A search towards alternative ways: replacing conventional chelation therapy against arsenic-induced female repro-toxicity: A review

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ABSTRACT

Arsenic is widely distributed in the earth’s crust and literally leaches in groundwater at high concentrations. Arsenicosis results from the long-term intake of contaminated water and poses the greatest public health threat from arsenic. Depending on the degree of exposure, its drastic effects might take years to manifest in developing skin lesions and peripheral neuropathy. Major radical changes occur with the onset of developmental toxicity and reproductive health hazards in both sexes. Arsenic causes cellular damage by promoting oxidative stress and reactive oxygen species (ROS) production, resulting in chromosomal aberration and DNA damage. Managing the health risks brought on by arsenic has now become a global challenge. Available therapeutic strategy against arsenicism is mainly concerned with using chelating agents that very often redistribute arsenic in the brain. This chelating treatment approach also needs a painful and invasive route that discourages affected individuals from continuing the therapy. Hence, we focused on alternative non-invasive strategies in managing female reproductive ailments in arsenicated animals. We used different micronutrients, herbal extracts, phytochemicals, etc. The studies established that arsenic-nutrients/arsenic-phytomolecules interaction efficaciously reduced toxicity levels by correcting the components of the s-adenosine-methionine pool (SAM) on the way of endorsing probable elimination of arsenic from the system. Natural non-enzymatic antioxidants like vitamin C, vitamin E, and selenium were investigated against arsenic toxicity and proved to be beneficial for scavenging free radicals. These nutrients also enhance female gonadal function by regulating ovarian steroidogenesis and gonadotropin levels by controlling the brain’s biogenic amines. Phyto compounds such as curcumin, N-acetyl cysteine (NAC), arjunolic acid, extracts of green tea leaves (Camellia sinensis), pectic polysaccharide (CCPS) from bitter gourd (Momordica charantia) and functional foods such as probiotics and spirulina have been studied. These have been shown to mitigate arsenic toxicity via regulating various inflammatory markers such as NF-κB, MT-1, TNF-α, IL-6, etc. The potential benefits of these were also shown against arsenic toxicity by upregulating Bcl-2 gene expression accompanied with suppression of pro-apoptotic genes BAX, p53 caspase-3, PARP, PCNA, AKT, etc., and eventually corrected female reproductive stress with improved fertility in arsenicated rats. Hence, these findings may pave the way for the development of a new drug as well as nutraceuticals with more effective and non-invasive therapeutic approaches for arsenic toxicity.

Keywords: Arsenic, reprotoxicity, SAM, antioxidant, oxidative stress, chelation, methylation, gonadotropin, genotoxicity.

INTRODUCTION

Arsenic is a naturally occurring element, present ubiquitously in the environment in both organic and inorganic forms. Arsenic contamination is a global concern and a significant risk factor in many nations, including India. Arsenic is absorbed into the body through ingestion or inhalation. The ingested arsenic is absorbed by the digestive tract, whereas the inhaled arsenic enters into the bloodstream through lungs, followed by redistribution to other organs. Drinking contaminated water, industrial operations, and smoking tobacco, are usually the greater source of arsenic exposure. As per the World Health Organization (WHO) guidelines on safe drinking water, the acceptable level of arsenic concentration in water is 10 µg/L, with a maximum allowable limit of 50 µg/L. At least 70 nations, home to an estimated 140 million people have drinking water that contains more arsenic than the WHO’s provisional recommended range of 10 µg/L. In various areas of the Bengal Delta Plain, notably Bangladesh and West Bengal, the chronic poisoning caused by high levels of arsenic in the water resulted in a public health emergency.

