Euphorbia lathyris L.-modulated hyperlipidemia induced by high-fat diet in an experimental animal model

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Background and objectives
Hyperlipidemia has been embroiled in atherosclerosis, which is the primary factor of heart disease and stroke. An important aim in the pivotal restrain of atherosclerosis awaits an affordable and safe drug that will fundamentally confirm to have a great effect where treatment is decisive. Humans have been served by a variety of drugs extracted from a natural source, and the most of these cures were provided by higher plants.
Materials and methods
The aim of this study is to investigate and assess the hypolipidemic effect of *Euphorbia lathyris* L. extracts on high-fat diet-induced hyperlipidemia in rats, and it shows a scientific proof for expansion of *E. lathyris* L. as a prospect naturalistic oral hypolipidemic drug. Rats were administrated orally with cholesterol in a dose of 100 mg/1 ml olive oil/1 kg five times a week for 12 consecutive weeks. This was accomplished by assessing the activities of particular biochemical parameters associated with liver functions in tissue of high-fat-diet group when compared with those of control one. Liver function tests, included aspartate aminotransferases; alanine and alkaline phosphatases; antioxidants and oxidative stress biomarkers, such as glutathione, catalase, and glutathione-S-transferase; total antioxidant capacity; nitric oxide; and lipid peroxide. In addition, lipid profile (cholesterol, total lipid, high-density lipoprotein-cholesterol, and triglyceride) were also evaluated. Moreover, histological examination of liver, heart, and kidney was done to prove the biochemical outcomes.
Results and conclusion
The present findings strongly clarify defects in all biochemical measurements, such as increase in the malondialdehyde level and liver function enzyme activities. However, reductions in enzymatic and nonenzymatic antioxidant in liver of hyperlipidemic rats were marked. On the contrary, treatment of hyperlipidemic rats with low and high dose of methanol and petroleum ether extract of *E. lathyris* natural product proved to have ameliorating effects in all biochemical parameters under investigation. The previous biochemical observations were also accompanied by histopathological examinations of the heart, liver, and kidney sections, which showed significant recovery of hepatocytes and amelioration in histological architectures of heart and kidney.
It could be concluded that the two extracts of *E. lathyris* showed improvement in liver function enzyme activities, oxidant–antioxidant biomarkers, and lipid profile activities in liver of high-fat-diet rats.

Keywords:
Euphorbia lathyris, histopathology, lipid profile, liver biochemical parameters

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Introduction

Cardiovascular diseases, including coronary artery diseases, stroke, and myocardial infarction, are the major reasons of morbidity and death rates in the world. Hypercholesterolemia is frequently associated with oxidative stress and release of inflammatory cytokines and results in the formation and accumulation of plaque deposits in the arteries, where it produces multiple structural and functional disturbances in the vascular wall that results in the development of atherosclerosis or coronary heart disease [1]. Hypercholesterolemia is a chronic inflammatory disease that begins by endothelial injury, followed by subintimal focal recruitment of circulating T lymphocytes and monocytes that heals by calcification and fibrosis and is the major cause of cardiovascular disease globally [2]. The leading risk factor for atherosclerosis is hyperlipidemia. Among the indicators of atherosclerosis development are deviated increases in serum triglycerides, total cholesterol, and low-density lipoprotein-cholesterol (LDL-C) levels; however, high serum high-density lipoprotein-cholesterol (HDL-C) is known to have a protective

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effect against the progression of atherosclerosis [3]. Oxygenation and modification of LDL-C to produce oxidized LDL-C, resulting in the aggregation of cholesterol in phagocytes and production of foam cells, boosting the progression of atherosclerosis, can be caused by reactive oxygen species [4]. Thus, decreased plasma cholesterol level could enhance the endothelial cell function and minimize vascular inflammation [5]. As flavonoids are known to be antioxidative and anti-inflammatory and exhibit many other health benefits as well, so the presence of a high flavonoid content is considered beneficial for health [6]. These active components (flavonoids) have an effect on the inflammatory procedures by means of their action on the implicated enzymes such as protein kinase C, cyclooxygenase, 5-lipoxigenase, and hyaluronidase [7]. Many medicinal plants have been used for the control of hyperlipidemia in past because of their anti-hyperlipidemic effect, but there was no scientific evidence of it [8]. *Euphorbiaceae* includes more than 1500 plant species [9]. Potential source of flavonoid compounds can be obtained by *Euphorbia lathyris*. *E. lathyris* (caper spurge) is a common biennial garden plant. It is prevalent in southern England but can occur throughout Europe, North America, and Australia. It is known by other names such as the Mole Plant, Gopher Spurge, and Myrtle Spurge [10].

For proving safety and efficacy of herbal medicines from simple biological assays to drug standardization along with toxicity and safety studies, there is continuous need of scientific research. The present study is carried out to explore the effects of seeds of *E. lathyris* in hypercholestremic rats.

Materials and methods

Chemicals and reagents

All chemicals and gallic acid used in the present study were of high analytical grade and were the products of Sigma (New York, USA), Merck (Hidenperg, Germany), BDH (London, England), Riedel de Hàen (Germany), and Fluka (Switzerland).

Euphorbia lathyris L. (seeds)

E. lathyris L. (seeds) was bought from local market. The extract of *E. lathyris* L. (seeds) was carried out on a quantity of seeds in which it was sufficient to yield about 20 g of crude extract. Seeds were ground and extracted with petroleum ether, and then the remainder was extracted by methanol at ambient temperature by percolation. Every extract was filtered and was evaporated to dryness under reduced pressure and totally freed from water by freeze drying and stored under freezing at −20°C until used.

Cytotoxicity

The MTT assay was developed by method of Bassyouni *et al*. [11] and used to determine the *in vitro* inhibitory effects of two test extracts on human hepatocellular carcinoma)HepG2) cell line. In brief, in each microplate well, cells (10×103) were seeded in RPMI 1640 growth medium, 1% antibiotic–antimycotic mixture (10 000 U/ml potassium penicillin, 10 000 mg/ml streptomycin sulfate, and 25 mg/ml amphotericin B), and 1% L-glutamine, and 10% fetal bovine serum was added in the 96-well plate at 37°C under 5% CO_2. After 24 h, media was aspirated, fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of extracts to give a final concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and 0.78 mg/ml. After 48 h of incubation, medium was aspirated, and 40 µl MTT was added to each well, and after 4 h, 140 µl of 10% sodium dodecyl sulfate in de-ionized water was added to each well and further incubated overnight at 37°C to solubilize MTT-formazan crystal. Doxorubicin, which is a known cytotoxic agent, was used as positive control in 100 mg/ml and gave 100% lethality under the same conditions. The optical density of each well was measured at 575 nm using a microplate multiwell reader, and the inhibition of cell growth (%) was calculated as $(1-T/C)\times100$, where C is the mean OD575 of the control group and T is that of the treated group [1]. The IC50 was determined from the dose–response curve. A statistical significance was tested between samples and negative synthesis and biological evaluation of some new triazolo [1,5-a] quinolone derivatives as anticancer and antimicrobial agents control (cells with vehicle) using independent t test by SPSS 11 program (SPSS Inc., Chicago, Illinois, USA).

Determination of total flavonoid (TFC), phenolic content, and free radical-scavenging activity of *E. lathyris* L. extracts was as follows:

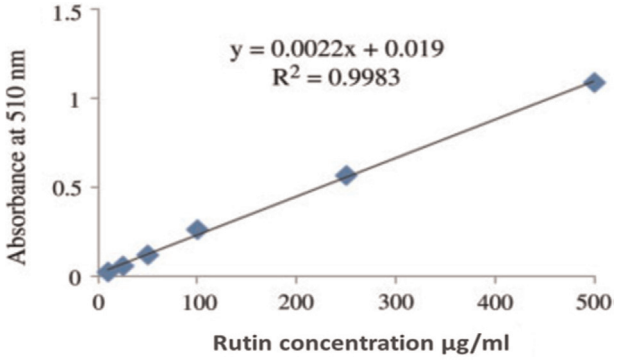
Determination of total flavonoid

Distilled water (100 µl) was added to each of the 96 wells, followed by 10 µl of 50 g/l $NaNO_2$ and 25 µl of standard or sample solution. After 5 min, 15 µl of 100 g/l $AlCl_3$ was added to the mixture; 6 min later, 50 µl of 1 mol/l NaOH, and 50 µl of distilled water were added. The plate was shaken for 30 s in the plate reader before absorbance measurement at 510 nm. All samples and standards were measured against an acetone reagent blank. Rutin was used as a standard at 5–250 µg/l to generate a calibration curve (average R^2=0.9990; Table 1 and Fig. 1).

Table 1 Well plate total flavonoid method

Concentration of rutin (μg/ml)	Absorbance
50	0.7
100	1
150	1.4
200	1.6
250	1.9
300	2
350	2.3

Figure 1



Representative standard curves for 96-well assays (flavonoid content).

