

Role of Artichoke Leaf Extract as Antioxidant in Nonalcoholic Fatty Liver Disease of Adult Male Albino Rats

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Abstract:

Background: The artichoke plant, scientifically known as *Cynara scolymus*, is often planted in Mediterranean regions. It has a notable abundance of antioxidants. This research aims to evaluate the medical capacity of artichoke in modulating biochemical oxidant and antioxidant enzymes, as well as liver biomarkers, in adult male albino rats with nonalcoholic fatty liver disease. **Methods:** The rats were allocated into three distinct cohorts for the purpose of the study: a control group, a group that was provided with a high-fat diet to induce hyperlipidemia, and a group that received treatment with artichoke leaf extract. Following the conclusion of the experimental phase, blood samples were obtained and subjected to analysis to assess the lipid profile, liver enzyme activity, as well as the levels of malondialdehyde (MDA) and glutathione peroxidase (GSH-PX). Additionally, liver specimens had been subjected to examination using a light microscope. **Results:** Artichoke extract improved blood serum lipids in rats with NAFLD. Triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and TC/HDL and LDL/HDL ratios decreased significantly. HDL values also rose. The treatment with artichoke extract to rats with NAFLD significantly reduced liver enzyme levels. In the group with non-alcoholic fatty liver disease (NAFLD), artichoke extract restored GSH and MDA levels to normal levels. **Conclusions:** The use of artichoke in rat models of non-alcoholic fatty liver disease (NAFLD) had a relation to diminished indicators of oxidative stress.

Key words: Artichoke Leaf Extract, Antioxidant, Nonalcoholic Fatty Liver Disease

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Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as the predominant etiology of chronic liver disease in numerous countries, primarily owing to its strong correlation with metabolic disorders such as diabetes and obesity (1). The most effective treatment strategies for this phenomenon encompass weight reduction, alterations in dietary patterns, and adjustments in lifestyle practices. Nevertheless, due to the multifactorial nature of NAFLD, the application of therapies targeting a single factor is of limited significance. Therefore, the utilization of the herbal medicine approach presents a potentially viable alternative owing to its multifaceted mechanisms of action (2).

The plant commonly known as artichoke (*Cynara scolymus*) is raised in Mediterranean regions and is renowned for its abundance of natural antioxidants, making it a valuable resource in herbal medicine (3). Artichoke has been suggested to possess choleric, hypocholesterolemic, and hypolipidemic properties (4). The nutritional and phytochemical composition of globe artichoke renders it a food that is widely recognized for its health benefits. The composition of the substance includes proteins, minerals, a minimal quantity of lipids, dietary fibers, and a significant concentration of phenolics (5). According to existing literature, the consumption of artichoke leaf extract (ALE) may lead to a reduction in cholesterol levels among individuals with hypercholesterolemia (6). Furthermore, it has been observed that ALE exhibits a reduction in the generation of reactive oxygen species, as well as the oxidation of low-density lipoproteins (LDL) and lipid peroxidation (7). This study's primary purpose is to evaluate the therapeutic efficacy of artichoke in modulating biochemical oxidants, antioxidant enzymes, and liver biomarkers

in adult male albino rats with nonalcoholic fatty liver disease.

Methods

The current study is true experimental research design that was carried out from January 2022 to March 2022 at Faculty of Medicine, Al-Azhar University. The Institutional Research Board (IRB), Faculty of Medicine, Al-Azhar University, Egypt, gave its approval to the study protocol (Approval Number: Pat_3Med.Research_0000003, Date: 2020). Thirty adult male albino rats of a local strain weighing between 150 and 180 g were bought from the Al-Azhar University Animal House Center in Cairo, Egypt. Rats were fed a normal pellet rodent diet along with unlimited access to water. The rats were housed for ten days prior to the experiment for adaption under conventional laboratory settings at $25\pm 2^{\circ}\text{C}$ and a regular light/dark cycle.

Preparation of Artichoke Leaves Aqueous Extract

One liter of distilled water was added to 100 grams of powdered artichoke leaves to create the extract, which was then stored at 60 degrees for 60 minutes. The aqueous extract was filtered, the filtrate dried at 50°C , and it was kept at 4°C until it was needed. Just before to being used in the animal experiments, the dry material was dissolved in distilled water.

