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Anti-obesity and Anti-fatty Liver Effects of *Cynara scolymus* L. Leaf Extract in Mice under Diet-induced Obesity

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Authors' contributions

This work was carried out in collaboration between all authors. Author EMAEA designed the study, wrote the protocol, carried out some laboratories work supervised the work, managed the analyses of the study and wrote the first draft of the manuscript. Author BA managed the literature searches and edited the manuscript. Author ZZ carried some laboratories work and performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Objective: In this study, we conducted an animal study to investigate the effect of Cynara leaf extract (CLE) on visceral fat and serum lipid profile in animals fed on high fat diet (HFD). In addition, we focused on gene expression of some lipogenesis and lipolysis enzymes to investigate the anti-obesity and anti- fatty liver mechanism of CLE.
Methods: Thirty male mice Swiss albino strain, at age of six weeks were classified into 3 groups. The first group was kept on standard normal diet and served as healthy control. Other animals received HFD for 4 weeks. These animals were assigned as HFD group and HFD + 5% *Cynara scolymus* leaf extract (CLE) -treated group.
Results: CLE supplementation significantly reduced both body weight and white adipose tissue

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(WAT) weight with significant decrease in serum cholesterol, LDL- C and triacylglycerol. While serum HDL-C was significantly increased as compared to HFD group. Moreover, in the histological analysis, CLE improved fatty liver.
Reverse transcription-polymerase chain reaction (RT-PCR) revealed that CLE up regulate the expression of mRNA of acetyl Co A (ACO) and hormone-sensitive lipase (HSL), in contrast, it suppressed the expression of fatty acid synthase (FAS) gene in the liver. While in WAT, the expressions of HSL gene was significantly upregulated at the main time FAS gene was down regulate when compared to HFD group.
Conclusion: These results suggest that CLE exerts anti-obesity and anti-fatty liver effects in high-fat diet-induced obese mice through suppressing lipogenesis in the liver, stimulating lipolysis in WAT.

Keywords: Cynara scolymus; ACO; HSL; fatty liver and lipid profile.

1. INTRODUCTION

Obesity, a pathological condition characterized by excessive accumulation of body lipids, is by far the most prevalent metabolic disease affecting hundreds of millions of people worldwide. The leading cause of this excessive lipid accumulation is a chronic positive energy balance combined with energy partitioning toward lipids [1].

Over the past few decades, obesity has become a global epidemic that affects diverse societies across developed and developing countries. Obesity rates correlate well with recent developments. These rapid environmental changes interact with pre-existing genetic tendencies, yet in a timescale so brief as to outstrip evolution [2].

Non-alcoholic fatty liver disease (NAFLD) is an alarming public health problem. The disease is one of the main causes of chronic liver disease worldwide and directly linked to the increased prevalence of obesity and type 2 diabetes mellitus in general population the worldwide prevalence of NAFLD has been estimated at 20-30%, also NAFLD has become the most prevalent cause of liver disease in western countries [3] The development of non-alcoholic steatohepatitis (NASH) and fibrosis identifies the risk group with an increased incidence of liver-related deaths. It includes a wide spectrum of liver complications ranging from simple steatosis to steatohepatitis (NASH); advanced fibrosis and cirrhosis. [4].

Fatty liver is a reversible condition in which triglycerides accumulate in large vacuoles in hepatocytes. Severe fatty liver is occasionally accompanied by inflammation, a situation that is referred to as steatohepatitis [5].

The potential sources of fats contributing to fatty liver include peripheral fats stored in adipose tissue that flow to the liver by way of the plasma no esterified fatty acid NEFA pool (pathway 1); fatty acids newly made within the liver through de novo lipogenesis (DNL) (pathway 2); and dietary fatty acids, which can enter the liver by two means: Through spillover into the plasma NEFA pool [6] (pathway 3) and through the uptake of intestinally derived chylomicron remnants (pathway 4) [7].

Abdominal adipose tissue is a complex organ and is composed of multiple distinct compartments and sub compartments, including subcutaneous fat and intra-abdominal fat, Intraperitoneal fat, which is also known as visceral adipose tissue (VAT), is considered a particularly important marker of metabolic risk [8-10].

The growing concern with obesity has led to an emphasis on the “undesirability” of white adipose tissue (WAT). Nevertheless, the tissue plays several key roles in mammalian physiology. The classical view of the function of WAT is that it provides a long-term fuel reserve which can be mobilized during food deprivation with the release of fatty acids for oxidation in other organs. Thus, the size of the adipose tissue stores increases in periods of positive energy balance and declines when energy expenditure is in excess of intake [11].

