The Ameliorative Effect of L-Carnitine on Experimentally Induced Liver Cirrhosis in Male Albino Rats

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Abstract: The present study was undertaken to explore the possible protective effect of L-carnitine if any against experimentally -induced liver cirrhosis in mature male rats using CCl_4. Fourty mature male rats were divided randomly into 4 equal groups each of ten. The first group (normal control group) was injected with olive oil (1.5 ml/kg b.wt.). The second group (CCl_4 cirrhotic group) were rendered cirrhotic by injecting CCl_4 (diluted 1: 7 in olive oil 1.5 ml /kg .b.wt.). While the third group were given L-carnitine (100 mg/kg.b.wt.) plus CCl_4. Whereas, the last group were given silymarin in a dose of 25 mg/kg b.wt. together with CCl_4 and used as standard. All treatments were given 3 times a week for 7 successive weeks. After the end of the study, all rats in all groups were sacrificed and their blood were collected in centrifuge tubes for preparation of serum which was kept at -20 ^{o}C until used for estimating various parameters. Liver function parameters (AST, ALT, ALP, total proteins, Albumin, globulins, total bilirubin, direct and indirect bilirubin). Kidney function parameters as serum creatinine, uric acid and urea. Lipogram (Triglycerides, total cholesterol, HDL-c and LDL-c). The obtained results revealed that cirrhotic non-treated rats showed a significant increase in serum activities of AST, ALT and ALP and total bilirubin as well as a significant decrease in serum total protein, albumin and globulins. On kidney function parameters CCl_4 afforded a significant increase in serum levels of creatinine, uric acid and urea. On lipogram, CCl_4 elicited a significant elevation in serum triglycerides. LDL-c and a significant decrease in serum total cholesterol and HDL-c. When compared with normal control group. L-carnitine when given to cirrhotic rats induced a non-significant decrease in serum AST, ALT , ALP and a significant increase in serum total proteins and globulins together with a slight decrease in serum total and direct bilirubin. On kidney function parameters L-carnitine induced a significant decrease in serum creatinine uric acid and urea compared with CCl_4 treated group and non-significant change, when compared with normal control group whereas, on serum lipogram L-carnitine afforded a significant decrease in HDL-c and LDL-c when compared with control and CCl_4 treated group respectively as well as a significant increase in serum triglycerides compared with CCl_4 treated group. On the other hand, the Co. administration of silymarin with CCl_4 elicited a significant decrease in serum AST, ALT, ALP and total bilirubin as well as a significant increase in T.P, Albumin and globulins compared with CCl_4 non treated group. On kidney function parameter, treatment of cirrhotic group with silymarin afforded a significant decrease in serum creatinine, uric acid and urea compared with CCl_4 non treated group. Whereas, on lipogram, silymarin induced a significant increase in serum HDL-c compared with CCl_4 non-treated group. From all previous results, it was clear that L-carnitine possesses a hepatoprotective activity against experimentally –induced liver cirrhosis by carbon tetrachloride.
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Key words: L-carnitine, carbon tetrachloride, silymarin and liver cirrhosis

Abbreviation: AST, ALT, ALP, HDL-c, LDL-c and TP.

1. Introduction:

Liver is a major parenchymal organ involved in many functional activities in the body, e.g metabolizing, detoxifying and regenerating functions ***(Mitra et al., 2001).***

Several functions are performed by the liver. It synthesizes essential circulating proteins, including albumin and selected clotting factors and is a major processor of circulating proteins such as lipoproteins. It is responsible for detoxification of many endogenous and exogenous substances, including bilirubin and xenobiotics. The liver may also be critical in modulating the immune system through its resident macrophyages and lymphocytes ***(Liu and Crawford, 2004).***

Carnitine is a cofactor for transformation of free long-chain fatty acids into acylcarnitine and for their subsequent transport into the mitochondrial matrix, where they undergo beta-oxidation for cellular energy production ***(Gregory and Kelley, 1998).*** They reported also that mitochondrial fatty acid oxidation is the primary fuel source in heart and skeletal muscle, pointing to the relative importance of this nutrient for proper function in these tissues.

Carnitine and its acetylated derivatives facilitate the β-oxidation and improve energy metabolism, minimize the toxic effects of free forms of long-chain fatty acids in and around mitochondrial membranes and prevent permeability transitions, there suppresses the release of free electrons that generate free radicals ***(Zhu, et al., 2008 and Mannelli et al., 2009).***

L-carnitine inhibits both the mitochondrial damage induced by oxidative stress and mitochondria-dependent apoptosis in various types of cell ***(Al-Majed 2007).*** Recent studies suggest that L-carnitine may play an important role in oxidative/antioxidative balance and has an antiperoxidative effect on several tissues ***(Bayraktar, et al., 2008 and Cayir et al., 2009, Pehlivan et al., 2009).***

This work aimed to assess the possible hepatoprotective effect of L-carnitine against acute hepatotoxicity induced by CCl_4 with special reference to their ameliorative effect on oxidative stress on liver tissues.

2. Material and Methods

L-Carnitine:

Is available as 250 mg capsule (Mepaco. Pharm. Co.), Egypt.

