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Hepatoprotective Effect of Silymarin and Propolis in Chemically Induced Chronic Liver Injury in Rats

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Hepatoprotection, DEN, silymarin, propolis, rat

ABSTRACT

Silymarin and propolis among phytochemicals that show anti-inflammatory, healing, antioxidant and several other promising properties. Our aim is to investigate protective effect of silymarin and propolis in chemically induced chronic liver injury in rats.

A total of 60 male albino rats (90±10 days old) were divided equally into ten groups: (6 rats per each group). The experiment lasted along four months, through which all groups except for group 1 and 6 were given silymarin and propolis. Liver injury was induced in groups 6, 7, 8,9,10 while other groups were injected with saline and oil of the same volume as following. Group 1: served as negative control, the rats were fed on basal ration and water *ad libitum*. Group 2 (silymarin 100 mg/ kg b.wt). Group 3 (propolis 100 mg/kg b.wt). Group 4 (propolis 150 mg/kg b.wt). Group 5 (propolis 200 mg/kg b.wt). Group 6 served as positive control (DEN+CCl_4). Group 7 (liver injury+ silymarin 100 mg/ kg b.wt). Group 8 (liver injury+ propolis 100 mg /kg b.wt). Group 9 (liver injury + propolis 150 mg/kg b.wt). Group 10 (liver injury + propolis 200 mg/kg b.wt). Blood samples were collected from all groups after four months and serum samples were separated for biochemical analysis of liver functions and tumor markers

Administration of DEN+CCl_4 significantly increased liver weight, relative liver weight, AST, ALT, ALP, GGT, AFP and CEA while, body weight, total protein, albumin and A/G ratio were markedly decreased. Treatment with silymarin and propolis caused improvement of liver function and reduction of tumor markers.

The results indicated a protective effect for silymarin and propolis against hepatic injury induced by DEN+CCl_4 that may be due to their ability to block the bioactivity of those hepatotoxicants and via their antioxidant properties.

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1. INTRODUCTION

Liver has an essential role in regulation of several physiological processes as storage, secretion and metabolism also, it detoxifies a variety of drugs and xenobiotics and plays a central role in clearing the chemicals therefore it is susceptible to the toxicity from these agents. That's why, Liver diseases remain to be serious health problems and its management is still a challenge to the modern medicine (Hamza, Al-harbi, 2015).

The chronic liver diseases are common worldwide and characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis that causes serious complications including portal hypertension, variceal bleeding, intractable ascites, and hepatic encephalopathy that resulted in hepatic failure which can eventually progress to liver cancer (Heindryckx, Gerwins, 2015).

Diethylnitrosamine (DEN) is an N-nitroso alkyl compound described as an effective hepatotoxic agent in experimental animals. DEN is found in a wide variety of foods such as cheese, soybeans, smoked, salted and dried fish, cured meat and alcoholic beverages. Metabolism of certain therapeutic drugs is also reported to produce N-nitrosodiethylamine. DEN became metabolically active by the action of cytochrome P 450 enzymes to produce reactive electrophiles, which increase

oxidative stress level leading to cytotoxicity and liver injury (Shaarawy et al., 2009)

Carbon tetrachloride (CCl4) is one of the xenobiotics that have been reported to induce acute and chronic tissue injuries and is a well-established hepatotoxin so it has been used extensively to study the hepatotoxicity in animal models by initiating lipid peroxidation, in addition to liver pathogenesis. Liver is particularly susceptible to oxidative stress due to the direct release of CCl4 metabolites and cytokines, which propagate inflammatory responese leading to hepatic steatosis, centrilobular necrosis, and cirrhosis in the liver (Faten et al., 2014)

