CHAPTER-10

DISCUSSION

10.0. DISCUSSION

Arsenic, an element approached toxicity as well as carcinogenicity through the multi-mechanistic way. Among these oxidative stress is the most often accepted and conventional mechanism attributed to arsenic intoxication. Though arsenic has a superior affinity towards the protein's thiol (-SH) group and hampers their functional utility. The oxidation states of various metals initiated and propagated the arsenic associated oxidative damage and that could be a foremost reason for ROS accumulation (Halliwell and Whiteman, 2004). ROS are the radical species originated inside the living cells owing to the molecular oxygen which constituted a distinctive electronic characteristic that aids in the superoxide anion ($O_2^{\cdot}-$) generation (Miller et al., 1990). This $O_2^{\cdot}-$ is well-known as the chief ROS which proceeds directly to stimulate the secondary ROS production via metal-enzyme catalyzed pathway (Valko et al., 2005) and these are peroxyl radicals, singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). A bunch of direct plus indirect mechanism has been well settled about arsenic handled ROS induction. The disintegration of electron-transport-chain (ETC) inside mitochondria is being guided for the $O_2^{\cdot}-$ stimulation by arsenic. In reality arsenic hampers the functionality of succinic dehydrogenase and perturbed the oxidative phosphorylation in mitochondria that subsequently mediates mitochondrial disorders by arising ROS (Corsini et al., 1999). However, molecular oxygen extends the hands towards dimethylarsine and produce dimethylarsinic radical following the attachment of an extra molecular oxygen to introduce dimethylarsinic peroxyl radical which has cellular deteriorative consequence (Yamanaka et al., 1990). Apart from these straightforward interventions about ROS synthesis by arsenic, the indirect evidence

has been soundly established too. The malfunction of cellular antioxidants including SOD, CAT and GPx in various organs and systems undoubtedly results from overproduction of ROS (Dash et al., 2018). Therefore, our five distinct experiments shown the idea of erasing arsenic aided deleterious and toxic outcomes by the amplification of NAC in different ways.

The hepatocyte is the prime entrance site for arsenic though this toxicant also distresses the delicate organs like uterus and ovary. The *in-vitro* experiment (Experiment-I) notified that As^{3+} along with H_2O_2 augmented the ROS formation that promoted lipid peroxidation suggested increased assembly of MDA-CD in hepatic and reproductive organs (Experiment-I; Table 5.1). Likewise, the other four experiments revealed the same trends of consequence wherein arsenic intensified various ROS synthesis coupled with lipid peroxidation which further accomplished with the advanced intensity of MDA along with CD in the ovarian-uterine organs (Experiment-II, Fig. 6.2A; Experiment-III, Fig. 7.2 A & B; Experiment-IV, Fig. 8.2 A, B, C & D; Experiment-V, Fig. 9.2 E & F). Elevated amplification of free radicals by arsenic related oxidative stress has the direct action on lipid peroxidation (Dey et al., 2018) and the same view was noted in these experiments where arsenic provoked the flooding of MDA and CD in metabolic and reproductive tissues. The amplified intensity of MDA and CD supported the perception that arsenic paired with H_2O_2 accelerates unnecessary production of ROS. Actually, the hydrogen molecule attached with the methylene group of an unsaturated fatty acid is more vulnerable for direct attack by arsenic and thereby created the molecules like MDA-CD (Flora, 2011). This resulted loss of functions of antioxidant enzymes i.e. SOD, catalase plus GPx which were stated as first stage defence system in contrary to

oxidative injury (Singh et al., 2013). From the outcome of Experiment-I, duration dependent action of arsenic plus H_2O_2 was explored. A remarkable suppression of antioxidant enzymes were explored via spectrophotometric study following arsenic and H_2O_2 (Experiment-I, Fig 5.2). More diminution was noticed when the liver and sex organs were settled for 6 hrs in the Kreb's solution containing these toxicants. From the zymographic analysis a diffused nature of SOD band was prominent in As^{3+} exposed tissues (Experiment-I, Fig. 5.3). However, more distorted band was found when the uterine and hepatic tissues were incubated together with As^{3+} and H_2O_2 for 6 hrs (Experiment-I, Fig. 5.3B and D). Similar views of outcome were observed regarding the deviation of cell defence enzymes by arsenic when explored through both spectrophotometric plus electrophoretic gel study in Experiment-II, III, IV and V (Fig. 6.3A & B; Fig. 7.3A, B, C & D, 8.3A, B, C & D and 9.2A, B, C & D respectively). Actually, this concomitant repression of SOD functionality was believed to be the inability to get rid of the excess superoxide anions these further contribute to the making of various radical products in collaboration with H_2O_2 (Knoefler et al., 2013). Arsenic supported SOD inactivation possibly attributed to the oxidative stress resulting in the enhancement of H_2O_2 formation indirectly. Another promising reason of arsenic aggravated inactivity of SOD may be interconnected with the altered amino acid residue cysteine on its surface (Acharyya et al., 2015). The reduced action of catalase promotes the build up of H_2O_2 inside the liver and sex organs. The mis-functionality of catalase by arsenic was accredited to the modulation at mRNA transcription level (Wang et al., 2012). Besides, the function of antioxidant enzyme GPx was noticeably diminished when executed spectrophotometric plus electrozymographic study in liver and sex organs in the entire experiments (Experiment-I, Fig. 5.2C, F & I; Fig. 5.3; Experiment-II, Fig.

