BRIEF OVERVIEW ABOUT CYCLOPHOSPHAMIDE TOXICITY AND POSSIBLE PROTECTIVE ROLE OF SELENIUM

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Abstract

Background: Cyclophosphamide (CP) is an important anticancer drug which belongs to the class of alkylating agents. CP is mostly used in bone marrow transplantation, rheumatoid arthritis, lupus erythematosus, multiple sclerosis, neuroblastoma and other types of cancer. CP was approved by Food and Drug Administration (FDA) for treatment of breast cancer, retinoblastoma, minimal change nephrotic syndrome in pediatric patients and ovarian adenocarcinomas. CP is used in the management of autoimmune illnesses like multiple sclerosis due to its potent immunosuppressive properties and prior to organ transplantation to reduce the risk of transplantation rejection. CP induced circulatory inflammatory cytokines are consistent with the recorded significant upregulation of nuclear factor kappa beta (NF-KB). CP induced hepatotoxicity is mediated by tissue damage which leads to generation of inflammatory mediators by the immune cells as well as by injured cells. The generated inflammatory mediators induce migration and infiltration of leukocytes into the site of injury and provoke the primary injury, increase vascular permeability and apoptosis of hepatocytes. CP administration induces a significant increase in serum levels of interleukin six (IL-6) as reported in previous studies. Selenium (Se) alleviated hepatocyte necrosis and deoxyribonucleic acid (DNA) damage in CP treated animals presented by improving liver functions and oxidative stress markers level, decreasing hepatic edema, portal congestion and sinusoidal dilatation. Se has a protective role against CP induced nephrotoxicity. At the level of kidney functions, Se decreases urea and creatinine levels beside decreasing the oxidative stress markers and increasing the antioxidants.

Keywords: Cyclophosphamide, Toxicity, Selenium

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Introduction

Cyclophosphamide is an important anticancer drug which belongs to the class of alkylating agents. CP is mostly used in bone marrow transplantation, rheumatoid arthritis, lupus erythematosus, multiple sclerosis, neuroblastoma and other types of cancer ***(1).***

Physical properties of cyclophosphamide:

Cyclophosphamide is a fine white odorless crystalline powder with a slightly bitter taste. The melting point of CP is 41-45°C. A 2% solution of CP has pH of 4 to 6 ***(2).***

Chemical Structure of cyclophosphamide:

Pavan et al. (3) reported that CP is an alkylating agent used in cancer treatment that attaches an alkyl group (CnH2n+1) to DNA. Molecular formula of Cyclophosphamide monohydrate is C7H15Cl2N2O2P.H2O and the systematic name is 2-[bis(2-chloroethyl) amino] tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate. The structural formula of cyclophosphamide monohydrate is shown in Figure (1).

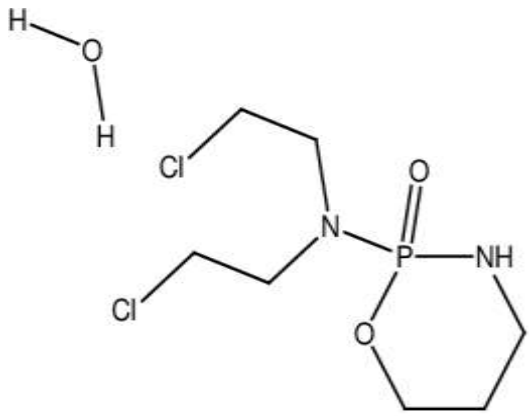


Figure (1): Chemical structure of cyclophosphamide monohydrate ***(3).***

Pharmacokinetics*:*

Absorption:

Cyclophosphamide is widely absorbed by oral, intravenous injection, skin contact and inhalation Urine samples from two nurses working in a cancer clinic were analysed for CP by gas chromatography after they had prepared the drug for treatment and they were positive ***(4).***

Distribution:

Cyclophosphamide has low plasma protein binding so it can diffuse through cell membranes and widely distributed in human body. CP is distributed with a volume of distribution of 30–50L, which approximates to the total body water ***(7).***

Metabolism:

Cyclophosphamide is a prodrug metabolized to both active and inactive metabolites. CP is metabolized initially in the liver by hepatic microsomal cytochrome p-450 to 4-hydroxy cyclophosphamide, which exists in equilibrium with its tautomer, aldo-phosphamide. Most of the aldo-phosphamide is then oxidized by the enzyme aldehyde dehydrogenase to create carboxy-cyclophosphamide. A small proportion of aldo-phosphamide freely diffuses into cells, where it is decomposed into two compounds, phosphoramide mustard and acrolein ***(6).*** Its metabolic profile is shown in figure (2).

