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

Sandip Chattopadhyay

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 arsenicosis 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.

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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.[1] 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.[2] 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.[3] 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.[4,5] 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.[6] Alarmingly arsenic level in the drinking water of this area were found to be 800 µg/l.[7]

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.[8] Trivalent arsenic is more hazardous than pentavalent arsenic, as shown by a plethora of studies.[9] Arsenic interferes with

Department of Biomedical Laboratory Science and Management, Molecular and Cellular Toxicology Laboratory, Vidyasagar University, Midnapore, India

Sandip Chattopadhyay, Department of Biomedical Laboratory Science and Management, Molecular and Cellular Toxicology Laboratory, Vidyasagar University, Midnapore, India, Email: sandipdoc@yahoo.com

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cellular energy production by attaching to sulphydryl (-SH) groups. It interferes with normal metabolic activities by occupying the position of metal ions like zinc and selenium in the biomolecules' binding sites.[10] 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.[9,11] Well-demonstrated underlying mechanisms of arsenic toxicity explored metabolic inhibition, oxidative stress, genotoxicity, epigenetic changes, and micro-RNA-dependent regulation.[12] 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.[13] The International Agency for Research categorises arsenic compounds on Cancer (IARC) as group-1 carcinogen to humans.[14] Chronic arsenic exposure found to cause malignancy of several organs including skin, bladder, liver, and kidney.[15] Furthermore, arsenic exposure has been linked to infertility and miscarriage in humans.[16-18] There is evidence from numerous epidemiological research on the menstrual cycle that heavy metal exposure affects reproductive function by altering the hormonal level.[19]

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)[20] 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.[21] Moreover, the release of arsenic from BAL-arsenic complexes during oxidation is potentially harmful to the system.[22] 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.[23] However, its effectiveness is limited by its inability to pass the cell membrane, which prevents it from chelating arsenic from intracellular compartments.[24] Meso-DMSA therapy has also been linked to other adverse effects, including gastrointestinal disorders, increased liver enzymes, placid neutropenia and skin allergies.[22] Hence, their long-term intramuscular administration with a number of mild to severe adverse effects makes the treatment less acceptable and less effective.[25] 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.[20] Arsenic causes oxidative stress, which can be mitigated by exogenous antioxidant supplementation since arsenic influences the intracellular antioxidant machinery.[26,27]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.[26] 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 Δ^5, 3β-HSD and 17β -HSD.[28] Arsenic treatment is associated with the suppression of testicular and ovarian steroidogenesis.[29] 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.[18,30-33] 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.[31, 34-45] 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).[33, 46-48] On the other hand, exposure to arsenic also found to affect the levels of biogenic amines, the critical regulators of gonadotropin synthesis and secretion.[49-52] 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.[35,53]

Moreover, superoxide radical production in female gonads is linked to arsenic intoxication, which could be one of the factors inhibiting the steroidogenesis.[54] Reduced uterine peroxidase activity, which is a marker for decreased plasma levels of estradiol in arsenicated rats, is also consistent with these findings.[35,53,55-58] Prolonged diestrous in rats treated with arsenic was evident in our previous studies that may be caused by low plasma levels of estradiol.[31,34-45] A significant ecrease in ovarian weight, gonadotropins regulate that along with uterine weight loss was evident by our studies

in accordance with other studies.[34-45,53,59] 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.[31,34-44] On the other hand, a significant reduction in the most crucial cellular antioxidant defence system non-protein thiols (NPSH).[36,37,42-44] as well antioxidant enzymes catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) has been reported for gonadal tissue.[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.[60,61] 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.[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, steroidogenic 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.[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.[53,57,58] The restoration of

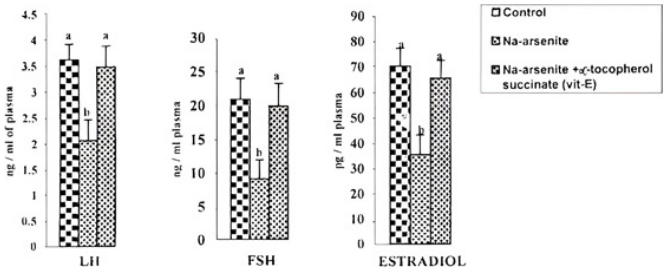

Figure 1: The supplementary effect of vitamin E on the plasma levels of LH, FSH, and estradiol in albino rats treated with sodium arsenite. The data was depicted as the mean ± SE (n=16). Bars sharing the same superscript within each group exhibited no noteworthy distinction among them ($p<0.001$)[53]

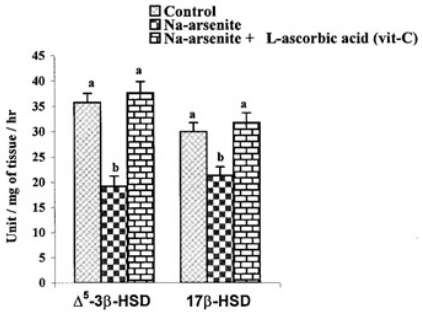

Figure 2: Alterations in the functions of Δ5-3β-HSD and 17β-HSD enzymes in rats treated with 10 ml of water containing 0.4 ppm sodium arsenite/ day, along with ascorbic acid (vitamin C), over a span of 28 days. A control group received the same 10 ml of water per day for the same duration. The results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

ovarian and uterine histoarchitecture during arsenic toxicosis further confirmed the therapeutic impact of selenium.[58] 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.[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 steroidogenesis, which supports the normalization of ovarian folliculogenesis and other normal functions of the ovary.[53,57, 58]

Vitamin B_9 (Folate) and B_{12} (Cobalamin) are necessary for maintaining one-carbon metabolism and are particularly important for arsenic methylation. Our research indicated that vitamin B_{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.[34] The mechanisms could be linked to the improvement of

