Effect of Carvedilol, Silymarin and Combination of Both on Carbon Tetrachloride-Induced Hepatic Toxicity in Rats

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Abstract: Background: hepatotoxicity is injury to the liver that is associated with impaired liver function caused by exposure to a drug or other chemical agents, such as those used in laboratories, industries and natural chemicals. The most commonly used hepatotoxic model is carbon tetracholoride (CCL4) because it gives the same hepatic changes in animal as in human. Silymarin is an isoflavonoid in origin used in patients suffering from hepatotoxicity as it has different properties that make it hepatoprotective drug. It has antioxidant and anti-inflammatory properties. Carvedilol is one of beta blockers that are used to decrease portal hypertension in cirrhotic patients. Carvedilol has antifibrotic, anti-inflammatory and antioxidant properties. Methods: 50 male albino rats were divided into five groups: group one received normal saline, group two received CCL4, group three received carvedilol and CCL4, group four received Silymarin and CCL4 and group five treated with carvedilol, silymarin and CCL4. After 5 weeks rats were scarified and parameters were measured in serum (AST, ALT, ALP and total bilirubin) in tissue (GSH, MDA and total protein). Liver was used for histopathological examination and assay of the change in tissue parameters.Result: CCL4 treated group showed significant elevation in all liver enzymes, total bilirubin and tissue MDA and significant decrease in GSH, total protein, with significant loss of hepatic architecture. In Silymarin, carvedilol and combination groups there were decrease in liver enzymes, total bilirubin and tissue MDA and increase in GSH, total protein and improvement of necrosis and inflammation in hepatic tissues. Results were more significant in combination group than with Silymarin and with carvedilol respectively. Conclusion: Silymarin has antioxidant and anti-inflammatory properties that showed protection more significant than carvedilol, and the combination of both arvedilol and Silymarin showed more significant results.
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Key words: $CC1_4$, silymarin, carvedilol.

1. Introduction

Liver, which is the major organ responsible for biotransformation of drugs, toxic chemicals and byproducts endogenous to the body, is also the primary target for detoxification of many endogenous and exogenous toxic chemicals **(*Luster et al,2001*).**

CCL_4 is one of the most widely used hepatic toxins for experimental induction of liver fibrosis and cirrhosis following hepatocellular necrosis in laboratory animals. Besides hepatocellular regeneration and inflammatory infiltration, proliferation of hepatic stellate cells and deposition of connective tissue are major features of liver histopathology ***(Jiang et al., 1992).***

Silymarin is used for treatment of several hepatic disorders ***(Hakova and Misuruva, 1993)*** and is mainly indicated for acute and chronic hepatitis, liver cirrhosis, fatty degeneration and toxic metabolic liver disease ***(feher et al., 1987)***. silymarin has antioxidant activity ***(Valenzuela and Garrido ,1994).***

Carvedilol treatment alone significantly enhances the antioxidant enzyme activities and glutathione levels and inhibited lipid peroxidation as compared to the control values. These finding support the premise that carvedilol can guard against the sequences of oxidative stress. Hence, the antioxidant properties of carvedilol are involved in its hepatoprotective mechanism. The powerful antioxidant activity of carvedilol has been examined previously in different oxidative stress situations ***(El–Demerdash , 2006)***.

2. Material and Methods

Drugs:

1-**Carvedilol**: 25 mg tablets from MAP (multi-apex pharma; prepared as a suspension using distilled water to a final concentration of 3.6 mg/ml and given in adose of 10 mg/kg, p.o. /day ***(Hamdy N and El-Demerdash, 2012).***

2-**Silymarin**: (50 mg/5ml) from medical union pharmaceuticals (Abu-Sultan, Ismailia, Egypt). Rats received 50mg/kg, p.o. /day for 5 weeks. ***(*Pradeep K, 2007*).***

Chemical:

Carbon tetracholoride (CCL_4): Liquid from, Elfaroina Company 153.8 Mr.

Animals:

50 male wistar albino rats (150-200gm) were selected for this study. They were obtained from the animal house, of pharmacology department of Al Azhar University.

Methods:

a-Animal grouping and design of the work:

Animals in this study were randomly divided into five groups each contain ten rats:-

1-Group (1): control normal rats received normal saline (2.78ml/ kg, p.o. /day).

2-Group (2): (CCL_4 intoxicated model): rats received CCL_4 in a dose of 1ml/ kg, p.o., twice weekly for 4 weeks ***(Mortezaee K .et al 2015)***.CCL_4 is prepared under surface of corn oil in ratio 1: 1 V/V ***(Basu, 2003)***.

3-Group (3): rats received carvedilol (10 mg/kg/day =2.78 ml/ kg, p.o. /day) for 5 weeks ***(Massart P.et al.1999).***Carvedilol administration started one week before CCL_4 administration.

4-Group (4): rats received Silymarin (50mg/kg,p.o./day)for 5 weeks ***(Pradeep K . et al.2007).*** Silymarin administration started one week before CCL_4 administration.

5-Group (5):rats received carvedilol (10 mg/kg/day =2.78 ml/ kg, p.o. /day) for 5 weeks and Silymarin (50mg/kg,p.o./day)for 5 weeks. Both were administrated one week before CCL_4 administration.**b-Biochemical studies:**

Collection of blood samples:

Blood samples will be collected from the retro-orbital venous plexus of rat eye by using heparinized capillary tubes. The collected bloodwill be then centrifugedat 3000 round/minute for 30 minutes. Then the serum will be transferred into clean vials and stored at -18°C for biochemical parameters determinationand the abdomens of the rats will be dissected and the livers will be excised to measure the following parameters:

(A) Biochemical measurements:

1- Serum parameters:liver function tests will be done by measuring Serum alanine amino transferase (ALT), Serum aspartate amino- transferase (AST), Alkaline phosphatase (ALP), totalserum bilirubin.

