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The Anti-Oxidant and Anti-Inflammatory Effects of *Elaeagnus Angustifolia L.* Extract on Cirrhotic Patients, a Randomized Placebo-Controlled Clinical Trial

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Keywords: Liver disease; Inflammation; Elaeagnus angustifolia L; Fibrosis; Antioxidant; Complementary medicine.

Abstract

Background: Cirrhosis, a prevalent manifestation of chronic liver disease, is associated with a high risk of morbidity and mortality. Previous research has examined the function of oxidative stress and the therapeutic effects of various herbs, albeit with ambiguous results. E. Angustifolia L.'s influence on malondialdehyde (MDA) and tumour necrosis factor- (TNF-) in cirrhosis was evaluated for the first time in the present investigation.

Methods: In this double-blind, placebo-controlled trial, 48 stable cirrhotic volunteers were randomised into two groups: the intervention group (N=24) which received Eleaegnus angostifolia L. and the control group (N=24) which received corn starch as a placebo. After 10 weeks of intervention, serum concentrations of MDA and TNF-, as well as biochemical markers, were evaluated.

Results: The level of MDA in the intervention group decreased statistically significantly among and across groups, whereas it rose in the control group. The decrease in TNF- was only statistically significant based on within-group analysis, not between-group analysis. In the intervention group, the level of alanine amino transferases (ALT) fell dramatically, whereas it rose in the control group.

Conclusion: Despite the statistically significant decrease in MDA level and the non-significant decrease in TNF-, it is difficult to draw a definitive conclusion regarding the anti-inflammatory benefits of Elaeagnus Angustifolia L. This may be owing to the limited sample size, short duration of the study, and diverse disease progression patterns.

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Background

Cirrhosis is the result of chronic liver diseases with many causes, including viral and autoimmune hepatitis, Wilson and hemochromatosis, Non-Alcoholic Steatohepatitis (NASH), and alcoholic liver diseases [1-3]. In its early stages, cirrhosis may be asymptomatic, but if left untreated, it can result in liver decompensation and substantial consequences related to structural and functional abnormalities. Complications of end-stage liver disorders include abnormal substance synthesis, such as albumin, coagulation factors, hormones accompanied by portal hypertension (P.HTN), and ascites with subacute bacterial peritonitis (SBP). Toxin accumulation and hepatic encephalopathy are two additional serious ramifications [4]. Among other concerns are metabolic abnormalities, such as aberrant glucose hemostasis and altered sex hormones metabolism, as well as protein-calorie malnutrition (PCM) with its significant manifestation "Frailty and sarcopenia" [5-6]. These unavoidable issues have detrimental effects on the quality of life of patients and place a large socioeconomic burden on them and their families [7-10]. The irreversibility of consequences and lack of therapeutic options, other than liver transplantation, need additional study to identify therapeutic approaches to prevent or ameliorate liver fibrosis. In light of the significance of uncontrolled cell death and the connection between hepatocyte apoptosis and fibrosis, it is essential to be acquainted with these processes and to study effective interventions in an effort to mitigate further pathological effects. Following the release of extracellular vesicles (EVs) and apoptotic bodies in response to hepatocyte stress, damage-associated molecular patterns are expressed (DAMPs). Activated stellate cells and proinflammatory macrophages promote and increase fibrosis through the release of interleukins, tumour necrosis factor- (TNF-), and transforming growth factor-B if left untreated (TGF-B). Activated T cells also accelerate fibrosis through tumour necrosis factor-related apoptosis by generating ligand or Apo 2 ligand (TRAIL/Apo2L) and F as ligand and its receptor (F as L) [11-13]. In contrast, the role of malondialdehyde (MDA) with its hybrid protein that adducts acetaldehyde (MAA) is extensively investigated in all chronic liver diseases, primarily alcoholic types [11-14]. An intense proinflammatory and profibrogenic response is generated in liver cells by the production of antibodies against MAA epitopes and its carrier protein. This occurs through the activation of a robust immunological response capable of exacerbating apoptosis and fibrosis in liver cells [11-14]. Consequently, reducing stress stimuli, modulating extracellular vesicle release, reducing their circulation, and focusing on their functional signalling role constitute the foundation of diagnostic and therapeutic treatments. In addition to pharmacological and non-pharmacological approaches, extensive research is being conducted on herbal remedies. Eleaegnus angostifolia L., often known as Oleaster or Russian olive, is native to western and central Asia, Iran, southern Russia, and Turkey, and is renowned for its medicinal properties. This plant's pain-relieving, antipyretic, antidiarrheal, and ulcer-healing properties are the basis of Iranian traditional medicine. It has also been utilised for the treatment of asthma, tetanus, rheumatoid arthritis, and kidney stones [12-22]. Several studies have compared the analgesic and anti-inflammatory properties of Russian olive to those of nonsteroidal anti-inflammatory medications from an experimental standpoint. The anti-inflammatory effects of Elaeagnus angostifolia L. on cyclophosphamide-induced nephrotoxicity and its protective effects against myocardial ischemia/repercussion injury in isolated rat hearts are documented in the relevant literature [23,24]. Flavonoid components, polysaccharides, sitosterols, cardiac glycosides, terpenoids, coumarins, phenol carboxylic acid, amino acids, saponins, carotenoids, vitamins, minerals, and tannins are responsible for its qualities [25]. The phenolic hydroxyl group, which chelates metals, reduces lipid peroxidation, and eliminates free radicals, is most closely associated with anti-oxidant action [25]. Some active components of E. angustifolia L. inhibit the expression of the genes encoding inflammatory cytokines and also decrease nuclear factor kappa-B (NF-kB) activity via the Peroxisome proliferator-activated receptor gamma (PPAR-) pathway [25,26].