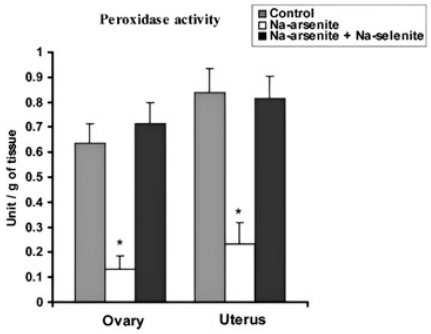

Figure 3: Alteration in the activity of ovarian and uterine peroxidase in arsenic treated rats followed by selenium administration. Data represented as mean ± SE (n=6), $p < 0.001$, and ANOVA followed by two-tailed student's t-test were used. The asterisk (*) on the bar indicates significant difference[58]

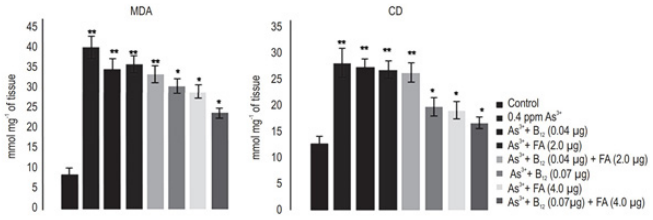

Figure 4: A dose-dependent protective effect of Vitamin B_{12} and folic acid co-administration on malondialdehyde (MDA) and conjugated dienes (CD) levels in uterine tissue of rats exposed to As3+ toxicity. Significantly higher levels of vitamin B_{12} (0.07 µg) and folic acid (4.0 µg), either individually or combined, demonstrated enhanced efficacy in providing the aforementioned protection. The data is based on six replicates for each group, and statistical analysis involved ANOVA followed by a two-tailed Student's t-test, where *$p<0.05$ and **$p<0.01$[34]

antioxidant defence systems partly through the removal of arsenic with the participation of the S-adenosine methionine pool (SAM) as vitamin B_{12} and B_9 are two important regulators of this pool.

Dietary Supplements

Glutathione (GSH) is an essential non-enzymatic antioxidant.[63,64] Few studies recognised GSH as a protective factor against oxidative damage to both male and female gametes in numerous investigations.[65] 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.[66,67] 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.[35]

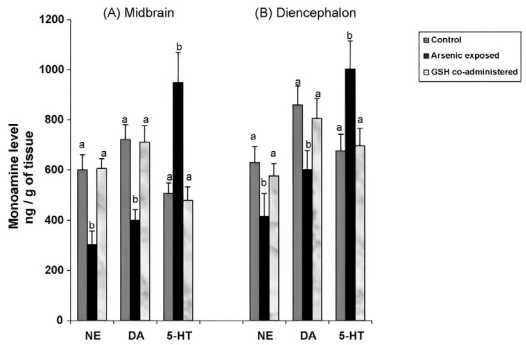

Figure 5: Level of brain monoamines in the midbrain (A) and diencephalon (B). The data shows mean ± SE (n= 12). Compared to the control, the differences were significant ($p < 0.001$). ANOVA followed by a two-tailed t-test for multiple comparisons was used. Bars with the same superscript within each group were not significantly different[35]

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.[68] Due to the presence of sulphur and sulfhydryl groups, it can be used as an intracellular glutathione precursor and a unique nutraceutical agent.[69] NAC acts as an effective free radical scavenger through the generation of endogenous glutathione.[70] 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

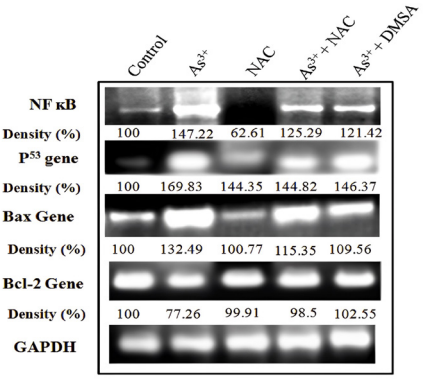

Figure 6: The impact of arsenic and NAC on the expression of pro-inflammatory and apoptotic genes in uterine tissue, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the reference gene. Arsenic led to an increase in the expression of NFκB, p53, and BAX genes, while decreasing the expression of the Bcl-2 gene. NAC brought about alterations in these gene expressions[37]

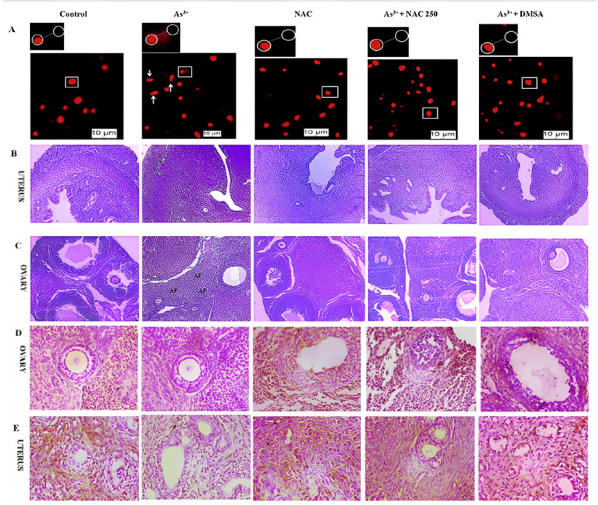

Figure 7: Impact of NAC on uterine comet assay in arsenic-exposed female rats (A). Arsenic-induced DNA damage in single cell apoptosis was observed, which was visibly mitigated by NAC post-treatment. Arsenic and NAC Effects on Uterine and Ovarian Histoarchitecture (B and C). Tissues were embedded in paraffin, 5 µm sections were stained and examined under a 400× 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[37]

