Zagazig J. Pharm. Sci., December, 2008 ISSN 1110-5089
Vol. 17, No. 2, pp. 6-16

BIOCHEMICAL AND HISTOPATHOLOGICAL STUDIES ON THE EFFECT OF SILYMARIN, CURCUMIN OR TAURINE ON CCl_4-INDUCED LIVER INJURY IN RATS

Shimaa M Elshazly, Mohamed NM Zakaria, Ahmed F Ahamed and Mohamed A Mohamed
Department of Pharmacology, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

ABSTRACT

The present study investigated the role of silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) in the prophylaxis against carbon tetrachloride (CCl_4) induced liver injury in rats. The substances used were administered 2 weeks before and 6 weeks along with CCl_4 (25 µl/100 gm). The levels of serum transaminases (ALT, AST), alkaline phosphatase (ALP), blood glucose and serum protein concentration were determined. In addition, histopathological examination of liver samples was carried out. Silymarin resulted in a significant reduction in ALT, AST, ALP and blood glucose levels and a significant elevation of total protein concentration. On the other hand, curcumin significantly elevated the levels of ALT, AST and ALP compared to cirrhotic rats. Taurine resulted in a significant reduction in AST and blood glucose with a significant elevation of ALT and ALP levels. Liver of silymarin pretreated rats showed few cells at the periportal area having necrotic changes and regeneration of large number of the hepatocytes. The bile ducts and ductules of the livers of curcumin pretreated rats showed moderate hyperplastic changes and the hepatic blood vessels were moderately congested. Mono-lobular cirrhosis, necrosis of the hepatocytes, sever congestion of the hepatic blood vessels and hyperplasia of bile ducts and ductules were found in livers of taurine pretreated rats. The present study demonstrates that silymarin can protect the liver against CCl_4-induced hepatic injury while, taurine or curcumin couldn't perform the same effect.

INTRODUCTION

The liver is one of the largest glands and the most complex organ in the body. It performs multiple functions, including the production of proteins and enzymes, detoxification, metabolic functions as well as regulation of cholesterol and blood clotting[1]. Hepatic cirrhosis is one of the most common causes of mortality worldwide. Incidence of cirrhosis is growing due to wide spread occurrence of chronic hepatitis as well as lack of an effective therapy for hepatic cirrhosis[2].

Silymarin has been reported to protect liver cells against a wide variety of toxins, including acetaminophen, ethanol, and D-galactosamine[3, 4]. Silymarin has also been found to protect liver cells against ischemia-induced injury[5], radiation[6] iron toxicity[7] and viral hepatitis[8].

Like silymarin, curcumin (turmeric) has been found to protect animal liver against a variety of hepatotoxic substances[9, 10]. The hepatoprotective effects of turmeric may be due to its potent antioxidant effects, including scavenging of such reactive oxygen species as superoxide anions and hydrogen peroxide, inhibition of lipid peroxidation and inhibition of the oxidation of low-density lipoprotein (LDL)[11].

Taurine is a non-protein amino acid reported to have a hepatoprotective effect. In addition, animal studies also demonstrated taurine's ability to make complex with and neutralize the xenobiotic effects of carbon tetrachloride and retinol[12]. The goal of this work was to clarify if there is any prophylactic effect for silymarin, curcumin or taurine aganist liver injury induced by CCl_4 in rats.

EXPERIMENTAL DESIGN

Drugs and chemicals:

Drug name	Dose	Reference	Source
Silymarin*	100 mg/kg	Soto et al., [13]	Sedico CO., Egypt
Taurine**	1 gm/kg	Gandhi and Mulky, [14]	Sigma Co., St. Louis, Mo, USA
Curcumin**	75 mg/kg	Nanji et al., [15]	Sigma Co., St. Louis, Mo, USA

(*) the drug was dissolved in normal saline.
(**) the drugs were suspended or emulsified in 10% gum acacia.
All other chemicals are of analytical grades.

Animals:

Adult male albino rats weighing about 150-200 gm provided by the Faculty of Veterinary Medicine, Zagazig University in Egypt were used. The animals were housed in cages with wood shaving bedding, 6-8 per cage. The animals were randomly divided (12-16 rats each) among groups according to following design: **Group (1):** served as normal control group and received liquid paraffin (0.3 ml/kg, i.p.) for 6 weeks. **Group (2):** served as cirrhotic control group and received CCl_4 3 times a week for 6 weeks in a dose of 25µl/ 100 gm., b.w, i.p. diluted 1:6 with liquid paraffin. **Group (3):** received silymarin (100 mg / kg, orally) for 2 weeks, followed by concurrent administration of silymarin together with CCl_4 for 6 weeks. **Group (4):** received curcumin alone in a dose (75 mg/kg, orally) for 8 weeks. **Group (5):** received curcumin (75 mg /kg, orally) for 2 weeks, followed by concurrent administration of curcumin together with CCl_4 for 6 weeks. **Group (6):** received taurine alone (1 gm /kg, orally) for 8 weeks. **Group (7):** received taurine (1 gm/kg, orally) for 2 weeks, followed by concurrent administration of taurine together with CCl_4 for 6 weeks.

Venous blood samples were collected in clean dry test tubes from the retro-orbital sinus of rats using

heparinized microcapillary tubes[16]. Serum samples were collected and used immediately for the determination of:

Serum AST level was determined by a colourimetric method[17] using a diagnostic kit supplied by **Plasmatek (Germany).**

Serum ALT level was determined by a colourimetric method[14, 18] using a diagnostic kit supplied by **plasmatek (Germany).**

Serum alkaline phosphatase was determined by an enzymatic colourimetric method[19] using a diagnostic kit supplied by **Teco diagnostics (USA).**

Serum glucose level was determined by an enzymatic colourimetric method[20] using a diagnostic kit supplied by **Elitech diagnostics (France).**

Total protein was determined by Biuret method an enzymatic colourimetric method[21] using a diagnostic kit supplied by **Biocon (Germany).**

Serum albumin was determined by Bromcresol green method an enzymatic colourimetric method [22] using a diagnostic kit supplied by **Biocon (Germany).**

In addition, animals were sacrificed and dissected; one lobe of the liver was removed, cut into longitudinal sections (2-4 mm. in thickness) and kept in 10% formalin for histopathological examination.

Statistical analysis:

Results are presented as mean ± standard error of the mean. Student's t-test for unpaired data at $p<0.05$ was used to determine the significance of the differences between control and treated groups[23]. Linear regression was used to determine the value of r^2 for the standard curve of aminotransferase enzyme concentration. $P<0.05$ or $p<0.001$ were used as statistical significance levels.

RESULTS AND DISCUSSION

I. Effect on alanine aminotransferase (ALT) enzyme:

1- Effect of CCl_4 administration (25 µl / 100 gm, I.P., 3 times weekly for 6 weeks) on ALT level in adult male rats:

The results presented in figure (1) illustrate that injection of CCl_4 (25µl / 100 gm, I.P.) three times weekly for 6 weeks to normal adult male rats induced a significant increase in the level of ALT (U/L) from 19.21±1.05 to 98.77±7.34 (by 414.15%) compared to normal control group.

2- Effect of 14 days pretreatment with silymarin (100 mg / kg), curcumin (75 mg / kg) or taurine (1 gm / kg) orally on ALT level in adult male cirrhotic rats:

As illustrated in figure (1), pretreatment of cirrhotic rats with silymarin (100 mg / kg, orally) for 2 weeks before and throughout CCl_4 administration significantly decreased ALT level (U/L) from 98.77±7.34 to 71.62 ± 4.4 (by 27.49%) compared to cirrhotic control group.