Determination of total phenolic content

The Folin–Ciocalteu colorimetric method was used for determination of total phenolic content (TPC) of each sample [12]. In brief, 100-μl extract (or gallic acid standard solution) was diluted with 400-μl de-ionized water in a glass culture tube, and 100-μl Folin–Ciocalteu reagent was then added. The mixture was mixed well and incubated at room temperature for 8 min before adding 1-ml 7% sodium carbonate and 0.8-ml de-ionized water. The mixture was blended well and incubated for 90 min at room temperature. Duplicates of the sample or standard (200 μl each in volume) were transferred to a 96-well plate, and absorbance was measured at 760 nm in a microplate reader. Total phenolic acid content was expressed as milligrams of gallic acid equivalents per 100 g of sample on a dry weight basis (mg GAE/100 g DW).

1,1-diphenyl-2-picryl-hydrazil free radical-scavenging activity

The free radical-scavenging activity of extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Abeer *et al.* [13]. *E. lathyris* L. extracts were screened at 100 μg/ml, whereas the most potent active extracts (gave more 90%) were assayed at 25–75 μg/ ml. In brief, 0.1-mmol/l solution of DPPH in methanol was prepared. Then, 1 ml of this solution was added to 3 ml of extract solution at different concentration (25–75 μg/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm in Asys microplate reader. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity.

$$\text{DPPH scavenging effect}(\%) = 100 - [((\text{A0} - \text{A1})/\text{A0}) \times 100].$$

Where A0 was the absorbance of the control reaction and A1 was the absorbance in the presence of the sample [14].

Acute toxicity studies

Wistar rats weighing 200–250 g were used. Acute oral toxicity testing was performed according to OECD-423 guidelines [15]. The animals were fasted overnight, provided only water after which extracts were administered to the animals orally at the dose level of 5 mg/kg body weight by gastric intubation, and the animals were observed for 24 h. If mortality was observed in two or three animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses, such as 50, 300, and 2000 mg/kg body weight. The animals were observed for any toxic symptoms such as behavioral changes, locomotion, and convulsions, and the mortality rates were recorded during 72 h.

Animals

Male Wistar albino rats (100 : 120 g) were selected for this study. They were obtained from the Animal House, National Research Centre, Egypt. All animals were kept in a controlled environment of air and temperature with access to water and diet.

Ethics

Anesthetic procedures and handling with animals were complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt and performed to make sure that the animals did not suffer at any stage of the experiment (approval no: 13115).

Doses and route of administration

Administration regimen of cholesterol was as follows: cholesterol was orally administrated at a dose of 30 mg/0.3 ml olive oil/1 kg animal five times a week for 12 consecutive weeks [2]. Low dose of both methanol and petroleum ether extracts of *E. lathyris* was orally administered at a dose of 29.16 mg/kg body weight

five times a week for four consecutive weeks [16]. High dose of both methanol and petroleum ether extracts of *E. lathyris* was orally administrated at a dose of 291.6 mg/kg body weight five times a week for four consecutive weeks [16].

Experimental groups

One hundred and ten male Wistar strain albino rats were used in this study. Animals were divided into 11 groups (10 rats each). Group 1 served as normal healthy control rats. Groups 2–5 were normal healthy rats orally administrated with low methanol extract, high methanol extract, low petroleum ether extract, and high petroleum ether extract. Group 5 served as the high-fat-diet group. Groups 6–11 were high-fat-diet rats administrated with low methanol extract, high methanol extract, low petroleum ether extract, high petroleum ether extract, as well as orlistat as a reference drug.

Sample preparations

Serum sample were obtained as follows: blood was collected from each animal by puncture in the subtongual vein in a clean and dry test tube. It was left for 10 min to clot and centrifuged at 3000 rpm at 4°C for serum separation. The separated serum was stored at −80°C for further determinations of liver function indices.

Liver tissues were homogenized in normal physiological saline solution (0.9N NaCl) (1 : 9 w/v). The homogenate was centrifuged at 4°C for 5 min at 3000 rpm, and the supernatant was used for estimation of liver function and marker enzymes and the antioxidant parameters.

Biochemical determinations

All animal groups were subjected to determination of the liver biomarker enzyme; liver function indices, such as aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase (AST, ALT, and ALP) activities; nonenzymatic and enzymatic antioxidants; glutathione-reduced (GSH); glutathione-S-transferase (GST); catalase enzyme; and total antioxidant capacity (TAC). Oxidative stress biomarkers, malondialdehyde (MDA), and nitric oxide (NO) were also assessed. Moreover, lipid profile picture (total lipid, triglyceride, cholesterol, and HDL-C) was estimated.

Estimations of AST and ALT and ALP in serum were carried out by the method of Reitman and Frankel [17] and Belfield and Goldberg [18], respectively.

Estimations of hepatic total protein content, MAD, NO, catalase enzyme, GSH concentration, TAC, and GST were carried out by the method of Bradford [19], Buege and Aust [20], Montgomery and Dymock [21], Aebi [22], Moron *et al.* [23], Koracevic and Koracevic [24], and Habig *et al.* [25], respectively.

Estimation of lipid profile parameters, such as total lipid, triglyceride, cholesterol, and HDL-C in serum, was carried out by the method of Zollner and Kirsch [26], Fassati and Prencipe [27], Richmond [28], and Burstein [29], respectively.

Histopathological analysis

Liver tissue slices were fixed in 10% formaldehyde and embedded in paraffin wax blocks. Sections of 5 μm thick were stained with hematoxylin and eosin and then examined under light microscope for determination of pathological changes [30].

Statistical analysis

All data were expressed as mean±SD of the 10 rats in each group. Statistical analysis was carried out by one-way analysis of variance, Co-stat Software Computer Program (USA, newwork version 8). Significance values between groups were set at *P* value less than or equal to 0.05.

DPPH free radical-scavenging activity values are expressed as mmol Trolox/100 g DW.

There was a positive correlation between the TPC and the DPPH free radical-scavenging activity in different parts of *E. lathyris*.

The study of Cai *et al.* [31] reported that medicinal herbs are declared as having stronger antioxidant activity and contain significantly more phenolics than common vegetables and fruits, which are considered as good natural sources of dietary antioxidants. The results of the present study indicate that the extract of *E. lathyris* has significant antioxidant activities, perhaps owing to its high contents of phenolics and flavonoids. Many reports have suggested that the antioxidant properties in *Euphorbiaceae* members are mainly owing to the presence of high contents of secondary metabolites, such as different types of flavonoids [32,33].

DPPH free radical-scavenging activity was measured using a modification method of Brand-Williams *et al.* [15]. In brief, 2 ml of different concentrations of sample extracts was allowed to react with 4-ml DPPH solution for 30 min in the dark after mixing. The absorbance at 517 nm was measured relative to the reagent blank, which was also incubated under the

same conditions. The percentage inhibition was calculated against a control and compared with a Trolox standard curve (60–200 μg/ml). The DPPH radical-scavenging rate (%) was calculated as follows.

Radical-scavenging rate (%)=(1−Ax×AtA0)×100, where Ax is the absorbance of test compounds, At is the absorbance of test compounds with 4 ml anhydrous ethanol as the blank reaction, and A0 is the absorbance of ethanol solution (95%, v/v) as the blank reaction. The absorbance at 517 nm was measured relative to a blank reagent. The percentage inhibition was calculated against a control and compared with a Trolox standard curve (60–200 μg/ml).

Results and discussion

Determination of IC50 (μg/ml) values of *Euphorbia lathyris* seed crude extract

The *in vitro* cytotoxic screening of the methanol and petroleum ether *E. lathyris* extracts against HEPG2 cell line is reported in Table 2. From these results, we indicated that the methanol extract and petroleum ether have low to moderate activity with IC50 of 250 and 294.5 μg/ml, respectively.

Determination of total flavonoid, phenolic content, and free radical-scavenging activity of *Euphorbia lathyris* L. extracts

Total flavonoid

Flavonoids are considered as significant antioxidants in the medicine herb *E. lathyris* [6]. It is found that the TFC in seeds of *E. lathyris* methanol extract was higher than TFC in petroleum ether extract. The range of TFC is from 12.8 to 19.5 mg RE/100 g DW. It is difficult to compare the current results with extracts of other plants, owing to the fact that research on flavonoids of *E. lathyris* different parts is still scarce. However, the reported flavonoid contents of other plants, like Chinese dwarf cherry seed (2.17 mg RE/g DW), Gordon Euryale seed (1.39 mg RE/g DW), platycodon root (0.95 mg RE/g DW), ginkgo seed (0.62 mg RE/g DW), or bitter apricot seed (0.90 mg RE/g DW) [34], in comparison with the *E. lathyris* TFC were higher. Flavonoids are known to have antioxidant and anti-inflammatory effects; moreover, they exhibit many other health benefits, so the high flavonoid content is considered beneficial for health [35,36]. *E. lathyris* is also a promising powerful source of flavonoids. However, the identification of flavonoids of *E. lathyris* stays under-investigated, and further research is warranted (Table 3).

