CARDIOPROTECTIVE AND NEPHROPROTECTIVE EFFECTS OF VITAMIN E IN MALE WISTAR RATS EXPOSED TO SODIUM ARSENITE

BY

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CERTIFICATION

We the undersigned hereby certify that ADEBAYO AYOMIDE SUCCESS (BMS2101366) carried out this research in the department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

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DEDICATION

I dedicate this project book to Almighty God, with whom strength has seen me through the whole process of the work. Also to my parents and siblings whose love, support and care has guided me.

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ABSTRACT

The disruption of cardiovascular and renal function by environmental toxicants remains a major concern in toxicological research, particularly when such agents interfere with biochemical markers essential for assessing organ integrity. Among these toxic agents, exposure to sodium arsenite poses a serious threat due to its ability to induce acute myocardial injury and impair renal filtration processes. This study evaluated the potential of vitamin E to counteract these toxic effects in male Wistar rats, focusing exclusively on validated clinical biomarkers of cardiac and renal injury. Rats exposed to sodium arsenite (Group C) showed a pronounced rise in cardiac troponin (59.24 ± 3.8 pg/mL) and CK-MB (62.30 ± 2.1 U/L), indicating significant myocardial cell damage relative to the control group. The nephrotoxic impact was equally evident, as reflected by elevated serum urea (24.49 ± 1.8 mg/dL) and creatinine (1.98 ± 0.1 mg/dL), demonstrating impaired glomerular filtration and reduced renal functional capacity.

Administration of vitamin E showed a clear dose-dependent protective effect. Rats receiving 25 mg/kg (Group D) and 50 mg/kg (Group E) of vitamin E along with sodium arsenite experienced significant reductions in cardiac troponin (48.65 ± 2.2 and 40.04 ± 2.9 pg/mL) and CK-MB (41.17 ± 2.9 and 31.02 ± 1.2 U/L), indicating decreased myocardial damage. Renal biomarkers also improved, with urea decreasing to 16.08 ± 1.6 and 10.20 ± 1.7 mg/dL, and creatinine dropping to 1.08 ± 0.1 and 0.88 ± 0.1 mg/dL in Groups D and E, respectively. These results highlight vitamin E's ability to protect both cardiac and renal functions despite ongoing toxic exposure. Overall, this study shows that vitamin E provides significant cardioprotective and nephroprotective effects, mainly by normalizing key biomarkers of myocardial and renal dysfunction in rats exposed to sodium arsenite.

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INTRODUCTION

1.0 Background of Study

Arsenic contamination remains a major global health concern, particularly in regions where industrial activities, agricultural practices, or natural geological deposits contribute to elevated levels of the toxin in water and soil. Sodium arsenite, one of the most toxic inorganic forms of arsenic, is widely recognized for its ability to induce oxidative stress and disrupt normal cellular physiology (WHO, 2018). Once absorbed, arsenic interferes with mitochondrial function, promotes the generation of reactive oxygen species (ROS), and impairs antioxidant defense systems, ultimately causing significant tissue injury (Jomova *et al.,* 2011).

The cardiovascular and renal systems are among the primary targets of arsenic toxicity because of their high metabolic activity and continuous exposure to circulating toxicants. Arsenic-induced oxidative stress has been linked to myocardial cell injury, altered membrane integrity, impaired contractility and the release of cardiac biomarkers such as CK-MB and cardiac troponin (Méndez-Armenta, 2011). Similarly, the kidneys suffer considerable functional decline due to glomerular and tubular damage, leading to elevated levels of urea and creatinine in systemic circulation (ATSDR, 2022). These biochemical changes provide measurable indicators of cardiac and renal dysfunction in arsenic-exposed organisms.

The use of antioxidants in mitigating toxin-induced tissue damage has gained significant attention. Vitamin E, a potent lipid-soluble antioxidant, plays a critical role in inhibiting lipid peroxidation, stabilizing cell membranes, and neutralizing free radicals (Atkinson, 2007). Its protective functions extend to various organs, making it a promising candidate for reducing oxidative stress-related injuries. Several studies have demonstrated that vitamin E supplementation can attenuate biochemical and structural changes induced by heavy metals and environmental toxins (Khan *et al.,* 2015). Given its strong antioxidant properties, vitamin E is hypothesized to counteract arsenic-induced toxicity by restoring normal physiological balance and protecting vital organs.

This study, therefore, explores whether vitamin E can offer meaningful protection against sodium arsenite-induced cardiac and renal dysfunction, using biomarkers of organ injury as major indicators of outcome.

1.1 Justification of the Study

Arsenic exposure remains widespread, particularly in low and middle-income regions, where contamination of drinking water and agricultural produce continues to pose significant health risks (WHO, 2018). Chronic exposure has been associated with increased rates of cardiovascular disorders, kidney disease, hypertension, neurological deficits, and certain cancers. Despite growing awareness of these risks, effective preventive and therapeutic strategies remain limited.

Vitamin E is capable of reversing or minimizing oxidative damage. However, the degree to which vitamin E can protect the heart and kidneys against sodium arsenite, especially at varying doses, has not been fully established. This makes it necessary to examine whether vitamin E can meaningfully modulate biomarkers of cardiac and renal injury in arsenic-exposed organisms.

Understanding this protective effect is essential for several reasons. First, it may contribute to improved management of arsenic toxicity, especially in populations with ongoing exposure. Second, it may strengthen scientific evidence supporting antioxidant therapy as an accessible and cost-effective intervention. Finally, insights gained from this study may support future applications of vitamin E in clinical toxicology and pharmaceutical research.

1.2 Aim of the Study

This study aims to evaluate the cardioprotective and nephroprotective effects of vitamin E in male Wistar rats exposed to sodium arsenite.

1.3 Objectives of the Study

The specific objectives of this study are to:

1. Evaluate the effects of sodium arsenite on cardiac biomarkers, specifically CK-MB and cardiac troponin**.**
2. Determine the impact of sodium arsenite on renal function, using urea and creatinin**e** levels as indicators.
3. Assess the ability of vitamin E to reduce arsenic-induced alterations in cardiac and renal biomarkers.
4. Compare the protective effects of different doses of vitamin E to establish whether its action follows a dose-dependent pattern.

CHAPTER TWO

LITERATURE REVIEW

2.0 Sodium Arsenite

Sodium arsenite (chemical formula: NaAsO2) is a water-soluble inorganic arsenic compound that exists as the sodium salt of arsenous acid. It is one of the forms of arsenic commonly encountered in environmental contamination, industrial processes, and toxicology research. Understanding sodium arsenite is important because arsenic remains a global public health concern as it contaminates drinking water in many regions, is found in certain industrial effluents, and can cause serious acute and chronic health effects even at low concentrations. They are primarily used as a pesticide, but has other uses such as hide preservative, antiseptic, dyeing, and soaps.

Sodium arsenite can be inhaled or absorbed through the skin. Along with its known carcinogenic and teratogenic effects, contact with the substance can yield symptoms such as skin irritation, burns, itching, thickened skin, rash, loss of pigment, poor appetite, a metallic or garlic taste, stomach pain, nausea, vomiting, diarrhea, convulsions, decreased blood pressure, and headache. Severe acute poisoning may lead to nervous system damage resulting in weakness, poor coordination, or “pins and needles” sensations, eventual paralysis, and death.

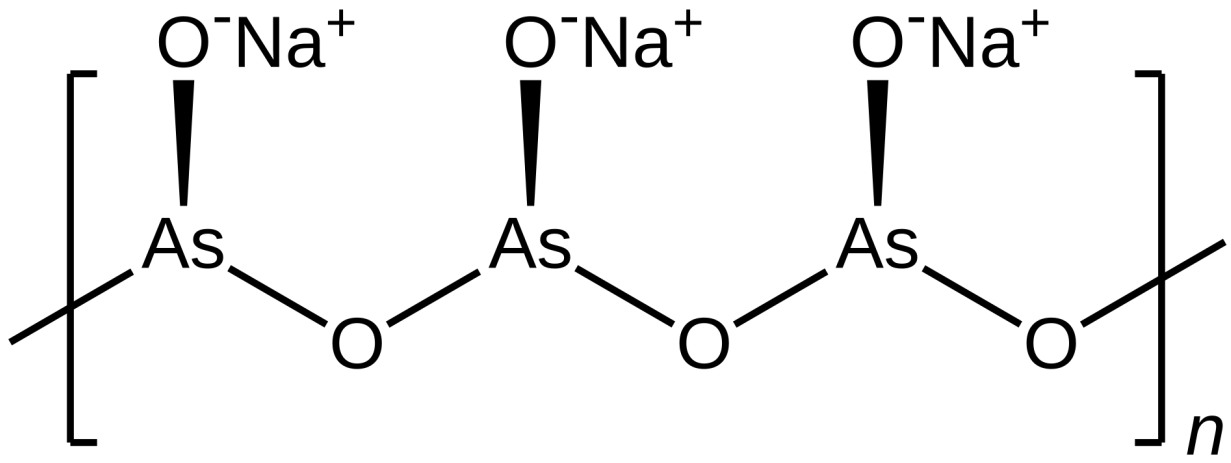

Fig 1: Sodium Arsenite ($NaAsO_2$) chemical structure

2.0.1 Chemical Nature and Sources

Sodium arsenite ($NaAsO_2$) is an inorganic compound that represents one of the most common and toxic forms of trivalent arsenic (As^{3+}). Chemically, it is the sodium salt of arsenious acid (H_3AsO_3) and is classified among the arsenite salts. It appears as a white or grayish powder that is highly soluble in water and slightly soluble in alcohol. Its solubility in aqueous medium facilitates the formation of various arsenite ions depending on the pH of the environment. In neutral or weakly acidic conditions, arsenious acid (H_3AsO_3) predominates, while in alkaline conditions, deprotonation occurs to form arsenite ions such as $H_2AsO_3^-$ or AsO_3^{3-}. Structurally, sodium arsenite contains arsenic in the +3 oxidation state, which is responsible for its high reactivity and toxicity. The arsenic atom is coordinated by three oxygen atoms in a trigonal pyramidal geometry, a structure that allows it to interact strongly with sulfhydryl (-SH) groups in proteins. This interaction is one of the biochemical bases of arsenic toxicity, as it can inhibit critical enzyme systems such pyruvate dehydrogenase complex, alpha-ketoglutarate dehydrogenase complex, thioredoxin dehydrogenase, gluthatione reductase e.t.c., involved in cellular respiration and antioxidant defense (Mandal and Suzuki, 2002). The compound has a molar mass of 129.91 g/mol and melts at approximately 315°C, when heated, it decomposes, releasing toxic arsenic oxides and sodium oxide vapors.

Sodium arsenite is readily oxidized to sodium arsenate (Na_2HAsO_4) when exposed to air or oxidizing agents. This oxidation is significant because arsenic exists in nature primarily in two oxidation states: arsenite [As(III)] and arsenate [As(V)]. Of these, arsenite compounds are generally more toxic, more mobile in groundwater, and more bioavailable than arsenate species. The As(III) oxidation state of sodium arsenite allows it to bind strongly to thiol groups in cysteine residues of enzymes, thereby interfering with pyruvate dehydrogenase, glutathione reductase, and other key metabolic enzymes. This mechanism underlies its use in research as a potent oxidative stress inducer and a model xenobiotic for studying metal toxicity in animal and cellular systems (Flora, 2011).

