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Carbon tetrachloride: A classic model for liver toxicity.

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ABSTRACT

Carbon tetrachloride (CCl4), an efficient hepatotoxin, is employed to trigger liver damage in experimental animals. CCl4 disrupts liver cell membranes and impairs the activity of endoplasmic reticulum and mitochondria. CCl4's liver cytotoxicity depends on its metabolism by ferric cytochrome P450. Trichloromethyl free radical is responsible for lipid peroxidation. Carbon tetrachloride (CCl4) interacts with triacylglycerols and phospholipids within subcellular compartments, initiating lipid peroxidation in hepatic parenchymal cells. Long term use of CCl4 can result in fatty deposits in the liver, fibrotic cells, as well as hepatic cancer. Reactive free radicals stimulate a range of biological processes, such as programmed cell death, necrosis, ferroptosis, and autophagy. A proper ratio of free radicals and antioxidants is essential for optimal physiological function. If the equilibrium favors free radicals, many pathological diseases can arise. In this review, we demonstrated the efficacy of carbon tetrachloride on liver delving into its role in oxidative stress, lipid peroxidation, inflammation and models of liver toxicity such as fibrosis and cirrhosis.

1. What is Carbon tetrachloride?

CCl4 is a colorless, transparent, fire-resistant, and volatile liquid. The molecule consists of four Cl− atoms surrounding a carbon atom in the center. It can develop natively or as a consequence of a number of chemical reactions. It is highly chemically stable [1]. Carbon tetrachloride (CCl4) has traditionally been used as a cleanser in domestic, industrial, and dry-cleaning applications, as well as in fire suppression and refrigeration systems, and as a propellant precursor. The bulk of its applications are currently forbidden due to their high toxicity and severe consequences. However, it is still used in certain businesses. CCl4 can easily enter the body by inhalation, ingestion, or cutaneous absorption.

The rate of uptake from the gastrointestinal system is prompt and heavily regulated by diet (for example, fat and alcohol accelerate CCl4 absorption in the intestine). CCl4 quickly reaches the body by inhalation, ingestion, and cutaneous absorption. The most common form of exposure is breathing, with respiratory uptake predicted to be an extremely high level in humans.

The process of absorption from the gastrointestinal tract is quick and significantly regulated by diet.

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Particularly, fat or alcohol stimulates CCl4 absorption in the intestinal tract [2].

It can also be purposely consumed as a suicide substance. CCl4 induces cellular damage in a variety of organs, most notably the liver, kidneys, and lungs [3-6]. Haloalkanes generate significant oxidative stress not only in vitro but also in the animal [7]. Carbon tetrachloride (CCl4) is a recognized hepatotoxin. Hepatic microsomal mixed-function oxidase is thought to activate the trichloromethyl free radical, CCl3·. This interacts with oxygen to create the highly reactive trichloromethyl peroxyl radical (CCl3OO·). CCl3OO· interacts with polyunsaturated fatty acids, causing lipid peroxidation. CCl3· binds to membrane lipids and protein components, resulting in covalent binding. CCl3OO· damages cells and promotes inflammation and fibroblasts [8-10]. CCl4 toxicity is caused by cytochrome P450's synthesis of the free radical CCl3 and other metabolites, not CCl4 itself directly. Finally, they damage cells by altering their structure by lipid peroxidation and other activities via several pathways. These free radicals can cause numerous organ malfunction, which can lead to severe illnesses [11].

2. Liver and Carbon tetrachloride efficacy.

The liver is a vital organ necessary for metabolism and detoxification in the human body [12]. The word hepatotoxicity stands for liver damage produced by specific chemicals. Hepatotoxicants refer to chemicals that cause liver injury. Hepatotoxins include chemical compounds (CCl4, paracetamol), natural poisons (aflatoxin, microcystins), remedies made from herbs (cascara sagrada, ephedra) and industry essentials such as (lead, arsenic). Other hepatotoxicants include excessive alcohol intake, hepato-viruses, different poisons, and medicines, which lead to the development of liver disorders [13].