Table 2 IC50 (μg/ml) values of *E*uphorbia *lathyris* seed crude extract

Crude extract	IC50 (μg/ml) (48 h exposure)
Methanol extract	250
Petroleum ether	294.5

Table 3 Concentration of flavonoid in two extracts of *Euphorbia lathyris* L.

Sample code	Concentration
Methanol extract	19.5
Petroleum ether extract	12.8

Phenolic content

The TPC of *E. lathyris* was 1.15 g GAE/100 g of dry sample using aqueous methanol [12]. This method extracted only the loosely attached or free and more readily soluble phenolic substances in samples; however, it could not be tightly joined to cell wall materials [37]. Furthermore, no further extraction or alkali hydrolysis was performed. However, the value obtained in a study of Adom and Liu [37] was considerably higher than those reported in the current study, which may be influenced by *E. lathyris* genotype.

In plants, phenolics provide a lot of health benefits, especially in the vegetables or corn [16]. Compared with the TPC in different parts of *E. lathyris*, TPC was significantly higher in the testa than those in the root, seed, and stem. The same results were gained in many other economically important fruits, including grape berry, apple, and olive, where their skins (or peels) hold TPC content higher than other tissues [38]. The TPC ranged from 290.46 to 117.44 mg GAE/100 g DW among different parts of *E. lathyris*. The present results were matched with the data reported by Liu *et al.* [34], who found that the total phenolic ranged from 0.57 to 280.46 mg GAE/g. Moreover, Cai *et al.* [31] found that phenolic compounds were the prevailing antioxidant components in Chinese medicinal herbs attributed to anticancer activity. In the current study, the high TPC content obtained from *E. lathyris* indicated its plentiful antioxidant source with health benefits (Tables 4 and 5).

Acute toxicity studies

Lethal dose of Euphorbia lathyris methanol extract

Acute toxicity study showing no toxicity, and only one dead rat, as shown in Table 6.

Table 4 Phenolic acids content of *Euphorbia lathyris* (mg gallic acid equivalent/100 g DW) seeds of *Euphorbia lathyris* L. (methanol)

Free phenolics	150.04±10.45[a]
Bound phenolics	46.00±2.44[b]
Total phenolics	196.50±5.54[c]

Values with different letters in a columns are significantly different ($P<0.05$, $n=3$).

Table 5 Phenolic acid content of *Euphorbia lathyris* (mg galic acid equivalent/100 g DW) seeds of *Euphorbia lathyris* L. (petroleum ether)

Free phenolics	65.04±3.45[a]
Bound phenolics	13.00±1.10[b]
Total phenolics	44.22±2.32[c]

Values with different letters in a columns are significantly different ($P<0.05$, $n=3$).

Table 6 Lethal dose of *Euphorbia lathyris* methanol extract

Dose (mg/kg b.wt.)	Rat number	Dead rat number	*Z*	*d*	*Z*×*d*
100	6	0	0	100	0
250	6	0	0	150	0
500	6	0	0	250	0
1000	6	0	0	500	0
2000	6	0	0	1000	0
3000	6	1	0.5	1000	500

Z is the mean of dead animals in two successive groups. dis the constant factor between two successive groups.
LD50=Dm–Σ(*Z*×*d*)/*n* LD50 of *Euphorbia lathyris* methanol extract=2916.7 mg/kg. 1/10 LD50=291.6 mg/kg.

Effect of methanol and petroleum ether extracts of *Euphorbia lathyris* on liver marker enzymes in serum of high-fat-diet rats

Table 7 indicates an insignificant difference in ALT, AST, and ALP enzyme activities in normal rats treated with methanol and petroleum ether extracts of *E. lathyris* compared with normal untreated one. However, high-fat-diet rats showed significant ($P\leq0.05$) increase in liver enzyme activities [ALT (95.25%), AST (108.75%), and ALP (227.87%)] as compared with normal control rats. On the contrary, high-fat-diet rats treated with low and high dose of methanol and petroleum ether extracts as well as oristat as reference drug declared significant increase in ALT, AST, and ALP enzyme activities compared with normal healthy rats (Table 1). With respect to the percentages of improvement, ALT enzyme activity recorded improvement percentage of 76%, in high dose of methanol extract administered to high-fat-diet rats, followed by reference drug (orlistat) (73.50%), and then high-dose petroleum extract (72.50%). However, low methanol dose extract recorded the lowest amelioration percentage (32%). Moreover, AST enzyme activity showed the highest improvement percentage (95.26%) in high petroleum ether dose administered to high-fat-diet rats and the lowest improvement percentage (52.11%) in low methanol extract-supplemented rats. Moreover, ALP enzyme activity showed the highest improvement percentage (65.67%) in orlistat reference drug administered to high-fat-diet rats and the lowest improvement percentage (31.31%) in low methanol extract.

Data represent mean±SD of 10 rats in every group. Data are expressed as U/ml. Statistical analysis is carried out using SPSS computer program (New York, USA, version 8) coupled with Co-Stat computer program, where unshared letters between groups are the significance value at *P* value less than or equal to 0.5.

An important source to identify new active pharmaceutical compounds is medicinal plants [17]. *E. lathyris* (caper spurge), a common biennial garden plant, is spread in southern England; however, it can exist throughout North America, Europe, and Australia [39]. Because of its high contents of hydrocarbons and fatty acids, it is used as a source of bio-fuel and biomass [40]. For treatment of hydropsy, coprostasis, ascites, amenorrhea, snakebite, and scabies, its seeds are prescribed in traditional Chinese medicine [41]. Moreover, it has toxicological effects of croton oil like *E. lathyris*. Prior biological studies have showed the antitumor effects of *E. lathyris* for curing esophagus cancer, leukocythemia, and skin cancer [42]. In the past decades, the phytochemical ingredients of the seed, aerial parts, and root of *E. lathyris* have attracted great attention, especially for the applications of human health. The presence of different metabolites have been shown in *E. lathyris*, such as diterpene, aesculetin, euphorbetin, kaempferol-3-glucuronide, daphnetin-sitosterol, vitexicarpin, daucosterol, artemetin, p-hydroxybenzoic acid, flavones, and flavonol glucosides [12,16,43,44].

Effect of methanol and petroleum ether extracts of *Euphorbia lathyris* on catalase, glutathione, glutathione-S-transferase, and total antioxidant capacity activities in the tissue of high-fat-diet rats

Table 8 clearly indicates insignificant difference in catalase, GST, and TAC activities, whereas significant decrease in GSH activity in normal rats treated with methanol and petroleum ether extracts of *E. lathyris,* compared with normal untreated ones. However, high-fat-diet rats showed significant ($P\leq0.05$) decrease in catalase (15.79%), GSH (181.93%), GST (85.78%), and TAC (0.91%) compared with normal control rats. Considering the percentages of improvement, GSH and GST enzyme activities recorded the highest improvement percentage of 31.95 and 44.93% in high-dose methanol extract administered to high-fat diet rats. Low methanol dose extract recorded the lowest amelioration percentage (16.17%) in GSH, whereas the low-dose petroleum ether extract showed the lowest improvement percentage (16.91%) in GST. High petroleum ether

Table 7 Effect of methanol and petroleum ether extracts of *Euphorbia lathyris* on alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase activities in the serum of different intoxicated therapeutic groups

Groups Parameters	Control	Controls treated				High-fat diet	High-fat treated				
		Low Meth. dose	High Meth. dose	Low Pet. dose	High Pet. dose		Low Meth. dose	High Meth. dose	Low Pet. dose	High Pet. dose	Ref. drug
ALP (U/ml)	115.15 ± 5.14^{f}	126.12 ± 4.12^{e}	127.45 ± 4.12^{e}	124.28 ± 3.72^{e}	125.15 ± 3.72^{e}	227.87 ± 4.73^{a}	191.82 ± 9.67^{b}	154.93 ± 4.26^{d}	179.15 ± 2.56^{c}	154.01 ± 4.26^{d}	152.25 ± 4.78^{d}
%[a]		−9.53	−10.40	−7.93	−8.68	−97.89	−66.58	−34.55	−55.58	−33.75	−32.22
%[c]							31.31	63.34	42.31	64.14	65.67
ALT (U/ml)	50.00 ± 3.74^{e}	52.30 ± 1.30^{cde}	53.50 ± 1.29^{cde}	51.21 ± 2.21^{de}	52.72 ± 2.21^{de}	95.25 ± 4.11^{a}	79.25 ± 3.77^{b}	57.25 ± 5.25^{cd}	75.5 ± 5.26^{b}	59 ± 5.29^{c}	58.5 ± 4.43^{cd}
%[a]		−4.60	−7.00	−2.42	−5.50	−90.50	−58.50	−14.50	−51.00	−18.00	−17.00
%[c]							32.00	76.00	39.50	72.50	73.50
AST (U/ml)	47.5 ± 2.38^{f}	49.1 ± 1.25^{f}	50.25 ± 1.29^{f}	48.13 ± 2.21^{f}	49.25 ± 2.21^{f}	108.75 ± 6.34^{a}	84.25 ± 3.77^{b}	68.5 ± 3.74^{d}	77 ± 2.64^{c}	63.5 ± 1.91^{b}	81.5 ± 3.46^{bc}
%[a]		−3.37	−5.79	−1.33	−3.68	−128.95	−76.84	−44.21	−62.11	−33.68	−70.53
%[c]							52.11	84.74	66.84	95.26	58.42

%[a], % change as compared with normal control rats; %[c], % of improvement. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Meth, methanol; Pet, petroleum.