From a chemical standpoint, sodium arsenite behaves as a weak base and can react with acids to liberate arsenious acid. It is deliquescent, meaning it can absorb moisture from the atmosphere, forming aqueous solutions that remain highly toxic and environmentally persistent. Its chemical stability under reducing conditions contributes to its accumulation in soils and sediments where oxygen availability is limited. Moreover, it can form complexes with organic ligands, further influencing its transport and bioavailability in environmental systems (Kinniburgh, 2002).

Sodium arsenite occurs both from natural and anthropogenic (human) sources. Naturally, arsenic is a metalloid found in over 200 mineral forms, mainly as sulfides and oxides such as arsenopyrite (FeAsS), realgar (As_4S_4), and orpiment (As_2S_3). Through the natural weathering of these minerals, volcanic emissions, and geothermal activity, arsenic compounds including arsenite species are released into soil, groundwater, and sediments. In groundwater systems, arsenite is the dominant form under reducing (oxygen-poor) conditions, while arsenate prevails under oxidizing conditions. Hence, in many parts of the world (e.g., Bangladesh, India, and parts

of Nigeria), geogenic processes involving arsenic-bearing minerals are major contributors to arsenic contamination in drinking water (Mukherjee *et al.,* 2006). Anthropogenically, sodium arsenite is produced for industrial and laboratory purposes. It is synthesized by reacting arsenic trioxide (As_2O_3) with sodium hydroxide (NaOH) according to the reaction: $\mathbf{As2O3 + 2NaOH \rightarrow 2NaAsO2 + H2O}$.

This compound has been historically used as a herbicide, pesticide, wood preservative, and rodenticide due to its strong biocidal properties. In the early and mid-20th century, sodium arsenite-based formulations were widely used in agriculture for controlling weeds and pests in cotton fields, orchards, and vineyards. However, its use has been drastically restricted or banned in most countries because of its persistence and toxicity to humans, animals, and the environment (ATSDR, 2007).

In industrial contexts, sodium arsenite may also be generated as a byproduct of metal smelting, glass manufacturing, and semiconductor production. Effluents from these activities often contain soluble arsenic compounds that contaminate nearby soils and water bodies. Improper waste disposal or accidental release from mining operations can further contribute to the mobilization of arsenite species. In particular, the leaching of tailings from gold and copper mines has been identified as a major source of arsenic pollution in surface and groundwater systems (Ravenscroft *et al.,* 2009).

Additionally, sodium arsenite is frequently employed in scientific research as a standard toxicant or oxidative stress-inducing agent. In toxicological and biomedical studies, it is used to model arsenic-induced neurotoxicity, cardiotoxicity, hepatotoxicity, and nephrotoxicity in laboratory animals such as Wistar rats. Its controlled application allows researchers to understand the biochemical and physiological effects of arsenic exposure and to evaluate the protective effects of antioxidants such as vitamin E and C (Flora, 2011).

Environmental sources also include the oxidation–reduction cycling of arsenic in aquatic and terrestrial ecosystems. Under reducing conditions, microbial activity can convert arsenate [As(V)] to arsenite [As(III)], thereby increasing its solubility and mobility. Agricultural runoff containing phosphate fertilizers can enhance this process because phosphate competes with arsenate for adsorption sites in soils, facilitating arsenite release into groundwater. Consequently, arsenite contamination remains a global concern, especially in regions with intensive mining or irrigation practices (Kinniburgh, 2002).

2.0.2 Metabolism and Mechanism of Action

Once sodium arsenite ($NaAsO_2$) enters the body, it undergoes a series of biochemical transformations aimed at detoxifying it or preparing it for excretion. Sodium arsenite is primarily absorbed through the gastrointestinal tract, skin, and respiratory surfaces, depending on the route of exposure. Because arsenite (As^{3+}) is highly soluble in water, it can readily cross cell membranes, especially by interacting with sulfhydryl (–SH) groups of membrane proteins (Cohen *et al.,* 2013).

After absorption, arsenite is transported in the bloodstream, where it binds strongly to red blood cell proteins and plasma components containing thiol groups. The liver plays a major role in arsenic metabolism. In hepatocytes, inorganic arsenic (As^{3+}) undergoes methylation, which converts it into less toxic and more excretable organic forms. This process is catalyzed by the enzyme arsenic (+3 oxidation state) methyltransferase (AS3MT)**,** using S-adenosylmethionine (SAM) as a methyl donor (Thomas, 2007). The metabolic pathway generally proceeds in two main steps:

a) Arsenite (As^{3+}) is first oxidized to arsenate (As^{5+})**.**
b) The resulting forms are then methylated sequentially to produce monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)**.**

These methylated derivatives are more water-soluble and are therefore readily excreted in urine. However, some intermediate metabolites, particularly **monomethylarsonous acid (MMA^{3+})**, are even more toxic than the parent compound and can cause oxidative damage to tissues (Vahter, 2002).

Although methylation is considered a detoxification mechanism, the generation of these reactive intermediates during the process can contribute to the compound's overall toxicity. In chronic exposure, the liver, kidneys, and other organs may accumulate arsenic, leading to systemic toxicity.

Mechanism of action

The toxic action of sodium arsenite arises mainly from its strong affinity for thiol (–SH) groups present in enzymes and structural proteins. By binding to these sulfhydryl groups, arsenite disrupts the structure and function of several key cellular enzymes, particularly those involved in energy production and antioxidant defense.

One of the primary targets of arsenite is the pyruvate dehydrogenase complex, an enzyme that catalyzes the conversion of pyruvate to acetyl-CoA in the mitochondria. When arsenite binds to the lipoic acid cofactor within this enzyme complex, it inhibits its activity, thereby blocking the tricarboxylic acid (TCA) cycle and impairing cellular respiration (Hughes, 2002). As a result, ATP synthesis is reduced, leading to energy depletion and eventual cell death.

Additionally, sodium arsenite interferes with oxidative phosphorylation by uncoupling the electron transport chain in mitochondria. This leads to the excessive generation of reactive oxygen species (ROS) such as superoxide anions and hydrogen peroxide. These ROS induce oxidative stress, causing lipid peroxidation, DNA strand breaks, and protein oxidation (Flora, 2011).

Arsenite also affects signal transduction pathways and gene expression, altering cellular responses to stress and apoptosis. For instance, it can activate transcription factors such as NF-κB and AP-1, which are linked to inflammation and cell proliferation. Chronic exposure to arsenite has been associated with carcinogenesis, as it promotes genomic instability, disrupts DNA repair processes, and enhances cell transformation (Wallace, 2008).

2.0.3 Toxicological Effects of Sodium Arsenite

Sodium arsenite is one of the most dangerous inorganic forms of arsenic, and its toxic effects have been widely documented in toxicology, public health, and environmental science. Once it enters the body, whether through contaminated water, food, or industrial exposure, it interferes with normal cellular processes and triggers a wide spectrum of harmful physiological changes. The toxicity of sodium arsenite is mainly driven by its ability to generate excessive reactive oxygen species (ROS), disrupt energy metabolism, and damage essential biomolecules (Jomova *et al.,* 2011).

A major toxicological feature of sodium arsenite is its strong capacity to induce oxidative stress**.** Normally, the body produces small amounts of ROS that are neutralized by antioxidants. However, arsenite causes a massive overproduction of ROS, which overwhelms the body's defense system and leads to lipid peroxidation, protein denaturation, and DNA damage (Valko *et al.,* 2005). This oxidative stress is considered the central mechanism through which arsenite harms vital organs. By damaging cell membranes and mitochondria, arsenite disrupts cellular energy production and increases cell vulnerability to apoptosis or necrosis.

The cardiovascular system is one of the primary targets of arsenite toxicity. Studies have shown that sodium arsenite can impair the structural integrity of cardiac muscle cells, alter ion channel functions, and trigger inflammatory responses within the myocardium (Méndez-Armenta, 2011). These changes often manifest as elevated levels of cardiac biomarkers such as CK-MB and cardiac troponin, indicating myocardial cell leakage and injury. Long-term exposure has been linked to hypertension, arrhythmias, atherosclerosis, and even heart failure (Moon *et al.,* 2012). This highlights the significant risk arsenite poses to cardiovascular health.

The kidneys are also highly susceptible to sodium arsenite toxicity because they play a major role in filtering blood and eliminating toxins. Arsenite accumulates in renal tissues, causing glomerular degeneration, tubular necrosis, and impaired reabsorption of essential molecules (ATSDR, 2022). As kidney function deteriorates, there is a marked increase in serum urea and creatinine, which are common indicators of renal impairment. Prolonged arsenite exposure can lead to chronic kidney disease, electrolyte imbalance, and reduced waste-excretion efficiency (Ratnaike, 2003).

Beyond the heart and kidneys, sodium arsenite also affects the nervous system, where it disrupts neurotransmitter balance and reduces neuronal survival. This may result in memory deficits, altered motor coordination, and increased susceptibility to neurodegenerative conditions (Bhattacharya et al., 2013). In the reproductive system, arsenite exposure has been associated with reduced sperm quality, hormonal imbalances, and impaired fertility. Pregnant exposure has been linked to fetal growth restriction and developmental abnormalities (Kumar *et al.,* 2014).

At the molecular level, sodium arsenite binds to sulfhydryl groups in proteins, inhibiting important enzymes involved in metabolism and antioxidant defense (Hughes, 2002). It also affects DNA repair mechanisms, causes chromosomal instability, and alters gene expression through epigenetic modifications such as abnormal DNA methylation (Ren *et al.,* 2011). These

molecular disruptions explain why long-term arsenite exposure is strongly associated with cancers of the skin, bladder, liver, and lungs.

2.0.3.1 Oxidative Stress-Independent Effects

Sodium arsenite, beyond generating oxidative stress, exerts toxicity through several mechanisms that do not directly involve free radicals. One key pathway is the inhibition of sulfhydryl-containing enzymes critical for energy metabolism, such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. By binding to thiol groups, arsenite disrupts mitochondrial ATP production, leading to energy depletion. This impairs essential cellular functions, including ion regulation and membrane stability, and contributes to organ dysfunction in metabolically active tissues such as the heart and kidney (National Research Council, 2001).

In addition, arsenite alters crucial cell signaling pathways that regulate growth, survival, and apoptosis. Sodium arsenite affects kinases, which can induce premature cell death or abnormal proliferation even in the absence of oxidative stress. Furthermore, arsenite triggers epigenetic modifications, including changes in DNA methylation and histone patterns, which disrupt gene expression involved in cell cycle control, stress response, and tissue repair. These alterations compromise the structural and functional integrity of organs, highlighting that arsenic toxicity is not solely dependent on oxidative mechanisms (Andrew *et al.,* 2008).

Sodium arsenite also disrupts cellular ion homeostasis, particularly calcium and sodium handling, which is critical for cardiac and renal function. Abnormal ion flux can lead to cellular swelling, contractile dysfunction, and necrosis, further impairing organ function. Combined with enzyme inhibition, signaling disruption, and epigenetic changes, these effects underscore the multifactorial nature of arsenic toxicity. Understanding these oxidative stress-independent pathways is essential for developing interventions that protect the heart and kidney from arsenite-induced damage (Flora *et al.,* 2012).

2.0.3.2 Dose and Duration Dependent Effects

The toxic effects of sodium arsenite are closely linked to both the dose and the duration of exposure. At low doses or short-term exposure, the body may partially compensate through detoxification mechanisms, including methylation of arsenic in the liver and excretion via the kidneys. However, even at subacute levels, prolonged exposure can accumulate arsenic in target tissues, particularly the kidneys, liver, and heart, leading to progressive organ dysfunction (Flora *et al.,* 2012). This study has shown that repeated exposure to low doses over several weeks can result in elevated levels of renal markers such as urea and creatinine, as well as cardiac biomarkers like CK-MB and troponin, indicating cumulative organ injury (Chattopadhyay *et al.,* 2015).