2- Liver homogenate parameters: Total protein in liver, malondialdehyde in liver and reduced glutathione in liver.

(B) Histopathological study:

To study theprotective effect of the tested drugs on hepatotoxicity; Fixed liver specimens will be embedded in paraffin cubes. Sections of 5–6µm in thickness will be cut and stained with Hematoxylin& Eosin (H&E) and Masson Trichrome (MT) then subjected to photomicroscopic examination**.**

3. Results

The effects of carvedilol (10mg/kg, p.o. / day) and Silymarin (50 mg/kg, p.o. /day) and combination of both on the levels of liver enzymes (ALT, AST and ALP) and serum total bilirubin: table (1) and figures (1), (2), (3) and (4):

- **CCL_4 (group 2)** significantly increased serum levels of ALT, AST, ALP and total bilirubin by about (371%, 220%, 1460% and 1790%) respectively compared to control group.
- **Carvedilol (group 3)** administration showed significant decrease in serum levels of ALT, AST, ALP and total bilirubin by about (45.5%, 57.5%, 88.8% and 45%) respectively compared to CCL4.
- **Silymarin (group 4**) showed significant decrease in serum levels of ALT, AST, ALP and total bilirubin by about (49.5%, 58.5%, 90.3% and 56.5%) respectively compared to CCL4.
- **Carvediloland Silymarin(group 5)** combined treatment exhibited more significant decrease in serum levels of ALT, AST, ALP and total bilirubin by about (52%, 60.5%, 90.7% and 62.5%) respectively compared to CCL4.

Table 1: Effects of carvedilol (10mg/kg, p.o. / day) and Silymarin (50 mg/kg, p.o. /day) and combination of both on the levels of liver enzymes (ALT, AST and ALP) and serum total bilirubin:

Parameter / Treatment	ALT (U/ml)	AST (U/ml)	ALP (IU/L)	Serum total bilirubin (mg/ dl)
Saline [1]	23.3 ±7.55	32.9 ±1.63	87.5 ±11.26	0.4100 ±0.06
CCL_4[2]	109.8 [a,b] ±1.468	105.31 [a,c,d,e] ±8.289	1365.3 [a,c,d,e] ±194..58	7.75 [a,c,d,e] ±0.317
CCL_4+ Carvedilol [3]	59.7 [a,b] ±4.45	44.65 [b] ±3.014	152.4 [b]±11.53	4.26 [a,b,d,e] ±0.303
CCL_4+ Silymarin [4]	55.3 [a,b] ±2.43	43.62 [b] ±2.52	131.8 [b] ±7.47	3.38 [a,b,c] ±0.308
CCL_4+ carvedilol + Silymarin [5]	52.5 [a,b] ±2.27	41.7 [b] ±3.112	126[b] ±7.64	2.91[a,b,c] ±0.182

Data are presented as means ± SEM.

[1] Control animals received saline (2.78ml/kg/day orally).

[2]CCL_4 (1 ml/kg b.w., orally) was given twice weekly for 4 consecutive weeks.

[3]Carvedilol (10 mg/kg/day =2.78 ml kg /day; orally) for 5 weeks**,** started one week before CCL_4 administration.

[4] Silymarin (7.5mg/kg,p.o./day)for 5 weeks**,** started one week before CCL_4 administration.

[5]Carvedilol &**Silymarin**adapting the same regimen and schedule of treatment as previously mentioned. Rats received Carvedilol

&Silymarin continuously for 5 weeks. Both administrated one week before CCL_4 administration.
a: Significantly different from control .
b: Significantly different from CCL_4 treated group.
c: Significantly different from CCL4+carvedilol treated group.
d: Significantly different from CCL4+Silymarin treated group.
e: Significantly different from CCL4+carvedilol+Silymarin treated group.
- Multiple comparisons were accomplished using one way ANOVA followed by Tukey-Kramer as a post-hoc test ($P \leq 0.05$).

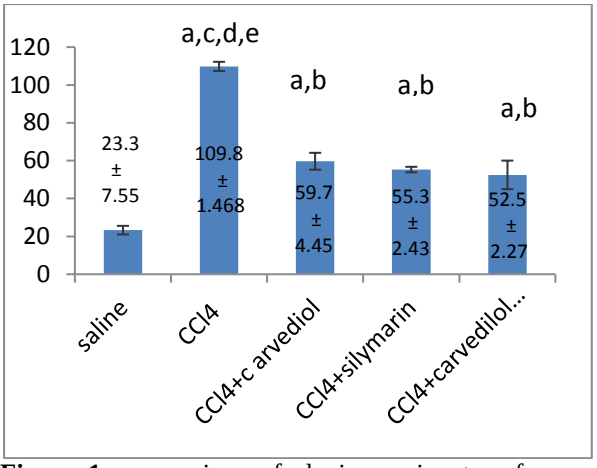

Figure 1: comparison of alanine amino transfrerase (ALT) (u/ml) in different studied groups.

