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*Original Article*

# Role of Vitamin D as Protective Agent against Induced Liver Damage in Male Rats

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**Abstract**

One of the main factors which played a key role in the prevention of liver disorders such as hepatic inflammation, fibrosis, and carcinogenesis would be the vitamin D axis. Therefore, the current research was designed to evaluate the role of Vitamin D (Vit D) as a protective agent against liver damage caused by Thioacetamide (TAA). In the current study, 18 male Wistar rats were randomly allocated into three equal groups (n=6): in group 1(G1) the animals were considered as the control group and did not receive any supplement in drinking water; in group 2 (G2) TAA was administrated to the drinking water at a dose of 300 mg/L; in group 3 (G3) TAA was administrated to the drinking water at a dose of 300 mg/L plus vitamin D at a dose of 0.5 mg/100g body (intraperitoneal) for 8 weeks. At the end of the experiment, the animals were sacrificed and the liver was dissected and removed for histopathology. Histopathological evaluations were used to evaluate the possible adverse effects of TAA on the liver. Several hepatic damages were observed in the G2 group such as lobular disorder, some degrees of degeneration in hepatocytes and enlargement of the hepatic capillaries, and focal necrotic areas. Hepatic fibrosis was observed around portal areas and central veins. Bridging fibrous septa were formed between portal veins. The recorded data in this study showed that Vit D has some beneficial effects in protecting the liver from fibrosis and toxic damages. The recorded data showed that liver damages in the G3 group were partially prevented or cured. In conclusion, it is evident that the Vit D played a pivotal role as an antioxidant and anti-fibrotic agent, therefore it would be the best supplement for liver protection against damages due to toxin entrance into the animal's body.

**Keywords:** Vitamin D, Liver fibrosis, Thioacetamide, Histopathological examinations

## 1. Introduction

Liver is the largest gland in the body, with diverse functions in the regulation of many physiological processes. Hence, serious liver disease may be the leading cause of death. One of these life-threatening conditions is drug-induced liver damage, which requires major clinical and monitoring measurments (1). Among these, Thioacetamide (TAA), as a white crystalline organosulfur, develops cytomegaly, which in turn disrupts liver function and causes carcinogenesis. This compound is utilized for the induction of acute liver failure in rat models (2). Chronic intraperitoneal (IP) or oral administration of TAA is applied to establish a reliable experimental model for cirrhosis and fibrosis in rodents (3). A single-dose administration of TAA generates lobular necrosis in animal models. The chronic administration of TAA results in hepatocellular carcinoma and cirrhosis. The TAA-induced toxicity can be attributed to its bioactivity via oxidase systems, especially CYP2E1 and FAD-dependent monooxygenases (4, 5).

Vitamin D (Vit D) as a secosteroid with an endocrine function is sequentially produced and exerts actions to establish skeletal and calcium homeostasis. The source

of Vit D intake goes back to food sources or endogenous synthesis in the skin. Exposure to ultraviolet B causes a stereoisomeric change in 7-Dehydrocholesterol (7-DHC), which leads to the production of pre-vitamin D3. It is affected by skin pigments, dermal 7-DHC stores and exposure to ultraviolet rays (6). Previous studies have tried to find a link between Vit D deficiency and liver disease, with a greater focus on liver fibrosis and liver function. Nonalcoholic fatty liver disease (NAFLD) has shown an inverse correlation of Vit D with aspartate aminotransferase (ALT) and aspartate aminotransferase (AST). A high doses consumption of Vit D can cause an improvement in liver function markers among adolescents with liver dysfunction (7, 8). The Vit D can be obtained from natural plant food sources containing Vit D2 (ergocalciferol) or through animal food sources containing Vit D3 (choleocalciferol). The main origin of Vit D3 goes back to its production in human skin by being exposed to natural sunlight (9, 10).

Therefore, this study was designed to investigate the possible role of Vit D as a protective agent against liver damage caused by thioacetamide (TAA) in rats.

## 2. Materials and Methods

### 2.1. Chemicals

All the chemicals were purchased from Sigma-Aldrich Co. (MO, USA), unless otherwise stated.