Higher doses of sodium arsenite produce more immediate and severe toxic effects. In the experimental work carried out on male Wistar rats, acute high-dose exposure causes rapid mitochondrial dysfunction, enzyme inhibition, and structural damage to organs, manifesting as necrosis in kidney tubules and cardiomyocyte injury (National Research Council, 2001). The release of intracellular enzymes such as creatine kinase-MB and cardiac troponin into circulation

occurs faster at higher doses, reflecting the intensity of myocardial injury. Similarly, kidney injury becomes pronounced, with elevated serum urea and creatinine levels, histological alterations, and impaired glomerular filtration. This shows that both the magnitude of arsenite exposure and the vulnerability of target organs determine the severity of toxicity.

Duration of exposure further influences the pattern of damage. Chronic exposure, even at moderate doses, often leads to subtle but cumulative effects, including fibrosis, altered gene expression, and epigenetic modifications in cardiac and renal tissues (Andrew *et al.,* 2008). These long-term changes may not be immediately apparent through biochemical markers but can compromise organ function over time. Conversely, short-term acute exposure produces rapid and over cellular injury, detectable through elevated biomarkers and histological damage.(ATSDR, 2007).

2.0.3.3 Renal Toxicity of Sodium Arsenite

The kidneys are particularly vulnerable because they filter arsenic from the blood, leading to accumulation in renal tubular cells (Sahni *et al.,* 2016). Chronic or high-dose exposure disrupts normal tubular function, causing electrolyte imbalances, reduced glomerular filtration, and impaired waste excretion. This is reflected in elevated serum urea and creatinine levels, which serve as key biomarkers of renal injury. The studies carried out on male Wistar rats have demonstrated that arsenite exposure causes tubular degeneration, glomerular shrinkage, and interstitial inflammation (Chattopadhyay *et al.,* 2015). The mechanism involves direct binding of arsenite to thiol groups in enzymes critical for cellular metabolism and energy production, leading to cellular dysfunction and necrosis. Additionally, arsenite can trigger apoptosis in tubular epithelial cells, further exacerbating kidney injury even before overt clinical signs appear.

Importantly, arsenic-induced nephrotoxicity is often dose and duration-dependent. Short-term, low-level exposure may cause mild tubular changes that are reversible if exposure ceases, whereas chronic exposure results in persistent structural and functional damage (Flora *et al.,* 2012). These toxic effects impair the kidney's ability to maintain homeostasis, contributing to systemic disturbances such as hypertension and fluid retention. The use of biomarkers like urea and creatinine in this study provides a sensitive means to monitor renal injury and evaluate protective interventions, such as supplementation with vitamin E.

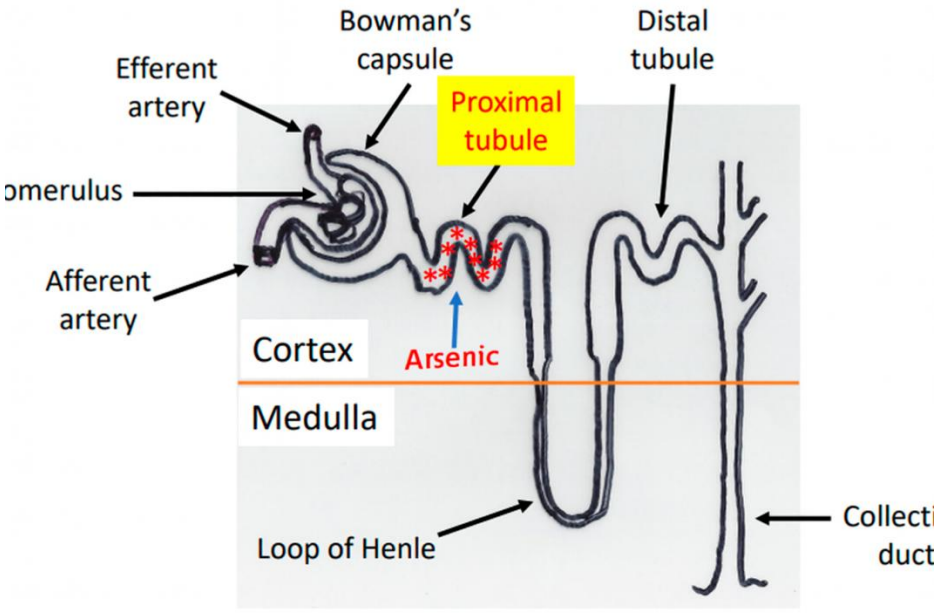

Fig 2: Illustration of Renal Nephron Segments with Arsenic Deposition in the Proximal Tubule

2.0.3.4 Cardiac Toxicity of Sodium Arsenite

Sodium arsenite also has significant cardiotoxic effects, affecting both the structure and function of the heart. Arsenite accumulation in cardiomyocytes disrupts mitochondrial energy metabolism, interferes with enzyme activity, and alters ion homeostasis, particularly calcium handling, which is essential for proper myocardial contraction (Andrew *et al.,* 2008). This disruption can lead to myocyte injury, reflected biochemically by elevated levels of cardiac biomarkers such as creatine kinase-MB (CK-MB) and cardiac troponin. In experimental studies with Wistar rats, arsenite exposure has been shown to cause structural changes, including myocardial fiber degeneration, vacuolization, and inflammatory infiltration (Chattopadhyay *et al.,* 2015).

Arsenic-induced cardiac dysfunction is also mediated by changes in cellular signaling pathways. For example, inhibition of survival pathways like PI3K/Akt increases susceptibility to apoptosis, while dysregulation of mitogen-activated protein kinases (MAPKs) contributes to inflammation and tissue remodeling. Functional consequences include reduced cardiac contractility, arrhythmias, and, in severe cases, heart failure. The cardiotoxic effects are both dose and time-dependent, with higher doses or prolonged exposure producing more extensive myocardial damage (Flora *et al.,* 2012). Measurement of CK-MB and cardiac troponin in serum provides a reliable indicator of myocardial injury and serves as a key outcome in this study evaluating protective interventions such as vitamin E supplementation.

2.1. Vitamin E Structures, Sources and Biological Roles

Vitamin E is a collective term for a group of naturally occurring compounds that possess antioxidant properties and play essential roles in maintaining cellular health. Chemically, vitamin E refers to a family of lipid-soluble compounds consisting of tocopherols and tocotrienols, which share a common chromanol ring structure but differ in the saturation of their side chains (Brigelius-Flohé & Traber, 1999). Among the eight naturally occurring forms (α-, β-, γ-, and δ-tocopherols and tocotrienols), α-tocopherol is the most biologically active and abundant form in human tissues. Structurally, each tocopherol molecule consists of a chromanol head with a hydroxyl group capable of donating hydrogen atoms to neutralize free radicals, and a phytol tail that anchors the molecule into lipid membranes. This dual structure enables vitamin E to act effectively as a chain-breaking antioxidant, particularly in protecting cell membranes from oxidative damage (Traber & Atkinson, 2007).

Vitamin E is mainly obtained through the diet since the human body cannot synthesize it. Natural sources of vitamin E include vegetable oils such as sunflower, safflower, and wheat germ oil, as well as nuts, seeds, whole grains, and green leafy vegetables. Animal products like eggs and butter also contain small amounts. The content of vitamin E in food largely depends on the oil composition and freshness, as the vitamin can be degraded by heat, light, and prolonged storage (Sen *et al.,* 2006). In the body, vitamin E is absorbed in the small intestine together with dietary fats and requires the presence of bile salts for efficient absorption. Once absorbed, it is incorporated into chylomicrons and transported via the lymphatic system to various tissues. The liver plays a central role in distributing α-tocopherol to the bloodstream through a specific protein called the α-tocopherol transfer protein (α-TTP), which ensures that the most active form is selectively retained and delivered to cells (Traber, 1999).

Biologically, the primary function of vitamin E is its antioxidant activity. It protects polyunsaturated fatty acids (PUFAs) in cell membranes from lipid peroxidation by donating a hydrogen atom to lipid radicals, thereby breaking the chain reaction of oxidative damage. In doing so, vitamin E is converted to a relatively stable tocopheroxyl radical, which can later be regenerated to its active form by other antioxidants such as vitamin C and glutathione (Niki, 2012). Vitamin E also participates in several cellular and physiological functions beyond the role it plays as an antioxidant. It helps to maintain membrane integrity and fluidity, modulates gene expression, and influences signal transduction pathways involved in cell growth and immune function. Studies have shown that adequate vitamin E intake supports the immune system, enhances reproductive health, and contributes to neurological protection by preventing oxidative stress in neurons (Burton, 2019).

Deficiency of vitamin E is relatively uncommon but can occur in individuals with fat malabsorption disorders, genetic defects in α-TTP, or extremely low-fat diets. Symptoms of deficiency may include muscle weakness, vision problems, impaired coordination, and neurological disorders due to oxidative damage in nerve tissues (Traber, 2014).

Overall, vitamin E is an essential micronutrient that serves as a first line of defense against oxidative damage in biological membranes. Its antioxidant and non-antioxidant roles contribute significantly to protecting vital organs such as the liver, heart, and brain against oxidative stress and toxic insults, such as those induced by sodium arsenite exposure.

2.1.1 Chemical composition and forms

Chemically, all forms of vitamin E possess a chromanol ring with a hydroxyl group (–OH) that can donate a hydrogen atom to neutralize free radicals, and a hydrophobic isoprenoid side chain that allows the molecule to embed within lipid membranes (Atkinson, 2007). This unique structural combination is what enables vitamin E to function effectively as a lipid-soluble antioxidant within biological systems.

There are eight naturally occurring forms of vitamin E, which are divided into two main groups: tocopherols and tocotrienols. Each group consists of four homologues - alpha (α), beta (β), gamma (γ), and delta (δ) - making up the total eight variants (Brigelius-Flohé, 1999). The difference between tocopherols and tocotrienols lies primarily in the saturation of their side chains. Tocopherols have a saturated phytyl tail, whereas tocotrienols contain an unsaturated isoprenoid tail with three double bonds, which slightly alters their physical properties and biological activities (Sen *et al.,* 2006). Among these, α-tocopherol is the most biologically active and predominant form in human and animal tissues. It is selectively retained and distributed by the α-tocopherol transfer protein (α-TTP) in the liver, which explains why it is considered the main form of vitamin E with physiological significance (Traber, 2014). Other forms, such as γ-tocopherol and tocotrienols, also exhibit potent antioxidant properties, but their distribution and metabolic fates differ from that of α-tocopherol.

Each form of vitamin E differs in the number and position of methyl groups on the chromanol ring, which influences its antioxidant potency and affinity for biological membranes. For instance, α-tocopherol has three methyl groups on the chromanol ring, while β-, γ-, and δ-forms have fewer. These subtle structural differences affect how efficiently each variant reacts with free radicals and interacts with other biomolecules (Burton & Traber, 1990). In addition to the naturally occurring forms, there are also synthetic forms of vitamin E, commonly known as all-rac-α-tocopherol (previously called DL-α-tocopherol). This synthetic variant is a racemic mixture of eight stereoisomers and is often used in supplements and fortified foods. However, the natural form (RRR-α-tocopherol) has been found to have higher biological activity because it is preferentially recognized by α-TTP and retained more efficiently in body tissues (Traber, 1996).