Its recommended dose for rat is 100 mg/kg b.wt., ***Yildirim et al. (2013).***

Silymarin powder was obtained from Madousag co., Germany. It was given orally using metallic stomach tube as oral gavage in a dose of 25 mg/kg b.wt. ***(Bhandari et al., 2003)***.

Experimental forty mature male rats weighting 110 -150 gm were obtained from laboratory animal research Unit, Faculty of Vet. Med. Zagazig University.They were divided into four equal groups each of 10 as follows:

1st group: Was given olive oil (1.5 ml/kg) given i.p 3 times a week for seven weeks and kept as normal control group.

2nd group: (cirrhotic group) given 1.5 ml/kg carbon tetrachloride (CCl_4) diluted in olive oil (1:7) injected i.p 3 times a week for seven weeks. ***(Özbek et al., 2006).***

3rd group: given L-carnitine diluted in distilled water given orally in a dose of 100 mg/kg +CCl_4 1.5 ml/kg: olive oil (1:7) 3 times a week for 7 weeks. ***(Yildirim et al., 2013).***

4th group: Given Silymarin (25 mg/kg.) diluted in distilled water orally +1.5 ml/kg CCl_4: olive oil (1:7) 3 times a week for seven weeks ***(Bhandari et al., 2003)***

All animals were observed daily and dead animals were subjected to post-mortem examination to determine the cause of death.

At the end of the experiment, all rats were overnight fasted and were sacrificed and blood samples were collected and allowed to clot and serum was separated by centrifugation at 3000 rpm for 10 minutes for determination of various biochemical parameters as follows.

Liver function parameters:

Aspartate and Alanine amino-transferase activity (AST) and ALT (***Tietz 1976),*** Alkaline phosphatase (ALP) ***(Belfield and Glodberg, 1971),*** Total protein ***Henry, (1964),*** serum albumin ***(Doumas and Biggs, 1976),*** serum globulin was calculated as difference between total proteins and albumin, serum total, direct and indirect bilirubin, ***Walters and Gerarde (1970).***

Kidney function parameters:

Serum creatinine, ***(Husdan & Rapoport, 1968),*** uric acid, ***Coalombe and Faurean, (1963)*** and Urea, ***(Talke & Schubert, 1965).***

Lipogram parameters:

Total cholesterol, ***(Allain, 1974)***, HDL-c ***(Burstein and Scholnick, 1973),*** LDL-c ***(Friedewald et al., 1972)*** and triglyceride by ***(Sugiura (1977).***

All parameters were assayed calorimetrically using readymade kits supplied by bioMerieux, France.

Data analysis:

Data were analyzed using computers Spss program version 21 ***(SPSS, 2001).*** The biochemical estimations results were reported as Mean±S.E.M (Standard error of mean). The statistical method was one way Anova test, LSD test (least significant difference). Significance probability levels of less than 0.05 were considered significant.

3. Results and Discussion

Liver diseases are considered to be a serious health problem, as the liver is an important organ for the detoxification and deposition of endogenous and exogenous substances. Steroids, vaccines and antiviral agents, which have been employed as therapies for liver diseases, have potential adverse effects, especially when administered for long terms. Therefore, herbal, natural products and traditional medicines with improved effectiveness and safety profiles are needed as a substitute for chemical therapeutics. It has been reported that a number of herbal and natural products have been shown to protect against liver injury and many possess one or a combination of antioxidant, anti-fibrotic, immune

modulatory and antiviral activities ***(Seeff et al., 2001; Lee and Jeong 2002 and Shin et al., 2006).***

Conventional or synthetic drugs used for liver diseases are sometimes inadequate and can have serious adverse effects. Therefore, there is a worldwide trend to go back to traditional medicinal plants and natural products. Many natural products of herbal origin are now in use for the treatment of liver ailments ***(Latha et al., 1999).***

Hepatic cirrhosis is a common disease that possess a serious threat to public health ***(Yao et al., 2005).*** There is no doubt that the Reactive Oxygen Species (ROS) play an important role in the pathological changes in the liver ***(Poli and Parola, 1997).*** Several endogenous protective mechanisms had been evolved to limit ROS and the damage caused by them ***(Sies, 1993).*** However, since this protection might not be completed, or when the formation of ROS is excessive, additional protective mechanisms of dietary antioxidant may be of a great importance ***(Ulicne et al., 2003).*** Therefore, many natural antioxidants agents had been proposed to prevent and treat hepatopathies induced by oxidative stress ***(Lieber, 1997 and Cervinkova and Drahora, 1998).***

Carbon tetrachloride induced free radical processes may cause an early inactivation of Ca^{2+} sequestering capacity of endoplasmic reticulum ***(Moore et al., 1996),*** CCl_4 treatment of hepatocytes caused a sustained elevation of intracellular Ca^{2+} by affecting Ca^{2+} regulation at the level of the endoplasmic reticulum, plasma membrane, and mitochondria. Such sustained elevation of intracellular Ca^{2+} had been associated with mitochondrial dysfunction, endonuclease activation, protease activation and phospholipase activation all of which may lead to irreversible cell injury ***(Orrenius et al., 1989).***

In the present study the possible hepatoprotective effect of L-carnitine has been evaluated against carbon tetrachloride induced hepatotoxicity and has been compared with silymarin (a standard hepatoprotective drug).

