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Hepatotreatment Effect of Silybum, Spirulina and Propolis Consumption Compared to Silymarin Drug in Experimental Rats

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ABSTRACT

The purpose of this study was to investigate whether Silybum, Spirulina, and Propolis may have a therapeutic impact when compared to the silymarin medication. .The results revealed that,Negative control showed significant lower in weight gain, feed efficiency ratio,serum HDL-c, total protein, albumin, globulin, catalase(CAT),superoxide dismutase (SOD) and Cytochrome P450(CYP450) and significant higher in serum liver enzyme (ALT,AST,ALP, γ GT),total bilirubin ,creatinine, urea ,uric acid,cholesterol,triglycerides,LDL-c, VLDL-c and cholesterol /HDLc, ,LDLc/HDLc and malondialdehyde (MDA) compared to negative control. Administration of Silymarin, Silybum, Spirulina Propolis and mixture of them to liver injured rats for both the treated or preventive group showed a significant decrease in serum liver enzyme (ALT,AST,ALP, γ GT),total bilirubin ,creatinine ,urea ,uric acid, cholesterol, triglycerides, LDL-c, VLDL-c and cholesterol /HDLc, ,LDLc/HDLc and (MDA) and significant increase in weight gain, feed efficiency ratio, HDL-c, total protein, albumin,globulin,CAT,SOD,andCYP450 in compared to positive control.Histopathological examination revealed that consumption of Silymarin, Silybum, Spirulina Propolis can lower pathological changes in injured.It can be recommended that consumption of Silymarin, Silybum, Spirulina Propolis and mixed them could manage treat liver injury rat by improve liver and renal function, lipids profiles ,antioxidant enzymes and Cytochrome P450.

***Keyword*:** Silymarin, Silybum, Spirulina Propolis Nutritional, liver, CCL4, rat.

INTRODUCTION

In the experimental study of hepatology, carbon tetrachloride CCl4 is a hepatotoxic chemical due to trichloromethyl, which induces necrosis of hepatocytes, activates inflammation, and promotes the development of hepatic fibrosis (Santoyo *et al.*, 2006).

As shown by Jacobs *et al.*, (2002), Silymarin (milk thistle) could prevent the absorption of toxins into hepatocytes and regulate the action on cellular and mitochondrial membrane permeability (Anna *et al.*, 2020).

Spirulina, a unicellular filamentous algae belonging to the Oscillatoraceae family, is a cyanobacterium that includes a high amount of proteins as well as vital nutrients such as carotenoids, vitamins, and minerals and is mostly utilised as a food additive (Khan *et al*., 2005b). It also contains numerous biological actions, including the prevention of anaemia, the reduction of medication genotoxicity, and the prevention of fatty liver disease, in addition to hypolipidemic and hypoglycemic effect. According to in vitro research, Spirulina has the potential to remove superoxide radicals and hydroxyl while also inhibiting lipid peroxidation (Mazo *et al*., 2004 Premkumar *et al.*, 2004 and Li *et al.*, 2007).

Propolis is a resinous substance of diverse colours and textures that *Apis mellifera* bees collect from various plant sources. It mostly contains resins, flavonoids, phenolic aldehydes (polyphenols), waxes, fatty acids, essential oils, pollen, organic matter, and other minerals. It also contains vitamins B, C, and E, as well as essential minerals and trace elements (Hegazi *et al.*, 2000). Propolis thus possesses anti-inflammatory, antibacterial, antiviral, immunomodulatory, antioxidant, and anti-proliferative activities (Almeida and Menezes, 2002).

Silybum marianum (*Cardus marianus*), commonly known as milk thistle or St. Mary thistle, is one of the oldest and thoroughly researched plants in the treatment of liver diseases (Pradhan and Girish, 2006). The active constituent of milk thistle is silymarin, a mixture of flavonolignans comprised of 4 isomers: silibinin, isosilibinin, silichristin, and silidianin. Most supplements are standardized according to their silibinin (often called silybin) content, the main component of the silymarin (Post-White *et al.*, 2007). Silymarin/silybin has being currently used for the treatment of cirrhosis, chronic hepatitis and liver diseases associated with alcohol consumption and environmental toxin exposure (Gazak *et al.*, 2004).

The purpose of this study Silybum, Spirulina, and Propolis could treat hepatic toxicity caused by carbon tetrachloride better than silymarin medication.

MATERIALS AND METHODS

Materials

1-Spirulina (biomass of cyanobacteria (blue-green algae) dried powder and silybum seeds(Cardus marianus) were obtained at a local herbal store in Cairo, and porpolis powder was purchased from an unique beehive retail market in Beni Suef.

The Research Institutes of Ophthalmology in Giza, Egypt, provided 70 male white rats (181±8 g). Rats were placed in plastic mesh cages in an air-conditioned unit with a 12 hour light/dark cycle. Rats were fed a conventional food

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and water ad libitum for a week before to experimentation to acclimate them. The experimental protocols were authorised in accordance with the Ethics Committee, Faculty of Specific Education, Mansoura University, Egypt's Guide for the Care and Use of Laboratory Animals.