2.1.2 Dietary sources and absorption

Vitamin E is an essential nutrient that must be obtained through the diet, as the human body cannot synthesize it. It is widely distributed in nature, particularly in plant-based foods, and its concentration in different foods largely depends on the oil content and the degree of processing or storage conditions. Among the natural sources, vegetable oils such as wheat germ oil, sunflower oil, safflower oil, olive oil, and palm oil are the richest sources of vitamin E, especially in the form of α-tocopherol (Sen *et al.,* 2006). Other good sources include nuts (such as almonds, hazelnuts, and peanuts), seeds (like sunflower and pumpkin seeds), and whole grains.

Green leafy vegetables such as spinach, broccoli, and kale also contain moderate amounts of vitamin E, although their levels are generally lower compared to oils and nuts. In addition, some animal-derived foods like eggs, butter, and fish provide smaller quantities of vitamin E. However,

the majority of the body's requirement is fulfilled through plant-based foods and vegetable oils. The vitamin content of foods can decrease during cooking, storage, and exposure to light or air, since vitamin E is sensitive to oxidation (Brigelius-Flohé, 1999).

In dietary supplements and fortified foods, vitamin E is often added as either natural α-tocopherol (RRR-α-tocopherol) or synthetic all-rac-α-tocopherol (DL-α-tocopherol). Although both forms are active, the natural form has greater bioavailability because it is more efficiently recognized and retained by the liver's α-tocopherol transfer protein (α-TTP).

Food Group	**Specific sources**
Vegetable oils	Wheat germ oil, sunflower oil, safflower oil, almond oil, olive oil, corn oil, soybean oil.
Nuts and Seeds	Sunflower seeds, almonds, hazelnuts, peanuts, pine nuts, Brazil nuts.
Vegetables	Spinach (boiled), broccoli, red sweet pepper, turnip greens, swiss chard, butternut squash, asparagus, fresh tomatoes.
Fruits	Avocado, mango, kiwi fruit, blackberries, mamey sapote, black currants, pulp.
Animal products and seafood	Abalone, goose meat, Atlantic salmon, rainbow trout, fish roe, eggs.
Fortified foods	Some breakfast cereals, margarines, and fruit juices.

Fig 3: Vitamin E food sources

The absorption of vitamin E occurs primarily in the small intestine, where it follows the same pathway as dietary fats. For effective absorption, the presence of bile salts, pancreatic enzymes, and dietary lipids is required to form micelles, which facilitate the incorporation of vitamin E into the intestinal mucosa (Atkinson, 2007). Once absorbed, vitamin E is packaged into chylomicron lipoprotein particles that transport dietary fats and are released into the lymphatic system before entering the bloodstream.

After reaching the circulation, vitamin E is taken up by the liver, where different forms are metabolized and sorted. The liver selectively retains α-tocopherol, the most biologically active form, through the specific binding of α-tocopherol transfer protein (α-TTP). Other forms of vitamin E, such as γ- and δ-tocopherols, are rapidly metabolized and excreted through bile or urine (Traber, 2014). The α-TTP-mediated transport ensures that α-tocopherol is distributed to

various tissues, including the brain, heart, liver, and reproductive organs, via lipoproteins like low-density lipoproteins (LDL) and high-density lipoproteins (HDL).

The efficiency of vitamin E absorption can vary depending on several factors, including dietary fat content, digestive health, and bile production. Conditions that impair fat absorption, such as cholestasis, cystic fibrosis, or pancreatic insufficiency, can lead to vitamin E deficiency even when dietary intake is adequate (Niki , 2012). Vitamin E is mainly derived from plant-based foods rich in oils, nuts, and seeds, and its absorption in the intestine depends on the presence of fats and bile salts. The liver's ability to selectively retain α-tocopherol plays a key role in regulating vitamin E status and ensuring its delivery to vital organs where it performs its antioxidant and protective functions.

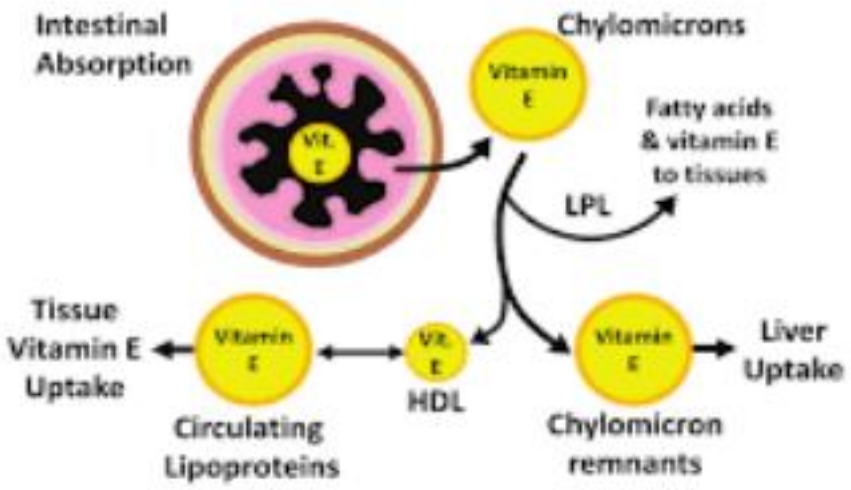

Fig 4: Biological diagram illustrating the absorption and metabolism of vitamin E

2.1.3 Physiological and Pharmacological Roles

Vitamin E is a lipid-soluble antioxidant that plays a central role in maintaining cellular integrity and protecting tissues from oxidative damage. Its primary physiological function is to scavenge reactive oxygen species (ROS) and prevent lipid peroxidation of polyunsaturated fatty acids in cell membranes. By neutralizing free radicals, vitamin E protects structural components of cells, including membrane phospholipids, proteins, and nucleic acids, from oxidative injury. This is particularly important in organs with high metabolic activity, such as the heart and kidneys, which are susceptible to oxidative stress induced by toxic compounds like sodium arsenite (Traber & Atkinson, 2007).

In the cardiovascular system, vitamin E stabilizes cellular membranes, reduces oxidative stress, and modulates inflammatory pathways, thereby contributing to cardioprotective effects. It has

been shown to decrease the levels of biomarkers associated with cardiac injury, such as creatine kinase (CK) and lactate dehydrogenase (LDH), and to preserve myocardial tissue integrity in animal models exposed to oxidative stress (Yuan *et al.,* 2014). Vitamin E also influences platelet aggregation and vascular function, which further supports its role in maintaining cardiovascular health.

In the renal system, vitamin E prevents nephrotoxicity by enhancing the activity of endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase. These enzymes work synergistically with vitamin E to neutralize ROS and reduce lipid peroxidation in kidney tissues, thereby protecting glomeruli and renal tubules from damage (Aziz et al., 2013). Vitamin E's role in maintaining renal cellular homeostasis is critical when tissues are exposed to environmental toxins like sodium arsenite, which can induce oxidative stress, inflammation, and apoptosis in renal cells.

Pharmacologically, vitamin E exhibits dose-dependent therapeutic effects, with experimental studies demonstrating its ability to ameliorate oxidative damage, reduce inflammatory markers, and improve organ function in models of toxicity. Its transport involves absorption in the small intestine, incorporation into chylomicrons, and distribution through lipoproteins to target organs, ensuring its bioavailability in tissues at risk (Brigelius-Flohé & Traber, 1999). By maintaining a balance between ROS production and antioxidant defense, vitamin E serves as both a protective and regulatory molecule, making it a promising agent for mitigating the cardiotoxic and nephrotoxic effects of sodium arsenite.

2.1.3.1 Membrane Stabilization

One of the most important physiological roles of vitamin E is its ability to stabilize cell membranes. Being a fat-soluble compound, vitamin E integrates into the lipid bilayer of cell membranes, where it protects the structural integrity of phospholipids and prevents excessive membrane permeability. In tissues such as the heart and kidney, this stabilization is particularly vital because it prevents the leakage of critical intracellular components like creatine kinase-MB and cardiac troponin from cardiomyocytes, as well as enzymes from renal tubular cells. By maintaining membrane fluidity, vitamin E ensures that cells can respond to mechanical and chemical stress without structural compromise. Additionally, mitochondrial membranes benefit from this stabilization, preserving energy production and preventing premature cell injury or death.

2.1.3.2 Anti-inflammatory Properties

Vitamin E also has significant anti-inflammatory and immunomodulatory effects. It modulates the expression of pro-inflammatory cytokines and reduces the activity of enzymes involved in inflammation, such as cyclooxygenase. In experimental studies, supplementation with vitamin E has been shown to reduce tissue infiltration by inflammatory cells and lower markers of inflammation in both cardiac and renal tissues exposed to toxins, such as sodium arsenite (Meydani *et al.,* 1998). This anti-inflammatory property helps prevent secondary tissue injury caused by the body's immune response. In essence, vitamin E not only protects cells directly but also reduces collateral damage resulting from inflammation, thereby preserving organ function.

2.1.3.3 Modulation of Cell Death Pathways

Another critical pharmacological role of vitamin E is its ability to modulate cell death pathways. Vitamin E influences apoptosis-related signaling, including the balance between pro-apoptotic and anti-apoptotic factors, helping to prevent unnecessary cell loss in stressed tissues. In the heart, this can prevent the excessive death of cardiomyocytes, thereby maintaining contractile function. Meanwhile, in the kidney, it protects tubular epithelial cells, preserving glomerular filtration and tubular reabsorption (Saeed *et al.,* 2022). This modulation of cell survival pathways complements vitamin E's structural and anti-inflammatory roles, contributing to overall organ protection.

2.1.3.4 Role in Ion Homeostasis and Mitochondrial Function

Vitamin E plays a crucial role in maintaining ion homeostasis, particularly calcium, in cardiomyocytes and renal cells. Proper calcium regulation is essential for cardiac contraction and relaxation cycles, as well as for signaling pathways in kidney cells. By preserving membrane integrity and modulating cellular pathways, vitamin E helps prevent calcium overload, which can lead to contractile dysfunction or cell death. Additionally, vitamin E supports mitochondrial function by maintaining membrane stability and energy production. Efficient mitochondrial activity is critical for ATP synthesis, which powers cellular processes in both heart and kidney tissues, ensuring that these organs function optimally even under toxic stress (Flora *et al.,* 2012).

2.2. Nephrotoxic Effects of Sodium Arsenite

Sodium arsenite ($NaAsO_2$) is a highly toxic metalloid that significantly compromises kidney function due to the kidneys' essential roles in filtering blood, eliminating metabolic wastes, regulating electrolyte balance, and maintaining fluid homeostasis. The kidneys' high metabolic rate and rich vascularization make them particularly vulnerable to arsenic accumulation and its toxic effects. Upon exposure, sodium arsenite disrupts the delicate balance between reactive oxygen species (ROS) and the body's natural antioxidant defenses, leading to oxidative stress. This oxidative imbalance targets lipids, proteins, and nucleic acids, impairing cellular integrity and kidney function (Tchounwou *et al.,* 2003). In addition to oxidative damage, arsenic can bind to thiol groups of key enzymes and proteins, altering their structure and function, which further contributes to renal dysfunction.

One of the critical consequences of sodium arsenite exposure is the impairment of renal filtration, tubular transport, and solute reabsorption. Excess ROS generated by arsenic interferes with Na^+/K^+-ATPase and other ion transporters, which are vital for electrolyte and water homeostasis in the nephron. Disruption of these mechanisms leads to imbalances in sodium, potassium, and chloride, resulting in fluid retention or loss, electrolyte disturbances, and systemic toxicity. Furthermore, arsenic interferes with mitochondrial function, reducing ATP production in renal cells. Since active tubular reabsorption and secretion are energy-dependent processes, decreased ATP availability weakens the kidney's ability to concentrate urine and reabsorb essential solutes, contributing to the accumulation of metabolic wastes such as urea, creatinine, and blood urea nitrogen (BUN) in the bloodstream (Kumar *et al.,* 2015). These functional impairments manifest as compromised renal excretory capacity, potentially leading to systemic metabolic imbalances.

