



## Role of Vitamin D Supplementation with Liv-52 on Inflammatory Biomarkers in Carbon Tetrachloride (Ccl<sub>4</sub>) Induced Liver Disease in Wister Rats

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 01 Nov 2023	<p><i>Introduction: Liver disease is a significant global health concern, with a wide range of etiologies and adverse effects on overall health. Among the various causes of liver disease, exposure to hepatotoxins such as carbon tetrachloride (CCl<sub>4</sub>) has been widely studied. Inflammatory processes play a crucial role in the development and progression of liver disease. Inflammatory biomarkers, serve as important indicators of liver injury and can provide insights into the underlying inflammatory response. Therefore, identifying effective interventions that can modulate these inflammatory biomarkers holds great promise for the management of liver disease. The goal of the current study was to examine the anti-inflammatory effects of vitamin D combined with Liv52 on liver disease caused by CCl<sub>4</sub>. Materials and Methods: Male Wistar rats were given CCl<sub>4</sub>, twice a week for nine weeks, Liv-52 (1 mL/kg b.w), and vitamin D (500 IU/kg b.w) orally for nine weeks. The effects of vitamin D supplementation along with Liv-52 on inflammatory markers were estimated in the serum samples. Results: When compared to control animals, it was discovered that the hepatotoxic carrying animals had higher serum concentrations of IL-6, TGF-<math>\beta</math> and TNF-<math>\alpha</math>. Supplementation of vitamin D (group III) and Liv-52 (group IV) treated rats showed significantly reduced the levels of IL-6, TGF-<math>\beta</math> and TNF-<math>\alpha</math> when compared with group II animals. The supplementation with combination of vitamin D and liv-52 more significantly reduced the levels of IL-6, TGF-<math>\beta</math> and TNF-<math>\alpha</math>. The comparative analysis between group III vs IV, the protective function of vitamin D in isolation is similar to the protection by Liv-52 in isolation, there is no marker difference between the two and both have significant protection effect. Conclusion: The results obtained indicate that the combination of vitamin D supplementation and Liv-52 exhibited beneficial effects on inflammatory biomarkers in CCl<sub>4</sub>-induced liver disease.</i></p>
<b>CC License</b> CC-BY-NC-SA 4.0	<b>Keywords:</b> Anti-Inflammatory Effect, Carbon Tetrachloride, Liver Disease, Vitamin D, Liv-52

## 1. Introduction

CCl<sub>4</sub> is one of the xenobiotics chemicals known to induce tissue injuries and hepatotoxin [1,2]. Lipid peroxidation (LPO) is the main factor in CCl<sub>4</sub>-induced liver damage, and free radicals are produced as a result of diminished enzymatic and non-enzymatic antioxidant activity. Hepatocyte lipid accumulation causes cellular vacuolization and fatty degeneration as the only minimally harmful effects. In earlier research, fibrosis, hepatic necrosis (cell death), and cirrhosis were noted when exposure levels were elevated [3]. The liver is vulnerable to damage caused by harmful molecules called free radicals, which are produced during oxidative stress. One major source of oxidative stress on the liver is the release of a chemical called CCl<sub>4</sub> [4]. This can lead to inflammation and damage to liver cells, including degeneration or death. Studies in humans have shown that exposure to high levels of CCl<sub>4</sub> through inhalation (250 ppm) or oral ingestion ( $\geq 110$  mg/kg) can cause chronic liver damage and the build-up of fat in the liver [4].