2-According to NRC(1995), the usual diet consisted of maize starch (497g/kg), casein (200g/kg), sucrose (100g/kg), corn oil (50g/kg), cellulose (30 g/kg), mineral mixture (100g/kg), vitamins mixture (20g/kg), and DL-methionine (3g/kg) acquired from El-Gomhuria Company for Chemicals

3-Carbon Tetrachloride was purchased from El-Gomhuria Company for Chemicals in El-Mansoura, Egypt (3 ml/kg body weight, 50% in olive oil).

According to Lee *et al*., the predicted dose for producing rat liver damage was 0.5ml/bw rat, delivered by back intraperitoneal injection (2005).

4-Silymarin (TMLegalon 140) tablets were acquired from El-Gomhuria Company for Chemicals in El-Mansoura City, Egypt, from a pharmacy.

Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt, provided 5-kits.

Methods

Experimental protocol

Next the period of adaptation Rats were divided into seven groups at random. The first group only followed the standard diet (-V).

The other six groups were fed a standard diet and injected intraperitoneally with two doses of CCl4 before being reclassified as the control group (+ve), the treated groups fed 200 mg/kg bw silymarin, 400 mg/kg bw Silybum, 400 mg/kg body weight spirulina, 300 mg/kg body weight propolis, and 300 mg/kg body weight a mixture of the above agents (100 mg/kg silymarin, 200 mg and 150 mg/kg of Propolis) by gastric gavage every day for eight weeks. Chapman *et al*. calculated the daily food intake, weekly body weight gain, and feeding ratio (FER).

The serum enzymes activity of liver] alanine and aspartate amino transferase (ALT and AST), alkaline phosphatas (ALP) and gamma glutamyltransferase (GT), total bilirubin, total protein, albumin (A) creatinine, urea, and uric acid, and serum lipid profiles such as cholesterol, high-density lipoprotein cholesterol (HDLc) Serum superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and cytochrome P450 levels were measured in accordance with Beuchamp and Fridovich. Sinha (1972), Draper and Hadley (1990), and Crespi *et al*., (2002) are three examples. Serum globulin, low density lipoprotein cholesterol (LDLc), very low-density lipoprotein cholesterol (VLDLc), albumin/globulin ratio (A/G), and athrogenic indexes were calculated using Coles (1974), Lee and Nieman (1996), Friedewald *et al*. (1972), and Castelli and Levitar (1973).

Histological study:

The liver was extracted and fixed in 10% neutral buffered formalin, then cleaned in xylene and embedded in paraffin. 4-5um tick sections were produced and stained with Hematoxylin and Eosin (H&E) for future histopathological evaluation (Bancroft *et al.*, 1996).

Analyses statistical

SPSS software was used to conduct statistical analysis on the gathered data.

Calculations were made using analysis of variance ANOVA and subsequent LSD (SPSS). According to Artimage and Berry, computer programmes vary (1987).

RESULTS AND DISCUSSION

Carbon tetrachloride CCl4 has been shown to be hepatotoxic in rats.

Table (1) shows that the Control (+ve) group had lower final weight, weight growth (%), and FER (p0.001) than the Control (-ve) group. Overall, all treated rat groups exhibited substantial increases in final weight, weight growth, weight gain (percent) and FER (p0.001) in comparison to the control (+ve) group. There was no significant difference in final weight, weight growth, weight gain (percentage) and FER between the mixed group and the negative control group.

Table 1. Nutritional indications for hepatotoxic rat groups after treatment with diets enriched with silymarin, silybum, spirulina, propolis, and Their mixture.

Variables Groups		Final Weight(g)	Weight Gain(g)	Weight Gain%	Food Intake(g)	(FER)
Negative control group		a 228.50 ±7.47	a 47.50 ±4.86	a 26.34 ±2.81	a 21.53 ±2.98	a 0.036 ±0.013
CCL4 induced-hepatotoxic groups	Positive control	d*** 193.80 ±3.61	f*** 12.20 ±4.80	f*** 7.32 ±2.39	ab 18.45 ±0.49	d*** 0.011 ±0.0065
	Silymarin	c* 206.50 ±4.11	e*** 20.90 ±1.66	e*** 11.33 ±1.02	a 19.43 ±0.56	cb*** 0.0111 ±0.008
	Silybum	ab 218.20 ±8.74	b** 36.00 ±1.76	b** 19.49 ±1.50	a 19.97 ±0.25	bc** 0.021 ±0.0135
	Spirulina	c* 205.80 ±5.34	d*** 24.30 ±1.70	d*** 13.39 ±0.91	a 19.25 ±0.54	ab 0.030 ±0.0017
	Propolis	bc* 212.60 ±5.93	c*** 30.00 ±2.36	c*** 16.43 ±1.28	a 19.49 ±1.50	b** 0.025 ±0.008
	Mixture	ab 227.60 ±8.94	a 44.10 ±2.28	a 24.17 ±1.34	a 20.46 ±0.32	a 0.035 ±0.011