Sodium arsenite also disrupts cellular signaling pathways that regulate antioxidant defenses, inflammation, and cell survival within the kidneys. By altering the expression and activity of transcription factors such as Nrf2, which regulates antioxidant response, and NF-κB, which modulates inflammation, arsenic amplifies oxidative stress and promotes apoptosis in renal cells (Jomova, 2011). Additionally, arsenic-induced oxidative stress impairs renal microvascular function, reducing glomerular filtration rate (GFR) and renal perfusion. It can also interfere with nitric oxide (NO) bioavailability, which is critical for maintaining vascular tone and renal blood flow, further exacerbating functional deficits. Chronic exposure can progressively weaken renal adaptive mechanisms, impair fluid and electrolyte balance, and increase susceptibility to systemic complications, including hypertension and cardiovascular strain.

Beyond oxidative stress, sodium arsenite interferes with hormonal regulation of kidney function. Arsenic has been shown to alter the renin-angiotensin-aldosterone system (RAAS), which regulates sodium and water balance, and may disrupt antidiuretic hormone (ADH) signaling, leading to further fluid imbalance and compromised urine concentration. Combined, these effects contribute to a multifactorial nephrotoxicity characterized by reduced excretory efficiency, impaired electrolyte homeostasis, energy depletion, and increased vulnerability to systemic metabolic disturbances. This underscores the importance of protective strategies, such as antioxidant supplementation with vitamin E, which can mitigate oxidative damage, restore cellular energy balance, and improve renal functional outcomes (Aziz *et al.,* 2013).

2.2.1 Mechanisms of Renal Injury

Sodium arsenite targets the kidney through multiple, converging mechanisms that together impair renal structure and function. The proximal tubule is the primary site of injury because it performs heavy reabsorption and concentrates toxicants, exposing its epithelial cells to high intracellular arsenic levels. Inside these cells, arsenite binds to thiol (-SH) groups on metabolic enzymes and transport proteins, disrupting mitochondrial enzyme complexes (for example pyruvate dehydrogenase) and impairing ATP production; energy failure quickly compromises active transport and cell viability (Robles-Osorio, 2015). Independent of energetic collapse, arsenite perturbs intracellular signalling (e.g., PI3K/Akt, MAPKs), promotes apoptotic programs, and alters autophagy, all of which lead to cell death or dysfunctional repair (Concessao, 2025). Arsenic also provokes endothelial and tubular inflammation: injured epithelial cells and infiltrating immune cells release cytokines and proteases that worsen tissue damage and favor fibrosis with time. Finally, defects in arsenic biotransformation (methylation pathway) or impaired urinary excretion increase local retention of toxic arsenic species, amplifying renal injury.

2.2.2 Biomarkers of Kidney Injury (urea, creatinine)

Urea and creatinine are the two most widely used biochemical markers for assessing renal function, primarily because of their strong relationship with glomerular filtration efficiency. Urea is the end-product of protein and amino acid catabolism, produced in the liver through the urea cycle and eliminated almost entirely by the kidneys through glomerular filtration. Under normal conditions, blood urea levels remain stable; however, when the kidneys are impaired, the filtration of urea declines, leading to elevated serum urea concentrations. This rise serves as an

early indicator of compromised renal clearance and is commonly associated with conditions such as acute kidney injury, dehydration, or toxicant exposure, including heavy metals like sodium arsenite, which impair filtration and tubular function (Adeyemi *et al.,* 2020).

Creatinine, on the other hand, is a more specific and reliable biomarker of renal injury because it is derived from the constant, non-enzymatic breakdown of muscle creatine phosphate. Creatinine production remains largely unaffected by dietary or metabolic fluctuations, making it a stable indicator of renal health. In a healthy kidney, creatinine is freely filtered by the glomerulus with minimal tubular reabsorption; therefore, even mild declines in glomerular filtration rate (GFR) lead to measurable increases in serum creatinine. Elevated creatinine levels are strongly associated with nephrotoxicity induced by agents such as sodium arsenite, which damages tubular cells and decreases filtration efficiency, resulting in the accumulation of creatinine in the bloodstream (Johnson *et al.,* 2019).

Together, urea and creatinine provide a comprehensive biochemical evaluation of renal dysfunction, especially in toxicological studies examining nephrotoxicants. While urea is more sensitive to external factors such as dietary protein intake and hydration, creatinine is more specific due to its stable production rate and direct relationship with GFR. In experimental toxicity models, including those involving sodium arsenite exposure, simultaneous elevation of both markers is often used to confirm kidney injury, correlating with histological evidence of tubular necrosis, glomerular shrinkage, or interstitial inflammation. These biomarkers therefore, remain indispensable tools for evaluating functional impairment, monitoring disease progression, and assessing the protective efficacy of agents such as vitamin E in nephroprotection study (Rahman *et al.,* 2020).

2.2.3 Structural and functional changes in kidney tissue

Histologically, arsenite exposure produces a reproducible spectrum of renal lesions that correlate with biochemical changes. Acute high-dose exposure commonly causes proximal tubular epithelial cell swelling, vacuolization, cytoplasmic degeneration, epithelial necrosis and luminal cast formation; glomeruli may show collapse or shrinkage and Bowman's space dilation in severe injury. Chronic exposure more often yields tubular atrophy, interstitial inflammation, tubular basement membrane thickening and progressive interstitial fibrosis changes that underlie chronic kidney disease (CKD) phenotypes in population studies (Robles-Osorio, 2015). Functionally, these structural lesions manifest as reduced GFR, impaired tubular reabsorption and secretion (manifesting as electrolyte disturbances and impaired urine concentrating ability), and proteinuria when glomerular structures become involved. Animal histology reports of sodium arsenite specifically describe congested blood vessels, proximal tubular degeneration, epithelial desquamation, inflammatory infiltration, and focal fibrosis findings that parallel raised urea/creatinine in those models.

2.2.4 Dose and Time-Dependent Nephrotoxicity

Severity and pattern of arsenite nephrotoxicity are strongly dose- and duration-dependent. High**,** short-term doses often produce acute tubular necrosis with rapid rises in serum urea and creatinine and overt histologic necrosis. By contrast, repeated low-to-moderate doses accumulate

and produce cumulative tubular and interstitial damage: mitochondrial dysfunction, chronic inflammation, and maladaptive repair that progress to fibrosis and long-term renal impairment (Flora *et al.,* 2012). Time also shapes biomarker profiles serum creatinine may lag behind histological injury in subacute exposures, while urinary markers and histology reveal early tubular stress. Importantly, recovery potential depends on exposure pattern: acute, limited exposures may allow tubular regeneration and partial recovery, whereas chronic exposures favor irreversible scarring and CKD risk.

2.3. Cardiovascular Effects of Sodium Arsenite

The cardiovascular system is highly sensitive to environmental toxins due to the heart and blood vessels' constant metabolic demands and critical role in maintaining systemic homeostasis. Exposure to toxic compounds such as sodium arsenite ($NaAsO_2$) has been shown to compromise cardiac function through a combination of oxidative stress, endothelial dysfunction, mitochondrial impairment, and disruption of ion homeostasis. One of the primary mechanisms of arsenic-induced cardiotoxicity is the generation of reactive oxygen species (ROS). Excess ROS overwhelms endogenous antioxidant defenses, resulting in the peroxidation of membrane lipids, oxidative modification of proteins, and DNA damage within cardiomyocytes. This oxidative injury not only weakens myocardial cells but also predisposes the heart to functional abnormalities, including impaired contractility, reduced cardiac output, and increased susceptibility to arrhythmias (Rai *et al.,* 2014).

Assessment of cardiac injury in both experimental and clinical settings often relies on biochemical markers, particularly cardiac troponins (cTn) and creatine kinase-MB (CK-MB). Cardiac troponins are highly specific indicators of myocardial cell damage, released into the bloodstream when the integrity of cardiomyocytes is compromised. Elevated cTn levels are therefore considered an early and sensitive marker of cardiac stress or injury. Similarly, CK-MB, an isoenzyme predominantly found in heart muscle, is released upon damage to cardiomyocyte membranes and serves as a reliable indicator of myocardial injury. Studies have demonstrated that arsenic exposure significantly increases circulating levels of both cardiac troponin (cTn) and CK-MB, reflecting subclinical cardiac dysfunction even in the absence of overt structural changes (Kumar et al., 2015). These biomarkers provide measurable evidence of arsenic-induced cardiotoxicity and are essential for understanding the functional consequences of exposure.

Sodium arsenite also disrupts endothelial function, which is central to regulating vascular tone, blood pressure, and tissue perfusion. Oxidative stress reduces the bioavailability of nitric oxide (NO), a key vasodilator, leading to endothelial dysfunction characterized by increased vascular resistance and elevated blood pressure. Impaired endothelium-dependent vasodilation promotes inflammation and adhesion of immune cells to the vascular wall, contributing to vascular rigidity and early atherogenic processes (Navas-Acien *et al.,* 2005). Endothelial damage, combined with ROS-mediated cardiac injury, places additional stress on the myocardium, impairing overall cardiovascular efficiency.

Mitochondrial function in cardiomyocytes is also compromised by arsenic exposure. Disruption of mitochondrial enzymes reduces ATP synthesis, which is essential for maintaining cardiac contractility and ion gradients. Dysfunctional mitochondria further enhance ROS generation, creating a self-perpetuating cycle of oxidative injury. This mitochondrial impairment, along with disruption of calcium and potassium ion channels, can result in arrhythmias, impaired myocardial conduction, and decreased pumping efficiency. Additionally, arsenic exposure can dysregulate neurohormonal systems such as the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system, leading to increased vascular tone, sodium retention, and elevated cardiac workload (Rai *et al.,* 2014).

2.3.1 Mechanisms of Cardiac Injury

Sodium arsenite damages the heart through several interlocking biological processes that converge on cardiomyocyte dysfunction and death. A primary mechanism is mitochondrial disruption: arsenite interferes with mitochondrial enzymes and electron transport, reducing ATP production and triggering the mitochondrial permeability transition that releases pro-apoptotic factors (cytochrome c), thereby activating intrinsic apoptosis pathways (Pace *et al.,* 2017). Closely related is disturbance of cellular signalling arsenite alters kinase cascades such as MAPKs and PI3K/Akt, shifting the balance toward pro-death signalling and impairing cellular repair responses (Ganie *et al.,* 2023). Arsenite also interferes with cardiac ion channels, notably repolarizing potassium currents, which destabilizes electrical activity and predisposes to arrhythmias and QT prolongation; this ion channel block is a distinctive pathway by which arsenic can cause sudden electrical instability even before frank structural damage appears (Wu *et al.,* 2014; Weinberg, 1960). In parallel, arsenite provokes endothelial injury and cardiac inflammation: endothelial dysfunction and local inflammatory mediator release worsen myocardial perfusion and promote remodeling. Finally, arsenite causes DNA damage and impaired repair, which together with oxidative and non-oxidative insults, produce myocyte degeneration, fibrosis and loss of contractile units. These mechanisms are dose- and time-dependent, such that acute high exposures may produce necrosis and arrhythmia, while chronic low exposures favor progressive dysfunction and remodeling.