Metabolism of certain Free radicals and reactive oxygen species (ROS) played a crucial role in development of liver diseases. As the unique vascular and metabolic features of liver, it exposed to absorbed drugs or xenobiotics in concentrated form. Drug-metabolizing enzymes detoxify many xenobiotics but bioactivate the toxicity of others. In case of bioactivation, liver is the first organ exposed to the damaging effects of newly formed toxic substance. Thus, protective measurements for liver are of particular interest. Considerable efforts are being made to obtain useful herbal medicines from documented medicinal plants for a wide variety of clinical conditions. Dietary antioxidants of natural products may serve as therapeutics to cope with liver damage against free radicals and ROS-induced liver diseases pathology and progression. Natural antioxidants in complex mixtures if ingested with the diet are more efficient than pure compounds in preventing oxidative stress-related pathologies due to particular interactions and synergisms by modulating antioxidant, drug-metabolizing, and repairing enzymes along with acting as signaling molecules in important cascades for cell survival (Bhadauria, 2012)

Chemoprevention is defined as the use of natural or synthetic chemical agents to reverse, suppress or prevent carcinogenic progression to invasive cancer (Sporn, Liby., 2005). Many chemopreventive agents are phytochemicals, namely non-nutritive plant chemicals that have protective or disease preventive properties (Seren etal., 2008; Mann et al., 2009; Glauert et al., 2010; EL-Mesallamy et al., 2011).

Numerous studies had been carried out to evaluate silymarin on patients with adverse liver conditions. Silybum marianum L. is a member of the family (Asteracae) widely used in traditional European medicine. Silymarin, a polyphenolic flavonoid isolated from milk thistle, primarily consists of four isomeric componds of active flavonolignans: silychristin, silydianin, and two groups of diastereoisomeric flavonolignans, silibinin, and isosilibinin. The silibinin, a flavanone, is the major and the most active component present in Silymarin that represents about 60–70%. Silymarin had clinical applications in treatment of toxic hepatitis, fatty liver, cirrhosis, ischaemic injury, radiation toxicity and viral hepatitis as a result of its antioxidative, anti-lipid-peroxidative, antifibrotic, anti-inflammatory, immuno-modulating and even liver regenerating effects. It protects against a wide range of carcinogen and tumor promoter-induced cancers (Kabiri et al., 2013).

Apitherapy or therapy with bee products (e.g. honey, pollen, propolis, fortified honey, and herb honey) is an old tradition that has been reviewed by researchers. These products, receiving renewed focus on their beneficial effects in a general “back to nature (Bhadauria et al., 2008).

Bees and bee products have long been recognized for their medicinal properties, often being sold as nutritional supplements and health products. There has been renewed interest in the medicinal properties of honey bee products which include antibacterial, antifungal, cytostatic, wound healing, anti-tumor effects and anti-inflammatory effects. Propolis (bee glue) is the generic name for the resinous substance collected by honeybees from various plant sources. It is rich in biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic and ketones several biological and pharmacological properties, such as immunomodulatory, antitumor, antimicrobial, antiinflammatory, antioxidant, among others. Several researchers have reported the antitumoral property of propolis in vivo and in vitro. Propolis antiproliferative activity on tumor cells has been demonstrated and some responsible compounds were isolated which inhibited the growth of hepatoma cells and arrested the tumor cells at S- phase,also it showed antioxidant activity and was cytotoxic to human hepatocellular carcinoma (Naama et al., 2010)

The aim of this study was to throw light on the protective role of silymarin and propolis against liver injury through measuring liver function, tumor markers and liver body weight index.

2. MATERIALS AND METHODS

2.1. Experimental animals:

Total number of 60 male albino rats aging 90±10 days old, weighing 110 : 150 gram were obtained from Egyptian company for production Vaccine, Sera and Drugs, and kept for a period of 14 days before starting the experiment to be adapted with the environment. The animals were housed in clean metal cages with a 12 hour day-night cycle, temperature of 22 ± 2.0°C and humidity of 45: 46%. The rats were fed with a balanced commercial diet (21% protein) and the drinking water provided *ad libtum*.

