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Research Article

CYNARA SCOLYMUS FOR RELIEVING ON NONALCOHOLIC STEATOHEPATITIS INDUCED IN RATS

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ABSTRACT

Objective: The current study was undertaken to evaluate the efficacy of the total crude aqueous methanolic extract of Cynara scolymus and its fraction against high fat diet-induced of nonalcoholic steatohepatitis (NASH) in adult female rats. Methods: Forty adult female Sprague Dawley rats were classified into 4 groups. The first group was kept on standard rodent chow and served as healthy control. The other groups received high fat diet (HFD) for 32 weeks for NASH induction. These animals were assigned as NASH-induced group, Cynara scolymus (CSM) extract-treated group and purified fraction (CSF) -treated group. Results: The results revealed significant increase in serum ALT activity, cholesterol, LDL and triglycerides levels as well as leptin and resistin levels. Additionally, serum NF-κB, TNF-α, Cox-2, CD 40 and HGF levels have been increased significantly, while, serum HDL and adeponectin levels have been decreased significantly in NASH-induced group compared with healthy control group. Conversely, treatment with CSM or CSF resulted in significant decrease in serum ALT activity, cholesterol, LDL and triglycerides levels as well as leptin and resistin levels. Serum NF-κB, TNF-α, Cox-2, CD40 and HGF levels also showed significant decrease. While serum HDL and adiponectin levels were significantly increased as a consequence of treatment with either CSM or CSF as compared to the untreated NASH-induced rats. The photomicrogrophs of liver section of rats treated with CSM or CSF extract confirmed the present improvement in the studied biomarkers. The results suggested that Cynara scolymus extract or its purified fraction possess hepatoprotective activity, hypolipidemic effect and anti-inflammatory property. Conclusion: Thus, our findings reinforce current advice recommending the consumption of natural products to modulate nonalcoholic steatohepatitis and its metabolic complications.

Keywords: *Cynara scolymus*, Nonalcoholic steatohepatitis, Insulin resistance, Inflammation, Hyperlipidemia, Rats.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a clinic pathologic entity increasingly recognized as a major health burden in developed as well as in developing countries. It includes a spectrum of liver damage ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and probable progression to cirrhosis [1]. The presence of NASH with cirrhosis has been documented in large series. Cirrhosis occurs in a minority of NASH patients, but the overall incidence has been reported to be as high as 26%. Progression of fibrosis as detected by liver biopsy has been reported to occur in 43% of NASH patients, while 54% of patients remained unchanged and 3% showed histologic improvement during a follow-up from 1 to 7 years [2]. In general, 30-50% of individuals with NASH will develop fibrosis, 15% will develop cirrhosis and 3% will progress to terminal liver failure [3]. Among the many causative factors of NASH, oxidative stress, lipid peroxidation and inflammation are considered the most probable causative factors [4]. NASH is believed to be a feature of metabolic syndrome because it is closely associated with visceral obesity, dyslipidaemia, insulin resistance, and type 2 diabetes mellitus [5].

Artichoke (*Cynara scolymus L.*), Asteraceae family (Compositae) is a plant that is widely grown in Mediterranean countries and is rich in natural antioxidants. It is not only a good food, known for its pleasant bitter taste, but also an interesting and widespread herbal drug [6]. Artichoke leaf contains up to 2% phenolic acids, mainly 3-caffeoylquinic acid (chlorogenic acid), plus 1.3-di-O-caffeoylquinic acid (cynarin), and caffeic acid; 0.4% bitter sesquiterpene lactones of which 47-83% is cynaropicrin; 0.11.0% flavonoids including the glycosides luteolin-7-β-rutinoside (scolymoside), luteolin-7- β-D-glucoside and luteolin-4-β-D-glucoside; phytosterols (taraxasterol); sugars; inulin; enzymes; and a volatile oil consisting mainly of the sesquiterpenes β-selinene and caryophyllene [7, 8].

The artichoke leaf extract has been used as hepatoprotective [9], antimicrobial [10] and cholesterol reducing purposes [11]. Artichoke has been found to decrease the production of reactive oxygen species, the oxidation of low-density lipoproteins [12], lipid peroxidation [9], and protein oxidation and increase the activity of glutathione peroxidase [13].

The aim of the present article is to investigate the efficacy of *Cynara scolymus* total methanolic extract (CSM) and its fraction (CSF) against high fat diet-induced NASH in adult female rats in attempt to understand their mechanisms of action, which may pave the way for possible therapeutic applications. This could be achieved through conducting routine biochemical analysis for liver functions, estimating the circulating levels of insulin resistance indices, evaluating serum levels of inflammatory markers. Histopathological investigation of liver sections was also carried out to confirm the biochemical analyses.

