

# Silymarin and Curcumin has a Potential Hepato-Protective Effect against Chemically-Induced Liver Dysfunction

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## Abstract

The liver is important, essential, and vital organ in the body due to their function in body. Detection of hepatic dysfunction depends on the lab tests such as: Elevated aspartate aminotransferase (AST) or alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Curcumin has been shown to protect liver in much models of liver injury by inhibiting oxidative damage. Twenty four adult male albino rats their average weight 150-200 g were divided into four group six rats in each group ; group1: served as a normal group, group 2:subjected to 0.5mg/kg ccl<sub>4</sub> dissolved in olive oil s.c every other day for 3 weeks, group 3:subjected to ccl<sub>4</sub>0.5mg/kg dissolved in olive oil s.c every other day in addition to Curcumin 200 mg/kg /d orally every day for 3 weeks, group 4: subjected to ccl<sub>4</sub>0.5 mg/kg dissolved in olive oil s.c every other day in addition to silymarin 200mg/kg orally every day for 3 weeks. The administration of ccl<sub>4</sub> cause hepatic dysfunction and increase in serum ALT, AST and ALP level. The administration of Curcumin and silymarin along with ccl<sub>4</sub> showed a decrease in serum ALT, AST and ALP level. The hepatic-protective effect of Curcumin and silymarin are relating to reduced liver enzymes as ALT, AST and ALP in hepatic dysfunction that produced by CCl<sub>4</sub> toxicity through an antioxidant effects, exerting anti-inflammatory and free radical diminish.

**Keywords:** Hepatotoxicity; Carbon tetrachloride; Liver transaminases; Silymarin; Curcumin

## Introduction

The Liver is largest organ in the body and it weighs about 2.5 kg of total body weight. The Liver is wedge shape organ with base to the right and its apex to left. It's located at right hypochondrium, epigastrium, and left hypochondrium. The liver consists of 2 lobes: a large lobe, and a small left lobe, and these lobes are separated by falciform ligament (which is a fold of peritoneum extending from anterior abdominal wall and diaphragm

to liver), fissure for ligamentumteres, and fissure for ligamentumvenosum [1].

The liver is the one of the most important organs in the body, it maintains the normal body activities with many essential functions which include drug detoxification, also the liver is considered the main site for metabolism inside the body, It organizes many metabolic reactions in the

body, as well as providing the body with major secretory functions (Figure 1) [1].

Liver dysfunction is related to the abnormality in the liver's ability to perform its normal functions, mainly due to exposure to various stimuli such as toxic substances, viral infections, and trauma, which eventually lead to massive damage to the hepatocytes as well as diminished functions of the liver [1].

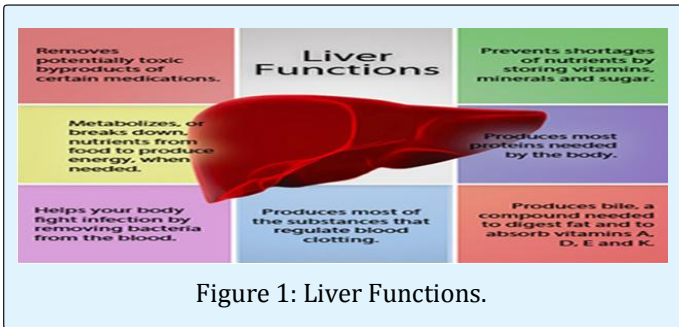


Figure 1: Liver Functions.

Chemicals are likely to induce liver injury. In the current study, the potential hepatoprotective activity of both silymarin and curcumin were investigated. Hepatic dysfunction is considered an indication of the abnormal functions of the liver.

#### Different Uses of Liver Function Tests such as

1. Screening: Detection method for liver dysfunction.

2. Pattern of disease: Useful into differentiate between acute viral hepatitis, Cholestasis liver diseases and chronic disorders.
3. Assess severity: To evaluate severity results of some diseases such as primary biliary cirrhosis.
4. Follow-up: useful in assessing response such as the treatment of autoimmune liver [2].

#### Classification of Liver Function Tests

1. Tests of ability of liver to transfer organic electrolytes and metabolism of drugs such as (urobilinogen, Serum bilirubin and urine bilirubin).
2. Tests that reveal the injury of liver cells which include (ALP, leucine amino peptidase, aminotransferase, glutamylTranspeptidase and 5-nucleotidase).
3. Tests the ability of liver to biosynthesis such as (prothrombin time, Serum proteins, serum ceruloplasmin, prealbumin, and albumin) [2].