6.3A & B; Experiment-III, Fig. 7.3A, B, C & D; Experiment-IV, Fig. 8.3A, B, C & D; Experiment-V, Fig. 9.2A, B, C & D). These evidences strictly suggested that arsenic aggravate oxidative stress which facilitates ROS formation and provides a resistant shield in the functionality of cellular antioxidant enzyme (Xu et al., 2017). However, GPx alone serves as a scavenger of lipid peroxides without any involvement of GSH via the supply of electron (Noctor et al., 2002).

NAC is acknowledged as the stabilizer of endogenous defence arrangement and protected from ROS by the formation of GSH. Alongside, the acetylated cysteine residue attached with nitrogen helps in the oxidation and biotransformation of different radicals (Samuni et al., 2013). Yi et al, proposed that NAC has the super power for direct interaction with H_2O_2 in company with other ROS especially hydroxyl radical, hence provided cellular protection against oxidative injury due to the existence of sulfhydryl group (Yi et al., 2014). Supply of NAC in distinguished way promoted the antioxidant aptitude by GSH re-synthesis and thereby re-stimulated the functionality of antioxidative enzymes (Experiment-I, Fig 5.2 & 5.3; Experiment-II, Fig. 6.3A & B; Experiment-III, Fig. 7.3A, B, C & D; Experiment-IV, 8.3A, B, C & D and Experiment-V, 9.2A, B, C & D accordingly in the entire groups) and diminished the course of MDA-CD too following arsenication (Experiment-I, Table 5.1; Experiment-II, Fig. 6.2A; Experiment-III, Fig. 7.2 A & B; Experiment-IV, Fig. 8.2A, B, C & D; Experiment-V, Fig. 9.2E & F). The exhausted GSH level following arsenic application led to deprive NPSH status in reproductive glands (Experiment-II, Fig. 6.2B; Experiment-III, Fig. 7.2C & D; Experiment-IV, Fig. 8.2E & F; Experiment-V, Fig. 9.2 G) whose level was further enhanced owing to the amplification of NAC. Therefore, diminution of NPSH level together with

GPx activity suggested the exhaustion of endogenous non-enzymatic GSH pool that ultimately united with assembly of H_2O_2 during apoptosis (Forman et al., 2009). Moreover, NAC is the originator of GSH that sustains the redox stability and thereby granted the protection in reply with arsenic originated ROS invention (Arouma et al., 1989) whereas GSH mitigated the arsenic mediated damage on female organs (Chattopadhyay and Ghosh, 2010). All these outcomes agreed with our earlier published investigations (Dash et al., 2018; Dash et al., 2020). However, NAC can be promptly deacetylated and thereby forms cysteine which performs as a substrate in the rate limiting steps of GSH biosynthesis (Corcoran et al., 1985) and improves the antioxidant capacity in the interior of cell.

The appearance of higher amplified band of LDH in arsenic applied group recommended the tissue necrosis during programmed cell death (Iglesias et al., 1988) and this disability was found from the total experiments (Experiment-II, Fig. 6.4A & B; Experiment-III, Fig. 7.3E & F; Experiment-IV, Fig.8.4A & B; Experiment-V, Fig. 9.3A & B correspondingly) except Experiment-I (as LDH assay was not performed in experiment-I). Indeed, elevated serum LDH also corroborated with the growth of collagen and fibroid modifications in the uterus (Dash et al., 2018). There is evidence that ROS liberated lipid peroxidation caused membrane damage that accelerated the drainage of LDH into blood (Drent et al., 1996). However, NAC confidently neutralizes the arsenic driven LDH liberation and prevented the cellular vulnerability of necrosis by various manners.