2.2. Chemicals and natural products

2.2.a. Diethyl nitrosamine (DEN) from sigma Aldrich was dissolved in saline given at a dose of 200 mg/kg b.wt / i.p. given once

2.2.b. Carbon tetra chloride (CCl_4) from Central drug house (CDH) was dissolved in olive oil given at a dose of 3 ml/ kg b.wt /sc two times/ week

2.2.c. Commercial colorimetric and ELISA kits

2.2.d. Silymarin as standard drug (80%): was obtained as a gift from Mepaco Company Egypt was dissolved in distalled water to reach a dose of 100 mg/ kg b.wt /oral daily

2.2.e. Bee propolis extract capsules (4:1): was obtained from Best naturals USA each capsule was opened and its content was dissolved in distalled water in three different doses 100, 150 and 200 mg/ kg b.wt/ oral daily

2.3. Experimental Design.

The whole period of experiment was four months, the first two weeks before induction of liver injury, all groups except control and liver injury group were protected by silymarin and propolis which lasted till the end of experiment**,** groups were divided as shown in table 1.

At the end of the experimental period, rats were weighted, left night fasted. Animals were euthanized, blood samples were collected from the retro-orbital plexus, and livers were excised, washed by saline, dried on filter paper and weighted.

Blood samples were collected in clean and dry wesserman tubes and left in slope position to be clotted at room temperature. The tubes were centrifuged at 3000 rpm for 15 minutes and clear serum samples were carefully separated then transferred into clean dry epindorffs and kept frozen at -20°C till be used for determination of serum levels of total protein according to the procedure described by (Doumas et al., 1981), albumin determined according to the method described by (Doumas et al., 1971), ALT, AST according to (Young, 1990), ALP according to (Tietz et al., 1983) and GGT (Moss et al., 1987), using commercial kits (Vitro Scient company Egypt), globulin, A/G ratio and relative liver weight were calculated. AFP and CEA were measured by chemiluminescent immunoassay according to (Sturgeon, 2002)

2.4. Statistical analysis: analysis was done by one way analysis of variance (ANOVA). Data were expressed as means± standard error (Means ± SE). $P<0.05$ was set as statistical significance according to SAS, (2002).

Table 1: Experimental Design

Group (1)	Negative control: kept on basal ration and water *ad libitum*
Group (2)	Control silymarin (100 mg/ kg b.wt) (Usmani, Kushwaha. 2010)
Group (3)	Control propolis (100 mg/ kg b.wt) (Eman, 2012)
Group (4)	Control propolis (150 mg/ kg b.wt) (El-kott, Owayss, 2008)
Group (5)	Control propolis (200 mg/ kg b.wt) (Bhadauria, 2012)
Group (6)	Positive Control (DEN 200 mg/ kg b.wt/i.p. and CCl4 3 ml/ kg b.wt/sc) (Hussain et al., 2012)
Group (7)	Liver injury + silymarin (100 mg/ kg b.wt)
Group (8)	Liver injury + propolis (100 mg/ kg b.wt)
Group (9)	Liver injury + propolis (150 mg/ kg b.wt)
Group (10)	Liver injury + propolis (200 mg/ kg b.wt)

3. RESULTS

Body weight of rats with liver injury without protection was significantly decreased as compared to negative control group at $p<0.05$ while, body weight of rats in groups (7,8,9,10) was better than positive control group at group (6) at $p<0.05$. Moreover, protection with propolis at a dose of (200 mg/kg b.wt) showed lowest decrease in body weight gain as compared to other doses and to silymarin. Liver weight and relative liver weight of rats with induced liver injury were significantly increased as compared to negative control group, protection with propolis at three different doses showed better effect as compared to silymarin at $p<0.05$ (Table 2).

Serum levels of total protein, albumin, and albumin/globulin ratio in positive control group were significantly decreased as compared to group (1) at $p < 0.05$. However, treatment with silymarin and propolis significantly increased serum levels of TP, Alb, A/G ratio as compared to rats treated only with DEN+CCl_4 at $p < 0.05$. .The propolis dose of 200 mg/kg b.wt showed the best effect on TP, Alb and A/G ratio as compared to negative control and positive control group at $p < 0.05$ as shown in Table (3).