#### Tests that Reveal the Injury of Liver Cells

##### Aspartate Aminotransferase & Alanine Aminotransferase

ALT is present in the liver, in Cytoplasm, but AST is found in a large variety of the tissue such as skeletal muscles, heart, Hepatic tissues and brain and it is also found in mitochondria and Cytoplasm of liver cells. In case of chronic liver disease also mitochondrial AST is increased (Figure 2) [2].

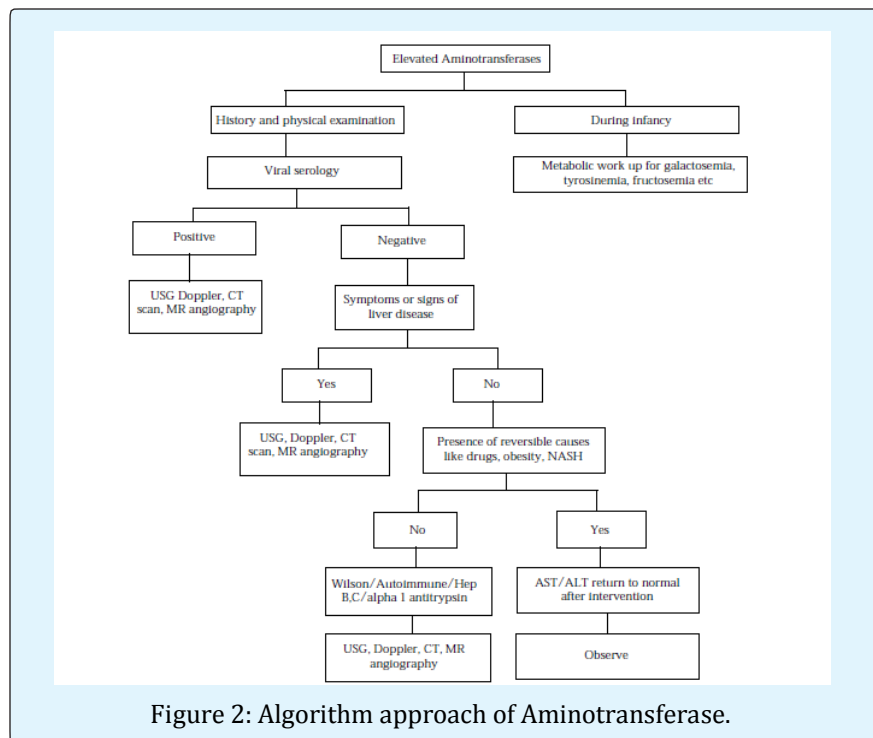


Figure 2: Algorithm approach of Aminotransferase.

### There are Three Types of Aminotransferases Elevations

1. Severe elevation of aminotransferases: (More than 20 times).  
An Increased level of Aspartate aminotransferase and Alanine aminotransferase if found in most liver diseases. In case of hepatitis, toxins or drugs which cause liver necrosis and circulatory shock leading to an increase in ALT and AST enzymes [2].
2. Moderate elevation of aminotransferases: (From 3 to 20 times).  
In case of acute hepatitis, newborn hepatitis, chronic hepatitis and autoimmune hepatitis, Hepatitis due to drug abuse, alcoholic hepatitis and as well as severe biliary tract obstructions cause moderate elevation of ALT and AST. An increased level of Alanine aminotransferase is usually higher more compared with Aspartate aminotransferase only in chronic liver disease [2].
3. Mild elevation of aminotransferases: (From one to three times).  
It's seen in cases of newborn hepatic inflammation that causes sepsis, extra-hepatic biliary atresia [EHBA], liver

cirrhosis, drug toxicity, muscle inflammation and muscular dystrophy.

### Alkaline Phosphatases Test

ALP level is usually normal or increased slightly in case of acute viral hepatitis. Hepatitis A can provide an image of cholestasis with noted and long-term Itching and high alkaline phosphatase level. High levels of serum and intestinal alkaline phosphatase (ALP) are found in patients with cirrhosis of the liver, especially with type O blood, and can be linked particularly to the disease within the liver instead of the extra-hepatic obstruction. Some diseases such as liver and bone metastasis can cause high levels of alkaline phosphatase. Also other diseases such as infiltrative liver disease, cysts, and amyloidosis can cause high alkaline phosphatase. High levels of the alkaline phosphatase are found in liver cirrhosis and hepatitis (Fig 3). In disease like Hypothyroidism, pernicious anemia, and decreasing zinc level may cause reduced alkaline phosphatase level. Drugs may cause increase in alkaline phosphatase level such furosemide, cimetidine, Anti-epileptic drug like (Phenobarbitone and Phenytoin).

