COMPARATIVE STUDY OF THE PROTECTIVE EFFECT OF SILYMARIN AND COLCHICINE IN INDUCED LIVER FIBROSIS

BY

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Abstract

The comparative protective effect of SM (200 mg/kg P.O daily), COL (0.1 mg/kg P.O daily) and mixture of SM and COL (200 mg/kg and 0.1 mg/kg P.O daily respectively) each given for 28 days on the CCl_4 liver fibrosis were studied. Collagen content in livers of animals treated with CCl_4 was increased compared to control and histopathological examination of liver samples by Masson's Trichome stain (MTC) showed that collagen accumulated in the portal area resulting in the formation of fibrotic tissues. SM has shown a significant recovered enzyme activities in all the changes observed in LF induced by CCl_4 rats, except for serum total cholesterol and serum alkaline phosphatase which were reduced only 35% and 80% respectively as compared with CCl_4 treated rats. The mixture of SM & COL was more potent in the hepato-protection than COL only, but SM was the best hepatoprotective drug.

Key words: Carbon tetrachloride (CCl_4); Liver Fibrosis (LF); Silymarin (SM) and Colchicine (COL)**.**

Introduction

The main causes of liver diseases are viral, autoimmune, drug/toxin, alcohol and nonalcoholic fatty liver disease (***Pinzani and Rombouts, 2004***). Liver damage goes through several stages which are fatty liver, liver fibrosis and liver cirrhosis. LF defined as the reversible wound-healing process that occurs as a result of a repeated injury and wide range of inflammatory reactions in the liver ***(Mera et al., 2014).*** LF results from chronic damage to the liver in conjunction with the excessive accumulation of extracellular matrix proteins including collagen ***(Seki et al., 2009).*** Early stages of fibrosis are reversible either by removal of the specific stimulus or by treatment with antifibrotic medications, whereas late stages, progressing to cirrhosis are less reversible (***Ellis and Mann, 2012*****).** CCl_4 induced hepatic injury as it used as experimental model for anti-inflammatory and hepatoprotective drug screening, promoting hepatic pathology similar to that observed in humans ***(Li et al., 2013).*** Acute administration of a large dose of CCl_4 causes severe necrosis, while chronic administration of lower doses

of CCl_4 frequently used to induce LF ***(Risal et al., 2012)***. SM used to regenerate liver cells damaged by alcohol or drugs, protect against industrial poisons, such as CCl_4 ***(Catalina et al., 2003)*.** Also, SM has an activity against lipid peroxidation as a result of free radical scavenging and the ability to increase the cellular content of GSH. In addition to its ability to regulate membrane permeability and to increase membrane stability in the presence of xenobiotic damage. SM inhibits the transformation of stellate hepatocytes into myofibroblasts, which are responsible for the deposition of collagen fibers leading to cirrhosis. COL effectively inhibits collagen synthesis and fibrosis in experimental animal models; COL is used in the following cases: primary biliary cirrhosis and alcoholic cirrhosis ***(Rodriguez et al., 1998)***. COL protects the liver of experimental animals against several hepatotoxins and inhibits polymerization of microtubules, a process that is believed to be required for collagen secretion. Thus, it is believed to work as an antifibrotic compound by two ways; the first is inhibition of the cellular secretion of procollagen leading to its intracellular accumulation ***(Mosnier et al., 1991)***, the second a stimulation of collagenase activity (**Hellstrom and Bivalacqua, 2000).**

Materials and Methods

Animals

Female albino rats obtained from the animal house of the Institute of Ophthalmology (Giza, Egypt). The animals acclimatized for period of two weeks to adapt themselves with the new location at the animal house. They housed under appropriate conditions of controlled humidity, temperature and light with free access to water and standard pellet rat diet. All animals received human care in compliance with the state authorities following the Egyptian rules of animal protection.

Chemicals

All chemicals from analytical and purified grade provided from Sigma-Aldrich Company. (St. Louis USA), El-Gomhoreya Chemical Co. (Cairo, Egypt), Merck (Darmstadt, Germany) and MUP "Medical Union Pharmaceuticals" (Ismailia, Egypt).