2.3.2 Biomarkers of cardiac injury (CK-MB, cardiac troponin)

Creatine kinase-MB (CK-MB) and cardiac troponins (cTnI, cTnT) are the principal circulating biomarkers used to detect myocardial injury. CK-MB is an isoenzyme primarily found in myocardium (though present in skeletal muscle to a lesser extent) and rises in serum when membrane integrity is lost; it typically appears within 4-6 hours after injury, peaks at ~24 hours, and returns toward baseline over 48-72 hours depending on injury size and clearance (Peela *et al.,* 2010). Cardiac troponins are structural proteins integral to the contractile apparatus and are highly specific for cardiac myocyte damage; they rise within 3-4 hours after injury, remain elevated longer than CK-MB, and are more sensitive and specific for small amounts of myocardial necrosis (Bachmaier *et al.,* 1995). Mechanistically, both markers are released when sarcolemmal integrity is breached (necrosis) or when irreversible membrane poration occurs during severe stress; minor reversible injury may not raise them.

2.4. Role of vitamin E in Nephroprotection

Sodium arsenite is a highly toxic environmental pollutant that exerts profound effects on renal physiology by disrupting filtration and excretory processes essential for maintaining metabolic balance. In the kidney, the two most clinically relevant biomarkers of renal dysfunction, serum creatinine and serum urea serve as sensitive indicators of how severely arsenic affects glomerular and tubular function. Under normal physiological conditions, creatinine is produced at a stable rate from muscle metabolism and is almost exclusively eliminated through glomerular filtration. Because it is not reabsorbed and minimally secreted, even small increases in serum creatinine indicate a marked decline in glomerular filtration rate (GFR). When sodium arsenite enters the system, it interferes with glomerular membrane integrity, reduces filtration efficiency, and triggers tubular obstruction or degeneration, leading to elevated serum creatinine levels (Mazumder, 2008). Similarly, urea, a metabolic waste product formed from protein breakdown is typically filtered and reabsorbed in a regulated manner in the renal tubules. Arsenic exposure disturbs this balance by damaging both the glomeruli and the tubular epithelium, causing reduced urea clearance and a significant rise in serum urea concentration (Flora, 2011). These elevations in urea and creatinine are hallmark indicators of nephrotoxicity and are widely used to assess the extent of renal injury following exposure to toxicants such as sodium arsenite. The worsening of these markers reflects impaired nitrogenous waste excretion, reduced blood filtration, and an overall decline in renal physiological performance.

Vitamin E provides nephroprotection primarily through its capacity to preserve renal structural integrity and maintain filtration functions that sodium arsenite would otherwise impair. Rather than acting only through classical antioxidant enzyme pathways, vitamin E directly supports membrane stability, cellular survival, and metabolic efficiency within renal tissues factors that are crucial to preserving normal creatinine and urea handling. In arsenic toxicity, elevated creatinine signifies disrupted glomerular filtration, but vitamin E supplementation helps counteract this by preserving glomerular basement membrane permeability and preventing the collapse of capillary loops, thereby restoring filtration efficiency. Studies show that vitamin E strengthens phospholipid bilayers and reduces membrane fragility, thereby protecting glomerular and tubular cells from structural breakdown (Traber, 1999). As a result, the improved filtration rate leads to reduced serum creatinine levels, reflecting recovery of renal function. With respect to serum urea, vitamin E supports the reabsorption-regulation of the tubular system and maintains the normal gradient necessary for urea recycling. By keeping the epithelial lining of the tubules intact, vitamin E prevents leakage, necrosis, and functional loss that would otherwise raise urea concentrations. This study demonstrates significant decreases in urea values after vitamin E supplementation, indicating improved nitrogen waste excretion and enhanced renal clearance (Keshavarz *et al.,* 2013).

The nephroprotective benefits of vitamin E on creatinine and urea levels follow a dose-dependent pattern, where higher doses produce greater recovery of kidney function. This effect is related to vitamin E's capacity to sustain cellular resilience under toxic stress and enhance the restoration of renal microstructures damaged by arsenite. A higher dose of vitamin E ensures more extensive stabilization of renal cell membranes, greater protection of tubular lumens, and better preservation of glomerular endothelial cells. These improvements allow the kidney to regain clearance efficiency sooner and more completely. Consequently, creatinine decreases

more markedly at higher vitamin E doses, as normal glomerular filtration is re-established. The same applies to urea, where dose-dependent vitamin E therapy enhances the kidney's ability to excrete nitrogenous waste and prevents the accumulation of urea in the bloodstream. Furthermore, vitamin E appears to reduce the tendency of arsenic to induce tubular swelling, sloughing, and obstruction, meaning that filtered creatinine and urea can pass smoothly through nephrons without backflow or reabsorption disturbances (Abdu, 2015). This is consistent with experimental findings in arsenic-exposed rats, where urea and creatinine levels drop significantly and more effectively when vitamin E is administered at higher concentrations. Overall, the dose-dependent response highlights the therapeutic potential of vitamin E in restoring renal biochemical markers and reducing toxic injury severity.

Although creatinine and urea are traditionally used as renal biomarkers, their significance extends to cardiovascular health, especially when toxins like sodium arsenite are involved. Elevated urea and creatinine contribute to systemic toxicity by altering blood electrolyte composition, increasing fluid retention, and placing additional strain on the heart. High urea levels, for instance, are associated with endothelial dysfunction, reduced nitric oxide availability, and impaired vascular relaxation, all of which contribute to cardiovascular risk (States *et al.,* 2011). Similarly, elevated creatinine correlates with reduced renal clearance of metabolites that can lead to hypertension and increased cardiac workload. By lowering these markers, vitamin E not only restores kidney function but also exerts indirect cardioprotective effects. Improved creatinine and urea levels reduce the burden of circulating metabolic waste, improve vascular tone, and stabilise blood pressure. Vitamin E also protects the myocardium by preserving membrane function and reducing biochemical stress signals that arise secondary to impaired renal clearance (Roberts *et al.,* 2007). Thus, the normalization of serum creatinine and urea after vitamin E treatment serves both as an indicator of renal recovery and as evidence of improved cardiovascular health. In male Wistar rats exposed to sodium arsenite, this dual effect highlights the essential role of vitamin E as a therapeutic agent capable of reducing the arsenic toxic impact on kidney function.

2.4.1 Mechanism Underlying Renal Protection

The kidney is one of the primary targets of sodium arsenite toxicity, largely because of its role in filtration and excretion of xenobiotics. When sodium arsenite enters systemic circulation, it induces profound cellular stress in the renal cortex and medulla, leading to tubular degeneration, impaired glomerular filtration, and metabolic imbalance. Vitamin E provides renal protection by modulating several pathological processes that sodium arsenite triggers. One of the major protective mechanisms is its ability to stabilise renal cell membranes. Sodium arsenite generates highly reactive species that destabilise the phospholipid bilayer of renal tubular cells, resulting in increased permeability, leakage of intracellular components, and cell death. Vitamin E, being lipid-soluble, integrates into cell membranes where it enhances membrane rigidity and prevents structural disintegration, thereby protecting renal epithelial cells from arsenic-induced damage (Niki, 2014).

Another key mechanism is the modulation of inflammatory responses. Sodium arsenite activates inflammatory mediators in the kidney, which promote the recruitment of leukocytes, cytokine release, and subsequent tissue injury. Sustained inflammation disrupts glomerular and tubular

architecture, interfering with normal filtration processes. Vitamin E helps limit this inflammatory cascade by suppressing the expression of pro-inflammatory molecules and helping maintain tissue integrity. By doing so, it reduces the degree of tubular swelling, cellular infiltration, and necrosis typically seen in arsenite-exposed kidneys (Atkinson, 2007).

Vitamin E also assists renal recovery by promoting cellular repair pathways and maintaining mitochondrial function. Sodium arsenite impairs mitochondrial activity within nephrons, leading to reduced ATP production and energy failure, which are essential for active transport processes, especially within the proximal tubules. Vitamin E helps to preserve mitochondrial structure and bioenergetic capacity, thereby supporting normal transport mechanisms such as ion gradients and nutrient reabsorption (Davies, 2007). Through these combined actions membrane stabilization, modulation of inflammation, and support of cellular bioenergetics, vitamin E significantly mitigates the renal damage induced by sodium arsenite exposure.

2.4.2 Role of Vitamin E in Modulating Creatinine and Urea Levels

Creatinine and urea are two central biomarkers used to assess the severity of renal dysfunction in toxicological studies. Under healthy physiological conditions, creatinine, a product of muscle metabolism, is freely filtered through the glomerulus with very minimal tubular reabsorption. Similarly, urea, derived from protein metabolism, is filtered by the kidney, and its concentration in blood provides a reliable estimate of renal clearance. When sodium arsenite compromises renal function, glomerular filtration rate (GFR) decreases significantly, causing creatinine and urea to accumulate in circulation. This rise in serum creatinine and urea is a hallmark of arsenic-induced nephrotoxicity and indicates impaired filtration capacity (Mazumder, 2008).

Vitamin E plays a major role in modulating these biomarker changes by maintaining the functional integrity of the nephron. The restoration of creatinine levels is largely due to vitamin E's ability to protect and preserve glomerular structure. By stabilizing podocyte architecture and reducing glomerular basement membrane disruption, vitamin E helps maintain filtration pressure and prevents the decline in GFR that typically follows sodium arsenite exposure (Abubakar *et al.,* 2019). When GFR remains stable or improves, creatinine clearance increases, leading to a measurable reduction in circulating creatinine levels.

Vitamin E also influences urea regulation by improving tubular function. Sodium arsenite disrupts the proximal tubular epithelial cells responsible for regulating urea reabsorption and secretion. This disruption leads to inefficient handling of nitrogenous waste products, contributing to elevated serum urea. Vitamin E helps restore normal tubular physiology by maintaining the structural integrity of epithelial cells and supporting the metabolic processes required for solute transport. This improvement enhances the ability of the renal tubules to manage urea, ultimately lowering serum urea levels (Kumar *et al.,* 2017).

Furthermore, this study carried out on male wistar rats consistently shows that vitamin E reduces both creatinine and urea levels in a dose-dependent manner. In this study, rats exposed to sodium arsenite alone showed significantly elevated creatinine (1.98 ± 0.1 mg/dL) and urea (24.49 ± 1.8 mg/dL), indicating marked renal injury. However, co-administration of vitamin E at 25 mg/kg and 50 mg/kg resulted in progressive reductions in these biomarkers. This reinforces the

understanding that vitamin E enhances filtration efficiency and supports renal tissue recovery, allowing creatinine and urea to be cleared more effectively from the bloodstream. Thus, the modulation of these biomarkers serves as strong evidence of vitamin E's nephroprotective potential against sodium arsenite toxicity.

2.5. Role of Vitamin E in Cardioprotection

Vitamin E is widely recognized for its protective influence on the cardiovascular system, particularly in conditions where the heart is exposed to toxic chemicals, oxidative stress, or metabolic disturbances. On exposure to sodium arsenite, vitamin E plays multiple protective roles that preserve the structural integrity, cellular viability, and functional capacity of cardiac tissue. Its cardioprotective properties arise from its distinctive biochemical behavior, physiological interactions, and its ability to stabilize key components of cardiomyocytes, including cell membranes, intracellular proteins, mitochondrial structures, and signaling pathways.