As far as the literature indicates, no investigations on the potential application of Eleaegnus angostifolia L for liver cirrhosis have been published to date. This study's objective was to evaluate the beneficial effects of Eleaegnus angostifolia L. on compensated cirrhotic patients by investigating the reduction of inflammatory indices such as TNF-alpha and MDA. Alterations in biochemical markers and liver function tests were also evaluated.

Methods

Ethical Declaration

This placebo-controlled, randomised, double-blind clinical trial was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The procedure was approved after consideration by the Shiraz University of Medical Sciences ethics committee (IR.SUMS.REC.1396.47). In addition, it was registered with the Iranian Registry of Clinical Trials (IRCT20100223003408N4). From July to December 2018, participants were recruited from the cirrhosis clinic of the Shahid Motahari outpatient polyclinic affiliated with Shiraz University of Medical Sciences. Preparing E. Angustifolia L. Powder entails: In the fall of 2018, the required E. Angustifolia L. fruit was obtained from the city of Tabriz, Iran. It was confirmed by the Herbarium of Tabriz University of Medical Sciences, Iran, and a voucher specimen was deposited in the Herbarium of Pharmacy Faculty for future reference (Voucher No. TBZ-fph721). The research team eliminated those that were spotted or immature. The remaining fruit was completely washed with tap water and separated before the research team ground the whole fruit, including peel, fruit, and seeds, using a clean mechanical grinder. The powder bulk was packed in sealed packages (each containing 15 g of Elaeagnus Angustifolia L.). As previously mentioned, corn starch was employed as a placebo. Similarly, the dry starch powder was prepared using a clean mechanical grinder. Both powders were held at -18C. Using Nutrient Broth Media, the microbial load of corn starch and E. angostifolia L. powders was studied. As the trial was blind, all powders were similarly packed.

Design of the study and patient selection

In this study, participants were recruited from the cirrhosis clinic at the associated Shahid Motahari outpatient polyclinic from July to December 2018. The objective of this study was to determine the effects of E. Angustifolia L. fruit on inflammatory mediators (MDA and TNF-a) and para-clinical laboratory tests in compensated cirrhotic adult patients (17-65 years) with a MELD score 20. Histories, physical examinations, biopsies, sonograms, hepatic fibro scans, and liver biopsies were utilised to confirm the diagnosis of cirrhosis. Patients with hepatocellular carcinoma (HCC), any underlying systemic disease (diabetes mellitus, hypertension, collagen vascular diseases, and any type of allergy), or genetic issues (Wilson diseases and hemochromatosis), a history of smoking, addiction, alcohol or antioxidant supplement use (vitamin E, vitamin C, lipoic acid, omega 3, fatty

acid, or soy extract), and pregnancy were excluded. Assuming a mean difference of 0.80, a standard deviation of 1, and a probability (test power) of 80%, the sample size was calculated to be 46 patients at a significance level of = 0.05 (using the type 1 error level of =0.05). (23 per each group). Concerning potential loss to follow-up and any evidence of probable adverse effects, a 15% safety margin was defined; hence, 26 patients were assigned to each group. The categorising of patients was accomplished by block randomisation.

The primary objective of our study was absolute changes in TNF MDA (end point minus baseline levels). The decreased values were viewed as advantageous consequences. Absolute change in serum levels of aspartate amino transferases (AST), alanine amino transferees (ALT), and alkaline phosphatase (Alp), Albumin (Alb), and total protein (T.prot) as liver function tests used as surrogate outcomes (LFT). Hemoglobin (Hb), white blood cells (WBC), platelets (plat), estimated sedimentation rate (ESR), and C-reactive protein (CRP) were also evaluated. Blood urea nitrogen (BUN), creatinine (Cr), calcium (Ca), fasting blood sugar (FBS), and lipid profile including triglyceride (TG), cholesterol (Chol), low density lipoprotein (LDL), and high density lipoprotein (HDL) were also evaluated in every patient.

Serum levels of TNF-, pg/ml, were determined using Enzyme-Linked Immunosorbent Assay (ELISA) kits (IBL, Germany) for human quantitative assessment, whereas MDA levels were determined using the thiobarbituric acid (TBA) method. Other paraclinical parameters were measured using normal automated procedures. All patients' body mass indices (BMI) were computed based on their weight and height2. The weights of the participants were determined using a calibrated scale (Omron, Korea) with an accuracy of 0.1 kg, and their heights were determined using a stadiometer with an accuracy of 0.1 cm, both without shoes.