Design of the work

A total of fifty five female albino rats (140-180 g) randomly divided into the five groups. Duration of the experiment was twenty eight days. The groups distributed as follows: ***Control group:*** rat injected with 2 ml/kg corn oil I.P twice weekly for four weeks and given 2 ml D.W daily P.O. ***Rats injected with CCL_4:*** rats injected with 2 ml/kg CCl_4 I.P, dissolved in corn oil (1:1, v/v), twice weekly for four weeks ***(Yachi et al., 2010). Rats injected with both SM & CCL_4:*** rats received 200 mg/kg SM suspension P.O daily ***(Li et al., 2012)*** and they also injected with 2 ml/kg CCl_4. ***Rats injected with both COL & CCL_4:*** rats received 0.1 mg/kg COL P.O dissolved in distilled water daily ***(Huang et al., 2015)*** and they also injected with 2 ml/kg CCl_4 I.P. ***Rats injected with SM, COL & CCL_4: r***ats received both 200 mg/kg SM plus 0.1 mg/kg COL P.O daily, for four weeks. Rats injected with 2 ml/kg CCl_4 I.P after 2 hr from the ingestion of SM and COL drugs.

Measuring parameters

Physical parameters

Organ index (liver & spleen) calculated as follows: Organ index = (organ weight/ rat body weight) X 100 ***(Yang et al., 2005).***

Biochemical serum analysis

At the end of experiment, fasted rats anesthetized by diethyl ether. Blood samples obtained from the retro-orbital junction. All photometric measurements carried out using Shimadzu spectrometer UV-1201 (Japan). Serum Tests: Total cholesterol **(*Watson, 1960*)**, the activity of alanine aminotransaminase (ALT) activity & aspartate aminotransaminase (AST) activity (**Reitman and Frankel 1957)**, alkaline phosphatase (ALP) activity (***Belfield and Goldberg, 1971)***, direct bilirubin (DB) and total bilirubin (TB) ***(Doumas et al., 1985)***.

Biochemical assay of liver homogenate

The liver was dissected, weighted. Tissue homogenates (20% w/v) made by homogenization with saline for one minute using TRI-R, homogenizer. Tubes centrifuged at 3000 rpm for 15 minutes. Assessment of fibrosis markers in Hydroxyproline (HP) content (***Reddy and Enwemeka 1996)***. Assessment of oxidative stress markers as determination of catalase (CAT) activity (***(Claiborne, 1985)***, determination of superoxide dismutase (SOD) (***Minami and Yoshikawa 1979)***, determination of glutathione-s-transferases (GST) (***Habig et al., 1974)***, determination of reduced glutathione (GSH) (***Beutler, 1963)*** and determination of malondialdehyde (MDA) content (***Uchiyama and Mihara, 1978)***.

Histopathological examination

Hematoxylin and Eosin (H&E) for routine histological examination, Masson's Trichome stain (MTC) for fibrosis markers (***Bancroft and Gamble, 2008)***.

Statistical analysis

All data expressed as mean ± standard error of mean ($\bar{\chi}$±SEM). Descriptive statistics were performed using Microsoft Excel 2010. All analysis & graphics were performed using Graph pad prism (windows version 5; Graph pad software 2007). Difference between means was assessed by ANOVA test. Differences were considered statistically significant at $P < 0.05$.

Results

The clinical characteristics as difference of body weight (BW), liver & spleen index as well as the serum levels of (ALT, AST, TC, ALP, DB & TB), hepatic homogenate (HP, MDA, SOD, GSH, GST & CAT) of the studied subjects will be showed in Tables (1, 2).

Concerning, the TC as shown in figure (1) was significantly higher in CCl_4, SM+CCl_4 and SM+COL+CCl_4 groups as compared to normal group. Also, it was significantly lower in SM+ CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to CCl_4 alone treated group. Also it was lower in COL+CCl_4 & SM+COL+CCl_4 groups as compared to SM+CCl_4 group.

Additionally, AST, ALT & ALP as shown in table (2) were significantly higher in CCl_4, SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to normal group. Also, it was significantly lower in SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to CCl_4 alone treated group.

Concerning the serum level of DB and TB as shown in figure (3), they were significantly higher in CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to normal group. Also, they were significantly lower in SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to CCL_4 alone treated group. Also, they were significantly higher in COL+CCl_4 as compared to SM+CCl_4 group.

