



Bombax ceiba flowers extract ameliorates hepatosteatosis induced by ethanol and relatively moderate fat diet in rats



A.F. Arafa^{a,*}, D.S. Foda^a, A.H. Mahmoud^a, N.S. Metwally^a, A.R.H. Farrag^b

^a Department of Therapeutic Chemistry, Pharmaceutical and Drug Industries Research Division, National Research Centre (NRC), El Bohouth st., Dokki, Giza, Egypt

^b Department of Pathology, Medical Research Division, National Research Centre (NRC), El Bohouth st., Dokki, Giza, Egypt

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ABSTRACT

Chronic excessive alcohol consumption could induce serious liver injury. In this study, therapeutic effect of aqueous methanol extract of *Bombax ceiba* L. flowers (BCE) (Family: Bombacaceae) was investigated against hepatic steatosis. This study included seven groups, and the research period was eight weeks. The first group served as control. The six remaining groups were divided into two categories, three groups in each. The first category was fed fat diet. The second category was fed fat diet and orally administered ethanol, which was given in graduate doses from 2 g/kg/d to 6 g/kg/d. Then, one group from each category was orally treated with the standard drug fluvastatin (2 mg/Kg/d). Another group was orally treated with BCE (200 mg/kg/d). The third group left untreated. The results revealed that BCE significantly decrease both the body and liver weight. The treatment with BCE extract also ameliorates the effect of alcohol induced increase of liver enzyme activities. In addition, the extract was significantly increased hepatic liver antioxidants and decreased malondialdehyde (MDA) level. Also, serum lipid profiles: triglycerides (TG), total cholesterol (TC) and low density lipoprotein (LDL) were significantly decreased after BCE treatment. Histopathological study showed fatty changes induced by alcohol which were improved by BCE treatment. These data suggest that the BCE has anti-inflammatory, antioxidant and anti-steatosis potential properties against alcohol induced liver damage. This may be due to the presence of flavonoids and other phenol compounds.

1. Introduction

Excessive alcohol consumption causes a wide spectrum of liver and other organ diseases. Alcoholic liver disease (ALD) has a complex and incompletely known pathogenic. The liver dysfunction ranges from fatty change to alcoholic hepatitis, cirrhosis and hepatocellular carcinoma [1]. In addition to the cumulative quantity of alcohol intake and alcohol addiction patterns, other factors such as gender, age, geographic area, nutritional factors, genetic background, immunological mechanisms and energy metabolism abnormalities also play a key role in the progression of alcoholic liver damage [2]. Dietary intake of fat and the development of fatty infiltration are important factors in the pathogenic of hepatosteatosis. The degree of fatty infiltration found in liver biopsy of alcoholic patients is a risk factor for the development of liver cirrhosis [3].

Ethanol is the principle ingredient in most of the syrups,

mouthwash, tincture, and alcoholic beverages. It is also a common ingredient in a range of products, from cosmetics and beauty products to paints and varnishes to fuel. Ethanol is effective in killing microorganisms like bacteria, fungi and viruses, so it is used in many hand sanitizers and personal care. Alcohol chemotherapy is the first choice treatment for cystic lesions and benign solid thyroid venous malformations [4]. In small doses it has a great medicinal value because of its effectiveness, ease of use, low cost and long shelf life. But some people tend to have ethanol abuse. In excessive dose, it induces severe liver injury in experimental animals and humans [5].

Three obvious enzymatic pathways shared in the process of ethanol oxidation [6]. The first pathway for the ethanol metabolism is dehydrogenase system. It is initiated by alcohol dehydrogenase (ADH), which oxidizes ethanol to acetaldehyde. Then, acetaldehyde enters the mitochondria where it is oxidized to acetate by aldehyde dehydrogenases (ALDH) giving rise to reactive oxygen species (ROS). In-

* Corresponding author.

E-mail address: azza_arafa63@yahoo.com (A.F. Arafa).

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Table 1
Changes in body weight, absolute liver weight and food intake of control, fat diet, ethanol-fat diet and treated rat groups.