Table 8 Effect of methanol and petroleum ether extracts of *Euphorbia lathyris* on catalase, glutathione-S-transferase activities, glutathione level, and total antioxidant capacity in the tissue of different therapeutic group

Groups Parameters	Control	Controls treated				High-fat diet	High-fat treated				
		Low Meth. dose	High Meth. dose	Low Pet. dose	High Pet. dose		Low Meth. dose	High Meth. dose	Low Pet. dose	High Pet. dose	Ref. drug
Catalase (U/g tissue)	46.47 ± 5.31^{a}	42.88 ± 5.14^{a}	45.88 ± 3.21^{a}	47.10 ± 2.14^{a}	47.59 ± 2.60^{a}	15.79 ± 2.03^{d}	28.28 ± 2.14^{c}	30.81 ± 3.66^{c}	26.31 ± 2.02^{c}	37.84 ± 2.54^{b}	28.79 ± 4.53^{c}
%[a]		7.73	1.27	−1.36	−2.41	66.02	39.14	33.70	43.38	18.57	38.05
%[c]							26.88	32.32	22.64	47.45	27.98
GSH (μg/mg protein)	419.95 ± 17.33^{a}	385.65 ± 12.08^{b}	387.64 ± 13.08^{b}	390.26 ± 6.67^{b}	393.26 ± 6.77^{b}	181.93 ± 15.51^{e}	249.83 ± 16.56^{d}	316.08 ± 14.01^{c}	259.11 ± 16.52^{d}	312.62 ± 9.18^{c}	315.98 ± 12.41^{c}
%[a]		8.17	7.69	7.11	6.36	56.68	40.51	24.73	38.30	25.56	24.76
%[c]							16.17	31.95	18.38	31.12	31.92
GST (μg/mg protein)	186.72 ± 5.50^{a}	173.13 ± 4.30^{abc}	175.13 ± 4.08^{abc}	177.12 ± 9.14^{ab}	178.37 ± 8.21^{ab}	85.78 ± 10.2^{e}	118.78 ± 6.64^{d}	169.67 ± 10.65^{bc}	117.35 ± 4.33^{d}	165.90 ± 8.94^{c}	165.32 ± 8.87^{c}
%[a]		7.30	6.21	5.42	4.47	54.06	36.39	9.13	37.15	11.15	11.41
%[c]							17.67	44.93	16.91	42.91	42.60
TAC (U/g tissue)	2.32 ± 0.46^{a}	2.08 ± 0.25^{a}	2.11 ± 0.27^{a}	2.04 ± 0.11^{ab}	2.05 ± 0.09^{ab}	0.91 ± 0.1^{e}	1.38 ± 0.1^{d}	1.455 ± 0.09^{d}	1.37 ± 0.06^{d}	1.79 ± 0.09^{bc}	1.54 ± 0.08^{cd}
%[a]		10.34	9.05	12.07	11.64	60.78	40.52	37.50	40.95	22.84	33.62
%[c]							20.26	23.28	93.36	37.93	27.16

%[a], % change as compared with normal control rats; %[c], % of improvement. Data are represented as means±SD of 10 rats in every group. Data are expressed as μg/mg protein for glutathione and glutathione-S-transferase, unit/g tissue for catalase enzyme and for total antioxidant capacity. Statistical analysis is carried out using SPSS computer program (version 8) coupled with Co-Stat computer program, where unshared letters between groups are the significance value at *P* value less than or equal to 0.5. GSH, glutathione; GST, glutathione-S-transferase; Meth, methanol; Pet, petroleum; TAC, total antioxidant capacity.

extract showed the highest improvement percentage in catalase enzyme activity (47.45%), whereas low petroleum ether extract recorded the lowest by 22.64%. In addition, low petroleum ether extract showed the highest improvement percentage in TAC (93.36%); in contrast, low methanol extract recorded the lowest amelioration percentage (20.26%) (Table 8).

One of the biological interest of *E. lathyris* is the variety of chemical compounds in this herb. The results of the current study reveal significant antioxidant activities of *E. lathyris* extract, which may be owing to high contents of phenolics and flavonoids. Certainly, due to the existence of major contents of secondary metabolites, including various flavonoid types, *Euphorbiaceae* members have been suggested to have antioxidant properties [32]. From the botanical secondary metabolites, hydrophilic antioxidants such as phenolics and flavonoids are the most abundant in plant [45]. Many research studies demonstrated that disturbances of hyperlipidemia, obesity, and lipid metabolism are attributed to increased production of oxygen free radicals [46]. Oxidative stress is formed as a result of the exhibited dysfunction of antioxidant defense system and accumulation of these radicals [47]. These radicals covalently bind to macromolecules and enhance the peroxidation of membrane lipids with great level of polyunsaturated fatty acids, consequently leading to the formation of lipid peroxides and multiple pathological changes [48]. Several antioxidant defense mechanisms are possessed by oxygen-dependent organisms against oxidative damage by prooxidants, which turn active oxygen molecules into nontoxic compounds. These defense systems include the antioxidant enzymes such as catalase, superoxide dismutase, GST, and glutathione peroxidase, as well as nonenzymatic compounds such as vitamins and GSH. The first line of defenses against free radicals is superoxide dismutase, which catalyzes the dismutation of superoxide anions into hydrogen peroxide (H_2O_2). CAT is a hemeprotein that catalyzes H_2O_2 to oxygen and water.

Effect of methanol and petroleum ether extracts of *Euphorbia lathyris* on lipid peroxidation and nitric oxide level in tissue of high-fat diet rats

Table 9 clearly indicates insignificant difference in NO activity in normal rats treated with methanol and petroleum ether extracts of *E. lathyris* compared with normal untreated one. On the contrary, lipid peroxidation normal rats treated with high methanol extract showed significant increase in lipid peroxidation activity (886.34%) compared with normal untreated one. However, high-fat-diet rats showed significant ($P \leq 0.05$) increase in lipid peroxidation and NO activities (2322.16 and 314.62%, respectively), as compared with normal control rats. High-fat-diet rats treated with low and high dose of both methanol and petroleum ether extracts showed significant decrease in lipid peroxidation and NO activities, as compared with control rats. In addition, low dose of petroleum ether extract showed the highest improvement percentage in both NO and lipid peroxidation (116.08 and 176%, respectively). However, the reference drug recorded the lowest amelioration percentage (81.56%) in NO activity, and low-dose methanol extract showed the lowest improvement percentage (145.62%) in lipid peroxidation activity (Table 9).

GSH caused detoxification of toxic compounds such as H_2O_2 and lipid peroxides directly or in glutathione peroxidase and GST [49,50]. However, the innate antioxidant defense systems are often scantly to resist the oxidative stress, which may play an important mediator for progressive damage to cell structures.

Consequently, method of inhibiting or curing atherosclerosis and decreasing the incidence of cardiovascular disease is by targeting of the hyperlipidemia by diet and/or lipid-lowering drugs.

