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Review Article

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Toxicity of carbon tetrachloride, free radicals and role of antioxidants

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Abstract: Several chemicals, including environmental toxicants and clinically useful drugs, cause severe cellular damage to different organs of our body through metabolic activation to highly reactive substances such as free radicals. Carbon tetrachloride is an organic compound of which chemical formula is CCl_4. CCl_4 is strong toxic in the kidney, testicle, brain, heart, lung, other tissues, and particularly in the liver. CCl_4 is a powerful hepatoxic, nephrotoxic and prooxidant agent which is widely used to induce hepatotoxicity in experimental animals and to create hepatocellular carcinoma, hepatic fibrosis/cirrhosis and liver injury, chemical hepatitis model, renal failure model, and nephrotoxicity model in recent years. The damage-causing mechanism of CCl_4 in tissues can be explained as oxidative damage caused by lipid peroxidation which starts after the conversion of CCl_4 to free radicals of highly toxic trichloromethyl radicals ($\bullet CCl_3$) and trichloromethyl peroxyl radical ($\bullet CCl_3O_2$) via cytochrome P450 enzyme. Complete disruption of lipids (i.e., peroxidation) is the hallmark of oxidative damage. Free radicals are structures that contain one or more unpaired electrons in atomic or molecular orbitals. These toxic free radicals induce a chain reaction and lipid peroxidation in membrane-like structures rich in phospholipids, such as mitochondria and endoplasmic reticulum. CCl_4-induced lipid peroxidation is the cause of oxidative stress, mitochondrial stress, endoplasmic reticulum stress. Free radicals trigger many biological processes, such as apoptosis, necrosis, ferroptosis and autophagy. Recent researches state that the way to reduce or eliminate these CCl_4-induced negative effects is the antioxidants originated from natural sources. For normal physiological function, there must be a balance between free radicals and antioxidants. If this balance is in favor of free radicals, various pathological conditions occur. Free radicals play a role in various pathological conditions including Pulmonary disease, ischemia / reperfusion rheumatological diseases, autoimmune disorders, cardiovascular diseases, cancer, kidney diseases, hypertension, eye diseases, neurological disorders, diabetes and aging. Free radicals are antagonized by antioxidants and quenched. Antioxidants do not only remove free radicals, but they also have anti-inflammatory, anti-allergic, antithrombotic, antiviral, and anti-carcinogenic activities. Antioxidants contain high phenol compounds and antioxidants have relatively low side effects compared to synthetic drugs. The antioxidants investigated in CCl_4 toxicity are usually antioxidants from plants and are promising because of their rich resources and low side effects. Data were investigated using PubMed, EBSCO, Embase, Web of Science, DOAJ, Scopus and Google Scholar, Carbon tetrachloride, carbon tetrachloride-induced toxicity, oxidative stress, and free radical keywords. This study aims to enlighten the damage-causing mechanism created by free radicals which are produced by CCl_4 on tissues/cells and to discuss the role of antioxidants in the prevention of tissue/cell damage. In the future, Antioxidants can be used as a therapeutic strategy to strengthen effective treatment against substances with high toxicity such as CCl_4 and increase the antioxidant capacity of cells.

Keywords: antioxidants; carbon tetrachloride; hepatotoxicity; nephrotoxicity; neurotoxicity; oxidative stress.

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Introduction

CCl_4 is a colorless, clear, fireproof, and volatile liquid substance. It has a carbon atom at its center and four Cl^- atoms around it. Besides naturally occurring, it can also

occur as a result of many chemical reactions. It has strong chemical stability, correspondingly resulting in an atmospheric half-life of 30 to 100 years [1]. While CCI_4 was widely used in the production of cleaning agents and solvents, in grain spraying and the synthesis of chlorofluorocarbons as an intermediate product, its production was reduced after its toxicity had been discovered. Although the harmful effects of products, various oils, varnish, polish, rubber waxes, insecticides, as resin solvent and in starting materials of organic compounds [2, 3]. CCl_4 enters the body easily through inhalation, ingestion and dermal absorption. Respiration is the primary way of exposure in which pulmonary absorption is estimated to be 60% in humans. The rate of absorption from the gastrointestinal system is rapid and greatly influenced by the diet (for example, fat or alcohol increases absorption of CCl_4 in the intestine) [4]. The average daily intake of CCl_4 for the general population is estimated to be 0.1 mg. After exposure to this toxic compound by ingestion, inhalation or dermal absorption, it spreads in the body with the highest concentrations through the liver, brain, kidney, muscle, fat and blood. Human data on the carcinogenic effects of CCl_4 is limited. However, it has been shown that CCl4 induces hepatocellular carcinomas by oral, inhalation and parenteral exposure in rodents. US Environmental Protection Agency classified CCl_4 in Group B2 as possibly carcinogenic to humans [5, 6]. Acute toxicity of CCl_4 has been obtained from many animal studies. Especially the studies on rats have shown that the lethal dose $(LD)_{50}$ is after acute oral intake and the body weight is within the range of 4.7–14.7 mL/kg, based on nutritional conditions and applied supplements [7]. The general population may be exposed to CCl_4, albeit in small amounts, from the surrounding air because CCl_4 easily vaporizes. Unfortunately, the interfusion of CCl_4 into the air, water, and soil as chemical waste cannot be controlled [8]. The first step in tissue/cell damage caused by CCl_4 is cytochrome P450-mediated transfer by transferring a single electron to the C–Cl bond; this leads to the formation ($\bullet CCl_3$), which is a carbon-centered radical and an intermediate metabolite, and then the transformation of it to the trichloromethyl peroxyl radical ($\bullet OOCCl_3$) in the presence of oxygen. These reactive free radical metabolites of CCl_4 initiate lipid peroxidation by reacting with polyunsaturated fatty acids (PUFA); or cause cell membrane disruption, leakage of microsomal enzymes, and thus cell damage by covalently binding to protein and fatty acids [6, 9–10]. Lipid peroxidation products are highly reactive and show significant biological effects which, depending on their concentration, cause selective changes in cell signaling, protein and DNA damage, and cytotoxicity. The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH) Among the many different aldehydes that may occur as secondary products during lipid peroxidation, there are structures such as malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE) [11, 12]. Although MDA appears to be the most mutagenic product of lipid peroxidation, 4-HNE is the most toxic one [13] (Figure 1). Carbohydrates are also affected by free radicals. Reducing sugars plays an important role in modifying proteins through the formation of advanced glycation end products in a non-enzymatic reaction called glycation. Glycation is a common mechanism found in many disorders, and molecular precursors, particularly reactive dicarbonyl metabolite methylglyoxal, are key to the development and accumulation of damage. It is known that biological products related to glycation are mainly related to aging, neurodegenerative disorders, diabetes and its complications, atherosclerosis, kidney failure, immunological changes, retinopathy, skin photo, osteoporosis, and progression of some tumors [14–16]. Proteins interact easily with free radicals due to the sensitive amino acids in their structure. The amino acids of cysteine, methionine and histidine are particularly sensitive to the attack and oxidation of the hydroxyl radical. Enzymes, where these amino acids are located in positions critical to the activity of the enzyme, enter the path of interaction with free radicals and the activity of the enzyme is disabled. Besides free radical oxidation of proteins can lead to changes in the three-dimensional structure of the proteins, as well as the cleavage, aggregation or cross-linking of the proteins [17–18]. DNA is the genetic material of the cell, and permanent damage to DNA can lead to changes (i.e., mutations) in the proteins encoded in DNA, which can lead to malfunction or complete inactivation of the affected proteins. DNA must remain intact for the viability of individual cells and even the whole organism. ROS is an important source of DNA damage that causes yarn breaks, removal of nucleotides and various changes of the organic bases of the nucleotides. Cells have developed repair mechanisms to correct naturally occurring changes in DNA. However, excessive changes caused by ROS can lead to permanent changes in DNA or damage to DNA. Cellular DNA damage plays a role in the etiology and progression of many different human disorders and diseases [17, 19]. CCl_4 causes disorders in the kidneys, lungs, testicle, and brain. Some chemicals, including various environmental toxicants and clinically useful drugs, can cause serious cellular damage in different organs of our body through metabolic activation with highly reactive substances such as free radicals [20–22].