However, administration of curcumin (75 mg/kg, orally) or taurine (1 gm / kg, orally) for 2 weeks before and during the CCl_4 administration resulted in a significant elevation of ALT level (U/L) from 98.77±7.34 to 239.49±22.29 by (142.47%) and from 98.77±7.34 to 242.95±20.08 (by 145.97%), respectively, compared to cirrhotic control group.

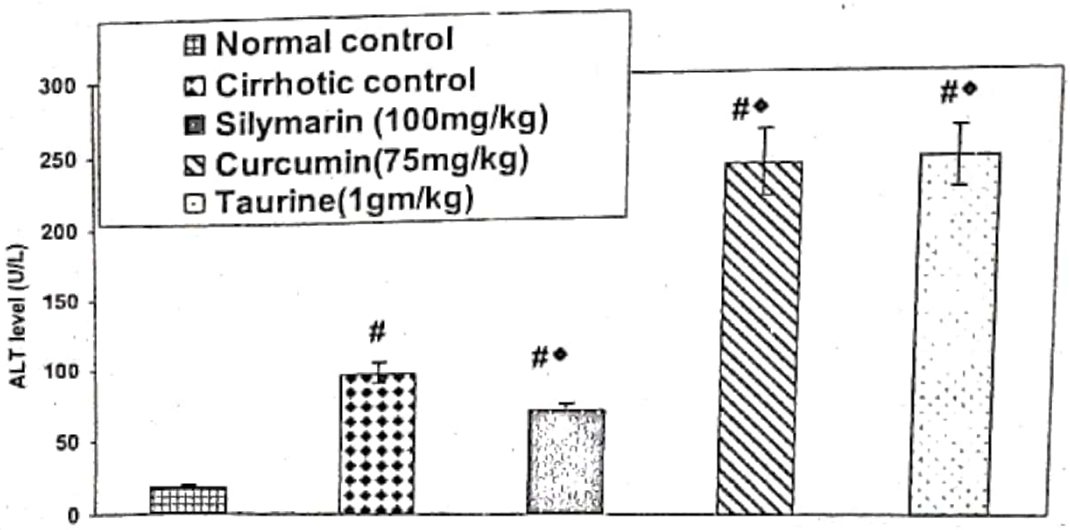

Figure (1): Effect of 14 days pretreatment with silymarin (100mg/kg), curcumin (75 mg/kg) or taurine (1gm/kg) orally on ALT level in adult male cirrhotic rats

Oral administration of curcumin in the previously mentioned dose to normal adult rats for 8 weeks induced a significant increase of ALT level (U/L) from 19.21±1.05 to 30.46±3.25 (by 58.55%) compared to normal control group. While, administration of taurine orally in the used dose to normal adult rats for the same period didn't significantly change ALT level compared to normal control group.

Zagazig J. Pharm. Sci., December, 2008
Vol. 17, No. 2, pp. 6-16
ISSN 1110-5089

II. Effect on aspartate amino transferase (AST):

1- Effect of CCl_4 administration (25µl/100 gm, I.P., 3 times weekly for 6 weeks) on AST level in adult male rats:

Administration of CCl_4 in the selected dose and period to normal adult male rats resulted in a significant elevation in AST level (U/L) from 63.08±5.37 to 425±29.53 (by 573.74%) compared to the normal control group as shown in figure (2).

2-Effect of oral pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) for 14 days on AST level in adult male cirrhotic rats:

As presented in figure (2), oral administration of silymarin or taurine in the selected doses for 2 weeks before and during time course of CCl_4 administration significantly reduced AST level (U/L)from 425±29.53 to 147.36±14.25 (by 65.32%) or from 425±29.53 to 256.33±20.1 (by 39.69%), respectively, compared to cirrhotic control group.

However, oral administration of curcumin in the previously administered dose level for 2 weeks before and throughout CCl_4 administration significantly increased AST level (U/L) from 425±29.54 to 1263.15±121.2 (by 197.21%) compared to cirrhotic control group.

Administration of curcumin or taurine in the previously mentioned doses for 8 weeks to normal adult male rats resulted in a significant increase of AST level (U/L) from 63.08 ± 5.37 to 93.15 ± 3.15 (by 47.67%) or from 63.08 ± 5.37 to 86.14 ± 4.4 (by 36.55%), respectively, compared to normal control group.

I. Effect on serum alkaline phosphatase level (ALP):

1-Effect of CCl_4 administration (25µl/100 gm, I.P., 3 times weekly for 6 weeks) on AST level in adult male rats:

As presented in figure (3), administration of CCl_4 intraperitoneally in the selected dose and period to normal adult male rats induced a significant elevation in ALP level (IU/L) from 80.92±3.13 to 251.10±7.9 (by 210.31%) compared to normal rats.

2-Effect of 14 days pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) orally on ALP level in adult male cirrhotic rats:

As graphically presented in figure (3), oral administration of silymarin in the previously mentioned dose for 2 weeks before and during time course of CCl_4 administration significantly decreased ALP level (IU/L) from 251.10±7.9 to 130.44±12.48 (by 48.1%) compared to cirrhotic control group.

However, administration of curcumin orally in the selected dose for 2 weeks before and along with CCl_4 administration caused a significant increase in ALP level (IU/L) from 251.102±7.9 to 282.52±27.8 (by 12.5%) compared to cirrhotic control group. Oral administration of taurine in the used dose for 2 weeks before and throughout CCl_4 administration didn't significantly change ALP level compared to cirrhotic control group.

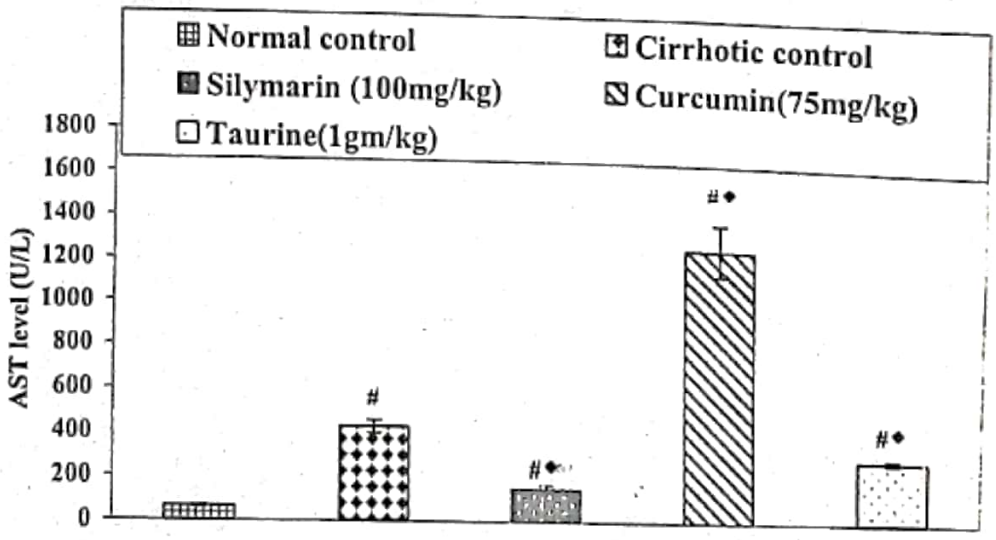

Figure (2): Effect of oral pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) for 14 days on AST level in adult male cirrhotic rats

*Significantly different from the corresponding mean value of normal control group at $p < 0.05$
*Significantly different from the corresponding mean value of cirrhotic control group at $p < 0.05$

Oral administration of curcumin or taurine in the selected doses for 8 weeks to normal adult rats didn't induce any significant effect in ALP level (IU/L) compared to normal control group.

