

# Recent interventions on carbon tetrachloride-mediated free radical toxicity in liver and kidney and role of antioxidants: a review

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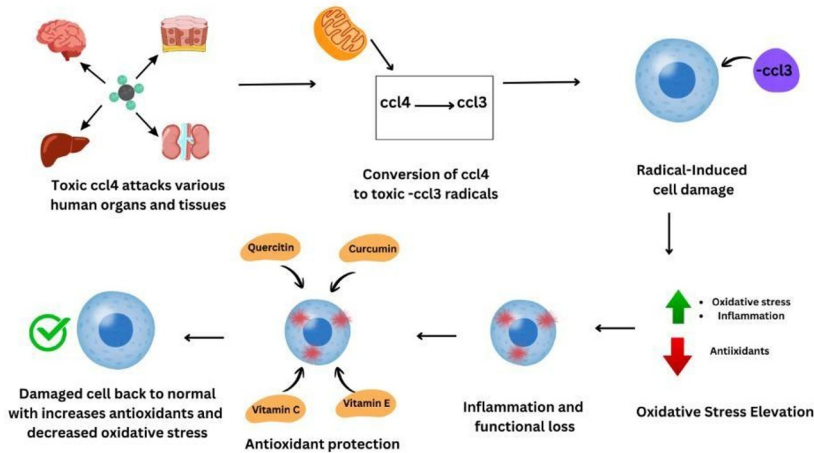
## ABSTRACT

By turning into very reactive free radicals, various substances, including environmental pollutants and some medications, damage cells in many organs. The most harmful of these is carbon tetrachloride (CCl<sub>4</sub>). It affects not only the liver but also the kidneys, brain, heart, lungs, and reproductive organs. CCl<sub>4</sub> is toxic to the liver and kidneys. It breaks down into more reactive radicals in the liver through the cytochrome P450 enzyme system. The main radicals produced are trichloromethyl (CCl<sub>3</sub>) and trichloromethyl peroxy (OCCl<sub>3</sub>). These radicals cause harm by producing lipid peroxidation, disrupting mitochondrial function, and ultimately leading to cell death through processes like ferroptosis, necrosis, and apoptosis. This imbalance between antioxidants and free radicals leads to ongoing oxidative stress. This stress is connected to long-term issues like cancer, neurological diseases, and liver fibrosis. While synthetic medications can

have side effects, plant-based natural antioxidants may neutralize these harmful radicals. They also offer anti-inflammatory, anti-cancer, and protective properties. This paper emphasizes the damaging effects of  $\text{CCl}_4$  on cell and tissue membranes and explores how natural antioxidants could help prevent this damage.

**KEYWORDS:** Carbon tetrachloride ( $\text{CCl}_4$ ), Free radicals, Oxidative stress, Lipid peroxidation Natural antioxidants.

## Graphical Abstract



## INTRODUCTION

Carbon tetrachloride ( $\text{CCl}_4$ ) is a colorless, volatile liquid that used to be common in cleaning solutions, refrigerants, pesticides, and varnishes. Its use has declined due to its toxic effects, but it can still be found in industrial waste and labs. Because  $\text{CCl}_4$  is very stable, it can remain in the environment for many years [36]. The compound can enter the body through breathing, swallowing, or skin contact, with the lungs being a main entry point. After exposure, it builds up in important organs like the liver, kidneys, brain, and fatty tissues. Research shows that even small amounts can cause serious damage, including liver cancer and organ failure in animals [33]. This has led the EPA to classify it as a possible human carcinogen. In the body,  $\text{CCl}_4$  is first processed in the liver, where enzymes convert it into a highly reactive radical ( $\text{CCl}_3\cdot$ ), when oxygen is present, these radical changes into  $\text{OOCCL}_4$ , which attacks cell membranes, proteins, DNA, and other cellular parts. This starts a harmful cycle of oxidative stress, leading to lipid peroxidation, DNA damage, protein dysfunction, and ultimately cell death [41].

