Viper's Bugloss (*Echium vulgare L*) Extract as A Natural Antioxidant and Its Effect on Hyperlipidemia

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ABSTRACT

Echium vulgare which is known as viper's bugloss is a species of flowering plant in the borage family of Boraginaceae. In this study, *Echium vulgare* was examined considering its phenolic and flavonoid contents, and the antioxidant activity of methanolic extract was investigated by the method called 2, 2-diphenylpicrylhydrazyl radical scavenging (DPPH) activity. High performance liquid chromatography (HPLC) was utilized to estimate the phenolic acids and flavonoid compounds. The data reported that the *Echium vulgare* methanol extract is a good source of total phenolic compounds, total flavonoids content and the antioxidant activity. The phenolic acids from *Echium vulgare* extract were estimated using HPLC, and the highest compounds were gallic acid, benzoic acid and isoferulic acid. Flavonoid compounds were the highest compounds in quercetrin and naringin. The results of the biological experiments illustrated that the concentrations 250 and 500mg/kg body weight from *Echium vulgare* had contained the polyphenols in the extract to maintain an ideal body weight, improve the complete blood picture, lipid profile and liver functions as Alanine (ALT) and Aspartate (AST) transaminoferase. From the results of histopathology, it could be observed that in all the groups fed orally on *Echium vulgare* extract at 250 and 500 mg/kg no histopathological changes were observed in heart and liver except for the positive control. In conclusion, the results obviously illustrated that the *Echium vulgare* extract had contained high amounts of phenolic contents and flavonoid compounds which improve the blood parameters, lipid profile and liver functions and also histopathological changes in heart and liver.

Key Words: Echium Vulgare, Phenolic And Flavonoid, Lipid Profile, Liver Functions, Histopathological

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INTRODUCTION

Viper's bugloss blueweed, blue devil, blue-weed, viper's bugloss, blue thistle; vipérine commune, vipérine vulgaire, bouquet bleue, herbe aux vipères, langue d'oie, herbe bleue, herbe piquante, vipérine Fe+2 to Fe+3 and stimulated iron efflux were collected from the liver [1].The English names "salvation Jane" and "Patterson's curse" are probably best applied to E. plantagineum [2] . The family Boraginaceae covers about 120 genera with reported 200 species [3]. The genus Echium comprises about 40 species of annual, biennial herbs, mainly distributed in the Mediterranean region [4].

Viper's bugloss (*Echium vulgare L.*) is a biennial herb occurring in weed communities in Europe [5]. The aerial parts (known as Echii herba) contain olyphenols and pyrrolizidine alkaloids [6, 7], and they have also been used as a purgative in traditional remedies and veterinary practices since a long time ago [8]. Aqueous extract of this plant, along with Potentilla anserina, are used as teas [9].

The Viper's bugloss is used in traditional folk medicine to treat cracked hands and heal wounds. It has also been a major wild honey plant [6]. Various parts of Echium

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species (including herbs, petals, roots and root barks), containing the wound healing, demulcent, diuretic, sedative and antioxidant properties, have been applied to treat rheumatic pains [10, 11]. The *Echium vulgare* is reported to be applied externally to treat wounds in traditional Turkish folk medicine [12]. Scientific researches have reported antibacterial, antiinflammatory, antiproliferative, antidepressant, antioxidant, antiviral, anxiolytic and cytotoxic activities in various species of Echium [13].

Oxidative stress can be defined as an inequality between creation of reactive oxygen species and a biological system's capability to detoxify reactive inter-mediates. As one of the consequences of oxidative stress, a free radical generation may cause severe damage to cells, and is associated with many diseases such as cancer, cardio-vascular diseases, neurological disorders, and metabolic diseases [14]. The use of antioxidant supplementation is beneficial to prevent these diseases.

Antioxidant capacity can be assessed by two main mechanisms. The first main mechanism discards the free radicals, and the second mechanism reduces the metal ions such as iron, cupper, and chromium [15]. Antioxidants are added to foodstuffs to prevent undesirable deteriorations. However, synthetic antioxidants such as butylated hydroxylanisole, butylated hydroxytoluene, and propyl gallate may be quite unsafe because of their side effects and toxicity to non- target organs, which are of concern.

Oxidative stress is implicated as a factor in a wide range of the acute and chronic diseases, such as cancer, cardiovascular disorders and neurodegenerative conditions. The balance between antioxidation and oxidation must be maintained if a biological system is to be preserved in a healthy state [16, 17]. Medicinal plants contain high levels of natural antioxidants, such as phenolic acids, flavonoids and tannins, exhibiting potent antioxidant activities [18, 19].