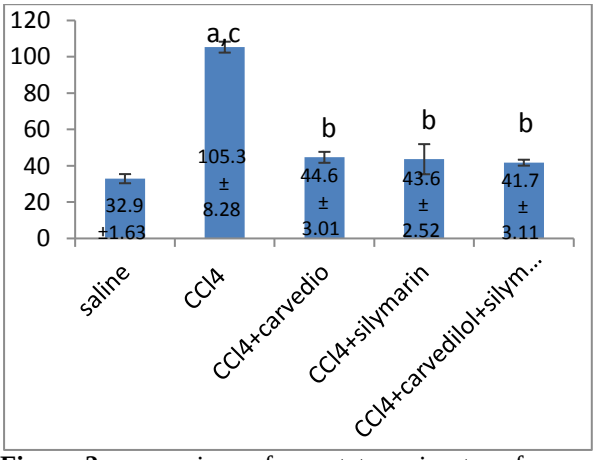

Figure 2: comparison of aspartate amino transfrerase (AST) (u/ml) in different studied groups.

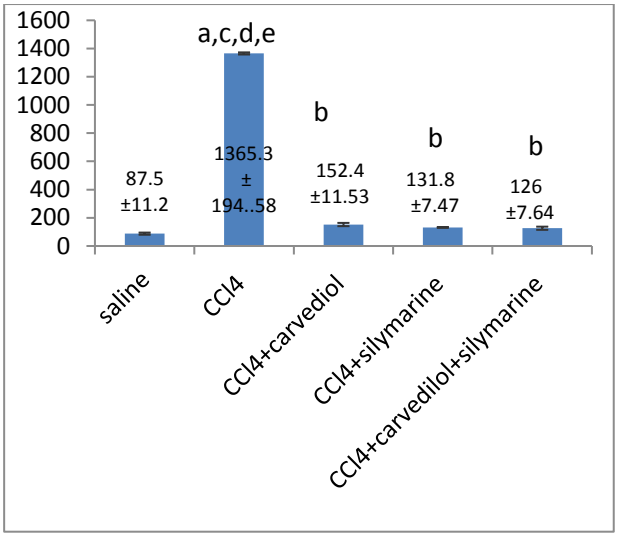

Figure 3: comparison of serum alkaline phosphatase (ALP) (IU/L) in different studied groups.

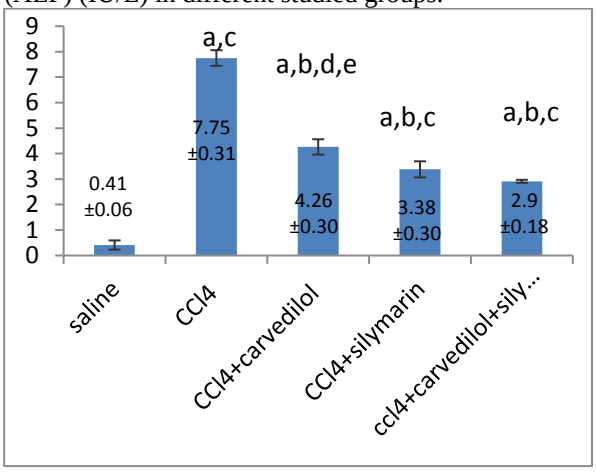

Figure 4: comparison of serum total bilirubin mg/dl in different studied groups.

The effects of carvedilol (10mg/kg, p.o. / day) and silymarin (50 mg/kg, p.o. /day) and combination of both on the levels of parameters detecting oxidative stress (tissue total protein, GSH and MDA): table (2) and fig (5),(6) and (7):

- **CCL_4 (group 2)** significantly decreased tissue total protein and tissue GSH by about (61% and 48.5%) respectively and increased tissue MDA by about 32.95% compared to control group.
- **Carvedilol (group 3)** administration showed significant increase in tissue total protein by about 83% and decrease in tissue MDA by about 33%, but non-significant increase in tissue GSH by about 12% compared to CCL4.

- **Silymarin (group 4**) showed significant increase in tissue total protein by about 94.5% and decrease in tissue MDA by about 44.5% and non-significant increase in tissue GSH by about 14.7% compared to CCL4.
- **Carvediloland silymarin (group 5)** combined treatment exhibited more significant increase in tissue total protein by about 79% and tissue GSH by about 16.5% and decrease in MDA by about 50.5% compared to CCL4.

Table 2: Effects of carvedilol (10mg/kg, p.o. / day) and Silymarin (50 mg/kg, p.o. /day) and combination of both on the levels of parameters detecting oxidative stress (tissue total protein, GSH and MDA):

Parameter / Treatment	Tissue total protein (gm/dl)	GSH (mmol/ gm tissue)	MDA (mmol/ gm tissue)
Control [1]	6.53 ±0.22	191.3 ±9.92	125 ±6.49
CCl_4 [2]	2.53 ^{a,c,d,e} ±0.31	98.6 ^{a,e} ±9.95	282.8^{a,c,d,e} ±10.94
CCl_4+ Carvedilol [3]	4.63^{a,b} ±0.33	110.2 ^{a} ±4.76	189.6^{a,b,e} ±21.56
CCl_4+ Silymarin [4]	4.92^{a,b} ±0.248	113.1 ^{a} ±3.08	157 ^{b} ±10.42
CCl_4+ carvedilol + Silymarin [5]	4.99 ^{a,b} ±0.27	114.8^{a,b} ±2.93	140^{b,c} ±9.31

Data are presented as means ± SEM.