All data were expressed as mean ± SD. All data were expressed as mean ± SD. Significant with control group * p < 0. 05 ** p < 0. 01 *** p < 0.001. Mean values in each column having different superscript (a b,c,d,)were significantly different at P < 0.05 . Mixture: silymarin, silybum, spirulina, propolis

The effect of all studied extracts on food intake (FI), body weight gain ratio (BWG %) and feed efficiency ratio (FER) were illustrated in table (1) and figures 2 and 3. It could be observed that CCL4-positive contral group rats group showed high significant decrease in body weight gain (g), body weight gain percentage (BWG %) and feed efficiency ratio (FER) compared to control negative group. El-Sayeda et al (2012) Saber et al., (2011) and Aldo Ferreira et al., (2010). reported similar results this finding is due to hepatotoxic effect of CCL4 that affects weight gain in those rats but observed that all cirrhotic groups fed on basal diet with different levels of spirulina (0.25, 0.5 and 1%) had significant increase in body weight gain (BWG %) and food efficiency ratio (FER) compared with control positive rats.In agreement with Shaker et al., 2010 and Soufy, 2012 treatment with silymarin, silybum extract and propolis showed significant increase in final weight (g), weight gain (g), food intake (g) and FER, similar results were obtained on treatment with spirulina the same as results reported by Osman et al., 2012. Moreover, treatment with a mixture of all extracts showed significant

increase in final weight (g), weight gain (g), weight gain (%) food intake (g) and FER.

The data in table (2) showed that the control (+ve) group had a significant increase in serum AST, ALT, ALP, GT, and bilirubin values when compared to the control (-ve) group. However, when compared to the control (+ve), all treated groups silymarin, silybum, spirulina, propolis, and a combination of them showed a significant decrease in serum AST, ALT, ALP, GT, and bilirubin values. The results of the group fed a mixture of them showed a significant decrease in serum AST, ALT, ALP, GT, and bilirubin when compared to the treated groups.

Weber et al., (2003) investigated the CCl4 induced hepatic toxic accompanied by elevation in serum levels of AST, ALT,ALP, and GT enzymes with increased levels of serum bilirubin, creatinine, urea, and uric acid, lipids profile, and increase Oxidative stress, thereby confirming the typical hepato toxic effects of CCl4 in rats. These findings are consistent with those of Ozturk et al., (2012), Albassam et al., (2017), and Fenclova et al., (2019), who found that the ameliorating effect of silymarin and silybum on liver enzymes (ALT, AST, and ALP) from hepatic damage induced by CCl4 was elevated in hepatotoxic rats, but rats treated with Silymarin or Silybum after CCl4 Saber et al., (2011) and Osman et al., (2012) discovered that spirulina appears to preserve the structural integrity of the hepatocyte cell membrane, as evidenced by a significant reduction in CCl4-induced elevation of serum enzymes. The decrease in serum enzymes could be attributed to their ability to prevent intracellular enzyme leakage via membrane stabi lising activity. Spirulina has been shown to have potent antioxidant and free radical scavenging properties. Spirulina's protective effect against CCl4-induced injury can be attributed to its high levels of antioxidants such as vitamins, carotenoids, and phycocyanin. In a similar study, Ozturk et al., (2012) agreed with our findings as they demonstrated significant elevations in serum creatinine, urea, and uric acid in rats administered only by CCL4 and reduced by consumption of silymarin. Demir et al., (2014) and You After treatment with diets supplemented with silymarin, silybum, spirulina, propolis, and a combination of these, the lipid profile of all hepatotoxic rats improved significantly.

Table 2. Serum liver enzymes and bilirubin levels in hepatotoxic male rats after treatment with diets enriched with silymarin, silybum, spirulina, propolis,and Their mixture.