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Cytochrome CYP2E1, CYP2B1, CYP2B2, and maybe CYP3A activate CCl<sub>4</sub> to produce the trichloromethyl (CCl<sub>3</sub><sup>\*</sup>) radical. This radical interferes with important physiological functions like lipid metabolism, likely leading to fatty degeneration, by binding to cellular components (nucleic acid, protein, and lipid). It also interferes with the reaction between CCl<sub>3</sub> and DNA. The trichloromethyl peroxy (CCl<sub>3</sub>OO<sup>\*</sup>) radical, a highly reactive species, can also be created when this radical reacts with oxygen. This substance starts the process of lipid peroxidation, which leads to the oxidation of polyunsaturated fatty acids, particularly those connected to phospholipids [5]. This leads to alterations in the ability of the mitochondria, endoplasmic reticulum, and plasma membrane to allow substances to pass through, resulting in a decrease in the amount of calcium within the cells, the trapping of calcium, and an imbalance of calcium levels. These changes further damage the cells.

An interesting and alluring chemical, vitamin D has recently attracted a lot of interest in medicine. Its immunomodulatory and anti-inflammatory potential may be similar to that of many chemicals found in nature (such as flavonoids), but its function in biology was chosen over a lengthy evolutionary pathway to reduce the negative effects of the immune response and the cell stress response. This molecule can be thought of as a prehistoric hormone whose main function is to promote survival. The goal of this research was to investigate the impact of vitamin D on protecting the liver from harmful effects caused by inflammation and changes in the immune system. The VDR (Vitamin D Receptor), a protein found in the nucleus of cells, plays a major role in how the body responds to vitamin D. When 25(OH)D binds to the VDR, it forms a complex with another protein called the RXR (Retinoid X Receptor). This complex then attaches to specific parts of the DNA known as VDRE (Vitamin D Response Elements). Together, the VDR and RXR act as regulators, turning on genes involved in maintaining healthy levels of calcium and phosphate in the body [6], as well as controlling cell growth, development, and the functioning of the immune system [7].

Two crucial genes, including the anti-aging gene Klotho and Nrf2 (nuclear factor erythroid 2-related factor 2), are activated by VDR and play various functions in preserving the integrity of cellular signalling networks. In order to maintain the usual reducing environment inside the cell, vitamin D, Nrf2, and Klotho all enhance cellular antioxidants [7]. The movement of the protein Nrf2 from the cytoplasm to the nucleus is caused by the interaction between the vitamin D and the VDR protein. A number of genes that have antioxidant function are activated by Nrf2. Risks of tissue damage brought on by oxidative stress rise when Nrf2 activity is insufficient. An important aspect of liver disease caused by toxins is the presence of abnormal oxidative stress, which results from an overproduction of reactive oxygen species (ROS) due to malfunctioning mitochondria. Another protein, known as Keap1, plays a role in regulating the activity of Nrf2. Keap1 is a transcription factor and negative regulator that controls Nrf2 activity to some degree [8]. Additionally, Keap1 regulates Nrf2's subcellular distribution, which is related to its antioxidant action. The Nrf2-antioxidant response pathway is initiated as a defensive mechanism in response to intracellular oxidative stressors. This response activates the production of specific genes and proteins to neutralize or eliminate toxins and ROS (reactive oxygen species) in the body.

When the body has an adequate amount of Vitamin D, the activities related to intracellular oxidative stress are decreased. However, if there is a deficiency in Vitamin D, it can lead to an increase in oxidative

stress and cellular damage through apoptosis. The presence of Nrf2 in cells is inversely related to the accumulation of ROS in mitochondria, which can lead to an increase in oxidative stress. Therefore, Nrf2 plays a crucial role in preventing oxidative stress and is regulated by Vitamin D [9]. Additionally, Vitamin D helps to maintain normal mitochondrial function and aids in the regulation of intracellular oxidation and reduction [10]. Additionally, studies on animal models with liver fibrosis both in the lab and in living organisms have demonstrated that vitamin D has a positive effect on reducing fibrosis as it can affect various stages of the fibrosis process, such as preventing the initial injury, reducing the activation and growth of cells that contribute to fibrosis, decreasing the build-up of extracellular matrix, and even promoting the breakdown of collagen and the activation of enzymes that break down matrix proteins [11].