Arsenic intensified ROS propagation and increased the propensity of apoptosis, subsequently necrosis in the hepato-uterine tissues by the degree of DNA injury which parallelly causes reduction of DNA fixation (Li et al., 2002). Experiment-I

claimed that the hepatic DNA was damaged drastically following the incubation of hepatic tissues with As^{3+} and/or H_2O_2 and that was confirmed from the comet test (Experiment-I, Fig. 5.5 A & B). The breakage of DNA strand was validated upon the creation of comet on the single DNA cell. More extreme breakage of DNA was viewed in As^{3+}, H_2O_2 and the mixture of As^{3+}-H_2O_2 group with elevation of tail extent in both 3 hrs and 6 hrs incubation time (Experiment-I, Fig. 5.5 A & B; Table 5.2). The residual four experiments viewed the same approach of exhausted DNA strand on account of arsenic inclusion. The DNA strand extrusion was expressed through DNA ladder assay and mostly comet assay (Experiment-II, Fig. 6.5 A1 & B; Experiment-III, Fig. 7.4B; Experiment-IV, Fig. 8.5; Experiment-V, Fig. 9.5). The dramatic changes of redox status by arsenic and subsequent elevation of ROS along with down-streaming of endogenous antioxidant capacity promotes the DNA damage including the breakage of single strand that further proceeds to double strand splitting at the moment of replication and base excision repair of DNA (Celino et al., 2009; Kligerman et al., 2010). The build-up of H_2O_2 in interior of cell and consequently inactivity of SOD and catalase also directly correlated with this category of DNA lesion (Celino et al., 2009). Evidences from previous study exhibited that arsenic advances ROS production and hastens the appearance of guanine oxidation products like 8-hydroxy-2′-deoxyguanosine (8-OHdG), 8-hydroxy-guanine (8-oxo-G), 8-oxo-2′-deoxyguanosine (8-oxodG) in the urine (De Vizcaya-Ruiz et al., 2009) and causes genomic instability. Not only that, arsenic also retards the DNA ligation process (steps of DNA renovation) and amazingly enhances the dysfunction of mRNA, protein and different enzymes concerned in this course (Snow et al., 2003). Introduction of NAC in As^{3+}-fed group sharply retarded the DNA lesion with reduced length of comet tail (Experiment-I, Table 5.2;

Experiment-II, Table 6.2; Experiment-III, Table 7.2; Experiment-IV, Table 8.2; Experiment-V, Table 9.3) as viewed from our experiments. Actually, NAC non-enzymatically reacts with ROS and removes and detoxifies these free radicals from the cell owing to the conformation of thiol group. A study explored that the potent antioxidant NAC inhibited oxidative DNA lesion (Andreassi et al., 2012) by maintaining endogenous pro/antioxidant ratio. This observation signified that NAC could mitigate DNA lesion and the outcome of our whole experiments documented that NAC fruitfully maintained the antioxidant enzymes status and counteracted redox enhanced DNA injury. NAC stimulates the yield of GSH and prevented the re-occurrence of free radicals owing to arsenic and thereby slow down the oxidative DNA injury (Dash et al., 2018).

Earlier study by Chattopadhyay et al, established that lengthy treatment of arsenic interrupted the propensity of steroidogenic enzymes i.e. 17β-HSD and Δ^5, 3β-HSD and thereby disruption of ovarian steroidogenesis was apparent (Chattopadhyay et al., 1999). Arsenic ingestion reflected that degradation of these important enzymes was coupled with gonadotropins and estradiol suppression. Here the experiments stated that arsenic lower the magnitude of gonadotropins (LH plus FSH) which further suppressed the estradiol production (Experiment-II, Fig. 6.6C, D & E; Experiment-III, Fig. 7.5A, B & C; Experiment-IV, Fig. 8.6A, B & C) and steroidogenesis via HSD enzymes (Experiment-I, Fig. 5.4A & B; Experiment-II, Fig. 6.6A & B; Experiment-III, Fig. 7.5D & E; Experiment-IV, Fig. 8.6D & E). Actually, arsenic resists the expressional tendency of estradiol owing to its binding with estradiol receptor (Bae-Jump et al., 2008). Surprisingly, this statement matches our current outcome (Experiment-V) wherein a massive loss of ER-α was seen in

ovarian-uterine tissue by arsenic inclusion (Experiment-III, Fig. 7.5F; Experiment-IV, Fig. 8.6F; Experiment-V, Fig. 9.8A). It was additionally illustrated and affirmed by immunohistochemical study (Experiment-V, Fig. 9.8B & C) where the numbers of ER-α was significantly negligible in arsenicated group. This declining nature of ER-α postponed the standard secretion of most essential ovarian hormone estradiol.