All referred cirrhotic adult patients (521) between the ages of 17 and 65 were evaluated for matching inclusion criteria over the course of four months. Notably, each patient was observed for a period of 10 weeks. 130 individuals with MELD scores below 20 were reassessed for exclusion until the target sample size (no. 49) was reached. 46 participants finished the trial.

Intervention and monitoring

All participants were fully informed of the purpose and protocols of the study and have signed and dated consent forms. Under the supervision of a gastroenterologist, the researcher explored and clarified potential adverse outcomes. Patients were also blinded to the treatment. The intervention group consumed 15 g /day of powdered whole fruit, while the control group consumed 15 g /day of corn starch as a placebo. Both groups were instructed to ingest 15 g of whole fruit powder or a placebo with one glass of water each night immediately following dinner for ten weeks to prevent gastrointestinal problems. All supplements were identically packed and supplied to patients during a 10-week period. The patients' adherence and compliance were monitored twice each week by telephone contact, and any reported adverse effects were documented. The patient was considered compliant if 90 percent of the supplied medication was swallowed. In addition, patients were instructed to visit the clinic to get the prepared packages on the day of their initial visit, on day 45 of the study for follow-up, and on the last day of the study. During the course of the trial, we asked patients not to alter their lifestyle or traditional therapies. In addition, a 24-hour meal recall was performed on all patients at the beginning and conclusion of the trial to ensure that their dietary intakes (micro- and macronutrients) were not significantly different. Utilizing the International Physical Activity Questionnaire, their physical activities were determined based on the metabolic equivalent of tasks calculated in 24 hours (IPAQ). There was no significant difference between the two groups in terms of physical activity.

Analytical statistics

All data was analysed using SPSS version 21.0 and presented as means standard error. Using the Kolmogorov-Smirnov test, the normality of the data was determined (K-S). The independent sample T-test was used to evaluate data with a normal distribution pattern, whereas the Mann-Whitney U-test was used to investigate data with a skewed distribution pattern. A P value of.... 0.05 was statistically significant.

Results

In this double-blind case-control clinical experiment, the medical records of 521 cirrhotic patients were reviewed retrospectively over the course of four months. 130 out of 521 individuals were reviewed, and 53 of those patients met the study's inclusion criteria (cirrhotic patient with MELD scores..... 20, no systemic disease, and similar socioeconomic conditions). 27 patients (15 female and 12 males with a mean age of 47 years) were randomly assigned to the intervention group (15 g of Eleaegnus Angostifolia per day) and 26 patients (10 female and 16 males with a mean age of 48 years) were assigned to the control group (using corn starch as placebo). Three patients in the intervention group did not complete the course; one had a new beginning of mild to moderate pruritus that was treated by an antihistamine after a few days, but the patient discontinued the study. The tow others exhibited availability issues. One patient in the control group had poor follow-up, while another had pyelonephritis. As depicted in Figure 1, 48 patients completed the evaluation, and their results were examined.

Before and after the investigation, body weight, height, and body mass indices were measured twice by two nurses using the same instrument, and the mean values for each index are included in this study. There were no significant differences between the two measurements for each index. **Table 1** displays baseline demographic and anthropometric information. The only statistically significant difference between groups was in height, although body mass indices did not differ significantly. Malondialdehyde and tumour necrosis factor-alpha levels were measured similarly in the same laboratory. Other para-clinical indices, such as liver function tests, lipid profile, and hematologic parameters, were evaluated in both groups in the same laboratory with the same methodology. Unfortunately, ESR and CRP measurements were not trustworthy due to a technical error. The remaining measures are illustrated in **Table 2**. Except for lipid profile (cholesterol, and low density lipoprotein), and Cr, which were greater in the Elaeagnus angustifolia group, there were no statistically significant variations between the case and control groups' baseline para-clinical data. (P values were..... 0.02, 0.01, and 0.04, respectively) Compared to the control group, the intervention group had a lower haemoglobin level (P value:.... 0.02). After describing to each patient the method of solution preparation, the time of intake, and the follow up programme (telephone number, availability, and outpatient appointment), samples were administered in a block random way. All patients' general conditions, daily activities, food programme, and drug compliance were monitored twice

Table 1: Pre and post-interventional demographic and basic anthropometric data of *Eleaegnus Angostifolia* (case) and *Starch* (control) pre and post intervention.