The harmful effects of  $\text{CCl}_4$  are not limited to fats; sugars and proteins are also at risk. Reducing sugars can bind to proteins in a process called glycation. This produces advanced glycation end-products (AGEs), which are linked to aging, diabetic

complications, and tissue injury. Proteins, especially those with delicate amino acids like cysteine and methionine, can undergo oxidation, losing their proper shape and biological activity. DNA is another major target [27]. Free radicals can break strands, alter bases, and trigger mutations that threaten cell survival. While the body normally produces reactive oxygen species (ROS) during energy metabolism and immune defense, small amounts are beneficial for functions like killing microbes and signaling repair [24]. However, excessive ROS, as seen during CCl<sub>4</sub> exposure, overwhelms the antioxidant defenses and creates oxidative stress [40]. To combat this, the body relies on enzymatic antioxidants such as SOD, CAT, and GSH-Px, along with non-enzymatic ones like glutathione, vitamins A, C, and E, and plant-derived polyphenols [28]. Together, these substances neutralize radicals and help maintain cellular balance. Recently, researchers have focused on natural compounds such as flavonoids, phenolics, and herbal extracts. These are viewed as safer and more effective options than synthetic drugs for controlling oxidative stress caused by CCl<sub>4</sub> and similar toxins. This review highlights how CCl<sub>4</sub> damages cells, triggers free radical cascades, and how natural antioxidants may serve as protective agents [42].

### **HEPATOTOXICITY OF CCL<sub>4</sub>**

The liver is the main detoxifying organ in the body. It filters toxins, regulates metabolism, and breaks down harmful compounds. However, these functions make it highly susceptible to damage from chemicals, particularly carbon tetrachloride (CCl<sub>4</sub>), which is a strong liver toxin [6]. Inside the liver, CCl<sub>4</sub> is processed by cytochrome P450 enzymes into reactive radicals (CCl<sub>4</sub> and OOCCL<sub>4</sub>) [32]. These radicals damage cell membranes, start lipid peroxidation, and create a chain reaction that disrupts protein production, fat metabolism, calcium balance, and the liver's ability to regenerate. This process leads to inflammation, scarring, cell death, and, in serious cases, liver failure. CCl<sub>4</sub> exposure triggers immune and inflammatory cells such as Kupffer cells, T cells, and neutrophils [11]. This increases the release of pro-inflammatory and fibrotic factors like TNF- $\alpha$ , IL-6, TGF- $\beta$ 1, and MCP- 1 [16]. At the same time, oxidative stress worsens: levels of markers like MDA, TBARS, H<sub>2</sub>O<sub>2</sub>, and ROS increase sharply, while antioxidant enzymes (SOD, CAT, GSH-Px, GST, GR) decrease. Pathological changes include ballooning degeneration of hepatocytes, fatty accumulation, bile duct proliferation, fibrosis, collagen deposits, immune cell infiltration, vascular injury, and calcification. Blood tests show elevated ALT, AST, GGT, and bilirubin, indicating hepatocyte damage and membrane leakage [21]. With long-term exposure, CCl<sub>4</sub> can lead to steatohepatitis, cirrhosis, and hepatocellular carcinoma (HCC), particularly in experimental models fed high-fat or Western diets [1]. On a cellular level, CCl<sub>4</sub> disrupts mitochondrial function, lowering membrane potential and uncoupling oxidative phosphorylation [38]. This increases ROS production and weakens ATP generation. These reactive species damage DNA, proteins, and lipids, limiting the cell's ability to repair itself [7]. Additionally, CCl<sub>4</sub> causes endoplasmic reticulum (ER) stress, raising levels of proteins like GRP78 and XBP1. These are indicators of a disrupted unfolded protein response, which further jeopardizes liver cell survival.

Fortunately, natural antioxidants have shown protective effects against these injuries [18]. They reduce oxidative and mitochondrial stress, restore antioxidant enzyme activity, decrease lipid peroxidation, and improve liver enzyme profile [24]. They also block inflammatory and fibrotic signals such as TGF- $\beta$ ,  $\alpha$ -SMA, and COL-1a1 [19]. Compounds like quercetin, curcumin, flavonoids, and vitamins C and E help maintain liver architecture and function, lessen fibrosis and cancer risk, and support recovery by neutralizing radicals and regulating stress pathways.