Alarmingly arsenic level in the drinking water of this area were found to be 800 µg/L. There are two oxidation states of arsenic: trivalent and pentavalent. Pentavalent arsenic is converted to its trivalent form inside the cells. As (III) leaves the cells into intracellular compartments in free form or as thiol conjugates. Trivalent arsenic is more hazardous than pentavalent arsenic, as shown by a plethora of studies. Arsenic interferes with
cellular energy production by attaching to sulphhydryl (-SH) groups. It interferes with normal metabolic activities by occupying the position of metal ions like zinc and selenium in the biomolecules' binding sites. It has been established that exposure to arsenic increases reactive oxygen species (ROS) nitric oxide, superoxide, and hydroxyl radicals, which damage DNA and proteins in addition to altering cellular architecture, permeability and cell viability. Well-demonstrated underlying mechanisms of arsenic toxicity explored metabolic inhibition, oxidative stress, genotoxicity, epigenetic changes, and micro-RNA-dependent regulation. Symptoms of arsenic toxicity in humans are primarily influenced by the duration and degree of exposure. Chronic exposure of arsenic is found to cause dermatological, cardiovascular, respiratory, hepatic, hematological, neurological, diabetic, and reproductive consequences in humans while extended exposure to arsenic damages various organs, acute exposure to arsenic causes nausea, vomiting, abdominal discomfort, and severe diarrhea.

The International Agency for Research categorises arsenic compounds on Cancer (IARC) as group-1 carcinogen to humans. Chronic arsenic exposure found to cause malignancy of several organs including skin, bladder, liver, and kidney. Furthermore, arsenic exposure has been linked to infertility and miscarriage in humans. There is evidence from numerous epidemiological research on the menstrual cycle that heavy metal exposure affects reproductive function by altering the hormonal level.

The most common method for treating arsenicosis is through chelation therapy. Dimercaprol, also known as 2,3-dimercapto-1-propanol or British anti-Lewisite, is the first chelating agent to be introduced for the elimination of arsenic (BAL) though it has several drawbacks. Arsenic redistribution to the brain, nephrotoxicity, hypertension, tachycardia, and a low therapeutic efficacy have all been observed with the chelating therapy. Moreover, the release of arsenic from BAL-arsenic complexes during oxidation is potentially harmful to the system. Another popularly used arsenic chelator is Meso-2,3-dimercaptosuccinic acid (DMSA), a common arsenic chelator with low toxicity that doesn’t cause arsenic to be redistributed to other organs like BAL. However, its effectiveness is limited by its inability to pass the cell membrane, which prevents it from chelating arsenic from intracellular compartments. Meso-DMSA therapy has also been linked to other adverse effects, including gastrointestinal disorders, increased liver enzymes, placid neutropenia and skin allergies. Hence, their long-term intramuscular administration with a number of mild to severe adverse effects makes the treatment less acceptable and less effective.

Therefore, research towards a safe therapeutic approach for the effective treatment of arsenic toxicity is vital and of constant interest due to the wide range of toxicity and serious health implications.

Recent studies demonstrate that arsenic-mediated toxicity can be successfully treated naturally without any negative side effects using phyto molecules and other natural compounds. Arsenic causes oxidative stress, which can be mitigated by exogenous antioxidant supplementation since arsenic influences the intracellular antioxidant machinery. Several natural dietary ingredients have been identified to possess antioxidant qualities that are helpful in controlling arsenic toxicity. The benefits of several nutrients, especially vitamins C, and E, selenium, bioactive phytochemicals which control the activity of glutathione and antioxidant enzymes, including catalase, superoxide dismutase, and glutathione peroxidase, have been demonstrated in their roles as protective mechanisms against oxidative stress caused by arsenic. Moreover, several nutrients and phytochemicals help in chelation through metabolism and further elimination of arsenic. This review mainly summarizes the potential of natural substances in reducing arsenic-induced female repro-toxicity based on our prior studies.

**Effect Of Arsenic On Female Reproductive Physiology**

Several investigations revealed that arsenication has an impact on the Hypothalamus- pituitary-ovarian axis. The two key enzymes in ovarian steroidogenesis are Δ5, 3β-HSD and 17β-HSD. Arsenic treatment is associated with the suppression of testicular and ovarian steroidogenesis. Numerous studies in accordance with our previous studies on animal have revealed that the reduction in gonadotropin level is associated with arsenication which leads to suppressed steroidogenesis followed by altered tissue morphology and a cessation of spermatogenesis as well as folliculogenesis.

Series of our studies noted that Arsenic toxicity resulted in altered utero-ovarian histo-architecture, follicular atresia associated with decrease in ovarian weight as well as distorted uterine layer accompanied by reduced number of secretory gland. We previously documented the low plasma gonadotrophin levels in arsenic toxicity that may be a result of hypertrophy of adrenal gland followed by increased secretion of glucocorticoids, which may eventually reduce the sensitivity of the gonadotroph cells in releasing gonadotrophin hormone (GnRH).