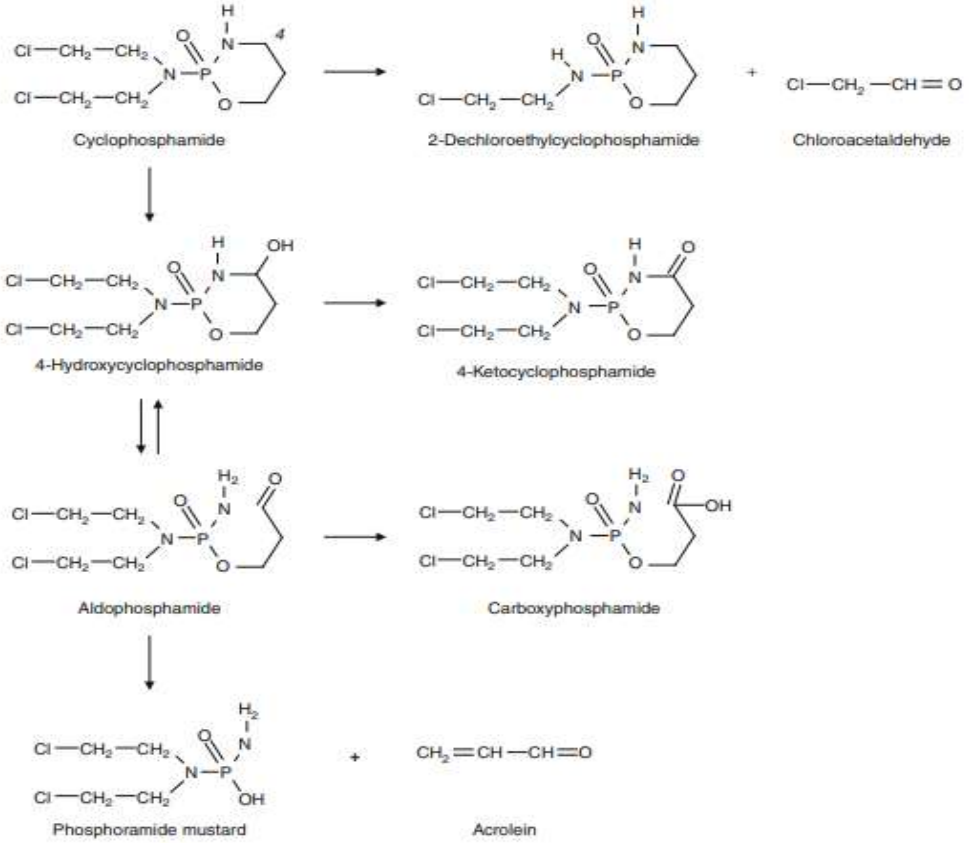
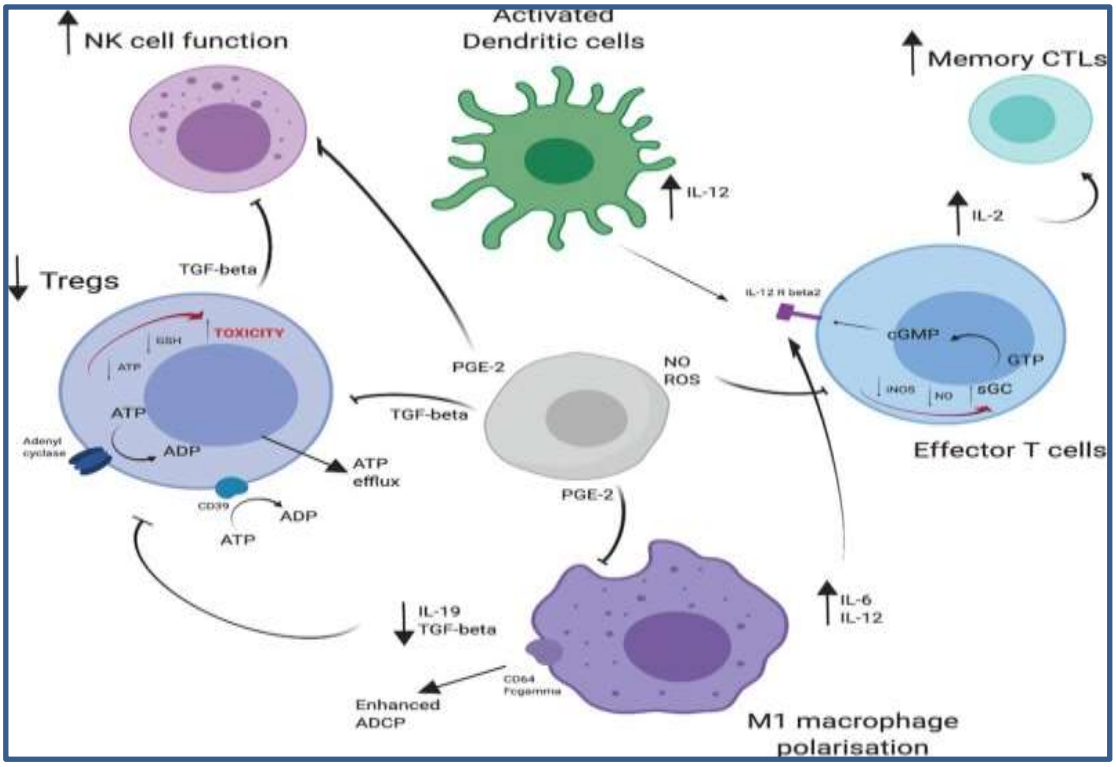