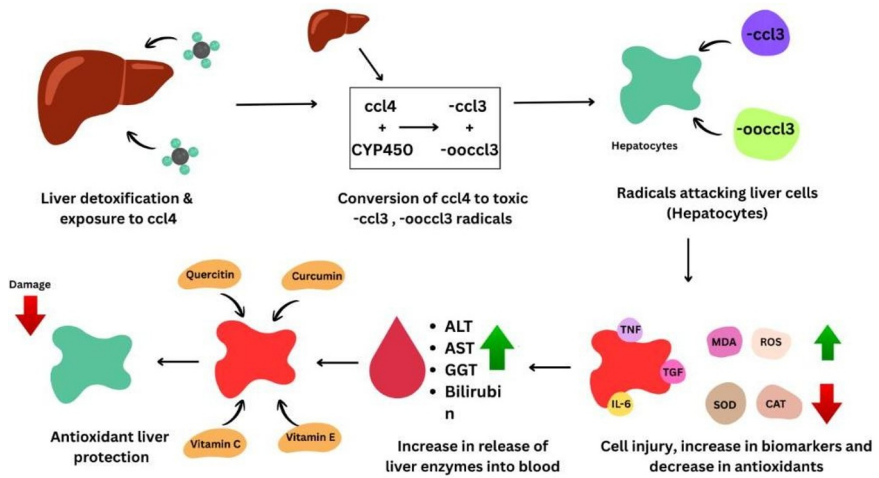


Fig.1 - Representation of ccl<sub>4</sub> mediated hepatotoxicity

Table-1 – Hepatotoxic characteristics of carbon tetrachloride

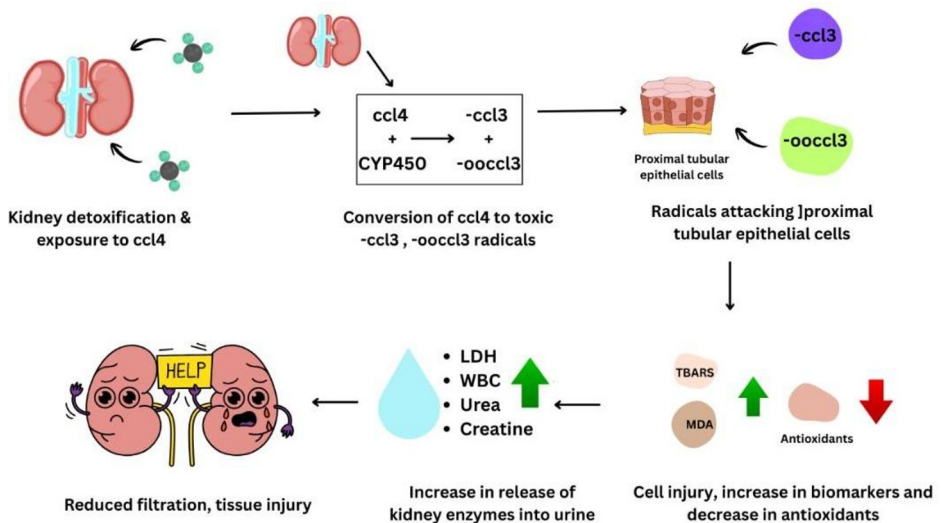
Action of CCl <sub>4</sub>	Details of action	Reference
Cellular Site of Attack	<ul style="list-style-type: none"> <li>• Endoplasmic membrane damage</li> <li>• Mitochondrial damage</li> </ul>	[12]
Mechanism of Action	<ul style="list-style-type: none"> <li>• Reductive activation by cytochrome P450</li> <li>• Formation of CCl<sub>4</sub> and haloalkylation</li> <li>• Formation of OOCCL<sub>4</sub> and lipid peroxidation</li> <li>• Reactive aldehydes formation and enzyme inhibition</li> <li>• Cellular hypomethylation</li> <li>• Loss of Ca<sup>2+</sup> sequestration and catabolic enzymes</li> <li>• Loss of energy supply</li> <li>• Recovery phase</li> <li>• Apoptosis</li> </ul>	[22]
Molecular Mediators	<ul style="list-style-type: none"> <li>• Tumor necrosis factor-α (TNF-α) &amp; interleukin-1</li> <li>• Transcription factors AP-1 &amp; NF-κB</li> <li>• Nitric oxide</li> <li>• Interleukins-6, -8, and -10</li> <li>• Transforming growth factors α &amp; β</li> </ul>	[15]
Lipid Homeostasis	<ul style="list-style-type: none"> <li>• Obstruction of lipoprotein</li> </ul>	[6]