The liver is involved in the detoxification and metabolic activities of various substances, both endogenous and exogenous, in the human body. Exposure to harmful chemicals may overload its antioxidant defense mechanism and induce hepatocellular damage because it is thought to be the primary focus of numerous substances processing [14]. As a result, it is critical to evaluate liver health and establish treatment strategies.

The liver is particularly vulnerable to carbon tetrachloride because it contains numerous enzymes that alter the chemical's structure. Some of the breakdown products may assault cell proteins and interfere with liver cell activity. Products that assault cell membranes may cause cell death. In mild cases, the liver becomes enlarged and painful, with fat accumulating inside the organ. In severe situations, liver cells might be injured or killed, resulting in a decline in liver function. Such effects are typically reversible if exposure is not too high or too lengthy [15]. It is hypothesized that CCl4 has an effect that varies according to dosage, exposure frequency and species susceptibility. Long-term exposure can frequently lead to chronic injury to the liver, which progresses to fibrosis, cirrhosis, and cancer [16]. As indicated in **Table (1)**, CCl4 is a hepatotoxin that destroys liver cell membranes and impairs the function of organelles such as the endoplasmic reticulum and mitochondria. Antioxidants lower the incidence of CCl4-induced hepatocellular cancer, hepatic fibrosis/cirrhosis, liver damage considered in **Figure (1),** and chemical hepatitis. Antioxidant agents are crucial for declining oxidative stress, mitochondrial stress, endoplasmic reticulum stress, and preventing macromolecular oxidation in the liver. Antioxidants reduce the detrimental impacts of CCl4, regulate biochemical modifications in liver tissue, and restore indices to normal values.

3. Metabolic activation of carbon tetrachloride.

It is standard procedure to cause hepatotoxicity in experimental animals by using carbon tetrachloride (CCl4). Hepatocellular necrosis with fat deposition is a hallmark of CCl4 hepatotoxicity. Prolonged toxic dosages of CCl4 frequently result in catastrophic liver failure when necrosis outpaces the liver's capacity to heal. Excessive CCl4 dosages cause nonspecific toxicity, which includes respiratory failure that leads to mortality and central nervous system depression [17]. The endoplasmic reticulum (ER) is damaged by free radicals composed by CCl4 and the implied molecule alone, resulting in lipid accumulation, reduced protein synthesis, and combined function oxidase activity [2]. CCl4, a component of the hepatotoxic class, functions via activating metabolic pathways. In the endoplasmic reticulum (ER), cytochrome p450 enzymes, particularly CYP2E1, change it to (CCl3•). Upon rapid reaction with molecular oxygen, CCl3• generates the highly reactive trichloromethyl peroxyl radical (CCl3OO•). The radical immediately interacts with lipids, producing lipid peroxidation metabolites [18]. The ER and mitochondria's polyunsaturated fatty acids, or PUFA, are more vulnerable to free radicals' oxidation. Lipid peroxidation is one of the primary ways that CCl4 uses to damage the liver [2].

4. Carbon tetrachloride and oxidative stress.

Liver injury is characterized by parenchymal cell necrosis, an increased inflammatory response, and alterations in the extracellular matrix (ECM) content [19]. Liver stellate cells (HSCs) and Kupffer cells (KCs), together with oxidative mediators, cytokines, and chemokines, all play important roles in liver injury [19]. Compounds generated by oxygen reduction, such as the atoms or molecules with electrons that have no pairings, are hazardous and particularly reactive since they can remove electrons from molecules in the vicinity. Oxidative intermediaries can be typically categorized as oxygen-centered radicals, and oxygen-centered non-radicals [20].
These molecules are synthesized during aerobic metabolism, a process essential for various physiological functions, such as signal transduction pathways and immune defense mechanisms mediated by neutrophils, eosinophils, and macrophages during inflammatory responses, among other critical activities [21].