	Mean ± SD				B.G*p-value
	Eleaegnus Angostifolia		Control		
M/ Female	15-Sep		Aug-16		-
Age (years old)	47.00 ± 7.95		48.62 ± 6.57		0.44
Weight (before) Kg**	64.83 ± 14.03	W.G*** P. value: 0.55	66.95 ± 11.51	W.G P. value: 0.96	0.57
Weight (after) Kg	65.00±13.67		66.95 ± 11.69		0.59
Height; M****	1.60 ± 0.08		1.65 ± 0.05		0.02*****
BMI (wt/ht²)****: before**	25.05 ± 5.02	W.G P. value: 0.41	24.36 ± 3.78	W.G P. value: 0.96	0.58
BMI (wt/ht²)****: After**	25.14 ± 5.06		24.35 ± 3.85		0.54

- SD; standard deviation, *B.G, between group, ** kilo gram, W.G, ***within groups,**** meter, ***** Significant
- ******Body mass index

Table 2: Pre interventional clinical data of Eleaegnus Angostifolia (case) and starch (control).

Variables	*Mean ± SD*		*p-value*
	Eleaegnus Angostifolia	**Control**	
MDA; µmol/ l	4.68± 1.14	4.09±0.43	0.1
TNF-α; mg/dl	7.94±3.46	6.67±2.05	0 .13
MELD score	15.20 ± 3.27	14.66± 2.63	0.65
AST; IU/l	45.33±33.08	47.08±22.81	0.22
ALT; IU/l	32.95± 20.05	33.91± 11.51	0.11
Alp; U/l	238.70±87.24	262.12±63.81	0.16
T. protein; g/dl	6.90±0.58	6.92±0.44	0.7
Albumin; g/dl	3.81±0.33	3.82±0.43	0.81
T. bili; mg/dl	1.35±0.66	1.27±0.59	0.74
D. bili; mg/dl	0.35±0.16	0.40±0.15	0.26
TG; mg/dl	103.25±44.46	84.75±29.08	0.09
T. cho; mg/dl	164.12±35.51	140.83±24.97	**0.02***
LDL; mg/dl	91.33±26.45	77.58±16.58	**0.03***
HDL; mg/dl	45.39±9.04	44.87±15.25	0.45
FBS; mg/dl	90.54±10.02	90.95±9.53	0.88
BUN; mg/dl	15.25 ± 7.37	14.13±5.31	0.76
Cr; mg/dl	1.02 ± 0.26	0.88±0.17	**0.04***
Ca; mg/dl	9.42 ± 0.43	9.25±0.37	0.12
WBC; 8100/ul	4.40 ± 1.19	4.89±1.41	0.32
Hb; g/dl	13.20 ± 2.10	14.45±1.50	**0.02***
*Platelet;*1000/ul*	124.16 ± 69.76	124.50±66.26	0.86
INR ratio	1.20 ± 0.25	1.26±.26	0.34

*Statistically significant, SD; standard deviation; µmol/ l; micromole, mg/dl; milligram per deciliter, IU/l; international unit per liter, U/l; unit per liter, gr/dl ; gram per deciliter, MDA: Malondialdehyde, TNF-A Tumor necrosis factor-alpha, no/ul; number per microliter, MELD; model of end stage liver diseases' AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, Alp; alkaline phosphatase, T. protein; total protein, T. bili; total bilirubin, D. bili; direct bilirubin, TG; triglyceride, Cho; cholesterol; LDL; low density lipoprotein, HDL; high density lipoprotein, FBS; fasting blood sugar, BUN; blood urea nitrogen, Cr; creatinine, Ca; calcium, WBC; white blood cell, Hb; hemoglobin, INR, international normalized ratio.

weekly by phone and once in the outpatient department (OPD) after 35 days (5 weeks). For greater tolerability, some patients dissolved the powder in 10 cc of water, rather than 15 cc, before administering it (less than10 percent). One patient in the intervention group was lost to follow-up due to mild to moderate itching, and one patient in the control group was lost to follow-up due to pyelonephritis. Two intervention group patients and one control group patient had inadequate follow-ups. In terms of their daily activities and nutrition, neither group exhibited any noteworthy modifications or variations.

Following the intervention (10 weeks later), both groups were reassessed using the same approach and measures. The within-group variable changes (post-pre values) and directions of both groups were evaluated. Both groups had varied changes with reverse direction (increment versus decrement). **Table 3** displays the results together with their directional changes (A for Elaeagnus angustifolia group and B for control group).

Changes in MDA, ALT, FBS, and Ca were significant (P. values 0.05) in both groups, but in opposing directions for MDA and FBS. TNF-, TG, and BUN levels decreased in both groups, however it was not statistically significant. MELD score, alkaline phosphatase, albumin, cholesterol, high density lipoprotein, and INR all showed modest increases.

The relevance of change directions, as indicators of disease improvement or progression, necessitates comparing the mean of variable changes between the two groups. **Table 4** displays the results. As observed in Table 4, when comparing the two groups, the changes in MDA, ALT, and Hb in the intervention group were statistically significant and indicated an improvement. The decrease in tumour necrosis factor alpha in both groups was not statistically significant.

Figure 2 depicts the changes in the main outcome variables (MDA and TNF-). Elaeagnus exhibited a decrease in MDA levels. Angustifolia, while an increase in the starch group suggests a positive effect of Elaeagnus on the oxidative marker MDA. Similarly minor decreases in TNF levels were found in both groups, however the Elaeagnus group was more affected.