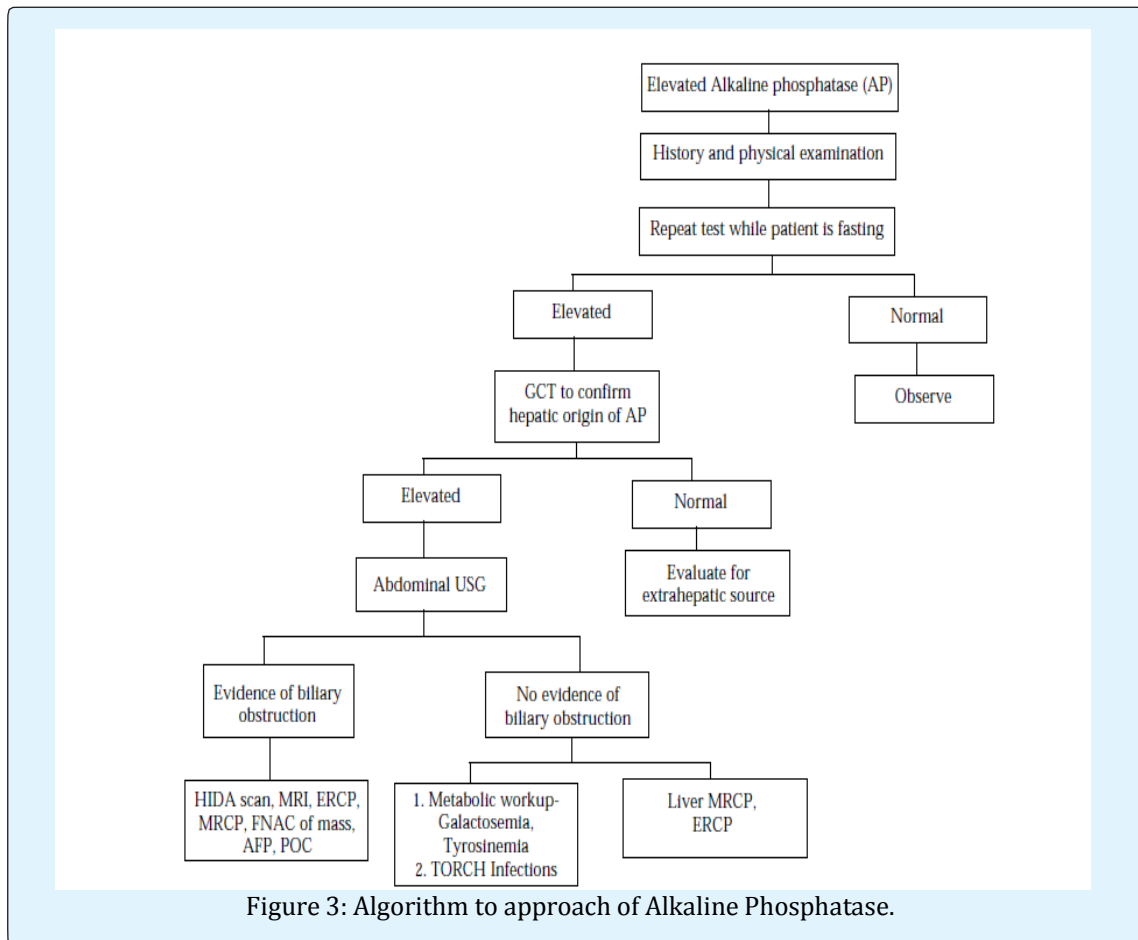


Figure 3: Algorithm to approach of Alkaline Phosphatase.

The present study was conducted to compare the antioxidative effects of Curcumin in comparison to that of silymarin in protecting the hepatotoxic injury caused by CCl<sub>4</sub> in rats.

## Materials and Methods

### Natural Product

Curcumin and Silymarin used as anti-oxidative stress in the rats used to protect liver against liver damage by carbon tetrachloride (CCl<sub>4</sub>).