The primary endogenous sources of reactive oxygen species (ROS) in the liver include mitochondria, cytochrome P450 metabolism, microsomes, and peroxisomes. These ROS can harm lipids, proteins, and DNA, affecting their functionality [19]. In addition, oxidative stress can modulate cellular pathways regulating gene transcription, protein expression, apoptosis, hepatic stellate cell (HSC) activation, and other processes that contribute to liver pathogenesis [21]. Oxidative damage mostly affects KCs, but it also affects HSCs and endothelial cells. As a result of oxidative stress, KCs produce a variety of cytokines, which promote inflammation and apoptosis. When ROS activates HSC, it stimulates collagen synthesis and accumulation [22]. Antioxidants, which mitigate or prevent the oxidation of susceptible substrates by neutralizing ROS, are critical in this context. The human body employs both enzymatic mechanisms [20] and non-enzymatic systems, including glutathione (GSH), to counteract oxidative damage and protect hepatic tissue [19].

Carbon tetrachloride (CCl4) causes oxidative stress in hepatic tissue, principally by disrupting the balance between oxidative and antioxidant systems, resulting in increased free radical generation and reduced antioxidant defenses [23]. Within hepatocytes, CCl4 suppresses the functionality of key antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione S-transferase (GST), and glutathione reductase (GR), while also reducing levels of endogenous antioxidants such as glutathione [24-27]. Moreover, CCl4 elevates protein carbonyl levels, a sign of protein oxidation, and raised malondialdehyde (MDA), known indicator of oxidative stress [28].

The administration of antioxidants mitigates oxidative stress by decreasing MDA, hydrogen peroxide (H2O2), thiobarbituric acid reactive substances (TBARS), and reactive oxygen species (ROS) in liver tissue, while enhancing the activity of SOD, CAT, GSH-Px and GR antioxidant enzymes. In models of CCl4-induced liver injury, Kupffer cells exhibit significantly upregulated expression of pro-inflammatory and pro-fibrotic mediators, including tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-2 (MIP-2), interleukin-1β (IL-1β), interleukin-6 (IL-6), transforming growth factor-beta 1 (TGF-β1), and nuclear factor-kappa B (NF-κB) p65 protein. CCl4-induced hepatic fibrosis, leads to a marked increase in the mRNA expression of alpha-smooth muscle actin (α-SMA) and collagen type I alpha 1 (COL-1a1), both are indicators of fibrotic processes in liver tissue [25, 27-29].

Administration of carbon tetrachloride (CCl4) significantly elevates serum concentrations of hepatic marker enzymes, reflecting their release from the cytoplasm into the bloodstream. Elevated levels of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), and bilirubin serve as critical indicators of compromised liver cell membrane integrity and cellular leakage, functioning as key diagnostic markers of hepatic dysfunction. Oral exposure to CCl4 disrupts liver enzyme profiles, increases triglyceride, total cholesterol, and low-density lipoprotein (LDL) cholesterol levels, while reducing pseudocholinesterase activity. Furthermore, CCl4 is a potent inducer of oxidative stress, nitrosative stress, endoplasmic reticulum stress, mitochondrial dysfunction, and inflammation, contributing to liver injury through the generation of free radicals derived from its metabolism.

5. Carbon tetrachloride and lipid peroxidation.

Throughout two primary mechanisms: the covalent binding of CCl4 intermediates to the cell constituents and elevated lipid peroxidation caused by the free radicals, carbon tetrachloride leads to cell destruction. These radicals interact with molecular oxygen, specifically targeting unsaturated fatty acids and causing oxidative destruction. This process predominantly affects lipids, notably unsaturated phospholipids, disrupting the integrity of the intracellular and plasma membranes [30]. The metabolism of carbon tetrachloride (CCl4) produces highly reactive free radicals, namely trichloromethyl (CCl3*) and trichloromethyl peroxyl (CCl3OO*), which can form covalent adducts with biological macromolecules, including proteins, nucleic acids, and lipids. In an atmosphere full of oxygen, CCl3* is converted into the more reactive CCl3OO*, which rapidly steals hydrogen from polyunsaturated fatty acids (PUFAs), triggering a chain reaction of lipid peroxidation that threatens the

integrity of PUFA-containing membranes [31].