[1] Control animals received saline (2.78ml/kg/day orally).
[2]CCL_4 (1 ml/kg b.w., orally) was given twice weekly for 4 consecutive weeks.
[3]Carvedilol (10 mg/kg/day =2.78 ml kg /day; orally) for 5 weeks**,** started one week before CCL_4 administration.
[4] Silymarin (7.5mg/kg,p.o./day)for 5 weeks**,** started one week before CCL_4 administration.
[5]Carvedilol &**Silymarin**adapting the same regimen and schedule of treatment as previously mentioned. Rats received Carvedilol & Silymarin continuously for 5 weeks. Both administrated one week before CCL_4 administration.

a: Significantly different from control .
b: Significantly different from CCL_4 treated group.
c: Significantly different from CCL4+carvedilol treated group.
d: Significantly different from CCL4+Silymarin treated group.
e: Significantly different from CCL4+carvedilol+Silymarin treated group.

- Multiple comparisons were accomplished using one way ANOVA followed by Tukey-Kramer as a post-hoc test ($P \leq 0.05$).

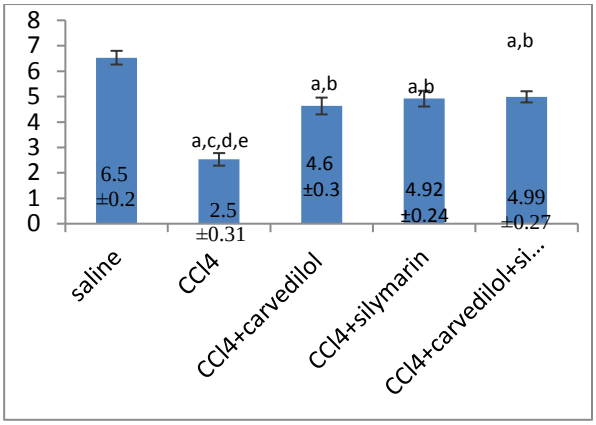

Table 5: comparison of tissue total protein (g/dl) in different studied groups.

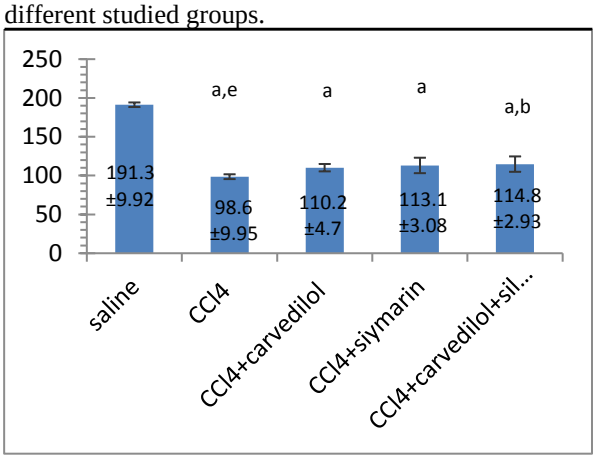

Figure 6: comparison of (GSH) in (mmol/gm tissue) different studied groups.

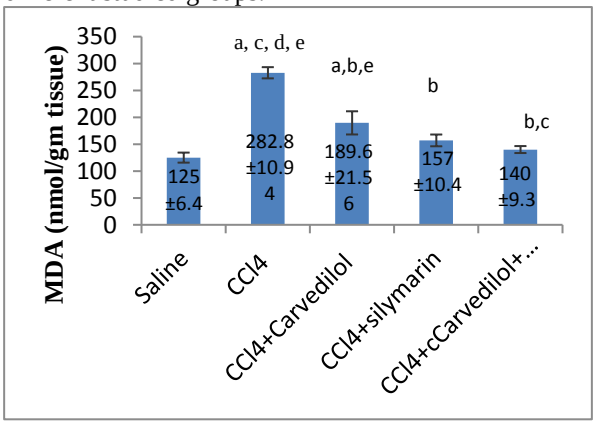

Figure 7: comparison of (MDA) in (nmol/gm tissue) different studied groups.

Histopathological findings:

In group 1(receivednormal saline)there was pathological study of section of this group confirmed the clinical serological parameters, showing normal hepatic architecture, normal hepatocytes, normal blood sinusoids and central vein (by H&E) and no excess fibrous tissue (by Mallory triochrome).

In group 2 (received CCL_4) showed diffuse loss of hepatic archticture, vaculated liver cells(ballooning), diffuse inflammatory cell infilteration, congested venulesand cholestasis (by H&E) and showedexcess fibrous tissue (by Mallory trichrome).

In group3 (received carvedilol and CCL_4) showing some pyknosis, little inflammatory cells and preserved hepatic architecture (H&E) andshowing no or mild fibrosis (by Mallory trichrome).

In group4 (received silymarin and CCL_4) showing normal liver architecture and congested hepatic central vein and normal hepatocytes (H&E) andno or mild fibrosis (by Mallory trichrome).

In group5 (received carvedilol, silymarin and CCL_4) showing normal hepatic architecture and hepatocytes and preserved hepatic architecture (H&E) and showing no excess fibrosis (by Mallory trichrome).

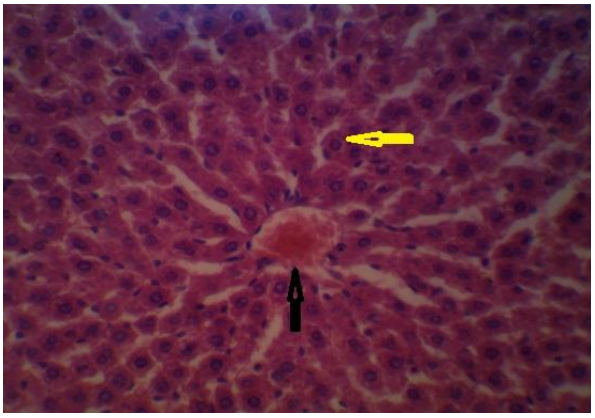

Figure 8:group 1(received normal saline) showing normal liver architecture and hepatocytes (H&E×400). Yellow arrow refers to normal hepatocyte and dark arrow refers to central vein.