Laboratory Design

Rats were split into three equally sized groups. Group I is the control group, which received a standard diet and distilled water orally; Group II is the NAFLD group, which received a lipogenic diet (high fat diet) consisting of a standard diet supplemented with 0.5% cholic acid, 20% sunflower oil, and 2% cholesterol for 3 weeks to produce hyperlipidemia; and Group III is the NAFLD treated with artichoke leaf extract (200 mg/kg body weight, once daily dose by stomach tube for 12 weeks) as previously described by Kim et al. (8)

Drawing blood and calculating biochemical variables:

After an overnight fast, blood samples were obtained at the conclusion of the trial using a retro-orbital puncture performed under isoflurane anesthesia. Sera were separated from blood samples using centrifugation at 4000 rpm for 10 minutes at 4°C, and then the remaining material was promptly frozen at -80°C for further biochemical parameter analysis. Triglycerides, HDL, and total cholesterol were all assessed colorimetrically whereas serum LDL was measured using Friedwald's equation (9). The activity of serum enzymes, including alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), and γ -glutamyl transpeptidase (GGT), were measured using colorimetric assays. The approach developed by Ohkawa et al. (10) was used to evaluate the amounts of malondialdehyde (MDA) in serum. The concentration of glutathione peroxidase (GSH-PX) in the blood stream was evaluated. The liver samples were subjected to histopathological investigation using the hematoxylin and eosin (H&E) staining method, allowing for examination under a light microscope.

Analytical Statistics

The Statistical Package for Social Scientists was used to conduct the statistical analysis (SPSS 25). (Armonk, USA, IBM) The data were presented as mean \pm SD, and the statistical significance was assessed using the SPSS software and Duncan post-hoc test. At P < 0.05, values were deemed statistically significant.

Table (1): Lipid profiles in study groups .

Parameter	GI (control group) (mean \pm SD)	GII (NAFLD group) (mean \pm SD)	GIII (NAFLD treated with artichoke) (mean \pm SD)
Triglyceride (mg/dl)	50 \pm 5	60 \pm 3*	48 \pm 6**
T. cholesterol (mg/dl)	83 \pm 4	123 \pm 8*	85 \pm 7**
LDL-C (mg/dl)	29 \pm 5	77 \pm 9*	29 \pm 4**
HDL-C (mg/dl)	45 \pm 3	34 \pm 2*	47 \pm 1.11**
VLDL (mg/dl)	10 \pm 1	12 \pm 1*	10 \pm 1.1**
Risk I (TC/HDL)	2 \pm 0.1	4 \pm 0.3*	2 \pm 0.10**
Risk II (LDL/HDL)	1 \pm 0.1	2 \pm 0.3*	1 \pm 0.08**

* Significant as compared with GI. ** Significant as compared with the GII.

Results

In comparison to the equivalent controls, NAFLD rats had a markedly elevated level of TG, TC, LDL, VLDL, and ratios of TC/HDL (risk I) and LDL/HDL (risk II), as well as a notable drop in HDL. The serum lipid profile of NAFLD rats treated with artichoke extract was improved as shown by the significantly lower values of TG, TC, LDL, and VLDL as well as the ratios of TC/HDL and LDL/HDL, which were both significantly elevated as seen in Table 1. The levels of ALT, aspartate aminotransferase AST, and GGT enzymes in the blood of rats with NAFLD were significantly higher compared to the control group. However, the administration of artichoke extract to rats with NAFLD resulted in a significant decrease in the enzymatic activity associated with this condition, as indicated in Table 2. The levels of GSH were significantly reduced, while MDA levels were markedly increased in the NAFLD group compared to the control group. However, after administering artichoke extract to the group with NAFLD, there was a significant improvement in GSH and MDA levels, approaching normal values. Additionally, rats with NAFLD displayed a significant increase in the activity of LDH enzyme in their blood compared to the control group. Nevertheless, the administration of artichoke extract to rats with NAFLD resulted in a significant decrease in LDH enzyme activity, as shown in Table 3.