MATERIALS AND METHODS

Plant materials

Preparation of Cynara scolomus total extracts (CSM)

The leaves of *Cynara scolomus* were collected from the experimental farm at Nubaria. Alexandria, Egypt on October 2009, air dried (3 kg) and extracted with 80 % methanol at room temperature for three times , followed by the removal of solvent under reduced pressure to obtain the crude aqueous methanolic extract (CSM) (26 % from the dried leaves).

Preparation of Cynara scolomus fraction (CSF)

300 g of CSM was subjected to silica gel column chromatography and eluted with solvent of increasing polarity (hexane/ethylacetate/methanol). The fractions eluted with ethyl acetate/methanol (1:1) were collected together to give a purified fraction (CSF) (120 g).

Animals

The present study was conducted on forty adult female *Sprague Dawley* rats weighing 120-150g obtained from the Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were maintained on standard laboratory diet and water *ad libitum* for two weeks before starting the experiment. All animals received human care and use according to the guide lines for Animal Experiments which were approved by the Ethical Committee of Medical Research, National Research Centre, Egypt. Steatohepatitis (NASH) was induced in rats by using high fat diet which provided

30% of its energy from fat, 35% from carbohydrate and 35% from protein (casein) for 32 weeks. Supplements of vitamins and minerals were also included [14].

Experimental set-up

The animals were classified into four groups with ten animals in each: (1) Healthy control group which was fed *ad-libitum* with an isocaloric regular rat chow [15], (2) Steatohepatitis (NASH) - induced group which was fed *ad-libitum* with high fat diet [14], (3) NASH -induced group orally treated with 150 mg/ kg b.wt. of CSM daily for 8 weeks. This dose was calculated from the chronic toxicity study for CSM (data not shown), and (4): NASH -induced group orally treated with 150 mg/ kg b.wt. of CSF daily for 8 weeks. This dose was calculated from the chronic toxicity study for CSF (data not shown).

At the end of the experimental period, the rats were fasted overnight and the blood samples were collected from the retro orbital plexus under diethylether anaesthesia [16]. The blood samples were left to clot and then centrifuged using cooling centrifuge at 1800 xg for ten minutes to obtain sera. The clear serum samples were stored at -20 ºC until analysis. After blood collection, all animals were rapidly killed and the liver tissues were dissected, washed in isotonic saline, then cut into small pieces (0.5x0.5cm) and fixed in 10% saline buffered formalin overnight for histological examination.

Biochemical assays

Serum alanine transaminase (ALT) activity was estimated colorimetrically using kit purchased from Quimica Clinica Aplicada S.A. Co., Spain, according to the method of Reitman and Frankel [17]. Serum cholesterol (Chol) concentration was determined colorimetrically using kit purchased from Stanbio Laboratory, Boerne, Texas, USA, according to the method of Allain et al. [18]. Serum LDL-cholesterol (LDL) concentration was assayed colorimetrically using kit purchased from Quimica Clinica Aplicada S.A. Co., Spain, according to the method of Assman et al. [19]. Serum HDL-cholesterol (HDL) concentration was measured colorimetrically using kit purchased from Stanbio Laboratory, Boerne, Texas, USA, according to the method of Lopez-Virella et al. [20]. Serum triglycerides (TG) level was determined colorimetrically using kit purchased from Stanbio Laboratory, Boerne, Texas, USA, according to the method of Fassati and Prencipe [21]. Serum adiponectin concentration was measured by enzyme-linked immunosorbent assay (ELISA) technique using kit purchased from AssayPro, USA, according to the method of Pannacciulli et al. [22]. Serum leptin level was measured by ELISA procedure using kit purchased from Ray Biotech Co., Georgia, USA, according to the method described by Petridou et al. [23]. Serum resistin concentration was determined by ELISA technique using kit purchased from Glory Science Co., Ltd, Veterans Blvd, Suite, USA, according to the method of Schaffler et al. [24]. Serum NF-κB p56 concentration was determined by ELISA technique using kit purchased from Glory Science Co., Ltd, Veterans Blvd, Suite, USA, according to the manufacturer's instructions. Serum Cox-2 concentration was determined by ELISA technique using kit purchased from Glory Science Co., Ltd, Veterans Blvd, Suite, USA, according to the manufacturer's instructions. Serum TNF-α concentration was measured by ELISA procedure using kit purchased from Ray Biotech Co., Georgia, USA, according to the method of Brouckaert et al. [25]. Serum CD40 concentration was measured by ELISA technique using kit purchased from Glory Science Co., Ltd, Veterans Blvd, Suite, USA, according to the manufacturer's instructions. Serum hepatocyte growth factor (HGF) level was quantified by ELISA procedure using kit purchased from Glory Science Co., Ltd, Veterans Blvd, Suite, USA, according to the method of Plum et al. [26].

Histopathological examination

Fragments of liver tissue previously fixed in 10% formalin saline were processed and submitted to hematoxilin and eosin (H&E) stain. SCHARLACH Rs stain was used for a more precise identification of fatty change. Histological variables were semiquantitated from 0 to 4+, including macro-and microvesicular fatty change, the foci of necrosis, portal and perivenular fibrosis as well as the inflammatory infiltrate.