Our study showed that the prophylactic treatment with L-carnitine (100 mg/kg orally), 3 times a week for 7 weeks in combination with carbon tetrachloride in olive oil (1:7, 1.5 ml/kg I/P) offered considerable protection to liver as evidenced from the levels of biochemical parameters (ALT, AST, ALP, Total proteins, albumin, globulins, and bilirubin in the serum). (Table 1) and (Figs. 1, 2 and 3). These results were supported by the hepatic histopathological changes. Moreover, the effect of L-carnitine on kidney function parameters as well as on lipogram were also studies in this work (Tables 2 & 3) and (Figs. 4 and 5).

CCl_4–induced hepatotoxicity in rats represented an adequate experimental model of cirrhosis in man and is used for the screening of hepatoprotective drugs ***(Al-Shabanah et al., 2000).*** Cytochrome P_{450} enzymes which are responsible for biotransformation of many drugs are suicidally inhibited by the reactive metabolites of CCl_4 ***(Athar et al., 1997).*** Trichloromethyl radical (CCl_3*) initially formed reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCl_{3OO}*) which is the probable initiator of lipid peroxidation ***(Bhat and Madyastha, 2000).***

Effect on liver enzymes (ALT, AST and ALP):

Hepatic cells participate in a variety of metabolic activities and contain a group of enzymes which have been used as markers for monitoring chemically liver damage. The enzymes alanine amino-transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are important enzymes that are often employed in assessing liver injury ***(Hukkeri et al., 2002). Sturgill and Lambert, (1997)*** reported also that serum activities of ALT and AST are the most commonly used biochemical markers of liver injuries.

In this study, chronic administration of CCl_4 (1.5 ml/kg I/P) 3 times a week for 7 weeks developed a significant hepatic damage which was observed from the substantial increase in the activities of serum ALT, AST and ALP. This is indicative of cellular leakage and loss of functional integrity of cell membrane of hepatocytes ***(Mukherjee, 2003).***

Our results were supported by ***Rajesh and Latha, (2004)*** who found that rats administered CCl_4 at a dose of 0.1 ml in ground nut oil (1:1, v:v) per 100 gm body weight through an intra-gastric tube twice a week for 2 months showed a significant liver damage evidenced by elevated serum activities of AST, ALT and ALP. Our results were supported by the results of ***(Achliya et al., 2004),*** they recorded that rats received suspension of CCl_4 in liquid paraffin (1:2, v:v, 1 ml/kg, S/C) for 8 days resulted in elevated ALT, AST and ALP levels.

Our results go hand in hand with those reported also ***Shanmugasundaram and Venkatoramon (2006).*** The authors tested the hepatotoxic effect of CCl_4 in olive oil (1:1, 2 ml/kg, S.C) for 2 days and noted that the activities of ALT, AST and ALP were significantly increased.

Similarly, ***Nevin and Vijayammal (2005)*** observed that there was a significant elevation in the activities of serum ALT, AST and ALP after CCl_4 administration.

The elevated AST, ALT and ALP activities obtained in this study due to injection of CCl_4 3 times a week for 7 weeks coincides with the explanation of

Rakhmanin et al., (1997) who observed that experimental hepatitis induced by CCl_4 in rats resulted in intoxicating action which was mediated via the activation of free radical oxidation of lipids with accompanying destabilization of biological membranes. Leading to leakage of these enzymes due to loss of functional integrity of cell membrane of hypatocytes ***(Mukherjee, 2003).***

Our findings were supported by the pathological changes evidenced in our study by extensive damage represented by cell death or steatosis or replacement with abundant fibrous tissue the latter consisted of mature collagen fibers which are deposited mainly in the interlobular tissue and invade hepatic lobules resulting in shrinkage and disappearance of the majority of the hepatic cells.

Kupffer cells hypertrophy and sometimes ladens with hemosiderin were seen. Sometimes, fibroblastic proliferation around individualized hepatic cells was encountered. The portal areas usually distorted by fibrosis and presence of numerous bile ductules with cholestasis. Telangectasis or hemorrhages were common.

These findings were reinforced by that reported by ***Atta et al. (2006),*** they showed severe degeneration and necrobiotic changes (centrolobular) in the hepatocytes in livers of rats intoxicated by CCl_4.

Özbek et al. (2004) found in CCl_4 –treated livers 1.5 ml/kg b.wt. i.p CCl_4 in olive oil (1: 7), drastic alterations represented by extensive ballooning degeneration. Ballooned hepatocytes were of different sizes and much larger than normal hepatocytes. Apoptotic bodies, cirrhotic nodules and hymphocytic infiltration in portal areas were frequently present. Profound steatosis, centrilobular necrosis, ballooning degeneration, nodule formation and fibrosis of the liver were also observed by ***Venukumar and Latha (2002).*** Similar changes were seen also by ***Yang et al. (2008),*** they reported that liver tissue in rats treated with CCl_4 revealed extensive liver injuries characterized by moderate to severe hepatocellular degeneration and necrosis around the central vein. Fatty changes, inflammatory cell infiltration, congestion, and sinusoidal dilatation when compared with normal liver tissues of the control.

