CHAPTER-12

SUMMARY

12.0. Summary

Arsenic contamination from different ways has become major trouble. Arsenic abruptly affects the agricultural production and creates a threat to human beings globally. Generally chronic arsenication manifested with several organ damage including cancer, infertility among male and female population, still birth, interrupted steroidogenesis, hyperpigmentation on skin, etc. Arsenic aided oxidative stress barely associated with interruption of redox balance and uncontrolled production of free radicals which further correlated with malfunctioning of internal antioxidants. This unbalanced condition led to cellular degradation, lipid peroxidation, cellular apoptosis, necrosis and over-synthesis of numerous pro-inflammatory markers. Hence, combating arsenic intoxication and its hazards needs quick therapeutic management. Application of traditional management policy has various degrees of dreadful side effects. In addition, an alarming condition has been found among the arsenic affected habitants about the long term painful and invasive therapy. Therefore, a novel non-invasive painless oral management with nutraceutical competency is required to combat against arsenic allied delinquencies among people. In this research work NAC was applied in various modes to mitigate arsenic intoxication and associated hepatic and reproductive dilemmas. In total experiments Wistar strain virgin female rats were utilized and the outcome of NAC was examined in several manners i.e. direct effect (*in vitro* study), protective (co-administration), preventive (pre-treatment) and remedial or curative (post-treatment) mode. Arsenic propagated metabolic toxicity was assayed by the assessment of SGOT, SGPT and creatinine. Oxidative stress markers were also assayed by the estimation of MDA, CD, NPSH and different antioxidant enzymes (SOD, catalase

and GPx). Cellular DNA fragmentation and single cell lysis were performed by comet assay. Arsenic aided tissue necrosis was assessed by LDH estimation. The status of gonadotropins (LH-FSH), estradiol, histo-architecture of ovary-uterus and steroidogenic enzymes (Δ^5, 3β-HSD, 17β-HSD) level were assayed. Arsenic mediated apoptotic and inflammatory response were measured by assessing Bcl-2, Bax, p53 and IL-6, TNF-α and NF-κB. Indeed, the status of MT-1, Hcy and vitamin B_{12} and folic acid were also analysed in the study.

Outcome of experiment-I disclosed the usefulness of NAC to resist sodium arsenite (0.6 ppm) and H_2O_2 (100 mM) assisted hepatic and ovarian-uterine hazards in duration dependent style. The antioxidant NAC was able to mitigate sodium arsenite and H_2O_2 driven hepatic-ovarian-uterine discrepancies when these tissues were incubated for 3 hrs and 6 hrs in Kreb's solution. Arsenic along with H_2O_2 responded oxidative stress was well managed by the inclusion of NAC. This antioxidant component finely mitigated MDA-CD status whether delicately induced the enzymatic function of SOD, catalase plus GPx in hepato-ovarian-uterine tissues. Besides, the interrupted ovarian steroidogenesis was also controlled by NAC. NAC may promote GSH synthesis for the functioning of antioxidant enzyme and minimized free radical formation. Additionally, the direct attachment of arsenic with sulfhydryl and hydroxyl group of NAC was believed to be imperative for protection against arsenication. Whether, 6 hrs incubation of these organs with NAC revealed to be effective in combating these toxicants with respect to the tissue incubated for 3 hrs.

Experiment-II offered the dose dependent response of NAC in combating sodium arsenite mediated uterine tissue distortion. Two doses of NAC were orally given by

gavage (50 mg and 100 mg per kg body weight) whereas 10 mg per kg body weight of sodium arsenite was given in the study models. Sodium arsenite was documented for over-synthesis of ROS in uterus thereby lowered the functioning of uterine antioxidant enzymes. The interrupted gonadotropins action, DNA breakage, ovarian-uterine aberrations by arsenication was brought back to normalcy with NAC. Here 100 mg per kg body weight of NAC was more effective to resist arsenite driven malfunctions than that of 50 mg NAC per kg body weight.