Free radicals can trigger lipid peroxidation by removing a hydrogen atom from PUFA, yielding a lipid radical (L). When the resulting radical reacts with molecular oxygen, it generates a lipid peroxyl radical (LOO·) that absorbs hydrogen from adjacent fatty acid side chains. This results in lipid hydroperoxide (LOOH). When exposed to metal ions, LOOH produces lipid alkoxyl radicals. LO· and LOO· can create reactive aldehydic compounds, such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) acrolein. Produced aldehydic chemical rapidly modifies proteins presented in the membrane and nucleic acids and is associated with the pathogenesis of a variety of reactive oxygen species and inflammation-related disorders. The consequence is an alteration in membrane structure that impacts its permeability, ion transport, and the metabolic reactions [32, 33]. MDA levels are considerably increased after CCl4 treatment [34].
In high alcohol-sensitive (HAS) rats receiving CCl4 (1 ml/kg), 4-HNE and MDA protein conjugates are generated and limited to the center of hepatocytes at 6 hours post-dose, extend to zone 3 at 24 hours with zone 3 necrosis, and are barely detectable at 36 to 72 hours after dosing.

Thus, these chemical aldehydic compounds modify proteins in a time-varying manner, triggering CCl4 damage [35]. Furthermore, treatment with carbon tetrachloride leads to a dependent upon dosage increase in DNA breakage and malondialdehyde deoxyguanosine (M1dG) conjugates, that is highly significant at 1 and 4mM. Moreover, CCl4 increases the amount of 8-Oxo-2'-deoxyguanosine, which leads to cytotoxicity at 4 mM after 2 hours of treatment. These implications indicate a higher possibility of genetic damage and cancerous potential [36].

6. Carbon tetrachloride and inflammation.

Cytochrome p450 in the liver tissue converts CCl4 into the extremely unstable free radicals trichloromethyl radical (•CCl3) and trichloromethyl peroxyl radical (•OOCCl3), leading to lipid peroxidation and cellar damage [31]. Free radicals may trigger an inflammatory response in the liver by stimulating macrophages and releasing cytokines that cause inflammation [37]. Hence, boosting the antioxidant pathway could be a critical method for saving hepatic cells during intense oxidative stress. Nrf2 is an important transcriptional nuclear factor that regulates several anti-oxidative genes [38]. Nrf2 is normally kept at minor ratio by creating a complex with Kelch-like ECH-associated protein 1 (Keap1), which then degrades it throughout a ubiquitin-proteosome pathway [39]. Certain stressors, including oxidative stress, induce the dissociation of Keap1 from Nrf2 by modulating cysteine residues on Keap1, facilitating Nrf2's translocation from the cytoplasm to the center of the cell. Within the nucleus, Nrf2 interacts with the cis-acting antioxidant response element (ARE) of various antioxidative genes, upregulating their expression as well as promoting cytoprotective effects [40].

7. Nitric oxide and Carbon tetrachloride role in liver injury.

At the molecular level, carbon tetrachloride can activate nitric oxide (NO), tumor necrosis factor (TNF)α, transforming growth factors (TGF)-α and -ß in the cell. These elements encourage cellular pathways leading to programmed cell death or fibrosis. Tumor necrosis factor-alpha (TNFα) triggers cell death, whereas transforming growth factors (TGFs) promote fibrogenesis [2, 41, 42].

Oxidative stress is stimulated through a variance allying reactive oxygen species (ROS) and antioxidants that shift in favor of the former. An elevation in reactive oxygen species levels diminishes the bioavailability of nitric oxide (NO). At the presence of oxidative stress, tetrahydrobiopterin levels are declining, leading to dissociation of nitric oxide synthase (NOS) and subsequent formation of superoxide. Excessive superoxide can be formed via specific enzymes that interact with nitric oxide (NO) to make catastrophic peroxynitrite (ONOO-), depleting nitric oxide (NO), that is harmful. Furthermore, the antioxidant enzyme superoxide dismutase (SOD) generates hydrogen peroxide, contributing to increased oxidative stress. This followed strategy is considered a popular contributor to the development of cardiovascular disorders [43, 44]. Cirrhosis model caused by CCl4 was developed to study the impact of declining oxygen levels due to NO availability. Cirrhosis is associated with elevated oxygen levels in the liver, as proved by declined hepatic cyclic guanosine monophosphate (cGMP) concentrations. Suppression of cyclooxygenase (COX) and xanthine oxidase (XO) as well as diminished superoxide dismutase (SOD) activity decreases oxygen levels, indicating that these enzymes contribute to excessive amounts of oxygen in cirrhosis process.