Table (4) showed that, rats with induced liver injury and without protection had a significant increase in serum levels of AST, ALT, ALP and GGT as compared to negative control group at $p < 0.05$. Protection with silymarin and propolis significantly decreased serum levels of AST, ALT, ALP and GGT as compared to positive contol group at $p < 0.05$. Also, Moreover, propolis induced an improvement of liver function than silymarin and that, propolis at dose of 200 mg/kg b.wt showed the best improvement at $p< 0.05$.

Serum level of AFP and CEA was significantly increased in rats treated with DEN+CCl_4 as compared to negative control at $p<0.05$, however protection with silymarin and propolis significantly decreased their levels as compared to positive control group at $p<0.05$. Propolis at dose of 200 mg/kg b.wt was the best protective dose as compared to other doses and to standard drug silymarin at $p<0.05$.

Table (2): Effect of silymarin and propolis on body weight, liver weight and relative liver weight in all treated groups.

Parameter / Group	Body wt (g)	Liver wt (g)	Relative liver weight
Group (1)	276.78±2.81c	7.80±0.14d	2.82±0.02f
Group (2)	333.25±3.20a	8.5±0.08c	2.56±0.02g
Group (3)	261.50±1.67d	7.48±0.12d	2.86±0.01f
Group (4)	292±5.60b	7.8±0.12d	2.67±0.01fg
Group (5)	228.12±2.63fg	6.4±0.07e	2.8±0.02f
Group (6)	208.38±4.43h	14.38±0.13a	6.9±0.11a
Group (7)	217.62±3.08gh	10.15±0.35b	4.66±0.1b
Group (8)	235.38±5.3ef	10.33±0.29b	4.38±0.03c
Group (9)	240.75±3.68e	9.78±0.37b	4.06±0.1d
Group (10)	255±3.21c	7.5±0.2d	2.94±0.07e

Values are means ± standard errors. Means in the same column carrying different letters are significantly different at($P<0.05$). Group(1): negative control, Group(2):control silymarin (100mg/kg b.wt), Group(3):control propolis(100mg/kg b.wt), Group(4):control propolis(150mg/kg b.wt), Group(5):control propolis(200mg/kg b.wt), Group(6): positive control (DEN+CCL_4), Group(7):liver injury+silymarin(100mg/kg b.wt), Group(8):liver injury+propolis(100mg/kg b.wt), Group(9):liver injury +propolis (150mg/kg b.wt) and Group(10):liver injury+proplis(200mg/kg b.wt)

Table (3): Effect of silymarin and propolis on serum protein and A/G ratio in all treated groups

Parameter / Group	TP (g/dl)	Alb (g/dl)	Glob (g/dl)	A/G ratio
Group (1)	6.83±0.16a	3.66±0.07b	3.17±0.1a	1.16±0.02a
Group (2)	6.81±0.12a	3.64±0.07b	3.17±0.1a	1.15±0.02a
Group (3)	6.85±0.13a	3.7±0.06b	3.15±0.07a	1.17±0.02a
Group (4)	6.74±0.05ab	3.7±0.02b	3.06±0.03a	1.21±0.01a
Group (5)	7.04±0.11a	3.89±0.05a	3.15±0.08a	1.23±0.02a
Group (6)	4.52±0.17g	1.27±0.08h	3.25±0.17a	0.4±0.04e
Group (7)	5.36±0.07f	2.02±0.07a	3.34±0.13a	0.61±0.05d
Group (8)	5.65±0.16ef	2.52±0.06f	3.13±0.17a	0.81±0.05c
Group (9)	5.98±0.13de	2.81±0.08e	3.17±0.1a	0.89±0.04bc
Group (10)	6.18±0.08cd	3.11±0.05d	3.18±0.04a	0.97±0.02b