Statistical Analysis

In the present study, all results were expressed as Mean + S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups [27]. Difference was considered significant when P value was < 0.05. The percent difference was calculated according to the following equation:

% difference = Treated group value – Control group value / Control group value X 100

RESULTS

(Table: 1) showed the effect of treatment with CSM and CSF on serum ALT activity and lipid profile in NASH-induced rats. The NASH-induced group showed significant increase in serum ALT activity (60.8 %) in comparison with the healthy control group. Conversely, treatment with CSM or with CSF produced significant decrease in serum ALT activity (-40.9% and -39.6% respectively) in comparison with the untreated NASH-induced group.

Table 1: Table shows the effect of treatment with CSM and CSF on serum ALT activity and lipid profile in NASH - induced rats.

Parameters Groups	ALT (U/L)	Cholesterol (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)
Healthy control group	35.4± 3.2	70.7 ± 1.7	41.3 ± 2.8	9.9 ± 0.2	64.8 ± 3.1
NASH – induced group	60.8±1.7[a] (71.7%)	124.7 ± 3.7[a] (76.3 %)	20.8± 1.1[a] (-49.6 %)	18.8 ± 1.1[a] (89.89 %)	95.8 ± 3.0[a] (47.8 %)
NASH +CSM treated group	40.9±1.7[b] (-32.7%)	78.7 ± 2.5[b] (-36.8 %)	30.9 ± 2.5[b] (48.5%)	12.8 ± 0.5 (-31.9 %)	75.8 ± 3.7[b] (-20.8 %)
NASH +CSF-treated group	38.6±2.1[b] (-36.5 %)	75.47 ± 3.7[b] (-35.4 %)	33.7±0.9[b] (62.01%)	10.5± 0.5[b] (44.14%)	72.5 ± 3.4[b] (-24.36.9 %)

a: Significant change at $P < 0.05$ in comparison with the healthy control group.

b: Significant change at $P < 0.05$ in comparison with NASH-induced group

(%): percent difference with respect to the corresponding control value.

The induction of NASH produced significant elevation in serum cholesterol, LDL and triglycerides levels (124.7%, 89.8% and 95.8% respectively) associated with significant decline in serum HDL level (-20.8%) in comparison with the healthy control group. On the other hand, treatment of NASH-induced group with CSM resulted in significant depletion in serum cholesterol, triglycerides levels and insignificant decrease in serum LDL level (-78.7%, -75.8% and -31.9% respectively) accompanied with significant rise in serum HDL level (48.5%) in comparison with the untreated NASH-induced group. Serum cholesterol, LDL and triglycerides levels were significantly decreased by -75.4%, -44.14% and -72.5 % respectively, while serum HDL level was significantly increased by 62.01% in NASH-induced group treated with CSF as compared to untreated NASH-induced group.

(Table: 2) showed the effect of treatment with CSM and its fraction (CSF) on serum adiponectin, leptin and resistin levels in NASH-induced rats. Significant increase in serum leptin and resistin levels (121% and 79.2%) accompanied with significant decrease in serum adiponectin level (-33.6%) were observed in NASH-induced group in comparison with the healthy control group.

Table 2: Table shows the effect of treatment with CSM and CSF on serum adiponectin, leptin and resistin levels in NASH-induced rats

Parameters Groups	Adiponectin (ng/mL)	Leptin (pg/mL)	Resistin (pg/mL)
Healthy control group	10.4 ± 0.42	343.2 ± 2.6	30.8 ± 0. 5
NASH – induced group	6.9 ± 0.2[a] (-33.6 %)	761.2 ± 2.5[a] (121 %)	55.2 ± 0.37[a] (79.2 %)
NASH + CSM-treated group	8.8 ± 0.3[b] (27.5%)	580.8 ± 2.4[b] (-23.6 %)	32.8 ± 0.37[b] (-40.5 %)
NASH +CSF-treated group	9.3 ± 0.2[b] (34.7 %)	576.4 ± 2.8[b] (-24.2 %)	30 ± 0.39[b] (-45.6 %)

a: Significant change at $P < 0.05$ in comparison with the healthy control group.

b: Significant change at $P < 0.05$ in comparison with NASH-induced group

(%): percent difference with respect to the corresponding control value.

In contrast, treatment of NASH-induced group with CSM or CSF resulted in significant decrease in serum leptin level (-23.6% and -24.2 % respectively) and resistin level (-40.5% and -45.6 % respectively) in concomitant with significant increase in serum adiponectin level (27.5 and 34.7% respectively) as compared to untreated NASH-induced group.