### Animals

24 adult male albino rats weighing (150-200 g) housed in individual cages 6 rats in each cage in a humidity, temperature and light controlled room- (12:12 h light: dark cycle) maintained on a standard laboratory diet and water ad libitum [3]. The Experimental protocol was approved by Ethics Committee MSA University.

### Experimental Design

The rats were randomly divided into 4 groups (6 rats per each).

Group I (Control group): subjected to 1ml olive oil administration by gavage for 3 weeks.

Group II (CCl<sub>4</sub> group): subjected to subcutaneous (s.c)

CCl<sub>4</sub> injection, 0.5 ml/kg of body weight in olive oil on every other day for 3 weeks CCl<sub>4</sub> (Sigma-Aldrich, Egypt).  
Group III (CCl<sub>4</sub> + Curcumin group): received CCl<sub>4</sub> by subcutaneous injection, 0.5 ml/kg in olive oil every other day for 3 weeks and 200 mg/kg of body weight Curcumin (Sigma-Aldrich, Egypt) dissolved in olive oil and given by gavage every day for 3 weeks.

Group IV (CCl<sub>4</sub> + Silymarin group): received CCl<sub>4</sub> by subcutaneous injection, 0.5 ml/kg in olive oil every other day for 3 weeks and 200 mg/kg of body weight and Silymarin (200mg/Kg/day) were orally given daily for 3 weeks [3].

### Biochemical Analysis of Aminotransferase Level

At the end of the experimental period, animals were anesthetized with sodium pentobarbitone (6mg/100 gr of body weight, IP). Blood were collected and serum separated by centrifuging at 825x *g* for 10 min. Serum was used for estimation of albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartateaminotransferase (AST) using specific diagnostic kits (Biodiagnostic, Egypt).

## Results

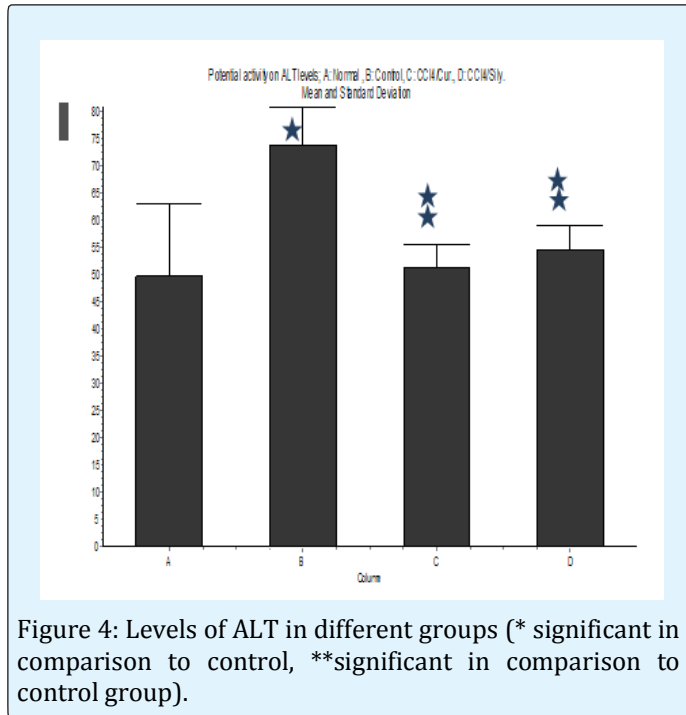
### Alanin Aminotransferase Levels

Animal No.	Normal	Control	CCl <sub>4</sub> + Curcumin	CCl <sub>4</sub> + Silymarin
1	38.5	75.1	49.1	49.4
2	38.3	75.3	44.7	49.2
3	71	80.2	51	59
4	40.6	78.6	52.6	54.3
5	50.2	72.1	56	58
6	59.2	60.5	55	57.3
Mean	49.63	73.63	51.4	54.6
S.D.	13.28	7.03	4.14	4.49

Table 1: Effect of CCl<sub>4</sub> (control), curcumin/CCl<sub>4</sub> and silymarine/CCl<sub>4</sub> group on ALT levels.

The control group showed a high significant elevation in the ALT levels in comparison to the normal group (P<0.001), were Curcumin/CCl<sub>4</sub> showed a high significant

reduction in comparison to the control group (P<0.001), also silymarine/CCl<sub>4</sub> showed a significant reduction in comparison to the control group (P<0.01) figure 4.