Concerning the effect of L-carnitine on serum AST, ALT and ALP activities in CCl_4 treated rats, the obtained results revealed that L-carnitine afforded non-significant increase and significant decrease in serum activities of ALT and ALP when compared with normal and CCl_4 treated group respectively. Whereas, AST showed a significant increase and decrease when compared with control and CCl_4 treated group respectively. These effects appear to be conceivable with the fact that L-carnitine possesses protective effect against oxidative stress, unlike CCl_4 which has a strong oxidative effect as previously explained. ***Yildirim et al. (2013)*** reported that treatment of hyperthyroid rats with both low dose 100 mg/kg) and high dose (500 mg/kg) L-carnitine for 10 days resulted in a marked increase in the activities of the antioxidant enzymes in the liver tissue (CAT, GPX and Myeloperoxidase) which were significantly lowered in hyperthyroid rats, indicating that the low-dose L-carnitine application was sufficient to prevent L-thyroxine –induced oxidative stress in rat livers.

Moreover, ***Zhu et al. (2008) and Mannelli et al. (2009)*** mentioned that L-carnitine go far beyond its role in the transport of fatty acids L-carnitine and its acetylated derivatives facilitate the β-oxidation and improve energy metabolism, minimize the toxic effects of free forms of long –chain fatty acids in and around mitochondrial membranes and prevent permeability transitions, there, suppresses the release of free electrons that generate free radicals which is well known to induce liver injury .

The discrepancy in the effect on serum AST is resorted to the fact that AST is found in many other organs besides the liver, including the kidneys, the muscles and the heart brain and pancreas having a high level of AST indicating that AST does not always indicate that there is a liver problem ***(Pratt and Kaplan, 2000).***

Effect on serum total proteins, albumin and globulins:

The liver synthesizes not only the protein it needs but also produces numerous export proteins. Among the later, serum albumin is the most important one. Export proteins are synthesized on polyribosomes bound to the rough endoplasmic reticulum of the hepatocyte, in contrast, proteins destined for intracellular use are synthesized on free rather than bound polyribosomes **(*Podolsky and Isselbacher, 1991).*** Immunoglobulin is synthesized by immunocytes and hyperglobulinemia is found in hepatocellular disorders, appearing as an inflammatory reaction of liver ***(Vandenberghe, 1996).***

In this study, administration of CCl_4 in olive oil (1:7, 1.5 ml/kg, I/P) for 7 weeks resulted in a decrease of serum protein, albumin and globulins. This revealed the decline in the liver synthetic function caused by CCl_4-induced fibrosis. This decline is due to the disruption and disassociation of polyribosomes from endoplasmic reticulum following CCl_4 administration ***(Clawson, 1989).***

Our results were supported by ***Rajesh and Latha (2004).*** The authors observed that the liver damage due to chronic administration of CCl_4 (0.1 ml/100 gm body weight) orally resulted in depression of total protein. Furthermore, ***Shih et al. (2005)*** found that CCl_4-induced hepatic fibrosis in rats (0.5 ml /rat

orally) twice a week for 8 weeks appeared to cause a decrease in the contents of serum total proteins and albumin.

Our results go hand by hand with those obtained by ***Nevin and Vijayammal (2005).*** Who found that the decrease in serum total protein and A/G ratio was an evidence of the CCl_4-induced liver damage.

A similar significant decrease, in serum total protein was noted by ***Achliya et al. (2004) and Shanmugasundaram and Venkataraman (2006).*** The authors attributed these results to the depression of liver ability to synthesize the protein due to CCl_4-induced hepatic damage.

Similar observations were also noted by ***Dang et al. (2007).*** The authors found that subcutaneous injection of CCl4 (3 ml/kg) in rats for 6 weeks resulted in a significant decrease in serum albumin and increase in serum globulins.

Further support is the results recorded by ***Lin and Lin (2006)*** who found that rats administered CCl_4 (20%, 0.2 ml/100 gm body weight) orally twice a week for 8 weeks showed a significant decrease in hepatic protein, albumin and A/G ratio.

The obtained results in this study revealed that CCl_4 elicited a marked decrease in serum total protein, albumin and globulins when compared with normal control group. Whereas, protection with L-carnitine afforded a significant increase in serum total proteins and globulins when compared with control and CCl_4 treated group. Whereas, a non-significant change was recorded in serum albumin compared with normal and CCl_4 treated group alone.

The increased serum total proteins represented by increased serum globulins in L-carnitine treated group might be possibly attributed to increased synthesis of globulins by immunocytes and hyperglobulinemia is found in hepatocellular disorders appearing as inflammatory reaction of liver, ***(Vandenberghe, 1996).***

Effect on bilirubin:

Our results revealed that CCl_4 elicited a significant increase in serum total and direct bilirubin when compared with normal control rats. Likewise, L-carnitine and silymarin exhibited a marked decrease in serum total and direct bilirubin of cirrhotic rats compared with cirrhotic non-treated rats reverting them to nearly their normal values except group treated with L-carnitine where the serum total bilirubin is still significantly higher than the normal control values.