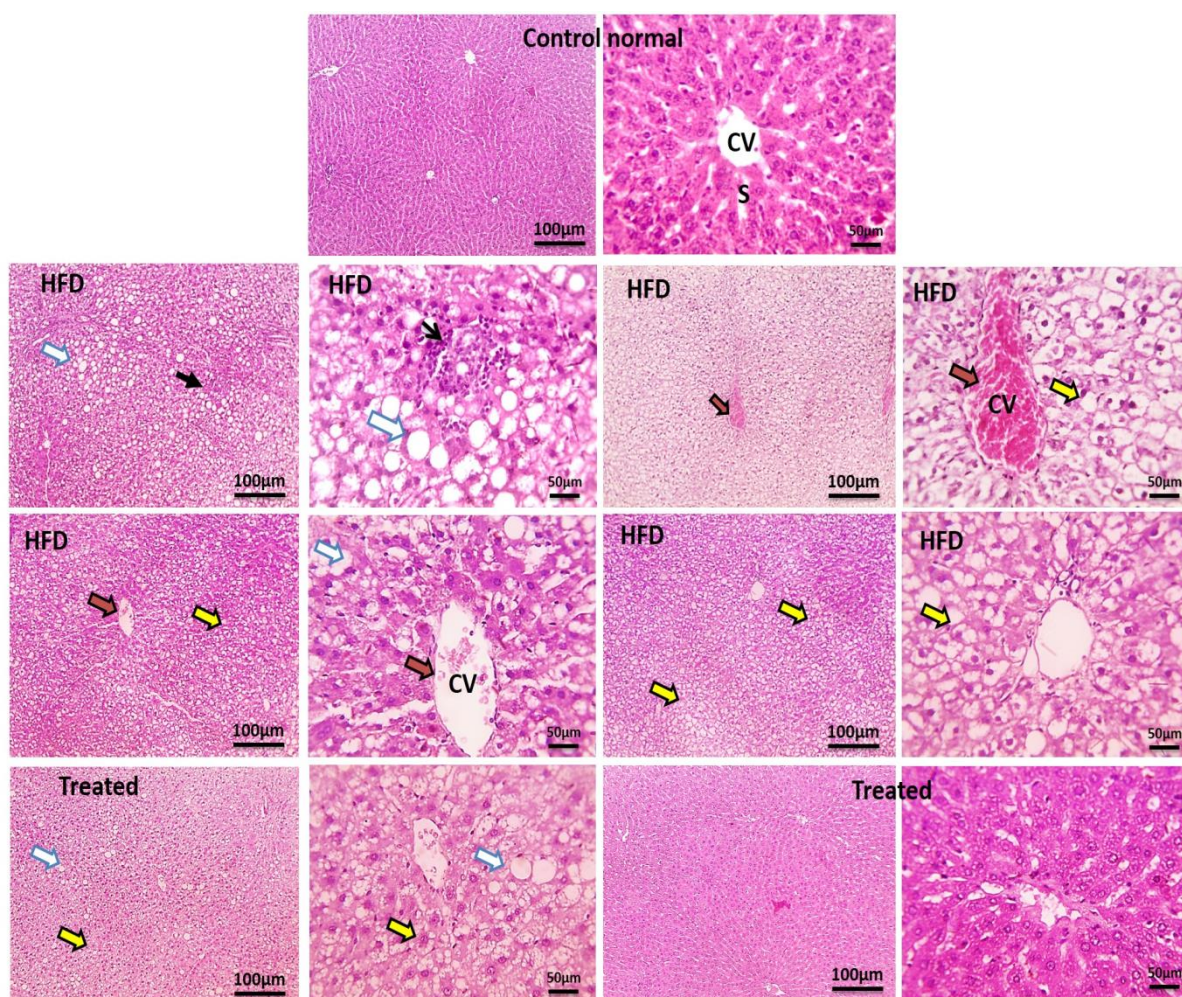


Fig.1: The microscopic examination of H&E-stained hepatic sections from the control rats reveals the presence of a normal histological arrangement in the hepatic lobules. The hepatic cords are seen to be regularly radiating around the central veins (CV), and the sinusoids (S) seem to be within the expected range of normalcy. The hepatic sections of rats fed a high-fat diet (HFD) exhibit widespread macrovesicular steatosis (shown by a thick white arrow) or ballooning degeneration of hepatocytes (indicated by a thick yellow arrow) accompanied with blocked sinusoids, dilated or congested central veins (indicated by a red arrow), and localized aggregation of leukocytic cells (indicated by a thin black arrow). The hepatic sections of the treated rats exhibit a widespread, moderate hydropic degeneration of hepatocytes, as shown by the thick yellow arrow. Additionally, there is a presence of very minor macrovesicular steatosis in a small number of hepatocytes, as indicated by the thick white arrow. These hepatocytes also have blocked sinusoids. Nevertheless, it is important to note that the remaining portions of the rats who received treatment had a histological picture that was within the normal range. At a low magnification of 100X with a bar length of 100, and at a high magnification of 400X with a bar length of 50, the observed images were analyzed.