Obesity prevalence is alarming globally and associated with the prevalence of chronic diseases such as cardiovascular diseases, diabetes mellitus, and cancer (colon, breast, and endometrial) [20-22]. The inhibition of human pancreatic lipase and amylase enzymes can be considered as a successful and relatively safe target for the obesity treatment by using orlistat and acarbose which are the only approved synthetic drugs [23]. Unfortunately, both drugs are accompanied by different side effects [24, 25]. Therefore, many effective alternative natural preparations with fewer side effects have been proposed for the treatment of obesity [26-28].

The aim of this investigation was to evaluate *Echium vulgare*'s leaves as a natural antioxidant and fractionation of phenolic contents and flavonoid compounds.

Biological experimentations were done to determine in hypercholesterolemic changes in separately fed orally on 250 and 500 mg/kg body weight of the extract. A complete blood picture, lipid profile, liver function, kidney function and pancreatic function were examined for four weeks.

MATERIALS AND METHODS

Materials:

Echium vulgare L. leaves were collected from Taif governorate, Saudi Arabia. The sample was grounded by household grinding machine.

The standard chemicals like phenolic acids and flavonoids compounds were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and the HPLC-grade solvents such as chloroform and methanol were purchased from Merck (Germany). Liquid paraffin, carbon tetrachloride and heparin sodium were purchased from Merck (Darmstadt, Germany).

Kits of liver functions and lipid parameters were obtained from Bicon Diagnosemittel GmbH and Co. KG Hecke 8 made in Germany.

Methods:

Preparations of methanol extract from *Echium vulgare* L. leaves

The *Echium vulgare* L. leaves were cut into small pieces and ground in grinding machine to fine powder, mixed with methanol (70%), and extracted for 24 h at 150 rpm at 25°C in a shaker. The resulting powder was packed in a glass bottle and stored at 4°C until needed.

Estimation of total phenolic acids and total flavonoids compounds

The Folin-Ciocalteu reagent was used to assay the phenolic content, based on Singleton's method which was slightly modified by [29]. Total phenolic content of the leaves was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE.g-1 DW) through the calibration curve with gallic acid.

Aluminum chloride method was used to determine the total flavonoid compounds [30]. The total flavonoid compounds were expressed in terms of quercetin equivalent (mg/g) [31].

DPPH Free Radical Scavenging Assay:

The stable 2,2-diphenyl-2-picrylhydrazyl (DPPH.) radical scavenging activity was determined by [32] with some modifications by [33]. The samples and references dissolved in ethanol (75%) were mixed with DPPH solution (1.5 ×10-4 M). Gallic acid and Quercetin were utilized as references.

Inhibition of DPPH in percent (%) was calculated as given below:

Inhibition % =A Control-A Sample /A Control × 100

Where

The control is the absorbance of the controlled reaction (containing all reagents except the test sample), and A Sample is the absorbance of the extracts/references.

Quantitative estimation of flavonoid compounds and phenolic acids by HPLC:

HPLC analyses were done using Dionex Ultimate 3000 liquid chromatography (Germany) including a diode array detector (DAD 3000) with 5 cm flow cell, a manual sample injection valve equipped with a 20 µl loop, and Chromeleon 6.8 system manager was used as the data processor. The separation was achieved by a reversed-phase Acclaim TM 120 C18 column (5 µm particle size, 4.6 x 250 mm) [34]. Moreover, phenolic acids were estimated by HPLC using the method of [35].

Biological experimental:

Female albino adult rats' (24 animals) weight ranged between (150-160g) fed on fat and basal diet consisted of 70 % corn starch, 10% casein, 10% corn seed oil, 4% salts mixture, 1% vitamins mixture, and 5% cellulose according to AOAC [36].

Experimental rats were fed on fat and basal diet for 15 days and randomly divided into four groups, six rats in each. The 1st main group was fed on basal diet for another 28 days and considered as the control negative rats. The 2nd main group was fed on basal diet plus 1% cholesterol and considered as the control positive.

The rats of 3rd and 4th groups were fed on basal diet and 1% cholesterol plus 250 and 500 mg /kg-1 body weight from *Echium vulgare* L. leaves' extract separately in normal saline of 5 ml/ kg-1 body weight, four times per week for four weeks.

At the end of the experiment, the blood samples were drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera afterwards, the sera were kept in a deep -freezer at -20°C until their analysis. The organs such as liver, heart, kidney and lung were immediately removed from the scarified rats, and they were gently pressed in the filter paper to free them from surface blood and weight.

Blood hemoglobin (Hb), Hematocrite (Ht) and platelets were determined including the whole blood sample by using the method described by [37]. Red blood cells (RBCs) and white blood cells (WBCs) were measured as recommended by [38]. Triglycerides, total lipids, total Cholesterol, HDL and (LDL) were determined according to the method of [39, 40].