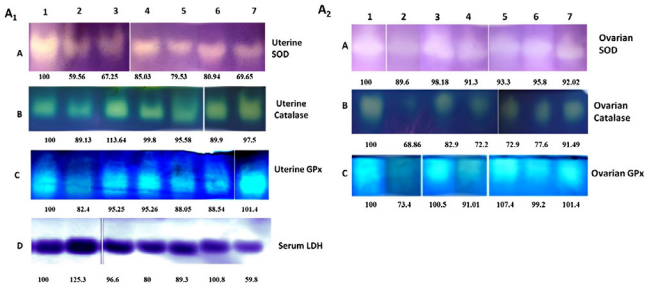

Figure 8: Uterine and ovarian tissue protection by spirulina and probiotics against As3+ toxicity. Expression of SOD, Catalase, GPx in uterine tissue (A, B, and C) was assessed via native gel electrophoresis. Serum LDH activity profiles were studied using agarose gel electrophoresis, and SOD, Catalase, 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[38]

of B_{12} and B_9 and supplying adequate cysteine residues to the methyltransferase enzyme to allow arsenic methylation during the arsenic elimination from the body.[36,37]

The photosynthetic cyanobacterium Arthrospira sp., popularly known as Spirulina sp., has recently received much attention because of its great ability for protein synthesis.[71] 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.[72]

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 also reduced. The recovery of reproductive function was supported by enhancing plasma gonadotropin and estradiol level while maintaining regularity in estrous cycle; Following DNA fragmentation study and comet assay, the geno-protective and antiapoptotic properties of spirulina were also confirmed.[31] The capacity to trap arsenic by extending its chelating properties together with the antioxidative activity in inhibiting NADPH-dependent superoxide and peroxyl-radical-induced lipid peroxidation production, may explain the potential mechanism underlying its therapeutic role.[73,74]

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.[75] A five-membered chelate compound with trivalent arsenic may emerge as a result of two vicinal equatorial -OH groups of arjunolic acid.[76] We found that co-treatment with arjunolic acid promotes cellular antioxidant actions and protects genetic material. Furthermore, the improvement in vitamin B_{12} 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.[39] In another study, we observed that arjunolic acid in association with B_{12} 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.[40] 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.[77] 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 B_{12}, 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.[41] Furthermore, an anticancerous property of curcumin has been established upon bioinformatics study (Figure 10).[41] 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, Gln161 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.[78]

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.[79] 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.[80] 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 onhypothalamic–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.[41] 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

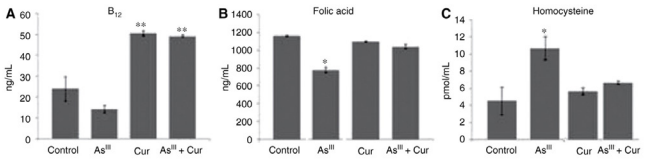

Figure 9: Impact of Curcumin on serum Vitamin B_{12}, Folic Acid, and Homocysteine (Hcy) Levels in arsenic-induced female rats. In the arsenic group (A–C), vitamin B12 and folic acid levels were low, while Hcy levels were high. Curcumin co-treatment led to elevated vitamin levels and reduced serum Hcy levels. Each dataset represents the mean ± SE (n=6). Statistical significance was determined using ANOVA followed by the Bonferroni post hoc test (*p < 0.05, **p < 0.01)[41]

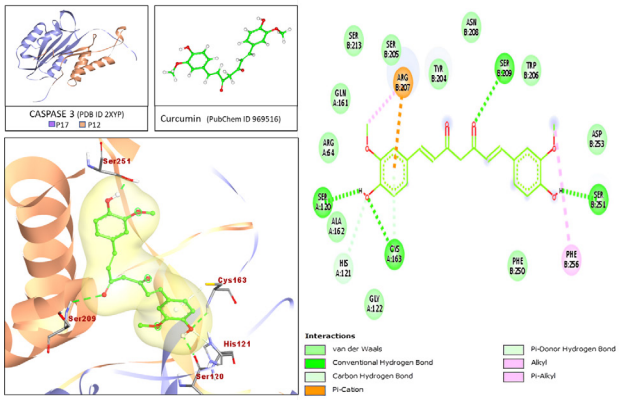

Figure 10: Molecular interaction of curcumin with caspase-3. 2D and 3D molecular docking image of curcumin with caspase-3. Curcumin shows hydrogen bond interaction with Ser120 and Cys163 of larger P17 subunit along with Ser209 and Ser251 of smaller P12 subunit. (Discovery Studio Visualizer)

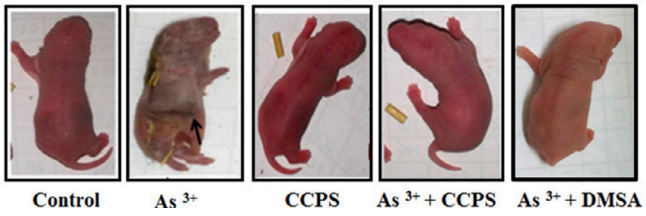

Figure 11: Curative potential of CCPS in arsenic induced female fertility. CCPS treatment results in the birth of healthy pups without deformities. An arrow highlights the contrast. arsenic-exposed pregnant rats give birth to unhealthy pups with deformities[41]