Furthermore, Ding et al. (2013) discovered a specific genetic circuit known as VDR/SMAD that plays a crucial role in regulating the formation of fibrous tissue in the liver. Additionally, the manuscript explores the use of VDR ligands as a possible treatment option for liver fibrosis, suggesting that the Vitamin D Receptor (VDR) acts as an endocrine checkpoint that oversees the healing process in the liver [12]. In 1955, Liv.52 is an herbal preparation used as a hepatoprotective supplementary product and it was distributed worldwide. Liv.52 helps to detoxify the body and protect the liver from harmful toxins found in food and drugs. This helps to keep liver enzymes at healthy levels, leading to improved liver function [13]. By reducing lipid peroxidation, Liv.52 is known to preserve damaged hepatocellular membranes. According to Malik et al. (1979), Liv.52's natural ingredients include 8 different compounds, including Capparis spinosa, Cichorium intybus, Solanum nigrum, Cassia occidentalis, Terminalia arjuna, Achillea millefolium, Tamarix gallica, and Mandur bhasma [14]. These compounds have powerful hepatoprotective properties against chemically induced liver toxicity. By defending the hepatic parenchyma and encouraging hepatocellular regeneration, it helps the liver regain its functional efficiency. Liv.52 is a supplement that focuses on detoxifying the body and protecting the liver from harmful substances such as medications, unhealthy diets, and high levels of liver enzymes. It is designed to support and maintain healthy liver function [15].

## **2. Materials And Methods Animal Care and Housing**

Male adult Wistar rats weighing between 150 and 200 g were obtained from Biogen Laboratory Animal Facility in Bangalore, Karnataka. The animal was kept in polypropylene cages under controlled environmental conditions of 23 ± 2 °C for the temperature and 50-70 % for relative humidity on alternate 12-hour light/dark cycles. All animals were given a regular pellet meal made by M/s. Hindustan Lever Ltd. in Mumbai, along with unlimited access to water. Before the trial began, the rats spent a week becoming used to the lab environment. The Institutional Animal Ethical Committee granted permission for and approved this study using Wistar rats (REG No. 765/03/ca/CPCEA) dated: 31.07.2018.

### **Experimental Design**

The study divided the animals into six groups, each with six animals. The first group served as the control group, while the second group received a mixture of CCl<sub>4</sub> and olive oil (1 mL/kg b.w., 50% CCl<sub>4</sub> in olive oil) twice a week to induce liver toxicity. Groups three and four received daily doses of Vitamin D (500 IU/kg b.w) and Liv-52 (1 mL/kg b.w), dissolved in distilled water, along with CCl<sub>4</sub> for nine weeks. The fifth group received daily injections of CCl<sub>4</sub>, Liv-52, and Vitamin D for nine weeks. The sixth group, also served as control, received daily doses of Vitamin D and Liv-52 dissolved in distilled water for nine weeks.

### **Measurement of Inflammatory Markers**

Inflammatory biomarkers, serve as important indicators of liver injury and can provide insights into the underlying inflammatory response. Therefore, identifying effective interventions that can modulate these inflammatory biomarkers holds great promise for the management of liver disease. In this study used a serum to analyze certain indicators of inflammation. We specifically looked at the levels of three proteins: interleukin 6 (IL-6), transforming growth factor (TGF-β), and tumour necrosis factor alpha (TNF-α). By using a method developed by Chan et al. in 1987 to measure the activity of these proteins [16].

### **Statistical Analysis**

In this study, the statistical analysis and calculations were performed using the SPSS software, specifically version 21.0, designed for Windows. To compare the means of different groups, both the

Duncan's Multiple Range Test (DMRT) and the one-way ANOVA approach were employed. Statistical significance was determined based on a P value of <0.05, indicating a threshold for significance.