Values are means ± standard errors. Means in the same column carrying different letters are significantly different at(P<0.05). Group(1): negative control, Group(2):control silymarin (100mg/kg b.wt), Group(3):control propolis(100mg/kg b.wt), Group(4):control propolis(150mg/kg b.wt), Group(5):control propolis(200mg/kg b.wt), Group(6): positive control (DEN+CCL_4), Group(7):liver injury+silymarin(100mg/kg b.wt), Group(8):liver injury+propolis(100mg/kg b.wt), Group(9):liver injury+propolis(150mg/kg b.wt) and Group(10):liver injury+proplis(200mg/kg b.wt)

Table (4): Effect of silymarin and propolis on serum liver enzymes in all treated groups.

Parameter / Group	ALT(U/l)	AST(U/l)	ALP(U/l)	GGT(U/l)
Group (1)	34.92±0.38f	247.02±3.7f	374.25±3.37f	3.3±0.12f
Group (2)	34.38±0.57f	250.38±2.24f	368.4±2.76f	2.61±0.04f
Group (3)	32.78±1.14f	243.1±2.14f	361.18±2.07f	2.71±0.07f
Group (4)	31.8±0.74f	237.26±1.63f	365.67±4.09f	2.79±0.05f
Group (5)	31.08±1.19f	232.08±1.73f	356.72±4.01f	2.82±0.1f
Group (6)	169.95±3.15a	500.22±11.82a	687.18±9.51a	13.25±0.67a
Group (7)	147.95±1.91b	403.08±7.25b	601.65±7.75b	9.79±0.41b
Group (8)	130.18±1.85c	368.25±6.49c	557.85±8.67c	8.43±0.25b
Group (9)	101.75±3.21d	344.78±8.12d	549.22±6.15d	6.33±0.18d
Group (10)	84.45±2.28e	317.7±7.35e	479.02±8.73e	4.78±0.14e

Values are means ± standard errors. Means in the same column carrying different letters are significantly different at(P<0.05). Group(1):control-ve, Group(2):control silymarin (100mg/kg b.wt), Group(3):control propolis(100mg/kg b.wt), Group(4):control propolis(150mg/kg b.wt), Group(5):control propolis(200mg/kg b.wt), Group(6):control positive(DEN+CCL_4), Group(7):liver injury+silymarin(100mg/kg b.wt), Group(8):liver injury+propolis(100mg/kg b.wt), Group(9):liver injury+propolis(150mg/kg b.wt) and Group(10):liver injury+proplis(200mg/kg b.wt)

Table (5): Effect of silymarin and propolis on AFP and CEA in all treated groups.

Parameter / Group	AFP (ng/ml)	CEA (ng/ml)
Group (1)	3.73±0.03f	1.46±0.03f
Group (2)	3.42±0.07f	1.38±0.01f
Group (3)	3.2±0.05f	1.29±0.01f
Group (4)	2.29±0.01f	1.25±0.01f
Group (5)	2.26±0.05f	1.21±0.01f
Group (6)	54.5±3.1a	9.61±0.76a
Group (7)	44.04±2.63b	7.1±0.14b
Group (8)	38.63±1.84c	6.05±0.16c
Group (9)	31.61±1.34d	5.39±0.26d
Group (10)	26.94±1.69e	4.2±0.1e

Values are means ± standard errors. Means in the same column carrying different letters are significantly different at(P<0.05). Group(1):negative control, Group(2):control silymarin (100mg/kg b.wt), Group(3):control propolis(100mg/kg b.wt), Group(4):control propolis(150mg/kg b.wt), Group (5):control propolis(200mg/kg b.wt), Group(6): positive control(DEN+CCL_4), Group(7):liver injury+silymarin(100mg/kg b.wt), Group(8):liver injury+propolis(100mg/kg b.wt), Group(9):liver injury+propolis(150mg/kg b.wt) and Group(10):liver injury+proplis(200mg/kg b.wt)

4. DISCUSSION

Liver disorders are one of the common recent problems affecting human health, that caused by many environmental polluted sources. The liver regulates many important metabolic functions, and continously exposed to xenobiotics because of its strategic placement in the body. Liver damage ranges from acute hepatitis to hepatocellular carcinoma, through apoptosis, necrosis, inflammation, immune response, fibrosis, ischemia, altered gene expression and regeneration (Shaker et al., 2010).