		Serum liver enzymes and bilirubin levels						
Variables Groups		**ALT m/ml**	**AST m/ml**	**ALP m/ml**	**γGT**	**Total Bilirubin**	**Direct Bilirubin**	**Indirect Bilirubin**
control –ve		f 42.99±2.59	f 31.63±3.51	a 155.07±12.05	f 25.0±4.69	c 0.61±0.12	d 0.46±0.03	e 0.15±0.04
CCL4 induced-hepatotoxic groups	Positive control	a*** 125.46±12.09	a*** 193.84±13.87	b*** 88.50±8.6	a*** 270.08±14.29	a*** 3.86±0.28	a*** 1.79±0.16	a*** 2.07±0.16
	Silymarin	d** 72.85±7.62	d*** 122.41±1.61	d** 54.2±5.22	bc** 173.94±11.61	b** 1.44±0.11	bc* 0.79±0.09	c** 0.65±0.09
	Silybum	c*** 83.14±8.34	b*** 163.18±12.4	bc*** 64.9±6.79	b * 183.75±13.61	b** 1.38±0.17	bc** 0.75±0.11	c*** 0.63±0.16
	Spirulina	cd** 74.18±7.43	bc*** 155.59±11.81	c*** 60.2±6.65	b* 181.32±11.94	b** 1.95±0.11	b** 1.02±0.13	b** 0.93±0.13
	Propolis	b*** 96.7±9.28	b*** 172.32±13.04	b*** 73.2±7.34	b** 192.15±14.18	b** 1.92±0.14	b** 1.06±0.16	bc** 0.86±0.17
	Mixture	de** 62.1±6.32	e** 74.97±5.5	de* 45.6±4.42	bc** 172.6±16.61	c 0.85±0.11	cd 0.58±0.10	de 0.26±0.04

All data were expressed as mean ± SD. All data were expressed as mean ± SD. Significant with control group * p < 0. 05 ** p < 0. 01 *** p < 0.001. Mean values in each column having different superscript (a ,b, c,d,.) were significantly different at P < 0.05. Mixture: silymarin, silybum, spirulina, propolis

The data in Table (3) indicated that the control Positive rat group had a substantial rise in blood A/G ratio, creatinine, urea, and uric acid(p<0.001&0.05), but a significant reduction in serum total protein and globulins (p0.05). Additionally, all treated groups fed silymarin, silybum, spirulina, propolis, or a combination of them demonstrated a significant decrease in serum A/G ratio, creatinine, urea, and uric acid, as well as a significant increase in serum total protein and globulin (p<0.001), when compared to the control positive group. The group given a combination of them had no significant difference in blood total protein, albumin, globulins, creatinine, urea, or uric acid levels as compared to the negative control group.

Table 3. Serum indicators of liver and kidney function in hepatotoxic rats fed diets enriched with silymarin, silybum, spirulina, propolis, and Their mixture.

Variables Group		**Total Protein(g/dl)**	**Albumin (g/dl)**	**Globulin (g/dl)**	**(A/G) Ratio**	**Creatinine (mg/dl)**	**Urea (mg/dl)**	**Uric acid (mg/dl)**
Negative control group		a 7.15±0.64	a 4.01±0.32	a 3.14±0.34	c 1.21±0.29	e 0. 46±0.03	b 35.03±3.22	b 2.47±0.85
CCL4 induced-hepatotoxic groups	Positive control	b** 5.19±0.32	a 4.12±0.32	c*** 1.76±0.41	a*** 1.94±0.33	a*** 1.12±0.19	a*** 54.96±2.72	a*** 4.57±0.13
	Silymarin	a 7.06±0.60	a 4.09±0.38	b* 2.65±0.56	b* 1.63±0.52	d* 0.61±0.04	b 34.88±3.34	b 2.55±0.16
	Silybum	a 6.87±0.38	a 4.16±0.36	ab 2.7±0.63	b* 1.66±0.61	c** 0.72±0.05	b 33.58±3.01	b 2.50±0.08
	Spirulina	a 7.13±0.11	a 3.98±0.25	ab 2.8±0.44	b* 1.43±0.29	d* 0.63±0.3	b 33.98±3.46	b 2.50±0.12
	Propolis	a 6.88±0.31	a 4.12±0.42	ab 2.75±0.53	b* 1.57±0.46	b** 0.92±0.05	b 34.59±2.72	b 2.48±0.14

All data were expressed as mean ± SD. All data were expressed as mean ± SD. Significant with control group * p < 0. 05 ** p < 0. 01 *** p < 0.001. Mean values in each column having different superscript (a ,b, c,d,.) were significantly different at P < 0.05 Mixture: silymarin, silybum, spirulina, propolis

Similar findings were found in the study of EL-Sayeda *et al.*, (2012), who discovered that all cirrhotic groups fed a basic diet containing varying levels of spirulina had significant reductions in total lipids, triglycerides, and

total cholesterol when compared to the positive control group of rats. Our findings appear to support the findings of Bashandy *et al.,* (2011), who discovered that spirulina significantly reduced HgCl2-induced hepatotoxicity and modulated the lipid profile via its antioxidant properties. However. In a human study conducted by Aldo Ferreira *et al.,* (2010), it was discovered that spirulina extract significantly improved the serum lipid profile in man. Furthermore, Mahran *et al.,* 1996 and El Menyiy *et al.,* 2016 reported that serum total cholesterol, triglycerides, and LDLc were significantly increased while HDLc was significantly decreased in rats injured with thioacetamide (TAA) compared with control rats, the injured rats and fed on aqueous extracts of propolis could significantly modulate the lipid contents by decreasing total lipids profile levels with significantly increase in HDLc,

According to the data in table (4), the positive control group had a substantial rise in blood cholesterol, triglycerides, LDLc, VLDLc, and atherogenic index ($p< 0.001$), whereas the negative control group had a significant drop in serum HDLc ($p< 0.001$). While all treated groups fed silymarin, silybum, spirulina, propolis, or a combination of these showed significant decreases in serum cholesterol, triglycerides, LDLc, VLDLc, and atherogenic index (p 0.05 & p 0.01, respectively) and significant increases in serum HDLc ($p< 0.05$) compared to the positive control group. In comparison to the negative control group, the group given a combination of them exhibited no significant difference in serum triglycerides, HDLc, or VLDLc.