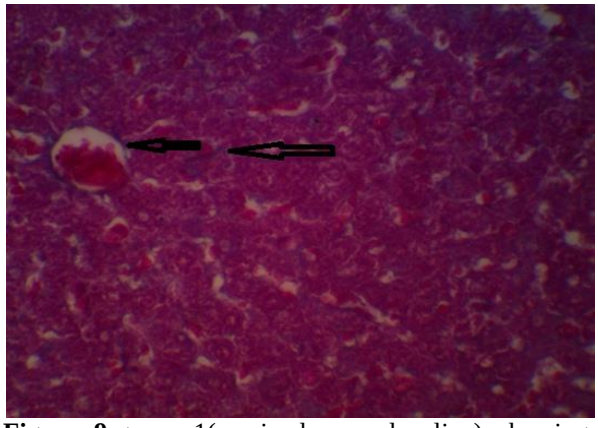

Figure 9:group 1(received normal saline) showing normal collagen fibers (Mallory trichrome x400). Black arrows are directed to fibrous tissue.

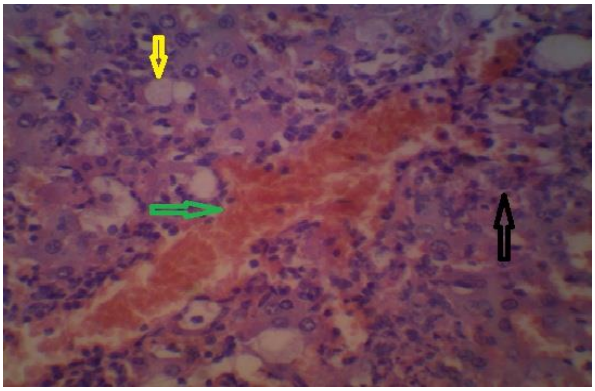

Figure 10: group2 (received CCL_4) showing loss of hepatic archticture, vaculated liver cells (ballooning) **(yellow arrows)**, diffuse inflammatory cell infilteration **(black arrows)** andcongested venules **(green arrows)** (H&E×400).

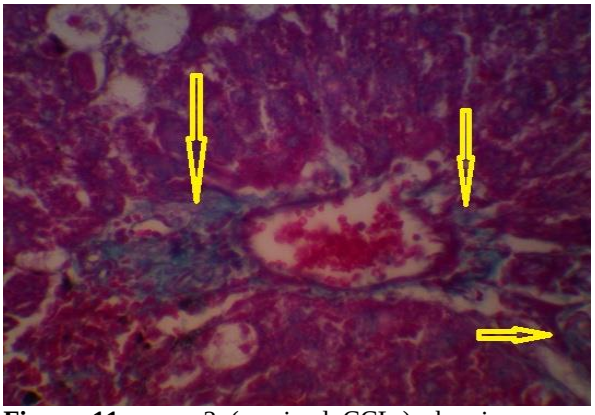

Figure 11: group2 (received CCL_4) showing excess fibrous tissue (yellow arrows(Mallory trichrome x400).

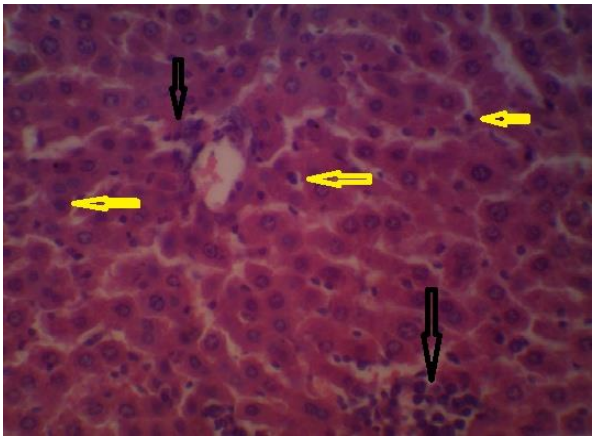

Figure 12: group3 (received carvedilol and CCL_4) showing some pyknosis (yellow arrows), little inflammatory cells (dark arrows) and preserved hepatic architecture (H&E×400).

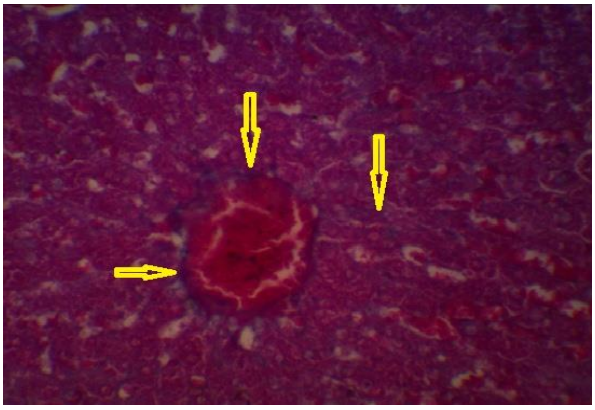

Figure 13: group3 (received carvedilol and CCL_4) showing no or mild fibrosis (yellow arrows (Mallory trichrome x400).