Much attention is given to the free radical-scavenging natural antioxidants to be served as potential, nontoxic agents. Many epidemiological studies detected that an increased intake of food wealthy in natural antioxidants increases the antioxidant activity of the body and diminishes the risk of different diseases. Herbal remedies or food supplements, especially for those with cholesterol at the border levels, are attractive alternatives to cure or inhibit hypercholesterolemia [51]. Consistent with the present results, Esin *et al.* [52] reported that the development of a progressive thickening of the artery wall arises from the accumulation of cholesterol oxidation products in the particles of LDL after they are oxidized is resulted by cholesterol oxidation. Vijayabaskar *et al.* [53] mentioned that the etiology of atherosclerosis is the significantly increased levels of plasma cholesterol in rats fed with CCT diet, which might damage the endothelial cell membrane lining the large arteries such as aorta. Moreover, Prado *et al.* [54] gave evidence that hypercholesterolemia has been extensively connected to endothelial cell dysfunction, as an early step in the atherosclerosis, and thus, increased release of NO, vascular production, and superoxide anions, which interact to produce peroxynitrite, a potent oxidant causing damage of multiple cell constituents. The results of both previous studies and the current

Table 9 Effect of methanol and petroleum ether extracts of *Euphorbia lathyris* on nitric oxide and malondialdehyde levels in the liver of different therapeutic group

	Control	Controls treated				High-fat diet	High-fat treated				
		Low Meth. dose	High Meth. dose	Low Pet. dose	High Pet. dose		Low Meth. dose	High Meth. dose	Low Pet. dose	High Pet. dose	Ref. drug
NO (μg/mg protein)	108.04±7.38^{d}	117.02±8.12^{d}	122.03±8.74^{d}	116.18±3.72^{d}	118.28±5.03^{d}	314.62±12.61^{a}	214.65±12.72^{b}	218.02±12.85^{b}	217.91±12.41^{b}	189.21±8.73^{c}	226.51±15.87^{b}
%^{a}		−8.31	−12.95	−7.47	−9.48	−191.21	−98.68	−101.80	−101.70	−75.13	−109.65
%^{c}							92.53	89.41	89.51	116.08	81.56
MDA (μg/mg protein)	735.31±53.16^{g}	884.12±38.56^{f}	886.34±39.92^{f}	804.10±17.6^{g}	805.14±18.3^{g}	2322.16±33.75^{a}	1251.37±81.79^{b}	1081.75±51.61^{de}	1195.5±29.96^{bc}	1028±29.23^{e}	1129.42±73.55^{cd}
%^{a}		−20.24	−20.54	−9.38	−9.50	−215.88	−70.17	−47.14	−62.58	−39.80	−53.60
%^{c}							145.62	168.70	153.22	176.00	162.21

%^{a}, % change as compared to normal control rats; %^{c}, % of improvement. Data are represented as means±SD of 10 rats in every group. Data are expressed as μg/mg protein for malondialdehyde and for nitric oxide. Statistical analysis is carried out using SPSS computer program (version 8) coupled with Co-Stat computer program, where unshared letters between groups are the significance value at *P* value less than or equal to 0.5. MDA, malondialdehyde; Meth, methanol; Pet, petroleum.

Table 10 Effect of methanol and petroleum ether extracts of *Euphorbia lathyris* on serum lipid profile in the different intoxicated therapeutic groups

Groups Parameters	Control	Controls treated				High-fat diet	High-fat treated				
		Low Meth. dose	High Meth. dose	Low Pet. dose	High Pet. dose		Low Meth. dose	High Meth. dose	Low Pet. dose	High Pet. dose	Ref. drug
Total lipid (mg/dl)	81.08±3.58^{f}	73.11±3.14^{g}	72.50±2.43^{g}	70.22±1.14^{g}	71.47±1.94^{g}	204.85±4.39^{a}	181.95±5.10^{b}	155.75±3.42^{d}	175.90±4.08^{c}	155.85±6.16^{d}	128.57±4.61^{e}
%^{a}		9.83	10.58	13.39	11.85	152.65	124.41	92.34	116.95	92.22	58.57
%^{c}							28.24	60.56	35.71	60.43	94.08
Cholesterol (mg/dl)	70.86±5.17^{d}	58.10±2.08^{e}	59.92±2.92^{d}	66.12±6.67^{d}	68.09±5.86^{d}	186.38±3.34^{a}	158.09±4.59^{b}	139.66±4.46^{c}	160.78±4.86^{b}	139.20±4.24^{c}	156.90±5.75^{b}
%^{a}		18.01	15.44	6.69	3.91	163.03	123.10	97.09	126.90	96.44	121.42
% ^{c}							39.92	65.93	36.13	66.58	41.60
Triglyceride (mg/dl)	69.26±3.38^{d}	65.12±4.30^{d}	64.46±4.47^{d}	62.20±3.14^{d}	63.38±3.70^{d}	147.90±3.56^{a}	118.90±3.41^{b}	92.82±4.97^{c}	145.81±3.80^{a}	96.90±4.84^{c}	93.39±7.79^{c}
%^{a}		5.98	6.93	10.19	8.49	−113.54	−71.67	−34.02	−110.53	−39.91	−34.84
%^{c}							41.87	79.53	3.02	73.64	78.70
HDL-cholesterol (mg/dl)	50.21±1.94^{ab}	51.11±2.25^{a}	51.58±2.52^{a}	53.12±2.11^{a}	52.36±2.34^{a}	19.92±2.14^{f}	32.92±2.02^{e}	45.65±3.03^{c}	40.92±3.54^{d}	46.25±3.30^{bc}	48.35±4.83^{abc}
%^{a}		−1.79	−2.73	−5.80	−4.28	60.33	34.44	9.08	18.50	7.89	3.70
%^{c}							25.89	51.24	41.82	52.44	56.62

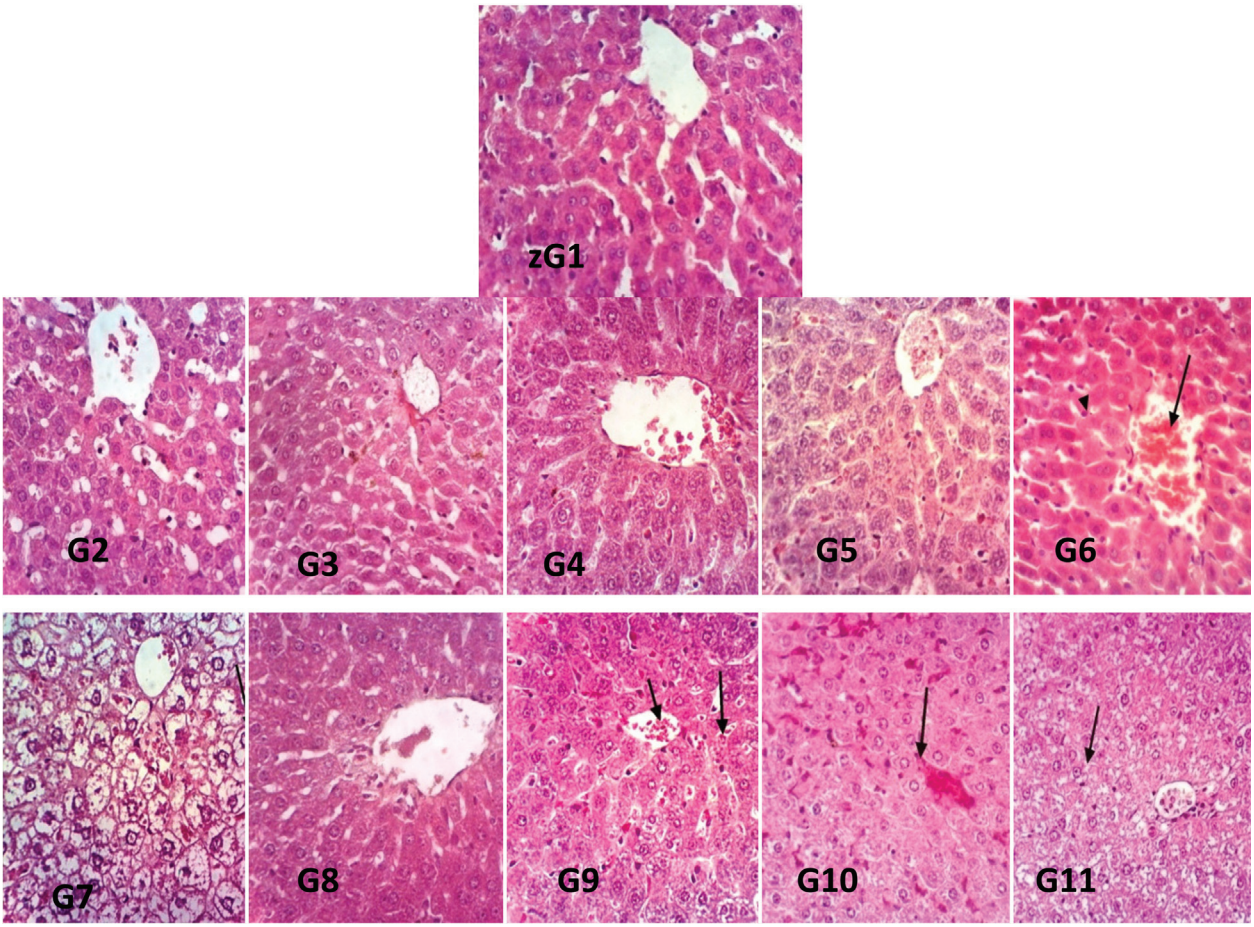
%^{a}, % change as compared to normal control rats; %^{c}, % of improvement. Data are represented as means±SD of ten rats in every group. Data are expressed as mg/dl for total lipid, cholesterol, triglyceride, and HDL-cholesterol. Statistical analysis is carried out using SPSS computer program (version 8) coupled with Co-Stat computer program, where unshared letters between groups are the significance value at *P* value less than or equal to 0.5. HDL, high-density lipoprotein; Meth, methanol; Pet, petroleum.

results of our study proposed the powerful beneficial health capabilities of *E. lathyris* in the animal model of hyperlipidemia, which could be linked to the antioxidant effects of its active substances such as antioxidants, vitamins, and phenolic compounds (ascorbic acid, tocotrienols, and tocopherols) along with the promoting of the total antioxidant defense system in the body. *E. lathyris* can direct challenge free radical before any significant oxidation can occur that prohibits antioxidants. *E. lathyris* retard or slow the oxidative processes resulting in MDA decrease (the lipid peroxidation nearby normal level and so inhibit the oxidative stress, as a chain-breaking antioxidant.