Similar findings were found in the study of EL-Sayeda *et al.,* (2012), who discovered that all cirrhotic groups fed a basic diet containing varying levels of spirulina had significant reductions in total lipids, triglycerides, and total cholesterol when compared to the positive control group of rats. Our findings appear to support the findings of Bashandy *et al.,* (2011), who discovered that spirulina significantly reduced HgCl2-induced hepatotoxicity and modulated the lipid profile via its antioxidant properties. However. In a human study conducted by Aldo Ferreira *et al.,* (2010), it was discovered that spirulina extract significantly improved the serum lipid profile in man. Furthermore, Mahran *et al.,* 1996 and El Menyiy *et al.,* 2016 reported that serum total cholesterol, triglycerides, and LDLc were significantly increased while HDLc was significantly decreased in rats injured with thioacetamide (TAA) compared with control rats, the injured rats and fed on aqueous extracts of propolis could significantly modulate the lipid contents by decreasing total lipids profile levels with significantly increase in HDLc,

Table 4. Serum lipids profile of hepatotoxic rats fed diets enriched with silymarin, silybum, spirulina, propolis, , and Their mixture.

Variables Groups		Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDLc (mg/dl)	HDLc (mg/dl)	VLDLc (mg/dl)	Cholesterol /HDLc	LDLC/ HDLc
Negative control		d 58.56±8.59	cd 30.81±5.75	e 20.17±1.33	a 32.23±2.52	cd 6.16±0.75	d 1.82 ± 0.25	D 0.63 ± 0.25
CCL4 induced-hepatotoxic groups	Positive control	a*** 112.68±6.94	a*** 111.70±6.43	a*** 79.1±7.86	d*** 11.24±1.87	a*** 22.34±1.28	a*** 10.02 ± 1.77	a*** 7.03 ± 1.45
	Silymarin	bc* 85.12±3.06	c 39.24±5.56	b*** 54.58±4.37	bc* 22.69±1.38	cd 7.84±0.51	bc** 3.79 ± 0.34	b** 2.40 ± 0.33
	Silybum	bc* 86.95±2.39	c 40.26±5.49	bc*** 51.71±7.78	ab 27.19±3.11	c 8.05±0.49	c** 3.19 ± 0.33	bc** 1.90 ± 0.31
	Spirulina	bc* 84.57±2.28	c 40.17±6.28	b*** 53.58±5.30	b* 22.97±1.32	c 8.03±0.45	c** 3.69 ± 0.33	b** 2.33 ± 0.16
	Propolis	b** 94.41±2.78	b** 66.40±8.90	b*** 57.97±3.32	b* 23.16±3.72	b* 13.28±1.78	b** 4.01 ± 0.50	b** 2.50 ± 0.31
	Mixture treated	bc* 84.03±1.43	c 38.94±5.0	cd** 49.15±1.02	ab 27.09±3.81	c 7.78±0.40	c** 3.1 ± 0.66	bc** 1.81 ± 0.68

All data were expressed as mean ± SD. All data were expressed as mean ± SD. Significant with control group * p < 0. 05 ** p < 0. 01 *** p < 0.001. Mean values in each column having different superscript (a ,b, c,d,.) were significantly different at P < 0.05 Mixture: silymarin, silybum, spirulina, propolis

The data in table (5) indicated that the control positive rat group had a substantial rise in serum malondialdehyde (MDA) levels but a significant reduction in serum catalase, superoxide dismutase (SOD), and cytochrome CYP450 levels (p0.001). On the other hand, all treatment groups given silymarin, silybum, spirulina, propolis, or a combination of them had a substantial rise in serum catalase, SOD, and CYP450 levels, but a significant drop in serum MDA levels (p0.01), as compared to the control positive group.

It is now generally accepted that oxidative stress due to increased ROS production has a role in the pathogenesis of hepatic injury induced by CCL4. Hepatocytes are continuously exposed to ROS and are protected from oxidative injury by a range of antioxidant pathways (Zhang and Morris., 2003). The state of oxidative stress exists when there is an imbalance between pro-oxidant and antioxidant chemical species.