II. Effect on serum blood glucose level:

1- Effect of CCl_4 administration (25 µl/100 gm, I.P., 3 times weekly for 6 weeks) on serum glucose level in adult male albino rats:

The results presented in figure (4), illustrate that intraperitoneal injection of CCl_4 in the selected dose and period to normal adult male rats significantly increased serum blood glucose level (mg/dl) from 78.67±2.9 to 102.25±5.36 (by 23.1%) compared to normal control group.

2- Effect of oral pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) for 14 days on serum glucose level in adult male cirrhotic rats:

As shown in figure (4), oral pretreatment of cirrhotic rats with silymarin or taurine in the selected doses for 2 weeks before and along with CCl_4 administration significantly reduced serum blood glucose levels (mg/dl) from 102.25±5.36 to 75.22±3.67 (by26.43%) and from 102.25±5.36 to 85.9±2.8 (by 16%), respectively, compared to cirrhotic control group. On the other hand, pretreatment with curcumin in the tested dose and for the same period didn't significantly affect serum glucose level compared to cirrhotic control group.

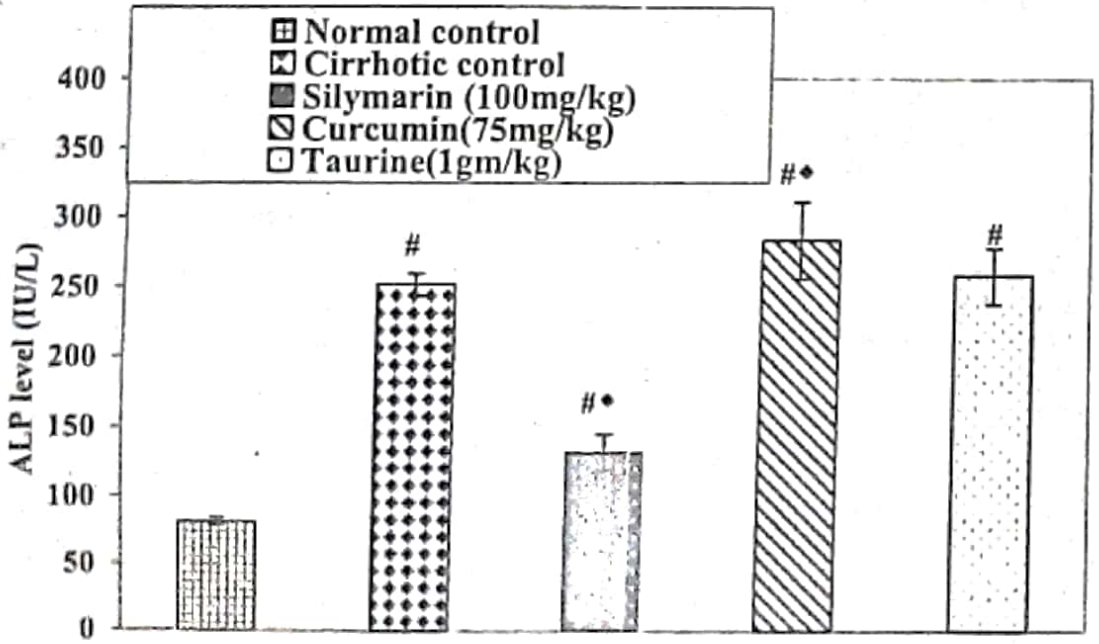

Figure (3): Effect of 14 days pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) orally on ALP level in adult male cirrhotic rats

*Significantly different from the corresponding mean value of normal control group at $p < 0.05$
•Significantly different from the corresponding mean value of cirrhotic control group at $p < 0.05$

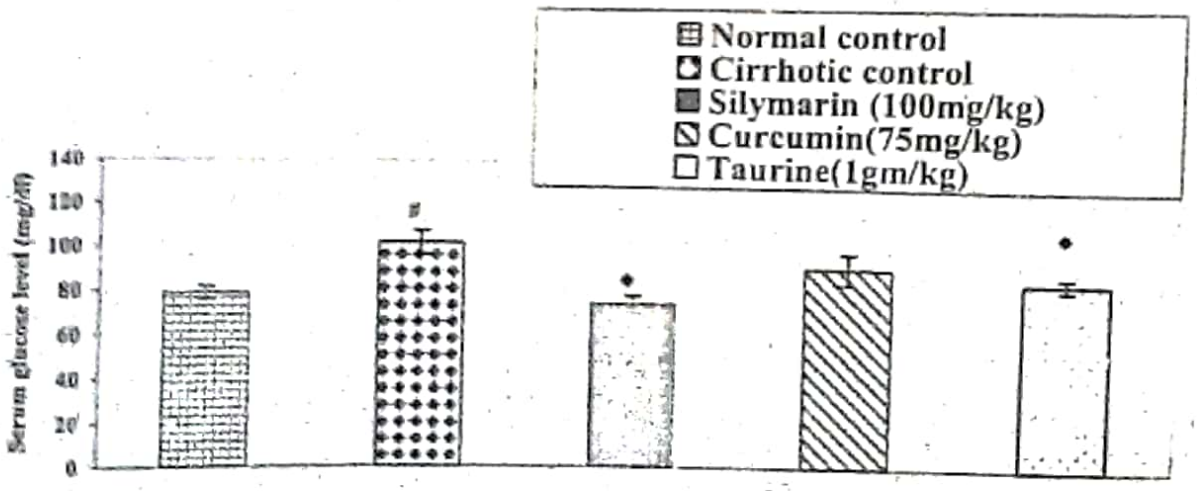

Figure (4): Effect of oral pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) for 14 days on serum glucose level in adult male cirrhotic rats

*Significantly different from the corresponding mean value of normal control group at $p < 0.05$
•Significantly different from the corresponding mean value of cirrhotic control group at $p < 0.05$

Oral administration of curcumin in the used dose for 8 weeks to normal rats didn't significantly change the serum glucose level as compared to normal control group. Moreover oral treatment of normal rats with taurine in the previously mentioned dose for the same period induced a significant elevation in serum glucose level (mg/dl) from 78.67±2.9 to 91.91±3.05 (by 16.8%) compared to normal rats.

III. Effect on serum proteins:

ISSN 1110-5089

1-Effect of CCl_4 administration (25 µl/100 mg, I.P., 3 times weekly for 6 weeks) on serum protein levels in adult male rats:

Intraperitoneal injection of CCl_4 in the previously mentioned dose and period to normal adult male rats produced a significant fall in total protein, albumin and globulin levels (gm/dl) from 7.63±0.22 to 6.2±0.08 (by 18.7%), from 3.83±0.15 to 2.64±0.11 (by 31.07%) and from 4.57±0.1 to 3.02±0.092 (by 33.9%), respectively, compared to normal control group. However, it didn't show any significant change on albumin/globulin ratio (A/G) as shown in figure (5).

2- Effect of oral pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) for 14 days on serum protein levels in adult male cirrhotic rats:

As presented in figure (5), oral pretreatment of cirrhotic rats with silymarin in the selected dose significantly elevated total protein, albumin and globulin levels (gm/dl) from 6.2±0.08 to 9.02±0.18 (by 45.48%), from 2.64±0.11 to 3.04±0.12 (by 15.15%) and from 3.02±0.09 to 5.91 ± 0.22 (by 95.69%), respectively compared to cirrhotic control group. However, it didn't significantly change the A/G ratio compared to cirrhotic control group.