Biomolecules such as proteins, lipids, nucleic acids, and carbohydrates are generally suitable for oxidation,

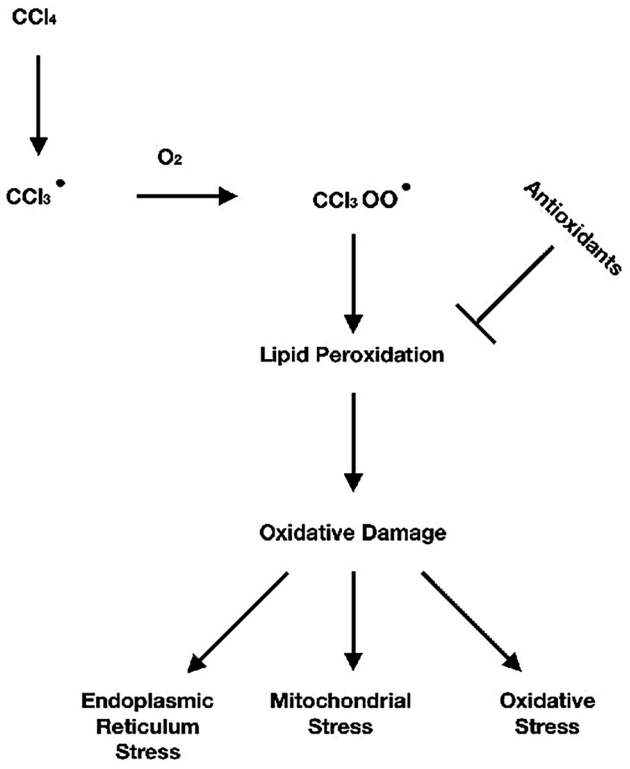

Figure 1: Reactive free radical metabolites of CCl_4 react with polyunsaturated fatty acids, initiating lipid peroxidation. This causes oxidative/mitochondrial, endoplasmic reticulum stress. Antioxidants, on the other hand, stop lipid peroxidation and prevent these stress situations from occurring.

which leads to a change in the structure of biomolecules. ROS, which has a comprehensive effect on cell physiology, is produced as a byproduct of normal cellular metabolism in the oxidative reaction process of the mitochondrial respiratory chain. In addition, ROS is produced as a cellular response to xenobiotics, cytokines, and bacterial invasion. Moderate ROS has positive effects such as killing invasive pathogens, wound healing and repair processes. However, Excessive ROS exposure impairs redox homeostasis. ROS has a short half-life and reacts with nearby molecules such as proteins, DNA, RNA, glucids or free fatty acids and initiates them as free radicals and changes their structure and/or functions. The resulting oxidative modifications of biomolecules are quite stable [23-24]. ROS in the cell changes the balance between oxidant/antioxidant status, leading to cell damage, apoptosis and cell death. In recent years, free radicals such as NO, $ONOO^-$, H_2O_2, $\mathbf{O^{\bullet}_2}$ and $^{\bullet}OH$ are the most important factors mediating oxidative stress and the cornerstone or precursor of some detrimental diseases [25, 26]. Free radicals are reactive chemical species that differ from other compounds since they have unpaired electrons in their outer orbits. Free radicals may damage cellular components [27]. Because of the unstable configuration in the outer orbit, it is then released by reacting with nearby biomolecules, such as carbohydrates, nucleic acids, proteins, and lipids. ROS mediates various intracellular signaling cascades. This type of damage caused by free radicals is called "oxidative stress". Another definition of oxidative stress is that the imbalance between oxidants and reductants (antioxidants) at the cellular or individual level resulting in favor of oxidants [26, 28–31]. Along with this, ROS also damages the organelles such as endoplasmic reticulum and mitochondrion [32, 33]. Accumulated ROS leads to ER dysfunction, thereby inducing ER stress. ROS causes unfolded and misfolded protein production, which further induces ER stress [34]. CCl_4 is one of the most powerful toxins widely used in scientific researches to produce experimental models in many pathophysiological conditions [35–38]. Cells have a complex antioxidant defense system that regulates cellular redox homeostasis to reduce or eliminate ROS damage. Antioxidants are classified as enzymatic and non-enzymatic antioxidants. Examples of non-enzymatic antioxidants are *glutathione*, *minerals*, *uric acid*, *bilirubin*, *melatonin*, *vitamins* (A, C, E), *carotenoids* (lycopene, β-carotene, zeaxanthin, lutein), *bioflavonoids* (quercetin, myricetin), flavone (e.g., apigenin, luteolin), flavonoids (e.g., taxifolin), flavan-3-ols (e.g. catechin, epigallocatechin), flavanone (e.g., hesperetin, naringenin), anthocyanidin (e.g., cyanidin, delphinidin), isoflavone (e.g., genistein, daidzein), *Hydroxycinnamates* (ferulic acid, caffeic acid, sinapic acid, *p*-coumaric acid). Enzymatic antioxidants function through a variety of enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GSH-Px), peroxiredoxins and glutathione S-transferase (GST). NADPH, GSH, and thioredoxin act together with these enzymes to defend against damage caused by ROS [26, 39, 40]. In the ROS production process, superoxide radicals are the primary reactive oxygen intermediate; SODs catalyze the rapid removal of superoxide radicals and are converted into H_2O_2. The intermediate H_2O_2 is then converted into water with CAT or GSH-Px. CAT is found in peroxisomes and contains iron [41, 42]. GSH-Pxs are found in cytosol, mitochondria, plasma, and nucleus. GSH-Pxs help prevents lipid peroxidation and maintains redox balance as well as intracellular homeostasis. Thioredoxin plays several key roles in maintaining the redox environment of the cell. In the defense against ROS, the thioredoxin system lowers H_2O_2 in cooperation with Trx peroxidases or glutathione [43]. Antioxidants do not only remove free radicals, they also have anti-inflammatory,

anti-allergic, antithrombotic, antiviral, and anticarcinogenic activities [44].