In the present study, it was observed that there was a significant decrease in SOD and catalase activities with significant increase in MDA (marker of lipid peroxidations) in the serum of rats administered with CCL4 only. Moreover, treatment of rats with spirulina extracts, silymarin, silbum marianum and propolis caused significant improvement in the serum levels of catalase and SOD activities and MDA compared to positive contral group group. Qureshi *et al.,* 2007 demonstrated significant reduction in liver content of GSH with significant elevation of lipid peroxidation in CCL4-treated rats.

In agreement with our study Osman *et al.,* 2012 proved the antioxidant effect of spirulina against hepatotoxicity, that was due to the anti-inflammatory and anti-oxidative properties of its constituent phycocyanin a biliprotein found in spirulina that exerts a scavenging action against reactive oxygen species as well as anti-inflammatory activity, also spirulina has other antioxidant and anti-inflammatory agents such as β carotene, phenolic compounds.

Table 5. Serum levels of many antioxidant enzymes and Cytochrome P450 (CYP450) in the negative control and hepatotoxic rat groups after treatment with diets supplemented with silymarin, silybum, spirulina, propolis, and a Their mixture.

Serum levels of many antioxidant enzymes and Cytochrome P450 (CYP450)					
Variables Group		MDA (Nmol/l)	CAT (U/ml)	SOD (U/ML)	CYP450
Negative control		c 2.60 ±0.71	a 28.00 ±2.58	a 1047.20 ±51.25	a 20.62 ± 1.48
CCL4 induced-hepatotoxic groups	Positive control	a*** 13.48 ± 1.13	e*** 3.90 ±0.48	fe*** 18.45 ± 0.49	e*** 1.11 ± 0.24
	Silymarin	b* 4.95 ±0.34	c*** 18.47 ±1.06	b** 846.39 ±24.95	a 19.05 ± 1.05
	Silybum	b* 5.32 ±0.48	cd** 15.78 ±2.01	c* 741.31 ±23.35	bc* 14.2 ± 0.48
	Spirulina	b* 5.30 ±0.45	c** 181.1 ±1.33	b** 837.97 ±30.07	b** 15.77 ± 0.71
	Propolis	b* 5.73 ±0.59	c** 16.35 ±2.40	d** 637.45 ±26.75	d** 12.13 ± 0.58
	Mixture	c 2.57 ±0.73	b* 22.43 ±1.03	e *** 473.45 ±34.12	cd** 13.4 ±1.21

All data were expressed as mean ± SD. All data were expressed as mean ± SD. Significant with control group * p < 0. 05 ** p < 0. 01 *** p < 0.001 Mean values in each column having different superscript (a ,b, c,d,.) were significantly different at P < 0.05 Mixture: silymarin, silybum, spirulina, propolis

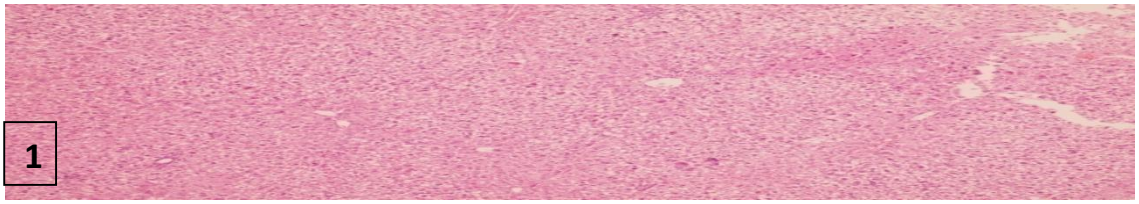
Concerning the antioxidant effect of aqueous propolis extract El-Khatib *et al*., 2002 published results similar to our results in the current study where they found that treatment with aqueous propolis extract normalize lipid peroxidation.

Shaarawy *et al.*, 2009 induced hepatic destruction by N-nitrosodiethylamine (NDEA) plus CCl4. In their experiment, liver function enzymes and hepatic lipid peroxidation (LPO) was found to be increased and superoxide dismutase (SOD), and GSH-dependent enzymes decreased. Pradhan and Girish 2006 also showed that another N-nistroso ALPyl compound, Diethylnitrosamine, induced alterations in the liver tissue and silymarin reversed these negative changes by improving antioxidant capacity. Protective effect of silymarin has also been shown on hepatic damage by many agents such as ethanol (Loguercio and Federico, 2003) and anti-tuberculosis drugs (Gazak *et al*., 2004).

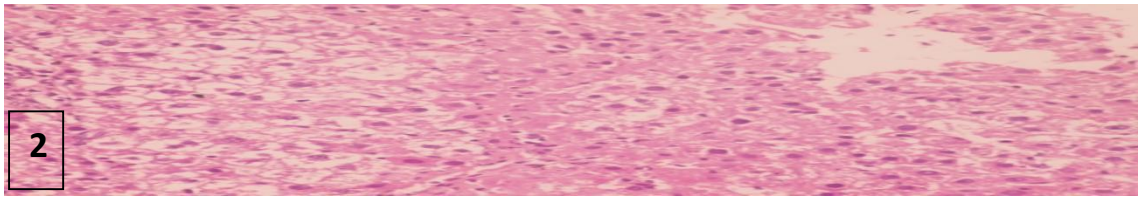
Varga *et al.*, 2004 made hepatotoxicity by cisplatin (CDDP) administration, which is a widely used anticancer drug. They showed that silymarin attenuated serum ALT, AST, liver nitric oxide (NO) and malondialdehyde (MDA) level, previously increased by CDDP.