Table (2): Effect of artichoke on liver enzymes in fatty liver albino rats (mean \pm SD).

Groups Parameter	GI (control group)	GII (NAFLD group)	GIII (NAFLD treated with artichoke)
ALT (IU/L)	39.60 \pm . 17	70.00 \pm .71*	34.40 \pm .10**
AST (IU/L)	42.46 \pm .29	80.00 \pm 39*	49.00 \pm . 16**
GGT (IU/L)	1.60 \pm 0.09	3.60 \pm .12*	1.59 \pm 0.08**

* Significant as compared with GI.

** Significant as compared with the GII.

Table (3): Effect of artichoke on (GSH) and (MDA) levels in fatty liver albino rats (mean \pm SD).

Groups Parameters	GI (control group)	GII (NAFLD group)	GIII (NAFLD treated with artichoke)
GSH (nmol/ml)	46.30 \pm 0.65	24.85 \pm 0.29*	47.25 \pm 0.56**
MDA (nmol/ml)	5.16 \pm 0.45	10.74 \pm 0.45*	5.29 \pm 0.22**
LDH (mg/dl)	316.20 \pm 0.06	608.00 \pm 0.95*	372.60 \pm 0.37**

* Significant as compared with GI.

** Significant as compared with the GII.

Histopathological studies of the liver in experimental groups of rats.

Microscopic H&E-stained hepatic slices of control rats reveal normal lobule structure with properly radiated hepatic cords surrounding central veins (CV) and normal sinusoids. In HFD rats, generalized macrovesicular steatosis (thick white arrow) or ballooning hepatocyte degeneration (thick yellow arrow) with blocked sinusoids, dilated or congested CV (red arrow), and localized leukocytic cell aggregation (thin black arrow). In treated rats, hepatic sections demonstrate widespread moderate hydropic degeneration of hepatocytes (thick yellow arrow) and very slight macrovesicular steatosis (thick white arrow) in a few hepatocytes with blocked sinusoids. Other portions of treated animals have normal histology.

Discussion

The findings of this research indicate that the administration of artichoke in rats with NAFLD led to a significant loss of blood cholesterol and LDL levels as compared to the control group. Additionally, the consumption of artichoke led to significant reductions in plasma triglyceride (TG) levels, as well as in the ratios of total cholesterol (TC) to HDL cholesterol, and LDL to HDL cholesterol. Previous studies have shown the lipid-lowering power of artichoke (4). Research conducted on cultured hepatocytes has shown that artichoke could impede the integration of ¹⁴C-labelled acetate into the non-saponifiable lipid fraction, hence leading to a reduction in cholesterol production.

Furthermore, it has been observed that chlorogenic acid, a bioactive compound found in artichoke, has lipid-lowering properties (4). Additionally, the inclusion of artichoke supplementation in a lipogenic diet resulted in a decrease in plasma LDL-cholesterol levels and a drop in the atherogenic index. As a result, Artichoke may have potential use in the management of coronary heart diseases in individuals with hyperlipidemia (11).

The current research observed that the supplementation of artichoke resulted in significant reductions in lipid peroxidation levels in the bloodstream, accompanied by an increase in the antioxidant capacity of the plasma. In this regard, there are published publications that are consistent with these findings. According to a meta-analysis of animal studies, the use of artichoke extract demonstrated a noteworthy elevation in the levels of superoxide dismutase, catalase, glutathione, and glutathione peroxidase in the liver. Furthermore, supplementation with artichoke extract led to a significant reduction in malondialdehyde levels in both the liver and plasma of animals with induced disease, compared to the control group. (12).