### 2.2. Animals and Experimental Design

In the current study 18 male Wistar rats (10-12 weeks with average weight of 200 ±25 g.) were randomly allocated into three equal groups (n=6): in group 1(G1) the animals considered as control group and did not received any supplement in drinking water; in group 2 (G2) TAA was administrated to the drinking water at a dose of 300 mg/L; in group 3 (G3) TAA was administrated to the drinking water at a dose of 300 mg/L plus vitamin D at a dose of 0.5 mg/100g body (intraperitoneal) for 8 weeks. At the end of experiment the animals were scarified and the liver dissected and removed for histopathological. At the end of the experiment, the animals were anesthetized using overdose of chloroform, samples of liver tissues were excised and fixed with 10% formalin for histopathological studies.

### 2.3. Histological Evaluations

Sections of 5 µm thickness from each tissue block were stained with Mason's trichrome (Abcam, MA, USA) to assess fibrosis in the liver. Two expert histopathologists and who were blind to the source group evaluated and scored liver fibrosis in all sections on an EVOS XL Core microscopy (Thermo Fisher Scientific) using 10 random non-overlapping fields from each slide at ×400 magnification. Additionally, quantitative measurement of collagen deposition (fibrosis index %) was done using Image J software (https://imagej.nih.gov/ij/) as previously described.

## 3. Results

According to liver sections in the G1 group, the hepatic lobules composed of polygonal and regular hepatocytes with normal cytoplasm and rounded vesicular nuclei. Such cells made up hepatic sinusoids and cords, present between each normal cord. Some Kupffer cells were distributed in the sinuses, the hepatocytes had only one nucleus, and others had binucleated status located around the central vein (Figures 1 and 2).

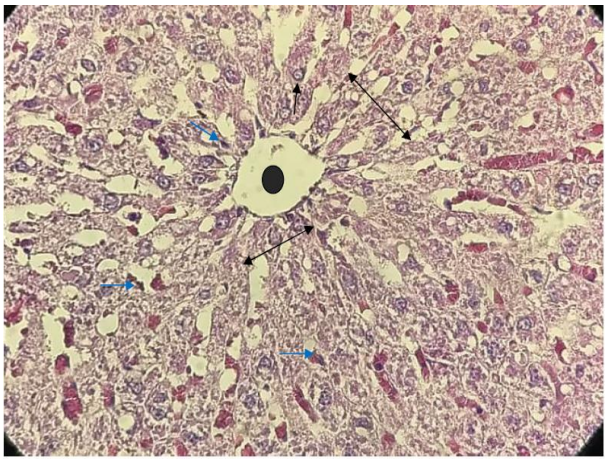

**Figure 1.** Section of normal liver showed the hepatocytes (⟷) around central vein (●) also kupffer cells (→) and hepatocyte binucleate (→) was obvious. H&E stain (40X)

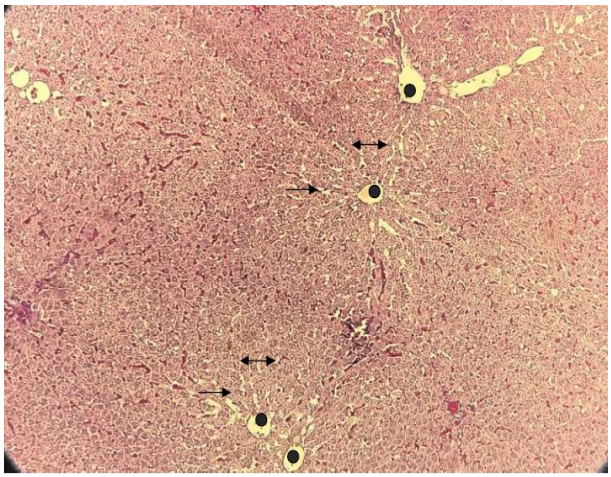

**Figure 2.** Section of normal liver showed hepatic cord (⟷) separated by smaller sinusoids (⟶) the hepatocyte around the central vein (●) H&E stain (10 X)