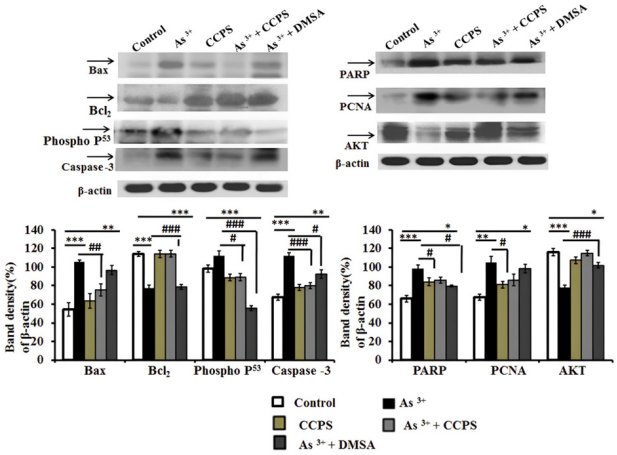

Figure 12: Protective effects of CCPS on changes in expression of protein Bax, Caspase-3, Bcl2, Phospho-p53, PCNA, PARP, and AKT in arsenic treated rats. The relative protein expression of β-actin, Bax, caspase-3, Bcl-2, Phospho-p53, PCNA, Parp, and AKT are illustrated in Figure 12. These findings are presented as the mean ± SE (N=6), analyzed through ANOVA followed by post hoc Dunnett test. Significance levels denoted as *p < 0.05, **p < 0.01, ***p < 0.001 compared to the control group, and #p < 0.05, ##p < 0.01, ###p < 0.001 compared to the arsenic-exposed group[41]

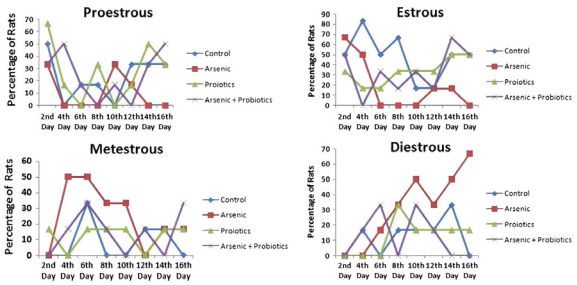

Figure 13: Estrous cycle pattern. The normal rhythmic estrous cycle pattern was disrupted, leading to prolonged diestrus in rats exposed to arsenic for 10 to 12 days. However, this irregularity was corrected and restored to a regular pattern in rats treated with probiotics alongside arsenic[43]

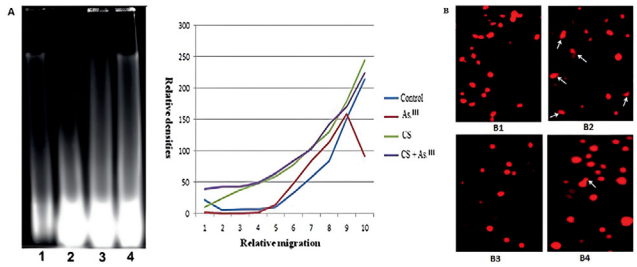

Figure 14: Influence of methanolic extract of CS on DNA fragmentation (Panel A) and comet assay (Panel B) in uterine cells of arsenic-treated female rats. In Panel A, the lanes are as follows. Lane 1: Control, Lane 2: Arsenic, Lane 3: CS, Lane 4: Arsenic + CS. In Panel B, the panels are denoted as: B1: Control, B2: Arsenic, B3: CS, B4: Arsenic + CS. Comet formation is indicated by arrows[44]

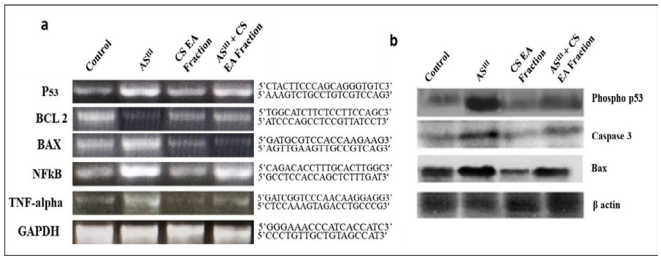

Figure 15: Curative potential of CS-EA fraction on apoptotic and pro-inflammatory gene expression in arsenic treated rat. Expression levels of p53, Bcl-2, BAX, NF-κB, and TNF-α were studied using PCR, with GAPDH as the reference gene (a). Furthermore, arsenic-induced apoptotic changes in protein expression, 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[45]

12). Genomic expression of uterine TNF-α, NF-κB, BAX and p53 could be regulated with the intervention of CCPS.[41]

Functional food and medicinal plant

Probiotics are living microorganisms that provide numerous health advantages to the host when given in appropriate quantities.[81] Various studies are witness of an oxidative stress reducing property of probiotics.[82] 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

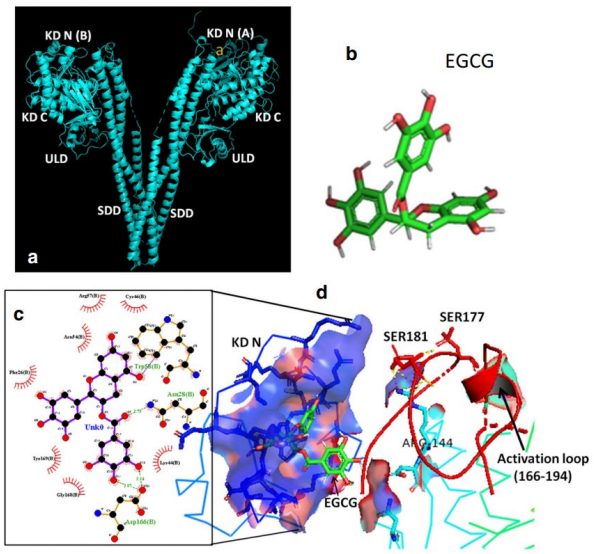

Figure 16: Human IκB kinase β Structure: (a) The structure comprises distinct domains, including the scaffold/dimerization domain (SDD), ubiquitin-like domain (ULD), N-terminal kinase domain (KD N), and C-terminal kinase domain (KD C). EGCG Three-Dimensional Structure: (b) Depicts the spatial arrangement 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[45]

levels of B_{12} 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.[43] 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.[43] 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).[83]