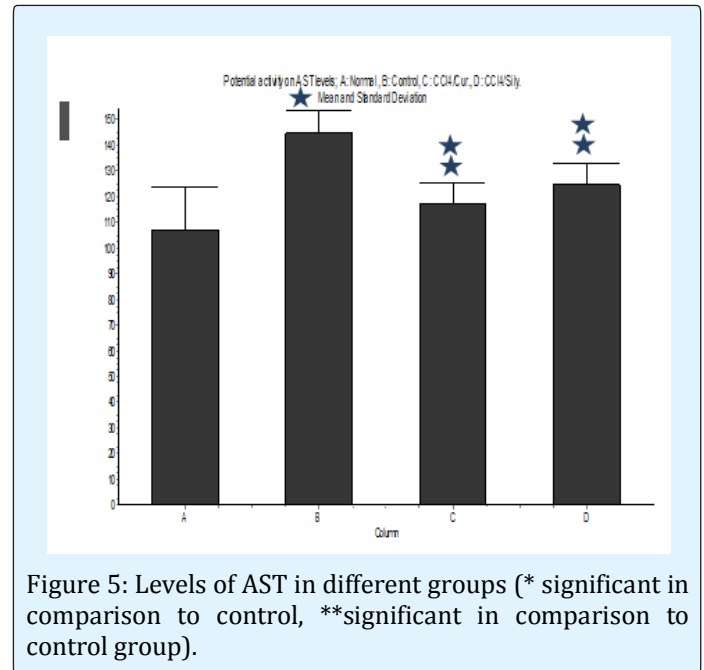


#### Asprtate Aminotransferase Levels

No.	Normal	Control	CCL <sub>4</sub> + Curcumin	CCL <sub>4</sub> + Silymarin
1	105	147	120	112
2	92.4	148	122.5	117
3	119	150	120.4	131
4	89.3	130	105	130
5	105	138	110	130
6	133	155	125.8	129
Mean	107.2	144.66	117.38	124.83
S.D.	16.45	9.07	7.9	8.18

Table 2: Effect of CCL<sub>4</sub> group (control), curcumin group and silymarine group on AST levels.

The control group showed a high significant elevation in the AST levels in comparison to the normal group ( $P < 0.001$ ), were Curcumin/CCL<sub>4</sub> showed a significant reduction in comparison to the control group ( $P < 0.01$ ), silymarine/CCL<sub>4</sub> showed a significant reduction in comparison to the control group ( $P < 0.05$ ) figure 5.

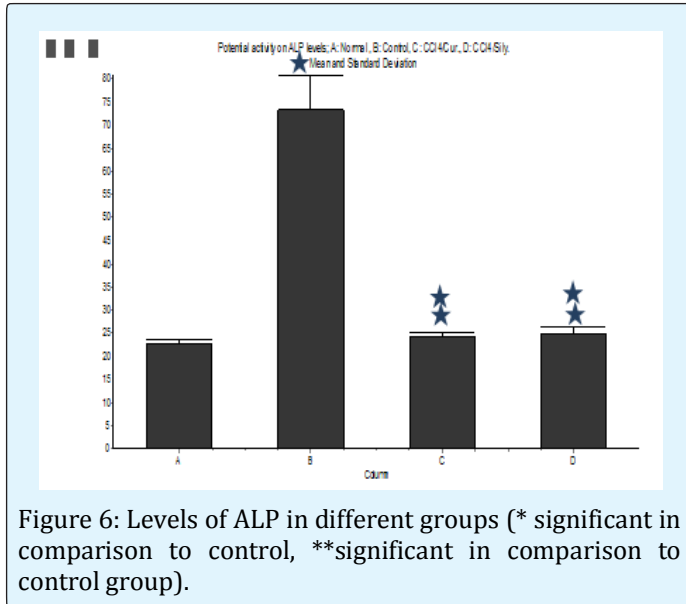


#### Alkaline Phosphatase Levels

No.	Normal	Control	CCL <sub>4</sub> + Curcumin	CCL <sub>4</sub> + Silymarin
1	21.9	70.8	23.3	23.6
2	22.5	72.9	23.8	27.2
3	23.3	77.7	23.9	23.6
4	21.1	60	25.9	23.3
5	24	80.3	24.2	24.5
6	22.5	78.3	24.9	26.4
Mean	22.55	73.33	24.33	24.76
S.D.	1.01	7.43	0.93	1.645

Table 3: Effect of CCL<sub>4</sub> group (control), curcumin group and silymarine group on ALP levels.