Concerning the hepatic hydroxylproline, as shown in figure (4) it was significantly higher in CCL_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to normal group. Also, they were significantly lower in SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to CCl_4 alone treated group. Also, they were significantly higher in COL+CCl_4 & SM+COL+CCl_4 as compared to SM+CCl_4 group. In addition it was lower in SM+COL+CCl_4 comparing to COL+CCl_4.

Additionally, the hepatic MDA as shown in figure (5) was significantly higher in CCL_4, SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to normal group. Also it was significantly lower in SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 comparing to CCl_4 alone treated group. While it was significantly higher in COL+CCl_4 & SM+COL+CCl_4 groups comparing to SM+CCl_4. Concerning the hepatic SOD, GST, GSH & CAT as shown in figure (6,7,8&9). They were significantly lower in CCl_4, SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to normal group. Also, they were significantly higher in SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups as compared to CCl_4 alone treated group. In addition, they were significantly lower in COL+CCl_4 & SM+COL+CCl_4 as compared to SM+CCL_4 group.

Additionally, the liver and spleen index as shown in figure (10) were significantly higher in all CCL_4treated groups comparing to normal one. Also they were significantly lower in SM+CCl_4, COL+CCl_4 & SM+COL+CCl_4 groups comparing to CCl_4 alone treated group.

Table (1): physical parameters in all studied groups ($\bar{\chi}$±SEM).

Factor	Controls	CCL_4			
		Alone	SM	COL	SM+COL
BW at beginning of experiment	144.60±1.93	162.2 ± 1.35	152.50± 1.91	156.62 ± 1.43	159.60±1.47
BW at end of experiment	162.20±1.94	154.80 ± 2.53	133.10 ± 1.89	142.10 ± 1.89	141.0 ± 2.26
Liver index	2.68 ±0.04	4.01 ± 0.12	3.07 ± 0.06	3.41 ± 0.12	3.48± 0.07
Spleen index	0.24 ±0.00	0.47 ± 0.02	0.28 ± 0.02	0.37 ± 0.01	0.32± 0.01

All results are expressed as mean ± SEM

Table (2): The levels of serum (ALT, AST, TC, ALP, DB & TB), hepatic homogenate (HP, MDA, SOD, GSH, GST and CAT) of the studied subjects.

Factor	Controls	CCl_4	SM-CCl_4	COL-CCl_4	SM-COL-CCl_4	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
TC(mg/dl)	54.0 ± 2.85	113 ± 2.70	93.8 ± 2.70	58. 0 ± 2.47	80.5 ± 2.20	< 0.05	< 0.05	NS	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
ALT(U/l)	21.60±0.96	70.70±1.24	30.40±1.86	50.30±3.74	39.00±1.23	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
AST(U/l)	28.90±1.05	66.90±2.06	31.40±0.90	46.40±1.22	33.80±1.22	< 0.05	NS	< 0.05	NS	< 0.05	< 0.05	< 0.05	< 0.05	NS	< 0.05
ALP(U/l)	48.60±0.92	108 ±4.12	68.90±1.1	65.90±1.77	60.80±1.85	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	NS	NS	NS
DB(mg/dl)	0.41±0.01	0.88±0.02	0.50±0.02	0.65±0.03	0.57±0.02	< 0.05	NS	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	NS	NS
TB(mg/dl)	0.65±0.02	1.54±0.03	0.77±0.02	0.93±0.03	0.84±0.02	< 0.05	NS	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	NS	NS
HP(µg/g)	150.2± 0.25	248 ± 3.15	159.1 ± 2.88	184.7± 3.30	171 ± 2.68	< 0.05	NS	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
MDA(nmol /g tissue)	61.24± 1.86	132.3 ± 3.87	73.38 ± 1.45	97.36 ± 1.86	85.4 ± 0.95	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
SOD(U/l)	8.90 ± 0.13	2.81 ± 0.19	7. 52 ± 0.10	5. 72 ± 0.11	6. 69 ±0.09	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
GSH(U/l)	0.613±0.022	0.16 ± 0.015	0.518±0.009	0.403±0.019	0.47± 0.011	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	NS	< 0.05
CAT(U/g tissue)	33.29 ± 0.58	12.74 ± 0.41	28.22 ± 1.12	20.39 ± 0.70	24.69±0.82	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
GST (µmol/min/g)	145.8 ± 2.02	73.53 ± 1.44	130.9 ± 2.71	88.85 ± 2.04	111.40±1.56	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