Accordingly, it is considered that both reducing WAT content and suppressing lipogenesis in the liver are very important for controlling fatty liver. Therefore, to prevent obesity, it is important to stimulate lipolysis in WAT and increase energy utilization in the liver and brown adipose tissue [12].

Cynara scolymus, L. (Artichoke) belonging to the Family Asteraceae, is used as an edible vegetable owing to its nutritive value and medicinal properties. It is a cultivated plant that grows in Egypt and many other countries. The aqueous extract of *Cynara scolymus*, L. leaves contains several polyphenolic compounds flavonoids and sesquiterpenes (cynarin, luteolin, isochlorogenic acid, chlorogenic acid, caffeic acid and quinic acid [13].

The artichoke leaf extract has been used as hepatoprotective, and cholesterol reducing purposes [14]. Luteolin and Caffeoylquinic acids are seen as the main bioactives of Artichoke extract Moreover, artichoke leaf extracts was proved to have anticarcinogenic, antioxidative, anti-inflammatory, antibacterial, anti-HIV, bile-expelling, and urinative activities as well as the ability to inhibit cholesterol biosynthesis and LDL oxidation [15].

Thus, in the present study, we conducted an animal study to investigate the efficacy of *Cynara scolymus L*. leaf extract (CLE) on visceral fat levels and hepatic lipid accumulation in mice with high fat diet-induced obesity. In addition, we focused on the expression of some genes related to beta-oxidation and lipogenesis in the liver and lipolysis in WAT to investigate the anti-obesity mechanism of CLE. Histopathological investigation of liver sections was also carried out to confirm the biochemical analyses.

2. MATERIALS AND METHODS

2.1 Materials

Casein was obtained from Sigma chemical company USA (Egyptian center). Starch vitamins for vitamins mixture and minerals used for salt mixture were obtained from Gomhoria chemical company Cairo, Egypt and Sigma chemical company USA (Egyptian center). *Cynara scolymus* leaf extract (CLE) was purchased from research centre of agriculture, Cairo Egypt.

The Kit used for Serum total cholesterol (TC), LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c) and triacylglycerol (TAG) determination were purchased from Diamond, Egypt.

2.2 Experimental Animals

Thirty male mice Swiss albino strain, at age of six weeks weighing 16- 21 g, were purchased from Helwan farm Mice were housed in care of laboratory animal house in the Faculty of Science, Ain Shames university, Cairo, Egypt. Ethics Committee at Ain Shames University, approved all the experimental procedures.

2.3 Test Environment

During the acclimation period, mice were housed and administered normal diet. During the test period, mice were housed in individual stainless steel wire mesh cages. They were kept in a regulated environment (25±1℃, 50±2% humidity), with 12 h light/dark cycles.

2.4 Experimental Design

Follow the acclimation for 1 week, albino mice were weighed and assigned to one of three groups so that the mean body weight of each group was uniform. The animals were divided into three groups, 10 mice each as following: Normal control: animals were fed on standard diet, Positive control group: animals were fed on a high-fat diet (HFD) [16], and the treatment group: animals were fed on HFD containing 5% CLE, Table 1. The animals were restricted-fed and given water *ad libitum* for one month.

Table 1. Composition of the experimental diet (g/100 g diet)

Ingredients	HFD	HFD +CLE
Casein	20.2	20.2
Alpha-potato starch	28.2	25.2
Sucrose	13.0	13.0
Corn oil	20.0	20.0
Lard	10.0	10.0
Cellulose	4.0	4.0
Mineral mixture*	3.5	3.5
Vitamin mixture*	1.0	1.0
dl-Methionine	0.3	0.3
CLE	--------	5.0
Total	100.0	100.0

(*): Mineral mixture and vitamin mixture according to American Institute of Nutrition (AIN-76)

2.5 Body Weight and Food Intake

During the experiment, the animals were weighed every day. Food intake was determined daily by determining the amount of feed remaining from the previous day, and the mean daily food intake for each animal was calculated.