	secretion • Dysfunctional Golgi apparatus • Increased triglyceride synthesis • Decreased $\beta$ -oxidation • Steatosis	
Oxygen Partial Pressure	• Normal O <sub>2</sub> pressure favors lipid peroxidation • Very low or high O <sub>2</sub> directs haloalkylation	[4]
Potentialiation of Toxicity	• Induction of CYP2E1, CYP2B1, CYP2B2	[20]
Beneficial Intervention	• Antioxidants & radical scavengers • Inhibition of cytochrome P450 & catabolic enzymes • Restoration of methylation • Preserve Ca <sup>2+</sup> sequestration • Kupffer cell inactivation via gadolinium	[12]
Other Haloalkanes	• Chloroform & phosgene formation • Bromine & iodine haloalkane dehalogenation	[26]

### NEPHROTOXICITY OF CCL<sub>4</sub>

The kidneys are vital for keeping the body's internal environment stable. They filter the blood, get rid of waste, balance fluids and electrolytes, and remove toxins [25]. Because they deal with harmful substances, they can easily suffer from toxic injury [11]. Carbon tetrachloride (CCl<sub>4</sub>) is one such chemical that can harm the kidneys, similar to other harmful agents like vancomycin and certain antibiotics. In research studies, CCl<sub>4</sub> is often used to cause kidney injury to investigate oxidative stress and kidney failure [17]. It tends to build up in kidney tissues more than in the liver, which shows it has a strong attraction to the renal system. When exposed to CCl<sub>4</sub>, kidney functions and blood chemistry change [33]. This leads to higher levels of urea nitrogen, creatinine, lactate dehydrogenase, bilirubin, and creatine kinase [45]. We also see more white blood cells, lymphocytes, and inflammatory markers, while red blood cell counts drop. This points to serious nephron injury that often goes unnoticed until more than half of the nephrons are damaged. Urinary changes include excess protein, creatinine, urea, albumin, and blood cells [37]. We also see higher urine specific gravity, indicating dehydration and decreased kidney function, along with a drop in urine pH that reflects acidosis or poor acid-base regulation. The cells in the proximal tubules are particularly at risk because they have a lot of cytochrome P450 enzymes, which turn CCl<sub>4</sub> into highly reactive free radicals like trichloromethyl and trichloromethyl peroxy [9]. These radicals harm mitochondria and lysosomes, disrupt membranes and proteins, increase intracellular calcium, impair energy metabolism, and eventually cause cell death. The resulting oxidative stress fuels inflammation and fibrosis, making kidney damage worse [34].

Carbon tetrachloride (CCl<sub>4</sub>) increases the production of reactive oxygen species while reducing the activity of important antioxidant defenses like superoxide dismutase, catalase, glutathione peroxidase, and glutathione [22]. This oxidative imbalance leads to lipid peroxidation, indicated by markers such as malondialdehyde and TBARS [44]. It also causes damage to DNA and proteins, activates apoptotic pathways involving caspase-3 and caspase-9, and triggers proinflammatory signaling through interleukins and tumor necrosis factor. Most of this inflammatory response is driven by the NF-κB pathway, which boosts cytokine production and causes further tissue injury. Histological studies of kidneys exposed to CCl<sub>4</sub> show notable changes, including thickening of the glomerular basement membrane, swelling of tubular cells with loss of their brush borders, necrosis in glomerular structures, blood vessel congestion, nuclear shrinkage, and disruption of normal tubular organization. These changes indicate both structural damage and reduced kidney function [35].



