in the opposition of arsenic persuaded utero-ovarian histological appearance. AF is remarked as atretic follicle.

9.3.9. Effect of NAC on ER-α signaling

From ELISA assay it has been affirmed that the ER-α signaling in uterus was deteriorated significantly after arsenic intake in contrast to control (Fig. 9.8 A). It was later authenticated by immuno-staining of uterus and ovary tissue section (Fig. 9.8 B & C). Arsenic lessened the quantity and expression of ER-α receptors in these female sex glands (Fig. 9.8 B & C). Following the dietary post-supplementation of NAC noticeably retrieved the ER-α receptor's signaling in utero-ovarian tissue (Fig. 9.8 A, B & C).

Figure 9.8

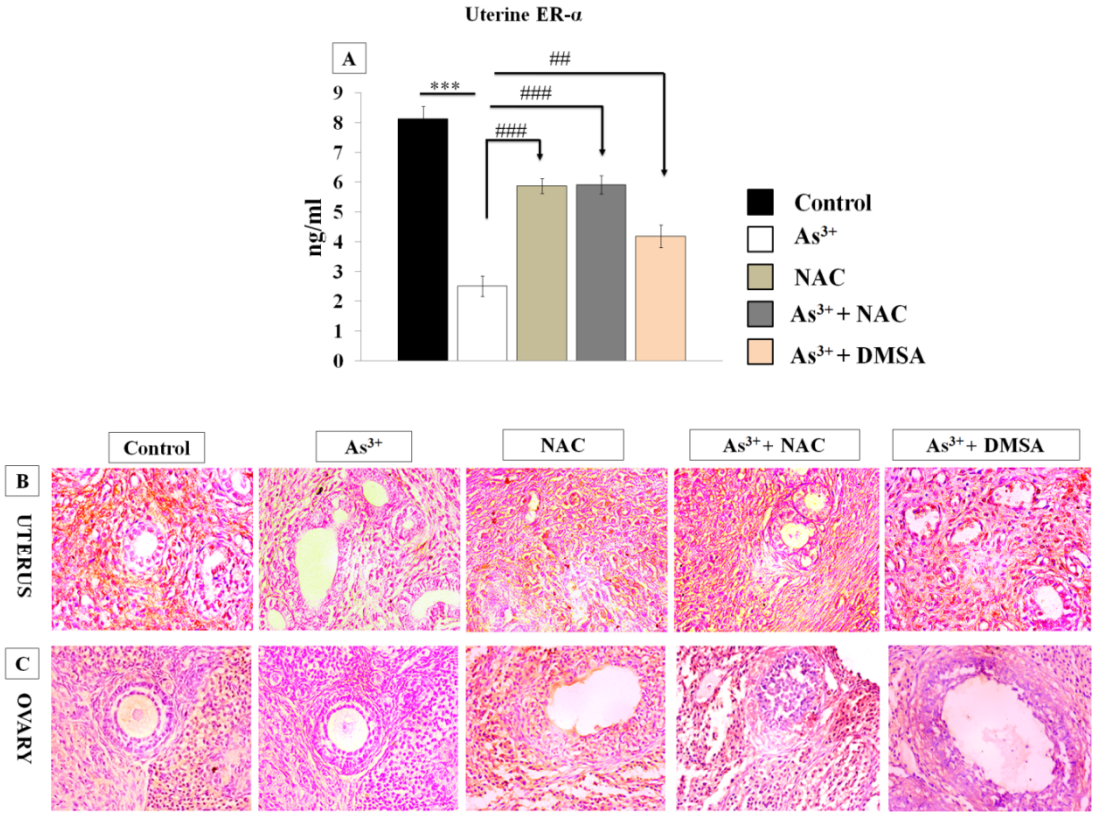

Figure 9.8: Effect of dietary NAC post-amplification in the resistance of arsenic provoked ER-α signaling in uterus (A) and its expression in utero-ovarian tissue (B & C). The results represent Mean ± SE (Standard Error), n=6. Data were evaluated using one-way-ANOVA following Dunnett's test (post-hoc). ***$p<0.001$ was considered for significance analysis when compared between control and As^{3+} group, whereas #$p<0.05$, ##$p<0.01$ and ###$p<0.001$ were considered for comparison between As^{3+} and rest of additional groups.