Although not definitively diagnostic, AST, ALT, and Alp values are suggestive of liver disorders (hepatocyte inflammation and biliary disorder). The results of Elaeagnus use. The Angustifolia are depicted in Figure 3. In both groups, there was a modest decrease in AST and an increase in Alp, although the direction of ALT alterations was different (decreased in Elaeagnus.Angustifolia, increasing in the group of starch)

Both groups' changes in calcium, haemoglobin, and INR were compared as nonspecific factors. Figure 4 also demonstrates the outcomes. Calcium and INR levels increased in both groups without statistical significance, however haemoglobin levels increased significantly in the Elaeagnus. Angustifolia group compared to the control group.

Table 3: Within groups' comparison of pre versus post intervention and the direction of changes in Eleaegnus Angostifolia and control groups.

Variables	*A: Eleaegnus Angostifolia*				*B: Control*			
	Pre.	Post	P. value	Dir.	Pre.	Post	P. value	Dir.
MDA; µmol/ l	4.68±1.14	4.17±1.01	0.00*	Dec**.	4.09±0.43	4.42±0.69	0.02*	Inc.***
TNF-α; mg/dl	7.94±3.46	7.15±3.62	0.02*	Dec.	6.67±2.05	6.56±2.92	0.8	Dec.
MELD score	15.20 ± 3.27	15.33± 3.74	0.63	Inc.	14.66± 2.63	15.25 ± 2.96	0.47	Inc.
AST; IU/l	45.33±33.08	43.04±20.74	0.83	Dec.	47.08±22.81	46.08±22.58	0.43	Dec.
ALT; IU/l	32.95± 20.05	27.08±16.09	0.01*	Dec.	33.91± 11.51	39.58±14.02	0.00*	Dec.
Alp; U/l	238.70±87.24	256.70±98.50	0.1	Inc.	262.12±63.81	279.79±80.55	0.19	Inc.
T. prot.; g/dl	6.90±0.58	6.95±0.47	0.39	Inc.	6.92±0.44	6.90±0.53	0.84	Dec.
Alb; g/dl	3.81±0.33	3.94±0.35	0.05*	Inc.	3.82±0.43	3.89±0.43	0.38	Inc.
T. bili; mg/dl	1.35±0.66	1.19±0.69	0.28	Inc.	1.27±0.59	1.24±0.35	0.76	Dec.
D. bili; mg/dl	0.35±0.16	0.38±0.19	0.36	Inc.	0.40±0.15	0.37±0.13	0.27	Dec.
TG; mg/dl	103.25±44.46	98.29±44.49	0.43	Dec.	84.75±29.08	84.41±25.48	0.93	Dec.
T. chol; mg/dl	164.12±35.51	165.54±32.90	0.84	Inc.	140.83±24.97	143.70±28.50	0.47	Inc.
LDL; mg/dl	91.33±26.45	92.45±29.68	0.85	Inc.	77.58±16.58	75.33±23.70	0.52	Dec.
HDL; mg/dl	45.39±9.04	48.00±10.06	0.6	Inc.	44.87±15.25	48.58±15.81	0.27	Inc.
FBS; mg/dl	90.54±10.02	98.50±15.00	0.01*	Inc.	90.95±9.53	97.29±18.08	0.03*	Inc.
BUN; mg/dl	15.25±7.37	14.64±6.20	0.59	Dec.	14.13±5.31	13.56±5.33	0.18	Dec.
Cr; mg/dl	1.02±0.26	0.98±0.26	0.12	Dec.	0.88±0.17	0.90±0.17	0.47	Inc.
Ca; mg/dl	9.42±0.43	9.67±0.32	0.00*	Inc.	9.25±0.37	9.40±0.43	0.00*	Inc.
WBC; 8100/ul	4.40±1.19	4.69±1.46	0.15	Inc.	4.89±1.41	4.74±1.28	0.34	Dec.
Hb; g/dl	13.20±2.10	13.36±2.24	0.31	Inc.	14.45±1.50	13.91±1.57	0.00*	Dec.
*Plat.;*1000/ul*	124.16±69.76	127.29±52.65	0.3	Inc.	124.50±66.26	121.41±70.60	0.54	Dec.
INR, ratio	1.20±0.25	1.27±0.40	0.2	Inc.	1.26±.26	1.34±0.36	0.39	Inc.

*Statistically significant, ** Decreased, *** Increased. The data are reported as *Mean ± SD* (SD; standard deviation).. µmol/ l; micromole, mg/dl; milligram per deciliter, IU/l; international unit per liter, U/l; unit per liter, gr/dl ; gram per deciliter, MDA: Malondialdehyde, TNF-A Tumor necrosis factor-alpha, no/ul; number per microliter, MELD; model of end stage liver diseases' AST; Aspartate aminotransferase, ALT; Alanine aminotransferase, Alp; alkaline phosphatase, T. protein; total protein, T. bili; total bilirubin, D. bili; direct bilirubin, TG; triglyceride, Cho; cholesterol; LDL; low density lipoprotein, HDL; high density lipoprotein, FBS; fasting blood sugar, BUN; blood urea nitrogen, Cr; creatinine, Ca; calcium, WBC; white blood cell, Hb; hemoglobin, INR, international normalized ratio.