The liver functions such as Alanine (ALT) and Aspartate (AST) transaminoferase were determined according to the method described by [41].

Pathological or histological examination of some organs:

The post-mortem examinations were done as soon as possible and the organ samples of the liver and heart were collected. Fixation was done in 10% dehydrated natural formalin, sectioned at 7µm, and stained with harries hematoxylin and eosin for histopathological examination [42].

Statistical analysis:

The obtained data were exposed to the analysis of variance. Duncan's multiple range tests at (P = 0.05) level were used to compare the means. The analysis was carried out using the PRO ANOVA procedure of Statistical Analysis System (SAS) [43].

RESULTS AND DISCUSSION

Evaluation of phytochemical extract of *Echium vulgare*

DPPH radical scavenging activities of *Echium vulgare* as compared with quercetine and gallic acid are shown in Table (1). DPPH assays of the methanol extract (70%) were 18.85, 27.02, 69.99 and 71.2%% in the 50, 100, 500 and 1000 µg/ml *Echium vulgare* extracts, while they were 48.95, 72.97, 90.38 and 92.61% in the quercetine. Meanwhile, gallic acid was 95.62, 96.29, 96.68 and 97.98%, respectively.

In the same table, it was observed that the *Echium vulgare* methanol extract (70%) had higher amounts of total phenolic compounds which was 16.82 mg GA/g, and total flavonoids content was 35.98 mg Quer_cetin /g. It could also be noticed that *Echium vulgare* methanol extract is a good source of total phenolic compounds, total flavonoids content and antioxidant activity.

Many medicinal plants had contained large amounts of flavonoids as antioxidants neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [44]. Flavonoids recently have received increasing attention because of some interesting new findings regarding their biological activities.

From pharmacological and therapeutic points of view, the antioxidants which interact well with free radicals scavenging and inhibiting the lipid peroxidation, are the most crucial [45]. However, the search for new plant-derived chemicals should thus be a priority in the current and future efforts towards sustainable conservation, and rational utilization of biodiversity [46].

Table 1. Total phenolic and flavonoid content of the *Echium vulgare* extract

Items	DPPH free radical scavenging activity			
	50µg/ml	100µg/ml	500µg/ml	1000µg/ml
Echium vulgare	18.85 ± 0.02	27.02 ± 0.06	69.99 ± 0.01	71.2 ± 0.005
Quercetine	48.95 ± 0.02	72.97 ± 0.08	90.38 ± 0.01	92.61 ± 0.01
Gallic acid	95.62 ± 0.01	96.29 ± 0.01	96.68 ± 0.01	97.98 ± 0.01
Total phenolic acids	16.82 ± 0.01 (mg GA/g)			
Flavonoid compounds	35.98 ± 0.03 (mg Quercetin /g)			

Quantitative HPLC estimation of phenolic and flavonoid contents:

HPLC was used to analyze 14 phenolic acids in methanolic extract from *Echium vulgare*, and the results are reported in Table (2). The phenolic acids from *Echium vulgare* extract were gallic acid, benzoic acid, isoferulic acid, chlorogenic acid, vanillic acid, catechol, salicylic acid, ferulic acid, catechin, P- hydroxy-benzoic acid, protocatechuic acid, alpha coumaric cid and p-coumaric acid. The antioxidative activity of phenolics contained in functional foods derives from the direct free radical scavenging activity, reducing activity and the indirect effect caused by the chelation of metal ions [47, 48].

The potential therapeutic attributes of numerous traditional medicinal plants may therefore largely be attributed to the phenolic compounds contained within them [49]. Some authors [49, 50] have demonstrated that the differences in the levels of the antioxidant activity may therefore be associated with the nature of the phenolic compounds involved, from phenolic acids to flavonoids, rather than with their contents [51].

For example, the radical scavenging properties of phenolic acids and their derivatives, such as esters, as well as flavonoids in plants may be derived from the number of hydroxyl groups the molecules contain [50, 52].