Hyperbilirubinemia is a very sensitive indicator to substantiate the functional integrity of the liver and the severity of necrosis which increases the binding, conjugating and excretory capacity of hepatocytes that is proportional to the erythrocyte degeneration ***(Singh et al., 1998).*** The liver damage induced by CCl_4 in this study resulted in a significant elevation in serum total and direct bilirubin. Because in cases of liver injury due to hepatotoxin, there is a defect in excretion of bile by the liver which is reflected by an increase in bile level in the serum ***(Rao, 1973).*** The increased amount of total bilirubin in the serum of rats given CCl_4 for 7 weeks might be most probably due to one of the following proposals: one of them resorted the increase due to hepatic uptake of free bilirubin from the blood or increase in hepatic β-glucuronidase ***(Acocella et al., 1968)*** due to hepatic insufficiency.

Gopinath and Ford (1972), in a study of experimental hepatopathies indicated that purely centrilobular lesions produced little hyper-bilirubimia, while moderate elevations occur if lesions include cells of the outer zone. Periportal lesions, however, that affects cell membrane integrity result in marked elevation in the serum bilirubin concentration. The other proposal is that the increase in bilirubin might be attributed to excessive erythrocyte hemolysis. ***Kaneko, (1980)*** stated that major elevations in total bilirubin are usually found only in hemolytic crisis and that increased concentration of bilirubin conjugates in the serum are indicative of severe hepatic involvement.

Our results were reinforced also by the results of ***Rajesh and Latha (2004).*** They observed that administration of CCl_4 for 2 months to rats induced a marked elevation in bilirubin concentration as an evidence of significant liver damage. The same observations were recorded by ***Achliya et al., (2004),*** they noted that rats treated with CCl_4 for 8 days developed a significant liver damage as evidenced by the elevated serum bilirubin. Our results were supported also with that of ***Nevin and Vijayammal (2005). Shanmygasundaram and Venkataraman (2006) and Shakir and Madhusudkan (2007),*** they recorded the same results obtained in this study. The decreased total bilirubin level observed in our study induced due to co administration of either L-carnitine or silymarin with CCl_4 could be possibly attributed to their antioxidative and anti-peroxidative effects ***(Cayir et al., 2009 and Pehlivan et al., 2009).***

Mourelle et al., (1989 b) noted that silymarin treatment was efficient in preventing biochemical alterations in CCl_4 induced live cirrhosis in rats. Liver cirrhosis was evidenced by significant increase in liver collagen, lipoperoxidation, serum activities of ALP, GGT, AST, glucose -6-phosphatase, bilirubin content and liver triglycerides (T.G). silymarin co-treatment (50 mg/kg b.wt.) completely prevented all the changes observed in CCl_4 cirrhotic rats, except for liver collagen content which was reduced only by 30% as compared to CCl_4 cirrhotic rats attributing these effects to the antioxidant and membrane-stabilizing actions of silymarin.

Effect on kidney function parameters:

The obtained results revealed that the administration of CCl_4 in its recommended dose 3 times a week for successive 7 weeks elicited a marked increase in serum levels of creatinine, urea and uric acid when compared with normal control group. Whereas, treatment of CCl_4 cirrhotic groups with either L-carnitine or silymarin in their recommended doses in the same manner afforded non-significant changes when compared with normal control and significant decrease when compared with cirrhotic non-treated group.

Our results coincides with the results of ***Lotfy (2009),*** he attributed these changes due to the damage and pathological changes observed in the kidneys in his study as a result of i.p injection of CCl_4 for 6 weeks. Since the kidney of cirrhotic rats showed nephritic changes in the renal parenchyma mainly degeneration or necrotic changes as well as fibrotic changes in the renal medulla. Our results were in accordance with ***Kaneko (1980).*** He found that increased urea production can occur in a variety of conditions such as renal shutdown resulting in insufficient urea excretion. Moreover, he added that the rate of urea formation depends on protein catabolism and an increase in blood urea formation depend on protein catabolism and an increase in urea nitrogen may reflect an accelerated rate of protein catabolism than decreased urea excretion. A hypothesis which appear to be accepted in our case since the liver suffered from degenerated changes as mentioned before. The increase in serum urea can be also attributed to a reduction in glomerular filtration rate as well as impairment of renal blood flow. Recently, ***Althnaian et al. (2013)*** reported that the i.p injection of CCl_4 in a dose of 1 ml/kg b.wt. diluted 1: 1 with paraffin oil for two successive days afforded a significant decrease in BUN level and a significant increase in uric acid level. The discrepancy in the level of BUN might be possibly attributed to difference in the dose of CCl_4 used and duration of administration.