According to microscopic findings of liver sections in the G2 group, the observations consisted of the following disorders: 1) distorted structure of hepatocytes surrounding the central vein, 2) focal hepatocyte necrosis, 3) vacuolated cytoplasm, 4) degeneration, 5) inflammatory cell infiltration, 6) aggregation of inflammatory and blood cells in the portal canal, 7) epithelial layer hyperplasia of bile duct lining, 8) edematosis with mild bleeding in the venous lumen, 9) degeneration of the endothelial lining of the portal vein, 10) portal vein and duct congestion, 11) portal triad and duct fibrosis, 12) portal triad referring to the thickness of the hepatic artery with bleeding, 13) absence of normal tissue stroma, 14) thrombosis around the central vein, 15) irregular dilated sinus hyperplasia of the bile duct, 16) lipid alterations,vacuolation of cytoplasm, 17) alterations probably in the form of steatohepatitis, 18) hepatocyte coaculative necrosis and 19) rupture extended to all hepatic structures (Figures 3, 4 and 5).

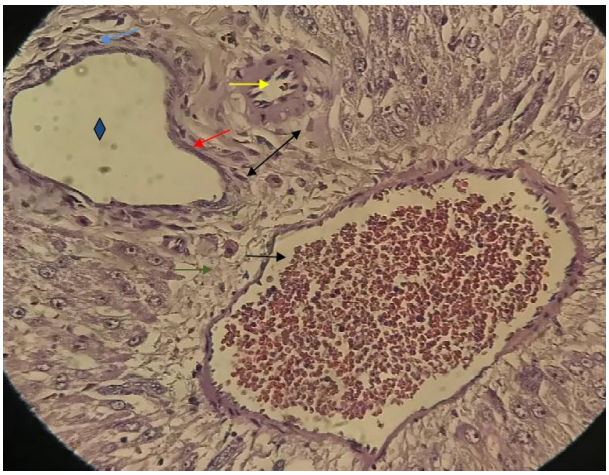

**Figure 3.** Section on liver from rat TAA (G2) showed focal necrosis (⟶) destruction and fibrosis (⟷) dilated portal veins (◆) yperplasia of lining epithelial layer (➔) and small dilated venule (➔) infiltration of inflammatory cell (➔) collagen fibers deposited (➔). H&E stain (40X)

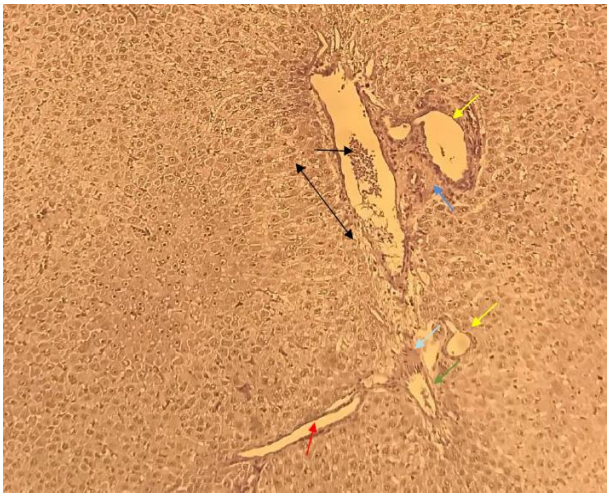

**Figure 4.** Section on liver from rat TAA (G2) showed congested portal tract (⟶) disorganized hepatocytes (⟷) mild area of fibrosis (➔) central vein (➔) more than one with degenerated lining layer, dilated sinusoids (➔) fibrous speta (➔) and the section revealed to liver steatosis H&E stain (10X)