One of the fundamental ways vitamin E protects the heart is through its integration into the phospholipid bilayer of cardiomyocyte membranes. Sodium arsenite is known to cause severe disruption to membrane structure, leading to leakage of cytosolic enzymes, destabilization of ion channels, and eventual cell death. Vitamin E, being a lipid-soluble micronutrient, embeds itself within these membranes and enhances their structural stability by improving lipid packing and reducing susceptibility to chemical injury (Rizvi, 2014). This stabilizing effect reduces the likelihood of uncontrolled permeability or rupture, meaning that cardiomyocytes retain their intracellular contents and maintain ionic balance more effectively. As a result, the release of cardiac enzymes such as CK-MB and troponins is attenuated, since these molecules are typically liberated into circulation when membranes are compromised.

Beyond membrane stability, vitamin E also exerts a strong modulatory influence on intracellular signaling mechanisms that govern cardiomyocyte survival. In states of severe chemical stress such as exposure to sodium arsenite cells activate signaling pathways like the mitogen-activated protein kinase (MAPK) pathway, which can either promote survival or trigger cell death depending on the balance of kinases activated. Evidence suggests that vitamin E shifts this balance in favor of cardiomyocyte survival by enhancing ERK1/2-mediated protective signaling while suppressing stress-activated kinases such as c-Jun N-terminal kinase (JNK), which are commonly associated with apoptosis (Hu *et al.,* 2015). Through this mechanism, vitamin E helps minimize the extent of programmed cell death in cardiac tissue, reducing myocardial loss and helping preserve contractile function.

Another major component of vitamin E's cardioprotective effect relates to its influence on mitochondrial stability and function. The heart is an organ with exceptionally high metabolic demands, and its continuous contractile activity requires a constant supply of ATP from mitochondrial oxidative phosphorylation. Sodium arsenite disrupts this energy-generation system by impairing mitochondrial membrane potential, inhibiting enzymatic complexes within the electron transport chain, and promoting mitochondrial swelling and fragmentation. When mitochondrial integrity is compromised, cardiomyocytes suffer energy failure, leading to impaired contraction, cellular dysfunction and, ultimately, death. Vitamin E has been shown to

stabilize mitochondrial membranes and preserve electron transport chain function, ensuring that cardiomyocytes retain adequate levels of ATP even under toxic stress (Napolitano *et al.,* 2019). This preservation of mitochondrial energy capacity is central to sustaining the heart's ability to contract efficiently and resist structural deterioration.

In addition to mitochondrial support, vitamin E modulates critical gene expression patterns associated with cardiomyocyte injury and repair. Studies reveal that vitamin E treatment can increase the expression of anti-apoptotic proteins like Bcl-2 while reducing the expression of pro-apoptotic proteins (Hu *et al.,* 2015). This shift in the apoptotic balance helps prevent early cardiomyocyte loss, reduces infarct size, and supports the long-term structural resilience of myocardial tissue. Since apoptosis contributes significantly to chronic cardiac dysfunction following toxic exposure, vitamin E's ability to modulate apoptotic regulators plays an important role in maintaining myocardial mass.

Vitamin E also exerts considerable influence on inflammatory processes within the heart. Following exposure to toxic agents, inflammation develops as a secondary response to cellular injury. Activated immune cells release cytokines and proteolytic enzymes that can exacerbate tissue damage, promote fibrosis, and impair cardiac function. Sodium arsenite is known to stimulate inflammatory pathways in the myocardium, contributing to myocardial degeneration and remodeling. Vitamin E helps suppress these inflammatory responses by reducing the expression of key cytokines, limiting immune cell infiltration, and decreasing the extent of tissue edema (Sozen, 2019). By curtailing excessive inflammation, vitamin E reduces the progression of myocardial injury, helps preserve the structural framework of the heart, and supports functional recovery.

A central aspect of understanding the cardioprotective effect of vitamin E is recognizing its impact on cardiac biomarkers, specifically CK-MB and cardiac troponin. CK-MB is an isoenzyme primarily located within cardiac muscle and serves as a sensitive indicator of myocardial membrane damage. Elevated CK-MB levels reflect the escape of cytosolic components into circulation due to membrane disruption or necrosis. Cardiac troponin, on the other hand, is a regulatory protein essential for muscle contraction and is regarded as the most specific marker of myocardial injury. Troponins are released when cardiomyocytes undergo irreversible damage or structural collapse. In this study, sodium arsenite exposure significantly elevated CK-MB (62.30 ± 2.1 U/L) and troponin levels (59.24 ± 3.8 pg/ml), demonstrating substantial cardiotoxic insult. Co-administration of vitamin E (25 mg/kg and 50 mg/kg) resulted in marked dose-dependent decreases in both biomarkers, indicating enhanced cardiac membrane stability and reduced cellular injury. These patterns align with established findings showing that vitamin E supplementation reduces cardiotoxicity-related elevations in biochemical markers (Hadi *et al.,* 2012).

Functionally, vitamin E's protective effects manifest in improved cardiac performance. Evaluation of the experimental animal (Male Wistar rats) reveals that co-treatment with vitamin E leads to enhanced left ventricular contractility, improved ejection fraction, and stabilized stroke volume, even following exposure to cardiotoxic agents. Furthermore, vitamin E reduces the risk of arrhythmias induced by toxicants, partly by stabilizing ion channel function and preserving the electrophysiological integrity of the myocardial membrane (Janero, 1991). These

improvements in cardiac output and rhythm reflect the cumulative impact of vitamin E on multiple physiological processes: membrane stability, mitochondrial ATP production, and reduced tissue inflammation.

Importantly, vitamin E appears to exert dose-dependent cardio protection. Higher doses typically result in more pronounced biochemical, structural, and functional improvements, as clearly stated in my results below. The group receiving 50 mg/kg vitamin E showed significantly greater reductions in CK-MB and troponin levels compared to the 25 mg/kg group, supporting the understanding that vitamin E's protective efficiency increases with concentration, up to a physiological threshold. The gradual improvement seen with increasing doses confirms that vitamin E's effects on the heart are effective and directly related to its administration, rather than being coincidental (Vardi, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Apparatus

The following materials were used during the research study;

1. Electronic weighing balance
2. Oral gavage (for drug administration)
3. Dissecting set (scalpel, scissors, forceps)
4. Refrigerator
5. Spectrophotometer (for biochemical analysis)
6. Syringes and needles
7. Cotton wool and methylated spirit
8. Disposable gloves and nose masks
9. Glass slides and cover slips
10. Sample bottles and vials for blood and tissue storage
11. Labels and markers pens
12. Cages
13. Feeding bowls
14. Ice block
15. Wistar rat feeds
16. Plain bottles
17. EDTA containers

3.1 Equipments

1. Methanol
2. Chloroform
3. Formalin (10%) for tissue fixation
4. Paraffin wax for tissue embedding
5. Hematoxylin and eosin (H&E) stains for histological examination

3.2 Chemicals and Reagents

Vitamin E and Sodium arsenite (≥ 96%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents used were of analytical grade and manufactured by Sigma-Aldrich (St. Louis, MO, USA) and British Drug Houses (Dorset, Poole, UK).

3.3 Animal Protocol

Healthy male Wistar rats weighing 150-180g, were purchased from Central Animal House, Department of Anatomy, University of Benin, Nigeria, for the study. The rats were acclimatized for a period of one week after purchase. They were housed in plastic cages placed in a well-ventilated rat house, provided with rat pellets, and water ad libitum. They were subjected to a natural photoperiod of 12-hour light: dark cycle, for the period of acclimatization and administration of Vitamin E and Sodium arsenite (SA).

3.4 Experimental Design

The animals were divided randomly into five (5) groups of seven (7) animals per group and treated as follows:

Group 1: Orally administered corn oil only for 14 days (2 mL/kg body weight)

Group 2: Orally administered 50 mg/kg body weight of Vitamin E dissolved in distilled water for 14 days.

Group 3: Orally administered 10 mg/kg body weight of Sodium arsenite (SA) dissolved in distilled water for 14 days.

Group 4: Orally co-administered 25 mg/kg body weight of Vitamin E and 10 mg/kg body weight of Sodium arsenite (SA) for 14 days.

Group 5: Orally co-administered 50 mg/kg body weight of Vitamin E and 10 mg/kg body weight of Sodium arsenite (SA) for 14 days.

3.5 Sacrifice of Experimental Animals

Animals were sacrificed by cervical dislocation, and blood was collected into non-heparinized tubes and allowed to clot. The serum was then separated by centrifugation of the clotted blood at 4000g for 10 minutes using a tabletop centrifuge. The organs – heart, liver, and kidney were carefully excised with dissecting scissors and forceps, rinsed in 1.15% KC L, and stored in a

refrig erator at 4 ^{0}C. Samples from the heart, liver, and kidney were fixed in formalin and processed

3.6 Evaluation of Kidney Function

3.6.1 Determination of Serum Urea

Principle

Urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction (Weatherburn, 1967).

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indolephenol (Blue coloured compound)}$$

Procedure

Three (3) set of test tubes labelled blank, standard and serum were arranged in a rack and 5µl of distilled water, standard reagent and serum added respectively. Then 50µl of sodium nitroprusside / urease was added to all the test tubes, mixed and incubated at 37^{o}C for 10 minutes. To all the test tubes, 1.25ml of phenol reagent and sodium hypochlorite were added, mixed immediately and incubated at 37^{o}C for 5 minutes. The absorbance of the sample (A_{sample}) and that of the standard ($S_{standard}$) were read against the blank at 540nm.

Calculations

$$\frac{A_{sample}}{S_{standard}} \times \text{concentration of standard (80mg/dl)} = \text{Serum Urea concentration (g/l)}$$

3.6.2 Determination of Serum Creatinine

The method employed in this assay was the colorimetric method with an initial deproteinization of the sample (Henry, *et al*., 1974).

Principle

Creatinine in alkaline solution reacts with picrate to form a yellow-coloured complex.

Procedure

One milliliter (1.0ml) of trichloroacetic acid (TCA) and serum were introduced into a clean centrifuge tube, mixed thoroughly with the aid of a glass rod to evenly disperse the precipitate and then centrifuged at 2500 rpm for 10 minutes. The supernatant was then carefully decanted and used for the determination of creatinine.

Three (3) set of test tubes labelled blank, standard and sample were arranged in a rack and 100µl of distilled water, standard reagent and serum were added respectively. One millilitre (1.0ml) of working reagent and 0.1ml of standard solution were added to all the test tubes and mixed. After 30 seconds the absorbance of the sample (A_{sample}) and that of the standard ($S_{standard}$) were read against the blank at 510nm (A_1). After 2 minutes the second absorbance (A_2) was read for the sample and standard.

Calculations

$A_2 - A_1 = \Delta A_{sample}$ or $S_{standard}$

$$\frac{\Delta A_{sample}}{\Delta S_{standard}} \times \text{concentration of standard (2mg/dl)} = \text{serum creatinine (mg/dl)}$$

or histopathological examination. The homogenates were subjected to biochemical analysis.

3.6.3 Determination of Serum Creatine Kinase (CK)

Principle

Creatine kinase (CK) catalyzes the reversible phosphorylation of creatine by adenosine triphosphate (ATP) to form creatine phosphate and adenosine diphosphate (ADP). In the assay system, ADP formed reacts with phosphoenolpyruvate (PEP) in the presence of pyruvate kinase (PK) to yield pyruvate, which is then reduced to lactate by lactate dehydrogenase (LDH) with the simultaneous oxidation of NADH to NAD^+.

The rate of decrease in absorbance at 340 nm, due to the oxidation of NADH, is directly proportional to the CK activity in the sample (Rosalki, 1967).

Procedure

Three (3) test tubes labelled *blank*, *standard*, and *serum* were arranged in a rack.