Table 2. Mice PCR primer sequences and conditions

Gene name	F/R	Sequence (5'-3')	Product size (bp)	Annealing temperature (ºC)	Primer concentration (µMolar)
FAS	F	CCATGGAGGAGGTGGTGATA	380	58	0.1
	R	CGTCTCGGGATCTCTGCTAA			
β-actin	F	ACTGCCGCATCCTCTTCCTC	399	60	0.2
	R	CTCCTGCTTGCTGATCCACATC			
HSL	F	GGAGCACTACAAACGCAAC	357	58	0.1
	R	TCCCGTAGGTCATAGGAGAT			
ACO	F	GGTGGTATGGTGTCGTACTTGA	294	62	0.1
	R	GAATCTTGGGGAGTTTATCTGC			

2.6 Measurement of Tissue Weight

At the end of the experimental period , mice were fasten for 12 hours and they were sacrificed, blood were collected for separation of serum after clotting and centrifugation at 4000 rpm for 15 minutes. Serum samples were stored in deep freezer at – 20℃ in plastic vial for determination of lipid profile. Liver and interscapular brown, mesenteric, epididymal, and retroperitoneal adipose tissues were removed. All samples excluding BAT samples were weighed. Each sample was cut into small pieces, dipped in Trizol reagent (Invitrogen, Carlsbad, CA), and stored at −80℃ until RNA extraction.

2.7 Biochemical Assays

Serum total cholesterol (TC) concentration was determined colorimetrically according to the method of Allain et al. [17]. Serum LDL-cholesterol (LDL) concentration was assayed colorimetrically according to the method of Assman et al. [18]. Serum HDL-cholesterol concentration was measured colorimetrically according to the method of Lopez-Virella et al. [19]. Serum triacylglycerol (TAG) level was determined colorimetrically according to the method of Buccolo [20].

2.8 Hepatic Histological Analysis

Suitable sections of liver were fixed in 10% formalin and processed for preparation of 5µm-thick paraffin section. These sections were sequentially stained with hematoxylin and Eosin (H & E) according to Bancroft et al. [21] to detect the presence of fat. This study was assessed by expert pathologists at Cairo University, Zoology Department.

2.9 RT-PCR

Total RNA was extracted from isolated mice organs using TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. cDNA was synthesized from 5 µg of total RNA using a TIANscript One Step reverse transcription-PCR (RT-PCR) kit (TIANGEN, China). The resulting cDNA was subjected to PCR reaction using EmeraldAmp® GT PCR Master Mix (Takara, Japan). Mice PCR primer sequences and conditions are demonstrated to Table 2. PCR reaction were performed using denaturation temperature of 95ºC for 30 seconds; annealing time of 45 seconds; and extension temperature of 72ºC for minute. PCR products were run on 2% agarose gel and then stained with ethidium bromide. Stained bands were visualized under U V light and photographed.

2.10 Statistical Analysis

SPSS package (version 21) was used for data analysis. All data were expressed as means standard deviation (SD) and standard error (S.E) of a minimum three or more replicates. The statistically significant differences were calculated by Student's t-test.

3. RESULTS

3.1 Body Weight and Food Intake

Table 3 shows the body weight and amount of food intake during the experimental period. There was no significant difference between the normal control and HFD+CLE groups regarding food intake, both the final body weight and body weight gain, liver and both WAT and BAT. Body weight gain in mice fed on HFD showed a significant increase ($P<0.05$) compared to control group. While animals fed on HFD+CLE showed a significant decrease ($P<0.05$) as compared to mice fed on HFD. In the main time, HFD group showed a significant increase in WAT as compared to HFD+CLE group. Liver weight showed a non-significant change in all groups.

(Table 4) showed the effect of CLE on serum lipid profile in HFD and (HFD+CLE) mice in comparison with health control group. Group fed on HFD produced highly significant elevation (P<0.001) in serum triacylglycerol, total cholesterol and LDL- C levels (40.76%, 50.9% and 41.52% respectively) associated with a highly significant decline (P<0.001) in serum HDL- C level (-56.31%) in comparison with the healthy control group. On the other hand, (HFD+CLE) group showed a highly significant depletion (P<0.001) in serum TAG, total cholesterol and LDL- C levels accompanied with significant rise in serum HDL level in comparison with the HFD group.

3.2 Hepatic Histological Analysis

Hepatic histological analysis data after the experiment period showed that there is no specific findings were observed during the hepatohistological examination of the healthy control mice (Fig.1-a). Histopathological investigation of liver tissue slides stained with H&E in mice fed on HFD showed moderate to severe macrovascular fatty changes, which were diffusely distributed throughout the liver lobules. Parenchymal inflammation with both acute and chronic inflammatory cells accompanying focal necrosis was also observed (Fig. 1-b and 1-c). In the HFD+CLE group, the development of fatty liver was apparently suppressed (Fig.1-d).