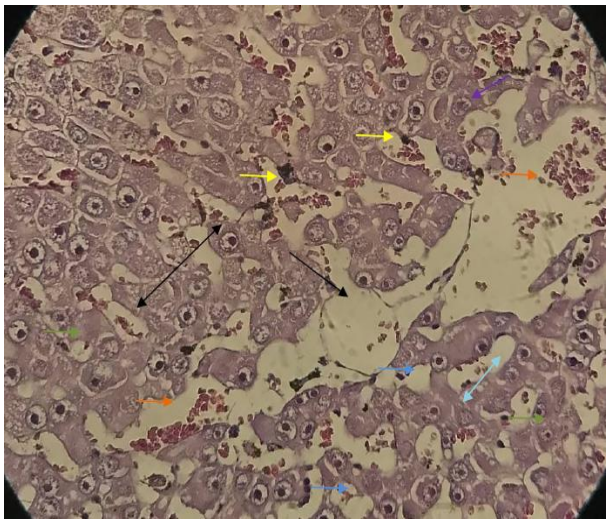

**Figure 5.** Section on liver from rat TAA (G2) showed congested portal tract (⟶) with accumulation of red blood cell (⟶) congested sinusoids (⟶) mild fatty changes (⟶) irregular hepatic cord (⟷) degeneration region (⟷) small kupffer cells (⟶) vacuolated cytoplasm (⟶) H&E stain (40X)

According to histopathological findings of liver sections in the G3 group, the observations consisted of the area of regeneration, plate or sheath-like hepatocytes in the liver stroma, the presence of inflammatory cells, mild blood vessel congestion, normal and sinusoidal scattering of Kupffer cells, endothelial layer with normal lining in the central vein, mild peripheral vacuoles, periportal area with normal view, the central vein with arranged cells, necrosis with mild status, hepatic lobules with hepatocyte strains, clearer cell boundaries, multiple binucleate hepatocytes despite the presence of fatty alterations, regeneration in liver sections, proliferation of inflammatory and kupffer cells, regularly hepatic cords spaced apart with normal sinuses, Kupffer cell foci with accumulation around portal triads, collagen fiber deposition and further moderate lymphocytes around the portal canal and central vein (Figures 6, 7, 8 and 9).

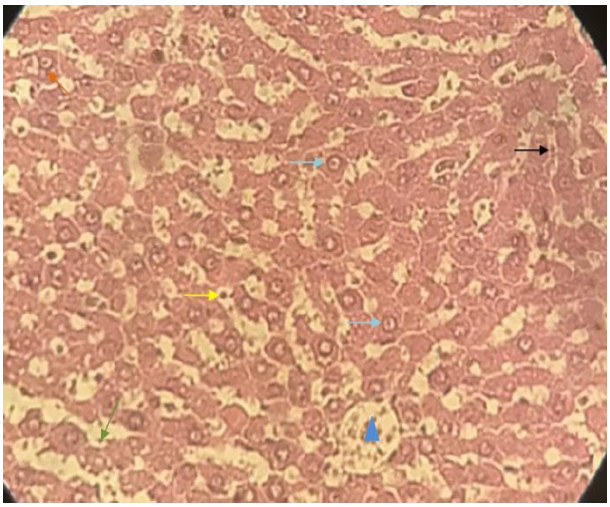

**Figure 6.** Section on liver of rat TAA and Vit D (G3) showed normal hepatocyte arranges as a cord (⟷) around central vein (▲), with vesicular nuclei (⟶) showed mitotic figures, few degenerated cells (⟶) normal kupffer cell (⟶), less dilated sinusoids (⟶) some hepatocyte binucleate (⟶) H&E stain (40X)

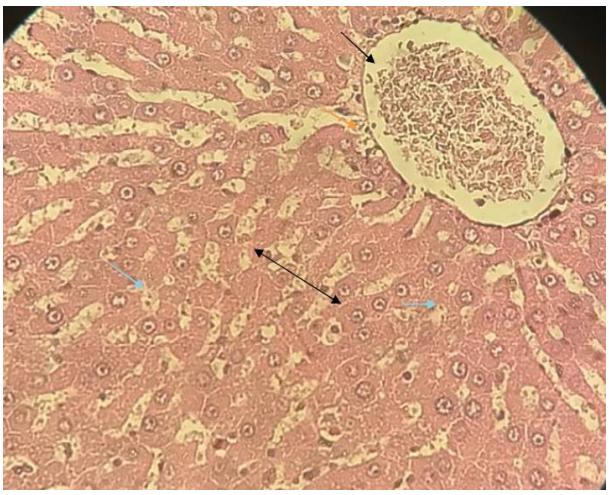

**Figure 7.** Section on liver from rat TAA & Vit D (G3) showed congested central vein (⟶), mild inflammatory cell (⟶) normal distribution of kupffer cells (⟶) also most of hepatocyte appear normal (⟷) H&E stain (40X)