The present investigation highlighted liver damage by a significant increase in serum ALT, AST, and GGT levels, which was further validated by histological analysis in the NAFLD group. The increase in elevation seen may be related to the liberation of these enzymes from the cytoplasm, which then enter the bloodstream after the rupture of the plasma membrane and cellular injury (3). The

findings of the current investigation demonstrated a statistically significant reduction in the blood levels of these enzymes within the group that received artichoke treatment. The observed impact may be ascribed to the presence of antioxidant components in artichoke extract, namely mono- and di-caffeoylquinic acid (cynarin and chlorogenic acid), caffeic acid, and flavonoids such as luteolin-7- β -rutinoside (scolymoside), luteolin-7- β -D-glucoside, and luteolin-4- β -D-glucoside. These compounds are predominantly found in *Cynara scolymus* extract (13–14).

The findings of the current research on the decrease in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and a significant improvement in the lipid profile among those who received *Cynara scolymus* extract, as opposed to those who were administered a placebo. These results coincide with a meta-analysis of intervention studies, indicating that the supplementation of artichoke may potentially lead to a reduction in blood levels of AST and ALT (15).

The observed effects may be ascribed to the active constituents present in *Cynara scolymus* extract, namely the caffeoylquinic acid derivatives cynarin and chlorogenic acid. The administration of artichoke extract resulted in a substantial reduction in the levels of blood cholesterol, triglycerides, and LDL-C in rats fed a high-cholesterol diet, as compared to the control group, with a p-value less than 0.05. The administration of artichoke extract resulted in the suppression of hepatic hydroxymethylglutaryl-CoA (HMG-CoA) reductase activity. Moreover, it has been shown that the extract has cardioprotective efficacy via its ability to enhance antioxidant activity (16).

Several studies have indicated that the extract of *Cynara scolymus* has the potential to decrease blood lipids through two mechanisms. Firstly, it directly affects

the biosynthesis of cholesterol. Secondly, it stimulates the bile production and secretion (17). The decrease in triglyceride levels can be attributed to enhanced glycemic control and a shift in the utilization of glucose without using lipids. The acetyl CoA produced from pyruvic acid enters the Krebs cycle, resulting in the full metabolism of glucose rather than the formation of triglycerides (17).

Cynara scolymus extract has been shown to have an effect in the reduction of glucose levels, namely by influencing glucose absorption. Antioxidant chemicals have been shown to exhibit the ability to delay the depletion of stomach and bowel functions. Additionally, these compounds have been seen to limit the activity of α -amylase and α -glucosidase enzymes in the intestines, hence impeding the breakdown of carbohydrates. Furthermore, they have been shown to hinder the transit of glucose from the bowels to the bloodstream (18).

The group that received *Cynara scolymus* exhibited a simultaneous improvement in liver enzymes and lipid profile, while no such changes were observed in the placebo group. The relationship between cholesterol metabolism and liver fat content remains significant regardless of body weight, suggesting that an increase in hepatic fat content is related with increased cholesterol production (19). The regulation of cellular cholesterol synthesis is mediated through the activation of sterol regulatory element-binding proteins (SREBPs), which are transcription factors located on the cell membrane. Among these factors, SREBPs are particularly abundant in the liver (20–21). The liver is the primary site of expression for sterol carrier protein 2 (SCP-2), also known as non-specific lipid-transfer protein. SCP-2 assumes a pivotal function in the intracellular transportation and metabolism of lipids. SCP-2 has been strongly associated with the pathogenesis of metabolic diseases linked to NAFLD, including obesity, atherosclerosis, Type 2 diabetes mellitus (T2DM), and gallstones.

Recent scientific research has shown that SCP-2 has a favorable influence on NAFLD by effectively modulating many components of lipid metabolism, including cholesterol, endocannabinoid, and fatty acid pathways (22). Therefore, it is postulated that the impacts of *Cynara scolymus* may manifest via the stimulation and engagement of these hepatic metabolic pathways (22).

One notable aspect of the research was its ability to provide insight into the possible impact of artichoke on biochemical and hepatic indicators in individuals with non-alcoholic steatohepatitis (NASH). Previous researches have examined the potential of this herb in the treatment of NASH; however, less attention has been given to investigating the specific effects of this herbal medication on the liver enzymes. Another notable aspect of the present research was its ability to evaluate the concurrent alterations in hepatic enzymes and lipid profile.

Conclusion

There was a correlation between the usage of artichoke in albino rats with NAFLD and an improved bio-metabolic profile as well as a reduction in signs of oxidative stress.

Conflict of interest

None of the contributors declared any conflict of interest.

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