NOS suppression did not have any impact on oxygen concentrations. Nevertheless, nitric oxide (NO) and oxygen (O_2) are controlled in endothelial cells in reversely. Thus, minimizing oxygen (O_2) can alleviate oxidative stress efficacy, which boosts nitric oxide (NO) bioavailability [45]. Nitric oxide (NO) is recognized for mitigating lipid peroxidation and oxidative stress in prolonged liver damage induced by carbon tetrachloride (CCl4). Using NO inhibitors, such as aminoguanidine (AG), intensified these pathological triggers. Conversely, administration of L-Arginine attenuated increases accumulation of collagen protein, bilirubin levels, and alkaline phosphatase activity, on the other hand, it did not significantly reduce lipid peroxidation. This limited effect is attributed to L-Arginine's inability to neutralize lipid (hydrophobic) free radicals, as NO is a transient water-soluble (hydrophilic) [46].

8. Carbon tetrachloride and fibrosis.

The development of liver fibrosis is a challenging process in which extracellular matrix proteins, notably collagen, accumulate, causing hepatic architecture to be distorted and liver function to be reduce [47]. This mechanism is begun, spread, and might be reversed by the behavior of hepatic stellate cells (HSCs), and this release fibro-genic proteins that trigger collagen synthesis via multiple cell types including portal fibrocytes, fibroblasts, and bone marrow-derived myofibroblasts [48]. The medical conditions listed function as triggers for the stimulation of Reactive Oxygen Species (ROS). In addition, pro-oxidants and oxidative lipid breakdown process lead to the discharge of profibrogenic growth factors, cytokines, and prostaglandins [49].

As a result, ROS has a crucial function in the first steps of fibrosis formation by amalgamating a variety of fibrosis cytokines not affected by Transforming Growth Factor Beta (TGF-β). Transforming Growth Factor Beta (TGF-β), is triggered by reactive radicals in rat hepatic stellate cells.[50]. Furthermore, research has shown that Transforming Growth Factor Beta (TGF-β) promotes ROS generation in fibroblasts. Research suggests that TGF-β induces ROS generation by activating nicotinamide adenine dinucleotide phosphate hydrogen

(NADPH) oxidase and changing the forth complex in the respiratory chain [51, 52]. Angiotensin II also induces stimulation of nicotinamide adenine dinucleotide phosphate hydrogen oxidase in liver, as demonstrated in prolonged liver injury models. Several investigations have shown that inhibiting angiotensin II production decreases hepatic fibrosis [53].

Oxidative stress in cirrhosis patients has also been well examined. Those in this category possess elevated pro-oxidant levels (e.g., serum MDA) but poor antioxidant levels (e.g., Red Blood Cells catalase, Superoxide Dismutase (SOD), and blood Reduced Glutathione GSH). Oxidative stress affects the activity of hemocytes. Cirrhotic Individuals have refinements in their red blood cells' outer layers triggered by oxidative conditions. The impact is visible in the patients with greater nitric oxide level [54]. The changes shown correspond to worsening Child-Pugh scores. Among the experimental approaches, liver fibrosis caused by carbon tetrachloride is a widely recognized strategy to exploring underlying processes and evaluating treatment medicines [55].

Prolonged CCl4 treatment leads to exacerbated stages of liver fibrosis, that is distinguished by substantial ECM deposition. Myofibroblasts are liable for the formation of ECM scars in fibrosis that substitute regular tissue with scars. The latent liver stellate cell is activated and changed into a myofibroblast, which produces extracellular matrix (ECM) and makes up the bulk of the myofibroblast variety [56]. The removal of harmful substances (CCl4) leads to unplanned fibrosis reversion. Throughout retraction, some myofibroblasts attain apoptosis, whereas other cells convert to a dormant state, similar to dormant HSCs but beyond them [57, 58]. Inactivated HSCs in the CCl4 and alcohol-induced fibrosis of the liver lead to reduced levels of fibrogenic agents. Hspa1a and Hspa1b, members of HSP70, are additionally reported to influence the longevity of HSCs in experiment models during liver fibrosis repair [58].