The body mass amongst the animals remains unchanged whereas enormously affected the dry weight of sexual glands (uterus & ovary) (Experiment-II, Table 6.1; Experiment-III, Table 7.1; Experiment-IV, Table 8.1 & Experiment-V, Table 9.2) and that is indicative of tissue-specific repro-toxicity of arsenic. Continuous arsenic treatment probably leads to the poor status of estradiol which may be liable for loss of sexual organs weight. The poor status of estradiol and gonadotropin signaling (Kulin and Reiter, 1973) furthermore contributes for the emergence of diestrous phase persistently (Experiment-II, Fig. 6.1; Experiment-III, Fig. 7.1; Experiment-IV, Fig. 8.1 & Experiment-V, Fig. 9.1) following arsenic introduction (Chattopadhyay et al., 2003). This notion suggested the potential destruction of usual structure of utero-ovarian tissue (Experiment-II, Fig. 6.7A & B; Experiment-III, Fig. 7.7A & B; Experiment-IV, Fig. 8.8A & B; Experiment-V, Fig. 9.7). Hence, the endometrial glands were drastically affected with simultaneous decline of endo-myometrium diameter which was documented after arsenication in animals (Experiment-II, Table 6.2; Experiment-III, Table 7.2; Experiment-IV, Table 8.2 & Experiment-V, Table 9.3). The ovarian discrepancies were also revealed because of arsenic feeding as there was relapsing of various ovarian follicles with consequent increase of atretic follicles (Experiment-II, Table 6.2; Experiment-III, Table 7.2; Experiment-IV, Table 8.2 & Experiment-V, Table 9.3). Estradiol eventually regulates the reproductive

cycles by maintaining the standard architecture of these important sex organs because of the vital action of this hormone may be restrained upon uterus (Patil et al., 1998). Arsenic exhibited a lower status of estradiol along with histological discrepancies in ovarian-uterine tissues. Both estradiol and gonadotropins naturally maintain the folliculogenesis process in ovary. Arsenic interrupts the discharge of estradiol from ovary with a hindrance of steroidogenic enzymes action (Hinshelwood et al., 1994). Arsenic responded oxidative stress also triggers the destruction in ovarian-uterine tissue (Sun, 1990). NAC amplification significantly retrieved the utero-ovarian deformation by changing the arsenic boosted oxidative strain which solely triggered the follicular distortion. This imperative role of NAC suggested the improvement of oocyte excellence followed by decreasing the quantity of atretic follicles (Cheraghi et al., 2016). Oxidative stress influenced follicular apoptosis in ovary was significantly refurbished by the application of NAC. Site by site increasing number of healthy and quality follicles with reduced follicular atresia in NAC co-treated group suggested that ovary is the primary target organ for NAC functioning (Dash et al., 2020). The possible assumption is that being an antioxidant, NAC improves the intracellular status of reduced glutathione and regulates hypothalamo-hupophyseal gonadal axis in the way of protecting ovary (Dash et al., 2018). The betterment of ovarian-uterine weight by the assistance of NAC guaranteed the mitigation of tissue specific necrotic and apoptotic lesion by arsenic and arsenic amplified free radicals. Likewise, NAC progresses steroidogenesis in female gonads and also maintains the normal status of plasma estradiol and gonadotropins that concomitantly improve the competency of ovarian graft and maintain the regular pattern of estrous cycle (Amorim et al., 2014). Therefore, NAC probably re-activates the functioning of steroidogenic enzymes i.e.

17β-HSD and Δ^5, 3β-HSD that re-initiates the folliculogenesis process and also regain the utero-ovarian weight (Dash et al., 2020). However, NAC supported the positive gene expression of ER-α and also increased their status and in our experiment an improved status of this receptor was documented in arsenic fed animals (Experiment-III, Fig. 7.5F; Experiment-IV, Fig. 8.6F; Experiment-V, Fig. 9.8A, B & C). This subsequently accommodated with the positive feedback for estradiol synthesis (Dash et al., 2020). Therefore, arsenic showed negative influence on reproductive-organs' weight followed by morphological deformities. Disrupted steroidogenesis and sexual organs' weight were renovated by the intake of NAC through diet.

The investigation by Acharyya et al, indicated arsenic supposed to be responsible for renal-hepatic toxicity through enhancing the circulatory status of creatinine, SGOT and SGPT (Acharyya et al., 2015). In Experiment-V a higher activities of serum SGOT and SGPT were found (Experiment-V, Fig. 9.4 A & B) following arsenication. A subsequent initiation of hepatic injury is possible by increasing discharge of cytochrome-c (Bustamante et al., 2005). Similarly, the malfunction of kidney was noticeable from the enhancement of serum creatinine status (Experiment-V, Fig. 9.4 C). Application of dietary NAC markedly reduced arsenic primed hepatic and kidney intoxication by minimizing the functionality of SGOT and SGPT along with the low level of creatinine respectively (Experiment-V, Fig. 9.4 A, B & C). It was earlier documented that NAC minimized the hepatic toxicity by reducing the discharge of these toxic enzymes into the circulation (Kannan and Flora, 2006). Similar study by Hemalatha et al, reported that NAC administration could postpone the arsenic primed toxicity in liver (Hemalatha et al., 2013).