Oral administration of curcumin in the selected dose for 2 weeks before and during the time course of CCl_4 injection didn't induce any significant change in total protein level, while significantly increased globulin level (gm/dl) from 3.02±0.09 to 4.53±0.11 (by 50%) compared to cirrhotic control group. However, it resulted in a significant decrease in albumin level and A/G ratio (gm/dl) from 2.64±0.11 to 1.91±0.19 by (27.65%) and from 0.66±0.05 to 0.45±0.04 (by 31.81%), respectively compared to cirrhotic control group.

Oral pretreatment of cirrhotic rats with taurine in the selected dose for 2 weeks before and during the time course of CCl_4 injection didn't induce any significant change in total protein and albumin levels. On the other hand, it induced a significant increase in globulin level and A/G ratio (gm/dl) from 3.02±0.09 to 3.42±0.16 (by 13.2%) or from 0.66±0.05 to 1.12±0.21 (by 69.69%), respectively compared to cirrhotic control group.

3- Effect of oral treatment with curcumin (75 mg/kg) or taurine (1 gm/kg) for 8 weeks on serum proteins levels in normal adult male rats:

As shown in figure (6), oral administration of taurine for 8 weeks to normal rats significantly reduced total protein level (gm/dl) from 7.63 ± 0.22 to 7.07 ± 0.15 (by 7.34%) compared to normal control group. Similar treatment with curcumin didn't significantly change total protein level. Oral administration of curcumin or taurine didn't show any significant change in albumin level. However, oral treatment with curcumin or taurine significantly decreased globulin level (gm/dl) from 4.57±0.1 to 3.65±0.15 (by 20.1%) or from 4.57±0.1 to 3.77±0.17 (by 17.5%), respectively compared to normal control group. Oral administration of curcumin or taurine produced a significant elevation in A/G ratio from 0.61 ± 0.08 to 1.15 ± 0.048 (by 88.5%) or from 0.61±0.08 to 0.96±0.08 (by 57.3%), respectively, compared to normal control group.

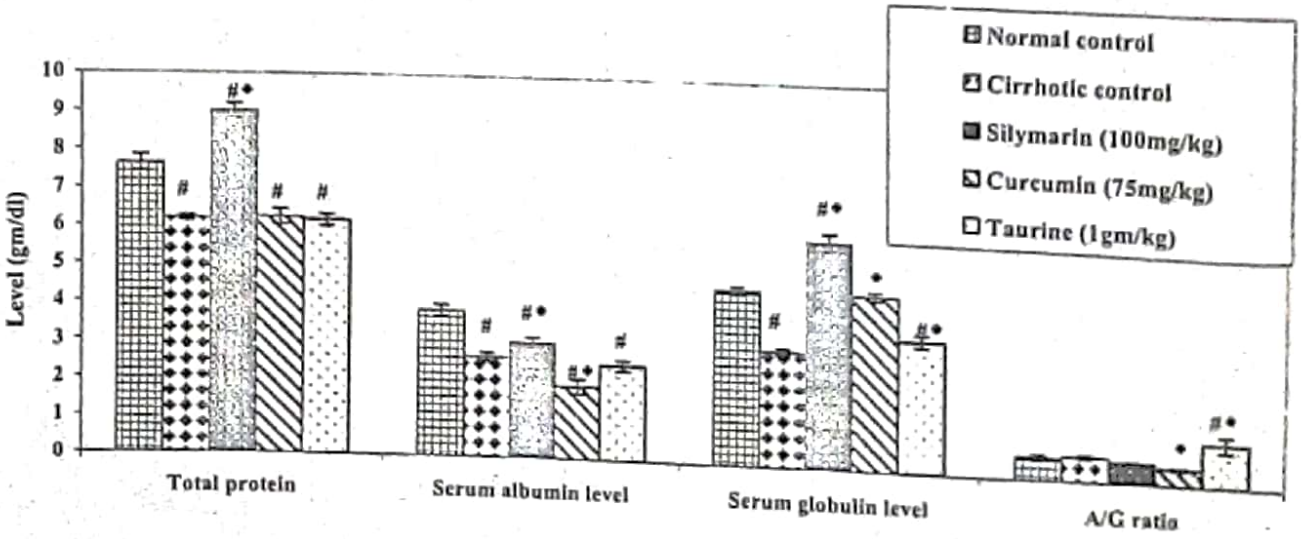

Figure (5): Effect of oral pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) for 14 days on serum protein levels in adult male cirrhotic rats

#Significantly different from the corresponding mean value of normal control group at $p < 0.05$
*Significantly different from the corresponding mean value of cirrhotic control group at $p < 0.05$

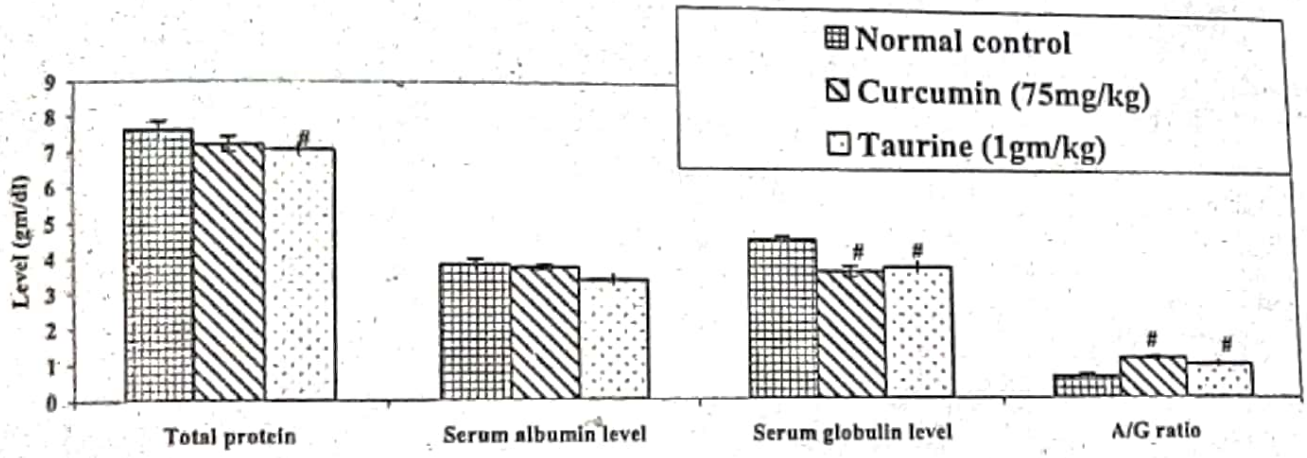

Figure (6): Effect of oral treatment with curcumin (75 mg/kg) or taurine (1 gm/kg) for 8 weeks on serum protein levels in normal adult male rats
#Significantly different from the corresponding mean value of normal control group at $p < 0.05$

Histopathological results:

Effect of CCl_4 administration (25 µl/100 gm, I.P., 3 times weekly for 6 weeks) on normal adult male rats:

Liver of normal rats which received liquid paraffin (0.3 ml/kg, I.P.) 3 times weekly for 6 weeks was apparently normal. However, in some examined cases, some hepatocytes showed hydropic degeneration (figure 7).

However, intraperitoneal administration of CCl_4 in the previously mentioned dose and period caused moderate enlargement of the liver. In addition, the examined liver had pale grayish colour, firm consistency and coarse granular surface. Most of the examined cases showed mono-lobular cirrhosis, represented by fibrous tissue encircling individual hepatic lobules (figure 8).