Discussion

In the present clinical investigation, the anti-inflammatory and anti-oxidant properties of E. angustifolia L. were studied for the first time in patients with cirrhosis. The primary outcome measures were the absolute changes of two inflammatory mediators, MDA and TNF-, relative to the placebo (starch). Changes in laboratory results and biochemical measurements (LFT, lipid profile, CBC, and INR) were added as surrogate outcome factors.

Oxidative stress is characterised by altered cellular and redox balance, resulting in cellular function adaptation, as well as activating downstream signalling pathways and irreversible chemical change of substances [27]. According to the literature, the role of plasma proinflammatory cytokines (MDA and TNF-) has been extensively studied, and elevated levels of MDA and TNF- have been observed in patients with inflammatory bowel disease (IBD), diabetes mellitus (DM), obesity, anxiety, and any type of acute or chronic liver disease, including viral hepatitis, steatohepatitis, NAFLD, and hypertension [28-31]. As the final stage of chronic liver diseases, hepatic cirrhosis is linked to an increase in free radicals, regardless of its origin. In cirrhosis, beneficial anti-inflammatory effects of several pharmaceutical and botanical medicines on alterations of these cytokines (mediators) have been studied [32]. In contrast, E. angustifolia has demonstrated therapeutic antioxidant effects in vivo and in vitro for hypertension, kidney stones, type 2 diabetes, ulcer healing, skin wounds, and obesity, albeit in small quantities and with variable efficacy [15-22,33,34]. In their study on mice, Farahbakhsh et al. observed the anti-inflammatory and anti-oxidant properties of E. angustifolia L. extract. Similar to the actions of indomethacin, the extract inhibited cyclooxygenase enzymes of types 1 and 2 (COX 1 and 2) in a separate research [22]. The research of Nikniaz et al. demonstrated a reduction in TNF- and an increase in anti-inflammatory factors such as interloukin-10 (IL-10) in knee osteoarthritis patients

who consumed 15g of whole fruit powder per day[18]. Notably, no interventional study evaluating the therapeutic effects of Russian olive on cirrhotic patients has been conducted to far. As a result, we were compelled to conduct this case-control study and evaluate the impact of this substance on antioxidant levels. Before intervention, the between-group p-values for MDA and TNF- were …0.10 and…. 0.13, respectively. There were no statistically significant differences in post-interventional p-values between groups (….0.09 and…. 0.82 for MDA and TNF-a, respectively), although within-group tests indicated statistically significant changes in MDA levels in both groups, albeit in reverse direction (decreased in interventional but increased in control). Logically, these decreased changes in the treatment group and increased changes in the control group can be attributed to the antioxidant benefits of Russian olive in the intervention group and the progressive nature of the disease in the control group, respectively. Both groups demonstrated decreased levels of TNF-. Although the intervention group had a greater shift, the difference was not statistically significant. The difference between our results and those of other research about the efficacy of different herbal or pharmaceutical components may be attributable to the nature and stage of the disease, the ethnicity of the participants, or the laboratory processes [35]. It should be noted that Russian antioxidant effects (phenolic and anthocyanosides) are depending on genotype (7genotypes) [28]. Variability in harvesting time is another compounding factor impacting antioxidant levels; the first 10 days of October are regarded as the optimal harvesting period [28]. In this study, E. Angustifolia L. fruit was obtained in the fall of 2018 from Tabriz, Iran. It was confirmed by the Herbarium of the Tabriz University of Medical Sciences, Iran, and a voucher specimen was deposited in the Herbarium of the Pharmacy Faculty for future reference. In light of the involvement of intestinal bacteria in liver parenchymal injury, other potential therapeutic activities of E. angustifolia L, such as antibacterial and antifungal, may be noteworthy [25,36].

Table 4: Between groups comparison of variable changes in Eleaegnus Angostifolia (cases) vs starch (control).