### 3. Results and Discussion

The result showed in table 1, the group that received CCl<sub>4</sub> (group II) showed a notable increase in their serum level of IL-6, with a statistical significance of P<0.001, compared to the control group. For rats in group III, administering vitamin D resulted in a significant decrease (P<0.001) in the amount of IL-6 in their blood compared to the control group (group II) that was given CCl<sub>4</sub>. The use of Liv-52 in group IV resulted in a significant decrease (P<0.001) in the amount of IL-6 present in the serum when compared to the control group treated with CCl<sub>4</sub> (group II). In contrast to the control group of rats that received CCl<sub>4</sub> therapy, the group that received a combination of vitamin D and liv52 had significantly lower levels of IL-6 in their blood (P<0.001). The comparative analysis between group III vs IV, the protective function of vitamin D in-isolation is similar to the protection by Liv-52 in-isolation, there is no marker difference between the two and both have significant protection effect. The results of the study revealed no significant variations in IL-6 levels between the control rats (group I) and the control rats given a combination of vitamin D and Liv-52 (group VI). This indicates that taking vitamin D and Liv-52 supplements does not negatively impact liver function.

**Table 1:** Vitamin D and Liv-52's anti-inflammatory effects on the level of IL-6 in the serum of experimental and control animals

Group Comparison	Mean ± SD	P Value	Statistical significance
I vs II	16.9±3.58 vs 86.93±2.36	p<0.001	Significant
II vs III	86.93±2.36 vs 33.2±3.33	p<0.001	Significant
II vs IV	86.93±2.36 vs 28.2±1.59	p<0.001	Significant
II vs V	86.93±2.36 vs 23.17±1.65	p<0.001	Significant
III vs IV	33.2±3.33 vs 28.2±1.59	p>0.13	Not significant
VI vs I	15.82±2.45 vs 16.9±3.58	p>0.47	Not significant

The group II rats that were administered CCl<sub>4</sub> had a statistically significant increase in TGF-β levels (P<0.001) when compared to the group I rats. There were significant decreases (P<0.001) in TGF-β level in the administration of vitamin D (group III) compared with group II rats. Similarly, in administration of Liv-52 (group IV) were significantly reduced (P<0.001) in TGF-β level compared with group II rats. In contrast to the control group of rats that received CCl<sub>4</sub> therapy, the group that received a combination of vitamin D and liv-52 had significantly lower levels of TGF-β in their blood (P<0.001). The comparative analysis between group III vs IV, the protective function of vitamin D inisolation is similar to the protection by Liv-52 inisolation, there is no marker difference between the two and both have significant protection effect. The results of the study revealed no significant variations in TGF-β levels between the control rats (group I) and the control rats given a combination of vitamin D and Liv-52 (group VI). This indicates that taking vitamin D and Liv-52 supplements does not negatively impact liver function (table 2).

**Table 2:** Vitamin D and Liv-52's anti-inflammatory effects on the level of TGF-β in the serum of experimental and control animals

Group Comparison	Mean ± SD	P Value	Statistical significance
I vs II	97.0±6.7 vs 567.6±40.1	p<0.001	Significant
II vs III	567.6±40.1 vs 122.0±10.0	p<0.001	Significant
II vs IV	567.6±40.1 vs 115.0±8.2	p<0.001	Significant
II vs V	567.6±40.1 vs 108.0±8.4	p<0.001	Significant
III vs IV	122.0±10.0 vs 115.0±8.2	p>0.46	Not significant
VI vs I	97.4±6.2 vs 97.0±6.7	p>0.96	Not significant

The result showed in table 3, the group that received CCl<sub>4</sub> (group II) showed a notable increase in their serum level of TNF-α, with a statistical significance of P<0.001, compared to the control group. For rats in group III, administering vitamin D resulted in a significant decrease (P<0.001) in the amount of