In recent years, there is an increase in analyzing the role of antioxidants in reducing the harm of toxic substances such as free radical-producing CCl_4. This study aims to discuss the damage of free radicals produced by CCl_4 on cell/tissue and the mechanism of action of natural antioxidant compounds.

Methodologies and literature search

PubMed, EBSCO, Embase, Web of Science, directory of open access journals (DOAJ), Scopus, and Google Scholar were searched using keywords of Carbon tetrachloride, carbon tetrachloride-induced toxicity, CCl_4-induced hepatotoxicity, CCl_4-induced renal Toxicity, CCl_4-induced nephrotoxicity, CCl_4-induced neurotoxicity, CCl_4-induced reproductive system, CCl_4-induced testis damage, oxidative stress, free radicals, antioxidants, and antioxidant therapy. A synthesis was obtained from the determined findings and results. In this review, no date limitation was made while scanning the articles. If possible, the last 5 years were preferred. But sometimes it was used in previously published articles.

Hepatotoxicity of CCl_4

The liver is a vital organ that performs a wide range of functions, including biotransformation and detoxification of endogenous and exogenous harmful substances and metabolic homeostasis [45–46]. It has been reported that numerous drugs and chemicals cause liver injury, which is generally considered to be the main cause of chronic liver disease [47]. CCl_4 belongs to the hepatotoxin class which plays a role after the metabolic activation. It is believed that CCl_4 usually enters hepatocytes and forms free radicals to cause peroxidation, which leads to disruption of liver structure and damage in liver function [48, 49]. The developmental stages of CCl_4-induced liver injury are summarized as follows; reductive dehalogenation, covalent binding of radicals, inhibition of protein synthesis, fat accumulation, loss of calcium homeostasis, apoptosis, and fibrosis [50]. Mechanisms such as activation of Kupffer cells, lipid peroxidation, reactive aldehydes, and nucleic acid hypomethylation along with the production of proinflammatory mediators are seen as supporting mechanisms for CCl_4 induced hepatotoxicity. In addition to the activation of Kupffer cells, it can activate macrophages, T lymphocytes and neutrophils that participate in liver inflammation [11, 51, 52]. CCl_4 is a strong hepatoxic and prooxidant agent widely used to induce hepatotoxicity and to create hepatocellular carcinoma, hepatic fibrosis/cirrhosis and liver injury, and chemical hepatitis model. In the acute toxic doses of CCl_4, fatal liver failure occurs when the regenerative capacity of the liver is exceeded [11, 49, 53, 54]. It started to be used to create a murine non-alcoholic steatohepatitis model with rapid progression of broad fibrosis and HCC, using the high-fat, high fructose and high cholesterol western diet with weekly low-dose intraperitoneal CCl_4 [55]. CCl_4 application causes significant pathological changes such as tremendous hepatocellular necrosis, bile duct proliferation, balloon degeneration, leukocyte infiltration (inflammation), vascular occlusion, loss of hepatic nodules structure, perisinusoidal space cords, increased collagen depositions, central vasodilation, cellular hypertrophy, hepatocellular fibrosis, fatty acid infiltration, and vascular degeneration and calcification [56–58]. CCl_4 increases oxidative stress in the liver. The mechanism underlying liver injury due to oxidative stress involves the imbalance of oxidation and antioxidant systems, thereby forms excessively free radicals and reducing antioxidant capacity [54]. CCl_4 causes a decrease in liver cells, in the activities of antioxidant enzymes such as CAT, SOD, GSH-Px, GST, GR, and Glutathione levels content to endogenous antioxidants [57–60]. CCl_4 increases the protein carbonyl content, which is a protein oxidation product, at the oxidative stress biomarker MDA level [61]. Antioxidants reduced MDA, H_2O_2, TBARS and ROS, which are markers of oxidative stress in liver tissue, and increased the activities of SOD, CAT, GSH-Px, GR antioxidant enzymes. (Table 1) It has been reported that CCl_4 activates proinflammatory cytokine-producing Kupffer cells and significantly upregulates the expressions of TNF-α, Monocyte chemoattractant Protein-1, Macrophage inflammatory protein-2, IL-1β, IL-6, TGF-21, which is a pro-fibrotic cytokine, and nuclear factor-mB p65 protein in the CCl_4 induced liver injury models. In the CCl_4-induced hepatic fibrosis model, it has been reported that the expression levels of α-SMA and COL-1a1 mRNA, which are the fibrotic markers in the liver tissue, were again upregulated [49, 58, 60, 61]. CCl_4 application significantly increases the concentrations of serum marker enzymes in the liver. It causes an increase in the amounts of enzymes in serum which are normally found in the cytoplasm (Table 1) An increase in Alanine Transaminase (ALT), AST: Aspartate Transaminase (AST), GGT, and Bilirubin levels are indicative of cellular leakage and loss of functional integrity of the liver cell membrane and these tests are critical determinants of