Experiment-III demonstrated the preventive motif of NAC in combating arsenic mediated reproductive ailments. NAC was given at 100 mg dose per kg body weight against 10 mg per kg body weight of sodium arsenite. Elevated ROS productions, lipid peroxidation, enzymatic malfunction, impaired steroidogenesis due to arsenic were profoundly managed by pretreatment of NAC. Surge of inflammatory markers like NF-κB, TNF-α plus IL-6 with subsequent down streaming of vitamin B_{12} plus folic acid all together increased the hazardous consequence of arsenic. NAC oral-application remarkably and significantly counteracted upon arsenic primed impairments and re-instigated endogenous enzymatic usefulness. Ovarian-uterine morphology was re-structured via improved estradiol synthesis and better ER-α signaling by NAC.

Results from experiment-IV projected the curative act of NAC; DMSA was separately or jointly given against sodium arsenite. NAC alone as well as conjointly with DMSA was extremely supportive to mitigate redox hazards along with their subsequent action through enabling antioxidant potentialities. The thiol content has been upgraded and circulatory LDH status was lessened at superior level by NAC. The gonadotropins and estradiol surge and ER-α upgradation due to NAC revealed

the theory that NAC promotes steroidogenesis that eventually supports the above said hormonal status. The MT-I expression was down-regulated by NAC with consecutive up-streaming of B vitamins (B_{12} and folic acid). This further may disseminate the arsenic exclusion from the system with minimization of Hcy surge by the proposition of NAC. The results from experiment-IV pointed that in many instances DMSA alone itself was incapable to execute significant outcome against arsenic wherein NAC and DMSA jointly exerted pronounced effect to antagonize arsenic driven malfunctions in model animals.

Experiment-V illustrated curative utility of NAC via dietary supply to abolish arsenic augmented hepato-renal and ovarian-uterine disorders. NAC was applied through dietary intervention at the dose of 250 mg per kg body weight to delineate the positive impact against arsenication. Arsenic responded oxidative stress was eliminated successively by dietary post-supplementation with NAC. Alteration of redox imbalance in arsenicated rats was balanced when NAC was supplied. The liver and kidney tissue aberrations have been renovated well with NAC. The steroidogenesis was recovered by repleting gonadotropins and estradiol by NAC. NAC also promoted ER-α signaling in ovarian-uterine tissues. The apoptotic and inflammatory response were significantly corrected after supplying NAC in diet by down-streaming of p53 and Bax gene regulation along with NF-κB plus TNF-α modulation. Besides, the specific chelating site of NAC may accelerate the progress of arsenic chelation and elimination of arsenic by involving SAM pool. However, NAC may spare the predisposition factor like cysteine for enzyme activation for arsenic methylation and upgrade the propensity of this process with consecutive renewal of B_{12} plus folic acid and controlled release of Hcy in circulation.

Therefore, the results and outcomes of the experiments executed that NAC is able to counteract the sodium arsenite assisted reproductive hazards. Arsenic guided oxidative stress was noticeably minimized by the application of NAC. Diminution of antioxidant enzyme functioning was refurbished by NAC. Not only that, NAC improved the steroidogenesis by influencing the function of estradiol and gonadotropins and thereby resulting in repairing of cellular structure of ovary and uterus. NAC also upgraded the equilibrium of B vitamins in the circulation and degraded the status of Hcy and MT-1. The excess production of pro-inflammatory markers and enhanced apoptotic progression by arsenic were controlled by NAC. This counteractive efficacy of NAC may due to its antioxidant property. NAC encourages glutathione synthesis inside the cell which is renowned to maintain cellular redox equilibrium. Thus, the functions of indigenous antioxidant enzymes become enhanced against cellular oxidative stress and synthesis of different radical compounds were postponed. Moreover, NAC contains sulfhydryl (-SH) groups which serve the site for metal chelation. Henceforth, it is supposed that NAC may bind arsenic its chelating site and may eliminate arsenic from the system. Alongside, NAC is the acetylated structure of cysteine amino acid and thereby it is required for arsenic bio-methylation. Actually, arsenic methyltransferase is a cysteine enrich enzyme and plays a crucial role in arsenic bioconversion. Therefore, NAC improved the functioning of this enzyme and enhanced arsenic clearance out of the system. However, our study highlights the harmless oral therapeutic treatment of NAC with antioxidant potency against arsenic allied health hazards.