Variables	*Mean ± SD*		*P-value*
	Eleaegnus Angostifolia	Control	
MDA; µmol/ l	-0.51 ±0.54	0.46± 1.02	0.00**
TNF-α; mg/dl	-0.78± 1.57	-0.10 ± 1.99	0.19
MELD score	0.12± 1.72	0.58± 2.74	0.57
AST; IU/l	-2.29 ± 34.02	-1.00 ± 18.38	0.50
ALT; IU/l	-5.87 ± 10.28	5.66 ± 10.89	0.00**
Alp; U/l	18.00 ± 92.19	17.66± 64.35	0.26
T. prot.; g/dl	0.05±0.34	-0.02 ±0.51	0.53
Alb; g/dl	0.12±0.27	0.06± 0.29	0.38
T. bili; mg/dl	-0.15 ± 0.51	-0.02 ± 0.43	0.56
D. bili; mg/dl	0.03 ± 0.22	-0.03± 0.14	0.21
TG; mg/dl	-4.95 ± 30.34	-0.33 ± 21.07	0.76
T. chol; mg/dl	1.41 ± 35.74	2.87 ± 19.38	0.43
LDL; mg/dl	1.12 ± 29.62	-2.25 ± 17.17	0.66
HDL; mg/dl	2.60 ± 9.92	3.70 ± 10.78	0.86
FBS; mg/dl	7.95 ± 14.56	6.33 ± 13.74	0.85
BUN; mg/dl	-0.60 ± 5.41	-0.57± 2.69	0.53
Cr; mg/dl	-0.03 ±0.11	0.01 ± 0.13	0.16
Ca; mg/dl	0.25 ± 0.34	0.15 ± 0.24	0.2
WBC; 8100/ul	0.29± 0.97	-0.15± 1.18	0.18
Hb; g/dl	0.15± 0.74	-0.54 ± 0.70	0.00**
*Plat.;*1000/ul*	*3.12 ± 34.02*	*-3.08 ± 20.65*	*0.26*
INR, ratio	0.07 ±0.22	0.07 ± 0.30	0.95

*Statistically significant, SD; standard deviation; µmol/ *l*; micromole, *mg/dl*; milligram per deciliter, *IU/l*; international unit per liter, *U/l;* unit per liter, *gr/dl* ; gram per deciliter, *MDA*: Malondialdehyde, *TNF-A* Tumor necrosis factor-alpha*, no/ul*; number per microliter, *MELD*; model of end stage liver diseases' *AST*; Aspartate aminotransferase, *ALT*; Alanine aminotransferase, *Alp;* alkaline phosphatase, T. *protein*; total protein, T. *bili;* total bilirubin, D. *bili*; direct bilirubin, *TG*; triglyceride, *Cho*; cholesterol; *LDL*; low density lipoprotein, *HDL*; high density lipoprotein, *FBS*; fasting blood sugar, *BUN*; blood urea nitrogen, *Cr*; creatinine, *Ca*; calcium, *WBC*; white blood cell, *Hb*; hemoglobin, *INR*, international normalized ratio

In liver disease, increased enzyme leakage is suggestive of liver cell inflammation, but their levels are often normal towards the end stage of liver damage due to cell depletion. E. angustifolia L. is considered to have beneficial anti-inflammatory effects on our patients with MELD scores of less than 20 based on a statistically significant decrease in ALT in the intervention group relative to the control group. Changes in AST levels were not statistically significant, which may be explained by the lower specificity of AST for estimating liver function [37]. There was no considerable increase in Alp. Level. According to the research, this may be related to an elevated calcium level and bone mineralisation in the interventional group [38]. Due to the short duration of the intervention and the extended half-life of albumin, a moderate change in albumin concentration was anticipated [39].

In this study, there were no statistically significant decreases in Bilirubin, BUN, or Cr, which may be owing to the anti-inflammatory and protective action of Russian Olive on renal function, as demonstrated in [22,24]. Increased levels of cholesterol, LDL, and FBS, while not statistically significant, may be attributable to the patients' improved appetite or the herb's side effects, which should be explored further. Hb, WBC, and platelet decreased in the control group, whereas they increased in the patients. It was statistically significant only for Hb, which may be attributable to the protective impact of Russian olive against the natural progressive decrements observed in the control group or its favourable effect on the bone marrow of the cases [40].

The beneficial anti-inflammatory effects of E. angustifolia L. on different diseases have been demonstrated in the literature [19-22, 35], as have the effects of herbs other than E. angustifolia L. on cirrhosis. This strongly suggests that the effect of E. angustifolia L. on cirrhosis has not been studied, which, on the one hand, is indicative of the novelty of our research and, on the other hand, demonstrates that the results are unlikely to be comparable on a one-to-one correspondence basis.

Though criteria such as severity levels, inflammation levels, and peroxidase levels have been the most prominent difficulties in cirrhosis research, inevitable confounding factors such as patients' age, gender, and diverse disease etiologies prevent us from reaching a definitive conclusion. Diverse types of antioxidants with variable biological activity in various animal species and diverse assessment techniques were additional challenges that made it difficult to compare the results of this study with those of others.

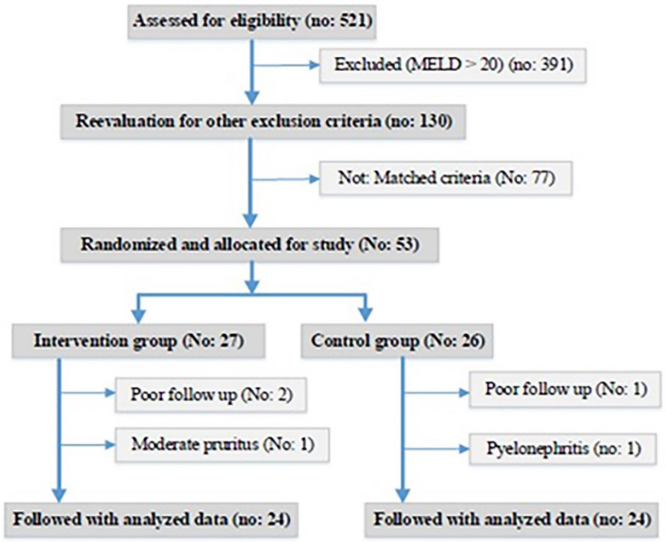

Figure 1: Consolidated standards of reporting trials (CONSORT) flow design.