TNF- $\alpha$  in their blood compared to the control group (group II) that was given CCl<sub>4</sub>. The use of Liv-52 in group IV resulted in a significant decrease ( $P < 0.001$ ) in the amount of TNF- $\alpha$  present in the serum when compared to the control group treated with CCl<sub>4</sub> (group II). In contrast to the control group of rats that received CCl<sub>4</sub> therapy, the group that received a combination of vitamin D and liv-52 had significantly lower levels of TNF- $\alpha$  in their blood ( $P < 0.001$ ). The comparative analysis between group III vs IV, the protective function of vitamin D in isolation is similar to the protection by Liv-52 in isolation, there is no marker difference between the two and both have significant protection effect. The results of the study revealed no significant variations in TNF- $\alpha$  levels between the control rats (group I) and the control rats given a combination of vitamin D and Liv-52 (group VI). This indicates that taking vitamin D and Liv-52 supplements does not negatively impact liver function.

**Table 3:** Vitamin D and Liv-52's anti-inflammatory effects on the level of TNF- $\alpha$  in the serum of experimental and control animals

<b>Group Comparison</b>	<b>Mean <math>\pm</math> SD</b>	<b>P Value</b>	<b>Statistical significance</b>
I vs II	20.0 $\pm$ 2.2 vs 84.3 $\pm$ 5.3	p<0.001	Significant
II vs III	84.3 $\pm$ 5.3 vs 28.6 $\pm$ 4.4	p<0.001	Significant
II vs IV	84.3 $\pm$ 5.3 vs 24.9 $\pm$ 3.7	p<0.001	Significant
II vs V	84.3 $\pm$ 5.3 vs 21.7 $\pm$ 2.1	p<0.001	Significant
III vs IV	28.6 $\pm$ 4.4 vs 24.9 $\pm$ 3.7	p>0.06	Not significant
VI vs I	21.0 $\pm$ 1.6 vs 20.0 $\pm$ 2.2	p>0.57	Not significant

Leucocytes move into damaged tissues as part of the complex defence system known as inflammation in order to eradicate possible tissue-damaging substances. In contrast to chronic inflammation, which is a continuous phenomenon that can cause tissue damage, acute inflammation is a temporary helpful response, especially in response to an infectious threat. A sign that the body's immune system is activating to fight off an infection or injury is the increase in certain genes responsible for inflammation and the decrease in genes that suppress inflammation in specific cell types. Depending on the type of cell, various cytokines or enzymes that produce inflammatory mediators may be upregulated at the transcriptional or post-transcriptional level. Inflammatory reactions can be controlled through the actions of various receptors such as toll-like receptors, signal transducers, and transcription factors [17]. These receptors act as triggers for specific signal cascades that ultimately lead to gene transcription. The level of activity of these receptors determines the extent of the inflammatory response.

A common reaction to various liver ailments, such as exposure to toxins or medicines, is liver fibrosis. Extracellular matrix (ECM) components are deposited excessively, which frequently results in hepatic dysfunction and even hepatocellular cancer. An important public health concern is liver fibrosis, which can develop into cirrhosis, portal hypertension, and hepatocellular cancer and result in higher morbidity and mortality rates [18]. Toxic substances are one of the causes of liver damage and fibrosis that cannot be disregarded. Inflammation, oxidative stress induction, and liver fibrosis are all generated by a variety of factors, including toxic effects from reactive metabolites, ROS, inflammatory reactions, and imbalances between cellular damage and defensive responses. CCl<sub>4</sub>, a frequently used laboratory reagent known for its toxicity that can cause liver lesions and liver fibrosis, has been used extensively in investigations pertaining to the liver [19].