Table 3. Final body weight, weight gain, food intake, liver weight, adipose tissue weight in mice fed on standard or HFD in different studied groups

Groups Parameters	Normal control	HFD	HFD+CLE	p value	Sig.
Final weight (g±SD)	20.23±0.45	26.10±0.81	21.17±0.49	0.01	**
Weight gain (g/day±SD)	2.05±.67	5.65±0.50	2.34±0.31	0.04	**
Food intake (g/day±SD)	2.55± 0.43	2.48±0.15	2.68±0.17	0.38	NS
White adipose (g± SD)	0.31±0.11	0.51±0.03	0.42±0.02	0.02	*
Brown adipose (g±SD)	0.16±0.48	0.27±0.06	0.26±0.03	0.84	NS
Liver weight (g±SD)	4.01±0.21	4.87±0.27	4.52±0.3	0.40	NS

∗ and ∗∗ indicate significantly different at P <0.05, P <0.01, respectively; NS: non- significant

Table 4. Serum lipid profile in different studied group

Groups Parameters	TAG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control				
Range	89.94-103.31	80.64-100.01	39.69-48-60	60.23-66.10
Mean± SE	98.39±1.92	88.58±2.30	43.00±1.56	63.91±1.4121
HFD				
Range	125.42-142.55	113.19-158.63	18.91- 21.24	88.29-100.46
Mean± SE	138.54±4.21	133.67±6.1	18.79±0.15	90.45±1.34
% Change	40.76	50.90	- 56.31	41.52
P_1<	0.001	0.001	0.001	0.001
HFD+CLE				
Range	91.71-113.71	88.14-110.16	40.26- 49.31	73.51-91.23
Mean± SE	97.14±2.69	94.13±1.81	46.33±1.08	81.11±0.33
% Change	-1.27	6.26	7.71	21.20
P_1<	N.S	N.S	N.S	0.05
P_2<	0.001	0.001	0.001	0.001

P< 0.05: significant, P<0.001: highly significant; N.S: non significant; % change with respect to control; P_1: in relation to control group, P_2: in relation to HFD group