Effect of methanol and petroleum ether extracts of *Euphorbia lathyris* on lipid profile activity in serum of high-fat-diet rats

There was insignificant difference in cholesterol activity in normal rats treated with low and high dose of methanol extract of *E. lathyris*, as well as insignificant change was recorded in both triglyceride and HDL-C activities in normal rats treated with methanol and petroleum ether extracts of *E. lathyris* compared with normal untreated one. However, significant decrease was recorded in normal rats treated with low and high dose of methanol and petroleum ether extracts of *E. lathyris* in total lipid activity (73.11, 72.50, 70.22, and 71.47%, respectively), as compared with normal untreated one. Moreover, significant decrease was showed in normal rats treated with low and high dose of methanol extract (58.10 and 59.92%, respectively) in cholesterol activity as compared with normal untreated one. High-fat-diet-treated rats showed significant increase in total lipid, cholesterol, and triglyceride activities (204.85, 186.38, and 147.90%, respectively) as compared with normal rats, whereas demonstrated significant decrease in HDL-C by 19.92% compared with control rats. High-fat-diet-administrated rats with both low and high dose of methanol and petroleum ether

Figure 2



Liver sections of control rats (G1), control rats treated with low dose of methanol extract (G2), control rats treated with high dose of methanol extract (G3), control rats treated with low dose of petroleum ether extract (G4), control rats treated with high dose of petroleum ether extract (G5), high-fat-diet rats (G6), high-fat-diet rats treated with low dose of methanol extract (G7), high-fat-diet t rats treated with high dose of methanol extract (G8), high-fat-diet rats treated with low dose of petroleum ether extract (G9), high-fat-diet rats treated with high dose of petroleum ether extract (G10), and high-fat-diet rats treated with reference drug (G11).

extracts recorded significant decrease in total lipid, cholesterol, and triglyceride levels, whereas significant increase in HDL-C was recorded compared with normal control rats. Considering the improvement percentage, reference drug recorded the highest improvement percentage in both total lipid and HDL-C (94.08 and 56.62%, respectively). However, low dose of methanol extract showed the lowest amelioration percentage in both total lipid and HDL-C (28.24 and 25.89%, respectively). High dose of petroleum extract recorded the highest amelioration percentage in cholesterol level (66.58%), whereas low dose of petroleum extract recorded the lowest improvement percentage in cholesterol level (36.13%). In addition, high dose of methanol extract showed the highest amelioration percentage (79.53%) in triglyceride activity, whereas low dose of petroleum extract recorded the lowest improvement by 3.02% in triglyceride level (Table 10).

Histopathological analysis

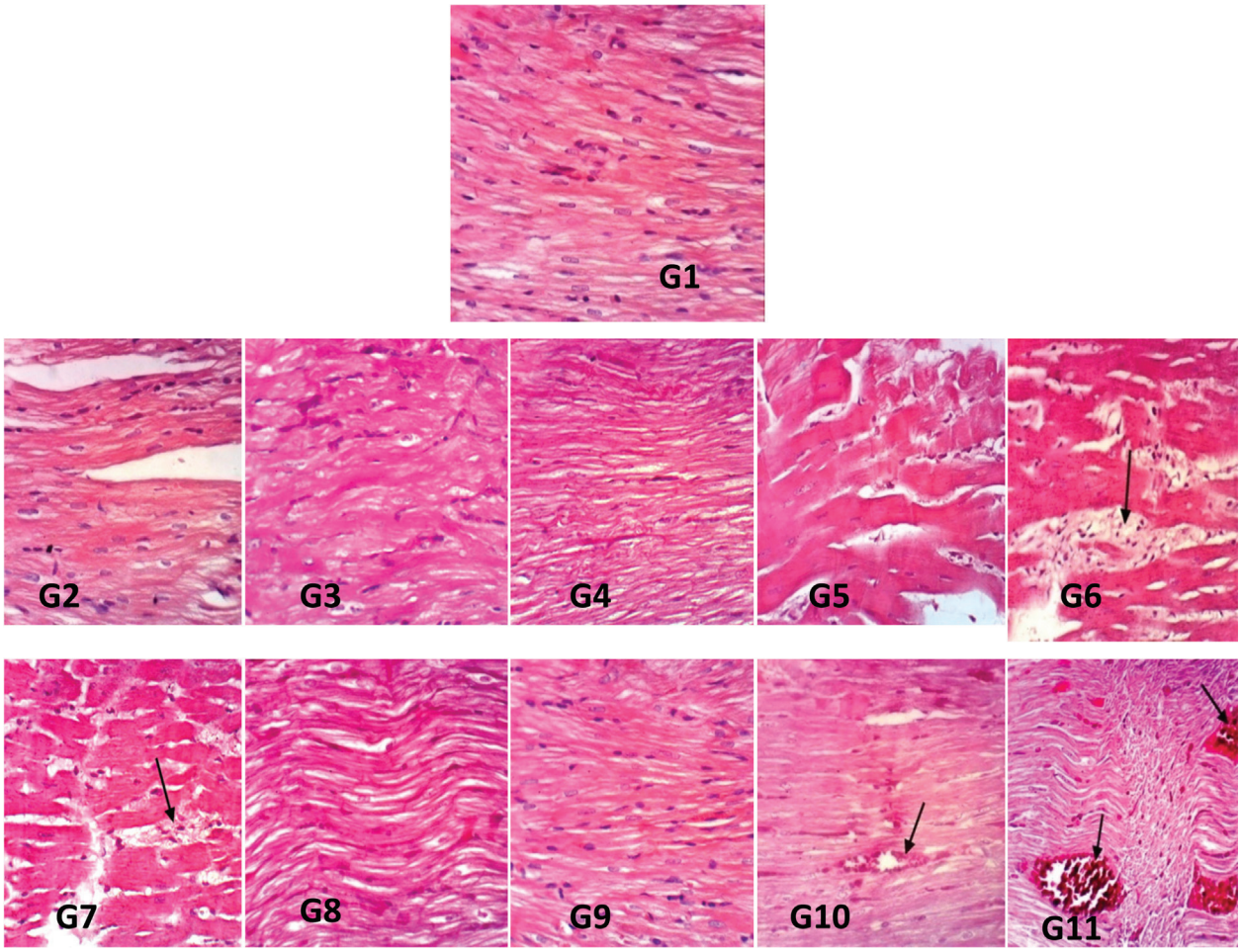
Effect of methanol and petroleum ether extracts of Euphorbia lathyris on hepatic architecture

Liver section of control rats, control rats treated with low methanol and petroleum ether dose, control rats treated with high methanol and petroleum ether extract showed liver with normal histological structure of hepatic lobule (hematoxylin and eosin, ×400) (Fig. 2, G1, 2, 3, 4, 5).

Liver section of high-fat diet rats showing cytoplasmic vacuolization of hepatocytes, congestion of central vein, Kupffer cells activation and hepatic sinusoids, necrosis of sporadic hepatocytes and hydropic degeneration of hepatocytes (hematoxylin and eosin, ×400) (Fig. 2, G6).