9. Carbon tetrachloride and cirrhosis.

Acute CCl4 exposure causes persistent liver damage because of harmful byproducts originating from cytochrome P-450 enzymes, specifically CYP2E1 in liver cells around the veins. Following multiple sessions of induction, frequent occurrences of prolonged injury led to centrilobular necrosis, inflammation, and hepatic stellate cell activation, reinforcing the creation of (ECM) and leading to structural variations which characterize cirrhosis [77, 78]. Lamson et al., [79] reported in 1926 for the inaugural moment that CCl4 exposure induced cirrhosis. Cameron et al. [80] followed up with a detailed analysis that defined the cellular structure and confirmed the model's usual parameters. Currently, to resemble cirrhosis, a model of carbon tetrachloride induction is often used [81]. To generate experimental cirrhosis, CCl4 must be given repeatedly, regardless of the species model or the mode of induction. The time gap between each dosage cannot be extended since wounded liver may heal itself, rendering the detrimental effects of the toxin [80].

In a study by P. Muriel et al., [82], cirrhosis develops in Wistar rats when they are young. Carbon tetrachloride (CCl4) is injected intraperitoneally thrice a week at a dose of 0.4 g/kg. Cirrhosis develops post two months of regular CCl4 administration, and the impact of the disease escalates after three months of induction; after four months, death was elevated (more than 80%); and the recurrence of cirrhosis rises with disease seriousness [83].Cirrhosis is regarded as entirely formed after two months of

constant CCl4 injection. It is marked by irregularities in various organs. Liver is usually augmented; but, during late stages of the disease, it is likely lesser than normal and has substantial nodules.

Enlarged spleen and ascitic fluid are frequently encountered. Mortality is significant (30-60%) in CCl4 cirrhosis models, and individual animals' reactions to the chemical are diverse; surprisingly, these traits also exist in people with cirrhosis [84]. CCl4-induced cirrhosis in rodents imitates the primary hallmarks observed in patients with cirrhosis: the liver is severely nodular, majority of animals have portal hypertension [85], centrilobular hepatic necrosis [86], and the typical structure gets substituted by nodules of renewing liver bounded by fibrous septa with developed bile ducts. Portocaval anastomosis occurs within the connective tissue septa [85], as it does in humans. However, a detailed analysis uncovers number of contradictions within the test model and the human analogue [84].

The CCl4 model of cirrhosis lacks centrilobular sclerosing hyaline necrosis, pericellular as well as interlaminar fibrosis, which are present in human cirrhosis triggered by long-term alcohol consumption. In conclusion, this type of artificial cirrhosis has some similarities with alcoholic human cirrhosis but differs significantly [84]. To assess potential antifibrotic substances, many of which have antioxidant and anti-inflammatory characteristics. CCl4 liver cirrhosis in rats is the most employed model before applying them in patients [81, 87].

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Table (1): Carbon tetrachloride toxic efficacy models in liver.

Research authors	Marker	Efficacy	Conclusion	Reference
1. Shu Dong, et al.,	CCl_4	Liver Fibrosis	In this study, CCl4's molecular processes were examined. CCL_4 metabolized a variety of biological processes, multi-targets, and throughout multi-pathways.	**[59]**
2. M. Boll, et al.,	CCl_4	Hepatotoxicity	In this study, the effects of CCl4 toxicity at medium to low doses are explored where lipid homeostasis is disrupted.	**[60]**
3. T Ohishi, et al.,	CCl_4	Hepatic Fibrosis	In this study, it was difficult to determine the responsible involved agent to cause hepatic fibrosis or degradation because they didn't determine the actual pathway of this process.	**[61]**
4. Lijun Zhang, et al.,	CCl_4	Liver fibrosis	In this study, Mangifera might reduce liver damage and inflammatory conditions, inhibit collagen buildup, and alter the mRNA levels of metabolism of bile acids and pro-fibrotic genes in CCl4-induced mouse livers. Mangifera inhibited the NF-κB pathway protein levels.	**[62]**
5. Marco Domenicali et al.,	CCl_4	Cirrhosis with Ascites	In this study, short cycles of CCl4 inhalation constitute a unique, secure, and reliable way to generate decompensated cirrhosis in mice. Intraperitoneal CCl4 injection results in abdominal adhesions, making it impossible to properly evaluate ascites, whereas subcutaneous CCl4 generates undesirable systemic inflammatory action.	**[63]**