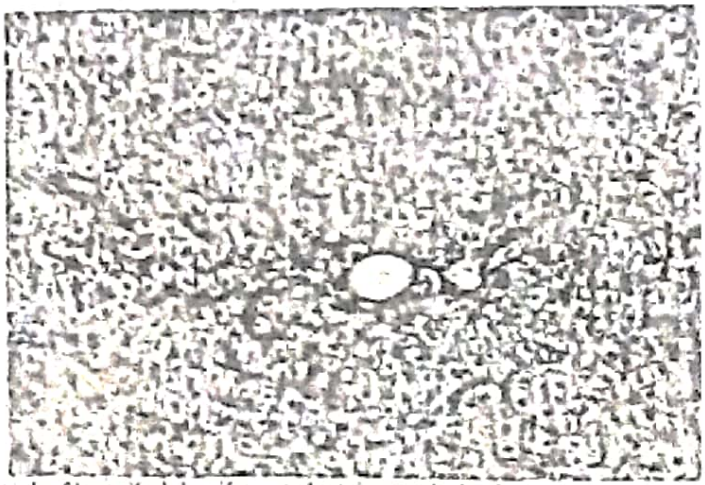

Figure (7): A photomicrograph of liver of adult male normal rat showing hydropic degeneration of some hepatocytes (H&E x 300)

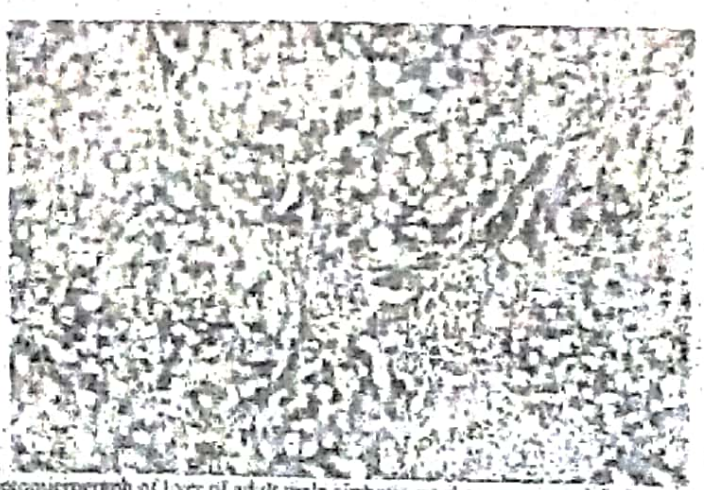

Figure (8): A photomicrograph of liver of adult male cirrhotic rat showing mono-lobular cirrhosis (H&E x 300)

ISSN 1110-5089

Effect of oral pretreatment with silymarin (100 mg/kg), curcumin (75 mg/kg) or taurine (1 gm/kg) for 15 days in adult male cirrhotic rats:

Most of examined cases of silymarin pretreated rats showed normal liver, with a few cells at the periportal area having necrotic changes (pyknosis of the nuclei and cytoplasmolysis) (figure 9). Regeneration of large number of the hepatocytes was also found. Some hepatocytes showed fatty changes.

On the other hand, oral pretreatment of cirrhotic rats with curcumin caused mild enlargement of liver with pale-grayish colour and fine granular surface. The bile ducts and ductules showed moderate hyperplastic changes and the hepatic blood vessels were moderately congested. Moderate numbers of the hepatocytes were apparently normal; however, mono-lobular cirrhosis was still present around cell infiltration (figure 10). In addition, oral pretreatment with taurine to cirrhotic rats induced some cirrhotic changes in some cases. Some of the examined cases showed mono-lobular cirrhosis, necrosis of the hepatocytes, severe congestion of the hepatic blood vessels and hyperplasia of bile ducts and ductules were also found (figure 11).

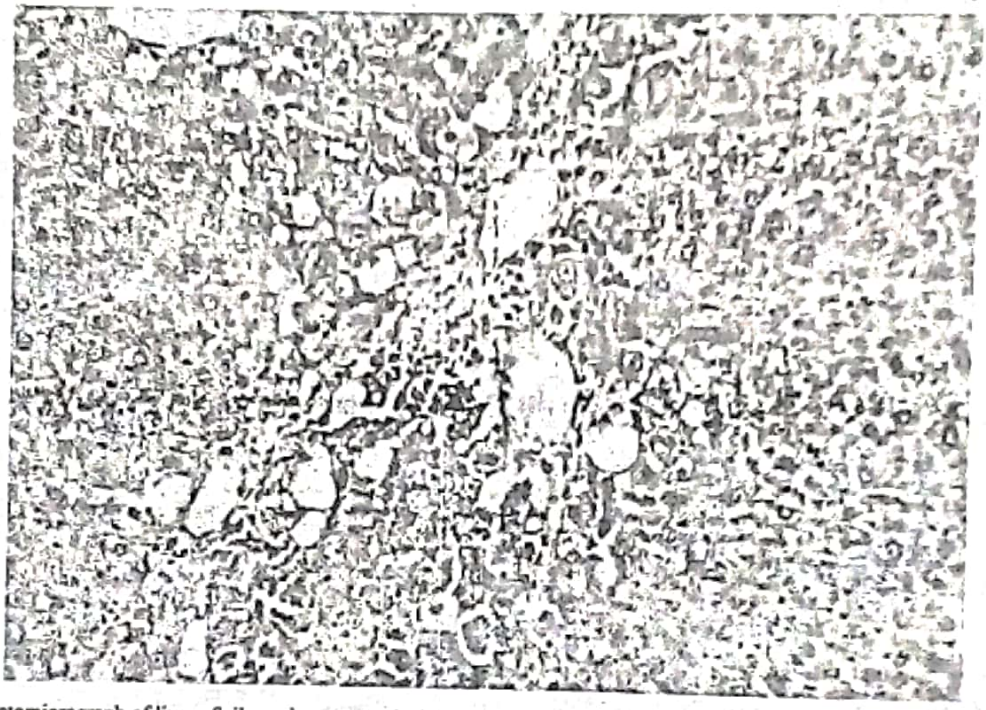

Figure (9): A photomicrograph of liver of silymarin-pretreated adult male cirrhotic rat for 15 days showing few cells with necrotic changes at the periportal area (H&E x 300)