Results are expressed as mean ± SEM, *p*1 for control and CCl_4, *p*2 for control and SM+CCl_4, *p*3 for control and COL+CCl_4, *p*4 for control and SM+COL+CCl_4 , *p*5 for CCl_4 and SM+CCl_4, *p*6 for CCl_4 and COL+CCl_4, *p*7 for CCl_4 and SM+COL+CCl_4, *p*8 for SM+CCl_4 and COL+CCl_4, *p*9 for SM+CCl_4 and SM-COL+CCl_4, *p*10 *for* COL+CCl_4 and SM+COL+CCl_4.

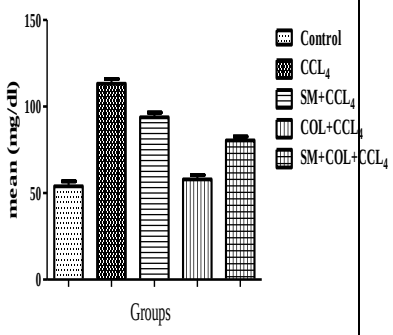


Figure (1): Serum TC level

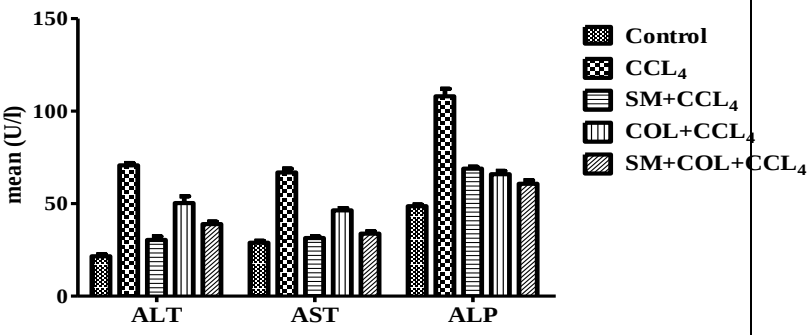


Figure (2): Serum ALT, AST & ALP enzymes**.**

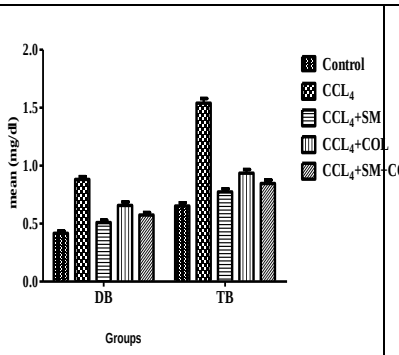


Figure (3): Serum DB & TB.

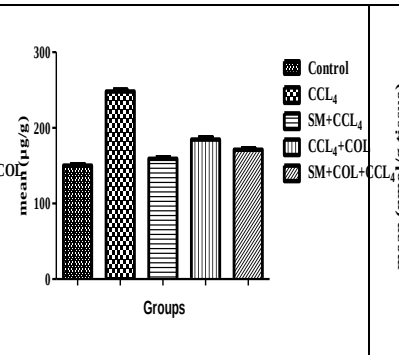


Figure (4): Hepatic HP content.

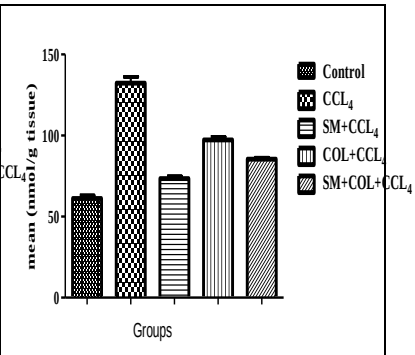


Figure (5): Hepatic MDA content.