Our results coincides also with ***Arroyo et al. (1996)*** they attributed the increase in creatinine to the occurrence of hepatorenal syndrome (HRS) which was observed in patients with advanced liver failure (acute or chronic). They stated that (HRS) type 1 is characterized by a rapid decline in renal function, defined as a doubling of serum creatinine to a level > 2.5 mg/dl or halving a creatinine clearance to < 20 ml/ min. within 2 weeks. The clinical presentation is that of acute renal failure, whereas, in type 2 (HRS) renal function deteriorates more slowly, with serum creatinine increasing to > 1.5 mg/dl or a creatinine clearance of < 40 ml/min. The clinical presentation is that of stable renal failure in a patient with refractory ascitis.

Table (1): Effect of CCl_4 (1.5 ml/kg), L-Carnitine, (100 mg/kg), Silymarin (25 mg/kg) on liver function parameters in male albino rats. Mean±S.E (n=10)

Groups	AST (u/L)	ALT (u/L)	ALP (Kind and king U/dl)	T.P (gm/dl)	Alb (gm/dl)	Serum globulins (gm/dl)	T. Bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)
Control	44.96 ± 2.99^{c}	45.34 ±2.15^{bc}	72.3 ± 3.01^{b}	10.62 ±0.33^{b}	4.04 ±0.21^{b}	6.58 ± 0.52^{b}	0.55 ± 0.14^{c}	0.12 ± 0.03^{abc}	0.43 ± 0.05^{bc}
CCL_4 treated	171.97 ± 8.14^{a}	61.55 ± 3.42^{a}	87.98 ± 2.30^{a}	4.23 ± 0.41^{c}	2.77 ±0.45^{c}	1.46 ± 0.12^{d}	1.78 ± 0.09^{a}	0.26 ± 0.06^{a}	0.52 ± 0.12^{b}
CCL_4 + L-Carnitine treated	158.27 ±6.83^{ab}	51.05 ± 2.30^{b}	65.6 ± 2.26^{b}	12.75 ± 0.32^{a}	3.14 ±0.63^{bc}	9.61 ± 0.76^{a}	1.11 ± 0.29^{b}	0.15 ± 0.05^{abc}	0.96 ± 0.10^{a}
CCL_4 + Silymarin treated	53.78 ± 5.95^{c}	43.91 ± 2.85^{c}	72.67 ± 2.2^{b}	10.03 ± 0.42^{b}	5.24 ±0.38^{a}	4.78 ± 0.33^{c}	0.61 ± 0.09^{c}	0.2 ± 0.05^{abc}	0.41 ± 0.04^{bc}

Values with different superscripts are significant at ($P \leq 0.05$)

Table (2): Effect of CCl_4 (1.5 ml/kg), L-Carnitine (100 mg/kg) and Silymarin (25 mg/kg) on some kidney function parameters in male albino rats. Mean±S.E (n=10)

Groups	**Creatinine** (mg/dl)	**Uric acid** (mg/dl)	**Urea** (mg/dl)
Control	0.56 ± 0.07^{c}	2.40±0.14^{b}	25.59±1.05^{b}
CCL_4 treated	1.30 ± 0.06^{a}	3.45 ± 0.24^{a}	33.25 ± 2.67^{a}
CCL_4 + L-Carnitine treated	1.08 ± 0.05^{b}	2.24 ± 0.44^{b}	23.78 ± 1.89^{bc}
CCL_4 + Silymarin treated	1.04 ± 0.15^{b}	1.99 ± 0.36^{bc}	25.05 ± 1.42^{b}

Values with different superscripts are significant at ($P \leq 0.05$)

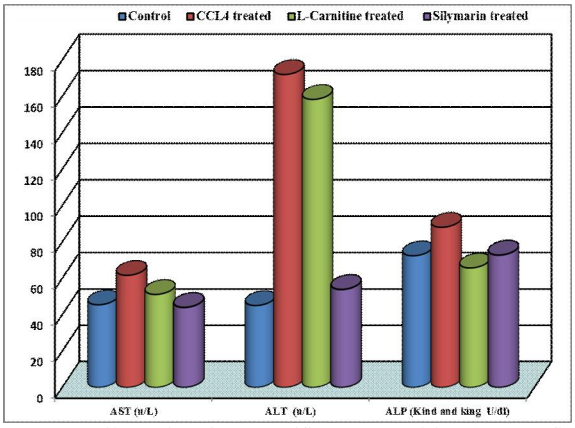

Fig. (1): Effect of CCl_4 (1.5 ml/kg), L-Carnitine (100 mg/kg) and Silymarin (25 mg/kg) on liver function parameters (ALT, AST and ALP (u/L)) in male albino rats.

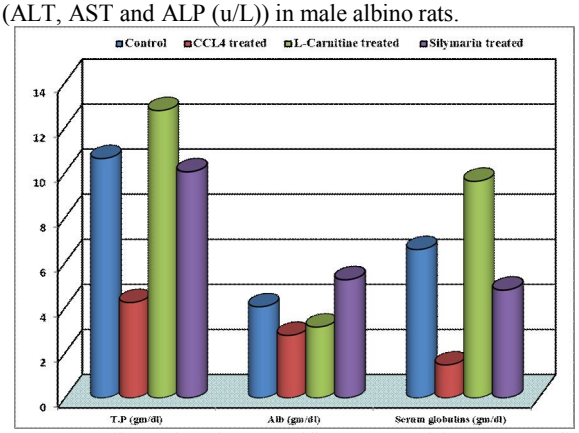

Fig. (2): Effect of CCl_4 (1.5 ml/kg), L-Carnitine (100 mg/kg) and Silymarin (25 mg/kg) on liver function parameters [TP., ALP and Serum globulins (gm/dl)] in male albino rats.