(Table: 3) showed the effect of treatment with CSM and CSF on serum NF-κBp56, TNF-α levels and Cox-2 activity in NASH-induced rats. Significant increase in serum NF-κBp56, TNF-α levels and Cox-2 activity (103.1%, 67.6% and 90.3% respectively) was recorded in NASH-induced group in comparison with the healthy control group. Conversely, the treatment of NASH-induced group with CSM or CSF caused significant decrease in serum NF-κB p56 level (-44.6% and -47.6 % respectively), TNF-α levels (-24.2% and -28.9 % respectively) and Cox-2 activity (-25% and -65.1 % respectively) as compared to the untreated NASH-induced group.

Table 3: Table shows the effect of treatment with CSM and CSF on serum NF-κB, TNF-α levels and Cox-2 activity in NASH-induced rats.

Parameters Groups	NF-κB (ng/mL)	TNF-α (Pg/mL)	Cox-2 (U/L)
Healthy control group	0.64 ± 0.04	58.1 ± 1.8	13.03 ± 0.4
NASH–induced group	1.3 ± 0.1[a] (103.1 %)	97.4± 1.2[a] (67.6 %)	24.8 ± 1,1[a] (90.3 %)
NASH +CSM-treated group	0.72± 0.02[b] (-44.6 %)	73.8 ± 1.5[b] (-24.2 %)	18.6 ± 0.3[b] (-25 %)
NASH+CSF-treated group	0.68± 0.03[b] (-47.6%)	69.2 ± 1.2[b] (-28.9 %)	16.2 ± 0.5[b] (-34.3 %)

a: Significant change at $P < 0.05$ in comparison with the healthy control group.

b: Significant change at $P < 0.05$ in comparison with NASH-induced group

(%): percent difference with respect to the corresponding control value.

The effect of treatment with CSM or CSF on serum CD40 and HGF levels in NASH -induced rats was illustrated in (Table: 4). The data revealed that the NASH–induced group showed significant increase in CD40 and HGF levels (95.4% and 88.5% respectively) in comparison with the healthy control group. Meanwhile, treatment of NASH-induced group with CSM or CSF resulted in significant decrease in serum CD 40 (-31% and -34.2% respectively) and HGF levels (-23.4% and-28.1%respectively) as compared to the untreated NASH-induced group.

Table 4: Table shows the effect of treatment with CSM and CSF on serum CD40 and HGF levels in NASH-induced rats.

Parameters Groups	CD40 (ng/L)	HGF (ng/L)
Healthy control group	377.2 ± 1.8	102.40 ± 1.6
NASH – induced group	737.2 ± 2.9[a] (95.4 %)	193.05 ± 1.4[a] (88.5 %)
NASH +CSM-treated group	508.4 ± 2.6[b] (-31%)	147.70 ± 1.6[b] (-23.4 %)
NASH +CSF-treated group	485.0± 1.4[b] (-34.2%)	138.70 ± 2.1[b] (-28.1 %)

a: Significant change at $P < 0.05$ in comparison with the healthy control group.

b: Significant change at $P < 0.05$ in comparison with NASH-induced group

(%): percent difference with respect to the corresponding control value.

Our histological study showed that there is no specific findings were observed during the hepatohistological examination of the healthy control rats (Fig.1-A). Histopathological investigation of liver tissue slides stained with H&E in rats fed with high fat diet for induction of NASH showed moderate to severe macrovesicular fatty changes, which were diffusely distributed throughout the liver lobule. Parenchymal inflammation with both acute and chronic inflammatory cells accompanying focal necrosis was also observed (Fig. 1-B and 1-C).

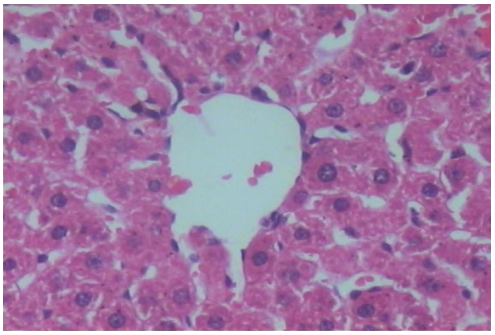

Fig. 1.A: It shows liver section of healthy control rat showing intact histological structure of the liver. Notice the central veins (CV), hepatocytes, and blood sinusoids.

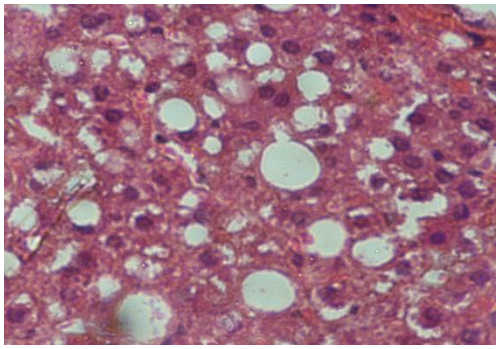

Fig. 1.B: It shows liver section of NASH induced rat showing a high degree of hepatocellular cytoplasmic vacuolation (macrovesicular and microvesicular steatosis).