Figure (2): Metabolism of cyclophosphamide. The inactivation pathways are depicted horizontally, while cyclophosphamide activation is shown vertically *(7).*

Elimination:
Cyclophosphamide and its metabolites are excreted in urine. Urinary elimination of CP and its metabolites almost completes 24 hours after the start of treatment. Less than 20% of the administered dose is eliminated unchanged in the urine. Renal excretion of CP or its metabolites accounts for between 30 and 60% of the total dosage. Phosphoramide mustard and carboxyphosphamide are the two main metabolites in urine. Little portion of the CP dose is excreted via faeces and expired air ***(5).***

Pharmacodynamics:

Mechanism of action:
Cyclophosphamide is a type of nitrogen mustard drug which exerts its effects through the alkylation of DNA. It is metabolized to an active form capable of inhibiting protein synthesis through DNA and RNA crosslinking ***(8).*** Most of the antineoplastic effects of CP are due to the phosphoramide mustard formed from the metabolism of the drug by liver enzymes like cytochrome P-450. Hepatic enzymes first convert cyclophosphamide to hydroxy cyclophosphamide and then subsequently metabolized to Aldo phosphamide. Aldophosphamide is cleaved to the active alkylating agent phosphoramide mustard and acrolein ***(8).***
The phosphoramide metabolite forms cross-linkages within and between adjacent DNA strands at the guanine N-7 position. These modifications are permanent and eventually lead to programmed cell death ***(9).***
In addition to antimitotic and antineoplastic effects, CP has immunosuppressive effects and selectivity for T cells. High-dose CP is used in eradication therapy of malignant hematopoietic cells. It increases the release of T-helper2 (Th2) cytokines including interleukin four (IL-4) and interleukin ten (IL-10) in the cerebrospinal fluid (CSF) and peripheral blood while decreasing the secretion of interferon-gamma and IL-12 ***(10).*** **Figure (3).**



Uses and administration:

Uses:
According to FDA, CP is mainly indicated for use in the treatment of malignant lymphomas stages III and IV, as designated by the Ann Arbor staging system. These may include Hodgkin and Non-Hodgkin lymphoma, lymphocytic lymphoma, small lymphocytic lymphoma, Burkitt lymphoma, and multiple myeloma ***(8).***
Cyclophosphamide is also FDA approved for treatment of breast cancer, disseminated neuroblastomas, retinoblastoma, minimal change nephrotic syndrome in paediatric patients, and ovarian adenocarcinomas. CP is also used in the management of autoimmune illnesses like multiple sclerosis due to its potent immunosuppressive properties and prior to organ transplantation to reduce the risk of transplantation rejection. ***(12).***

Administration:
The dosage of CP varies greatly depending on clinical implication. This drug may be prescribed at low dose, 1–3 mg/kg per day (40–120 mg/m^2) that is mostly administered orally or pulse dose intravenously (recommended), 15–40 mg/kg (600–