Many herbal, medicinal and pharmaceutical plants and their extracts are widely studied by many researchers. Silybum marianum (milk thistle) had been used to treat liver diseases since the 16th century. Its major constituents are flavonoids, silibinin, silidianin, silichristin and isosilibinin of which silibinin is the biologically most active compound and used for standardization of pharmaceutical products. Silymarin had clinical applications in the toxic hepatitis treatment, fatty liver, cirrhosis, ischaemic injury, radiation toxicity and viral hepatitis owing to its antioxidative, anti-lipid-peroxidative, antifibrotic, anti-inflammatory, immuno-modulating and even liver regenerating effects (Hamza, Al-Harbi, 2015).

Propolis is one of the most promising natural products presenting not only therapeutic action, but also a prophylactic one. It contains more than 300 compounds from different groups. It contains mostly a mixture of polyphenols, flavonoids (major ingredients), phenolic acids and their esters, caffeic acid and their esters, phenolic aldehydes and ketones. Propolis had been used in folk medicine all over the world. It had anti-inflammatory, immunoregulatory, bacteriostatic, and even antibacterial activities. propolis had been demonstrated to play an important role in preventing liver injury, oxidative stress, apoptosis and necrosis (Abd-El Mawla, Osman, 2012).

Results in table (2) showed that, the greatest decrease in body weight was observed in the experimental group treated with DEN+CCL4 that may be due to the fact that chronic liver injury is a deblitating disease, decreasing appetite and food intake. Hussain et al., (2012) also reported marked loss in body weight and increase of liver weight in rats treated with DEN+ccl4. In the experimental group treated with propolis and silymarin had also a decrease in body weight, but the loss was statistically significantly smaller in comparison with the experimental group treated with DEN+CCL4 only, hence silymarin and propolis improve appetite and food intake so reduction in body weight became smaller.

Moreover, liver weight and relative liver weight were higher in positive contol group than other groups which may be a reason of series of inflamation, fibrosis and cirrhosis that liver pass through during exposure to DEN+CCl4 which also cause proliferation and hyperplasia of hepatocyte. Silymarin and propolis decreased liver weight and liver/body weight index. Relative liver weight is an important parameter in judging the pathological condition of the liver. Therefore, lowering in the relative liver weight of rats by those natural products is an indication of the pathological improvement of the liver as they had anti-inflammatory proporities as described by (Abd-El Mawla, Osman, 2012; Hamza, Al-Harbi, 2015) .

Data in table (3) showed that, serum total protein, albumin and A/G ratio were markedly decreased in rats treated with DEN+CCL4 as compared to negative control that may resulted from liver disorders, which are accompanied by increased rate of catabolism rather than impairment of synthesis due to the damaging effect of DEN+CCL4. One of the suggested mechanisms is the cleavage of CCl4 which lead to the formation of highly unstable free radicals as CCl3 or CCl3O2 and peroxides initiation as reported by (Shaker et al., 2010). Those free radicals are capable of damaging biological molecules such as proteins that have an impact on cell activities as well as membrane functions and structure this was in accordance with (Hassan et al., 2014). Protection with silymarin and propolis increased serum protein, albumin and A/G ratio as they have regeneration ability to hepatocyte and are rich with flavonoids and polyphenols which are powerful antioxidant that act as scavengers of free radicals that cause damage to protein and albumin.