Vitamin D is a crucial steroid hormone that plays a vital role in reducing inflammation and fibrosis [20]. Vitamin D act as proinflammator and reduce the inflammation by following three ways. Firstly, the VDR, also known as the vitamin D receptor, is a type of protein that belongs to a larger group of proteins known as nuclear receptors. This protein plays a crucial role in how the body responds to vitamin D. When a form of vitamin D called 25(OH)D<sub>3</sub> binds to the VDR, the protein joins with another protein called the retinoid X receptor (RXR) to create a complex. This complex then attaches to specific regions on certain genes, known as vitamin D response elements (VDRE), which causes changes in those genes that are sensitive to vitamin D. The fibrotic response in HSCs is now suppressed by these elements' molecular activation of gene expression. This is accomplished by inhibiting the recruitment of SMAD3, a transcription factor in the TGF signalling pathway, to regulatory areas of pro-fibrotic genes.

Secondly, vitamin D activate two important genes such as, Nrf2 and the anti-aging gene Klotho play a variety of roles in preserving the reliability of cellular signalling networks. In order to maintain the usual reducing environment inside the cell, vitamin D, Klotho, and Nrf2 all enhance cellular antioxidants [21]. Thirdly, the target genes recruited by VDRE are those involved in cell proliferation, differentiation, and immune system cells in addition to calcium and phosphate homeostasis [21]. Dendritic cells, macrophages, T and B lymphocytes can activate 25(OH) D to calcitriol and induce a signalling pathway, which mediates either intracellular activity (i.e., intracrine) or cell-to-cell communication (i.e., paracrine) to induce changes in neighbouring cells. These immune system cells also express CYP27B1 and VDR. Mitogen-activated protein kinases (MAPKs) play a crucial role in transmitting inflammatory signals within cells. One of the most important MAPKs in this process is p38. It has been discovered that vitamin D also plays a role in regulating intracellular signaling, and there are multiple interactions between the VDR/RXR and MAP kinase signaling pathways. The outcome of these interactions can vary depending on factors such as the stimulus, the type of cell, and the response. Research has shown that vitamin D can inhibit p38 MAP kinase, which in turn prevents certain cells called monocytes from producing proinflammatory cytokines like IL-6 or TNF. This helps to reduce inflammation in the body [22].

It has been discovered that the inhibition of the protein p38 in monocytes is caused by an increase in the activity of an enzyme called MAPK phosphatase-1 (MKP1). This enzyme dephosphorylates p38, which reduces its activation. A similar process has been observed in prostate cells, where the activation of another enzyme, MKP5, caused by vitamin D, suppresses the production of another protein called IL-6 mRNA. This happens because vitamin D activates a complex of proteins called VDR/RXR, which bind to a specific region in the DNA called a VDRE in the MKP5 promoter. This binding enhances the transcription of the MKP5 gene. Vitamin D and its receptor complex VDR/RXR also have anti-inflammatory effects by interacting with other transcription factors such as nuclear factor kappa B (NF- $\kappa$ B) and nuclear factor of activated T cells (NFAT), which also control signaling pathways. As the inflammatory process continues, more chemical messengers and signals are created, leading to a rise in oxidative stress and harm to cells. These chemical messengers and signals include IL-6, TGF- $\beta$ , and TNF- $\alpha$ . For the communication between the many cells involved in inflammatory responses, the IL-6 group of cytokines is particularly important. These cells include various types of white blood cells (such as different types of T-cells, macrophages, dendritic cells, granulocytes, and B-lymphocytes) as well as cells from connective tissue and blood vessels (fibroblasts and endothelial cells). Macrophages and lymphocytes produce TNF- $\alpha$  during an inflammatory response and it plays major roles in both local and systemic effects on various body tissues. One key factor in the inflammatory response and the production of pro-inflammatory cytokines caused by lead (Pb) damage is the activation of the mitogen activated protein kinase (MAPK) [23].