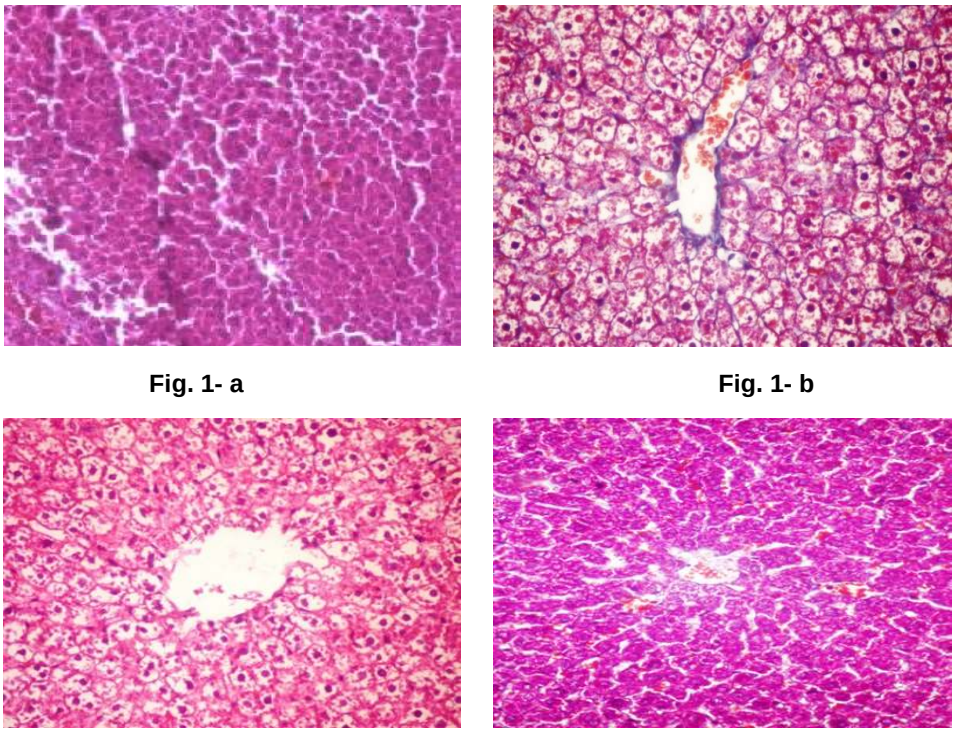

Fig. 1- a **Fig. 1- b**

Fig. 1-c **Fig. 1- d**

Fig. 1. Photomicrographs of H&E stained hepatocyte sections where (a) represent a normal hepatic lobules of the healthy control mice; (b) & (c) photos represent histopathological investigation of liver tissue in mice fed on HFD showed moderate to severe macrovascular fatty changes distributed throughout the liver lobules accompanying with focal necrosis. Photo (d): represent HFD+ CLE group, showed a reduction in fat deposits in liver tissues

3.3 Real-time Quantitative RT-PCR

Fatty acid synthase, hormone –sensitive lipase and acyl- Co A oxidase gen expression level was measured in both liver and white adipose tissue in HFD and HFD+CLE groups using RT-PCR. Data represented in Figs. (2a & 2b) and Table 5 showed that the expression of hepatic genes involved in lipogenesis such as Fatty acid synthase (FAS) in the HFD+CLE group was significantly lower than that in the HFD group. Hormone-sensitive lipase (HSL) was significantly unregulated in liver of HFD+CLE group.

For WAT, hormone-sensitive lipase was significantly unregulated in epididymal adipose tissue in the HFD+CLE. Expression of Fatty acid synthase (FAS) in the HFD+CLE group was significantly lower than that in the HFD group. The expression of genes related to beta-oxidation, such as acyl-CoA oxidase (ACO), was not significantly different between the two groups, although their expressions were higher in the HFD+CLE group. These results suggest that CLE has anti-obesity effects in high-fat diet-induced obese mice through suppressing lipogenesis in the liver, stimulating lipolysis in WAT.

4. DISCUSSION

Obesity is a chronic disease with serious health consequences, but weight loss is difficult to maintain through lifestyle intervention alone [22]. It is the result of a positive energy balance, whereby energy intake exceeds expenditure, resulting in the storage of energy, primarily as lipids in white adipocytes [23].

The number of people with obesity and obesity-related diseases, such as diabetes mellitus, hypertension, coronary artery disease, fatty liver and cancers, has increased at an alarming rate all over the world [24-25]. Consequently, the idea of developing antiobesity drugs with no undesirable side effect has become a hot topic [26]. Herbal medicine has been looked at as a complementary treatment [27-28]. So in the

present study we investigated the antiobesity effects of *Cynara scolymus* L.

Bundy et al. [29] and Lattanzio et al. [15] reported that, *Cynara scolymus* L contains some constituents as Cynarin and luteolin which play a crucial role in inhibiting cholesterol and triglycerides synthesis. This finding in agreement in our study, where CLE supplementation significantly reduced body weight, body weight gain, and WAT weight without affecting energy intake (i.e., food intake). It is known that obesity develops when energy intake exceeds energy expenditure. So it's clear that the consumption of CLE for one month caused a suppressive effect on weight gain and visceral fat accumulation in mice Therefore, CLE was believed to exert anti-obesity effects by increasing energy expenditure.

In the present study, feeding mice with high fat diet for (4weeks) led to increasing the serum triacylglycerol (TAG), total cholesterol (TC) and low density lipoprotein (LDL) levels were observed compared to those of the normal group (normal diet) whereas the HDL levels was decreased, with histopathological changes showed moderate to severe macrovascular fatty changes, which were diffusely distributed throughout the liver lobules. These histological assessments of hepatic injury are consistent with the elevated serum lipid concentrations.

Feeding mice on HFD with CLE improved the lipid profile by decreasing serum TAG, TC, and LDL-C levels accompanied with significant increase in serum HDL-C. Additionally, histopathological investigation of liver tissue of this group indicated a reduction in macrovesicular steatosis and microvesicular steatosis. Similar results were obtained with other previous studies [30-31]. However, highly significant decrease of serum LDL-C and an increase of HDL-C in the treated groups are agreed with Cieslik et al. [32] who reported decline tendency in total cholesterol, LDL and VLDL when diets were supplemented with *Cynara scolomus* flour.