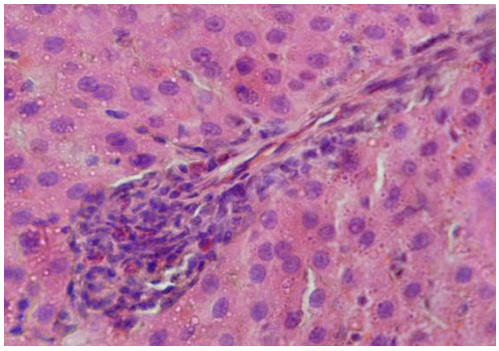

Fig. 1.c: It shows liver section of NASH–induced rat showing parenchymal inflammation with both acute and chronic inflammatory cells accompanying focal necrosis.

Histological examination of liver tissues of NASH-induced group treated with CSM, showed significant reduction in fatty infiltration as compared with that in the untreated NASH-induced group (Fig. 1-D). Interestingly, histological investigation of liver tissues of NASH-induced group treated with the CSF revealed significant improvement in the degree of liver fatty changes which appeared like the healthy control group (Fig. 1-E).

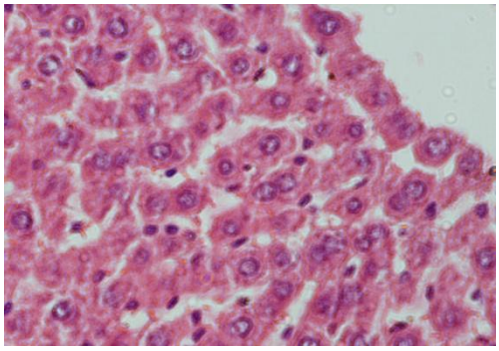

Fig. 1.D: It shows liver section of NASH-induced rat treated with CSM showing significant reduction in fat deposits in liver tissues.

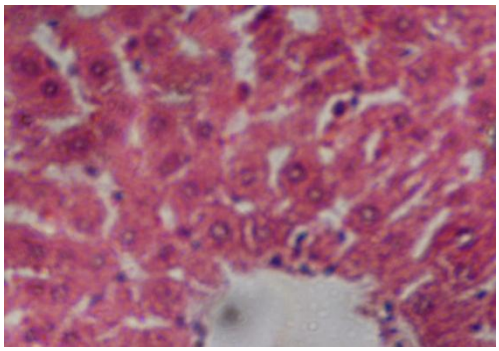

Fig. 1.E: It shows liver section of NASH –induced rat treated with CSF showing that the degree of liver steatosis was improved and the histologic feature was restored to nearly normal (H & E x 300).

Histopathological examination of liver sections of healthy control rats stained with CSHARLACH Rs stain showed negative stain (Fig. 2-A). Moderate macro- and microvesicular fatty changes in the periportal zone in the liver of NASH–induced rats were detected (Fig. 2-B), whereas in NASH-induced rats treated with CSM, few of macro and microvesicular fatty changes were observed (Fig. 2-C). Meanwhile, no fatty infiltration was seen in liver of NASH-induced rats treated with CSF (Fig. 2-D).

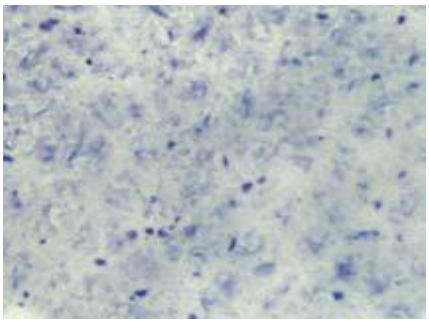

Fig. 2.A: It shows liver section of healthy control rat showing normal histological structure of the liver. The reaction is negative and the hepatocytes are slightly swollen with centrally placed nuclei. No fatty change is seen.

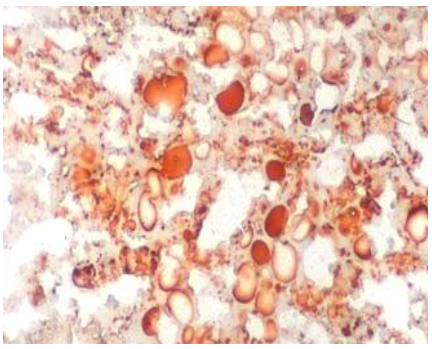

Fig 2.B: It shows liver section of NASH–induced rat showing the positive reaction in the macro and microvesicular fatty infiltration.

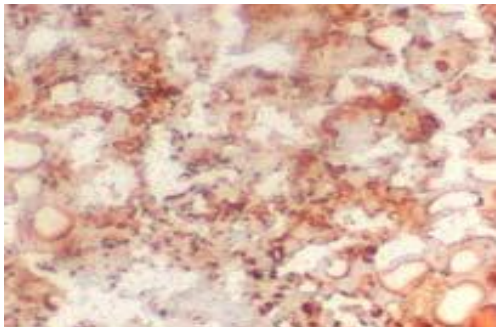

Fig. 2.C: It shows liver section of NASH–induced rat treated with the CSM showing significant reduction in fatty deposits in liver tissues and the reaction is negative in most areas of the lobules.