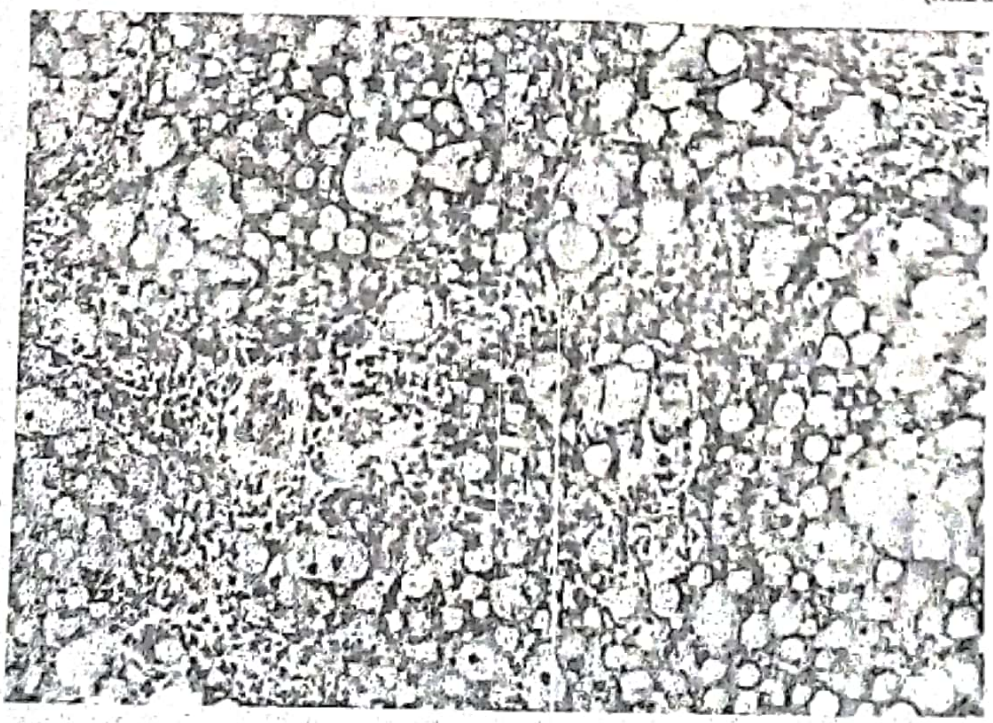

Figure (10): A photomicrograph of liver adult male cirrhotic rat pretreated with curcumin for 15 days showing mono-lobular cirrhosis around some lobules accompanied with round cell infiltration (H&E x 300)

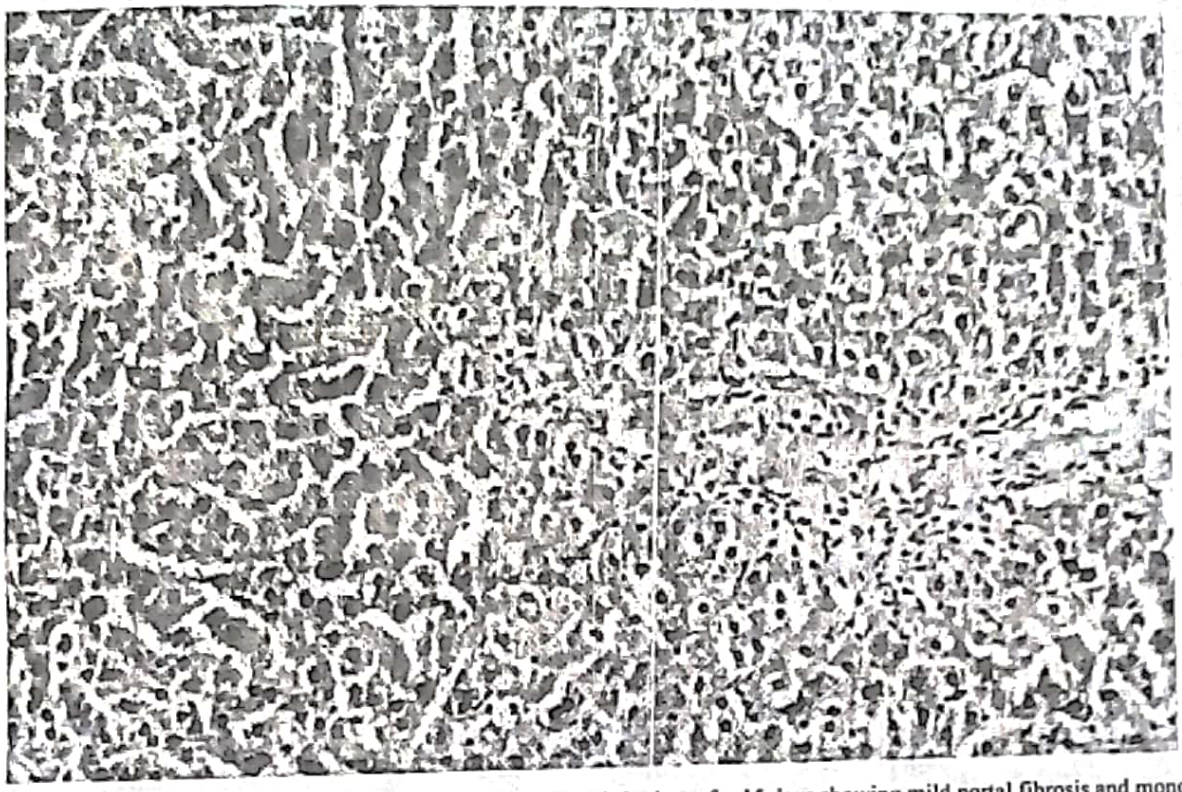

Figure (11): A photomicrograph of liver of taurine pretreated adult male cirrhotic rat for 15 days showing mild portal fibrosis and mononuclear cell infiltration (H&E x 300)

Effect of oral administration of curcumin (75 mg/kg) or taurine (1 gm/kg) for 8 weeks on normal adult male albino rats:

The examined livers of curcumin treated normal rats were apparently normal. However, moderate number of the hepatocytes showed increased mitotic activity represented by megahepatocytes, enlarged nucleus and nucleoli and double nucleated hepatocytes (figure 12). Livers of taurine treated normal rats were apparently normal. The changes observed in taurine treated normal rats were similar to those observed in curcumin treated normal rats.

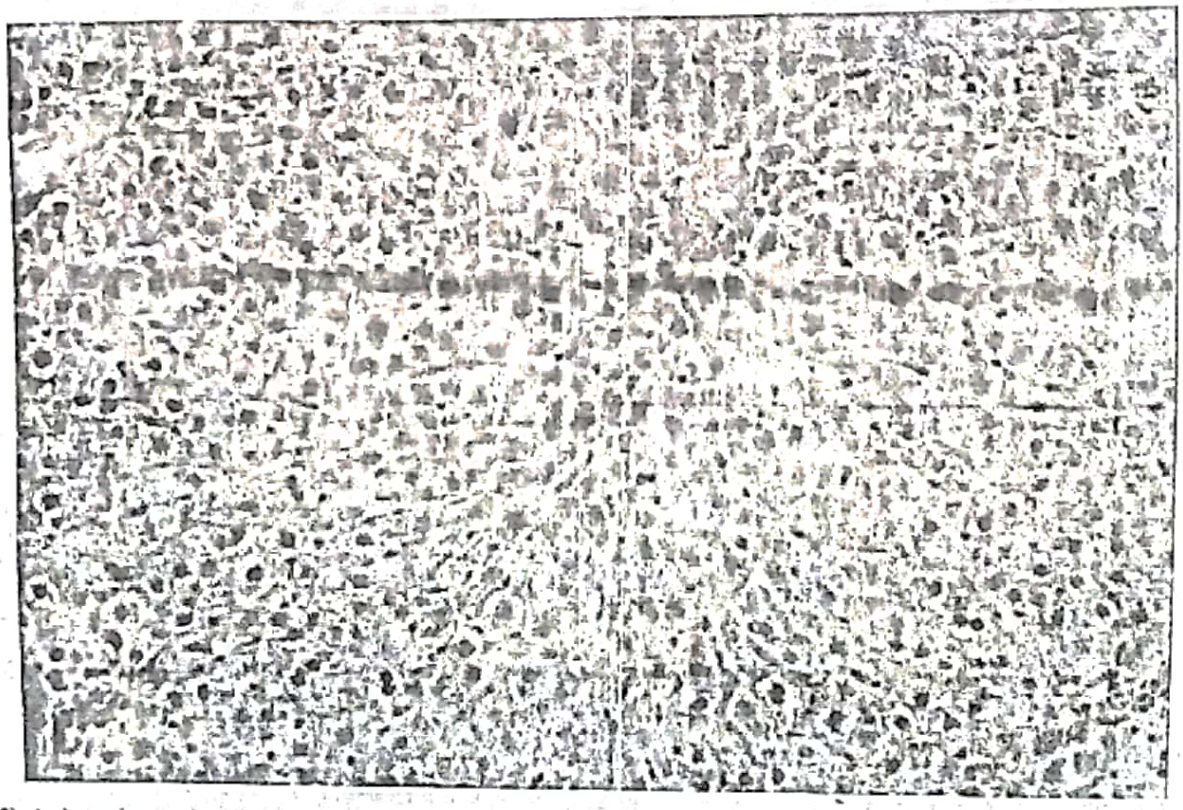

Figure (12): A photomicrograph of liver of normal rat treated with curcumin for 8 weeks showing megahepatocytes with enlarged nucleus and nuclei and double nucleated hepatocytes (H&E x 300)