Therefore, these extract has a strong hypo-triglyceridemic and hypo-cholesterolemic effects. This effects may be due to that *Cynara scolomus* contains some constituents as cynarin and luteolin which play a crucial role in inhibiting cholesterol and triglycerides synthesis. Luteolin by beta glucosidase in digestive tract could cause inhibition up to 60% of cholesterol synthesis [29].

These results also agree with Daniel, [33] who conclude that; this extract contains active compounds as flavonoids which have hypolipidemic effect. These compounds could not only increase the breakdown of cholesterol to bile salts and enhance their elimination through increased bile production and flow but they also inhibit the internal production of cholesterol in liver [34]. Cholesterol metabolism was associated with liver fat content independent on body weight, implying that the more fat the liver contains, the higher is cholesterol synthesis [35]. The accumulation of fat, mainly TAG, within the hepatocyte is a prerequisite for the development of NAFLD [36].

Non-alcoholic fatty liver disease (NAFLD) is one of the major causes of liver disease and is linked to the obesity and metabolic disease epidemics. NAFLD is characterized by excessive lipid accumulation in the liver accompanied by metabolic dysfunction and hepatic cell degeneration. It is considered to be the liver manifestation of insulin resistance. Hyperlipidaemia (increased blood triglycerides and/or cholesterol) is frequently associated with metabolic diseases and is also associated with fatty liver disease [37].

Table 5. Effect of CLE on mRNA levels in the liver and WAT in mice fed on HFD and HFD + CLE

Groups	HFD	HFD+CLE	p value
Gens			
Liver			
FAS	1.00±0.211	0.172±0.032	0.018*
HSL	1.00±0.182	2.121±0.177	0.011*
ACO	1.00±0.172	1.370±0.339	0.517
White adipose tissue			
FAS	1.00±0.047	0.617±0.0.97	0.024*
HSL	1.00±0.025	1.773±0.229	0.028*

The data represent the mean ±S.E values (n =10); (*) Significant different at P <0.05

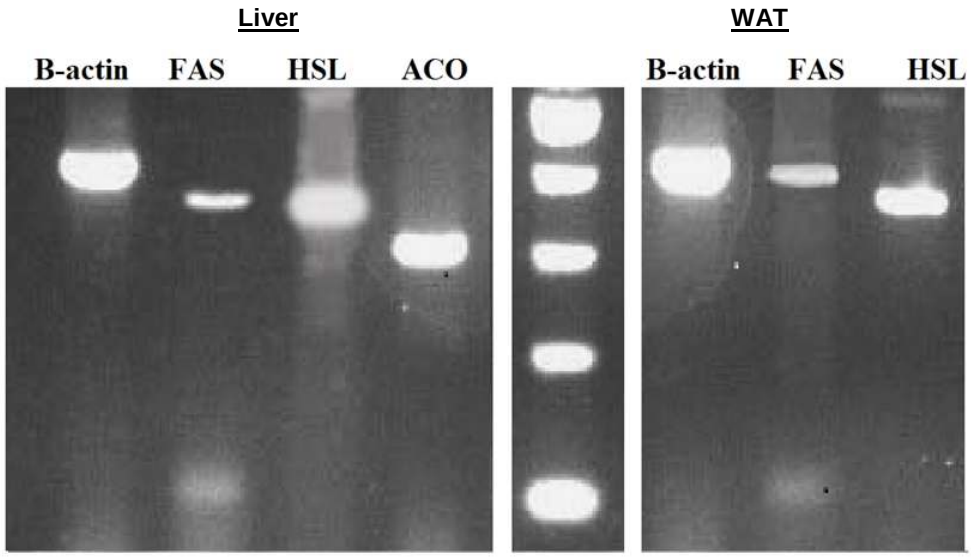

Fig. 2-a. PCR products of genes expression profile in different tissues of mice fed on HFD+CLE (2% agarose gel, stained with ethidium bromide. Stained bands were visualized under U V light and photographed)