1,500 mg/m^2) every 3–4 weeks. Furthermore, in bone marrow transplantation high dose of CP is used, >120 mg/kg (>5,000 mg/m^2) which is more frequently administered over 2–4 days as conditioning for bone marrow transplantation ***(13).***
Mesna is a prophylactic cytoprotective drug administered orally or by IV to reduce the effects of hemorrhagic cystitis in patients treated with high-dose CP. ***(13).***

Adverse effects:

The main adverse side effects of CP are associated with hemorrhagic cystitis, amenorrhea, myelosuppression, alopecia and spells of nausea and vomiting ***(14).***
The acrolein metabolite causes heightened vascular fragility and dilatation, irritation of the bladder mucosa, the release of pro-inflammatory mediators like tumour necrosis factor-alpha, IL-1 beta, and endogenous nitric oxide leading to haemorrhage. Prolonged exposure to acrolein may increase the severity of hemorrhagic cystitis ***(15).***
Cyclophosphamide can cause bone marrow depression and myelosuppression. There are also reports of cardiotoxicity, pulmonary toxicity, veno-occlusive liver disease, and secondary malignancies in some cases of CP use. Myocarditis, pericardial effusion with cardiac tamponade, pneumonitis, and respiratory failure are possible risks. Higher dosages aggravate the side effects and increase mortality ***(15).***
The alkylating effects of CP may also affect the processes of oogenesis and spermatogenesis, leading to sterility in both sexes. The risk of sterility is time and dose-dependent and irreversible in some patients ***(1).***

Contraindications:

Cyclophosphamide is not recommended in patients with allergies or hypersensitivity reactions to the drug or any of its metabolites, as well as adverse interactions with other chemotheraputic drugs. CP should not be used in patients with disorders that impair urine flow because it increases risk of developing hemorrhagic clot retention ***(16).***
Cyclophosphamide is associated with high teratogenicity, miscarriages, fetal losses, fetal malformations and have serious effects on fetal growth and development if they are taken during the period of organogenesis or before that ***(17).***

Toxicity:

Cyclophosphamide toxicity can be acute or chronic and correlates with the development of leukopenia, thrombocytopenia and anemia. These conditions may lead to the occurrence of recurrent infections and may interfere with wound healing ***(18).***
Nephrotoxicity and hepatotoxicity are considered two main side effects, as the kidney and liver are important organs responsible for metabolism and excretion of CP and its reactive metabolites ***(18).***

Hepatotoxicity:

Cyclophosphamide induced hepatotoxicity is mediated by tissue damage which leads to generation of inflammatory mediators by the immune cells as well as by injured cells. The generated inflammatory mediators induce migration and infiltration of leukocytes into the site of injury and provoke the primary injury, increase vascular permeability and apoptosis of hepatocytes**.** CP administration induce significant increase in serum levels of tumor necrotizing factor alpha (TNF-a) and IL-6 as reported in previous studies. Through binding to their receptors, IL-1β, IL-6 and TNF-α elicit potent pro-inflammatory actions and activate the pro-apoptotic caspase cascade **(19).**
Cyclophosphamide induced circulatory inflammatory cytokines are consistent with the recorded significant upregulation of NF-KB. Increased reactive oxygen species generation is known to play an important role in CP-induced hepatotoxicity and nephrotoxicity**.** CP increases protein carbonyl content which increases the level of free radicals**.** Free radicals formed by CP can attack lipids and cause serious changes in membrane structure and function ***(20).***
Conjugation of acrolein with glutathione (GSH) results in depletion of the intracellular GSH level, and other antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx). Elevated malonyl dialdehyde (MDA) level indicates that CP-induces oxidative stress and lipid peroxidation in the liver and kidney tissue. CP e can cause hepatotoxicity by low dose and from first dose so initial and follow-up liver function tests should be monitored in all patients receiving CP treatment. CP induces hepatotoxicity with elevation of liver enzymes Alanine aminotransferase (ALT), Aspartate transaminase (AST) and Lactate Dehydrogenase (LDH) oxidative stress markers, inflammatory cytokines and necrosis of liver cells ***(21).***
The severity of liver injury by CP ranges from mild elevations in liver enzymes to acute liver injury or to massive, fatal hepatic necrosis due to sinusoidal obstruction syndrome. The sinusoidal obstruction syndrome induced by cyclophosphamide is probably related to the direct toxic effect of CP on sinusoidal cells in the liver, causing their necrosis and release into the sinusoids, obstruction and obliteration of hepatic veins ***(22).***
Histological examination of liver tissue showed hydropic degeneration in hepatocytes, especially in the acinar region, coagulation necrosis, steatosis in hepatocytes, and hyperemia in the central vein and sinusoids ***(23).***