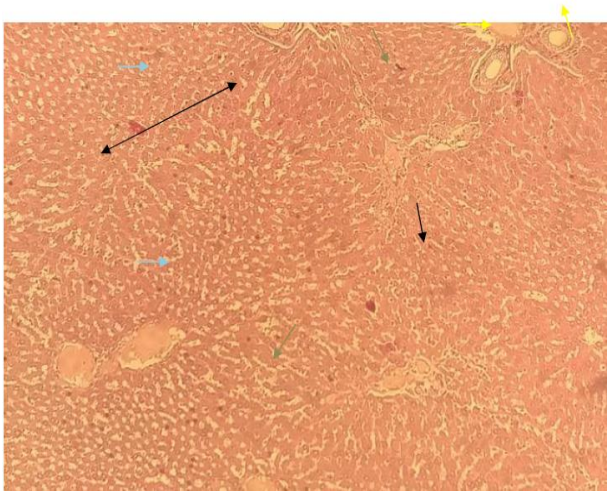

**Figure 8.** Section on liver from rat TAA & Vit D (G3) showed normal regeneration of hepatocytic cell ( ) mild inflammatory cell ( ) normal sinusoids ( ) normal blood vessel ( ) mild hemorrhage ( ) H&E stain (10X)

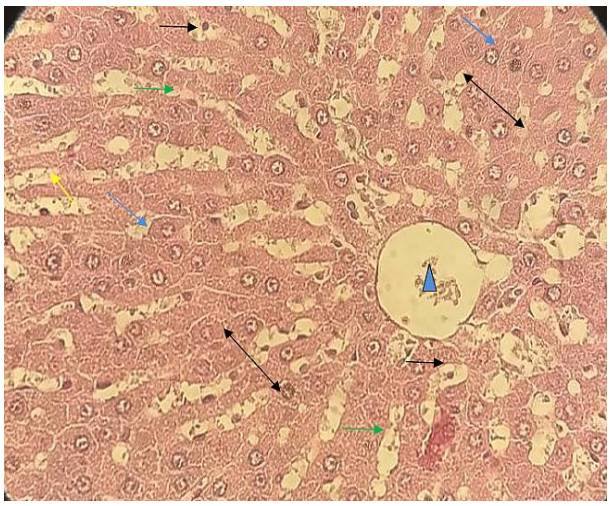

**Figure 9.** Section on liver from rat TAA & Vit D (G3) showed normal polygonal hepatocytes arranges as cords ( ) around normal central vein ( ) normal kupffer cells ( ) less dilated sinusoids ( ) and normal cell boundries ( ) moderat deposition of collagenous fibers ( ) H&E stain (40X)

## 4. Discussion

According to the observations of liver sections related to control rats (G1) treated with normal saline, the liver lobule had typical architecture with no alterations, confirming no injury caused by normal saline, consistent with Farokhi, Farkhad (11) and Aboonabi, Rahmat (12). Based on evidence obtained from the animals treated with TAA (G2 group), several induced damages were recorded in the liver sections. Such changes might be attributed to TAA toxicity and free radical formation and reactive oxygen species (ROS) production, resulting in cell membrane destruction, lipid peroxidation and liver dysfunction. The recorded histological and histopathological changes were as follows: necrosis, congestion and degeneration. Ansil, Nitha (13) reported that TAA-induced an extensive ROS formation which can devastate the antioxidant defense system and cellular components like proteins, lipids and DNA, thereby disturbing cell function and structure.