Liver Examination Histopathological Findings

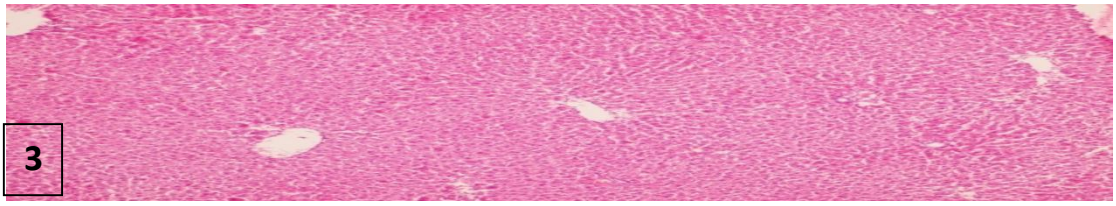
The negative group's liver tissue exhibited the usual histological structure of the hepatic lobule (pic 1). Intact liver architecture—intact central vein or portal tract—no steatosis observed—no necroinflammatory damage identified Microscopically, the liver tissue of the positive control (+ve) demonstrated a region of coagulative necrosis in hepatocytes with infiltration of mononuclear inflammatory cells, cytoplasmic vacuolization of hypatocytes, necrosis of sporadic hepatocytes, and inflammatory damage (pic 2) Examined slices of liver tissue from all injured rats treated with silymarin, silybum, spirulina, propolis, or a combination of these herbs revealed a virtually normal histological structure in pictorial form (3, 4, 5, 6 ,7)



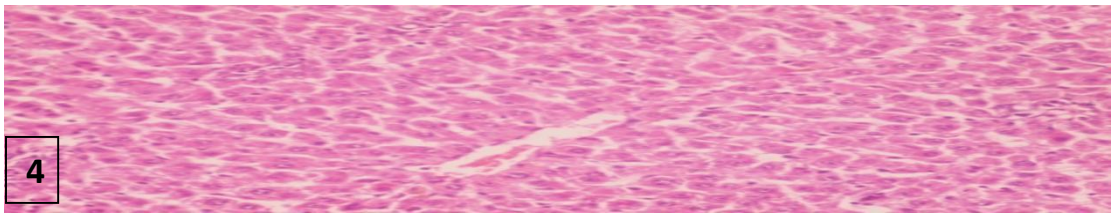
Pict 1. liver specimens showing a=loss of normal liver architecture vacuolated hepatocytes, focal necrosis and sinusoidal congestion (CCL4-untreated rats group, x100).



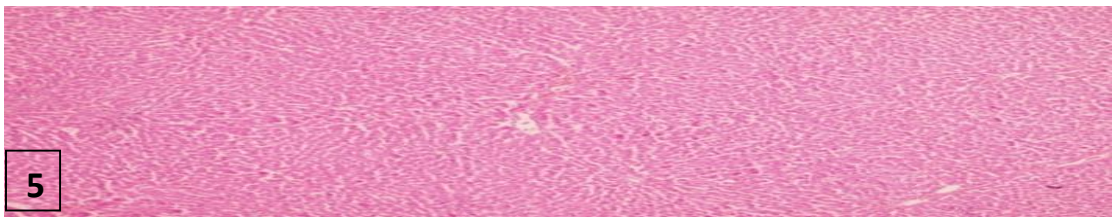
Pict 2. area of coagulative necrosis in hepatocytes with mononuclear inflammatory cells infiltration (positive control (CCL4-untreated rats group, x400),



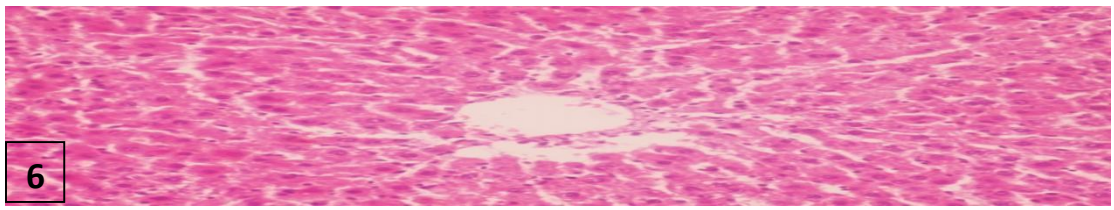
pict 3. nearly normal liver architecture with normal hepatocytes and Kupffer cells (Silymarin-treated group, x100).