liver function (Table 1). Oral exposure to CCl_4 alters liver enzymes as well as increases triglyceride, total cholesterol, LDL-cholesterol levels and reduces pseudocholinesterase values. Along with this, the level of lipogenic transcription factor SREBP-1 is upregulated to target lipogenic enzyme FAS activity [7, 54, 61, 62]. It is one of the main factors causing oxidative stress, nitrosative stress, endoplasmic reticulum stress, mitochondrial stress, inflammation, and hepatic damage mediated by free radicals derived from CCl_4. Cytochrome P450 enzyme is involved in the process of CCl_4-induced liver injuries. Cytochrome P450 in hepatocytes catalyzes CCl_4 to produce highly reactive $\bullet CCl_3$ and $\bullet OOCCl_3$. It is suggested that CCl_3O_2 creates alkylation reaction by inactivating the enzymes directly through membrane proteins and covalent bonds with the first mechanism or stimulates the membrane lipid peroxidation which causes liver steatosis, fibrosis or cirrhosis, by affecting the membrane fatty acids with the second mechanism. The fact that CCl_4 increases lipid peroxidation disrupts especially endoplasmic reticulum and mitochondria. Besides, this radical reacts with nucleic acids and proteins and damages cellular processes. The formation of an adduct between the DNA and CCl_3 is also triggered [7, 49, 53, 63–64]. Peroxides, which are the peroxidation products, inhibit protein synthesis and activity of some enzymes. Following these events, free radical production exceeds the antioxidant defenses in the liver; this results in oxidative destruction of the cell membranes and severe tissue damage. Free radicals are agents that cause or at least aggravate liver injury that can cause chronic liver diseases such as liver fibrosis and cirrhosis [65, 66]. The liver contains a large number of mitochondria and it is the main source for free radicals. In the CCl_4-induced liver injury, significant reduction is observed in the mitochondrial complex 1 and 2 activities [61]. Free radicals produced by CCl_4 disrupt the integrity and stability of the mitochondrial structure causing mitochondrial dysfunction. When the mitochondrial permeability transition pore is opened, the mitochondria swell and thus results in low mitochondrial membrane potential (MMP), which is a sensitive index used to assess mitochondrial function. Mitochondrial dysfunction causes unbound oxidative phosphorylation in energy respiration. A significant number of electron leaks occur from the non-separated electron transport chain. The leakage of the electron into the final electron acceptor during the electron transport enables it to bind to oxygen (O_2) and is considered as the main ROS source. High ROS level initiates lipid peroxidation, consumes free radical scavengers, breaks down the body's antioxidant system and leads to blasting oxidative stress in the body [67, 68]. Increased ROS based on CCl_4 may cause tissue damage through lipid peroxidation and increase Tissue Inhibitor of Metalloproteinase-1 expression, decrease EGF expression, and cause liver fibrosis due to the accumulation of collagen in the liver [60]. The prominent pathological feature of liver fibrosis is an excessive accumulation of extracellular matrix (ECM) [69]. CCl_4 increases α-SMA-positive myofibroblast-like cells, which are considered to be a suitable marker of hepatic fibrosis in the liver, and again, increases the hyaluronic acid (HA), laminin (LN), collagen type 3 (Col III), collagen type IV (Col IV) levels significantly. Also, it increases the level of MMP-9, one of the MMPs which can play an important role in predicting and repairing the condition of liver injury and inflammation [58, 61]. It has been observed that CCl_4 application indicated a quite significant increase in the AKT, MAPK STAT3, and TGF-b expression and that the Nrf2 expression, which is an important transcription factor that regulates the expression of a group of detoxifying and antioxidant defense genes in the liver, decreased significantly [47, 70, 71] (Table 1). CCl_4 causes upregulation of the proapoptotic protein Bax and downregulation of the antiapoptotic protein Bcl2. Similarly, CCl_4 increases Fas/FasL expression and increases the activity of caspase-3 and-8 and cytochrome P450 2E1, which leads to liver apoptosis. It binds to the Fas ligand and forms the signal complex causing death by Fas-associated protein with death domain (FADD) and then activates caspase-8, which leads to activation of caspase-9 and 3 [60, 70, 71] (Table 1). CCl_4 induces endoplasmic reticulum stress. It induces glucose-regulated protein of 78 kDa (GRP78), total X-Box Binding Protein 1 (XBP1t), added X-Box Binding Protein 1 (XBP1s), jointless X-Box Binding Protein 1 (XBP1s) [72]. As a result, CCl_4 damages the membrane of liver cells and prevents the proper functioning of organelles such as endoplasmic reticulum and mitochondria since it is a hepatotoxin. Antioxidants reduce the risk of CCl_4-induced hepatocellular carcinoma, hepatic fibrosis/cirrhosis, liver injury, and chemical hepatitis. Antioxidants gain importance by reducing oxidative stress, mitochondrial stress, endoplasmic reticulum stress and preventing macromolecular oxidation in liver tissue. Antioxidants neutralize the harmful effects of free radicals induced by CCl_4 on liver cells and modulate biochemical changes in liver tissue and pull parameters to physiological limits (Table 1).

Nephrotoxicity of CCl_4

The kidney is an important organ that is necessary for the maintenance of homeostasis by the body, the regulation of the extracellular environment such as detoxification and

Table 1: Protective effects of antioxidants against CCl_4-induced hepatotoxicity.

Animal model	Organ	Treatment	Outcomes	Effect	References
Mice (CCl_4, 10 mL/kg/b.w, i.p)	Liver	*Taxifolin*	AST↓, ALT↓, SOD↑, GSH-Px↑, GST↑, MDA↓	Taxifolin alleviates acute liver injury caused by CCl_4 in mice.	[31]
Mice and rats (CCl_4)	Liver	*Thymosin β4 (TB4)*	AST↓,ALT↓,SOD↑, GSH-Px↑, GST↑, MDA↓, TNF-α ↓, IL-1β ↓, Hydroxyprolinecontents↓	TB4 shows a hepatoprotective effect against liver injury in mice and rats induced by CCl_4.	[49]
Rats (CCl_4, 0.2 mL/10 g/b.w)	Liver	*E. ulmoides Extract (ILF-RE)*	SOD↑, CAT↑,GSH-Px↑, GST↑, AST↓, γ-GT ↓ , ALT↓, TG↓, Total Cholesterol↓, CHOP↓, p-PERK ↓, p-eIF2↓, SREBP-1↓, FAS↓,	ILF-RE may be a potential therapeutic agent for preventing/treating CCl_4-induced chronic hepatic dysfunction.	[54]
Rats (CCl_4, 0.5 mL/kg b.w)	Liver	*Rumex hastatus*	AST↓, ALP↓γ-GT ↓ , ALT↓, TG, Total Cholesterol, SOD↑, CAT↑, GSH-Px↑, GST↑, H2O2↓, TBARS↓ ,GST↑, GSR↑, GR↑, POD↑	*Rumex hastatus* strengthens the defense mechanism of antioxidants in treatment and can play a therapeutic role in diseases mediated by free radicals	[57]
Rats (CCl4, 1 mL/kg b.w)	Liver	*Averrhoa carambola L. (Oxalidaceae) roots (EACR)*	AST↓, ALP↓ , ALT↓, Hyp ↓,SOD↑,GSH-Px↑, GST↑, MDA↓, TBARS↓ ,GST↑, GSR↑, GR↑, Col-I↓HA↓, LN↓, Col III↓, Col IV↓, α-SMA↓ , TIMP-2, TGF-β1, Smad2↓, Smad4↓, Smad7↓, Bax/Bcl-2 ↓, caspase-3/caspase-3	EACR decreases liver fibrosis in CCl_4 treated rats. EACR is anti-fibrotic, antioxidant, and anti-apoptotic.	[58]
Rats (CCl_4, 3 mL/kg)	Liver	*Rutin*	AST↓, ALT↓, IL-6↓, MEK5↓, FADD↓, Bcl2↑, Bcl-xl↓, EGF↑, JAK↓	CCl4 application causes alteration in the expression of IL-6/STAT3 pathway genes, leading to hepatotoxicity. Rutin reverses these expression changes and protects against CCl4-induced hepatotoxicity.	[60]
Rats (400 mg/kg, i.p)	Liver	Naringenin	ALP↓, γ-GTP ↓ , ALT↓, GLYCOGEN↑, GST↑, MMP-9↑, MMP-2↑ , CTGF↑, Col-I↑, MMP-13↑, NF-κB↓, IL-1β↓, IL-10↓, TGF-β↓	Naringenin prevents oxidative stress and inflammation pathways, thus fulfilling its antifibrotic effects.	[66]
Mice (CCl_4, 15.95 g/kg, i.p)	Liver	*Salidroside*	SOD↑, CAT↑, GST↑, MDA↓, GOT↓, GPT↓, ROS↓, Gadd45a↓, Makp7↓, Rras2↓	Salidroside protects the liver from CCl_4-induced injuries and oxidative stress by maintaining mitochondrial function.	[68]
Rats (CCl4, 1 ml/kg b.w)	Liver	Silymarin, Vitamin E and Curcumin	ALT↓, MAPK↑, Nrf2↑, AKT↓, STAT3↓, Smad-2↓, TGF-β↓	Vitamin E, silymarin, curcumin combination can be used as a hepatoprotective agent against hepatotoxic substances.	[73]
Mice (CCl_4, 1 mL kg^{-1}, b.w)	Liver	*Pithecellobium dulce*	ALP↓ , ALT↓, ROS↓,SOD↑, CAT↑, GSH-Px↑, GST↑, LHP↓TBARS↓, PC↓, GSR↑, GR↑,GSSG↓, Total thiols↑, CYP P450↑, CYP2E1↑	AEPD protects the murine liver against oxidative degradation caused by CCl4, possibly due to its antioxidant properties.	[74]
Rats (CCL_4, 1 mL/kg, b.w)	Liver	*N-acetyl cysteine (NAC)*	AST↓, ALP↓, ALT↓, MDA↓	It has been seen that NAC has a protective effect against the toxicity of Cl_4.	[75]