The excess yield of free radicals by arsenic acts as second messenger and stimulates the genes expression liable for the formation of various pro-inflammatory cytokines (Guzik et al., 2003; Schulze-Osthoff et al., 1995). Hence, arsenic initiates the inflammatory response. NF-κB remains in inactive state in the core of cytosol and attached with inhibitor κB (IκB) (Makarov, 2000). The protein named IκBα is usually engaged for NF-κB activation whereas IκBβ is responsile for sustaining an activated condition (Makarov, 2000). Under inflammatory response plus oxidative stress NF-κB is being activated and migrated towards the nucleus and can be identified by the promoter part of the gene which further stimulates the commencement of various chemokines, cytokines like interleukin-8 (IL-8), interleukin-6 (IL-6), TNF-α etc (Libermann and Baltimore, 1990; Kunsch et al., 1994; Perveen et al., 2019). In this investigation the expression of three essential pro-inflammatory markers i.e. TNF-α, NF-κB and IL-6 were enhanced following arsenication (Experiment-III, Fig. 7.6C, D & E; Experiment-V, Fig. 9.9 A, B & C). This incidence documented by the group of previous author (Dash et al., 2020). Arsenic perhaps activates the phosphorylation process of IκB that further regulates the movement of IKK primed degradation of IκB (Verma et al., 1995). This causes ultimate stimulation of NF-κB expression since IκB level in cytoplasm is depleted (Liu et al., 2014) following its progressed phosphorylation because of arsenic. NAC supplementation in our study (Experiment-III & V) protected arsenic stimulated over activity and expression of these pro-inflammatory markers to a considerable level (Experiment-III, Fig. 7.6C, D & E; Experiment-V, Fig. 9.9 A, B & C). However, NAC possibly suppresses the action of enzymes responsible for the activation of IκB (Farid et al., 2005). Therefore, NF-κB responded signaling pathway is noticeably adjusted and thereby prevents the release of TNF-α and IL-6

into the circulation (Pathak et al., 2015). Moreover, the redox balance along with cytokines flooding were reported to be modulated by the predisposition of NAC that in succession regulates the cell signaling process, thus NF-κB propagated cellular inflammation could be abolished (Sadowska et al., 2007). Alternatively, earlier study explored that NAC curtails the cytokine's attraction to their specific receptor and resulting in the attenuation of TNF-α triggered cell signaling progression (Hayakawa et al., 2003). Besides, the diminution of these cytokine's mRNA expression by NAC also supports the inactivation of NF-κB. Hence, NAC amplification could cut down yielding ROS and consequently diminish oxidative stress which is accomplished with the reduced signaling of these inflammatory cytokines.

Arsenic propagated oxidative stress was accompanied with over expression of Bax and p53 gene, while down regulation of the Bcl-2 gene (Experiment-V, Fig. 9.9 C). This downstream regulation of Bcl-2 gene owing to oxidative trauma may promote the cellular apoptosis in mitochondria. Actually Bcl-2 helps in the upholding of mitochondrial status for regulating cellular death (Kitazawa et al., 2010). The downward plus upward expression was viewed in our investigation for Bax, p53 and Bcl-2 gene respectively following the supervision of dietary NAC (Experiment-V, Fig. 9.9 C). Actually, arsenic primed programmed cell death is dominated by NAC that withstands the mitochondrial death propagation to manage apoptosis (Sun et al., 2018). NAC perhaps encourages the cells survival with the engagement of protein called Akt that postpones the apoptosis via phosphorylation (Woo et al., 2003). Also, NAC inhibits the effectivity of CDK (cyclin dependent kinase) and cyclin D

and thereby detained the spontaneous cell cycle at the stage of G1 and subsequently arrests the DNA synthesis (Liu et al., 1999).

Metallothionein (MT) belongs to the proteins of metal binding family; bears minimum molecular weight and is loaded with cysteine residues (Klaassen et al., 2009). MT consisted huge numbers of thiol groups and the loaded cysteine residues which have direct affinity to the oxidants and thereby resumed as antioxidant and quenched ROS perceived oxidative pressure (Bell and Vallee, 2009; Qu and Waalkes, 2015). The multipurpose function of MT has been invented earlier where MT involves in maintaining homeostasis of various metals and also accelerates the detoxification gateway of inorganics (Klaassen et al., 2009). Arsenic itself manipulates the expression of MT (Qu et al., 2009; Qu et al., 2013). This outcome supports our present results wherein arsenic propagated the MT-I expression in hepatocyte (Experiment-IV Fig. 8.7C). Previous documentation by other authors also recommended that arsenic fastens the hepatic MT-I appearance (Perveen et al., 2019; Dey et al., 2018). The feasible hypothesis behind such activation is that arsenic stimulates MTF-1 (Metal activated Transcription Factor-1) which directly binds with MT-I resulting in mRNA expression of this protein (He and Ma, 2009). As previously mentioned that arsenic has a great attraction towards thiol group thus arsenic is supposed to bind with MT-I molecules enriched with –SH group and subsequently enhances the expression of MT-I. Additional study by Peng et al, successfully ascertained that the promoter part of MT-I gene opens for the binding of NF-κB, hence activated NF-κB further evokes MT-I expression (Peng et al., 2007). Arsenic permitted ROS over synthesis and sustained NF-κB activation might proceed for MT-I expression in our experimental models (Experiment-IV Fig.