The most prevalent CS polyphenol, EGCG, possesses antioxidant properties that reduce the cardiotoxicity caused by arsenic in rats.[83] 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.[44] Furthermore, ethyl acetate fraction of green tea shows its anti-inflammatory and antiapoptotic role

during arsenic intoxication through its capacity to regulate various gene and protein expression.[45] Epigallocatechin gallate (EGCG), the active component of green tea, can block TNF-α through IKK activation.[84] 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.[44,45]

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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REFERENCES

1. Mehta M, Hundal SS. Effect of sodium arsenite on reproductive organs of female Wistar rats. *Arch Environ Occup Health*. 2016;71(1):16-25. DOI:10.1080/19338244.2014.927346.
2. Saha JC, Dikshit AK, Bandyopadhyay M, Saha KC. A review of arsenic poisoning and its effects on human health. *Crit Rev Environ Sci Technol*. 1999;29(3):281-313. DOI:10.1080/10643389991259227.
3. Steinmaus C, Carrigan K, Kalman D, Atallah R, Yuan Y, Smith AH. Dietary intake and arsenic methylation in a US population. *Environ Health Perspect*. 2005;113(9):1153-9. DOI: 10.1289/ehp.7907.
4. Brammer H, Ravenscroft P. Arsenic in groundwater: a threat to sustainable agriculture in South and South-east Asia. *Environ Int*. 2009;35(3):647-54. DOI: 10.1016/j.envint.2008.10.004.
5. UNICEF. Arsenic primer: guidance on the investigation and mitigation of arsenic contamination. New York: UNICEF Water, Sanitation and Hygiene Section and WHO Water, Sanitation and Hygiene and Health Unit, United Nations Children's Fund (UNICEF). 2018. ISBN: 978-92-806-4980-2.
6. National Research Council. Arsenic in drinking water. 1999. ISBN-10: 0-309-07629-3.
7. Kinniburgh DG, Smedley PL. Arsenic contamination of groundwater in Bangladesh, vol. 2: final report. British Geological Survey. 2001. ISBN: 085273849.
8. Dopp E, Hartmann LM, Recklinghausen U, Florea AM, Rabieh S, Zimmermann U *et al*., Forced uptake of trivalent and pentavalent methylated and inorganic arsenic and its cyto-/genotoxicity in fibroblasts and hepatoma cells. *Toxicol Sci*. 2005;87(1):46-56. DOI: 10.1093/toxsci/kfi218.
9. Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ. Arsenic exposure and toxicology: a historical perspective. *Toxicol sci*. 2011;123(2):305-32. DOI: 10.1093/toxsci/kfr184.
10. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. *World Allergy Organ*. 2012;5:9-19. DOI: 10.1097/WOX.0b013e3182439613.
11. Muzaffar S, Khan J, Srivastava R, Gorbatyuk MS, Athar M. Mechanistic understanding of the toxic effects of arsenic and warfare arsenicals on human health and environment. *Cell Biol and Toxicol*. 2023; 39(1):85-110. DOI:10.1007/s10565-022-09710-8.
12. Ratnaike RN. Acute and chronic arsenic toxicity. *Postgrad Med J*. 2003;79(933):391-6. DOI: 10.1136/pmj.79.933.391
13. Rao CV, Pal S, Mohammed A, Farooqui M, Doescher MP, Asch AS, Yamada HY. Biological effects and epidemiological consequences of arsenic exposure, and reagents that can ameliorate arsenic damage in vivo. *Oncotarget*. 2017;8(34):57605. DOI: 10.18632/oncotarget.17745
14. Mukherjee A, Sengupta MK, Hossain MA, Ahamed S, Das B, Nayak B, Lodh D, Rahman MM, Chakraborti D. Arsenic contamination in groundwater: a global perspective with emphasis on the Asian scenario. *J Health Popul Nutr*. 2006:142-63. PMID: 17195556
15. Briffa J, Sinagra E, Blundell R. Heavy metal pollution in the environment and their toxicological effects on humans. *Heliyon*. 2020;6(9):e04691. DOI: 10.1016/j.heliyon.2020.e04691.
16. Martinez VD, Vucic EA, Becker-Santos DD, Gil L, Lam WL. Arsenic exposure and the induction of human cancers. *J Toxicol*. 2011;2011: 431287. DOI: 10.1155/2011/431287.
17. Wang X, Zhang J, Xu W, Huang Q, Liu L, Tian M, Xia Y, Zhang W, Shen H. Low-level environmental arsenic exposure correlates with unexplained male infertility risk. *Sci Total Environ*. 2016;571:307-13. DOI: 10.1016/j.scitotenv.2016.07.169.
18. Zargari F, Rahaman MS, KazemPour R, Hajirostamlou M. Arsenic, oxidative stress and reproductive system. *J Xenobiot*. 2022;12(3):214-22. DOI: 10.3390/jox12030016.
19. Rami Y, Ebrahimpour K, Maghami M, Shoshtari-Yeganeh B, Kelishadi R. The association between heavy metals exposure and sex hormones: A systematic review on current evidence.