Nephrotoxicity:

The mechanism of CP nephrotoxicity includes urine sediment abnormalities, electrolyte imbalances and most commonly a decline in the glomerular filtration rate. CP induces nephrotoxicity via its toxic

metabolites which produce free radicals and cause oxidative stress that elevate oxidant contents and deplete antioxidants. The nephrotoxic potential of CP is confirmed by elevation of kidney function markers urea and creatinine. CP urotoxicity may cause dose-limiting side effects, for example, haemorrhagic cystitis ***(23).***

Histologically CP treated rat kidneys show glomerular nephritis, interstitial edema and cortical tubular vacuolization. Lysosomal enzymes activities decrease and protein content increase in the kidneys of CP treated rats. Decrease in the activities of lysosomal enzymes may contribute to renal damage ***(23).*** Figure (4).

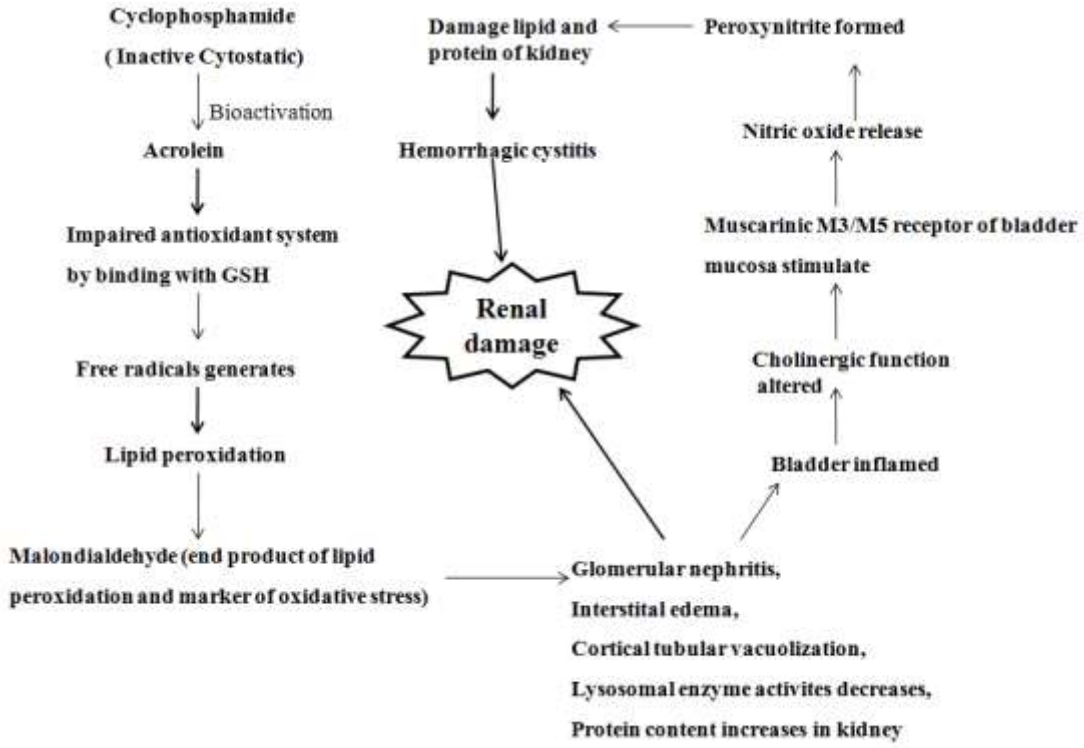


Figure (4): Mechanism of cyclophosphamide nephrotoxicity ***(22).***

Cardiotoxicity:

Cyclophosphamide induced cardiotoxicity is dose dependent. It affects between 7 and 28% of patients taking the drug. The use of CP is associated with fatal cardiomyopathy, myocarditis and cardiac tamponade. ***(24).***

Selenium :

Selenium is a necessary trace element in organisms including all animals, which is specified in Sec as the 21st amino acid. Selenium deficiency can lead to extensive inflammation and even liver cancer ***(25).***

Chemical structure:

Sodium Selenite is an inorganic form of the trace element Se with potential antineoplastic activity. Its molecular formula is Na2SeO3. Figure (5).