8.7C). Treatment with NAC conferred a noteworthy down regulation of MT-I expression in arsenic fed animals (Experiment-IV Fig. 8.7C). The ROS quenching aptitude of NAC may prohibit the stimulatory appearance of MT-I. Alongside, oxidative stress granted NF-κB activation was also showed downward regulation following NAC application which might be accredited to descending motif of MT-I expression in our arsenic chellenged group. But a contradiction was there in preceding study by Brandao et al, wherein NAC was regarded as stimulator of MT (Brandao et al, 2006). Another observation by Kadota et al, declared NAC deducted MT level in cultured adipocyte cell (Kadota et al., 2017). Moreover, NAC may influences for yielding arsenic-MT complex and thus suppresses metal delivered toxicity plus MT expression.

Homocysteine (Hcy) is a sulfhydryl group bearing amino acid and its intensity was abruptly elevated after the entry of arsenic inside the system (Experiment-IV, Fig: 8.7D) whereas a noteworthy reduction of vitamin B_{12} plus B_9 was documented (Experiment-III, Fig. 7.6A & B; Experiment-IV, Fig. 8.7A & B; Experiment-V, Fig. 9.6A & B) after the inclusion of arsenic via water which recommends the obstruction in arsenic elimination from the billiary system (Kile and Ronnenberg 2008; Hall et al., 2009). This finding is matched with the results of previous study by Perveen et al (Perveen et al., 2019). The peak intensity of Hcy and downward motif of vitamin B_{12} plus B_9 have the detrimental result on Hcy metabolism (Jacques et al., 2001). Actually, these B vitamins regulate the Hcy concentration in circulation wherein any elevation of these two vitamins minimizes the circulatory level of Hcy. However, there is a close relationship between methionine and folate cycle for Hcy metabolism (Fratoni and Brandi, 2015). At first vitamin B_6 contributes for the

makeover of Hcy into cystathionine followed by cysteine and then vitamin B_{12} serves for re-methylation of Hcy to methionine which is driven by methionine synthase and 5-methyltetrahydrofolate (Swart et al., 2013). Hence, adequacy of B vitamins is crucial for the lessening of higher Hcy concentration. Hyperhomocysteinemia surely contributes with ROS production and encourages the synthesis of various pro-inflammatory cytokines (Poddar et al., 2001), thus in our experiment these vitamins increased the expression of TNF-α, NF-κB and IL-6 in arsenic fed group (Experiment-III, Fig. 7.6C, D & E; Experiment-V, Fig. 9.9A, B & C). Alongside, enhanced serum Hcy led to disrupt oocyte maturation with an interruption of developing follicles, possibly because of lower estradiol level (Perveen et al., 2019). NAC treatment curtailed the elevated serum Hcy level in arsenicated animals (Experiment-IV, Fig: 8.7D) this outcome is same with previous study of Hildebrandt et al (Hildebrandt et al., 2015). In reality a redox pair is there between cysteine and cysteine residue of Hcy and also an uninterrupted exchange is going on between plasmatic rich part (disulfide and cysteine) and protein-albumin bound part inside Hcy. NAC enhances the replacement of albumin and disulfide binding of Hcy and thus promotes the clearance of plasma rich fraction through the urine (Hultberg et al., 1994; Urquhart et al., 2006). Moreover, these two vitamins are the crucial component required for arsenic methylation procedure by the participation of a well-known methyl contributor called S-adenosyl methionine (SAM) along with reduced glutathione and this total process is carried out by methyltransferase enzyme (Nakamura, 2011). Henceforth, the required circulatory status of B_{12} and B_9 should be sustained for arsenic biomethylation as it is the merely fundamental way for arsenic excretion (Tice et al., 1997). Here, we noticed that NAC post-supplementation by diet significantly repleted the circulatory status of B_{12}