**Fig 2:** Representation of ccl<sub>4</sub>mediated Nephrotoxicity

**Table-2– Nephrotoxic characteristics of carbon tetrachloride**

ACTION	MECHANISM DETAILS	SPECIFIC EFFECTS	REFERENCE
Radical Generation	Cytochrome P450 cleaves CCl <sub>4</sub> into CCl <sub>3</sub> - and Cl- free radicals	Alkylation of renal proteins/DNA; initiates chain reactions	[12]
Lipid Peroxidation	Radicals react with unsaturated fatty acids in membranes	↑ MDA levels; tubular membrane disruption and necrosis	[33]
Antioxidant	ROS overload	↓ GSH, SOD, CAT,	[2]

Depletion	consumes defenses; MT scavenging	GPx; ↑ protein carbonyls, NO	
Structural Pathology	Direct tubular/glomerular insult	Acute tubular necrosis, hypertrophy, dilatation, fibrosis	[26]

## ROLE OF ANTIOXIDANTS

Studies show that antioxidants can help reduce kidney damage caused by carbon tetrachloride (CCl<sub>4</sub>) [8]. Their protective effects are linked to plant-derived phenolic compounds, restoring natural antioxidant enzyme activity, suppressing inflammatory cytokines, and reducing lipid peroxidation and other signs of oxidative stress [14]. This suggests that antioxidants may be a useful strategy for preventing or treating CCl<sub>4</sub>-induced nephrotoxicity and aiding kidney recovery. Besides affecting the kidneys, CCl<sub>4</sub> is also known to harm the nervous system [5]. The brain, as the body's central control system, is very sensitive to toxic substances. CCl<sub>4</sub> presents particular risks due to its lipophilic nature [31]. This property allows it to cross cell membranes easily, accumulate in fatty tissues, and penetrate the blood-brain barrier, making direct contact with neural structures possible. Once inside the brain, CCl<sub>4</sub> is broken down into free radicals that cause oxidative stress and lead to cellular damage [3]. The risk is greater because the brain is rich in polyunsaturated fatty acids and consumes a lot of oxygen, making it very vulnerable to lipid peroxidation. At the same time, its natural antioxidant defenses are relatively limited compared to other organs, increasing its susceptibility to CCl<sub>4</sub>-induced neurotoxicity.

Unlike the liver or skin, the brain has a very limited ability to regenerate. This means injuries from oxidative stress, inflammation, or toxic substances often last a long time or cannot be reversed. Brain tissue also has high levels of iron, which can speed up the formation of free radicals through reactions that worsen oxidative damage. The main process behind CCl<sub>4</sub>-induced neurotoxicity is the creation of the trichloromethyl radical (CCl<sub>3</sub>). These radicals attack the endoplasmic reticulum, causing an abnormal buildup of lipids, disrupting protein synthesis, and impairing detoxification processes. As cell membranes get damaged, inflammatory signals increase, and neurons undergo apoptosis. At the same time, CCl<sub>4</sub> reduces key brain antioxidant defenses like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), while raising levels of oxidative stress markers such as malondialdehyde (MDA) and nitric oxide (NO) [43]. These changes clearly show the progression of oxidative damage. Interestingly, research findings on CCl<sub>4</sub>'s impact on acetylcholinesterase (AChE)—the enzyme that controls acetylcholine, a neurotransmitter vital for memory, learning, and attention—are inconsistent. Some studies find decreased AChE activity, while others note an increase. This suggests that CCl<sub>4</sub> disrupts cholinergic signaling in complex and context-sensitive ways. Since changes in AChE function are closely linked to neurodegenerative diseases like Alzheimer's, where high enzyme activity lowers acetylcholine levels, CCl<sub>4</sub> exposure might have serious effects on brain health [39]. Additionally, CCl<sub>4</sub> activates neuroinflammatory pathways by increasing cytokines like TNF-α, IL-1β, IL-6, and TGF-β1. This action worsens tissue injury and neuronal dysfunction. Over time, the combined effects of oxidative stress and inflammation can result in neuron loss, axonal degeneration, and disruption of essential neural networks [20].