DISCUSSION

Our results confirm the previous reports of Bhakta et al.[24] who revealed that subcutaneous administration of CCl_4 induced significant hepatocellular damage as evident from a significant elevation of serum activities of ALT, AST and ALP levels. It was reported that the metabolism of CCl_4 resulted in oxidative stress, which induces the production of reactive oxygen intermediates (ROIs) in both hepatocytes and Kupffer cells[25]. These ROIs in turn lead to activation of NF-κB, which mediates the expression of cytotoxic cytokines, involved in inflammation[26]. In addition, oxidative stress caused by CCl_4 resulted in reduction of glutathione under conditions in which increased lipid peroxidation was reported. During induction of cirrhosis enzymes scavenging free radicals such as glutathione reductase, peroxidase and catalase are decreased[27, 28, 29].

The present investigation demonstrated that treatment with CCl_4 significantly decreased total protein, albumin and globulin levels.

Albumin is the single most abundant serum protein and is exclusively synthesized by the liver[30]. Two possible reasons exist for why albumin synthesis rates may be reduced in cirrhosis. The first reason is that in patients with ascites, albumin might enter directly into ascitic compartment. The second one is that a defect might exist in cellular transport of albumin in cirrhotic patients[31].

Results of the present study indicated that oral pretreatment of rats with silymarin caused a significant reduction in ALT, AST and ALP. In addition, it caused regeneration of large number of hepatocytes with formation of new hepatic lobules as presented in histopathological results. It could be postulated that the hepatoprotective action of silymarin may be due to: first: the anti-lipoperoxidative action that it is the most important factor as a hepatoprotective activity of silymarin and its constituents related to lipid metabolism[32]. The second possibility is the regulatory effect towards membrane permeability which leads to an increase of membrane stability against xenobiotic injury. Thirdly the regulatory effect towards nuclear expression by exerting steroid like effect could be also possible[33]. In addition, Silymarin is able to stabilize cell membrane through its anti-oxidant action that can scavenge free radicals and increase intracellular content of reduced glutathione and superoxide dismutase[34]. This action protects membrane against lipid per-oxidation and damage by free radicals[35, 36].

Furthermore, cytoprotective effect of silymarin may be mediated through suppression of NF-$\kappa\beta$ activation[37]. The possibility for how silymarin inhibits NF-$\kappa\beta$ activation is that NF-κB requires sequential phosphorylation and degradation of I-$\kappa\beta$. Silymarin blocks $I_K\beta$ phosphorylation and degradation, and it consequently, inhibits the release of mediator activated by activation of NF-$\kappa\beta$ as TNF-α (tumor necrosis factor) and interleukin[38].

In the present study, pretreatment of cirrhotic rats with curcumin for 15 days before and throughout CCl_4 administration produced a significant elevation in ALT, AST, ALP and globulin level. However, it caused significant reduction in albumin and A/G ratio. In addition, histopathological results showed mild enlargement of liver and mono-lobular cirrhosis was still present compared with the control cirrhotic rats. This expected effect of curcumin on CCl_4 hepatotoxicity could be through the enhancement of toxicity, oxidative stress and lipid per-oxidation[39]. This could be partially attributed to the elevation of copper level which was observed in individuals with advanced hepatocellular liver disease and in CCl_4 treated rats[40]. Since it was reported that curcumin in the presence of copper turns into a pro-oxidant and enhanced DNA and chromosomal damage[41, 42], the effect which leads to the enhancement of CCl_4 toxicity.

On the other hand, pretreatment of cirrhotic rats with taurine caused a significant elevation in ALT, globulin and A/G ratio. However, it caused a significant reduction in AST and blood glucose levels. Moreover, histopathological study showed regeneration of a large number of hepatocytes but mono-lobular cirrhosis and necrosis of the hepatocytes was still detected. It has been reported that taurine pretreatment enhanced the CCl_4 induced increase in hepatic Ca^{2+} content[43]. Taurine modulates many Ca^{2+} dependent processes [44, 45]. The cellular Ca^{2+} stores in mitochondria and endoplasmic reticulum are known to be regulated by taurine which also controls the activity of two transport systems present in the plasma membrane of cells that are responsible for the efflux of Ca^{2+} from cell: the Ca^{2+}/Mg^{2+} ATPase and Na^{+}-Ca^{2+} exchanger[46]. Ca^{2+} increased the Fe^{2+}-induced NADPH dependent lipid peroxidation in hepatic microsomes and as a result, it causes increment of hepatic lipid peroxidation and liver damage[43].

CONCLUSION

From the present study it could be concluded that pretreatment of rats with silymarin may protect or at least delay the incidence of liver cirrhosis induced by CCl_4 in rats. On the other hand, curcumin or taurine couldn't protect liver from the same injury induced by CCl_4 in rats.

REFERENCES

1- Marsano LS, Mendez C, Hill D, Barve S and Mc-Clain C: Alcoh. Resear. and Heal.; 27: 247 (2003).
2- Bruck R, Hershkoviz R, lider O, Aeed H, Zaidel L, Matas Z and Barg J: J. Hepatol.; 24: 731 (1996).
3- Muril P, Garciapina T and Perez-Alvarez V: J. Appl. Toxicol.; 12: 439 (1992).
4- Chrungoo VJ, Singh K and Sigh I: Ind. J. Exp. Biol.; 35: 611 (1993).
5- Wu CG, Chamuleau RA and Bosch KS: Incl. Mol. Pathol.; 64(5): 259 (1993).
6- Kropacova K, Misurova E and Hakova H: Radiats. Biol. Radioecol.; 38: 411 (1998).
7- Pietrangelo A, Borella F and Casalgrandi G: Gastroenterol.; 109: 1941 (1995).