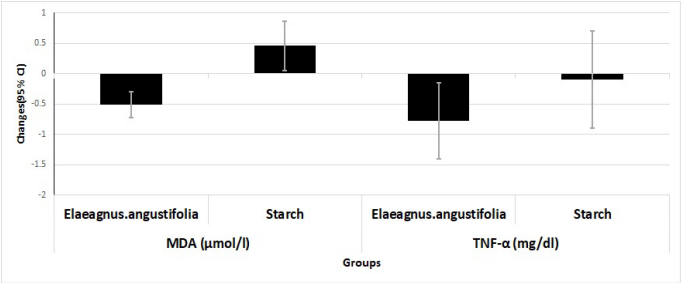

Figure 2: The changes of malondialdehyde (MDA) and tumor necrosis factor- α (TNF- α) levels in Elaeagnus. Angustifolia and control groups.

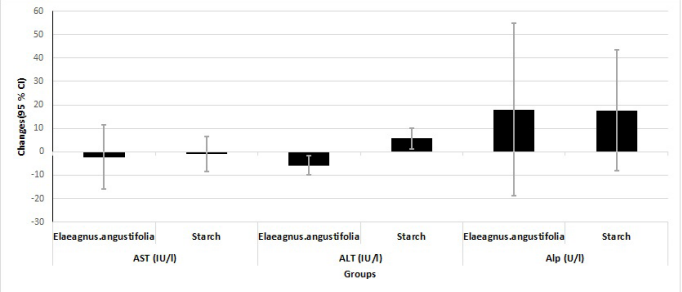

Figure 3: The changes of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels in Elaeagnus. Angustifolia and control groups.

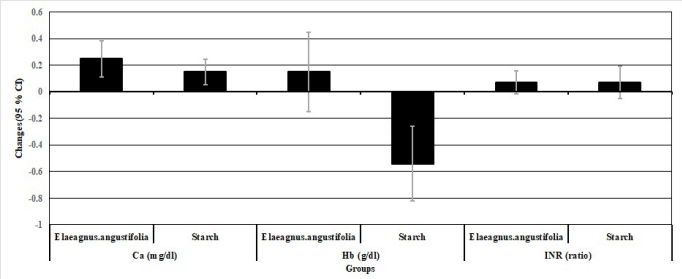

Figure 4: The changes of calcium (ca), hemoglobin (Hb) and INR, international normalized ratio (INR) in Elaeagnus. Angustifolia and control groups.

Conclusion

After consuming E. angustifolia L. powder, a statistically significant difference in MDA and ALT levels was identified based on between-group analysis, which may be indicative of an improvement in disease progression through modifying oxidative stress pathways. Taking into mind the limits and restrictions of this study, the findings of this study urge the conduct of additional controlled clinical trials with longer durations and multiple observations on a larger number of patients. Other pro-inflammatory signs and causes that are symptomatic of liver problems and their amelioration can be the subject of additional research.

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Conflict of interest

The authors declare that they have no competing interests.

Abbreviations

NASH: Non-Alcoholic Steatohepatitis; P.HTN: Portal Hypertension; SBP: Sub-Acute Bacterial Peritonitis; PCM: Protein-Calorie Malnutrition; DAMPs: Damage-Associated Molecular Patterns; TNF- α: Tumor Necrosis Factor-A; TGF-*B*: Transforming Growth Factor-*B;* MDA: MD Amalondialdehyde; PPAR-γ: Peroxisome Proliferator-Activated Receptor Gamma; NF-kB: Nuclear Factor Kappa-B; MELD: Model of End Stage Liver Disease; HCC: Hepatocellular Carcinoma; AST: Aspartate Amino Transferases; ALT: Alanine Amino Transferees; Alp: Alkaline Phosphatase; Alb: Albumin; T.prot.: Total Protein; LFT: Liver Function Tests; Hb: Hemoglobin; WBC: White Blood Cell; ESR: Platelet (plat), and Estimated Sedimentation Rate; CRP: C - Reactive Protein; BUN: Blood Urea Nitrogen; Cr: Creatinine; Ca: Calcium; FBS: Fasting Blood Sugar; TG: Triglyceride; Chol: Cholesterol; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; ELISA: Enzyme-Linked Immunosorbent Assay; TBA: Thiobarbituric Acid; BMI: Body Mass Indices; IPAQ: International Physical Activity Questionnaire; OPD: Outpatient Department.

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