Parameters	Groups						
	1- Control	2- Fat diet	3- Ethanol-fat diet	4- Fat diet- Fluvastatin	5- Fat diet- BCE	6- Ethanol fat diet-Fluvastatin	7- Ethanol fat diet BCE
Initial body weight (g)	133.33 ± 5.50	132.67 ± 3.19	133.33 ± 5.39	133.67 ± 4.46	132.50 ± 6.09	132.00 ± 5.10	132.17 ± 4.96
Final body weight (g)	236.00 ± 16.32 ^(2,3,4,5,6,7)	309.33 ± 18.19 ^(1,3,4,5,6,7)	390.50 ± 17.40 ^(1,2,4,5,6,7)	279.83 ± 13.29 ^(1,2,3,6,7)	274.17 ± 18.23 ^(1,2,3,6,7)	361.83 ± 14.16 ^(1,2,3,4,5)	354.00 ± 10.08 ^(1,2,3,4,5)
Body weight gain (%)	43.50 ± 0.84 ^(2,3,4,5,6,7)	57.11 ± 0.57 ^(1,3,4,5,6,7)	65.86 ± 0.76 ^(1,2,4,5,6,7)	52.23 ± 1.21 ^(1,2,3,6,7)	51.67 ± 1.35 ^(1,2,3,6,7)	63.52 ± 1.57 ^(1,2,3,4,5)	62.66 ± 0.71 ^(1,2,3,4,5)
Absolute liver weight (g)	4.95 ± 0.24 ^(2,3,4,5,6,7)	6.75 ± 0.43 ^(1,3,4,5,6,7)	7.72 ± 0.52 ^(1,2,4,5,6,7)	5.93 ± 0.43 ^(1,2,3)	5.72 ± 0.44 ^(1,2,3)	5.75 ± 0.40 ^(1,2,3)	5.92 ± 0.36 ^(1,2,3)
Relative liver weight (g/100 gbw)	2.09 ± 0.13 ^(6,7)	2.18 ± 0.17 ^(6,7)	1.98 ± 0.14 ^(6,7)	2.11 ± 0.14 ^(6,7)	2.08 ± 0.15 ^(6,7)	1.59 ± 0.15 ^(1,2,3,4,5)	1.67 ± 0.16 ^(1,2,3,4,5)
Food intake (g/day/rat)	27.35 ± 3.12 ^(3,4,5,6,7)	29.15 ± 2.73 ^(3,6)	33.24 ± 3.43 ^(1,2,4,7)	29.76 ± 2.47 ^(1,3)	29.85 ± 2.19 ^(1,3)	31.32 ± 2.15 ^(1,2)	30.94 ± 3.23 ^(1,3)

Data are represented as Mean ± SD of 8 rats in each group. Significant at P < 0.05.

creased oxidative stress is well recognized to be an essential factor in the pathogenic of hepatic injury [7]. The second major pathway to oxidize ethanol is the microsomal ethanol oxidizing system (MEOS), which involves the cytochrome P450 enzyme CYP2E1. The MEOS pathway is stimulated in individuals who consume alcohol chronically. In addition, ethanol can be oxidized and this oxidation pathway requires the presence of hydrogen peroxide [8].

Since oxidative stress and metabolite induced inflammatory factors are incorporated in the development of ALD, specific antioxidants potentially are useful for alleviation of ethanol induce oxidative stress and prevent this pathogenesis [9].

In mammals, a sophisticated antioxidant system, both enzymatic and non-enzymatic, has been developed to remain the redox homeostasis in the liver and deals with oxidative stress under physiological conditions. Therefore, the enzymatic antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase and non-enzymatic antioxidant such as reduced glutathione are affected and used as indicators to evaluate the level of oxidative stress [10]. Among alcoholics, the altered lipid profile is one of the crucial factors of fatty liver. Fluctuation in the gene expressions that are involved in the synthesis or degradation of fatty acids, triglycerides, and/or cholesterol may be as a result of differential regulation at the post-transcriptional levels [11].

Bombax ceiba Linn. is known as silk cotton tree and it belongs to family Bombacaceae. The plant is widely cultivated in China, Pakistan, India, and Austria and was introduced into Egypt several decades ago as a shade tree and an ornamental plant [12]. For use in foods, plant extracts are nutritionally more relevant and have obvious advantages in safety [13]. It is well-reputed in traditional systems of medicine for treatment of diarrhea, fever, and ulceration of bladder as well as kidney [14]. Different parts of *Bombax ceiba* (*B. ceiba*) has shown to possess many biological properties predominantly antioxidant, antimicrobial, anti-inflammatory, antihyperlipidemic, and hypoglycemic activities [15].

The correct pathological mechanisms of alcoholic liver disease remain ambiguous and no new accepted drugs are now on the market. In spite of that, ameliorating hepatic steatosis and oxidative stress are considered to be a promising strategy for ALD treatment. So, the aim of this study is to evaluate the therapeutic effect of the Egyptian BCE flowers extract upon chronic alcohol consumption induced hepatosteatosis in rats fed fat diet.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals used in the experiments were of analytical grade. Kits preformed for the quantitative determination of different parameters were purchased from Greiner Diagnostic GmbH- Germany and Bio-diagnostic Company, Egypt. Lescol XL drug (fluvastatin as the active ingredient) is a product purchased by Novartis, Switzerland.

2.2. Plant materials

Bombax ceiba (*B. ceiba*) flowers were collected from Orman botanical garden, Giza, Egypt, in January, 2016. The flowers were kindly authenticated by a botanist at the National Research Centre. A voucher specimen (No. 3241) was deposited in the herbarium of National Research Centre, Doki, Giza.

2.3. Preparation of aqueous methanol extracts of BCE

Shade dried *B. ceiba* flowers (1 Kg) were refluxed with aqueous methanol (70%W/V). The extracted solutions were combined, filtered,

Table 2
Effect of BCE on hepatic enzyme activities of control, fat diet, ethanol-fat diet and treated rat groups.