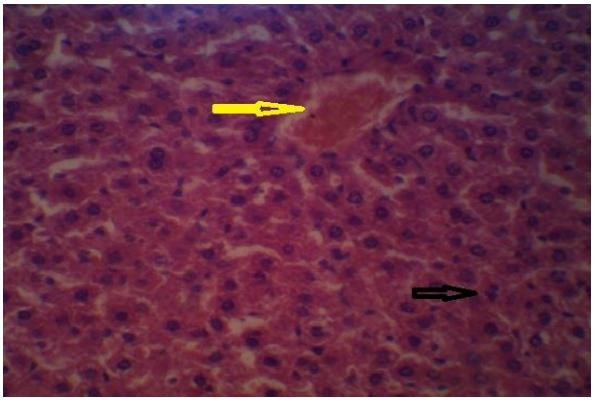

Figure 14: group4 (received Silymarin and CCL_4) showing normal liver architecture, congested hepatic central vein (yellow arrow) and little pyknotic cells (dark arrows) (H&E×400).

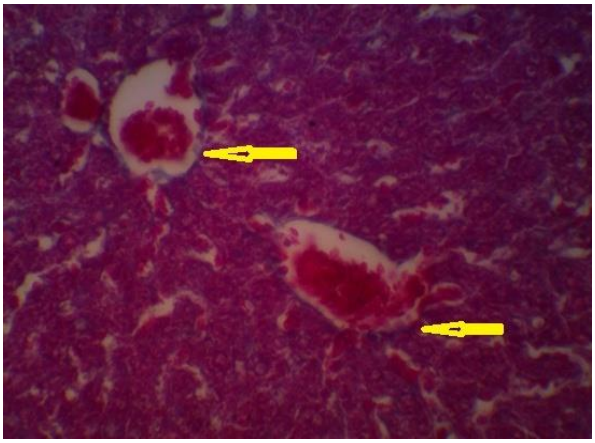

Figure 15: group4 (received Silymarin and CCL_4) showing no or mild fibrosis (yellow arrows) (Mallory trichrome x400).

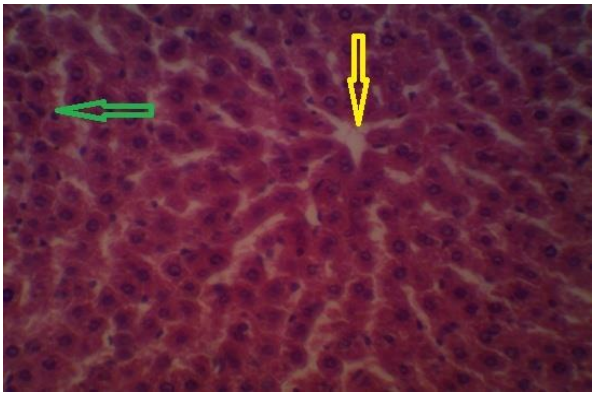

Figure 16: group5 (received carvedilol, Silymarin and CCL_4) showing normal hepatic architecture and hepatocytes, normal central vein (yellow arrow) and little pyknotic cells (green arrow) (H&E×400).

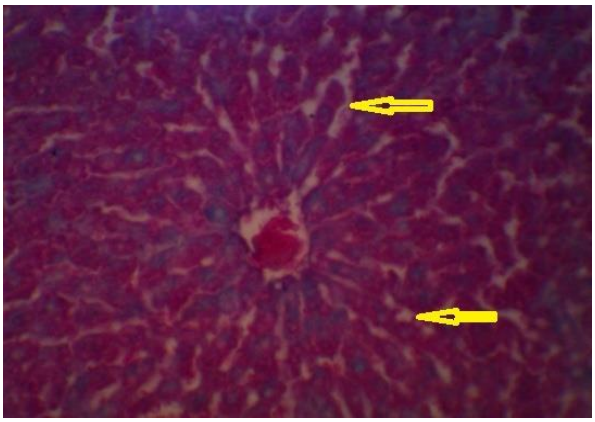

Figure 17: group5 (received carvedilol, Silymarin and CCL_4) showing no fibrosis (yellow arrows) (Mallory trichrome x400).

4. Discussion:

Liver has an important role in detoxification and is a primary target organ for many toxic chemicals and inflammatory processes that participate in a number of pathological (necrosis and fibrosis) conditions. Liver has protective and repair events following exposure to hepatotoxic chemicals and other inflammatory diseases which can affect the liver damage ***(Mora et al., 2010)***.

Carbon tetrachloride (CCl_4) is a hepatotoxin, causing liver necrosis, fibrosis and cirrhosis when administered. Lipid peroxidation occurred in carbon tetrachloride administered induced hepatotoxicity. Also covalent binding of the compound to cellular macromolecules may contribute to the damage. Kupffer cells may be involved in the hepatotoxicity of carbon tetrachloride, as a source of cytotoxic factors, such as active oxygen species leading to hepatocellular damage ***(Mora et al., 2010).***

Many natural and artificial agents possessing anti-oxidative properties have been proposed to prevent and treat hepatopathies induced by oxidative stress. There

is increasing evidence for the hepatoprotective role of flavonoids, from some herbs as (Silymarin) possess a wide range of anti-oxidant properties in vitro, such as inhibition of lipid peroxidation, Flavonoids inhibit the cytochrome P450 enzymes and are also known to reduce the hepatotoxicity of carbon tetrachloride ***(Kumarappan et al.,2010)***.