The control group showed a high significant elevation in the ALP levels in comparison to the normal group ( $P < 0.001$ ), were Curcumin/CCL<sub>4</sub> showed a high significant reduction in comparison to the control group ( $P < 0.001$ ), also silymarine/CCL<sub>4</sub> showed a high significant reduction in comparison to the control group ( $P < 0.001$ ) figure 6.



The effect of the Administration of CCl<sub>4</sub> on liver enzymes Produced significant increase in Alanine Transaminases Level and Aspartate Transaminases level as well as Alkaline Phosphatase Level. The administration of antioxidant Curcumin on CCl<sub>4</sub> Intoxicated liver showed a high protective activity reducing the levels of the transaminases with a high significant manner. The administration of antioxidant Silymarin on CCl<sub>4</sub> Intoxicated Liver showed also a significant reduction of the elevated transaminases level. Comparison between protection ability of curcumin and silymarin showed No significant difference (P>0.05).

## Discussion

Investigations regarding antioxidants are performed mainly for their ability to prevent the cardiovascular diseases and lung and liver damage which caused by the CCl<sub>4</sub> which induce the production of reactive oxygen species, peroxides, and cytokines [4]. Curcumin has been shown to protect liver in much models of liver injury by inhibiting oxidative damage. Therefore, this study was designed to study detailed mechanism of protection provided role of Curcumin against CCl<sub>4</sub> that induced the liver damage in mice, where measurements of biochemical, histological and immunological [4].

Serum transaminases such as AST, ALT and ALP show functional activity of liver. The increase in activities of these enzymes indicates a generalized effect due to any toxin substance. Curcumin administration before CCl<sub>4</sub> challenge in a significant reduction in serum levels of

these enzymes (AST, ALT and ALP). It has been reported for Curcumin to give the protection against such changes in formaldehyde and monosodium glutamate which induced hepatic toxicity and oxidative stress in mice [4].

The protective effects of the Curcumin in reduction levels of liver markers are well connected with the histological findings in this study. Supplementation with Curcumin rate dropped and the severity of CCl<sub>4</sub> in the relevant histological changes. Lipid peroxidation may cause changes in the membrane fluidity and permeability and increase rate of degradation of the protein, which finally lead to cell lysis. The prevention of the spread of the oxidation of polyunsaturated fatty acid is defined as the main role by which Curcumin is used to prevent oxidative damage by interfering with [4].

Curcumin supplementation showed an antiperoxidative effect in rat liver tissues via significantly decreasing the CCl<sub>4</sub> which induced increase of hepatic malondialdehyde levels. Effect of Curcumin can be interpreted via direct free radical scavenging properties which suggests that by maintaining cellular integrity, it can protect against damage caused by endotoxin [4]. The scavenging effect of Curcumin is to reduce oxidative stress caused by endotoxin in rat brain and that the behavior of the disease in mice, also the inhibition chemotaxis of leukocyte via Curcumin had reduced malondialdehyde levels, thus modulating oxidative stress. [4].

Host cells are protected from oxygen radical derived from injury of naturally occurring free radical scavengers and paths antioxidants, including Curcumin, vitamins A, C, E and, glutathione, superoxide dismutase, catalase, and glutathione peroxidase. However, antioxidant defense mechanism fails, either because of excessive free radical scavenging enzymes or decreased activity, or both, causing lipid peroxidation [4]. Decrease in the activities of superoxide dismutase and reduced GSH levels was observed in the liver tissues from CCl<sub>4</sub> challenged rats [4].

This may lead to obstruction dismutation of superoxide anions and detoxification of H<sub>2</sub>O<sub>2</sub> inefficient, leading to the formation of OH·. Ions promote membrane lipid peroxidation which leads to oxidative damage in many tissues. It has been shown to increase tumor necrosis factor alpha over and again to play a role in the CCl<sub>4</sub> that induced liver injury [4]. The potential of Curcumin to maintain the structure of the liver along with a significant reduction in biochemical indicative oxidative stress measurements suggest the use of Curcumin as an effective

preventive strategy for the management of CCl<sub>4</sub>-induced injury in the liver tissue [4].