Biol Trace Elem Res. 2022:200:3491-3510. DOI: 10.1007/s12011-021-02947-0.
20. Susan A, Rajendran K, Sathyasivam K, Krishnan UM. An overview of plant-based interventions to ameliorate arsenic toxicity. *Biomed Pharmacother*. 2019;109:838-52. DOI: 10.1016/j.biopha.2018.10.099.
21. Mückter H, Lieb B, Reich FX, Hunder G, Walther U, Fichtl B. Are we ready to replace dimercaprol (BAL) as an arsenic antidote?. *Hum Exp Toxicol*. 1997;16(8):460-5. DOI: 10.1177/096032719701600807.
22. Aaseth J, Skaug MA, Cao Y, Andersen O. Chelation in metal intoxication—principles and paradigms. *J Trace Elem Med Biol*. 2015;31:260-6. DOI: 10.1016/j.jtemb.2014.10.001.
23. Kosnett MJ. The role of chelation in the treatment of arsenic and mercury poisoning. *J Med Toxicol*. 2013;9:347-54. DOI: 10.1007/s13181-013-0344-5.
24. Flora SJ. Metal poisoning: threat and management. *Al Ameen J Med Sci*. 2009;2(2):4-26. ISSN: 0974-1143. http://ajms.alameenmedical.org/ArticlePDFs/AJMS4S.4-26.pdf.
25. Flora SJ, Mittal M, Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J Med Res*. 2008;128(4):501-23. PMID: 1910644.3.
26. Khandker S, Dey RK, Islam AM, Ahmad SA, Al Mahmud I. Arsenic-safe drinking water and antioxidants for the management of arsenicosis patients. *Bangladesh J Pharmacol*. 2006;1(2):42-50. DOI: 10.3329/bjp.v1i2.487.
27. Yu H, Liu S, Li M, Wu B. Influence of diet, vitamin, tea, trace elements and exogenous antioxidants on arsenic metabolism and toxicity. *Environ Geochem Health*. 2016;38:339-51. DOI: 10.1007/s10653-015-9742-8.
28. Simpson E, Lauber M, Demeter M, Means G, Mahendroo M, Kilgore M, Mendelson C, Waterman M. Regulation of expression of the genes encoding steroidogenic enzymes in the ovary. *J Steroid Biochem Mol Biol*. 1992;41(3-8):409-13. DOI: 10.1016/0960-0760(92)90366-q.
29. Sarkar M, Chaudhuri GR, Chattopadhyay A, Biswas NM. Effect of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. *Asian J Androl*. 2003;5(1):27-31. PMID: 12646999.
30. Chattopadhyay S, Pal SG, Chaki S, Debnath J, Ghosh D. Effect of sodium arsenite on plasma levels of gonadotrophins and ovarian steroidogenesis in mature albino rats: duration-dependent response. *J Toxicol Sci*. 1999;24(5):425-31. DOI: 10.2131/jts.24.5_425.
31. Khatun S, Maity M, Perveen H, Dash M, Chattopadhyay S. Spirulina platensis ameliorates arsenic-mediated uterine damage and ovarian steroidogenic disorder. *Facets*. 2018;3(1):736-53. DOI: 10.1139/facets-2017-0099.
32. Renu K, Madhyastha H, Madhyastha R, Maruyama M, Vinayagam S, Gopalakrishnan AV. Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity. *Life Sci*. 2018;212:37-58. DOI: 10.1016/j.lfs.2018.09.045.
33. Ghosh D. Effect of sodium arsenite on adrenocortical activity in immature female rats: Evidence of dosedependent response. *J Environ Sci*. 1999;11. ISSN: 1001-0742.
34. Deb B, Maity M, Maiti S, Pan B, Perveen H, Dash M, et al., Abrogation of sodium arsenite driven uterine antioxidant exhaustion and tissue impairment: Role of B12 and folate. Journal of Environmental Biology. 2018; 39(5):581-91. DOI: 10.22438/jeb/39/5/MRN-677.
35. Chattopadhyay S, Ghosh D. Role of dietary GSH in the amelioration of sodium arsenite-induced ovarian and uterine disorders. Reproductive Toxicology. 2010; 30(3):481-8. DOI: 10.1016/j.reprotox.2010.05.002.
36. Dash M, Maity M, Dey A, Perveen H, Khatun S, Jana L, et al., The consequence of NAC on sodium arsenite-induced uterine oxidative stress. Toxicology Reports. 2018; 5:278-87. DOI: 10.1016/j.toxrep.2018.02.003.
37. Dash M, Dey A, Chattopadhyay S. Mitigation of arsenic driven utero-ovarian malfunction and changes of apoptotic gene expression by dietary NAC. Ecotoxicology and Environmental Safety. 2020 Aug 1;199: 110675. DOI: 10.1016/j.ecoenv.2020.110675.
38. Khatun S, Chattopadhyay SC. Combined Dietary Action of Spirulina and Probiotics Mitigates Female Reproductive Ailments in Arsenicated Rats. J Pure Appl Microbiol. 2019;13(2):1175-84. DOI: 10.22207/JPAM.13.2.57.
39. Maity M, Perveen H, Dash M, Jana S, Khatun S, Dey A, et al., Arjunolic acid improves the serum level of vitamin B 12 and folate in the process of the attenuation of arsenic induced uterine oxidative stress. Biological trace element research. 2018; 182: 78-90. DOI: 10.1007/s12011-017-1077-0.
40. Maity M, Perveen H, Chattopadhyay S. Dual role of Arjunolic acid and B12 in mitigating sodium arsenite induced malfunction of female reproductive organs. International Journal of Pharmaceutical Sciences and Research. 2019; 10(7):3219-3227. DOI: 10.13040/IJPSR.0975-8232.10(7).3219-27.
41. Perveen H, Chattopadhyay S, Maity M, Dash M, Islam SS. Involvement of proinflammatory cytokines and metallothionein in the repairing of arsenic-mediated uterine tissue damage by curcumin. Journal of basic and clinical physiology and pharmacology. 2019; 30(4). DOI: 10.1515/jbcpp-2017-0179.
42. Perveen H, Dey A, Nilavar NM, Chandra GK, Islam SS, Chattopadhyay S. Dietary CCPS from bitter gourd attenuates sodium arsenite induced female reproductive ailments cum infertility in wistar rats: anti-inflammatory and anti-apoptotic role. Food and Chemical Toxicology. 2019;131: 110545. DOI: 10.1016/j.fct.2019.05.053.
43. Chattopadhyay S, Khatun S, Maity M, Jana S, Perveen H, Dash M, et al., Association of vitamin B 12, lactate dehydrogenase, and regulation of NF-κB in the mitigation of sodium arsenite-induced ROS generation in uterine tissue by commercially available probiotics. Probiotics and antimicrobial proteins. 2019; 11:30-42. DOI: 10.1007/s12602-017-9333-3.
44. Dey A, Chattopadhyay S, Jana S, Giri MK, Khatun S, Dash M, et al., Restoration of uterine redox-balance by methanolic extract of Camellia sinensis in arsenicated rats. Acta Biologica Szegediensis. 2018; 62(1):7-15. DOI: https://doi.org/10.14232/abs.2018.1.7-15.
45. Dey A, Perveen H, Khandare AL, Banerjee A, Maiti S, Jana S, et al., Arsenic-induced uterine apoptotic damage is protected by ethyl acetate fraction of Camellia sinensis (green tea) via Bcl-2-BAX through NF-κB regulations in Wistar rats. Environmental Science and Pollution Research. 2021; 28:41095-108. DOI: 10.1007/s11356-021-13457-w.
46. Chattopadhyay S, Ghosh D. The involvement of hypophyseal-gonadal and hypophyseal-adrenal axes in arsenic-mediated ovarian and uterine toxicity: modulation by hCG. Journal of Biochemical and Molecular Toxicology. 2010; 24(1):29-41. DOI: 10.1002/jbt.20309.
47. Christian JJ. Actions of ACTH in intact and corticoid-maintained adrenalectomized female mice with emphasis on the reproductive tract. Endocrinology. 1964;75(5):653-69. DOI: 10.1210/endo-75-5-653.