Several studies have shown a potential link between vitamin D therapy and the reduction of inflammatory markers in individuals with hepatitis C and non-alcoholic fatty liver disease (NAFLD). Salter et al. (2013) found that serum levels of IL-6 were elevated in hepatitis C patients and that vitamin D therapy led to a significant reduction in these levels over time [24]. Crespo et al. (2001) also found that over-expression of TGF- $\beta$  and TNF- $\alpha$  in hepatic tissue of NAFLD patients was reduced with vitamin D treatment [25]. Additionally, research conducted by Kim et al. (2013) found that activation of the 25(OH)D<sub>3</sub> signaling pathway through natural or synthetic analogs of vitamin D reduces the expression of IL-6, TGF- $\beta$ , and TNF- $\alpha$  [26]. A study by Zhang et al. (2012) also found that high fat diet (HFD) fed rats had elevated levels of IL-6, TGF- $\beta$ , and TNF- $\alpha$ , which leads to chronic inflammation and the development of NAFLD. However, the study also found that vitamin D and its nanoemulsion significantly reduced these proinflammatory markers compared to HFD fed rats [22].

Infectious hepatitis patients treated with Liv-52 have faster healing, faster liver function recovery, and protection and regeneration of the liver parenchyma. Liv-52 stops cell membrane deterioration because of its antioxidant activity. Cytochrome P-450 is normalised by Liv-52. Additionally, it encourages the removal of acetaldehyde, a toxic intermediate result of alcohol metabolism, protecting the liver from alcohol-related harm. In cases of chronic drinking, the preparation lowers lipotropic activity and avoids fatty liver infiltration. Liv-52 reduces the progression of the disease in precirrhotic situations, stops further liver damage, and enhances quality of life [27]. In 1990, Dhumal and Patel conducted a study to assess the effect of Liv-52 on liver toxicity caused by Carbon Tetrachloride in rabbits. The results of

their research indicated that Liv-52 had a protective effect on the liver by reducing inflammation markers, such as IL-6 and TGF- when administered orally to rats exposed to CCl<sub>4</sub>.

In this study, we discovered that animals with liver damage had elevated levels of the inflammatory markers IL-6, TGF- $\beta$ , and TNF- $\alpha$ . Treatment with vitamin D significantly reduced these levels by activating anti-inflammatory and immune response mechanisms, as well as increasing cellular antioxidants. Similarly, treatment with Liv-52 also reduced these levels by boosting the antioxidant system. Combining vitamin D and Liv-52 in rats with liver fibrosis caused by CCl<sub>4</sub> exposure resulted in even greater reductions of IL-6, TGF- $\beta$ , and TNF- $\alpha$ , likely due to the combination of different anti-inflammatory and antioxidant effects from both substances. These results indicate that vitamin D and Liv-52 play a significant role in reducing inflammation caused by toxins like CCl<sub>4</sub>.

#### **4. Conclusion**

The present study investigated the role of vitamin D supplementation in combination with Liv-52 on inflammatory biomarkers in Wister rats with carbon tetrachloride (CCl<sub>4</sub>)-induced liver disease. The results obtained indicate that the combination of vitamin D supplementation and Liv-52 exhibited beneficial effects on inflammatory biomarkers in CCl<sub>4</sub>-induced liver disease. The study found that rats treated with vitamin D supplementation and Liv-52 showed a significant reduction in inflammatory biomarkers compared to the control group. These biomarkers included proinflammatory of IL-6, TGF- $\beta$ , and TNF- $\alpha$ . The combination treatment led to a significant decrease in the levels of these biomarkers, suggesting an anti-inflammatory effect. The findings of this study support the potential role of vitamin D supplementation in combination with Liv-52 as a therapeutic strategy for mitigating inflammation in CCl<sub>4</sub>-induced liver disease. The combination treatment exhibited anti-inflammatory effects by reducing the levels of pro-inflammatory cytokines and improving liver function parameters. These results suggest that vitamin D supplementation in combination with Liv-52 may have hepatoprotective properties in liver disease associated with inflammation. However, further research is necessary to elucidate the underlying mechanisms responsible for these effects and to determine optimal dosages and treatment durations.

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#### **Conflict of interest**

The authors state that there is no financial or personal involvement that could influence the publication of this manuscript.

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