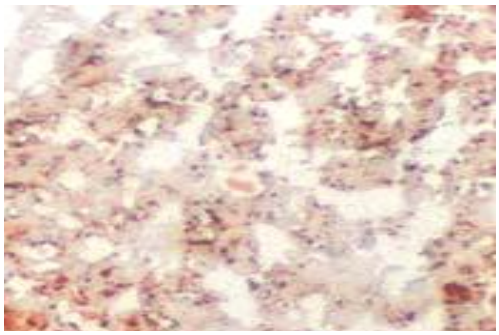

Fig. 2.D: It shows liver section of NASH–induced rat treated with CSF showing that the reaction is negative indicating the improvement of fatty infiltration (SCHARLACH Rs x 300).

Mean fatty infiltration in the NASH-induced group was 3 (Table: 5). Fat deposit in this group was classified as macrovesicular. Mean fatty infiltration in the NASH-induced group treated with CSM or CSF was 1, and fat deposit was mixed. Fatty infiltration in the treated groups was significantly lower than that in the untreated NASH-induced group ($P < 0.05$).

Table 5: Grades of fatty infiltration in the different studied groups

Groups	Rats (n)	Steatosis grades			
		0	1	2	3
Healthy control group	10	10	-	-	-
NASH – induced group	10	-	-	3	8
NASH +CSM-treated group	10	8	-	2	-
NASH +CSF-treated group	10	9	-	1	-

DISCUSSION

The result of the present study revealed marked increase in serum ALT activity in NASH group which is in agreement with Hooper et al. [28]. Both aminotransferases (AST and ALT) are highly concentrated in the liver and the increasing serum ALT activity is considered a consequence of hepatocyte damage in NASH patients [29]. A growing body of evidence supports the possibility that insulin resistance associated with adipose tissue inflammation and hepatic microvascular dysfunction as shown in our histological findings might actually contribute to the development and/or progression of ALT activity in serum [30].

Treatment of NASH group with CSM extract or CSF fraction induced remarkable depletion in serum ALT activity. In addition, both of these treatments led to an improvement in the histological feature of the liver of the treated rats as shown in our results. These effects could be attributed to the active ingredients in *Cynara scolymus* crude extract and fraction which are known as caffeoylquinic acid derivatives (cynarin and chlorogenic acid). These compounds have been proved to be effective in decreasing serum ALT activity [31] *via* their strong hepatoprotective effect and antioxidant capacity.

The current results showed marked increase in serum cholesterol, triglycerides and LDL-cholesterol in concomitant with significant decrease in serum HDL level in NASH group. These results coincide with Adams et al. [32]. 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 [33]. Cellular cholesterol synthesis is regulated by activation of membrane bound transcription factors, designated sterol regulatory element-binding proteins (SREBPs) which are the most abundant in the liver [34] and the excess of cellular cholesterol is esterified by the acyl CoA-cholesterol acyltransferase (ACAT) [35]. The high level of cholesterol synthesis and the increased SREBP-2 activity has paradoxically been shown in subjects with NASH [36].

In NASH disease, the ability of insulin to inhibit the production of very low density lipoproteins (VLDL) is impaired [37]. This results in hyperinsulinemia, and hypertriglyceridemia, which in turn lead to lower HDL cholesterol concentration [38]. This explains the diminished HDL serum level and the high triglycerides level in NASH group in the current study. The histopathological results of the present study showed macrovesicular and microvesicular steatosis. Hepatic accumulation of triglycerides has been associated with the development of macrovesicular steatosis of the liver. Since the inhibition of mitochondrial fatty acid metabolism is considered to result in microvesicular steatosis [39], secondary accumulation of cytosolic triglycerides and phospholipids in the presence of initial mitochondrial damage may explain the development of a mixed type of liver steatosis over time.

The insufficient elimination of triglycerides, probably caused by hepatic insulin resistance [40] may also contribute to the development of NASH. Triglycerides are progressively reduced by the action of lipoprotein lipase (LPL), eventually resulting in intermediate-density lipoproteins (IDLs) and low-density lipoproteins (LDL) with relatively high cholesterol content [41]. LDL circulates and is absorbed by the liver through binding of LDL to LDL receptor [42]. In addition, NAFLD ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) is strongly associates with insulin resistance, which caused inflammatory cytkokine tumor necrosis factor-alpha (TNF-α) to be over expressed in the liver. TNF-α activates cholesterol synthesis and inhibits cholesterol elimination through bile acids, which together contribute to increase LDL-cholesterol and decrease HDL-cholesterol [37].