On the other hand, exposure to arsenic also found to affect the levels of biogenic amines, the critical regulators of gonadotropin synthesis and secretion. We found a decreased level of norepinephrine (NE) and high levels of serotonin in the midbrain and diencephalon resulted from arsenic toxicity that may have contributed to the decrease in plasma gonadotrophins.

Moreover, superoxide radical production in female gonads is linked to arsenic intoxication, which could be one of the factors inhibiting the steroidogenesis. Reduced uterine peroxidase activity, which is a marker for decreased plasma levels of estradiol in arsenicated rats, is also consistent with these findings. Prolonged diestrous in rats treated with arsenic was evident in our previous studies that may be caused by low plasma levels of estradiol. A significant decrease in ovarian weight, gonadotropins regulate that along with uterine weight loss was evident by our studies.
in accordance with other studies.\textsuperscript{34}-53,59 Decreased levels of gonadotrophins and estrogen in the plasma may cause the relative weight reduction of these female reproductive organs in arsenic-treated rats. Additionally, our previous investigations suggest that AS (III) toxicity is associated with a significant elevation of gonadal malondialdehyde (MDA) and conjugated dienes (CD) which are linked with lipid peroxidation.\textsuperscript{31,34-44} On the other hand, a significant reduction in the most crucial cellular antioxidant defence system non-protein thiols (NPSH).\textsuperscript{36,37,42-44} as well antioxidant enzymes catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) has been reported for gonadal tissue.\textsuperscript{31,34,36-45} These events indicate an increased localized oxidative stress by arsenic and an imbalance between free radicals and antioxidants. Oxidative DNA damage from trivalent arsenicals and the inhibition of DNA repair systems may both result from oxidative stress. Arsenic (III) may affect the hypomethylation of uterine DNA at the expenditure of S-adenosyl methionine (SAM), a well-known methyl donor in the metabolism of arsenic. Arsenicals and its methylated derivative caused severe DNA damage and necrotic and apoptotic tissue death, and this may be further impacted by changes in antioxidant scarcity in the cell.\textsuperscript{50,61} Also, we noted that significantly elevated LDH (Lactate dehydrogenase) level in arsenic-exposed rats may significantly contribute to fibrosis in the organ by promoting collagen deposition.\textsuperscript{34}

**Effect of Natural Compound on Female Repro Toxicity Induced by Arsenic**

In recent findings, nutritional therapy seems to be an effective and affordable approach. Several vitamins, minerals, proteins, and antioxidants are found to help detoxify arsenic toxicity. Natural compounds, including nutrients provide defense against the toxic effects of arsenic in following possible ways: (i) by regulating the hypothalamic-pituitary-ovarian axis (ii) by methylating and chelating As (iii) by accelerating arsenic removal from the system (iv) via antioxidant action, which provides protection against radical species. During the process of methylation S-adenosylmethionine (SAM) serves as a methyl group donor to facilitate detoxification. Nutrients are beneficial for smooth operation of SAM pool.

**Nutrients**

Vitamin C or ascorbate, Vitamin E or tocopherol and Selenium (Se) are examples of non-enzymatic antioxidants. In our previous study, we found the therapeutic role of these nutrients against arsenic-induced female reprotoxicity in rodents. Selenium, Vitamin C and E were helpful in restoring the gonadal weight, steroidalogenic activities, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels along with estradiol (Figure 1, 2). This incident allied with the maintenance of normal estrous cyclicity.\textsuperscript{53,57,58} Vitamin C and selenium supplements decreased the levels of arsenic in the tissues and blood; however, vitamin E had no effect on arsenic removal.\textsuperscript{53,57,58} The restoration of arsenic-induced collagen deposition.