and B_9 in arsenic incorporated animals (Experiment-V, Fig. 9.6A & B) and thus alleviated sodium arsenite directed reproductive malfunctions and tissue necrosis (Perveen et al., 2019). The enzyme named arsenic methyltransferase is enriched with cysteine, so continuous availability of cysteine is imperative for arsenic bio-methylation (Hayakawa et al., 2005). Interestingly, the pre-acetylated structure of cysteine is NAC (Samuni et al., 2013), hence NAC accordingly furnishes the cysteine residue status for the persistent function of methyltransferase enzyme. Therefore, the notion should be established that NAC sustains the enzymatic process of arsenic bio-transformation by enhancing the concentration of B_{12} and B_9 (predisposition factor of SAM pool) in circulation (Dash et al., 2020). Besides, there is another pathway that entails non protein GSH for arsenic clearance. Hence, the cell's susceptibility is increased towards harmful metalloid like arsenic whenever there is decreased status of GSH (Shimizu et al., 1998). It has been ascertained by this present evaluation that NAC may improve the GSH level and that is connected with the reduced degree of arsenic primed oxidative stress followed by abolition of arsenic from the system.

Another metal chelating component has been assigned here in assistance with NAC named meso-2, 3- dimercaptosuccinic acid (DMSA) in the nullification of arsenic primed malfunction (Experiment-IV). DMSA comes from mercapto family and has strong affinity towards water and consisting of dithiol moiety which has binding capacity to metals. Various clinical trials explored that use of this metal binding component is very safe rather than other conservative metal ligands from the sight of renal excretion of metal (Miller, 1998). It has been established for its fruitful participation in restraining the poisonous effect of arsenic in both human and non-

humans (Gubrelay et al., 1998). Although DMSA alone itself is a metal binder because of double sulfhydryl group, since earlier study noticed that DMSA with additional metal binder NAC implicated more obvious effect against arsenic (Flora, 1999). The combined association of these pairs exert negative action to oppose arsenic mediated hepatic and brain oxidative stress and also influence arsenic elimination. Indeed, NAC in cooperation with DMSA could be sufficient enough in suppressing lead intoxication and hence, might be treated as preventive plus therapeutic mediator (Pande et al., 2001). DMSA regulated GSH production in arsenic fed rats has already been proposed by prior study of Flora (Flora, 1999). Thus, experiment-IV revealed the antagonistic action of DMSA and NAC alone plus combination in mitigating malicious outcome of arsenic. Here DMSA alone documented beneficial role against arsenite though in some context like comet numbers and corresponding tail length (Experimet-IV, Table 8.2), hormone (LH-FSH, estradiol) and 17β-HSD enzyme level and ER-α status (Experimet-IV, Fig. 8.6A, B, C, D & F) and in MT-I level (Experiment-IV, Fig. 8.7C) DMSA alone was unable to execute significant effect against arsenic. This drawback is perhaps associated with the intestinal imbalance and extracellular distribution of DMSA, because this bio-molecule is not able to penetrate the cell membrane. Hence, it is not capable to remove heavy metals from intracellular space by chelation (Flora and Pachauri, 2010; Yadav et al., 2014). The absorption rate is very low about 20% when given orally and major amount of this drug remain bound with plasma protein albumin. Therefore, only a limited amount remains in free form. Within 24 hours of oral application of DMSA about 10-25% is excreted as DMSA-cysteine disulfide conjugates through urine and remaining amount is mostly removed via faeces (Aposhian, 1983; Asiedu et al., 1995; Bradberry and Vale, 2009). Though NAC is a

hydrophilic thiol antioxidant but it has membrane-permeable potency (Arakawa et al., 2006; Arakawa and Ito, 2007) and it can act within the cells (Zafarullah et al., 2003). This easy penetration of NAC may be attributed to the deacetylation of NAC at the exterior of cells to form cysteine which further can be easily permeable via cell membrane (Samuni et al., 2013). But rather DMSA together with NAC has been reflected more obvious results against cellular oxidative condition exerted by arsenic and this have been acknowledged before (Flora, 1999). NAC together with DMSA conjointly suppressed oxidative stress and ROS production; significantly diminuted DNA lesion; improved steroidogenesis and B vitamins status in our arsenic fed animals. Hence, the existing findings matched the previous established study wherein NAC in adjacent with DMSA assisted better corrective outcome against arsenic (Flora, 1999).

From the entire experiments it has been estimated that 100 mg NAC per kg body weight has been assessed to be better in its function instead of 50 mg NAC per kg body weight in defending arsenic primed abnormalities. In utmost outcomes NAC at this dose was more reproducible against arsenication. However, our experiment involved in the rats with NAC at the dose of 250 mg per kg body weight was assessed by dietary application or curative mode against arsenication. Among all these conditions the most excellent result was accomplished when the two essential chelating components were administered together in arsenic consumed models. Even if NAC has outstanding aptitude to work against arsenic through various manners but the worthy functional consequences might be established by preventive plus curative mode especially while supplied through diet. This would be beneficial in

understanding the therapeutic directory of NAC to mitigate arsenic aided female repro-deformities.