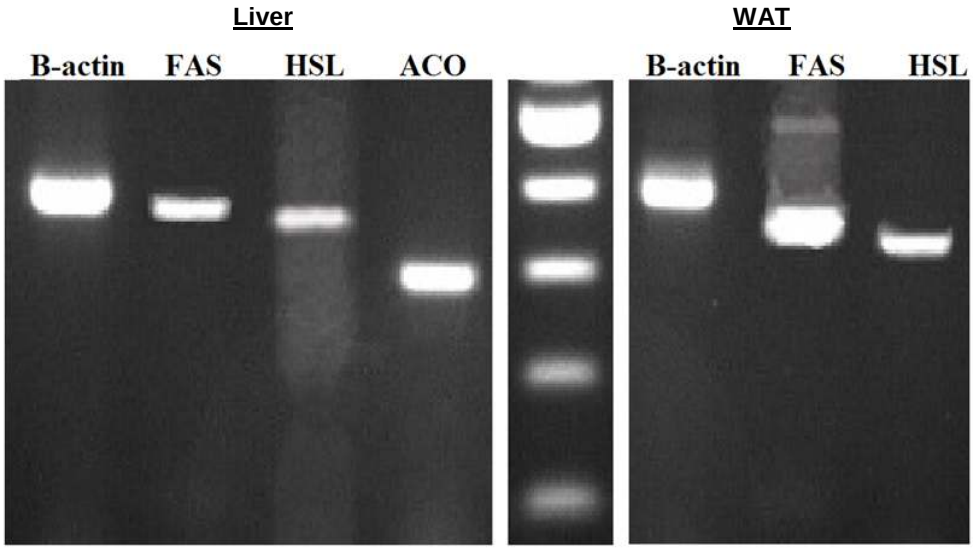

Fig. 2- b. PCR products of genes expression profile in different tissues of mice fed on HFD (2% agarose gel, stained with ethidium bromide. Stained bands were visualized under U V light and photographed)

The current results suggest that the CLE could be helpful in decreasing the incidence of several fatty liver diseases through a reduction in TAG, TC and LDL- C and an increase in HDL level.

In addition, CLE supplementation significantly modulates the expression of specific enzymes involved in lipogenesis and lipolysis processes in liver and WAT.

CLE leads to increase in gene expression of Acyl-CoA oxidase (ACO) (which catalyses the first and rate-determining step of the peroxisomel β-oxidation of fatty acids [38]) and Hormone-sensitive lipase (HSL) (Its the rate-limiting step in cleaving fatty acids from the triglyceride molecule [39]) and decrease the expression of fatty acid synthase (FAS) (rate-limiting enzymes in fatty acid biosynthesis, in the liver). These results

revealed that Cynara leaf extract significantly up regulate the mRNA level of ACO and mRNA of HSL, in contrast, it suppressed the expression of FAS in the liver compared to HFD group.

For adipose tissue, the expressions of HSL in WAT was significantly upregulated while FAS was down regulate. These results suggest that CLE exerts anti-obesity and anti-fatty liver effects in high-fat diet-induced obese mice through suppressing lipogenesis in the liver, stimulating lipolysis in WAT. These results are similar to the anti-obesity effects of isoflavones compounds [40-42]. Liu et al. [43] also reported the lipid-lowering potential of luteolin in palmitate-stimulated HepG2 cells. Also they record a reduction of FAS expression and an increase of CPT-1 expression, as a consequence of the luteolin-mediated AMPK activation and ROS inhibition. Therefore, the active ingredient of CLE may be an flavonoids and luteolin.

Therapeutic options for NAFLD are limited to medications that reduce the risk factors. Therefore, suppressing hepatic lipid accumulation, the first step of the pathogenesis of NAFLD, appears to be very important for preventing this hepatic disorder. In this study, CLE suppressed the development of fatty liver by preventing the accumulation of fat in hepatocytes as a results of downregulation of genes related to lipogenesis.

This result supports the possibility that CLE supplementation provides the dual effects of preventing both obesity and hepatic disorders. It is believed that flavonoids and luteolin content of CLE are promising compounds for preventing obesity and fatty liver disease.

These finding seem to be in accordance with the results of Azevedo et al. [44] who has previously shown that luteolin-7-glucoside given in the diet for 1 week improved plasma lipid profile. Here we demonstrate that these effects are accompanied by an increase in liver expression of fatty acid β-oxidation related genes as well as a decrease in the rate limiting enzyme of fatty acid synthesis. These effects are consistent with the decrease in LDL and total cholesterol observed in the serum of animals fed with CLE and indicate that this extract may, in addition to improving serum lipid profile, be beneficial in the prevention of NAFLD and other metabolic diseases.

5. CONCLUSION

The current study suggest that leaf extract of *Cynara scolymus* L. could be helpful in decreasing the incidence of several fatty liver disease through a reduction in TC, LDL and TG and an increase in HDL level. In addition, it appears to exert these effects through suppressing lipogenesis in the liver and promoting lipolysis in white adipose tissue.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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