![Figure 1](image1.png) **Figure 1**: The supplementary effect of vitamin E on the plasma levels of LH, FSH, and estradiol in albino rats treated with sodium arsenite. The data was depicted as the mean ± SE (n=16). Bars sharing the same superscript within each group exhibited no noteworthy distinction among them (p<0.001)\textsuperscript{53}

![Figure 2](image2.png) **Figure 2**: Alterations in the functions of Δ5-3β-HSD and 17β-HSD enzymes in rats treated with 10 ml of water containing 0.4 ppm sodium arsenite/ day, along with ascorbic acid (vitamin C), over a span of 28 days. A control group received the same 10 ml of water per day for the same duration. The results are presented as the mean ± SE (n=8). Bars with matching superscripts within each group exhibited no noteworthy variation between them (p < 0.001)\textsuperscript{57}

This finding can be illustrated by the significant antioxidant function of vitamin E, which controls membrane fluidity and lipid peroxidation to manage the oxidative stress caused by arsenic.\textsuperscript{62} While vitamin C and selenium, along with their antioxidant capabilities (Figure 3), might have direct regulatory effects on the hypothalamic-pituitary-ovarian axis by enhancing the activity of dopaminergic neurons and/or suppressing that of serotonergic neurons. They may also exert further direct stimulatory effects on ovarian steroidalogenesis, which supports the normalization of ovarian folliculogenesis and other normal functions of the ovary.\textsuperscript{53,57,58}

Vitamin B\textsubscript{9} (Folate) and B\textsubscript{12} (Cobalamin) are necessary for maintaining one-carbon metabolism and are particularly important for arsenic methylation. Our research indicated that vitamin B\textsubscript{12} and folic acid are essential for the detoxification of arsenic. The elevated levels of uterine peroxide, MDA, and CD were also controlled (Figure 4) along with decreased serum LDH activity, revealing a preventive effect against uterine fibrosis and necrosis development. These vitamins also rejuvenated; and dramatically recovered uterine secretory cells and endometrium in arsenicated rodents.\textsuperscript{34} The mechanisms could be linked to the improvement of
antioxidant defence systems partly through the removal of arsenic with the participation of the S-adenosine methionine pool (SAM) as vitamin B12 and B9 are two important regulators of this pool.

Dietary Supplements

Glutathione (GSH) is an essential non-enzymatic antioxidant. Few studies recognised GSH as a protective factor against oxidative damage to both male and female gametes in numerous investigations. GSH plays a significant role in arsenic removal from cells via stimulating biliary excretion of arsenate (As V) through the production of arsenic-GSH conjugates. In arsenic-induced reprotoxicity, our study demonstrated the ameliorative effect of GSH. The supplementation of GSH in arsenicated female rats showed a regulatory influence on biogenic amines accompanied by recovery of gonadotropin and estradiol levels (Figure 5), maintenance of catalase and peroxidase activity together with decreased MDA level in uterine tissue. These were connected with better restoration of utero-ovarian histomorphology; however, the exogenous supplementation of glutathione does not show any effect on plasma and tissue arsenic content that reflects its limited ability to excrete arsenic in its trivalent form.

The combined involvement of GSH in restoring the hypothalamic-pituitary-ovarian axis and its direct antioxidative potential on female reproductive organs serves as evidence of the ameliorative role of GSH on arsenic-induced reprotoxicity.

N-acetyl L-cysteine (NAC) is the acetylated form of the amino acid cysteine having anticancer and antioxidant properties. Due to the presence of sulphur and sulfhydryl groups, it can be used as an intracellular glutathione precursor and a unique nutraceutical agent. NAC acts as an effective free radical scavenger through the generation of endogenous glutathione. We examined utero-ovarian damage by arsenic toxicity. Our research indicated that dietary NAC supplementation considerably reduced the reproductive organs’ damage associated with utero-ovarian genotoxicity owing to its antioxidant, anti-inflammatory, and antiapoptotic effects (Figures 6, 7); Moreover, sulfhydryl (-SH) and hydroxyl (-OH) groups could possibly act as chelating sites for arsenic; NAC has been reported to modulate the functionality of the SAM pool by raising the circulating levels
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It could also supply adequate cysteine residues to the methyltransferase enzyme to allow arsenic methylation during the arsenic elimination from the body. The photosynthetic cyanobacterium Arthrospira sp., popularly known as Spirulina sp., has recently received much attention because of its great ability for protein synthesis. It is also known that spirulina produces the blue, water-soluble pigment known as C-phycocyanin (C-PC). The interest in C-PC has been emerging owing to its antioxidant, anti-inflammatory, and anticarcinogenic effects.