Parameters	Groups						
	1- Control	2- Fat diet	3- Ethanol-fat diet	4- Fat diet- Fluvastatin	5- Fat diet- BCE	6- Ethanol fat diet-Fluvastatin	7- Ethanol fat diet- BCE
AST (U/ml)	42.55 ± 3.28 ^(2,3,4,7)	68.95 ± 4.69 ^(1,3,4,5,6)	93.10 ± 6.39 ^(1,2,4,5,6,7)	55.21 ± 3.68 ^(1,2,3,5,6,7)	46.19 ± 4.05 ^(2,3,4,7)	47.70 ± 4.83 ^(2,3,4,7)	66.68 ± 6.40 ^(1,3,4,5,6)
% of improvement	–	62.04	118.80	29.75	7.88	12.10	56.71
ALT(U/ml)	15.39 ± 1.90 ^(2,3)	21.87 ± 1.48 ^(1,3,4,5,6,7)	25.91 ± 3.55 ^(1,2,4,5,6,7)	32.29	53.49	106.70	62.09
% of change	–	42.11	68.36	10.46	5.39	12.93	9.49
% of improvement	–	–	–	52.57	47.50	55.43	58.87
ALP (U/L)	154.45 ± 15.47 ^(2,3,4,5,6,7)	205.83 ± 13.53 ^(1,3)	241.56 ± 16.84 ^(1,2,4,5,6,7)	185.11 ± 14.28 ^(1,3)	183.79 ± 12.19 ^(1,3)	191.67 ± 21.22 ^(1,3)	194.27 ± 15.22 ^(1,3)
% of change	–	33.27	56.40	19.85	18.99	24.10	25.78
% of improvement	–	–	–	13.41	14.24	32.30	30.62

Data are represented as Mean ± SD of 8 rats in each group. Significant at P < 0.05.

and then concentrated by a rotary evaporator under reduced pressure to remove the methanol solvent. The extract was then subjected to lyophilization for removing the excess water yielding 100 g of crude dark-brown sticky BCE and stored at -20°C for use.

2.4. Experimental design

2.4.1. Animals

Fifty six adult female Wistar rats weighing 130 ± 5 g were obtained from the animal house of National research Centre. Animals were maintained in clean cages under normal laboratory conditions ($22 \pm 50^{\circ}\text{C}$, 12 h light/dark cycle). They were allowed for a standard pellet diet and free access to water. The experimental work on rats was performed with the approval of the Animal care and Experimental Committee, National Research Centre, Cairo, Egypt (Approval no: 18085).

The animals were divided into seven groups of eight animals each. Group1: control and given only standard pellet diet. Group2: Fat diet (Fed 22% fat/kg diet). Group3: Ethanol-fat diet (fed fat diet 22% fat/kg diet + Ethanol). Group 4: Fat diet group treated with standard drug fluvastatin. Group 5: Fat diet group treated with BCE. Group 6: Ethanol-fat diet treated with standard drug fluvastatin. Group 7: Ethanol-fat diet treated with BCE.

During the duration of the experiment, daily food intake and weekly body weights were recorded. At the end of treatment period, blood samples were drawn from the animals via puncturing the retro-orbital venous plexus with a thin sterilized capillary tube. Blood specimens were subjected to serum separation and stored at -20°C for biochemical analysis. Then, rats were sacrificed by decapitation, livers dissected out, cleaned and weighed. Some samples were rinsed and homogenized in a phosphate buffer (pH, 7.4) and centrifuged at 4000 rpm at 4°C for 15 min. The obtained supernatants were used for biochemical assays. Other liver tissues were immediately removed and fixed with neutral formalin solution 10% for histological examination.

2.4.2. Doses and route of administration

The oral daily administration route was used in the study. The starting dose was $2\text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ and increased up to $6\text{ g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$ [16]. The dose of BCE was selected to be 200 mg/kg/day for 30 consecutive days according to the previous study [14]. Whereas; fluvastatin dose (2 mg/Kg/day) was selected based on the clinical application from previous studies on human and experimental animals [17].

2.4.3. Biochemical analysis

2.4.3.1. Measurements of blood alcohol concentration. Blood alcohol levels were carried out on samples obtained from tail veins of animals using a commercial kit (Bio STC- Science and Technology Center, Mohandeseen, Giza). This enzymatic test for alcohol utilizes the coenzyme nicotinamide adenine dinucleotide (NAD) and alcohol dehydrogenase (ADH). The formation of NADH can be measured quantitatively by the increase in absorbance at 340 nm.

2.4.3.2. Assays of serum liver enzyme activities. Serum transaminases (AST&ALT) activities were determined by a colorimetric method according to Reitman and Frankel [18]. Serum alkaline phosphatase (ALP) activity was determined by enzymatic colorimetric method according to Belfield and Goldberg [19].