Treatment of NASH group with CSM or CSF produced marked decrease in serum cholesterol, triglycerides and LDL levels accompanied with significant increase in serum HDL. Additionally, histopathological investigation of liver tissue of the treated groups indicated a reduction in macrovesicular steatosis and microvesicular steatosis. These results coincide with Lattanzio et al. [43] who declared that, the active compunds in *Cynara scolomus* extract represented by caffeic acid, chlorogenic acid, cynarin, cynaroside, scolymoside and have been found to affect cholesterol metabolism. Daniel, [44] reported that, *Cynara scolomus* extract has anticholesterolemic action by decreasing rate of cholesterol synthesis in the liver and other tissue and this 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 [45]. However, highly

significant decrease of plasma LDL and an increase of HDL in the treated groups are agreed with Cieslik et al. [46] who reported decline tendency in total cholesterol, LDL and VLDL when diets were supplemented with *Cynara scolomus* flour. Moreover, Taylor [47] showed a decrease from 10% to 15% in total cholesterol LDL and ratio of LDL to HDL cholesterol in serum due to treatment with *Cynara scolomus* leaves extract. This could be explained as; this extract contains active compounds as flavonoids and caffeoylquinic acid 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 [48]. Furthermore, *Cynara scolomus* extract may work through the indirect inhibition of enzyme hydroxyl methyle glutryle – CoA (HMG-CoA) which avoid problems occur with strong direct inhibitors of HMG-coA reductase during long treatment. The indirect inhibition was supported by the fact that *Cynara scolomus* extract effectively blocked insulin-dependent stimulation of HMG-coA-reductase, a key enzyme in cholesterol synthesis and HMG-coA reductase inhibitors generally reduce cholesterol, LDL and triglycerides levels in serum [49].

The present data showed marked decrease in serum adiponectin level in NASH group. It has been shown that adiponectin is found in relatively high circulating levels but it is decreased in patients with NASH and in clinical manifestations associated with insulin resistance such as metabolic syndrome (MS) and type 2 diabetes mellitus [50]. In addition, plasma adiponectin levels correlated inversely with the markers of systemic oxidative stress, and oxidative stress is known to be a feature of liver disease. Many studies hypothesized that oxidative stress has been demonstrated in conditions such as NAFLD and NASH due to the increased levels of free fatty acids and consequent increased levels of free radicals [51]. In cultured adipocytes, under oxidative stress condition, the suppressed mRNA expression and secretion of adiponectin were detected. This could be attributed to the decreased gene expression of adiponectin under this condition [52].

Treatment of NASH group with CSM or CSF showed marked increase in serum adiponectin level. It has been demonstrated that *Cynara scolomus* extract contains natural antioxidants such as caffeoylquinic acid derivatives and flavonoids [53] that can regulate mRNA expression and secretion of adiponectin [52].

Serum leptin level showed significant increase in NASH group in the present study. Leptin is released into the circulation by mature adipocytes in response to changes in body fat mass and nutritional status. It has varied metabolic effects with the most significant of these being related to body weight and energy expenditure [54]. In NASH patients, leptin levels are elevated and are directly correlated with the severity of steatosis [55]. The presence of hepatic steatosis despite the presence of hyperleptinemia suggests the development of leptin resistance [56]. In addition, leptin levels have been reported to be associated with oxidative stress conditions which enhance reactive oxygen species (ROS) formation in accumulated fat. This leads to the elevated adipose nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that leads to dysregulated production of leptin [52].

Treatment of NASH group with CSM or CSF resulted in appreciable decrease in serum leptin level as compared to the untreated NASH group. *Cynara scolomus* active constituents (caffeic acid and chlorogenic acid) could reduce plasma cholesterol and triglycerides levels and this leads to a decrease in plasma leptin and an increase in adiponectin levels [57].

Serum resistin level in NASH group showed significant increase in comparison with the healthy control group. This result is in agreement with Pagano et al. [58] who reported that patients with NASH are characterised by high serum resistin level. A major target organ of resistin is the liver, where resistin induces insulin resistance and increases glucose production. Resistin is related to hepatic fat content and insulin resistance [59]. It has been suggested that resistin may contribute to hepatic steatosis by promoting insulin resistance and the increased resistin levels in NASH patients are related to histological severity of the disease [60]. Underlying liver damage and the progression of pure fatty liver to NASH and fibrosis, the hepatic stellate cells produce a variety of cytokines, including resistin. Daniel reported that a genetic polymorphism in the promoter region of the resistin gene may be an independent predictor of circulating resistin level. Hence, it is possible that a gene polymorphism(s) may be responsible for the high resistin levels in NASH disease [58].