We explored detoxifying property of spirulina in arsenic induced reprotoxicity. Spirulina administration decreased uterine MDA and CD generation linked to lipid peroxidation and restored antioxidant enzyme status in utero-ovarian tissue (Figure 8). The necrotic biomarker LDH expression was observed, which was visibly mitigated by NAC post-treatment.

**Figure 7:** Impact of NAC on uterine comet assay in arsenic-exposed female rats (A). Arsenic-induced DNA damage in single cell apoptosis was observed, which was visibly mitigated by NAC post-treatment. Arsenic and NAC Effects on Uterine and Ovarian Histoarchitecture (B and C). Tissues were embedded in paraffin, 5 μm sections were stained and examined under a 400x microscope. Arsenic-induced disruptions in uterine glands and endometrium were restored by NAC. Arsenic-triggered follicular atresia was also corrected by NAC. Immunohistochemistry of Ovary and Uterus (D and E). Arsenic reduced ER-α levels, which were subsequently restored in both organs with NAC supplementation.

**Figure 8:** Uterine and ovarian tissue protection by spirulina and probiotics against As3+ toxicity. Expression of SOD, Catalase, GPx in uterine tissue (A, B, and C) was assessed via native gel electrophoresis. Serum LDH activity profiles were studied using agarose gel electrophoresis, and SOD, Catalase, GPx, and LDH bands were developed specifically. Lane distribution. Lane 1: Control; Lane 2: Arsenic; Lane 3: Probiotics; Lane 4: Arsenic + Probiotics; Lane 5: Spirulina; Lane 6: Arsenic + Spirulina; Lane 7: Arsenic + Spirulina + Probiotics.

**Biologically active compounds**

Arjunolic acid, a triterpenoid saponin derived from *Terminalia arjuna*, is well recognized for its potent antioxidative and free radical quenching ability in the treatment of dyslipidemia, haematological disorders, cardiac disorders, and hypertension. When considering the ability of arjunolic acid as metal chelator and antioxidant it prevents metal-induced toxicity in the organs. A five-membered chelate compound with trivalent arsenic may emerge as a result of two vicinal equatorial -OH groups of arjunolic acid. We found that co-treatment with arjunolic acid promotes cellular antioxidant actions and protects genetic material. Furthermore, the improvement in vitamin B12 and folate store was seen and this may be accompanied by the prevention of necrotic tissue damage, possibly by removing arsenic from the uterine tissue. Additionally, higher-level serum homocysteine (Hcy) due to arsenic was controlled by the administration of arjunolic acid. In another study, we observed that arjunolic acid in association with B12 could lessen oxidative stress by maintaining the normal cellular level of metallothionine which is vital for metal homeostasis as well as in the cellular defence system.

Consequently, we come to interpret that the therapeutic action of arjunolic acid may be ascribed to its chelating capabilities, its modulatory role on SAM pool, and the correction of arsenic induced oxidative damage in either direct or indirect means.

Turmeric (Curcuma longa L.), an Indian spice, also popular for its bioactive compound curcumin, a yellow-orange pigment with anti-inflammatory and antioxidant potential. Our study found that curcumin intervention in arsenic-treated rats strongly supports cellular antioxidant system and the promotion of ovarian steroidogenesis through the detoxification of arsenicated tissue. The stabilization of the SAM pool, majorly regulated by vitamin B12, folate, and Hcy, may be the components of the possible mechanism of arsenic detoxification (Figure 9). Curcumin also showed its anti-inflammatory role by modifying the transcription of uterine NF-kB and restricting the proinflammatory cytokines, TNF-α and IL-6 signaling. Furthermore, an anticancerous property of curcumin has been established upon bioinformatics study (Figure 10). Using AutoDock 4.2,
a molecular docking analysis (Figure 10) was conducted on caspase-3 protein (PDB ID- 2XYP) with the ligand curcumin (PubChem ID 969516). Curcumin displayed hydrogen bond interactions with ser120 and cys163 of the larger P17 subunit and ser209 and ser251 of the smaller P12 subunit. Cys163, which forms the active site of caspase-3, was identified as the main amino acid involved. Furthermore, curcumin exhibited hydrophobic interactions with Arg64, Ser120, His121, Gly122, Ala162, Glu161 of the p17 subunit, and Tyr204, Ser205, Trp206, Arg207, Asn208, Ser213, Phe250, Asp253, Phe256 of the p12 subunit. The binding energy was calculated as -5.99 kcal/mol, and the RMSD value was found to be 0. Caspase-3 belongs to the aspartate-specific cysteine protease family and serves as a crucial initiator of the apoptotic pathway. The active sites of caspase-3 typically involve Cys163 and His121 residues within the larger p17 subunit. Curcumin demonstrated potential interactions with the sulfhydryl group of Cysteine (Cys163) and the imidazole ring of Histidine (His121), thereby intervening with the catalytic sites of caspase-3. This interaction suggests that curcumin may exert inhibitory action, resulting in the down-regulation of caspase-3. Previous studies have also evident a similar fashion of effect of curcumin.