2.4.3.3. Assays of some antioxidant defense markers. Liver glutathione (GSH) content and glutathione-S-transferase (GST) activity were determined according to the methods of Beutler et al. [20] and Habig and Pabst [21], respectively. The activity of enzymatic antioxidant

Table 3
Effect of BCE on some antioxidant enzymes of control, fat diet, ethanol -fat diet and treated rat groups.

Parameters	Groups						
	1- Control	2- Fat diet	3- Ethanol-fat diet	4- Fat diet- Fluvastatin	5- Fat diet- BCE	6-Ethanol fat diet-Fluvastatin	7- Ethanol fat diet BCE
GSH (mmol/g tissue)	257.53 ± 10.48 ^(2,3,4,5,7)	205.28 ± 9.35 ^(1,4,5,6,7)	201.97 ± 11.49 ^(1,4,5,6,7)	238.22 ± 6.77 ^(1,2,3)	242.73 ± 4.43 ^(1,2,3)	249.95 ± 10.63 ^(2,3)	239.89 ± 5.48 ^(1,2,3)
% change	-	-20.29	-28.56	16.05	18.24	23.76	18.78
% of improvement	-	-	-	12.8	14.54	18.63	14.72
GST (U/g tissue)	16.06 ± 2.36 ^(2,3,6,7)	10.57 ± 1.28 ^(1,4,5,6,7)	10.22 ± 0.87 ^(1,4,5,6,7)	14.32 ± 0.95 ^(2,3)	15.19 ± 1.41 ^(2,3,6)	12.97 ± 1.26 ^(1,2,3,5)	13.76 ± 1.56 ^(1,2,3)
% change	-	-34.18	-36.36	35.48	43.71	26.91	34.64
% of improvement	-	-	-	23.35	28.77	17.12	22.04
SOD (U/g tissue)	170.29 ± 12.65 ^(2,3)	145.46 ± 8.39 ^(1,7)	143.59 ± 14.65 ^(1,5,7)	155.36 ± 15.73	166.28 ± 6.43 ^(2,3)	159.91 ± 15.42	163.61 ± 7.03 ^(2,3)
% change	-	-14.58	-15.68	6.81	14.31	11.37	13.76
% of improvement	-	-	-	5.81	12.23	9.58	11.76
MDA (nmol/g tissue)	845.08 ± 38.98 ^(2,3)	1106.51 ± 59.65 ^(1,3,4,5,6,7)	1210.06 ± 57.86 ^(1,2,4,5,6,7)	839.16 ± 43.35 ^(2,3)	842.07 ± 32.75 ^(2,3)	818.12 ± 33.80 ^(2,3)	832.19 ± 31.03 ^(2,3)
% change	-	30.94	43.19	-24.16	-23.90	-32.39	-31.23
% of improvement	-	-	-	31.63	31.29	46.38	44.71
CAT (U/g tissue)	9.02 ± 1.24 ^(2,3,4)	4.14 ± 0.48 ^(1,4,5,6,7)	3.64 ± 1.37 ^(1,4,5,6,7)	7.18 ± 1.16 ^(1,2,3)	8.22 ± 0.93 ^(2,3)	7.96 ± 0.45 ^(5,3)	8.00 ± 0.45 ^(2,3)
% change	-	-54.10	-59.65	73.43	98.55	118.68	119.78
% of improvement	-	-	-	33.70	45.23	47.89	48.34

Data are represented as Mean ± SD of 8 rats in each group. Significant at P < 0.05.

(SOD) was assayed by the method of Kakkar et al. [22]. The activity of (CAT) was assayed by the method of Aebi [23]. Liver Lipid peroxidation was measured by estimating the levels of malondialdehyde (MDA) using a thiobarbituric acid reaction method [24].

2.4.3.4. *Assays of serum lipid profile.* Triglycerides (TG) were determined according to the method of Rifai et al. [25]. Total cholesterol (TC) and low density lipoprotein (LDL) were determined by the method of Deeg and Ziegenhorn [26].

2.4.3.5. *Liver histological examination.* Sections of 6 µm thickness were prepared and stained with hematoxylin and eosin as described by Afifi [27]. The stained sections were observed under the light microscope, cytoplasm stained in shades of pink to red and the nuclei gave blue color.

2.5. *Statistical analysis*

Data were analyzed by with SPSS (Statistical Package for the Social Sciences) version 9.05 software (USA). All values were expressed as the mean ± SD. Significant differences between the groups were statistically analyzed by one way analysis of variance (ANOVA) followed by Turkey’s multiple comparison post hoc test. A statistical difference of P < 0.05 was considered significant.