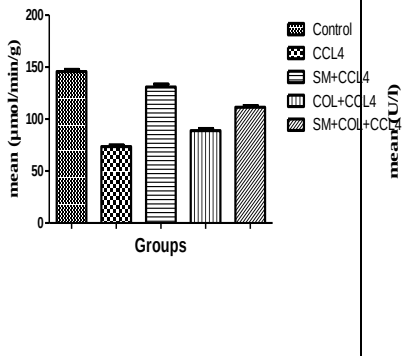


Figure (6): Hepatic GST enzyme.

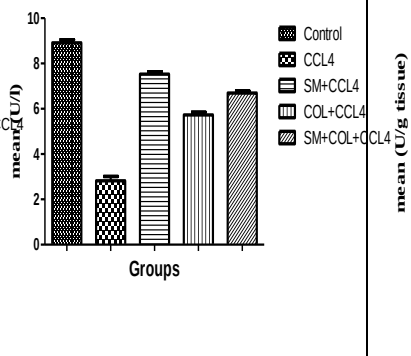


Figure (7): Hepatic SOD enzyme activity.

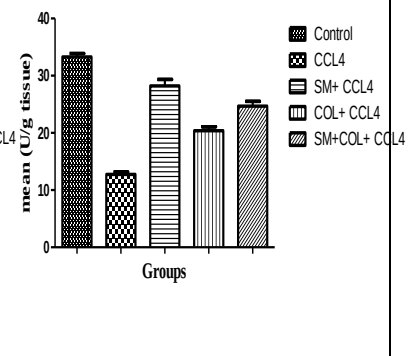


Figure (8): Hepatic CAT enzyme activity.

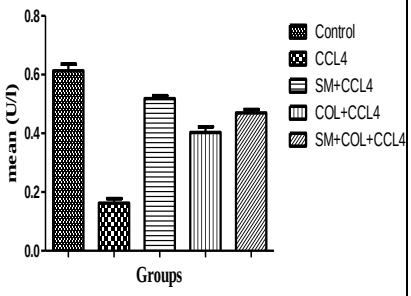


Figure (9): Hepatic GSH.

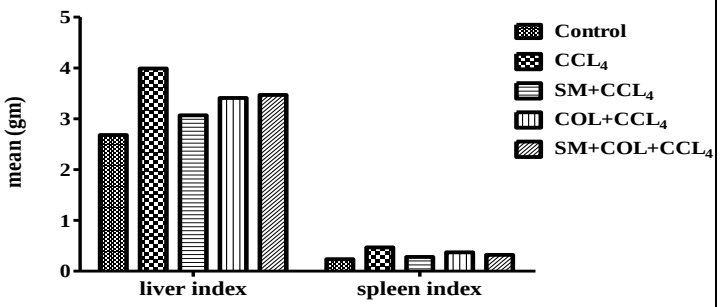
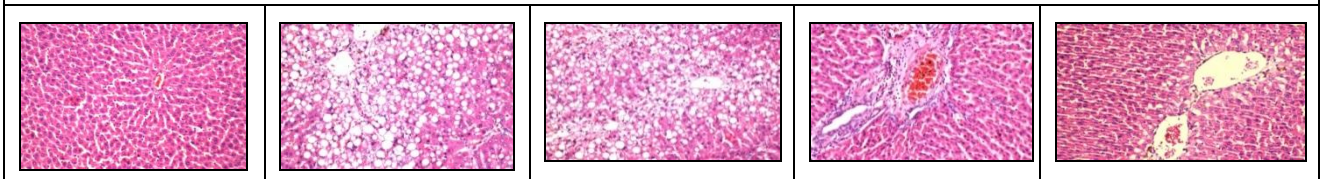


Figure (10): liver and spleen index.

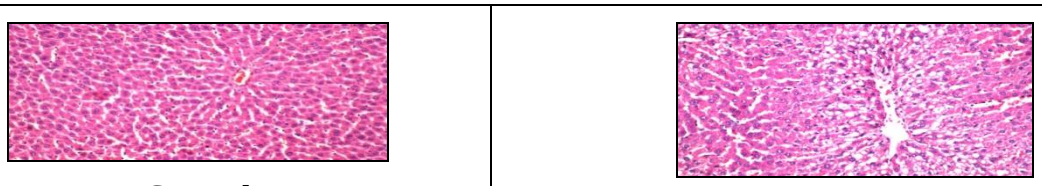
The Histopathological liver examination: Hematoxylin and Eosin (H&E) for routine histological examination will be showed in table (3).