Our results revealed also that the administration of silymarin for 7 weeks to rats suffering from liver damage induced by CCl_4 caused a significant decrease in serum urea and creatinine compared with cirrhotic non-treated rats. These results agree with that recorded by ***Gaedeke et al. (1996)*** and ***Bokemeyer et al. (1996).*** They observed that silibin prevented cisplatin –induced glomerular and tubular nephrotoxicity in rats as measured by blood urea nitrogen (BUN), creatinine and fibronectin and histopathological changes in renal tubules. The antioxidative and scavenging free radicals and inhibiting lipid peroxidation might be probably the cause of decreased urea and creatinine concentration.

The significant decrease in urea, uric acid and creatinine in cirrhotic rats treated with either L-carnitine or silymarin compared with CCl_4 treated group seems conceivable to be due to their antioxidative and antiperoxidative effect as previously mentioned.

Effect on lipogram:

Our results revealed that the administration of CCl_4 for 7 weeks afforded a significant increase in serum triglycerides, LDL-c and a significant decrease in HDL-c and total cholesterol when compared with normal control rats. While treatment of cirrhotic rats with L-carnitine and silymarin elicited also a non-significant decrease and increase in serum total cholesterol when compared with normal control and cirrhotic non-treated rats respectively. On HDL-c L-carnitine and silymarin induces a significant decrease and non-significant increase when compared with normal and CCl_4 treated rats respectively.

L-carnitine elicited a non-significant change and a significant decrease in LDL-c of control and CCl_4 treated group respectively. Meanwhile, silymarin afforded a significant increase in serum LDL-c compared with control group and a slight decrease when compared with CCl_4 group.

On serum triglycerides, both L-carnitine and silymarin exhibited a non-significant decrease and a significant increase when compared with CCl_4 and normal control groups respectively.

Silymarin had been reported to protect liver cells from a wide variety of toxins including CCl_4. The mechanisms which provide silymarins hepatoprotective effect are many and varied, including antioxidant activity ***(Halim et al., 1997),*** inhibiting lipid per-oxidation ***(Rui, 1991).*** The hypocholesterolemic effect of silymarin obtained in this study coincides with that recorded by ***Schriewer and Rauen (1977).*** Who reported that silibin induced a decrease in cholesterol synthesis *in vitro* on rat liver homogenates. Whereas, ***Skottova and Krecman (1998),*** found that silymarin normalized the clearance of LDL-c in perfused livers from rats fed a high cholesterol-diet. ***Krecman et al. (1998)*** recorded also that silymarin provided a significant protection against dietary induced hypercholesterolemia.

Moreover, silymarin exerted anti-atherosclerotic effects in rabbits fed high cholesterol diet ***(Bialecka, 1997).*** Further support to our results was obtained by ***Somogyi et al. (1989),*** who found that silymarin when given in doses of 450 mg daily for 7 months to 14 hyperlipidemic outpatients induced a decrease in total cholesterol and an increase in HDL-c levels. Moreover, it has been reported by ***Locher et al. (1998)*** that silibin inhibited peroxidation of LDL-c *in vitro*.

On a similar basis, the hypocholesterimic effect induced by L-carnitine inhibits both the mitochondrial damage induced by oxidative stress and mitochondrial – dependent apoptosis in various types of cells ***(Al-Majed, 2007 and Aleisa et al., 2007).*** Recent studies suggest that L-carnitine may play an important role in oxidative / antioxidative balance and has an antipe-roxidative effect on several tissues ***(Cayir et al., 2009; Bayraktar et al., 2008 and Pehlivan et al., 2009 and Zaied, 2014).***

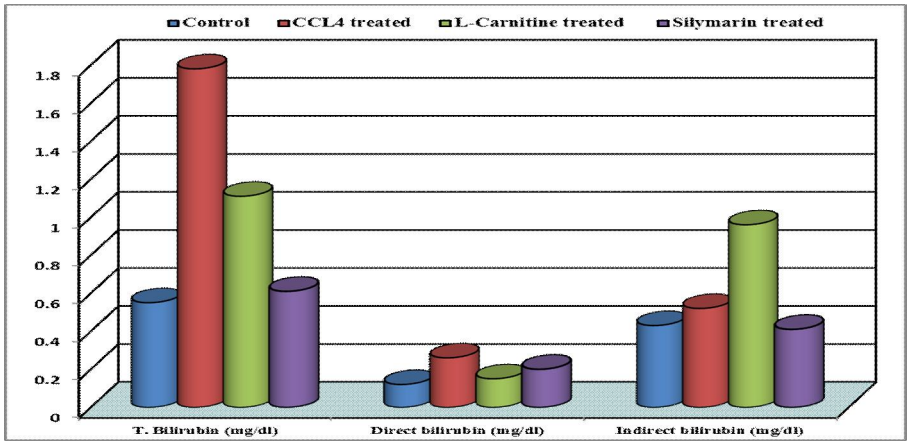

Fig. (3): Effect of CCl_4 (1.5 ml/kg), L-Carnitine (100 mg/kg) and Silymarin (25 mg/kg) on liver function parameters [**T. Bilirubin**., **Direct** and **Indirect bilirubin** (mg/dl)] in male albino rats.