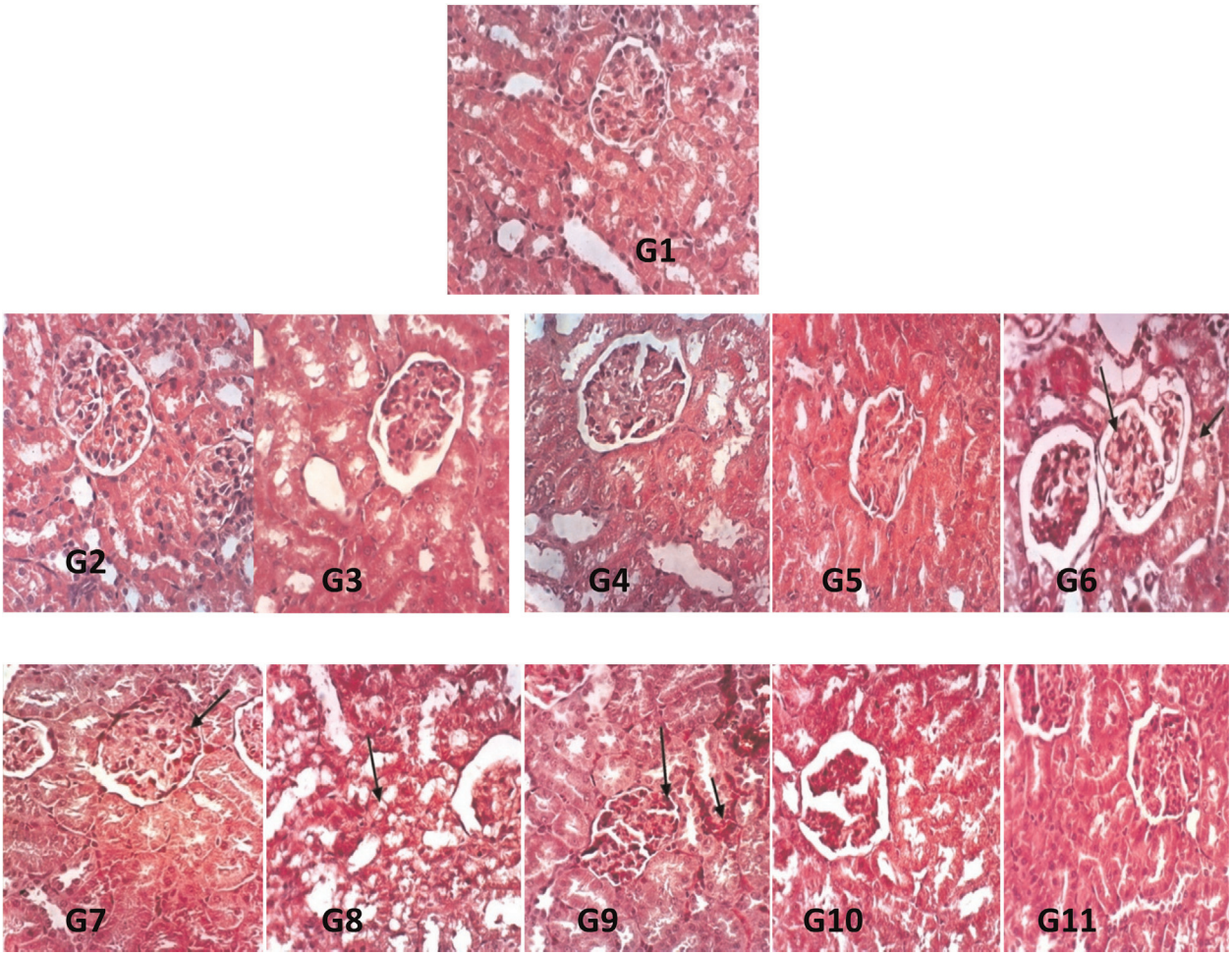
Liver section of high-fat diet rats treated with low methanol ether extract showing hydropic

Figure 3



Heart sections of control rats (G1), control rats treated with low dose of methanol extract (G2), control rats treated with high dose of methanol extract (G3), control rats treated with low dose of petroleum ether extract (G4), control rats treated with high dose of petroleum ether extract (G5), high-fat-diet rats (G6), high-fat-diet rats treated with low dose of methanol extract (G7), high-fat-diet rats treated with high dose of methanol extract (G8), high-fat-diet rats treated with low dose of petroleum ether extract (G9), high-fat-diet rats treated with high dose of petroleum ether extract (G10), and high-fat-diet rats treated with reference drug (G11).

Figure 4



Kidney sections of control rats (G1), control rats treated with low dose of methanol extract (G2), control rats treated with high dose of methanol extract (G3), control rats treated with low dose of petroleum ether extract (G4), control rats treated with high dose of petroleum ether extract (G5), high-fat-diet rats (G6), high-fat-diet rats treated with low dose of methanol extract (G7), high-fat-diet rats treated with high dose of methanol extract (G8), high-fat-diet rats treated with low dose of petroleum ether extract (G9), high-fat-diet rats treated with high dose of petroleum ether extract (G10), and high-fat-diet rats treated with reference drug (G11).

degeneration of hepatocytes (hematoxylin and eosin, ×400) (Fig. 2, G7).

Liver section of high-fat diet rats treated with high methanol ether extract showing slight congestion of central vein and hepatic sinusoids (Fig. 2, G8).

Liver section of high-fat diet rats treated with low petroleum ether extract showing no histopathological changes (hematoxylin and eosin, ×400) (Fig. 2, G9).

Liver section of high-fat diet rats treated with high petroleum ether extract showing slight congestion of hepatic sinusoids (hematoxylin and eosin, ×400) (Fig. 2, G10).

Liver section of high-fat diet rats treated with orlistat as reference drug showing slight hydropic degeneration of hepatocytes and slight congestion of central vein (hematoxylin and eosin, ×400) (Fig. 2, G11).

Effect of methanol and petroleum ether extracts of Euphorbia lathyris on heart architecture

Heart section of control rats, control rats treated with low methanol and petroleum ether dose, control rats treated with high methanol, and petroleum ether extract showed normal histological structure of cardiac myocytes (hematoxylin and eosin, ×400) (Fig. 3, G1, 2, 3, 4, 5).

Heart section of high-fat-diet rats showed vacuolation of the sarcoplasm of cardiac myocytes, intermyocardial edema, and intermyocardial hemorrhage (hematoxylin and eosin, ×400) (Fig. 3, G6).

Heart section of high-fat-diet rats treated with low methanol extract showed slight intermyocardial edema

and vacuolation of the sarcoplasm of cardiac myocytes (hematoxylin and eosin, ×400) (Fig. 3, G7).

Heart section of high-fat-diet rats treated with high methanol extract showed focal necrosis of cardiac myocytes (hematoxylin and eosin, ×400) (Fig. 3, G8).

Heart section of high-fat-diet rats treated with low petroleum ether extract showed congestion of myocardial blood vessels (hematoxylin and eosin, ×400) (Fig. 3, G9).

Heart section of high-fat-diet rats treated with high petroleum ether extract showed slight congestion of myocardial blood vessels (hematoxylin and eosin, ×400) (Fig. 3, G10).

Heart section of high-fat-diet rats treated with orlistat as a reference drug showed congestion of myocardial blood vessels (hematoxylin and eosin, ×400) (Fig. 3, G11).

Effect of methanol and petroleum ether extracts of Euphorbia lathyris on kidney architecture

Kidney section of control rats, control rats treated with low methanol and petroleum ether dose, control rats treated with high methanol and petroleum ether extract showed normal histological structure of renal parenchyma (hematoxylin and eosin, ×400) (Fig. 4, G1, 2, 3, 4, 5).

Kidney section of high-fat-diet rats showed endothelial lining glomerular and vacuolation of epithelial lining renal tubules focal inflammatory cell infiltration (hematoxylin and eosin, ×400) (Fig. 4, G6).

Kidney section of high-fat-diet rats treated with low methanol extract showed no histopathological changes (hematoxylin and eosin, ×400) (Fig. 4, G7).

Kidney section of high-fat-diet rats treated with high methanol extract showed slight congestion of glomerular tuft (hematoxylin and eosin, ×400) (Fig. 4, G8).

Kidney section of high-fat-diet rats treated with low petroleum ether extract showed vacuolation of epithelial lining renal tubules (hematoxylin and eosin, ×400) (Fig. 4, G9).

Kidney section of high-fat-diet rats treated with high petroleum ether extract showed no histopathological changes (hematoxylin and eosin, ×400) (Fig. 4, G10).

Kidney section of high-fat-diet rats treated with orlistat as a reference drug showed no histopathological changes (hematoxylin and eosin, ×400) (Fig. 4, G11).

Based on the results of both biochemical and histological, it can be suggested that the therapeutic effects of *E. lathyris* like several medicinal plants are usually related to its antioxidant compounds.

Conclusion

It could be concluded that the two extracts of *E. lathyris* declared improvement of liver function enzyme activities, oxidant–antioxidant biomarkers, and lipid profile levels in high-fat-diet-supplemented rats.

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Conflicts of interest

There are no conflicts of interest.

References

1 Balanescu S, Calmac L, Constantinescu D, Marinescu M, Onut R. Systemic inflammation and early atheromaformation: are they related? Maedica (Buchar) 2010; 5:292–301.

2 Ibrahim HB, Magda KE, Maha ZR, El-Sherbiny M, Azza AM, Hanan FA, *et al.* Hypolipidemic and anti-atherogenic effect of sulphated polysaccharides from the Green Alga Ulvafasciata. Int J Pharm Sci Rev Res 2015; 31:1–12.

3 Qi HM, Huang LY, Liu XL, Liu DM, Zhang QB, Liu SM. (Antihyperlipidemic activity of high sulfate contentderivative of polysaccharide extracted from Ulvapertusa (Chlorophyta). Carbohydr Polym 2012; 87:1637–1640.