In harmony with above proposed consequence it might be ascertained that NAC has a utility in the prohibition of arsenic allied different malicious action on reproduction, hepatic dis-integrity, inflammatory condition, apoptosis and interruption of vitamin B_{12} plus B_9 status. The protective, preventive and curative roles of NAC have been well revealed following the testing of different parameters which could be regulated and assisted by arsenic. The separate oxidative stress markers were up-regulated when feeding of arsenic was significantly and remarkably perturbed following the inclusion of NAC in diet. The antioxidant protection in cell, pro-inflammatory marker's status, arsenic guided apoptosis progression, all were notably re-structured and modulated with NAC application. The antioxidant properties of NAC overcame all of the malicious and deleterious circumstances which were aided by arsenic plus oxidative stress. NAC controlled inflammatory condition in tissue and also down-regulated apoptosis propagation by arsenic. Alongside NAC amplified the levels of B vitamins followed by restricting the Hcy surge which further guided for renal elimination of arsenic via attributing SAM pathway. Moreover, antioxidant streaming of NAC re-structured the healthy state of ovarian-uterine morphology by the re-stimulation of estradiol production. Therefore, the conclusion may be furnished that NAC is the fruitful bio-molecule for erasing arsenic driven malfunctions in reproductive system. The probable mechanistic approach of NAC in antagonism of arsenic is speculated below:

Figure 10.1

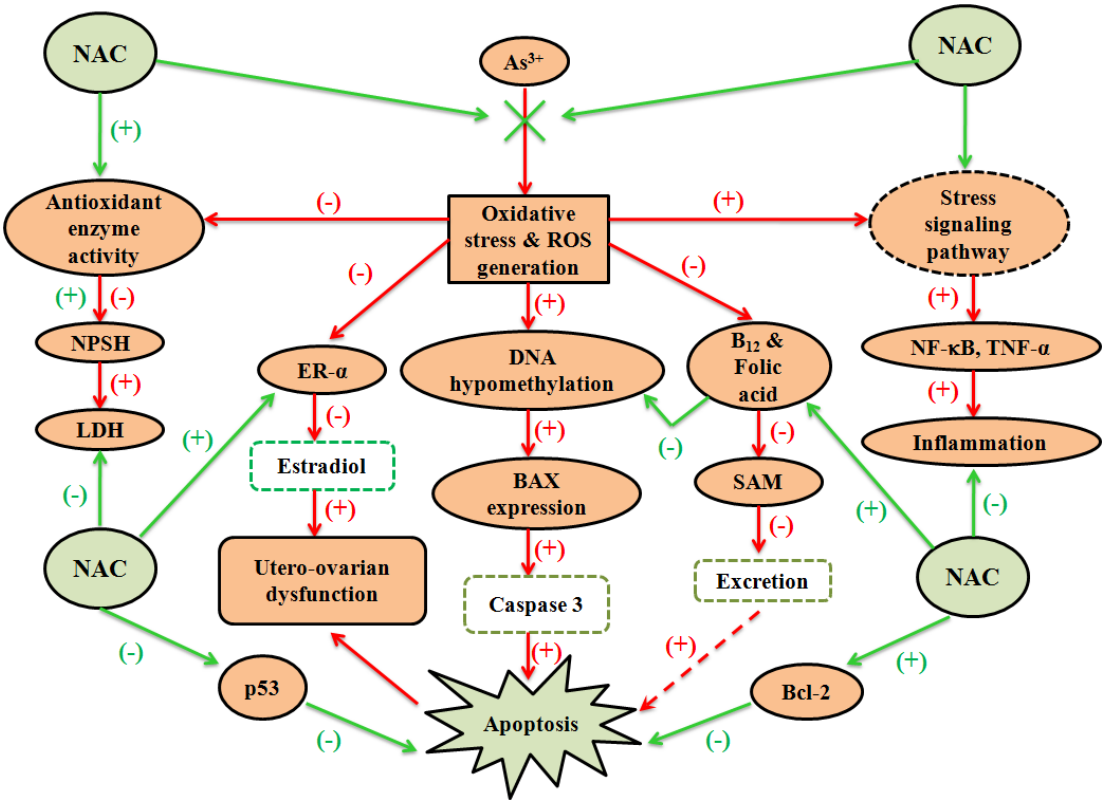

Figure 10.1: Probable speculation of NAC functions in opposition of arsenic driven organ toxicity. The red coloured sign of (+) and (-) referred to arsenic action while green coloured (+) and (-) sign indicated the utility of NAC un-favoured of arsenic.