It has been demonstrated that polysaccharides from *Momordica charantia*, commonly known as bitter gourd are traditionally used in India and other parts of the Indian subcontinent due to its various biological and therapeutic activities. Recently, it has been studied that polysaccharide of *M. charantia* is useful in exerting its hypolipidemic effect via the regulation of peroxisome proliferators-activated α-receptor (PPARα) gene expression. Our study elucidates the potential arsenic detoxifying role of pectic polysaccharide (CCPS) isolated from *M. charantia*. We found that the arsenicated rats supplemented with dietary CCPS exhibited successful fertility, with pup survival and unaffected birth weight and without birth defects among the CCPS supplemented groups (Figure 11). A regulatory influence of CCPS on hypothalamic–pituitary–gonadal axis was supported by its ability to correct gonadotropin level, ovarian steroidogenesis and improved ovarian histomorphology connected with upregulated expression of uterine estrogen receptors alpha (ERα). Utero-ovarian oxidative damage was controlled with improvement in the SOD, catalase, GPx level and the NPSH. Moreover, uterine expression of proteins linked with apoptosis, BAX, phospho-p53, caspase-3, PARP, and PCNA expression were shown to be downregulated where as an upregulation of antiapoptotic protein Bcl-2 and AKT expression appeared upon Western blot analysis (Figure 12).
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levels of B12 after administration of probiotics to arsenic-fed rats, which ultimately speeds up the biliary excretion of trivalent arsenic in the methylated form using the SAM pool.

Findings additionally indicate an elevation plasma estradiol influenced by improved gonadotropin status, improved gonadal function, and restored normal estrous cyclicity (Figure 13) in the arsenicated rodents after probiotic supplementation.

Probiotics’ important roles in reducing uterine oxidative stress and preventing arsenic-induced reproductive injury may be attributed to their metal-chelating and antioxidant activities.

Green Tea or *Camellia sinensis* L. belonging to Theaceae family, is consumed as a beverage that has gained popularity recently for its beneficial functional compounds. The major phytochemicals with therapeutic potential are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG).

The most prevalent CS polyphenol, EGCG, possesses antioxidant properties that reduce the cardiotoxicity caused by arsenic in rats. We discovered that the methanolic extract of green tea is essential for the improvement of the uterine antioxidant defense system and the restoration of normal utero-ovarian histoarchitecture; also it protects against arsenic-induced genotoxicity and necrotic damage as shown in Figure 14 and 15.

Furthermore, ethyl acetate fraction of green tea shows its anti-inflammatory and antiapoptotic role levels of B12 after administration of probiotics to arsenic-fed rats, which ultimately speeds up the biliary excretion of trivalent arsenic in the methylated form using the SAM pool.

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**Figure 13:** Estrous cycle pattern. The normal rhythmic estrous cycle pattern was disrupted, leading to prolonged diestrus in rats exposed to arsenic for 10 to 12 days. However, this irregularity was corrected and restored to a regular pattern in rats treated with probiotics alongside arsenic.