Antioxidants are chemicals found in a variety of natural food sources. Moreover, other studies mentioned the metabolization of TAA to TAA-S-oxide and acetamide instantly after administration. Then, the TAA-S-oxide can attach to all cell macromolecules in charge of alteration in cell permeability and $Ca^{2+}$ adsorption. This defect in calcium content causes nuclear enlargement and mitochondrial dysfunction, ultimately leading to liver necrosis (14, 15). These results are in line with other reports, which confirmed the role of TAA in further periportal infiltration, further ductal proliferation, further hepatocyte vacuolization, acidophilic body formation, and nuclear vacuolization along with large nucleoli (16, 17). Furthermore, previous studied reported the function of TAA as an electrophilic agent capable of forming s-oxide covalently attached to lysine contributing to adduct formation with the aid of sulfhydryl groups, thereby reducing protein content and so exerting serious injury (18). The reactive TAA metabolites covalently attached to lipids and proteins inside the cell not only exert oxidative stress, but also causes glutathione depletion and finally cell damage. Other abnormalities in this regard include changes in proteins related to cell cycle, inhibition of enzymatic function, protein folding and prevention of mitochondrial or chaperone function, meaning a serious cytotoxicity (18).

The recorded data of the current study introduced Vit D as a main player in prevention of TAA pathogenesis. The results obtained from liver sections in G3 group (TAA and VD) showed normal hepatocyte restoration and regeneration in the liver stroma, mild congestion of blood vessels, which probably due to the role of Vit D as an antioxidant and anti-fibrotic agent in the prevention of the liver damage. Similarly, Abramovitch, Dahan-Bachar (19) reported the prevention of liver fibrosis by the Vit D in thioacetamide-treated rats under both in vivo and in vitro conditions.

In a similar study, Özerkan, Özsoy (20) introduced the Vit D as an anti-fibrotic and antioxidant agent capable of preventing the rat liver damage, and reported that the Vit D treated rats experienced at least partial prevention of all aspects of liver injury.

The Vit D exerts antioxidant impacts both directly and indirectly. Directly, it is a membrane antioxidant. Indirectly, it relates to antioxidant defense system through the up-regulation of endogenously available antioxidant effectors such as catalase, superoxide dismutase, glutathione peroxidase and glutathione (21, 22).

Seif and Abdelwahed (23) found that the VD pre-treatment improved necro-inflammatory and apoptotic stigmata in the rat model of hepatic ischemia-reperfusion injury (IRI).

In a study conducted by Adelani, Ogadi (24), the dietary Vit D reduced hepatic inflammation and oxidative stress through a decrease in the formation of cytokines and lipid peroxidation.

The mechanisms of action of Vit D have been repeatedly conducted in the liver fibrosis. It can decrease the production of collagen and profibrotic indices in LX-2 cells, hepatic stellate cells (HSCs) and mesenchymal multipotent cells (MMCs). The VD supplementation caused a significant decline in extracellular matrix (ECM) deposition and also fibrotic score in various animal models induced with liver injury (25). The Vit D has attracted immense attention of the scientific community owing to its proprietary performances in immune function, cell proliferation and differentiation, cardiovascular activities, and calcium/phosphate homeostasis. The relationship between Vit D deficiency and liver pathophysiology has been investigated by many researchers. Some researchers have shown a high prevalence of Vit D deficiency in various liver diseases (26, 27).

The Vit D is a group of fat-soluble secosteroids, the main action of which is to promote the skeletal mineralization. It is a prominent actor with numerous biological potentials, such as anti-metastatic, anti-invasive, pro-differentiation, anti-proliferative, anti-angiogenic and pro-apoptotic activities. It should be mentioned that the Vit D may exhibit anti-inflammatory and antioxidant performances (28, 29).

The active Vit D 3 exerts its biofunctions through the nuclear Vit D receptor (VDR). It is a phosphoprotein receptor with high affinity capable of linking with 1,25OHD3 and regulating the expression of genes implicated in multiple cell process such as differentiation, proliferation, immunomodulation and apoptosis. Accordingly, VDR expression in the liver may have an inverse correlation with the liver damage severity (30).

## Authors' Contribution

Study concept and design: M. K. H.
Acquisition of data: M. K. H.
Analysis and interpretation of data: M. K. H.
Drafting of the manuscript: M. K. H.
Critical revision of the manuscript for important intellectual content: M. K. H.
Statistical analysis: M. K. H.
Administrative, technical, and material support: M. K. H.

## Ethics

The current research was approved by the Ethics Committee of the University of Misan, Maysan, Iraq.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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