the elimination of toxic metabolites and drugs. Therefore, the kidney can be accepted as the main target organ for exogenous toxic substances [76, 77]. CCl_4 is on the list of nephrotoxic drugs and chemicals such as Acetylaminofluorene, Diethylnitrosamine, streptozotocin, amikacin, amoxicillin, amphotericin B, amoxicillin, benzylpenicillin, cefotaxime, ceftazidime, cirozino, amitromin sulfadiazine, vancomycin, captopril, furosemide, hydralazine, hydrochlorothiazide, losartan, acetazolamide mannitol, acetaminophen, warfarin, and risperidone [6, 66, 67]. Recently,

CCl_4 started to be used to induce experimental renal failure model, experimental nephrotoxicity model, and oxidative stress in the kidneys. After the application of CCl_4 in rats, it has been seen that CCl_4 is distributed in higher concentration in the kidney compared to the liver and CCl_4 has a high affinity to kidney tissue [6, 78, 79]. CCl_4 adversely affects kidney function. CCl_4 exposure slows kidney function and increases Blood Urea Nitrogen (BUN), Creatine Kinase (CK-NAC), Lactate Dehydrogenase (LDH), Total bilirubin, Total protein, creatinine concentration, creatinine clearance, protein, albumin, WBCs, Platelet, Mean% lymphocytes, Mean% granulocytes, Mean% monocytes levels in the blood and lowers RBC. An increase in these parameters causes nephrotoxicity. High creatinine and urea levels are indicatives of serious damage to the structural integrity of the nephrons. It does not increase until at least half of the kidney nephrons are damaged or destroyed [80–84]. Urine analysis provides important information about if the kidneys functioning properly or not. In the urines of CCl_4-applied rats, urine specific gravity, RBC, WBC count, protein, urea, creatinine, Albumin, urobilinogen, and LDL increased. Increased specific gravity indicates dehydration, renal artery steatosis, severe fibrosis, renal necrosis, renal toxicity, and glomerular damage. (Table 2). Besides, CCl_4 reduces urine pH level [81, 85, 86]. Proximal tubular cells of the kidney are quite sensitive to CCl_4 toxicity due to high cytochrome P450 content. The trichloromethyl and trichloromethyl peroxyl free radicals that are formed after this substance is metabolized by cytochrome P450 cause cell damage. It has been indicated that when CCl_4 is exposed, free radicals formed by oxidative stress cause kidney injury [21, 87, 88]. Proximal tubular toxicity develops due to direct nephrotoxic effects such as mitochondrial dysfunction, lysosomal hydrolase inhibition, phospholipid damage, and increased intracellular calcium concentration. Oxidative stress has a significant effect on uremia, kidney failure, and other kidney diseases. Renal oxidative stress is often the result of the upregulation of proxy-to-enzyme-dependent ROS production and the exhaustion of antioxidants together. Depletion or inactivation of antioxidants leads to accumulation of endogenous ROS within cells. It activates ROS, MAPK, P53, and possibly P21, leading to renal tubular cell death. Then, ROS contributes directly or indirectly to the fibrotic process through increased inflammation. Fibrosis and inflammation itself may return to the pathway and further increase ROS formation or stimulate the production of cytokines and growth factors [42]. Radicals produced by CCl_4 damage cell membrane, lipids, proteins, and DNA in kidney tissue cells [6, 84]. Altering the antioxidant status with CCl_4 or increasing free radicals causes nephropathies. In many studies, it has been reported that CCl_4 application significantly decreases SOD, GSH-Px, GST, GR, CAT activities, and GSH levels in renal tissues. After CCl_4 administration, an increase in lipid peroxidation products (MDA, LPO, TBARS), an increase in DNA damage and an increase in protein oxidation product were found in kidneys. Oxidative stress caused by excessive ROS production often leads to kidney inflammation and fibrosis through various signaling pathways. (Table 2) CCl_4 also increases the production of classic inflammatory cytokines such as IL-1, IL-2, and TNF-α, but also increases the activity of caspase 9 and caspase 3, among the important enzymes of apoptosis, defined as programmed cell death [5, 82, 83, 89–94]. Caspases are involved in apoptosis subclassified by effect mechanisms based on initiator caspases such as caspase 9 or caspase 3. CCl_4 increases the activity of caspase 9 and caspase 3, which can induce apoptosis by stimulating proapoptotic Bax and inhibiting anti-apoptotic Bcl-2 proteins [82]. Cytokines such as IL-1β, IL-2, IL-6, and TNF-α are released by leukocytes and renal tubular cells and are associated with inflammation pathogenesis in acute kidney injury. Inflammatory processes are mainly activated by NF-B, which practically modulates cytokine production and thus increases the production of inflammatory cytokines [21, 82, 93]. CCl_4 makes histopathological changes in kidney tissue. In the kidneys of CCl_4-applied rats, histopathological findings such as glomerular basement membrane thickening, interstitial inflammation, cellular infiltration, tubular cell swelling, vasocongestion, pyknotic nucleus, medullary vascular congestion and glomerular necrosis, atrophy, brush border loss, separation of epithelial cells in proximal, and distal tubules have been indicated [22, 84, 90, 94, 95]. In recent years, various studies have been conducted in the prevention and treatment of CCl_4-induced renal toxicity. As a result of these studies, it has been seen that antioxidants have an important role in reducing or removing renal toxicity. Preventive effects of antioxidants against renal oxidative stress induced by CCl_4 have been attributed to high phenol levels. Antioxidants used in the studies indicate that they can protect against CCl_4-induced nephrotoxicity by increasing the activity of antioxidant enzymes or the levels of non-enzymatic antioxidants (Table 2).

Neurotoxicity of CCl_4

The brain is an important organ that assists the body's normal activities and contains various physiological functions [96]. The fact that CCl_4 is lipophilic enables it to access to cells easily. Therefore, it is accumulated in many

Table 2: Protective effects of antioxidants against CCl_4-induced nephrotoxicity.