3. *Results*

3.1. *Effect of BCE on body weight, absolute liver weight and food intake*

At the end of the experiment, it was found that ethanol-fat diet group recorded significant increase in the percentage of body weight gain compared to all other groups. While, there was no significant difference in the percentage of body weight gain between ethanol-fat diets BCE treated group and ethanol fat diet-standard drug treated group. The absolute liver weight was significantly increased in both fat diet and ethanol-fat diet groups compared to the control group. A significant decrease was also observed in absolute liver weight in both BCE treated groups compared to their correspondence; fat diet and ethanol-fat diet untreated ones, respectively. However, slight changes were recorded in relative liver weights among major groups. In addition, there was no significant difference in food intake between fat diet group and control. While, a significant difference was observed in food intake among major groups compared to control (Table 1).

3.2. *Effect of BCE on some biochemical markers in serum*

A more significant increase in the percentage change of AST, ALT and ALP levels was observed in ethanol-fat diet group than fat diet one as compared to control as shown in Table 2. However, ethanol-fat diet BCE treated group recorded higher percentage of improvement in the activities of the AST, ALT and ALP enzymes than that recorded in fat diet BCE treated group. Similar results were observed in the percentage of improvement of the AST, ALT and ALP enzyme activities upon treatment with standard drug.

3.3. *Effect of BCE on enzymatic antioxidant status in liver*

The ethanol fat diet and Fat diet groups showed significant decrease in the activities of GST, SOD and CAT enzymes and reduced the content of GSH as compared to control group. This decreasing was accompanied by a significant increase in MDA level in these groups. Treatment of fat diet group with BCE recorded more significant percentage of improvement in the levels of GSH, GST and SOD than that recorded in the

Table 4
Effect of BCE extract on lipid profiles of control, fat diet, ethanol fat diet and treated rat groups.

Parameters	Groups						
	1- Control	2- Fat diet	3- Ethanol-fat diet	4- Fat diet- Fluvastatin	5- Fat diet- BCE	6-Ethanol fat diet-Fluvastatin	7- Ethanol fat diet- BCE
TG (mg/d)	198.75 ± 21.57 ^(2,3)	235.13 ± 18.82 ^(1,4,5,6,7)	254.25 ± 16.99 ^(1,4,5,6,7)	205.86 ± 14.59 ^(2,3)	210.95 ± 14.88 ^(2,3)	202.73 ± 12.34 ^(2,3)	210.45 ± 9.33 ^(2,3)
% change	-	18.30	27.92	3.58	6.14	2.02	5.89
% of improvement	-	-	-	14.73	12.17	25.92	22.04
TC (mg/d)	50.56 ± 6.76 ^(2,3,6,7)	63.98 ± 8.49 ^(1,3)	87.66 ± 7.06 ^(1,2,4,5,6,7)	59.41 ± 4.58 ^(3,6,7)	57.20 ± 6.80 ^(3,6,7)	69.22 ± 6.76 ^(1,3,4,5)	69.67 ± 4.63 ^(1,3,4,5)
% change	-	26.54	73.38	17.50	13.13	36.91	37.80
% of improvement	-	-	-	9.03	13.41	36.47	35.58
LDL (mg/d)	21.53 ± 1.64 ^(2,3,4,5)	32.94 ± 4.34 ^(1,3,4,5,6,7)	37.58 ± 3.53 ^(1,2,4,5,6,7)	28.18 ± 3.85 ^(1,2,3,6,7)	26.78 ± 2.47 ^(1,2,3,6)	21.85 ± 2.10 ^(2,3,4,5)	22.41 ± 2.25 ^(2,3,4)
% change	-	53.00	74.55	30.89	24.38	1.49	4.09
% of improvement	-	-	-	22.11	26.52	73.06	70.46

Data are represented as Mean ± SD of 8 rats in each group. Significant at P < 0.05.

ethanol fat diet BCE treated group. While, ethanol fat diet BCE treated group showed a significant increase in the percentage of improvement in CAT and MDA levels more than the values recorded in Fat diet BCE treated group as shown in Table 4.

3.4. Effect of BCE on lipid profiles

In Table 3, the ethanol-fat diet group showed more significant percentage changes in the levels of the TG, TC and LDL than that of fat diet group as compared to the control one. However, the BCE as a treatment agent showed a more significant increase in the percentage of improvement on the levels of the TG, TC and LDL in ethanol-fat diet treated group than the fat diet BCE treated group. Also, the standard drug treatment groups recorded the same results of percentage of improvement in the levels of TG, TC and LDL.

3.5. Effect of BCE on histopathological study

Liver sections of control group show the normal architecture of the hepatic lobules and normal portal tract. The central vein lies at the center of the lobule surrounded by cords of hepatocytes. The hepatocytes show strongly eosinophilic granulated cytoplasm, round nuclei. Sinusoids are often seen between the strands of hepatocytes (Fig.1) (A& B). Fat-diet group shows hydropic degeneration, congested portal tract associated with mild inflammatory infiltration, fatty change and vacuoles in the hepatocytes (Fig.1) (C&D). Ethanol fat-diet group shows disturbance of the hepatic lobules associated with hydropic degeneration, vacuoles of fatty change in the hepatocytes, the hepatocytes around the dilated congested vessels appeared variable in shape and size, congested portal tract associated with mild inflammatory infiltration and necrosis of the hepatocytes degeneration (Fig. 1) (E&F).