48. Kamel F, Kubajak CL. Modulation of gonadotropin secretion by corticosterone: interaction with gonadal steroids and mechanism of action. Endocrinology. 1987;121(2):561-8. DOI: 10.1210/endo-121-2-561.
49. Crowley WR, Feder HH, Morin LP. Role of monoamines in sexual behavior of the female guinea pig. Pharmacology Biochemistry and Behavior. 1976; 4(1):67-71. DOI: 10.1016/0091-3057(76)90177-5.
50. Everitt BJ, Fuxe K. Serotonin and sexual behaviour in female rats. Effects of hallucinogenic indolealkylamines and phenylethylamines. Neuroscience Letters. 1977; 4(3-4):215-20. DOI: 10.1016/0304-3940(77)90141-0.
51. Nock B, Feder HH. Noradrenergic transmission and female sexual behavior of guinea pigs. Brain Research. 1979;166(2):369-80. DOI: 10.1016/0006-8993(79)90222-1.
52. Wise PM, Rance N, Selmanoff M, Barraclough CA. Changes in radioimmunoassayable luteinizing hormone-releasing hormone in discrete brain areas of the rat at various times on proestrus, diestrous day 1, and after phenobarbital administration. Endocrinology. 1981;108(6):2179-85. DOI: 10.1210/endo-108-6-2179
53. Chattopadhyay S, Ghosh S, Debnath J, Ghosh D. Protection of sodium arsenite-induced ovarian toxicity by coadministration of L-ascorbate (vitamin C) in mature wistar strain rat. Archives of environmental contamination and toxicology. 2001; 41:83-9. DOI: 10.1007/s002440010223.
54. Wang TS, Huang H. Active oxygen species are involved in the induction of micronuclei by arsenite in XRS-5 cells. Mutagenesis. 1994; 9(3):253-7. DOI: 10.1093/mutage/9.3.253.
55. Chattopadhyay S, Misro M, Ghosh S, Debnath J, Ghosh D. Supplementary effect of α-tocopherol succinate (vitamin E) on sodium arsenite-induced ovarian steroidogenic function and plasma levels of gonadotrophins in mature albino rats. Toxic Substance Mechanisms. 2000;19(3):137-50. DOI:10.1080/107691800300119356.
56. Tagatz G, Fialkow PJ, Smith D, Spadoni L. Hypogonadotropic hypogonadism associated with anosmia in the female. New England Journal of Medicine. 1970; 283(24):1326-9. DOI: 10.1056/NEJM197012102832407.
57. Chattopadhyay S, Misro M, Ghosh S, Debnath J, Ghosh D. Supplementary effect of α-tocopherol succinate (vitamin E) on sodium arsenite-induced ovarian steroidogenic function and plasma levels of gonadotrophins in mature albino rats. Toxic Substance Mechanisms. 2000;19(3):137-50. DOI:10.1080/107691800300119356.
58. Chattopadhyay S, Pal S, Ghosh D, Debnath J. Effect of dietary co-administration of sodium selenite on sodium arsenite-induced ovarian and uterine disorders in mature albino rats. Toxicological Sciences. 2003;75(2):412-22. DOI: 10.1093/toxsci/kfg194.
59. Kulin HE, Reiter EO. Gonadotropins during childhood and adolescence: a review. Pediatrics. 1973; 51(2):260-71. PMID: 4572068.
60. Bae ON, Lim KM, Noh JY, Kim KY, Lim EK, Chung JH. Methylated organic metabolites of arsenic and their cardiovascular toxicities. Toxicological Research. 2008:161-7. DOI: 10.5487/TR.2008.24.3.161.
61. Salnikow K, Zhitkovich A. Genetic and epigenetic mechanisms in metal carcinogenesis and cocarcinogenesis: nickel, arsenic, and chromium. Chemical research in toxicology. 2008 ;21(1):28-44. DOI: 10.1021/tx700198a.
62. Flora SJ, Bhadauria S, Kannan GM, Singh N. Arsenic induced oxidative stress and the role of antioxidant supplementation during chelation: a review. Journal of Environmental Biology. 2007;28(2):333. PMID: 17929749.
63. MARTINOVIC J. Effects of progesterone and estradiol benzoate on glutathione dependent antioxidant enzyme activities in the brain of female rats. Gen Physiol Biophys. 1999; 18:35-44. PMID: 10378119.
64. Shila S, Subathra M, Devi MA, Panneerselvam C. Arsenic intoxication-induced reduction of glutathione level and of the activity of related enzymes in rat brain regions: reversal by dl-α-lipoic acid. Archives of toxicology. 2005; 79:140-6. DOI: 10.1007/s00204-004-0614-8.
65. Luberda Z. The role of glutathione in mammalian gametes. Reprod Biol. 2005; 5(1):5-17. PMID: 15821775.
66. Gregus Z, Gyurasics Á. Role of glutathione in the biliary excretion of the arsenical drugs trimelarsan and melarsoprol. Biochemical pharmacology. 2000; 59(11):1375-85. DOI: 10.1016/s0006-2952(00)00263-x.
67. Csanaky I, Gregus Z. Role of glutathione in reduction of arsenate and of γ-glutamyltranspeptidase in disposition of arsenite in rats. Toxicology. 2005; 207(1):91-104. DOI: 10.1016/j.tox.2004.09.002.
68. Flora SJ. Arsenic-induced oxidative stress and its reversibility. Free Radical Biology and Medicine. 2011 Jul 15;51(2):257-81. DOI: 10.1016/j.freeradbiomed.2011.04.008.
69. Staal FJ, Ela SW. Glutathione deficiency and human immunodeficiency virus infection. The Lancet. 1992; 339(8798):909-12. DOI: 10.1016/0140-6736(92)90939-z.
70. Baumgardner JN, Shankar K, Hennings L, Albano E, Badger TM, Ronis MJ. N-acetylcysteine attenuates progression of liver pathology in a rat model of nonalcoholic steatohepatitis. The Journal of nutrition. 2008; 138(10):1872-9. DOI: 10.1093/jn/138.10.1872.
71. Soni RA, Sudhakar K, Rana RS. Spirulina–From growth to nutritional product: A review. Trends in food science & technology. 2017; 69:157-71. DOI: 10.1016/j.tifs.2017.09.010.
72. Fernández-Rojas B, Hernández-Juárez J, Pedraza-Chaverri J. Nutraceutical properties of phycocyanin. Journal of functional foods. 2014; 11:375-92. DOI: 10.1016/j.jff.2014.10.011.
73. Bhattacharjee SU, Pal SU. Additive protective effects of selenium and vitamin E against arsenic induced lipidemic and cardiotoxic effects in mice. Int J Pharm Pharm Sci. 2014; 6(5):406-13. ISSN-0975-1491.
74. Zheng J, Inoguchi T, Sasaki S, Maeda Y, McCarty MF, Fujii M, et al., Phycocyanin and phycocyanobilin from Spirulina platensis protect against diabetic nephropathy by inhibiting oxidative stress. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2013; 304(2):R110-20. DOI: 10.1152/ajpregu.00648.2011.
75. Pal PB, Ghosh S, Sil PC. Beneficial effect of naturally occurring antioxidants against oxidative stress–mediated organ dysfunctions. Bioactive Natural Products: Chemistry and Biology. 2014:199-240. DOI: 10.1002/9783527684403.ch8.
76. Sinha M, Manna P, Sil PC. Protective effect of arjunolic acid against arsenic-induced oxidative stress in mouse brain. Journal of Biochemical and Molecular Toxicology. 2008; 22(1):15-26. DOI: 10.1002/jbt.20209.
77. Gupta SC, Patchva S, Koh W, Aggarwal BB. Discovery of curcumin, a component of golden spice, and its miraculous biological activities. Clinical and experimental pharmacology and physiology. 2012; 39(3):283-99. DOI: 10.1111/j.1440-