Animal model	Organ	Treatment	Outcomes	Effect	References
Rats (CCl_4, 3 mL/kg b.w)	Kidney	Ferulic acid	SOD↑, CAT↑, GSH-Px↑, TBARS↓, H_2O_2↓, PC↓, GST↑	Ferulic acid effectively quenches free radicals, inhibits lipid peroxidation and improves antioxidant status in tissues.	[5]
Mice (CCl_4, 1 mL/kg, i.p)	Kidney	*Allium jesdianum Boiss*	BUN↓, Creatinine↓, CAT↑, GST↑, MDA↓, GST↑	*Application of the hydroalcoholic extract of Allium jesdianum Boiss may prevent nephrotoxicity caused by CCl_4.*	[22]
Mice (CCl_4, 1 mg/kg)	Kidney	*Glycyrrhiza glabra L (GG)*	WBC↓, RBC↑, Urea↓, Creatinine↓, SOD↑, CAT↑	GG has a nephroprotective effect and has indicated that it can be used to improve structural changes in the kidney due to CCl_4-induced toxicity.	[80]
Mice (CCl_4, 1.5 mL/kg)	Kidney	*Zingerone*	BUN↓, Creatinine↓, SOD↑, CAT↑,GSH-Px↑, TBARS↓, GST↑, GSR↑, IL-1β↓, IL-2↓, TNFα↓	*Zingerone significantly alleviated CCl_4-induced renal toxicity.*	[82]
Rats (CCl_4, 3 mL/kg b.w/i.p)	Kidney	*Rutin*	Urea↓, Creatinine↓, Uric acid↓, ↓SOD↑, CAT↑,GSH-Px↑, MDA↓	*Rutin partly overcame CCl_4-induced nephrotoxicity by showing antioxidant effect.*	[83]
CCl_4 (3 mL/kg b.w)	Kidney	*Sonchus asper* (SA)	Urea↓,Creatinine↓,Creatinine clearance↑, Protein↓, Albumin↓, Urobilinogen↓, SOD↑, CAT↑, GSH-Px↑, TBARS↓ H2O2↓, GST↑, GSR↑	SA protects kidneys by relieving CCl_4-induced oxidative stress in rats.	[85]
CCl_4 (1 mL/kg b.w)	Kidney	*Raphanus sativus* Seeds (RSME)	Urea↓, Albumin↓, Creatinine↓, Protein↓, SOD↑, CAT↑, GSH-Px↑, GST↑, H_2O_2↓, TBARS↓, GST↑, GSR↑	*RSME shows that it can relieve the damage occurred due to CCl_4 in the renal tissue of rats.*	[86]
Rats (CCl_4, 1 mL/kg)	Kidney	*Curcumin + Vitamin E*	Urea↓, Albumin↓, Creatinine↓, T. Protein↓, SOD↑, CAT↑, GSH-Px↑, GST↑, TBARS↓, H_2O_2↓, PC↓, GST↑	*Vitamin E and curcumin combination can be considered as an important combination in fighting against potentially oxidative stress and CCl_4-induced nephrotoxicity.*	[95]

organs, including the brain [97–99]. On the other hand, the facts that CCl_4 is lipophilic lead it to cross the blood-brain barrier, quickly taken up by the brain, accumulate in the brain and thus lead to neurotoxicity [100, 101]. Various CCl_4 poisoning studies have been indicated that CCl_4 causes free radical formation in many tissues including the brain [102]. The brain is rich in polyunsaturated fatty acids and is more susceptible to lipid peroxidation due to an unusually high oxygen consumption rate. Polyunsaturated fatty acids and aerobic metabolic activity of the brain increases the sensitivity of this organ to peroxidative damage induced by free radicals after CCl_4 ingestion [103, 104]. Compared to other organs of the body, the brain's antioxidant defense system activity is relatively lower and more susceptible to oxidative stress [105]. The disadvantage of the brain compared to the other organs is that it is not capable of regenerating the damage caused by neuroinflammatory progressions resulting from increased ROS production and that many neurotransmitters are autoxidation to create ROS [101, 106]. Another opinion which argues that the brain tissue is vulnerable to oxidative stress or free radicals is about the fact that brain is rich in iron and therefore playing a catalytic role in the production of oxygen-free radicals [107]. This mechanism works as follows. CCl_4 releases its neurotoxic effects through free radical ($\bullet CCl_3$) which leads to membrane lipid peroxidation. Free radicals produced from CCl_4 and the main molecule damage the endoplasmic reticulum, which leads to the accumulation of lipids, decreased protein synthesis and mixed-function oxidase activity [59, 101]. Peroxidation of the membrane phospholipids causes the loss of membrane integrity, an increase in inflammatory markers and finally stimulated cell death [108, 109]. In addition to inhibiting the activities of antioxidant enzymes such as SOD, GSH-Px, CAT based on CCl_4 toxicity, the indicators of processing towards the oxidative stress in

neurodegenerative diseases decrease in GSH level and increase in lipid peroxidation product MDA and NO levels [110, 111] (Table 3). The findings on the effect of CCl_4 on acetylcholinesterase (AChE) enzyme are different. Some studies have reported that CCl_4 decreases AChE activity [6, 112, 113] and some studies have reported that CCl_4 increases AChE activity [107]. In fact, there are contradictions. AChE plays a role in the hydrolysis inside the choline of the acetylcholine, which is a basic neurotransmitter of the central nervous system. Acetylcholine (ACh) is the main neurotransmitter of the cholinergic system associated with cognitive functions such as spatial and episodic memory, working memory, learning, and modulation of cerebral blood flow. In some neurological disorders, such as Alzheimer's disease, acetylcholinesterase is excessively activated in the synapses, thereby acetylcholine levels in the brain significantly reduce, leading to impaired neurotransmission and thus memory loss and other adverse effects [114–116]. The AChE enzyme is a target of carbamates used as pesticides and organophosphates (insecticides and nerve agents) in the treatment of Alzheimer's disease (approved drugs such as donepezil, rivastigmine, and galantamine), and these are inhibitors of AChE enzyme [117]. While organophosphates and carbamates bind irreversibly to the AChE enzyme, reversible binding of the drugs used in the treatment of Alzheimer's disease to the AChE enzyme makes them advantageous. After all, drugs used in the treatment of Alzheimer's disease are not successful enough. Although there are different views, the AChE enzyme is targeted and altered by CCl_4 in both conditions [117]. CCl_4 increases inflammation. Proinflammatory mediators have been found to increase levels of TNF-α, IL-1β, IL-6, and TGF-1β [118, 119]. CCl_4 application is a neurotoxic agent that reduces antioxidant capacity in brain tissue and leads to increased inflammation (Table 3). To summarize, CCl_4 exposure of brain tissue causes oxidative stress due to disruption of balance in prooxidant/antioxidant homeostasis in neurons. Oxidative stress causes free radical formation, which is potentially toxic for neurons. Excessive free radical formation damages neuron loss and lipids, proteins and DNA, which trigger axonal damage, so free radicals cause neurotoxicity. Oxidative stress plays a role in the progression of Alzheimer's disease, Huntington disease, Spinocerebellar ataxia, amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease and other neurodegenerative diseases. Also, Free radicals contribute to protein misfolding, glia cell activation, mitochondrial dysfunction, and then cellular apoptosis. Antioxidants neutralize the harmful effects of CCl_4-induced free radicals on neurons. Some antioxidants pull the parameters to physiological limits by modulating biochemical changes in neurons [15, 120, 121] (Table 3).