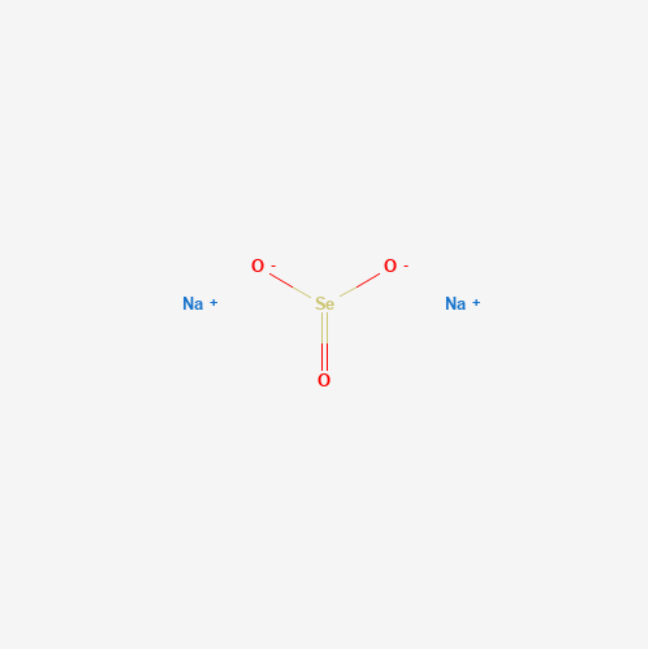


Figure (5) Sodium selenite chemical structure ***(26).***

Sources:

Selenium is a trace mineral that exists in minimal concentrations in the body but can play an essential role in human health. Brazil nuts, seeds, mushrooms, fish, seafood, meat and poultry are among the foods high in Se. In plant food, Se appears in the organic form as selenomethionine and in supplementation as selenate and selenite.; both the organic and inorganic forms have greater than 90% bioavailability ***(27).***

(a)

(b)

(c)

Pharmacokinetics:

Absorption:

Organic forms of Se such as selenomethionine and selenocysteine are absorbed in the small intestine through the active sodium dependent transport system. Inorganic selenate crosses the intestinal brush border membrane where absorption occurs via the sodium-facilitated and energy-dependent system. Alternatively, selenite is absorbed by non-mediated passive diffusion, with a slow absorption rate compared to amino acid–bound Se compounds ***(28).***

Distribution:

Tissue Se supply is dependent on the plasma Se transporter seleno-protein P (SELENOP). The liver, a key organ in whole-body Se homeostasis, is where the majority of SELENOP is produced. The differential tissue distribution of Se may occur because of differential SELENOP uptake ***(29).***

Metabolism:

Selenium has one of these three fates according to ***(29).***

Incorporation to selenoprotiens P, selenoenzymes such as glutathione peroxidase, type 1-iodothyronine deiodinase and thioredoxin reductase.

Binding by non-specific plasma proteins such as albumin or globulin metabolites

Hepatic methylation into nontoxic metabolites.

Excretion:

Selenium is excreted in urine, the process of converting 'metabolically active' Se to urinary metabolites is in competition with the use of that Se for the synthesis of selenoproteins, especially selenoprotein P. This competition appears to take place principally in the liver ***(30).***

Pharmacodynamic properties of selenium:

Mechanism of Action:

Selenium exerts its biological functions via molecules called selenoproteins, containing the amino acid selenocysteine. The human genome encodes 25 selenoproteins. Se plays a role in forming several biologically significant molecules/molecular families in humans and other mammals. ***(31)***