Table (4) showed that, Liver function enzymes (ALT, AST, ALP and GGT) were significantly increased in rats with induced liver injury as compared with negative control. The elevation of liver enzymes is a sensitive marker of liver injury as, treatment with DEN and CCl4 has been shown to induce extensive necrosis and

inflammatory infiltration, clusters of hepatocytes, bile duct proliferation and marked atypia (Abd EL- Hamid et al., 2013), hepatic damage caused by those two toxicants reflects instability of liver cell metabolism that lead to leakage of theses enzymes to circulation. Liver is the main site of DEN metabolism, the generation of ROS in the liver is recognized as an important contributor in DEN-induced damage (Faten etal., 2014). CCl4 is bio-transformed by cytochrome P450 (CYP) enzyme system in the endoplasmic reticulum to produce trichloromethyl free radicals. Trichloromethyl free radicals (CCl3•) then combine with cellular lipids and proteins in the presence of oxygen to form trichloromethyl peroxyl radical, which further attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl peroxyl free radical leads to elicitation of lipid peroxidation (LPO) and destruction of Ca2+ homeostasis, resulting in cell death (Talib, 2012; Kokou et al., 2014). ALP indicates alteration in biliary flow. GGT is an enzyme embedded in hepatocyte plasma membrane, mainly in the canicular domain and its liberation in serum indicate damage of cell and thus injury to liver. It is important to point out that, GGT activity is one of the best indicator of liver damage reported by (Hussain et al., 2012)

Furthermore, silymarin sucessfully reduced the elevated liver enzymes as it decreased liver damage and protect hepatocyte from toxicant as they reduce entry of toxicants to hepatocyte, maintaining integrity of membrane and scavenging free radicals that cause instability of hepatocyte memebrane thereby suppressing leakage of enzymes (Shaarawy et al., 2009). Silymarin is an antioxidant flavonoid complex derived from the herb milk thistle (Silybum marianum), has the ability to attenuate free radicals elevation, chelates metal ions, inhibits lipid peroxidation and prevents liver glutathione depletion (El-Hawary et al., 2011).

Propolis showed better hepatoprotective proporities than silymarin and the best protection achieved when propolis used at a dose of (200 mg/kg b.wt).The protective ability of propolis resulted from its modulatory effects on antioxidative enzymes, which, in turn, suppress the production of free radicals and reduce subsequent liver damage. Furthermore, the high content of polyphenols and flavonoids in propolis contributes to free radical scavenging and antioxidation activities (Barlak et al., 2015)

Table (5) showed that, AFP and CEA were markedly increased in rats treated with DEN+CCL4. The same results were also found by (Song et al. 2013; Ramadan et al., 2014). α-fetoprotein (AFP) an oncofetal serum protein, that progressively lost during development, such that it is virtually absent from the healthy adult. It had long been recognized that exposure of rats to certain toxicants like DEN causes an elevation of circulating AFP levels as, the newly regenerated and altered cells during liver injury leads to newly expression and production of AFP from these cells, these findings coincide with (Kadasa etal., 2015). Carcino embryonic antigen (CEA), a member of the immunoglobulin supergene family, is a 180–200kDa heavily glycosylated protein used clinically as a tumor marker to detect recurrence of many types of tumors. It functions as an adhesion molecule that can form both homotypic and heterotypic aggregates between cells. CEA is cleared from the circulation by the liver with significant traces taken up by the spleen and lungs. (Srigopalram and Ajayraa, 2012). It has long been recognized when the rat liver injury induced by DENA appeared, the CEA content in serum elevated (Shahat et al., 2015).

AFP and CEA were decreased by silymarin and propolis and their levels were mostly decreased when propolis used at a dose of (200 mg/kg b.wt) as, they attenuate damaging effect of DEN+CCl4 so reduced regeneraion and proliferation of hepatocyte and production of new AFP and reduced hyperplasia of cells and size of liver as mentioned in table (2)

5. CONCLUSION

Silymarin and propolis showed hepatoprotective effect against chronic liver injury induced by DEN+CCl_4 and were capable of maintainig body and liver weight, protecting hepatocyte from those toxicant sucessfully and that, propolis at a dose of (200 mg/kg b.wt) showed the best effect so, we recommend using of those natural products as chemopreventive strategy to solve liver problems and maintain its vitality.

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