However, liver sections of fat diet-standard treated group show structure of the hepatic and portal lobules appeared more or less like normal, congested portal tract associated with mild inflammatory infiltration and the hepatocytes around the dilated congested vessels appeared variable in shape and size (Fig. 2) (A, B&C). Fat diet BCE treated group shows normal structure of the hepatic and portal lobules (Fig. 2) (D&E). While, Ethanol-fat diet standard treated group shows the structure of the hepatic and portal lobules appeared more or less like normal (Fig. 3) (A&B). Ethanol- fat diet BCE treated group shows the structure of the hepatic lobule appeared more or less like normal, congested portal tract associated with mild inflammatory infiltration (Fig. 3) (C&D).

4. Discussion

Alcohol addiction represents the main causes of chronic liver diseases and is associated with high mortality in many developed countries [28]. Chronic ethanol consumption results in decreasing mitochondrial protein synthesis, impairment of the oxidative phosphorylation system, decline in ATP synthesis, leakage of ROS which induce the activation of intrinsic apoptotic pathways [29,30]. In vitro, HL7702 hepatic cell was establishing of steatosis model to evaluate the lipid-lowering effects of certain polyphenols [31]. Also, 4-nonylphenol is correlated with hepatic steatosis as non alcoholic models in rats [32].

The Phytochemical compositions of methanol extract of the Egyptian B. ceiba flowers indicate the presence of alkaloids, flavonoids, glycosides, saponine, polyphenols, tannins, proteins, carbohydrates, lipids, amino acids and coumarins [33,34]. These valuable compounds offer the potent antioxidant and hypolipidemic properties of BCE in the liver tissues [35]. In our study, we examined the efficacy of these constituents in curing the alcohol addiction that were accompanied by fat intake in comparison to the effect of the standard drug; Fluvastatin.

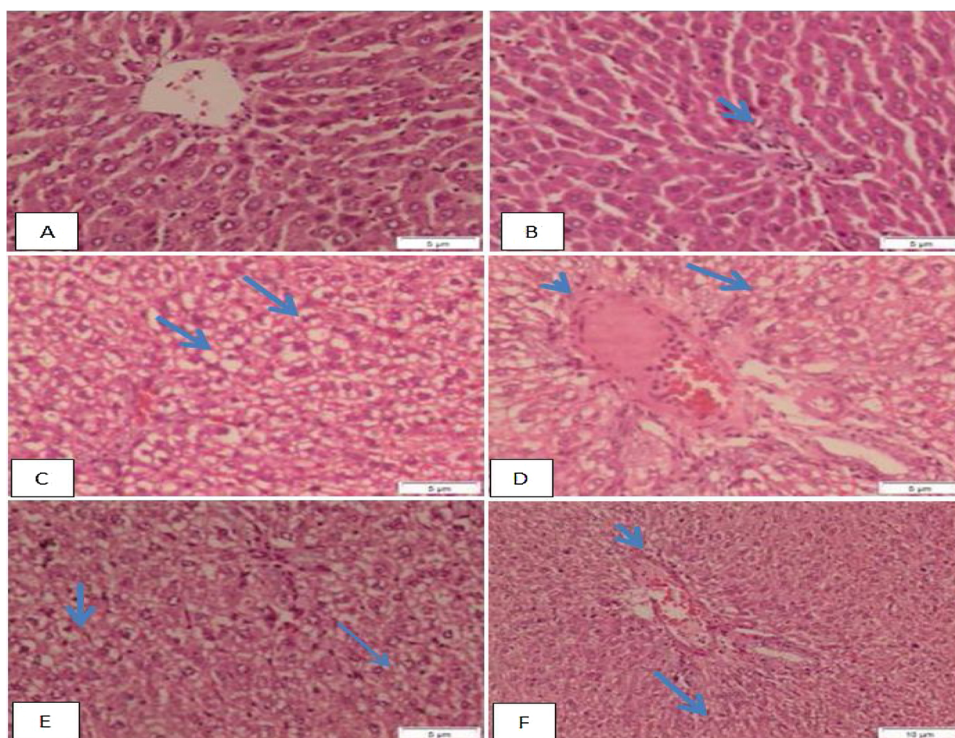


Fig. 1. Micrograph of liver sections of A) control group shows normal architecture of the hepatic lobules, central vein lies at the center, hepatocytes shows strongly eosinophilic granulated cytoplasm, round nuclei and sinusoids are often seen between strands of hepatocytes., B) control rat shows the normal architecture of the portal tract (arrow)., C) Fat-diet group shows hydropic degeneration, and vacuoles in the hepatocytes (arrow)., D) Fat-diet group shows congested portal tract associated with mild inflammatory infiltration (arrowhead), fatty change and vacuoles in the hepatocytes (arrow)., E) Ethanol fat-diet group shows disturbance of the hepatic lobule associated with hydropic degeneration (thick arrow), and vacuoles of fatty change the hepatocytes (thin arrow) and the hepatocytes around the dilated congested vessels appeared variable in shape and size., F) Ethanol fat-diet group shows congested portal tract associated with mild inflammatory infiltration (arrowhead), necrosis of the hepatocytes degeneration, and vacuoles in the hepatocytes (arrow) (H&E stain, bar: 10 µm).