H&E showing Fatty change



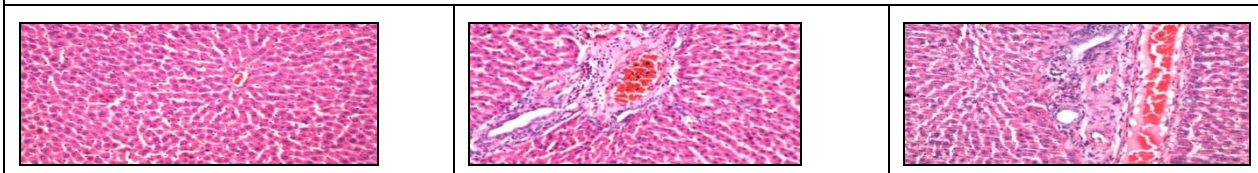
Control | **CCl_4** | **$SM+CCl_4$** | **$COL+CCl_4$** | **$SM+COL+CCl_4$**

H&E showing necrobiosis



Control | **CCl_4**

H&E showing inflammatory cells infiltration in portal area



Control | **CCl_4** | **$COL+CCl_4$**

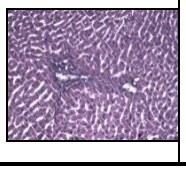
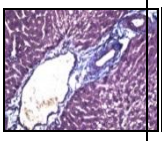
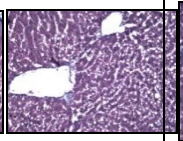
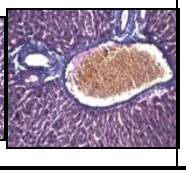
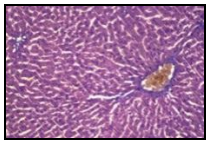
Table (3): The Histopathological liver examination for fibrosis marker by H&E.

Histopathological alteration by H&E	Control	CCl_4	SM+CCl_4	COL+CCl_4	SM+ COL+CCl_4
Fatty change	-	++	++	-	+
Necrobiosis	-	++	-	-	-
Portal infiltration reaction	-	++	-	+	-

Where (+++) for severe with range 75-100%, (++) for moderate with range 50-75%, (+) for mild with range 25-50% and (-) for Nil with range 0-25%.

This study showed H&E liver staining in SM group, showed a moderate fatty change in few hepatocytes only, and so SM protect the liver from necrosis, so it is considered an anti-inflammatory drug. Also, COL group, showed a mild dilatation and congestion in central and portal veins with few inflammatory cells infiltration in the portal area, so SM protect the liver from necrosis, so it is considered an anti-inflammatory drug. Finally, the mixture of SM+COL, showed a mild fatty change in few hepatocytes.

Table (4): The Histopathological liver examination for fibrosis marker by MTC.

Histopathological alteration by MTC	Control	CCl_4	SM+CCl_4	COL+CCl_4	SM+COL+C Cl_4
Blue color of collagen & fibrosis					

This study showed MTC, in CCl_4 group showed extensive accumulation of connective tissues resulting in the formation of continuous fibrotic septa, nodules of regeneration and noticeable alterations to the central veins, compared to those of the healthy controls. COL protected liver tissues, showed a reduced collagen deposition. SM only and the mixture of both COL+SM protected tissues, showed a negative collagen deposition.

2. Discussion

The liver damage produced by the administration of CCl_4, was evidenced by the characteristic pattern of histological and biochemical alterations. Both COL & SM significantly prevented the serum, liver homogenates & histological alterations induced by CCl_4. The presence of LF was evidenced by histological and biochemical determinations in liver tissues. COL given at the dose of 0.1mg/kg rat' day' along with CCl_4 partially inhibits collagen synthesis. COL has an antifibrogenic effect and significantly reduced the amount of collagen as compared to that found in rats receiving CCl_4 only. Also, SM co-treatment partially prevents CCl_4 induced LF.