Carvedilol is beta blocker drug, possess both ROS-scavenging and ROS-suppressive effects and its use is associated with reduction in oxidative stress that is cardinal in the pathogenesis of hepatotoxicity. The anti-oxidant and anti-fibrotic effects of carvedilol were used to protect against carbon tetracholoride induced hepatotoxicity ***(Hamdy and El-demerdash, 2012).***

In the present study, control group received normal saline for five weeks, showed no changes in parameters levels. In histopathological examination there was no change in normal liver pattern, no inflammation, any necrosis or fatty changes.

Carbon tetrachloride (CCL_4) is a common model used to induce hepatotoxicity used in the experimental study of liver diseases ***(Shenoy et al., 2001).*** This intoxication results in the stimulation of lipid peroxidation and the production of free radicals ***(Basu, 2003)*** which causes necrosis of hepatocytes, induces inflammation, and promotes the progression of hepatic fibrogenesis***(Fu et al., 2008).***

In the present study, CCL_4 significantly elevated levels of plasma ALT, AST, ALP and bilirubin. Decrease in the total tissue protein content was recorded following CCL_4 treatment. Increase in plasma level of ALP in CCL_4 treated rats could be due to its increased synthesis in presence of elevated biliary pressure and subsequent increase in bilirubin, and these results agree with that obtained by ***Moreiraet al., (2014)*** who tested the protective effect of bixin on carbon tetrachloride-induced hepatotoxicity in rats and CCL_4showed marked elevation in ALT, AST and reduced GSH significantly.

Liver sections of CCL_4 treated rats were characterized by significant intracellular lipid accumulation, ballooning of hepatocytes, infiltration with inflammatory cells and hepatocyte necrosis. These histopathological changes agree with previous reports on CCL_4 induced hepatotoxicity ***(Moreiraet al., 2014).***

There is decrease in tissue reduced glutathione in CCL_4 treated rats and increase in tissue malondialdehyde. The impairment in the liver function markers was coincided with a significant increase in the liver lipid peroxidation products, as malondialdehyde (MDA) and a decrease in their reduced glutathione (GSH) and these results agree with ***El-Maddawy and Gad (2012),*** whom studied the hepato-renal protection of Silymarin against CCL_4 in comparison with vitamin E in rats. Their results showed that CCL_4 significantly increased serum ALT, AST, ALP and tissue MDA and significantly decreased tissue GSH.

The serum levels of (ALT and AST) reflect the physiological state of the liver. They are changed according to the distorsion of liver, resulting from cellular injury of the organ caused by toxic metabolites and diseases ***(Patrick-Iwunanyanwe et al., 2007).***

Results of the present study indicated that CCL_4 caused an increase in serum levels of the diagnostic enzymes (ALT and AST) in rats that received CCL_4 as compared to the control group. Such elevation suggests that toxication was able to reach the liver and induce a detectable damage, as previously reported by ***Hukkeri et al., (2002)***who proved the elevation in the plasma level of cytoplasmic and mitochondrial enzymes due to liver injury induced by CCL_4. This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage ***(Shaarawy et al., 2009).***

Carvedilolis a nonselective beta-blocker with potent antioxidant and free radical scavenging properties that is used in the treatment of portal hypertension.

In the present study, treatment of rats with carvedilol started one week before CCl_4 administration. It was found that carvedilol significantly counteracted the hepatotoxic effect of CCL_4 as indicated by significant decrease of serum levels of (AST, ALT, ALP and total bilirubin) compared to CCL_4intoxicated group and these results agree with that obtained by **Araújo Júnior RFd., et al 2016,** who found that carvedilol treatment (5 mg/kg) during the alcohol exposure protocol was associated with reduced AST, ALT, MDA, and GSH. They explained the hepato-protective effect of carvedilol by that, carvedilol can reduce the oxidative stress, inflammatory response and fibrosis in ethanol-induced liver injury in a rat model by down-regulating signaling of Kuppfer cells and hepatic stellate cells (HSCs) through suppression of inflammatory cytokines.

These results agree also with that obtained by***Hamdy and El-demerdash, (2012)*** who co-treated rats with carvedilol(10mg/kg, orally) daily for 6weeksafter CCL_4 induction of chronic hepatotoxicity fortwo weeks to toAntifibrotic effects of carvedilol in chronic carbon tetrachloride-induced liver damageand found that treatment of animals with carvedilol significantly counteracted the changes in liver function and histopathological lesions induced by CCL_4. Also, carvedilol significantly counteracted lipid peroxidation, GSH depletion, and reduction in antioxidant enzyme activities; glutathione-S-transferase and catalase that was induced by CCL_4. In addition, carvedilol ameliorated the inflammation induced by CCL_4as

indicated by reducing the serum level of acute phase protein marker.

Also these results agree with **Anuradha, and Krishnamoorthy,2012,** who used carvedilol(5 mg/kg b.wt/ day) to detetectif carvedilol can ameliorate the hepatotoxicity induced by lead acetate and found that carvedilol decreased AST, ALT, ALP, total bilirubin and increased total protein.

These results disagree with**Ibrahim., et al 2010,** whom studied themodulating effect of carvedilol on doxorubicin-Induced cardiomyopathy and hepatic damage using carvedilol 1mg/kg 7 times over a period of 4 weeks including a dose before doxorubicin 1st dose and found that serum ALT was significantly increased and histopathological findings showed more liver damage than control group and doxorubicin group.

There are many differences between this study and our study including the use of doxorubicin instead of CCL_4, carvedilol dose was 1mg/ kg 7 times over aperiod of 4 weeks instead of 10 mg/ kg every day for 5 weeks in our study, also they gave a single dose of carvedilol before doxorubicin and we gave seven doses of carvedilol before CCL_4 and their research was mainly to study the cardioprotective effect of carvedilol against doxorubicin.