After one month of ethanol ingestion, the body weight of both fat diet and ethanol-fat diet groups were significantly higher than control. These observations may be due to hyperlipidemia and the effect of extra calories as ingesting excessive ethanol [36]. Treatment with BCE reduces the percentage of body weight gain of both fat-diet and ethanol-fat diet treated groups compared with the untreated ones. This explains that the extract may be preventing the pathological mechanisms susceptible for excessive fat accumulation and weight gain due to the presence of bioactive components like alkaloids and glycosides. In addition, the extract is possibly increasing leptin sensitivity, providing anorexic effect and increasing energy expenditure [35,37]. The liver is the primary organ responsible for ethanol metabolism and it is susceptible to alcohol's toxic effects. In the present study, the elevated levels of serum enzymes such as AST, ALT and ALP were observed after consumption of alcohol. The leakage of these enzymes into the blood reflected liver damage [38]. However, BCE treatment significantly decreased the content of these serum parameters in both treated groups. This illustrates the integration of plasma membrane, as well as repair of hepatic tissue damage.

In our study GSH, GR, SOD and CAT enzyme levels depleted by ethanol and fat intake as compared to control group. Treatment with BCE restored the normal hepatic concentrations of these studied antioxidant enzymes, which suggests the ability of active constituent of the extract to interact directly with ROS and scavenge free radicals [39]. Also, treatment with BCE significantly decreased MDA level by stopping the formation of lipid peroxide from fatty acids [40]. Our results were in line with the previous studies which have shown that the watermelon juice had hepatoprotective effects after excessive ethanol consumption in rats [41].

Many factors such as ethanol consumption, nutritional status, ethanol metabolism and genetic makeup can lead to hepatostestosis [42]. Also, hepatostestosis can be induced in people without alcohol

abuse history and may appear in those who have metabolic disorders especially obese ones [43].

In our study, alcohol induced liver steatosis was confirmed by significant increase in TG, TC and LDL in fat-diet and ethanol fat-diet groups compared to control group. This increase may be due to disturbance in lipid metabolism and lipid homeostasis [44]. These changes are the result of an adaptive mechanism to resist the fluidizing effect of ethanol [45]. Treatment with BCE significantly lowered the levels of lipid profiles due to the inactivation of acetyl-coA carboxylase as a result of adenosine monophosphate kinase activation that mediates thermogenesis and fatty acid synthesis inhibition [46]. Mangiferin, which is a polyphenol compound found in BCE upregulates proteins for mitochondrial bioenergetics and down regulates proteins controlling de novo lipogenesis [47].

Histopathological results indicated that in control group, there were neither lipid droplets nor steatosis distributed in the hepatic lobules, which approved the maintenance of the lobular structure integrity. On the other hand, liver sections of fat-diet and ethanol fat-diet groups showed different extents of steatosis in the hepatic lobules with mild inflammatory infiltration. Treatment with BCE alleviated the hepatic steatosis and reduced infiltrations of inflammatory cells in the hepatocyte.

5. Conclusion

BCE can be a safe treatment for the alcohol addiction cases accompanied by fat intake. The extract succeeded in decreasing the disease complications in addition to potentiating the antioxidant defenses in the liver. Further studies are needed for applying the extract as a recommended drug for ALD.