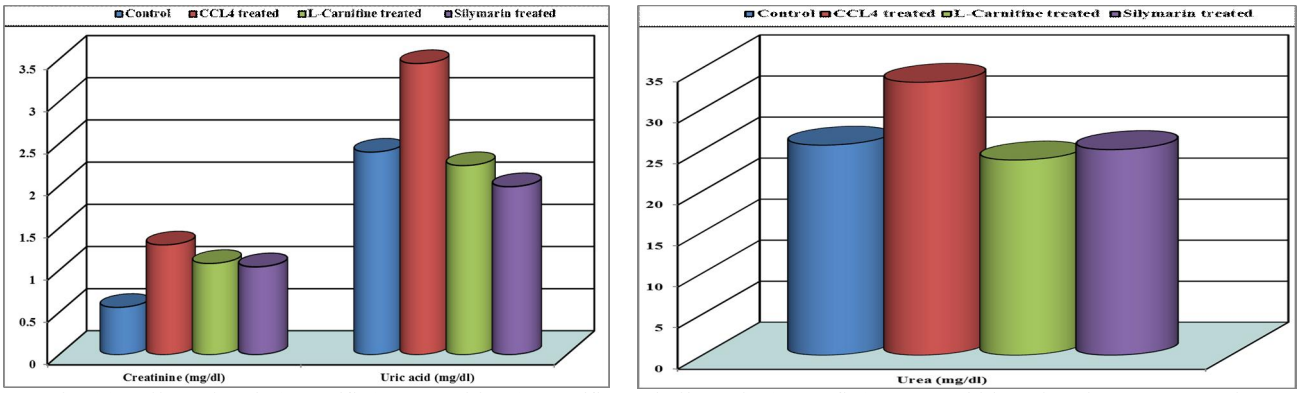

Fig. (4): Effect of CCl_4 (1.5 ml/kg), L-Carnitine (100 ml/kg and Silymarin (25 mg/kg) on some kidney function parameters in male albino rats

Table (3): Effect of CCL_4 (1.5 ml/kg), L-Carnitine (100 mg/kg) and Silymarin (25 mg/kg) on libogram of male albino rats. Mean±S.E (n=10)

Groups	Cholesterol (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	Triglycerides (mg/dl)
Control	106.78±8.98 [a]	188.97±6.03[a]	95.01±3.10[c]	136.06±4.68[c]
CCL_4 treated	77.74 ± 6.91 [c]	136.81 ± 5.09 [cd]	137.86 ± 5.14 [a]	197.38 ± 14.15 [a]
CCL_4 + L-Carnitine treated	83.66 ± 6.89 [abc]	147.42 ± 7.90 [c]	98.90 ± 5.71 [c]	175.72 ± 14.28 [ab]
CCL_4 + Silymarin treated	93.98 ± 5.85 [abc]	177.36 ± 8.76 [ab]	124.01 ± 6.51 [ab]	167.0 ± 11.82 [ab]

Values with different superscripts are significant at ($P \leq 0.05$)

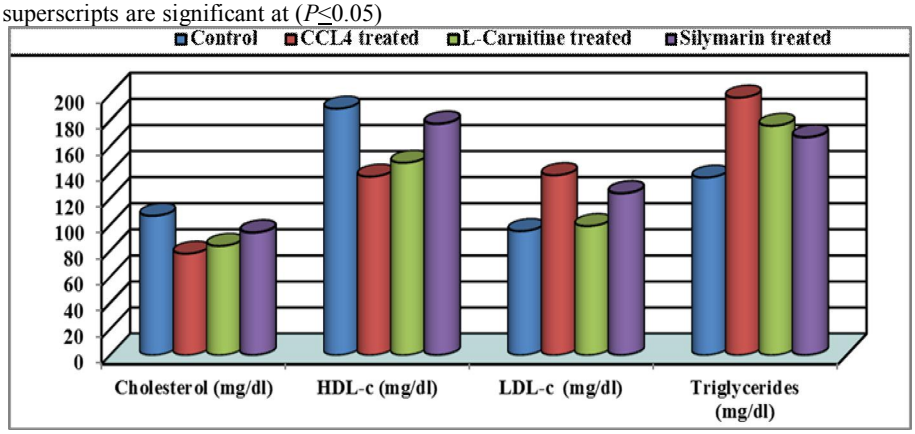

Fig. (5): Effect of CCL_4 (1.5 ml/kg), L-Carnitine (100 mg/kg) and Silymarin (25 mg/kg) on libogram of male albino rats.

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