1681.2011.05648.x.
78. Wang JB, Qi LL, Zheng SD, Wu TX. Curcumin induces apoptosis through the mitochondria-mediated apoptotic pathway in HT-29 cells. Journal of Zhejiang University Science B. 2009; 10:93-102. DOI: 10.1631/jzus.B0820238.
79. Raish M. Momordica charantia polysaccharides ameliorate oxidative stress, hyperlipidemia, inflammation, and apoptosis during myocardial infarction by inhibiting the NF-κB signaling pathway. International journal of biological macromolecules. 2017; 97:544-51. DOI: 10.1016/j.ijbiomac.2017.01.074.
80. Chao CY, Huang CJ. Bitter gourd (Momordica charantia) extract activates peroxisome proliferator-activated receptors and upregulates the expression of the acyl CoA oxidase gene in H4IIEC3 hepatoma cells. Journal of Biomedical Science. 2003; 10:782-91. DOI: 10.1159/000073966.
81. Sanders ME. Probiotics: considerations for human health. Nutrition reviews. 2003; 61(3):91-9. DOI: 10.1301/nr.2003. marr.91-99.
82. Mishra V, Shah C, Mokashe N, Chavan R, Yadav H, Prajapati J. Probiotics as potential antioxidants: a systematic review. Journal of agricultural and food chemistry. 2015; 63(14):3615-26. DOI: 10.1021/jf506326t.
83. Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 1998; 7(4):351-4. PMID: 9568793.
84. Yang F, Oz HS, Barve S, De Villiers WJ, McClain CJ, Varilek GW. The green tea polyphenol (–)-epigallocatechin-3-gallate blocks nuclear factor-κB activation by inhibiting IκB kinase activity in the intestinal epithelial cell line IEC-6. Molecular Pharmacology. 2001; 60(3):528-33. PMID: 11502884.