6. José I Fortea, et al.,	CCl_4	Cirrhosis	In this study, the updated protocol described by Regimbeau et al. outperformed the commonly used but poorer protocol presented by Runyon et al., indicating its value. Certainly, the CCl4-2xWk strategy achieved the quick onset of developing cirrhosis conditions in rats over a 12-week period of CCl4 exposure in a very efficient as well as predictable way, with no exacerbating fatality.	**[64]**
7. Kentaro Toriumi, et al.,	CCl_4	Hepatic injury	In this study, oxidized DAG is thought to be responsible for abnormal PKC activation observed during oxidative stress. It has been proven through Vitamin E usage that DAG-O(O)H generated during the degradation of lipids activates the PKC/NF-kB pathway as well as contributes to the progression of hepatic damage.	**[65]**
8. Scholten D, et al.	CCl_4	Liver fibrosis	In this study, CCl4 is useful for liver research. The model is stable and produces extremely consistent outcomes. IP injection is the most effective induction approach.	**[66]**
9. H B ANDERVONT	4-*o*-Tolylazo-*o*-Toluidine and Carbon Tetrachloride	Hepatomas	In this study, an azo dye, 4-o-tolylazo-o-toluidine, and CCl4 were delivered to strain 03H mice that were free of the mammary tumor agent. The azo dye affected the females more than the males, on the other hand both sexes got affected by CCl4 induction of hepatoma.	**[67]**
10. Kasuke Nagano, et al.,	CCl_4	CCl4 carcinogenicity	In this study, the dependent upon concentration production of benign as well as malignant HCC in rats and mice of both genders, as well as adrenal pheochromocytomas in mice of both genders demonstrated CCl4 carcinogenicity after a 2-year respiratory consumption of CCl4 vapor. Cirrhosis strongly caused rat HCC, but mouse liver adenomas appeared at 5parts per million with no progressive alterations in liver cells.	**[68]**
11. McGregor D, et al.,	CCl_4	Hepatotoxicity, genotoxicity,	In this study, in Kupffer cells, CYP2E1 and CYP2B1r2B2 boost the	**[69]**

		and carcinogenicity	DNA binding of NF-KB. Nevertheless, CCL4 produces c-fos and c-jun TNF-a is a powerful inducer that sustains c-jun expression, and the amount of AP-1 grows in the course of time. These could be significant events because overexpression of unmodified c-jun is adequate for rat fibroblast alterations, and AP-1 is thought to be associated with the growth and differentiation of cells.	
12. Recknagel RO, et al.	CCl_4	Liver toxicity	In this study, the problem of secondary mechanisms evoked by the metabolism of CC14 is complex and difficult. It's believed that focusing efforts on clarifying the nature of secondary mechanisms will yield unexpected novel and significant discoveries into the biochemical and cell physiological mechanisms linking hepatotoxic agent metabolism to their ultimate detrimental consequences for liver cells.	**[17]**
13. Tarun Kumar Dua, et al.,	CCl_4	Hepatotoxicity	In this study, it focused on the preventive role of probiotics against CCl4-induced liver damage. CCl4 radicals target proteins and lipids, causing liver injury, reducing the quantity of proteins in the outer layers of cells, and lipid peroxidation. Relying on the demonstrated favorable outcomes of preclinical investigations, probiotics or probiotic combinations could be a successful strategy for both minimizing and curing CCl4-induced liver damage.	**[70]**
14. Chidiebere Emmanuel Ugwu, et al.,	CCl_4	Hepatotoxicity	In this study, a range of phytochemicals in plant products have been shown to have hepatoprotective action against CCl4-induced damage experimental models.	**[71]**
15. Mary K Manibusan, et al.,	CCl_4	Toxicity	In this study, according to scientific research, liver carcinogenesis caused by CCl4 seems to be secondary to the bad chemicals ‘effects. CCl4 led to an elevation of cell division, as occurs by regenerative cell proliferation secondary to necrotic	**[72]**