Treatment of NASH group with CSM or CSF produced remarkable decrease in serum resistin level. Hepatoprotective effect of *Cynara scolomus* leaves extract may be assumed to be related to inducing glutathione peroxidase, besides its direct antioxidant properties which may be useful for the prevention of oxidative stress that exerts an impact on endogenous expression of resistin in the adipocyte [31]. Polyphenolic compounds in *Cynara scolomus* extract may be responsible for the suppression of hydrogen peroxide-induced oxidative stress [61]. By this way, CSM and its fraction (CSF) might reduce serum resistin level.

The present results showed significant increase in serum NF-κB p56 level in NASH group. High oxidative stress status in the liver of NAFLD patients with steatohepatitis may lead to modulation of Kupffer cell function, through activation of transcription factors such as NF-κB [62]. NF-κB then translocates from the cytoplasm to the nucleus to activate the inflammatory cytokines perturbing the inflammatory cycle [63].

Treatment of NASH group with CSM or CSF recorded marked decrease in serum NF-κB p56 level. The inhibition of NF-κB activation correlated with suppression of inhibitor of NF-κB (IκBα) phosphorylation and degradation, p65 nuclear translocation, and NF-κB-dependent reporter gene transcription. *Cynara scolomus* components mainly luteolin and apignen have been found to block IκBα phosphorylation and degradation [64] and in turn it could reduce NF-κB level. Serum TNF-α level showed significant elevation in NASH group as compared to the healthy control group. This could be attributed to the oxidative stress and stimulation of kupffer cell as well as stellate cell to secrete inflammatory cytokines such as TNF-α [65]. Moreover, it has been found that NAFLD patients have elevated plasma levels of lipopolysaccharide-binding protein (LBP) which are further increased in patients with NASH. This increase is related to a rise in TNF-α gene expression in the hepatic tissue which supports a role of endotoxemia in the development of steatohepatitis [66].

Treatment of NASH group with CSM or CSF led to significant decrease in serum TNF-α in comparison with the untreated NASH group. This effect could be attributed to the presence of luteolin and apignen in *Cynara scolomus* extract which could inhibit the inflammatory cytokine production in lipopolysaccharide-induced TNF-α production [67].

Significant increase in serum Cox-2 activity was recorded in NASH group in the current work. This could be explained as the oxidative stress which triggers lipid peroxidation and cytokines production such as TNF-α and interleukin (IL)-6 in the steatotic liver could mediate inflammatory recruitment directly or indirectly *via* activating NF-κB with upstream consequences that include cyclooxygenase-2 activity [68].

Treatment of NASH group with CSM or CSF produced significant decline in serum Cox-2 activity. *Cynara scolomus* extract with its active consitutent (luteolin and apignen) has been found to block NF-κB expression [64]. COX-2, which mediates prostaglandin synthesis during inflammation, is induced by NF-κB [69]. Thus, the inhibition of NF-κB by *Cynara scolomus* extracts contributes in the inhibition of Cox-2 activity.

The present data showed significant increase in serum CD40 level in NASH group. This result is in agreement with Ercin et al. [70]. Soluble CD40 was not only correlated with BMI, but was also more strongly related to lipid peroxidation [71]. Circulating sCD40 was believed to derive predominantly from platelets associated with platelet activation and lipid peroxidation during oxidative stress conditions. Thus oxidative stress plays a role in increasing platelet

CD40 expression [72]. Besides that, the upregulation of CD40 is mediated by TNF-α which stimules platelet activation *via* interaction with its platelet receptors. TNF-α has been shown to enhance oxidative stress *via* NADPH oxidase activation and TNF-α upregulated platelet CD40 *via* arachidonic acid-mediated oxidative stress [73]. Treatment of NASH group with CSM or CSF resulted in significant deplation in serum CD40. Luteolin in *Cynara scolomus* extract may be responsible for this effect. Luteolin could inhibit CD40 ligand expression by activated basophils [74].

The present results showed significant increase in serum hepatocyte growth factor (HGF) level in NASH group. This result is in consistent with that of Koutsogiannis et al. [75]. It has been demonstrated that HGF mRNA produced by nonparenchymal cells increases in NASH patients [76]. In NASH, the activation of Kupffer cells and macrophages within liver tissue increased the production of NF-κB which induced the expression of HGF and consequently its level [4].

Treatment of NASH group with CSF resulted in marked decrease in serum HGF as compared to the untreated NASH group. Luteolin and apignen in *Cynara scolomus* have been found to block NF-κB expression [64] and in turn they could indirectly reduce the stimulant of HGF expression and consequently its level [4].

The current study shed lights on the potential role of CSM and CSF in management of nonalcoholic steatohepatitis. The active contituents of *Cynara scolomus* namely flavonoids and caffeoylquinic acid may be responsible for this effect. These compounds have been proved to have hepatoprotective activity, hypolipidemic effect, antioxidant capacity and antiinflammatory property. Beside that, these compounds could modulate insulin resistance status associated with nonalcoholic steatohepatitis. Therefore, *Cynara scolomus* could have possible therapeutic application in chronic diseases accompanied with insulin resistance and severe inflammation.

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