8- Mc-Partland JM: **Complem. Med. Inter.**; 40 (1996).
9- Deshpande UR, Grdre SG and Raste AS: **Ind. J. Exp. Biol.**; 36: 573 (1998).
10- Park EJ, Jeon HC, Ko G, Kim J and Sohn DH: **J. Pharm. Pharmacol.**; 52: 437 (2000).
11- Choiu JW and Wei HC: **Zhongcoayoa**; 15: 197 (1983).
12- Nakashima T, Taniko T and Kuriyama K: **Jpn. J. Phamacol.**; 32: 583 (1982).
13- Soto CP, Perez BL, Favari LP and Reyes JL: **Biochem. Physiol. Pharmacol. Toxicol. Endocrinol.**; 119(2): 125-129 (1998).
14- Gandhi VM and Mulky MJ: **Ind. J. Pharmacol.**; 25: 237-239 (1993).
15- Nanji A.A, Jokelainen K., Tipoe G.L., Rahemtulla A., Thomas P. and Dannenberg A.J.: **Am. J. Physiol. Gastrointest. Liver Physiol.**; 284 (2): G321-G327 (2003).
16- Grad SC and Chengelis CP: Animal Models in Toxicology, (eds.), Marcel Dekker, New York; 21-164 (1992).
17-Rietman S and Frankel S: **Am. J. Clin. Pathol.**; 28(1): 56-63. Cited from: Elitech diagnostics kit for the determination of AST and ALT enzymes (1957).
18- Bergmeyer HU: Standardization of enzyme assays. **Clin. Chem.**; 18(11): 1305-1311 (1972).
19-Young DS, Pestaner LC and Gibberman V: **Clin. Chem.**; 21(5): D431-D432 (1975).
20-Trinder P:.**Ann. Clin. Biochem.**; 6: 24-27. Cited in: Elitech diagnostics kit for the determination of blood glucose (1969).
21-Chromy V and Fischer J: **Clin. Chem.**; 23(4): 754-756 (1977).
22-Doumas BT, Waston WA and Bigg HG: **Clin. Chim. Acta.**; 31(1): 87-96 (1971).
23-Armitag P and Berry G: Statistical Methods in Medical Research. Blackwell Scientific Publications, Oxford (1994).
24-Bhakta T, Pulok K, Tapan K and Pal M: **J. Ethnopharmacol.**; 66: 277 (1999).
25-Adams DO, Kao K, Fark F and Pizzo SU: **J. Immunol.**; 124: 293 (1980).
26- Baeuerle PA and Baltimore D: **Cell**; 86: 13 (1996).
27- Camps J, Bargallo T, Gimencz A, Alie S, Caballeria J, Pares A and Javen J: **Clin. Sci.**; 83: 695 (1992).
28- Sanz N, Diez-Fernadez C, Fernandez-Simone L, Alvare Z and Cascales M: **Carcino.**; 16(7): 1585-1593 (1995).
29- Boigk G, Stroedter L, Herbst H, Waldschmidt J, Riecken EO and Shuppan D: **Hepatol**; 26: 643 (1997).
30- Parving HH, Ranek K and Lassen NA: **Scand J Clin Lab Invest**; 37: 643 (1977).
31- Ballmer PE, Walshe D, Mc-Nurlan MA, Watson H, Brunt PW and Garlick PJ: **Hepatol.**; 18: 292 (1993).
32- Skottova N and Krecman V: **Physiol. Res.**; 47: 1 (1998).
33-Valenzulea A and Garrido A: **Biol. Res.**; 27: 105 (1994).
34- Lang I, Deak G, Muzes G, Pronal L and Feher J: **Biotechnol. Ther.**; 4: 263 (1993).
35- Mourelle M, Favari L and Amezcua JL: **J. Appl. Toxicol.**; 8: 351 (1988).
36- Letteron P, Labbe G, Degott C, Berson A, Fromenty B, Delafrge M, Larrey D and Pessayre D: **Biochem. Pharmacol.**; 39(12): 2027 (1990).
37-Manna SK, Kuo MT and Aggarwal BB: **Oncogene**; 18(30): 4371 (1999).
38- Frank N, Knauft J, Amelung F, Nair J, Wesch H and Bartsch H: **Mutat Res**; 523-524 (2003).
39-Frezza EE, Gerunda GE, Farinati F, De-Mario N, Galligioni A, Plebani F, Giacomin A and Van-Thiel DH: **Hepatogastroenterol.**; 41(4): 367 (1994).
40-Antunes LM, Araujo MC, Dias FL.and Takahashi CS: **Carcinog. Mutagen.**; 19: 1 (1999).
41- Araujo MC, Dias.FL and Takahashi CS: **Teratog. Carcinog. Mutagen.**; 19: 9 (1999).
42-Nakashima T, Taniko T and Kuriyama K **Jpn. J. Phamacol.**; 33(3): 515 (1983).
43-Huxtable RJ: **Physiol. Rev.**; 72: 101 (1992).
44-Porter DW and Martin WG: **Comparative Physiology**; 106: 309 (1993).
45-Lombardini JB: **J. Neurochem.**; 51: 200 (1988).
46-Igisu H, Izumi K, Goto I and Kino K: **Pharmacol.**; 14: 362 (1976).

Received: July 26, 2008
Accepted: August 30, 2008

Zagazig J. Pharm. Sci., December, 2008
Vol. 17, No. 2, pp. 6-16

ISSN 1110-5089

دراسات كيميائية حيوية ومجهرية لتأثير السليمارين، الكركومين، تورين على الأصابة الكبدية المحدثة بواسطة رابع كلوريد الكربون فى الجرذان

شيماء مصطفى الشاذلى، محمد نجيب محمد زكريا، أحمد فهمى أحمد، محمد عبد العال محمد

قسم الفارماكولوجى- كلية الصيدلة- جامعة الزقازيق – الزقازيق – مصر

أوضحت هذه الدراسة التأثير الوقائى لكل من السليمارين ، الكركومين أو التورين على كبد الجرذان بعد تكرار الحقن فى التجويف البريتونى لرابع كلوريد الكربون بجرعة ٢٥ميكروليتر/١٠٠ جم ٣ مرات اسبوعيا لمدة ٦ أسابيع. و ذلك بعد تجريع العقاقير المستخدمة لمدة ١٥ يوم قبل و أثناء حقن رابع كلوريد الكربون. و قد أظهرت النتائج أن تجريع السليمارين للجرذان المصابة بجرعة ١٠٠ مجم/كجم أحدث أنخفاضا حقيقيا فى مستويات كل من أسبارتات أمينو ترانسفيراز، الانين أمينو ترانسفيراز، الفوسفاتيز القاعدى، الجلوكوز.وكما أحدث أرتفاعا جوهريا فى مستويات كل من البروتين الكلى، الزلال، الجلوبيولين. بالأضافة إلى ذلك أحدثت الوقاية بالسليمارين إنخفاضا واضحا فى التاثير التليفى المستحدث برابع كلوريد الكربون و التى أصبحت قاصرة على تضخم طفيف فى الكبد. كما أن المعالجة المسبقة للكركومين بجرعة ٧٥ مجم/كجم لمدة ١٥ يوم قبل و أثناء حقن رابع كلوريد الكربون أحدثت زيادة فعلية فى مستويات أسبارتات أمينو ترانسفيراز، الانين أمينو ترانسفيراز، الفوسفاتيز القاعدى، الجلوبيولين. و لكنها أحدثت انخفاضا حقيقيا فى مستوى كل من الزلال، نسبة الزلال / الجلوبيولين. و لقد أظهر الفحص المجهرى لخلايا الكبد بعض التضخم مع وجود حبيبات على سطح الكبد ولكن بدت بعض خلايا الكبد طبيعية . بالرغم من ذلك وجد تليف حول الخلايا المرشحة. إعطاء التورين بجرعة ١ جم/كجم لمدة ١٥ يوم قبل و أثناء حقن رابع كلوريد الكربون أحدث انخفاضا حقيقيا فى مستوى أسبارتات أمينو ترانسفيراز. ومن ناحية أخرى أحدث زيادة فعلية فى مستوى كل من الانين أمينو ترانسفيراز، الجلوبيولين، نسبة الزلال / الجلوبيولين. و لقد أظهر الفحص المجهرى للكبد وجود بعض التغيرات التليفية فى بعض الحالات. و لكن فى بعض الحالات الأخرى أظهرت خلايا برنشيمية طبيعية مع تجدد عدد كبير من خلايا الكبد. و على ضوء النتائج السابقة اتضح أن اعطاء السليمارين كوقاية أدى الى تأخير حدوث مرض التليف الكبدى فى الجرذان المصابة وهوما لم يحدث بواسطة عقارى الكركومين أو التورين.