These harmful processes are closely tied to many neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), and spinocerebellar ataxias. Key mechanisms such as mitochondrial dysfunction, excessive activation of glial cells, protein misfolding, and continuous overproduction of reactive oxygen species (ROS) drive this gradual decline in neurons [29].

Fortunately, several natural antioxidants have shown protective effects against these problems. They can aid in restoring antioxidant enzyme activity, limiting oxidative damage, and reducing inflammation. This helps protect neurons and maintain normal brain function. Some compounds have even been found to bring altered biochemical markers back to near baseline levels, showing their potential to reduce CCL4-induced neurotoxicity.

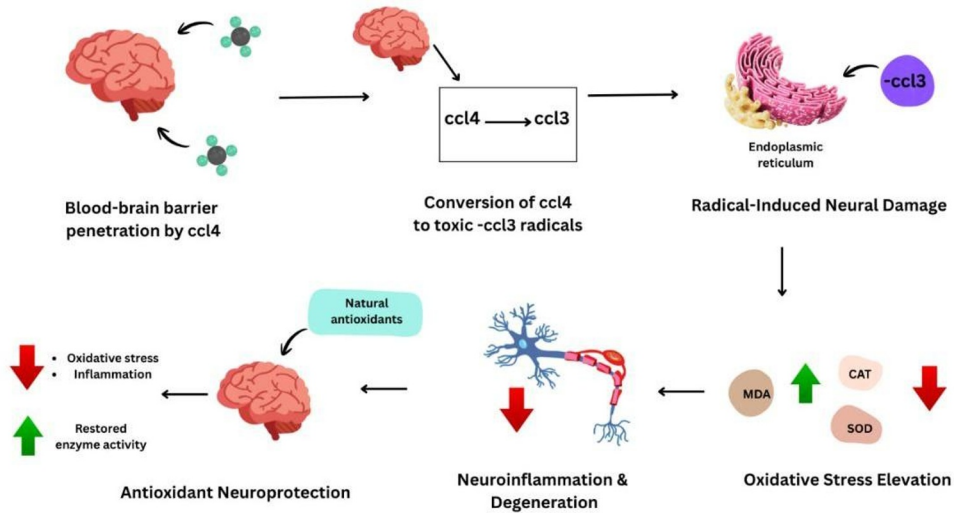


Fig.3 – Role of antioxidant in CCL4 Toxicity

Table-3 Antioxidant properties of against hepato and nephrotoxicity

Antioxidant	Target Organ	Key Effects on Oxidative/Physiological Markers	Reference
L-arginine	Kidney	↓ MDA; ↑ GSH, SOD, CAT, GPx, GST, GR; normalization of creatinine levels	[13]
(-) Epicatechin	Liver	↓ MDA; ↑ GSH, CAT, GPx, GST; ↓ ALT and	[30]

		AST enzymes	
CoQ10 & Silymarin	Liver & Kidney	↓ MDA, NO, APOP; ↑ SOD, CAT, GSH; ↓ ALT, AST, ALP	[23]
Pomegranate Peel Extract (PPE)	Kidney	↓ Urea & creatinine; ↑ SOD, CAT, GPx mRNA expression; ↓ caspase-3 & collagen levels	[10]

## CONCLUSION

Carbon tetrachloride (CCl<sub>4</sub>) is a highly toxic compound. It can damage several organs, including the liver, kidneys, brain, testis, heart, and lungs, by disrupting their normal functions. In experimental studies, it is often used to induce organ injury, especially liver diseases such as fibrosis, cirrhosis, chemical hepatitis, liver cancer, and kidney failure. The toxicity of CCl<sub>4</sub> mainly comes from the production of free radicals. These free radicals trigger lipid peroxidation, DNA and protein damage, inflammation, oxidative stress, and programmed cell death, which leads to significant cellular injury. Antioxidants can help reduce this toxicity by neutralizing harmful free radicals. Plant-based antioxidants are particularly promising because they are naturally abundant and typically have fewer side effects. In the future, these antioxidants may offer a new way to boost cellular defense systems and protect against the harmful effects of toxic agents like CCl<sub>4</sub>.

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