CCl_4-induced testicular toxicity

Testicles produce sperm by balancing the self-renewal and differentiation of spermatogonial stem cells during male reproductive life [125]. Male sexual dysfunction is caused by various problems related to alcoholism, some drugs, aging, drug addiction, and smoking, sperm concentration caused by toxic chemicals, motility, and hormonal imbalance [126, 127]. Heavy metals such as lead, cadmium, and uranium have a similar effect on testicles that disrupt spermatogenesis through mechanisms involving the induction of lipid peroxidation, depletion of ROS cleansers, and disruption of testicular antioxidant enzyme activity [128].Studies have shown that both oxidative stress and changes in the antioxidant enzyme system are the two most important factors leading to reproductive dysfunction [129] (Table 4). One of the target organs in CCl_4 toxicity is the testicle, the reproductive organ [130]. In neonatal rats exposed to CCl_4 by oral and inhalation, decreased chance of survival in newborns, decreased fertility in rats, decreased sperm production in male rats, and degenerative changes in testicles have been observed [131]. In experimental studies, it has been reported that low or high dose CCl_4 exposure causes oxidative tissue damage and lipid peroxidation in testicles, oxidative DNA damage, DNA insertions, chromosomal abnormalities, and genetic mutations. Besides, it has been also indicated that histopathological changes in testicular tissue occurred due to CCl_4 toxicity. Oxidative stress is considered to be one of the main causes of DNA damage in germ cells. Normally, the male has a balance between the reproductive system, ROS formation, and antioxidant activity. However, increased ROS in sperm disrupts sperm or seminal plasma antioxidant defense mechanisms and may cause oxidative stress. CCl_4 decreased GSH-Px and CAT levels while increasing the MDA level. Antioxidants reduced MDA, H_2O_2, TBARS, and ROS, which are markers of oxidative stress in testicular tissue, and increased the activities of SOD, CAT, GSH-px, GR antioxidant enzymes [75, 132–134] (Table 4). The development of spermatozoa, from spermatogonial stem

Table 3: Protective effects of antioxidants against CCl_4-induced neurotoxicity.

Animal model	Organ	Treatment	Outcomes	Effect	References
Rats (CCl_4, 2 mL/kg b.w)	Brain	*Pleurotus ostreatus*	SOD↑, CAT↑, GSH-Px↑	*Extract of P. ostreatus relieves the oxidative damage caused by CCl_4 in the brain of Wistar rats.*	[107]
Rats (CCl_4)	Brain	*Aqueous extract of Bryophyllum pinnatum (AEFP)*	SOD↑, CAT↑, AChE↑, ADA↑, GSH-Px↑, NO↓, MDA↓, TSH↑, NSPH↑	*AEBP's ability to destroy free radicals demonstrates its preventive role against short-term memory effect caused by CCl_4.*	[101]
Rats (CCl_4, 10% solution, 1.25 mL/kg p.o.)	Brain	*Alcesefoliside*	SOD↑, CAT↑, GSH-Px↑, AChE↑, MDA↓, GST↑	*Alcesefoliside has a neuroprotective effect against CCl_4-induced brain toxicity in rats.*	[112]
Rats (CCl_4, 2 mL/kg /b.w)	Brain	Flaxseed oil	SOD↑, CAT↑,GSH-Px↑, GST↑, NO↓, MDA↓, TNF-α↓, IL-6↓, TGF-β1↓, IL-1β↓	Flaxseed oil has indicated antioxidant and anti-inflammatory effects against CCl_4 toxicity.	[118]
Rats (CCl_4, 2 mL/kg/b.w)	Brain	Grape seed oil (GSO)	SOD↑, CAT↑, GSH-Px↑, GST↑, NO↓, MDA↓, TNF-α↓, IL-6↓, TGF-β1↓	GSO has a neuroprotective effect against CCl_4-induced brain injury.	[119]
Rats (CCl_4, 1 mL/kg, i.p)	Cerebrum, Cerebellum	Vanillin	SOD↑, CAT↑, AChE ↓,GST↑, NO↓, MDA↓	Vanillin blocks the oxidative brain injury caused by CCl_4 in rats.	[122]
Rats (CCl_4, 1 mL/kg)	Brain	Watermelon juice or ursodeoxycolic acid (UDCA)	MDA↓	Watermelon juice protects brain tissue from CCl_4 toxicity.	[123]
Rats (CCl_4, 2 mL/kg b.w)	Brain	*Cape gooseberry (Physalis juice)*	SOD↑, CAT↑,GSH-Px↑, GST↑, GR↑	*Physalis juice can be effective in preventing neurotoxicity and shows antioxidant and anti-apoptosis properties.*	[124]

cells, is regulated by various hormones and this process is controlled by the hypothalamic-pituitary-testicular axis. ROS accumulation in the testicles induces hypogonadism [133]. Peroxidation of sperm lipids destroys the structure of the lipid matrix in the membranes of the spermatozoa and it is associated with rapid intracellular ATP loss leading to axonemal damage, decreased sperm motility and increased mid-piece morphological defects [135]. Spermatozoa require a high PUFA content to provide the necessary fluidity to the plasma membrane during fertilization. However, this makes spermatozoa particularly vulnerable to ROS attacks, which are associated with decreased fertility [135–139]. CCl_4 has indicated seminiferous tubule necrosis, edema, and fiber accumulation, also slope and damage in walls. These effects are thought to result from the production of oxygen radicals that exceed the antioxidant capacity of stressed tissue. As a result of CCl_4-induced toxicity, a significant increase was observed in the percentage of abnormalities in sperm head morphology [140]. Seminal ROS reduces sperm motility and disrupts sperm morphology. Kalla and Bansal observed severe spermatogenic cycle destruction, including loss of germinal epithelium, empty germ cells, and constriction in tubular structures after the 20th day of initiation of CCl_4 in rats [141]. CCl_4 caused germ cell loss in seminiferous tubules of rat testicles, inhibition of mitosis, partial disappearance of the interstitium, and structural deterioration of sertoli cells [142]. CCl_4 application causes significant decreases in body weight and weights of testicles, epididymides and accessory sex glands, as well as reducing Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and testosterone levels (Table 4). It also increases estrogen and prolactin levels. In the male reproductive system, prolactin and estrogens antagonize the effects of testosterone, causing infertility in males [143]. High levels of estrogen may directly affect spermatogenesis through the disruption of gonadal atrophy secretion. Hyperstimulation of hypothalamic estrogen receptors may affect the gonadotrophin-releasing hormone (GnRH) pulse, which directly regulates GnRH gene expression at the GnRH neuron level. It can be induced by stimulating P450, which catalyzes the production of estrogen from androgen. Besides, it has been explained that testosterone secretion may be impaired by excessive oxidative stress and degeneration of Leydig cells [75, 132, 144, 145]. In CCl_4 application, significant histopathological findings such as necrosis, degeneration, desquamation, organism, reduction in germinal cells,

Table 4: Protective effects of antioxidants against CCl_4-induced testicular toxicity.