Hydroxyproline levels in tissue hydrolysates are a direct measure of the amount of collagen present (***Colgrave et al., 2012)***. The present study showed a significantly increased hepatic HP content in the (CCL_4, $COL+CCl_4$ & $SM+COL+CCl_4$) groups when compared with the control group. This study was in agreement with those of ***(Yang et al., 2012***) whom reported that increased expression of hepatic HP is another liver index that represents the degree of LF. Also, the present study showed a significantly decreased hepatic HP content in the (SM, COL and the mixture of SM & COL) groups when compared with the CCL_4 group. This study was in agreement with ***(Li et al., 2011***).

Our data revealed that CCl_4 treatment significantly increased levels of MDA and decreased levels of SOD, CAT, GSH & GST activities in liver tissues. These results were similar to the previous reports (***Breikaa et al., 2013).*** Administration of COL or SM also prevented the increase in liver peroxidation caused by CCI_4. Lipid peroxidation is one of the primary events of $CC1_4$ induced liver damage **(Hernandez-Gea and Friedman, 2011).** The changes produced by CCl_4, in the plasma membrane structure and stability are associated with alterations in its lipid composition and these changes are followed by the increases in lipid peroxidations produced by its free radical metabolites. The total protection against MDA increase could be an effect of COL on plasma membrane of the hepatocytes.

The overproduction of ROS in hepatocytes may cause cell death by damaging DNA, proteins, lipids and carbohydrates **(Khan and Ahmed, 2009)**. The imbalance between the production of ROS and antioxidant defense causes oxidative stress (OS), leading to significant physiological challenges. Hepatic damage induced by CCl_4 is associated with OS due to CCl_4 induced FR production ***(Wang et al., 2011).*** This study was in agreement with ***(Nagata et al., 2007)*** who reported that the mechanism of FR damage included ROS induced peroxidation of the polyunsaturated fatty acid, causing further oxidation of membrane, lipids and proteins. This study was in agreement with ***(Khan et al., 2009)*** who reported that result showed significant reduction in GSH contents as well as significant depletion in the activity of phase II metabolizing enzymes; GST & GSH (***Gumieniczek, 2005)*** and have an agreement with investigation following CCl_4 intoxication ***(Manna et al., 2007).*** SOD is known to be reduced markedly in CCl_4 induced hepatic damage ***(Chen et al., 2007)*** while OS could be ameliorated via the elevation of hepatic SOD level ***(Tirkey et al., 2005).*** This study was in agreement with ***(Kiruthiga et al., 2010)*** who reported that administration of SM significantly protected SOD, CAT, GSH & GST activities by directly scavenging ROS which in turn lowering serum cholesterol and lipid peroxide. There is no doubt that SM has antioxidant like activity and this could be due to the presence of various flavonoids, as reported with ***(Xiao-hui et al., 1997)***.

This study showed H&E liver staining in a control group of rats showed a normal architecture with both central and portal veins. Also, H&E for liver examination of CCl_4 exposed rats resulted in moderate necrosis in hepatic parenchyma; severe fatty changes; mild dilatation in central and portal veins as well as inflammatory cells infiltration; fibrosis and degenerative changes in the portal area. These results are in agreement with ***(Turkdogan et al., 2003)***.

In conclusion, our findings indicate that SM & COL drugs have potent antifibrotic activities. These results were in agreement with ***(Kershenobich et al., 1988)*** who reported that COL is thought to act on collagen accumulation in two ways; the first an inhibition of the cellular secretion of procollagen leading to its intracellular

accumulation, the second a stimulation of collagenase activity. Our findings also indicate that SM and/or COL may be useful in the protection and prevention of hepatic toxicity in CCl_4 treated rats, recovered enzyme activities in the liver (decreased hepatic damage), improved cellular injuries & also have potent antioxidant activities that might protect the liver and improve the symptoms of liver injuries by scavenging the ROS to overcome the oxidative damage caused by CCl_4 in artificially induced hepatic injury.