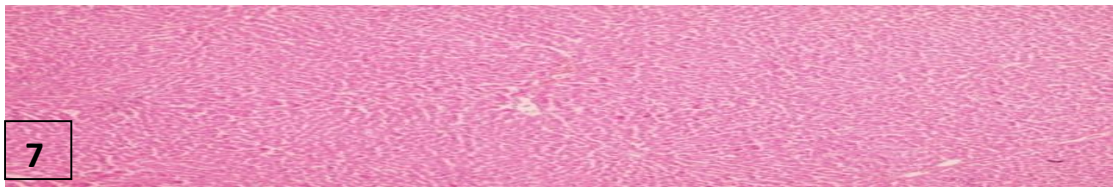
Pict 4. Liver specimens showing a= normal central vein, normal hepatocytes, hepatic cords, sinusoids and Kupffer cells (Silybum-treated group, x400).



Pict 5. normal liver architecture with mild sinusoidal congestion (Spirulina-treated group, x100).



Pict 6. Liver specimens showing a= portal regeneration of hepatocytes with residual degeneration with marked infiltration of sinusoids with mononuclear inflammatory cells (Propolis-treated group, x400).



Pict 7. Totally normal liver architecture with regenerated hepatocytes and normal Kupffer cells (mixture-treated group, x400)

The microscopical examination of liver from CCL4 administered untreated rats revealed a more severe and persistent change in degeneration, swelling of hepatocytes, loss of liver tissue structure, severe dilatation and vascularization, indicating liver injury (Picture 2). This result was previously published by El-Khatib *et al.*, (2002), Shaker *et al.*, (2010), and Soufy, (2012). Furthermore, treatment with silymarin, silybum (pict.3-4-5-6). In agreement with these pathological findings, Ozturk *et al.*, (2012) and Abenavoli *et al.*, (2018) discovered that treatment with silbum marianum and silymarin significantly reduced the liver histopathological findings. EL-Sayeda *et al.*, (2012) and Hotta *et al.*, (2020) discovered that microscopic examination of the livers of fibrotic rats fed a basic diet containing spirulina (0.25 and 0.5 percent) with subcutaneous injection of CCl4 shows mild degenerative change and mild swelling of hepatocytes. Ours is the first study to show a significant improvement in serum liver and renal function, lipid profile, antioxidant enzyme, and CYP450 by a combination of spirulina, album, propolis, and silymarin.

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التأثير العلاجي للسمية الكبدية لتناول السيليبوم والسبيرولينا والبروبوليس بالمقارنة بعقار السيليمارين في فئران التجارب

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تهدف الدراسة لمعرفة التأثير العلاجي والوقائي للسيليمارين،والسيليبوم والسبيرولينا و البروبوليس ومخلوطهم على الاصابة الكبدية في الفئران أظهرت نتائج المجموعة الضابطة الموجبة انخفاض معنوي في قيم الزيادة في الوزن ،ونسبة كفاءة التغذية ومستوي السيرم من عالية الكثافة والبروتين الكلي والألبومين والجلوبيولين وسوبرأكسيد ديسموتازوالكتلاز والسيتوكروم وزيادة معنوية في وظائف الكبد والبيليروبين والكرياتينين واليوريا وحمض اليوريك والكوليسترول والدهون الثلاثية واللليبوبروتينات منخفضة الكثافة ومنخفضة الكثافة جدا ومؤشر تصلب الشراين والمانولداهيد بالمقارنة بالمجموعة الضابطة السالبة كما أظهرت النتائج عند تناول كل من سيليمارين و سيليبوم و سبيرولينا والبروبوليس وخلطهم للفئران المصابة بالكبد للمجموعة المعالجة و الوقائية انخفاض معنوي في إنزيم الكبد والبيليروبين وظائف الكلى الكرياتينين واليوريا وحمض اليوريك والكولسترول والدهون الثلاثية واللليبوبروتينات منخفضة الكثافة ومنخفضة الكثافة جداومؤشرتصلب الشرايين والمانولداهيد وزيادة معنوية في زيادة الوزن ونسبة كفاءة التغذية واللليبوبروتينات مرتفعة الكثافة والبروتين الكلي والألبومين والجلوبيولين والكتالاز والسوبراكسيد دسموتاز والسيتوكروم بالمقارنة بالمجموعة الضابطة الموجبة كما أظهرت نتائج الفحص الهستولوجيا أن تناول كل من سيليمارين و سيليبوم و سبيرولينا و البروبوليس ومخلطهم خفض من التغيرات المرضية للانسجة وتوصي الدراسة بضرورة تناول السيليمارين وسيليبوم وسبيرولينا البروبوليس ومخلطهم لعلاج الاصابة الكبدية لتحسين وظائف الكبد والكلى و دهون الدم ومضادات الأكسدة. و السيتوكروم

الكلمات المفتاحيه :السيليبوم-السبيرولينا–البروبوليس- السيليمارين-رابع كلوريد الكربون .