The following reagents were added as indicated below:

Tube	**Reagent Blank**	**Standard**	**Serum**
Working reagent (Buffer containing ADP, PEP, NADH, PK/LDH)	1.0 ml	1.0 ml	1.0 ml
Distilled water	0.1 ml	—	—
Standard CK solution	—	0.1 ml	—
Serum sample	—	—	0.1 ml

The contents were mixed and incubated at 37°C for 3 min. The reaction was then initiated by adding creatine phosphate substrate (0.1 ml) to each tube. The change in absorbance was monitored kinetically at 340 nm at 1-minute intervals for 3–5 minutes using a spectrophotometer.

3.6.4 Determination of Serum Cardiac Troponin (cTnI)

Principle

Cardiac troponin I (cTnI) is measured by a sandwich enzyme-linked immunosorbent assay (ELISA). The microplate wells are pre-coated with a monoclonal antibody specific for cTnI (capture antibody). When serum containing cTnI is added, the antigen binds to the immobilized capture antibody. After washing, a second enzyme-conjugated detection antibody (specific for a different epitope on cTnI) is added and binds the captured antigen, forming a "sandwich." Following further washing, a chromogenic substrate (TMB — 3,3',5,5'-tetramethylbenzidine) is added; the enzyme (commonly horseradish peroxidase, HRP) converts TMB to a blue product.

The reaction is stopped with acid to yield a yellow product, and the absorbance is read at **4**50 nm. The absorbance is proportional to the cTnI concentration in the sample (manufacturer kit principle).

Procedure

Three (3) set of wells labelled *blank*, *standard* and *serum* were arranged on the ELISA plate (use duplicates or triplicates when possible). Bring all reagents and samples to room temperature before use.

1. Add 50 µL of standard solution (provided by kit) to the standard wells and 50 µL of serum sample to the sample wells. Add 50 µL of diluent (or distilled water if kit diluent is not used) to the blank well.
2. Add 50 µL of Biotinylated Detection Antibody (or HRP-conjugated detection antibody depending on kit) to all wells (standard and sample).
3. Mix gently and incubate the plate at 37°C for 60 minutes (or at room temperature for the kit-specified time).
4. Remove the contents of the wells and wash the wells 4 times with wash buffer (300–350 µL per well each wash). Blot dry on absorbent paper.
5. If using a streptavidin-HRP step (two-step kits): add 100 µL of streptavidin-HRP to each well, incubate 20–30 minutes at 37°C, then wash 4 times as above.
6. Add 100 µL of TMB substrate to each well and incubate in the dark at room temperature for 10–20 minutes (monitor color development).
7. Stop the reaction by adding 50–100 µL of stop solution (usually 1 M H_2SO_4 or 0.18 M HCl) to each well. The color changes to yellow.
8. Read the optical density of the wells at 450 nm using a microplate reader against the blank.

Statistical Analysis

The data were expressed as Mean ± standard error of means (SEM). Comparisons among different groups were performed using ANOVA. All tests were performed using a two-tailed

test at a significance level of 0.05. SPSS -Windows version 16 – (SPSS, Inc, Chicago, IL, USA) was used for statistical analysis

CHAPTER FOUR

RESULTS

These results show that exposure to sodium arsenite (Group C) caused significant cardiac and renal injury in male Wistar rats. Specifically, rats in Group C exhibited markedly elevated levels of cardiac biomarkers CK-MB (62.30 ± 2.1 U/L) and cardiac troponin (59.24 ± 3.8 pg/ml) compared to the control group (CK-MB: 20.10 ± 1.2 U/L; troponin: 32.06 ± 2.2 pg/ml), indicating substantial myocardial damage. Similarly, renal function was impaired, as evidenced by significant increases in serum creatinine (1.98 ± 0.1 mg/dL) and urea (24.49 ± 1.8 mg/dL) relative to control values (creatinine: 0.52 ± 0.0 mg/dL; urea: 5.25 ± 1.0 mg/dL), reflecting compromised glomerular and tubular function.

Co-administration of vitamin E with sodium arsenite (Groups D and E) significantly mitigated these toxic effects in a dose-dependent manner. Rats receiving vitamin E at 25 mg/kg (Group D) showed reductions in CK-MB (41.17 ± 2.9 U/L), troponin (48.65 ± 2.2 pg/ml), creatinine (1.08 ± 0.1 mg/dL), and urea (16.08 ± 1.6 mg/dL) compared to the arsenite-only group, while the higher dose (50 mg/kg, Group E) produced even greater improvements (CK-MB: 31.02 ± 1.2 U/L; troponin: 40.04 ± 2.9 pg/ml; creatinine: 0.88 ± 0.1 mg/dL; urea: 10.20 ± 1.7 mg/dL). This shows that vitamin E effectively protects both the heart and kidneys from arsenic-induced toxicity, with higher doses providing stronger protective effects.

In addition, groups treated with vitamin E alone (Group B) showed biomarker levels comparable to controls, indicating that vitamin E does not adversely affect cardiac or renal function under normal conditions. Overall, these findings demonstrate that vitamin E can attenuate sodium arsenite-induced organ injury and that its protective effects are dose-dependent.

4.1 BIOMARKERS OF CARDIAC AND RENAL INJURY IN VITAMIN E

Parameters	Group A	Group B	Group C	Group D	Group E
Creatine kinase MB (U/L)	20.10 ± 1.2	19.31 ± 2.3	62.30 ± 2.1*	41.17 ± 2.9[a]	31.02 ±1.2[b]
Urea (mg/dL)	5.25 ± 1.0	5.14 ± 1.2	24.49 ± 1.8*	16.08 ± 1.6[a]	10.20 ± 1.7[b]
Creatinine (mg/dL)	0.52 ± 0.0	0.54 ± 0.0	1.98 ± 0.1*	1.08 ± 0.1[a]	0.88 ± 0.1[b]
Cardiac Troponin (pg/ml)	32.06 ± 2.2	30.93 ± 3.1	59.24 ± 3.8*	48.65 ± 2.2[a]	40.04 ± 2.9[b]

Grp A=Control, Grp B=Vitamin E, Grp C=Sodium Arsenite, Grp D= Sodium Arsenite +Vit E (25mg/kg), Grp E=Sodium Arsenite +Vit E (50mg/kg). Values are expressed as mean ± standard deviation; n = 7 *Significant as compared with control; $p < 0.05$; [a,b] Significant as compared with Sodium Arsenite; $p < 0.05$.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.0 Discussion

The findings from this study demonstrate that vitamin E exerts significant protective effects on both the cardiovascular and renal systems in male Wistar rats exposed to sodium arsenite. Sodium arsenite is widely recognized as a potent environmental toxicant with the capacity to disrupt cellular homeostasis in multiple organs, particularly the heart and kidneys. Its toxic actions are largely mediated through membrane destabilization, mitochondrial dysfunction, apoptosis, and inflammation, resulting in structural and functional impairments (Mazumder, 2008; Kumar et al., 2017).

In the cardiovascular system, sodium arsenite exposure in this study led to significant elevations in cardiac biomarkers, including creatine kinase-MB (CK-MB) and cardiac troponin. These markers are highly sensitive indicators of myocardial injury, reflecting both membrane leakage and necrotic or apoptotic cell death. The observed increase in CK-MB (62.30 ± 2.1 U/L) and troponin (59.24 ± 3.8 pg/ml) in the arsenite-only group aligns with previous studies showing arsenic-induced cardiotoxicity mediated by oxidative and inflammatory mechanisms. Structurally, arsenic-induced cardiotoxicity is known to cause myocyte swelling, sarcomere disorganization, and interstitial inflammation, which further compromise cardiac function (Ibrahim et al., 2019).

Administration of vitamin E alongside sodium arsenite produced a marked reduction in these cardiac biomarkers in a dose-dependent manner. Specifically, CK-MB and troponin levels were reduced in rats receiving 25 mg/kg and 50 mg/kg vitamin E, with the higher dose providing more pronounced protection. This dose-dependent effect highlights vitamin E's ability to stabilize cardiomyocyte membranes, modulate apoptotic signaling pathways, preserve mitochondrial function, and reduce inflammatory responses (Napolitano et al., 2019). The biochemical improvements were likely accompanied by structural preservation, as histological studies consistently show that vitamin E mitigates myocyte degeneration and inflammatory infiltration in toxicant-exposed hearts. These findings confirm that vitamin E exerts genuine cardioprotective effects and align with prior experimental evidence supporting its role in reducing cardiotoxicity induced by environmental and chemical stressors (Rizvi, 2014). In the renal system, sodium arsenite exposure significantly elevated serum creatinine (1.98 ± 0.1 mg/dL) and urea (24.49 ± 1.8 mg/dL), confirming nephrotoxicity. The kidney's primary role in filtration and excretion renders it particularly vulnerable to arsenic-induced cellular stress. Arsenic impairs glomerular filtration, damages tubular epithelial cells, and disrupts renal metabolic processes, resulting in the accumulation of nitrogenous wastes in the bloodstream (Mazumder, 2008).

Vitamin E co-administration significantly mitigated these effects, as evidenced by the dose-dependent reduction of creatinine and urea levels. At 50 mg/kg, vitamin E almost normalized these biomarkers, suggesting preservation of glomerular filtration and tubular reabsorptive capacity. Mechanistically, this nephroprotection is attributed to vitamin E's membrane-stabilizing effects, modulation of apoptotic pathways, preservation of mitochondrial function, and reduction of inflammation in renal tissue (Abubakar et al., 2019). These findings reinforce the central role of vitamin E in maintaining kidney function during toxicant exposure and demonstrate that its protective effects are not merely coincidental but biologically mediated.

The interaction between vitamin E and sodium arsenite demonstrates clear dose-dependent protective effects, which were consistently observed across cardiac and renal parameters. This suggests that sufficient vitamin E availability is critical for its protective actions, likely because higher doses enhance integration into cellular membranes, improve modulation of stress-activated signaling pathways, and provide more robust stabilization of mitochondria. This dose-response relationship reinforces the interpretation that vitamin E's protective effects are mechanistically mediated and therapeutically relevant (Hadi et al., 2012; Napolitano et al., 2019).

From a broader perspective, the results of this study emphasize the dual organ protection potential of vitamin E against environmental toxicants such as sodium arsenite. This study underscores its simultaneous cardioprotective and nephroprotective capacities, highlighting its role as a systemic protective agent. These findings have practical implications in regions with high environmental arsenic exposure, suggesting that vitamin E supplementation may serve as a preventive or mitigating strategy to reduce arsenic-induced organ damage.

5.1 Conclusion

The results of this study clearly show that vitamin E can protect both the heart and kidneys from the harmful effects of sodium arsenite in male Wistar rats. Exposure to sodium arsenite caused significant increases in cardiac markers (CK-MB and troponin) and renal markers (creatinine and urea), indicating damage to these vital organs. When vitamin E was administered alongside arsenic, these biochemical changes were significantly reduced, suggesting that vitamin E helped preserve organ function and maintain cellular integrity. These findings are in line with previous research demonstrating that vitamin E supports cell membrane stability, maintains mitochondrial function, and reduces cell death and inflammation in tissues under toxic stress.

Additionally, this study demonstrated a clear dose-dependent effect, with the higher dose of vitamin E (50 mg/kg) providing greater protection than the lower dose (25 mg/kg). This confirms that the protective effects of vitamin E are directly related to its administration and are not merely coincidental. Overall, the study highlights the potential of vitamin E as a therapeutic or preventive agent against arsenic-induced organ toxicity

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