4 Liua X, Sunc Z, Zhangb M, Menga X, Xiab X, Yuana W. Antioxidant and antihyperlipidemic activities of polysaccharides from sea cucumber *Apostichopus japonicas*. Carbohydr Polym 2012; 90:1664–1670.

5 Liang YT, Wong WT, Guan L. Effect of phytosterols and theiroxidation products on lipoprotein profiles and vascularfunction in hamster fed a high cholesterol diet. Atherosclerosis 2011; 219:124–133.

6 Dragland S, Senoo H, Wake K, Holte K, Blomhoff R. Several culinary and medicinal herbs are important sources of dietary antioxidants. J Nutr 2003; 133:1286–1290.

7 Wallis TE, Churchill JA. Text book of pharmacognosy. London, 1946. vi + 504 pp.

8 Shamim T, Ahmad M, Mukhtar M. Antihyperglycemic and hypolipidemic effects of methanolic extract of euphorbia prostrataonalloxan induced induced diabetic rabbits. Eur Sci 2014; 7881:1857–7431.

9 Herrera A. Crassulacean acid metabolism-cycling in *Euphorbia milii*. AoB Plants 2013; 103:645–653.

10 Ioannidis AS, Papageorgiou KI, Andreou PS. Exposure to *Euphorbia lathyris*latex resulting in alkaline chemical injury: a case report. J Med Case Reports 2009; 3:1752–1947.

11 Bassyouni M, Abu-Baker FA, Mahmoud SM, Moharam K, El-Nakkady MS. Synthesis and biological evaluation of some new triazolo[1,5-a]quinoline derivatives as anticancer and antimicrobial agents. RSC Adv 2014; 4:24131–24141.

12 Surveswaran S, Cai YZ, Corke H, Sun M. Systematic evaluation of natural phenolic antioxidants from133 Indian medicinal plants. Food Chem 2007; 102:938–953.

13 Abeer YI, Khaled M, Salwa ME. Screening of antioxidant and cytotoxicity activities of some plant extracts from Egyptian Flora. J Appl Sci Res 2011; 7:1246–1258.

14 Oktay M, Gülçin I, Küfrevio I. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensmittel-Wissenchaft Tech 2003; 36:263–271.

15 Brand-Williams W, Cuvelier ME, Berset C. Use of a free-radical method to evaluate antioxidant activity. Food SciTechnol 1995; 28:25–30.

16 Zhang FL, Luo YH, Wei XY, Wang N. Non-terpenoid constituents from the seeds of *Euphorbia lathyri*. J Trop Subtrop Bot 2009; 17:298–301.

17 Reitman S, Frankel S. Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am J Clin Path 1957; 28:56–63.

18 Belfield A, Goldberg. Colorimetric determination of alkaline phosphatase (Alp) activity enzymes. J Clin Chem Clin Biochem 1971; 12:561.

19 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248–254.

20 Buege JA, Aust SD. Microsomal lipid, peroxidation. In Flesicher S, Packer L, eds. Methods in enzymology. New York: Academic Press; 1978; 52:302–310.

21 Montgomery HAC, Dymock JF. The determination of nitrate in water. Analyst 1961; 86:414–416.

22 Aebi H. Catalase in vitro methods.Antioxidant effects of black rice extract through the induction of superoxide dismutase and catalase activities. Enzymology 1984; 105:121–126.

23 Moron MS, Depierre JW, Mannervik B. Level of glutathione, glutathione reductase and glutathone-S-transferase activities in rat lung and liver. Biochem Biophys Act 1979; 582:67–78.

24 Koracevic D, Koracevic G. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001; 54:356–361.

25 Habig W, Pabst M, Jakoby W. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249:7130–7139.

26 Zollner N, Kirsch KZ. Colorimetric method for determination of total lipids. J Exp Med 1962; 135:545–550.

27 Fassati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28–2077.

28 Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem 1973; 19–1350.

29 Burstein M. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Lipid Res 1970; 11:583–595.

30 Hirsch C, Zouain CS, Alves JB, Goes AM. Induction of protective immunity and modulation of granulaomatoushypersenstivity in mice using PIII, an anionic fraction of schistosomamansoni adult worm. Parasite 1997; 115:21–28.

31 Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinesemedicinal plants associated with anticancer. Life Sci 2004; 74:2157–2184.

32 Subhan N, Alam MA, Ahmed F, Awal MA, Nahar L, Sarkar SD. In vitro antioxidant property of the extract of *Excocaria agallocha* (Euphorbiaceae). DARU-J Faculty Pharm 2008; 16:149–154.

33 Kadri A, Gharsallah N, Damak M, Gdoura R. Chemical composition and in vitro antioxidant propertiesof essential oil of *Ricinus communis*L. J Med Plant Res 2011; 5:1466–1470.

34 Liu H, Qiu N, Ding H, Yao R. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. Food Res Int 2008; 41:363–370.

35 Xiao ZP, Peng ZY, Peng MJ, Yan WB, Ouyang YZ, Zhu HL. Flavonoids health benefits and their molecular mechanism. Mini-Rev Med Chem 2011; 11:169–177.

36 Ni QX, Xu GZ, Wang ZQ, Gao QX, Wang S, Zhang YZ. Seasonal variations of the antioxidant composition in ground bamboo sasa argentea striatus leaves. Int J Mol Sci 2012; 13:2249–2262.

37 Adom KK, Liu RH. Antioxidant activity of grains. J Agric Food Chem 2002; 50:6182–6187.

38 Szakiel A, Paczkowski C, Pensec F, Bertsch C. Fruit cuticular waxes as a source of biologically activetriterpenoids. Phytochem Rev 2012; 11:263–284.

39 Alexander SI, Konstantinos IP, Petros SA. Exposure to *Euphorbia lathyris* latex resulting in alkaline chemical injury: a case report. J Med Case Reports 2009; 3:1–3.

40 Escrig PV, Iglesias DJ, Corma A, Primo J, Primo-Millo E, Cabedo N. Euphorbia characiasas bioenergycrop: a study of variations in energy value components according to phenology and water status. J Agric Food Chem 2013; 61:10096–10109.

41 Liao SG, Zhan ZJ, Yang SP, Yue JM. Lathyranoic acid A: first secolathyranediterpenoid in nature from *Euphorbia lathyris*. Org Lett 2005; 7:1379–1382.

42 Itokawa H, Ichihara Y, Watanabe K, Takeya K. An antitumor principle from *Euphorbia lathyris*. Planta Med 1989; 55:271–272.

43 Hou XR, Wan LL, Zhan ZJ, Li CP, Shan WG. Analysis and determination of diterpenoidsinunprocessed and processed *Euphorbia lathyris* seeds by HPLC-ESI-MS. J Pharmaceut Biomed Anal 2011; 1:197–202.

44 Jiao W, Lu L, Deng MC, Shao HW, Lu RH. Study on chemical constituents in seeds of Euphorbia lathyris. Chin Traditional Herb Drugs 2010; 41:181–187.

45 Srivastava A, Greenspan P, Hartle DK, Hargrove JL, Amarowicz R, Pegg RB. Antioxidant andanti-inflammatory activities of polyphenolics from Southeastern US range blackberry cultivars. J Agric Food Chem 2010; 58:6102–6109.

46 Rehman S, Mahdi A, Hasan M. Tracemetal induced lipid peroxidation in biological system. SFRR (Indian) Bull 2003; 2:12–18.

47 Giao MS, Sanjose G, Muniz P, Perez R, Kosinska M, Pintado ME, Malcata FX. Protection of deoxyribose and DNA from degradationby using aqueous extracts of several wild plants. J Sci Food Agri 2008; 88:633–640.

48 Shyamala MP, Venukumar MR, Latha MS. Antioxidant potential of the syzygium Aromaticum (Gaert.) Linn. (Cloves) in rats fed withhigh fat diet. Indian J Pharma 2003; 35:99–103.

49 Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacol 2003; 57:145–155.

50 Hamilton CA, Miller WH, Al-Benna S, Brosnan MJ, Drummond RD, Mcbride MW, Dominiczak AF. Review srategies to reduce oxidative stress in cardiovascular disease. Clin Sci 2004; 106:219–234.

51 Deng R. Food and food supplements withhypocholesterolemic effects. Recent Pat Food Nutr Agric 2009; 1:15–24.

52 Esin E, Necat Y, Ozgur A. High density lipoprotein and it's dysfunction. Open Biochem J 2012; 6:78–93.

53 Vijayabaskar P, Sethupathy S, Somasundaram S. A comparative study on the atheroprotectivepotential of heparin and atorvastatin inhypercholesterolemicrats. AJBR 2008; 2:120–127.

54 Prado CM, Ramos SG, Elias J, Rossi M. Turbulent blood flow plays an essentiallocalizing role in the development of atherosclerotiClesions in experimentally inducedhypercholesterolaemia in rats. Inter J Exp Pathol 2008; 89:72–80.