9.3.10. Effect of NAC on inflammatory markers and gene expression in uterus

A noteworthy expansion of serum inflammatory markers e.g. TNF-α and NF-κB were seen because of arsenic ingestion when contrasted with control (Fig. 9.9 A & B). Post-administration of NAC via diet prohibited their expression remarkably to

their usual condition (Fig. 9.9 A & B). The gene modulation of NF-κB was stimulated and concurrently the apoptotic gene Bax expression and p53 gene expression were up-regulated too (Fig. 9.9 C). But Bcl-2 gene modulation was diminished due to arsenication which is an anti-apoptotic gene (Fig. 9.9 C). The deviation of above said genes expression was normalized after post-supplementation of dietary NAC (Fig. 9.9 C).

Figure 9.9

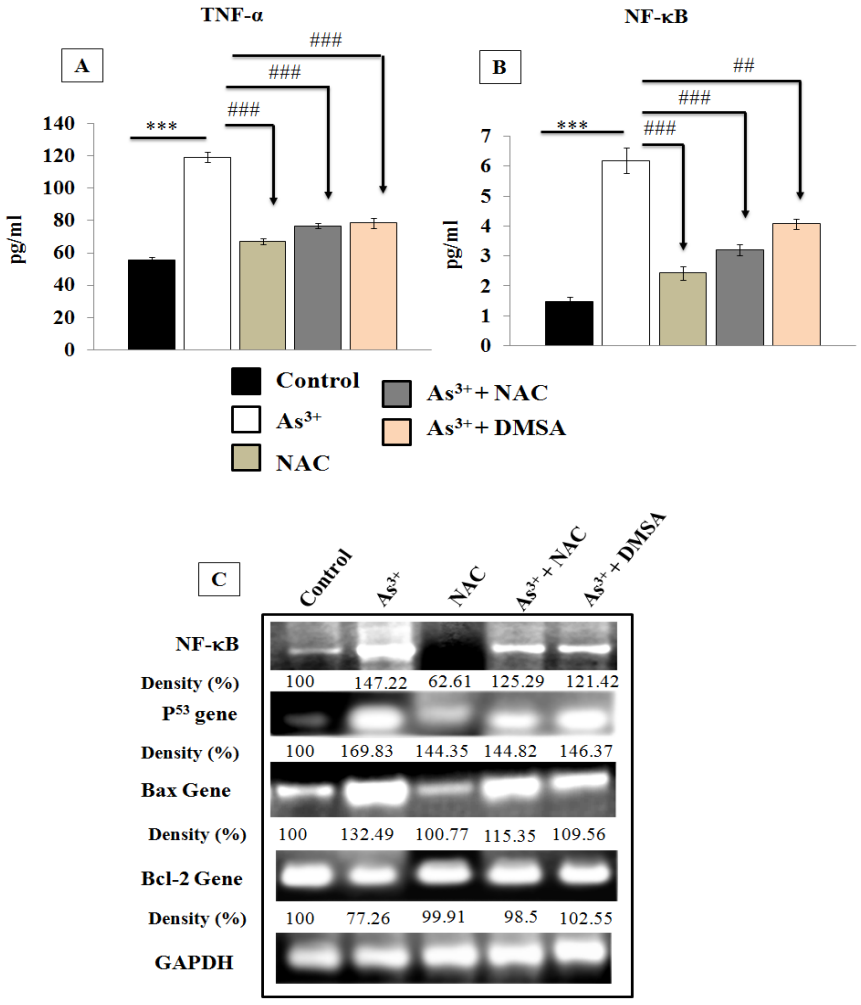

Figure 9.9: Effect of dietary NAC post-administration to alienate arsenic directed over expression of inflammatory sensors (A & B) and gene modulation (C) in uterus. The results represent Mean ± SE (Standard Error), n=6. Data were evaluated

using one-way-ANOVA following Dunnett's test (post-hoc). ***$p<0.001$ 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.