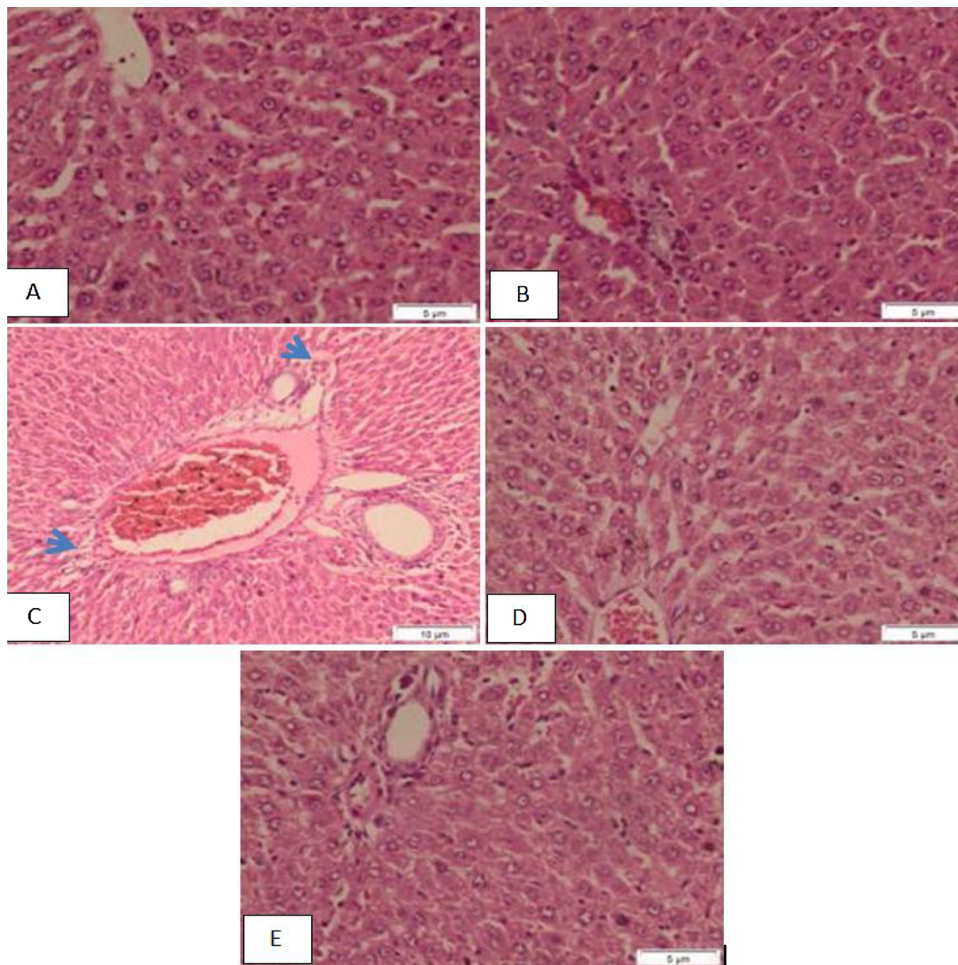


Fig. 2. A, B) Fat-diet standard treated group shows structure of the hepatic and portal lobules appeared more or less like normal., C) Fat-diet standard treated group shows congested portal tract associated with mild inflammatory infiltration (arrowhead) and the hepatocytes around the dilated congested vessels appeared variable in shape and size., D, E) Fat-diet BCE treated group shows the normal structure of the hepatic and portal lobules (H&E stain, bar: 5 µm).

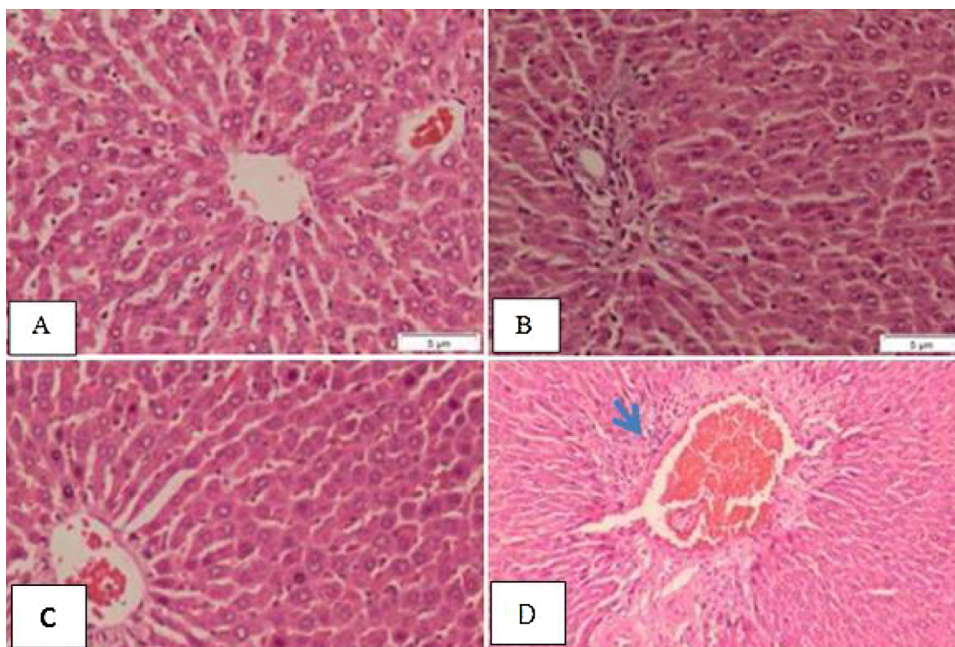


Fig. 3. Micrograph of liver section of A, B) Ethanol fat-diet standard treated group show the structure of the hepatic and portal lobules appeared more or less like normal., C) Ethanol fat-diet BCE treated group shows the structure of the hepatic lobule appeared more or less like normal., D) Ethanol fat-diet BCE treated group shows congested portal tract associated with mild inflammatory infiltration (arrowhead) (H &E stain, bar: 5 µm).

Conflict of interest

The authors declare that there are no conflicts of interest.

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