**Figure 14:** Influence of methanolic extract of CS on DNA fragmentation (Panel A) and comet assay (Panel B) in uterine cells of arsenic-treated female rats. In Panel A, the lanes are as follows. Lane 1: Control, Lane 2: Arsenic, Lane 3: CS, Lane 4: Arsenic + CS. In Panel B, the panels are denoted as: B1: Control, B2: Arsenic, B3: CS, B4: Arsenic + CS. Comet formation is indicated by arrows.

**Figure 15:** Curative potential of CS-EA fraction on apoptotic and pro-inflammatory gene expression in arsenic treated rat. Expression levels of p53, Bcl-2, BAX, NF-κB, and TNF-α were studied using PCR, with GAPDH as the reference gene (a). Furthermore, arsenic-induced apoptotic changes in protein expression, such as Phospho p53, caspase 3, and BAX, were rectified by the CS-EA fraction, as evidenced by Western blot analysis (b) with β-actin as the control.

**Figure 16:** Human IkB kinase β Structure: (a) The structure comprises distinct domains, including the scaffold/dimerization domain (SDD), ubiquitin-like domain (ULD), N-terminal kinase domain (KD N), and C-terminal kinase domain (KD C). EGCG Three-Dimensional Structure: (b) Depicts the spatial arrangement of EGCG. Inhibitor and EGCG Binding Site: (c and d) Present a focused view of KD N, highlighting the binding site for the inhibitor and EGCG. Initiation of Kinase Activity: Kinase activity commences with phosphorylation of Ser177 and Ser181 in the activation loop. EGCG obstructs other amino acids within the active site. EGCG Interaction: EGCG forms interactions, including hydrogen bonds (2.24 and 2.87 Å) with Asp166, hydrogen bonding (2.75 Å) with Asn28, and bonding with Trp58.

12). Genomic expression of uterine TNF-α, NF-κB, BAX and p53 could be regulated with the intervention of CCPS.

**Functional food and medicinal plant**

Probiotics are living microorganisms that provide numerous health advantages to the host when given in appropriate quantities. Various studies are witness of an oxidative stress reducing property of probiotics. We employed probiotics cocktail of bacterial strain of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Bifidobacterium bifidum* along with yeast, *Saccharomyces boulardii*. Our investigation confirms an increase in circulating levels of B12 after administration of probiotics to arsenic-fed rats, which ultimately speeds up the biliary excretion of trivalent arsenic in the methylated form using the SAM pool.

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during arsenic intoxication through its capacity to regulate various gene and protein expression.\(^4\) \(^5\) Epigallocatechin gallate (EGCG), the active component of green tea, can block TNF-\(\alpha\) through IKK activation.\(^6\) This highlights the crucial role of EGCG as a natural anti-inflammatory agent. Our bioinformatics and molecular modeling data (Figure 16) indicate that EGCG binds to the KD N terminal site. The activation of IKK kinase begins with the phosphorylation of SER177 and SER181 in the activation loop. By blocking other amino acids within the active site, EGCG may partially regulate kinase activity, thereby restoring it to basal levels necessary for proper physiological functioning. The well-known antioxidant qualities of green tea and its indirect impact on SAM pool due to its favourable effect on plasma folate and vitamin B12 levels suggest a potential therapeutic strategy for arsenic-induced reprotoxicity.\(^4\),\(^4\) 6

**CONCLUSION**

In conclusion, this review has highlighted the potential of natural non-enzymatic antioxidants, dietary supplements, phytocompounds, and functional foods to mitigate the reprotoxic effects of arsenic exposure on female reproductive health. Studies established that natural compounds such as vitamin C, vitamin E, selenium, curcumin, NAC, arjunolic acid, green tea leaf extracts, CCPs from *Momordica charantia*, spirulina and probiotics could contribute in improving various parameters of reproductive toxicity, including utero-ovarian function, reproductive hormones, and restoration of utero-ovarian histoarchitecture. These findings suggest that these natural compounds and functional foods may hold promise as safe and effective approach to mitigating the harmful effects of arsenic exposure on female reproductive health. Overall, this review underscores the need for continued research into natural compounds and functional foods as potential therapeutic agents for mitigating arsenic-induced reprotoxicity in females. Further investigation may provide novel therapeutic strategies for managing the reprotoxicity caused by arsenic exposure and ultimately protects the reproductive health of women exposed to arsenic.

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