Animal model	Organ	Treatment	Outcomes	Effect	References
Rats (CCl_4, 3 mL/kg b.w)	Testis/Serum	*Rutin*	FSH↑, LH↑, Testosterone ↑, Sperm counts↑, sperm motility↑, sperm abnormality↑	*It has been discovered that Rutin has a protective effect not only against ROS-mediated oxidative stress based on CCl_4-induced toxicity but also against testicular/fertility deterioration.*	[83]
Rats (0.25 mL kg^{-1})	Testis	Cinnamon (*Cinnamomum zeylanicum*)	SOD↑, CAT↑, GSH-Px↑, GST↑, Sperm motility↓, Epididymal sperm concentration↓	It has been indicated that cinnamon has a protective effect against cellular damage in male reproductive organs induced by CCl_4.	[139]
Rats (CCl_4, 1 mL/kg)	Testis	*Quercetin*	CAT↑,GSH-Px↑, MDA↓, GST↑, Sperm motility↓, Epididymal sperm concentration↓	*It has been discovered that Quercetin has a mitigating effect on abnormalities in sperm shapes, testicular histopathological lesions and CCl_4-induced damages in apoptosis. This effect of Quercetin is an inhibitor on CYP activity as well as the removal of free radicals and the suppression of LPO.*	[140]
Rats (2 mL/kg b.w)	Testis	*Teucrium polium*	SOD↑, CAT↑, GSH-Px↑, TBARS↓, FSH↑, LH↑, Testosterone↑, Sperm motility in epididymis ↑, Sperm motility in epididymis↑, Sperm motility in testicles↑, Sperm count in epididymis	The mitigating effect of *T. polium* on sperm parameters, sex hormones, oxidative stress, and histopathological disorders has been proved and also it has been concluded that it can protect the reproductive system of male rats from CCl_4-induced damage.	[144]
Rats (2 mL CCl_4/kg b.w)	Testis	*Physalis peruviana* L.	SOD↑, CAT↑, GSH-Px↑, LPO↓, GST↑, GR↑, FSH↑, LH↑, Testosterone↑, Caspase 3↓	*It clearly shows that P. peruviana juice strengthens the defense mechanism of antioxidants against reproductive toxicity of CCl_4 and provides evidence that water can play a therapeutic role in diseases and infertility of free radical origin.*	[147]
Rats (2 mL CCl_4/kg/ b.w) granatum)	Testis	Pomegranate (Punica)	SOD↑, CAT↑,GSH-Px↑, TBARS↓, GST↑, GR↑, FSH↑, LH↑, Testosterone↑	It has been concluded that pomegranate juice strengthens the defense mechanism against CCl_4-induced reproductive toxicity and may play a therapeutic role in diseases based on free radicals.	[148]
Rats (CCl_4, 1 mL/kg)	Testis	*Jurenia dolomiaea (JDEE)*	SOD↑, CAT↑,GSH-Px↑, LPO↓, GST↑, GR↑, H_2O_2↓, Testosterone↑	*JDEE has shown an antioxidant effect against CCl_4-induced oxidative stress in testicles of rats.*	[149]
Rats (CCl_4, 1 mL/kg b.w)	Testis	*Berberis integerrima* Bge. root (MEBIR)	CAT↑, LPO↓, GST↑, GR↑, MDA↓, Testosterone↑	*It has been concluded that the protective effects of MEBIR on testicular damage caused by CCl4 have been due to the antioxidant effects of bioactive compounds.*	[150]
Rats (5 mL/kg)	Testis	Geranylgeranylacetone (GGA)	Testosterone↑, LDH↑, ALP↑, MDA↓, T-AOC↑, Hsp 70↑, Gonadosomatic index↑	GGA increased HSP70 expression. GGA reversed testicular damage due to its antioxidant effects.	[151]

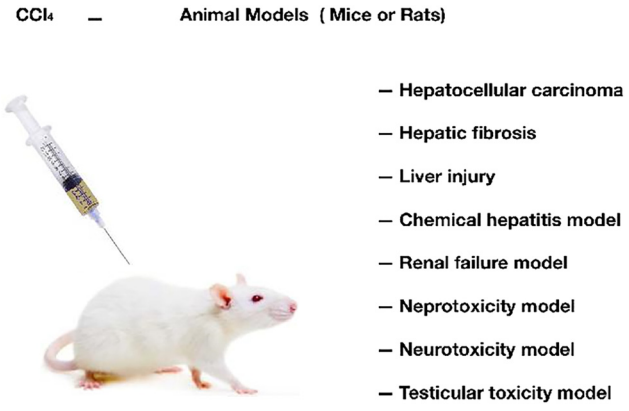

Figure 2: CCl_4 is used in experimental research in animal model development. Revealing free radicals in experimental research, CCl_4 plays a critical role in cellular damage, tissue inflammation. Therefore, it provides an important advantage for drug research. As a result of these studies, it is learned about the potential benefits of drugs on humans.

spermatogenesis arrest, and significant decreases in ST, GCLT and Johnsen's testicle score diameters were determined. It causes histopathological damage in the testicles and an increase in the apoptotic index of the testicles [146]. It has been indicated that CCl_4 increased the number of caspase 3 positive cells in rat testicles. This shows that the mechanism of cell death involves caspase 3 activation. Massive necrosis in the testicles and, consequently, oxidative stress activate caspase 3 and increase apoptosis [127]. Some antioxidants have been used to prevent testicular oxidative stress, hormonal disorders, apoptosis, and sperm abnormalities. These antioxidants have been shown to prevent oxidative stress, hormonal disorders, apoptosis, and sperm abnormalities. The removal of ROS from the testicles has been attributed to the presence of phenolic and polyphenolic compounds that may have different functional properties, such as prevention of the formation of free radicals and chain-breaking activity [145] (Table 4).

Conclusion

CCl_4 is strong toxic in the kidney, testicle, brain, heart, lung, other tissues, and particularly in the liver. It disrupts the functions of these tissues. CCl_4 is a strong hepatoxic, nephrotoxic, and prooxidant agent widely used to induce hepatotoxicity and to create models of hepatocellular carcinoma, hepatic fibrosis/cirrhosis and liver injury, and chemical hepatitis, renal failure model, and nephrotoxicity model in experimental animals (Figure 2). CCl_4 is an important source of free radicals. Excess free radicals in the cell can lead to many harmful effects, including lipid peroxidation, DNA modification and protein oxidation, resulting in cell damage, increased inflammation, apoptosis and cell death. The way to reduce or eliminate these CCl_4-induced negative effects is the antioxidants that act as shields. It is promising because of antioxidants extracted from plants, rich sources, low diversity side effects from all antioxidants investigated in CCl_4 toxicity. In the future, Antioxidants can be used as a therapeutic strategy to strengthen effective treatment against substances with high toxicity such as CCl_4 and increase the antioxidant capacity of cells.

Highlights

What is current knowledge?
- CCl_4 is free radical source and a strong toxic substance.
- CCl_4 is the cause of oxidative stress, mitochondrial stress, endoplasmic reticulum stress.

What is new here?
- Promising targets have been reviewed in reducing and treating the toxicity of CCl_4.
- Antioxidant compounds react with free radicals from CCl_4 and are involved in reducing cell damage. Thus, antioxidant intake can help maintain normal physiological function.

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