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دراسة مقارنة التأثير الوقائي لكل من السيليمارين و الكولشيسين فى تليف الكبد المحدث للسادة الدكاترة

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يتناول هذا البحث دراسة التأثير الوقائي لكل من السيليمارين و الكولشيسين فى تليف الكبد المحدث والإجهاد المؤكسد الناتج عن حقن رابع كلوريد الكربون مقارنة بالمجموعة الضابطة من البحث. وقد تم قياس بعض العوامل مثل العوامل الفيزيائية وتشمل وزن الجسم، ومعامل الكبد، ومعامل الطحال. العوامل االكيموحيوية (اختبارات على المصل) وتشمل إنزيمات الألانين أمينوترانسفيراز، الأسبرتات أمينوترانسفيراز، الفوسفاتاز القلوى، البليروبين الكلى والبليروبين الغير مباشر والكولستيرول الكلى. ومعايرات إحيائيه على جناسة وتشمل مؤشرات إنزيمات الجلوتاثيون أس ترانسفيرازيس، الكتالاز، سوبرأكسيد ديسميوتيز، جلوتاثيون المختزل ومستويات المالون داى الدهيد. وكذلك بحث التغيرات النسيجية لتليف الكبد المحدث.

أجريت الأبحاث على خمسة وخمسين من إناث الجرذان تتراوح أوزانهن من١٤٠إلى١٨٠جم وقسمت إلى خمسة كما يلى: ١. المجموعة الأولى: تتكون من الجرذان التى تمثل المجموعة الضابطة. ٢. المجموعة الثانية: تتكون من الجرذان التى تم حقنها برابع كلوريد الكربون فى الغشاء البريتونى مرتين أسبوعيا. ٣. المجموعة الثالثة: تتكون من الجرذان التى تم حقنها بالسيليمارين (٢٠٠ مجم/كجم عن طريق الفم) مرة يوميا ، ثم تم حقنها برابع كلوريد الكربون فى الغشاء البريتونى مرتين أسبوعيا. ٤. المجموعة الرابعة: تتكون من الجرذان التى تم حقنها بالكولشيسين (٠.١ مجم/كجم عن طريق الفم) مرة يوميا ، ثم تم حقنها برابع كلوريد فى الغشاء البريتونى مرتين أسبوعيا. ٥. المجموعة الخامسة: تتكون من الجرذان التى تم حقنها بخليط من السيليمارين والكولشيسين عن طريق الفم مرة يوميا لمدة أربعة أسابيع، ثم تم حقنها برابع كلوريد الكربون.

أوضحت الدراسة النتائج التالية بعد مرور أربعة أسابيع:

١. رابع كلوريد الكربون أحدث إرتفاعاً ملحوظاً بوظائف الكبد كلها ذا دلالة إحصائية مقارنة بالمجموعة الضابطة، بينما الكولشيسين حمى الكبد من التليف المحدث برابع كلوريد الكربون، والخليط من السيليمارين والكولشيسين أحدثا إنخفاضاً لكنه أفضل من الكولشيسين بمفرده، لكن السيليمارين أحدث إنخفاضاً ملحوظاً ذا دلالة إحصائية.
٢. رابع كلوريد الكربون أحدث إجهاد مؤكسد بالكبد ذا دلالة إحصائية، بينما الكولشيسين حمى الكبد من التليف المحدث برابع كلوريد الكربون، والخليط من السيليمارين والكولشيسين أحدثا إنخفاضاً لكنه أفضل من الكولشيسين بمفرده، بينما السيليمارين أحدث إنخفاضاً ملحوظاً ذا دلالة إحصائية.
٣. رابع كلوريد الكربون أحدث تليف للكبد ذا دلالة إحصائية بالهيدروكسيبرولين وأيضاً عند فحص الباثولوجى فى الماسون ترايكوم لوحظ تركيز الكولاجين على خلايا الكبد، بينما الكولشيسين حمى الكبد من التليف المحدث برابع كلوريد الكربون، والخليط من السيليمارين والكولشيسين أحدثا إنخفاضاً لكنه أفضل من الكولشيسين بمفرده، بينما السيليمارين أحدث إنخفاضاً ملحوظاً ذا دلالة إحصائية.
٤. رابع كلوريد الكربون أحدث تغيرات بنسيج الكبد عند فحص الباثولوجى بالهيماتوكسيلين والأيوسين، حيث أرتفع عدد الخلايا الدهنية، الخلايا الميتة، والإرتشاح بداخل أنسجة الكبد. بينما السيليمارين بمفرده والخليط مع الكولشيسين كلاهما أحدثا إنخفاضاً بعدد الخلايا الميتة والإرتشاح بداخل أنسجة الكبد.