			tissue destruction, at a point at which the rate of genetic destruction is rising, it can overpower the DNA repair processes, triggering a surge of mutagenicity and probably cancer.	
16. Dejan Popović, et al.,	CCl_4	Acute liver injury	In this Study, the use of anthocyanins from the extract of bilberry is a valuable consideration in minimizing the hepatotoxic and pro-inflammatory drug efficacy and avoiding or slowing the growth associated with numerous chronic liver illnesses such as ALD, NASH, and NAFLD.	**[73]**
17. Rolf Teschke	CCl_4	Liver injury	In this study, Acute intoxication with CCl4 is a clinical dilemma. Therapeutic strategies are crucial to improve CCl4 removal. CCl4 radicals assault cellular structures, causing apoptosis and cell necrosis. There are two techniques for reducing these harmful episodes. While these two techniques largely aid in the reduction of liver impairment, forceful diuresis is required to prevent CCl4-related renal harm.	**[3]**
18. Eman A. Sayed et al.,	CCl_4	Liver fibrosis	In this study, the pro-fibro genic signals mediated by CCl4 were suppressed by propolis and prevented the fatal consequences of liver fibrosis.	**[74]**
19. K.R. Ritesh et al.,	CCl_4	Hepatotoxicity and OS in rat brain.	In this study, CCl4 is also a neurotoxic substance which leads to oxidative impairment in the brain. The OS result due to CCl4 efficacy on the liver was considered lower than in the brain.	**[75]**
20. Ramesh K Gupta et al.,	CCl_4	Acute liver toxicity	In this study, the hepato-prophylactic effects of ethanolic fruit extract of S. xantho-carpum were confirmed by histopathological and bio-chemical investigations.	**[76]**

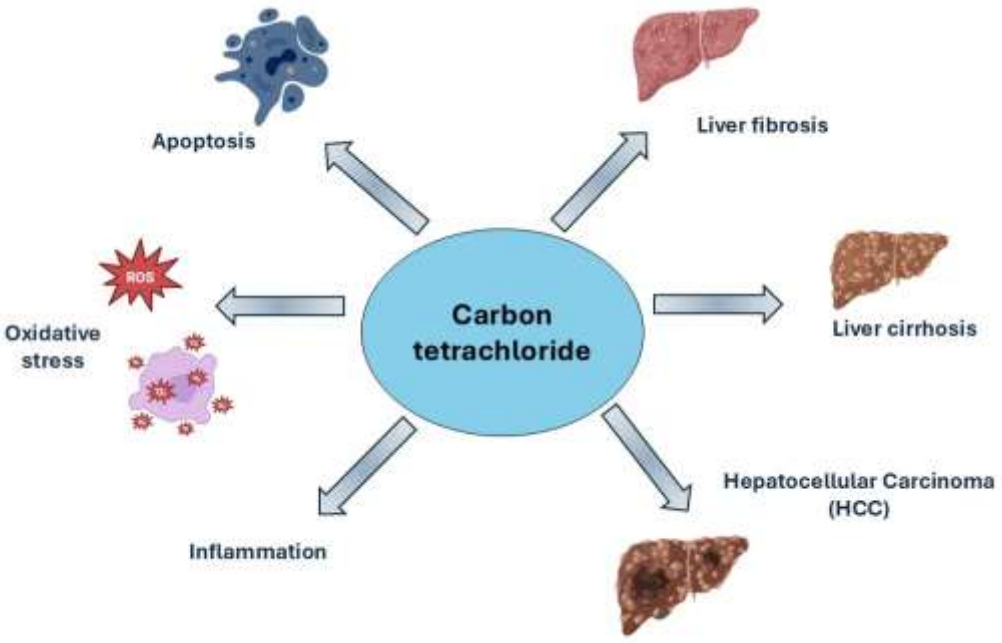

Figure (1): Carbon tetrachloride efficacy on liver.