Reactive oxygen species (ROS) are implicated in the pathogenesis of most liver diseases, including ischemia/reperfusion injury, endotoxemia, chronic hepatitis C, alcoholic and non-alcoholic fatty liver disease and cholestasis ***(Rost et al.,2007).***

In the present study, carvedilol co-treatment with CCL_4treated group non-significantly counteracted the GSH depletion and significantly counteracted increase MDA level induced by CCL_4that agree with ***Hamdy and El-demerdash, (2012).*** And there is also significant increase in total protein study which agrees with that obtained by ***Anuradha and KrishnamoorthyP, (2012).***

The powerful antioxidant activity of carvedilol has been examined previously in different oxidative stress situations ***(EI-Demeerdash, 2006; Arozal et al., 2010).***

Carvedilol therapeutic actions could not be fully explained by adrenoreceptor blockade. Numerous studies have provided evidence that carvedilol has various other properties including antioxidant action, calcium channel antagonism, anti-inflammatory actions ***(Romeo et al., 2000; Kalinowski et al., 2003; Bellenger et al., 2004; Kostka and Tykarskia, 2009).***

Histopathological study of this group confirmed these results, showing some pyknosis, little inflammatory cells and preserved hepatic architecture (H&E) andshowing no or mild fibrosis (by Mallory trichrome).

Silymarinoffers good protection in various toxic models of experimental liver diseases in laboratory animals. It acts as an antioxidative, antilipid peroxidation ***(Hubert et al, 2011)***, antifibrotic, anti-inflammatory, membrane stabilizing and immunomodulatory***(Pradhan and Girish, 2006).***

In the present study Silymarin treatment, started one week before CCL_4 administration showed marked protective properties. There is decrease in (AST, ALT and ALP) also decrease in total bilirubin with CCL_4 treated group than CCL_4 treated rats alone and these results agree with ***Freitag et al., (2015)*** who studied the ameliorationof carbon tetrachloride induced hepatotoxicity in rat by tanarize feronia limonia, Silymarin showed decrease in serum levels of AST, ALT and ALP and had many antioxidant properities. This hepatoprotective effect of Silymarin is due to membrane stabilizing action, free radicals scavenging properties, inhibition of lipid peroxidation and modulation of hepatocyte Ca++ ***(Flora et al., 1998; Farghali et al., 2000).***

In the present work, Administration of Silymarin significantly reduced the activity of liver enzymes in CCL_4 induced rats, a finding which agree with those shown before by ***Pradeep et al., (2007)***and are almost definitely suggestive of protection of the structural integrity of the hepatocytes membrane or regeneration of damaged liver cells by test samples ***(Patrick-Iwuanyanwu et al.,2007).***

Administration of Silymarin to CCL_4 treated rats was significantly able to reduce the activities to liver enzymes, liver MDA levels and to increase their GSH levels non-significantly and total protein significantly and these results agree with that obtained by ***Elmaddawy and Gad, (2012),*** whom studied the hepato-renal protection of Silymarin against CCL_4 in comparison with vitamin E in rats. They used Silymarine 10mg/ 100 g b.w. p.oand found asignificant decrease in serum ALT, AST, ALP activities and liver GSH and significant decrease in liver MDA in Silymarin treated group.

The elevated level of GSH in liver with Silymarin protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species and/or neutralizes reactive intermediate species generated from exposure to CCL_4 ***(Gupta and Singh, 2007).***

In comparison between rats treated with Silymarin and CCL_4 and rats co-treated with carvedilol and CCL_4, both groups gave good results in protection of liver. There were non-significant changes in measuring (ALT, AST, ALP, tissue reduced glutathione, tissue MDA and total protein) but there was significant increase in serum total protein in Silymarin group than carvedilol group.

In the present study, Silymarin showed more significantresults in decreasing (serum AST, ALT, ALP, total bilirubinand tissue MDA) and increasing tissue GSH and total protein in comparison with carvedilol group as Silymarin is a standard drug that showed the prominent protection of liver and these results agree with ***Ghosh et al., (2016)***who studied the protective effect Silymarin on the kidney and the liver againist thioacetamide induced toxicity and showed significant decrease in srum level of ALT, AST and ALP and elevated GSH level.

Histopathological findings of this group confirmed these results, showing normal liver architecture, congested hepatic central vein, normal hepatocytes (H&E) andno or mild fibrosis (by Mallory trichrome).

In the present study, treatment with Silymarin and carvedilol for 5 weeks started one week before CCL_4 administration significantly counteracted the hepatotoxic effect of CCL_4. There is decrease in (AST, ALT and ALP), also decrease in total bilirubin when compared with CCL_4 treated group, carvedilol treated group alone and Silymarin treated group alone.

Administration of carvedilol and Silymarin to CCL_4 treated group was significantly able to reduce the activities of liver enzymes, liver MDA levels and increased significantly GSH levels and total protein more than carvidelol treated group alone and Silymarin treated group alone.

Histopathological study of this group confirmed these results, showed normal hepatic architecture and hepatocytes and preserved hepatic architecture (H&E) and showed no excess fibrosis (by Mallory trichrome).

So, as a conclusion, Silymarin which is herbal in origin, showed more significant results than carvedilol but these results also indicated that carvedilol had good role in hepatoprotection that antagonized CCL_4 induced hepatotoxicity. The treatment using Silymarin and carvedilol gave more significant results than the treatment using only one of them.

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