Evaluate combined effect of curcumin against carbon tetrachloride induced liver disorders in mice. Chronic liver injury by carbon tetrachloride is well established animal model of cirrhosis of the liver. Reactive oxygen species and oxidative stress has been shown to play very important role in the etiopathogenesis of fibrotic changes of the liver, and it appears that antioxidant therapy in vivo to be effective in preventing or reducing fibrosis and chronic liver damage [4].

Hepatic enzymes like AST, ALT and ALP present in high concentrations in liver under normal conditions. When there is a hepatic necrosis or membrane damage, will be the release of these enzymes in the circulatory system, as evidenced by the high enzyme levels in the blood [4].

High levels of these enzymes found in the liver, indicates every sign was observed in carbon tetrachloride treated mice, or either indicates liver damage caused by hepatotoxins. Curcumin treatment for carbon tetrachloride rats caused an improvement toxic effect of carbon tetrachloride and restore some signs above the normal level. This effect may be free-radical scavenging of curcumin and the results obtained in this study are consistent with previous findings activity [4]. In carbon tetrachloride toxicity, also increased the synthesis of cholesterol. Significant decrease the concentration of Total cholesterol and triglycerides in serum of carbon tetrachloride and Curcumin administered in the rats [4].

This due to Curcumin possesses hypocholesterolemic action and this could be due to a decrease in absorption of cholesterol or an increase in HDL cholesterol [4]. Increased malondialdehyde levels in group treated with carbon tetrachloride and increase oxidative stress compared with group of control [4].

The Curcumin used as treatment in large restoration of antioxidant enzymes activities as well as the concentration of non-enzymatic antioxidant glutathione in the liver. However, curcumin along with vitamin E treatment showed significant protection of curcumin alone [4]. The combined administration of curcumin and vitamin E can be considered as potentially anti-oxidative stress and toxicity of the liver caused via carbon tetrachloride [4].

This design of experimental studies has shown that treatment of CCl<sub>4</sub> induced liver injury with curcumin leads

to an increase in serum levels of liver enzymes as AST, ALT and ALP. For that, in this study was confirmed the hepatotoxicity induced by CCl<sub>4</sub> by significant increases of serum levels of AST, ALP, and ALT after accurate analysis of these enzymes [5]. But after Administration of CUR in rats which have liver dysfunction induced by CCl<sub>4</sub> we showed a recovery from this injury and that is significant evidence by decreasing in activities of ALT, ALP, and AST in serum in period time around 3 weeks of treatment by curcumin [5].

This study was reported that the hepatic protective effects of curcumin are intermediated by its anti-oxidant protect ability and the scavenging activity of free radicals [6]. In addition, the activity of curcumin is 10 times more effective than anti-oxidant activity of vitamin E, and also it has been shown that curcumin was decreased the levels formation of hepatic lipoperoxide in both types of injury as acute cases or chronic cases of CCl<sub>4</sub> injuries [6].

The administration of silymarin alone or in combination with curcumin to treated CCl<sub>4</sub> -induced liver dysfunction in rats was founded a significant reducing the elevation of ALT in all groups of rats in this study. Such elevation of ALT level was suggested that the toxicity of CCl<sub>4</sub> was possessed ability to reach liver and inducing significant detectable damage to it [7]. The elevation of ALT level was by releasing of this enzyme from the cytoplasm into blood circulation after plasma membrane rupture and cellular damage. Administration of silymarin, and curcumin are significantly reduced the activity of liver enzyme ALT in CCl<sub>4</sub> intoxicated rat.

The previous experimental studies [9,10] had shown that treatment with CCl<sub>4</sub> leads to an increase in serum levels of AST, ALT and ALP. The hepatotoxicity of CCl<sub>4</sub> was confirmed in our study by significant increases of serum levels of AST and ALT. Administration of CUR in rats with hepatic injury, induced by CCl<sub>4</sub> caused a recovery from injury, as evidenced by decrease in the activities of ALT and AST in serum within 3 weeks of treatment.

## Conclusion

The administration of Antioxidants Silymarin or Curcumin has a Hepato-protective effect against Liver Dysfunction induced by CCl<sub>4</sub>. The hepatic-protective effect of Curcumin and silymarin are relating to reduced liver enzymes as ALT, AST and ALP in hepatic dysfunction that produced by CCl<sub>4</sub> toxicity through an antioxidant effects. Moreover, further studies are needed to compare the

ability of Curcumin and silymarin to protect the structure and the function integrity of the liver.

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