Table (1): Biological functions of selected selenoproteins ***(32)***

Selenoprotien	Biological function
Glutathione peroxidases	Reduces lipid peroxides formed during food digestion in the alimentary tract. Protects against peroxidation of lipids, which are incorporated into biological membranes and lipoproteins. Plays a part in the process of organism defense against oxidative stress
Thioredoxin reductase	Reduces nucleotides during DNA synthesis. Maintains the intracellular redox homeostasis. Participates in the prevention and repair of damage caused by H2O2 oxidative stress
Iodothyronine deiodinase	Participates in the production and inactivation of active thyroid hormones. Determines normal metabolism of thyroid hormones
Selenoprotein P	Protects against oxidizers. Prevents liver necrosis and lipid oxidation. Protects against free radicals. Transports Se to other tissues (brain, kidney, testes, erythrocytes) Plays main part in Se organification and metabolism
Selenoprotein W	An intracellular transporting medium for Se Metabolizes muscle. Prevents excessive oxidation
Selenophosphate synthetase	Catalyzes the reaction of selenophosphate synthesis. Participates in biosynthesis of selenophosphate from selenide and ATP
Selenoprotein R	Contains antioxidant properties. Participates in methionine metabolism. Participates in protein repair

Protective role of selenium against the toxic effects

selenium seems to be one of the most appealing agents to be examined in relation to its protective role against the toxic effects induced by different harmful factors, both chemical and physical. But its effect depends on many factors, such as its chemical form as well as the applied dose and experimental model, so supplementation must be performed taking proper precautions to obtain the best results and avoid the toxicity of Se itself. Se plays a preventive role against the formation of free radicals and reactive oxygen species, and thus prevents the development of many chronic diseases. Se acts as a cofactor of three enzyme groups, the first group includes glutathione peroxidases (GPx), which are responsible for the reduction of hydrogen peroxide (H2O2) and organic peroxides using an electron donor-reduced glutathione. For example, GPx1 peroxidase is responsible for the protection of cells against oxidative stress, while gastrointestinal peroxidase (GPx2) in turn protects against lipid peroxides resulting from lipid peroxidation. GPx3 reduces peroxides in plasma and other body fluids, while GPx4 reduces phospholipid peroxides, protects against oxidative stress and is responsible for the integrity of chromatin in sperm. Se plays a significant role in the anti-cancer protection of the organism ***(33).***

The second important group are thioredoxin reductases (TrxR), which are a reducing substrate in reactions with thioredoxin (Trx). At the cellular level, together with thioredoxin (Trx) and Nicotinamide adenine dinucleotide phosphate (NADPH), they form the so-called thioredoxin system, which is responsible for the regulation of redox and H2O2 by mitochondria. Moreover, by combining with thioredoxin (Trx), it forms a complex that can protect the human body against cardiovascular diseases ***(34).***

The third group; iodothyronine deiodinases which is responsible for the conversion of thyroid hormones. Enzymes are responsible for activating and deactivating thyroid hormones, such as when iodine is removed from thyroxine, which is converted into triiodothyronine, and then into diiodothyronine**.** Se level in patients with chronic liver disease is lower than healthy people, especially in patients with advanced chronic liver disease such as hepatitis, cirrhosis, and liver cancer. The reason for liver damage is mainly related to extensive inflammation and oxidative stress, generated by excessive ROS production, which promotes liver diseases. Due to the unique chemical reactivity of selenocysteines, several selenoproteins have been reported to mitigate and repair liver damage and prevent liver necrosis. ***(35).***

Se supplementation inhibits carcinogen induced covalent DNA adduct formation and reduces the oxidative damage to DNA, lipids and proteins. Many showed that Se inhibits pro-oxidant enzyme NADPH oxidase and decreases the free radical production by activated macrophages and decreases enzymatic and non-enzymatic lipid perioxidation. The discussed mechanisms may be responsible for the attenuation of CP-induced oxidative stress and the subsequent DNA damage ***(36)*.**

Selenium has a protective role against CP induced nephrotoxicity. At the level of kidney function Se decreased urea and creatinine levels beside decreasing the oxidative stress markers and increasing the antioxidants. Histologic examination revealed a significant improvement of CP induced inflammatory foci, congested blood vessels, narrowed Bowman's capsule space, glomerular compaction, hyalinized material accumulation in renal tubules, and local sloughing of tubular epithelial cells. Se even preserved the normal kidney histology except for minor local changes. Se is protective against CP induced cardiotoxicity and ovarium toxicity in rats. Se has also been proved to possess many beneficial pharmacological properties: anti-hyperglycemic, anti-hyperlipidemic, hepatoprotective, antiulcer and antidepressant. Se has a major protective role against : cisplatin induced nephrotoxicity and gonadal toxicity, cadmium hepatotoxicity and ovarium toxicity, hepatotoxicity and nephrotoxicity of aluminum, mercury and arsenic and lead and chromium induced neurotoxicity ***(37)*.**

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