Table 2. Phenolic acid identified in *Echium vulgare* using HPLC analysis

Phenolic compounds	Concentration (mg/g)	Phenolic compound	Concentration (mg/g)
Gallic acid	10.85	Salicylic acid	0.44
Benzoic acid	5.23	Ferulic acid	0.43
Isoferulic acid	2.96	Catechin	0.29
Ellagic acid	1.38	P- hydroxy-benzoic	0.26
Chlorogenic acid	1.36	Protocatechuic acid	0.24
Vanillic acid	0.97	Alpha coumaric cid	0.17
Catechol	0.47	P-Coumaric acid	0.16

Flavonoid compounds were determined by HPLC, and the results are reported in Table (3). From the results, it could be noticed that the flavonoid compounds from *Echium vulgare* extract were quercetrin, naringin, rutin, hesperetin, hesperidin, naringenin, quercetin and apigenin which had amounts of 2.17, 1.92, 0.38, 0.37, 0.20, 0.069, 0.024 and 0.023 mg/g, respectively. Flavonoids have beneficial effects on human health with significant antioxidant amounts, and chelating properties in the human diet. Over the years, they have been found to be an important part of the human diet, and are considered to be active principles in some medicinal plants.

The antioxidant activity of flavonoids is efficient in trapping superoxide anion (O2·-), hydroxyl (OH·), peroxyl (ROO·) and alcohoxyl (RO·) radicals [53].

Table 3. Flavonoid content in *Echium vulgare* using HPLC analysis

Flavonoid compounds	Concentration (mg/g)
Quercetrin	2.17
Naringin	1.92
Rutin	0.38
Hesperetin	0.37
Hesperidin	0.20
Naringenin	0.069
Quercetin	0.024
Apigenin	0.023

Biological investigation:

The effect of different concentrations of *Echium vulgare* on the body weight in the rats

The data in Table (4) showed that the average body weight gain was 126 g in negative control, while it was 156.6 g for the positive control. The data present in Table (4) showed that, the body weight gain decreased to 91.8 g and 77.6 g as an average daily decrease, and to 1.53g and 1.29 g when fed on cooked black rice mill which is 10% and 20% compared with the positive control. These results illustrated that the concentrations of *Echium vulgare* which contained the polyphenols in the extract, maintain an ideal body weight, and also are considered to be efficient in the treatment of free radical related disorders.

Table 4. Effect of different diets on body weight in the rats

Groups	Body weight			
	Initial (g)	Final (g)	Gain (g)	Daily gain(g)
Negative control	153.2 ± 2.58^{a}	279.2 ± 4.43^{a}	126 ± 2.44^{b}	2.10 ± 0.04^{b}
Positive control	155.0 ± 2.70^{a}	312.0 ± 1.58^{d}	156.6 ± 2.70^{a}	2.61 ± 0.05^{a}
Echium vulgare 250 mg	153.8 ± 3.49^{a}	245.8 ± 1.92^{b}	91.8 ± 5.10^{b}	1.53 ± 0.08^{c}
Echium vulgare 500 mg	155.4 ± 2.6^{a}	233.0 ± 2.34^{c}	77.6 ± 4.04^{c}	1.29 ± 0.07^{d}

Effect of different concentration from *Echium vulgare* on complete blood picture in the rats:

The data present in Table (5), showed that the hemoglobin was higher in the group fed orally on *Echium vulgare* at 500 mg/kg (13.8 g/dl) compared with the negative and positive controls which was 12.7 and 11.8 g/dl in them. The results indicated that the hematocrit was lower in the positive control (which was 35.3%) than in the negative control (which was 38%), and it was increased in the group fed orally on *Echium vulgare* at 500 mg/kg (which was 41.4%). From the results, it could be found that the red blood cells were increased by feeding orally on *Echium vulgare* extract at 250 and 500 mg/kg (7.22 and 8.04 m/cm) whilst, in negative and positive controls they were increased by 6.97 and 6.45 m/cm, respectively. White blood cells were happened to increase in the positive control group by 10.4 cm, in the negative control by 5.67cm, and in the other groups fed orally on *Echium*

vulgare extract at 250 and 500 mg/kg by 8.33cm and 6.53 cm, respectively. Finally, it was found that the platelets were lower in the positive control (which were 588.3 cm) than in the negative control (which were 753.3 cm), and they were increased in orally fed on *Echium vulgare* extract at 250 and 500 mg/kg to 876.3 and 919.3 cm, respectively.

Table 5. The effect of different concentration from *Echium vulgare* on complete blood picture in the rats

Groups	Hemoglobin (g/dl)	Hematocrit (%)	Red blood Cells (m/ cm)	White blood cells (cm)	Platelets (cm)
Negative control	12.7 ±0.8^{ab}	38.0 ±2.4^{ab}	6.97 ±0.30^{ab}	5.67 ±0.93^{b}	753.3 ±37.8^{b}
Positive control	11.8 ±1.03^{b}	35.3 ±3.1^{b}	6.45 ±0.43^{b}	10.4 ±1.05^{a}	588.3 ±54.7^{c}
E. vulgare 250 mg	13.2 ±0.87^{ab}	39.5 ±2.6^{ab}	7.22 ±0.53^{ab}	8.33 ±2.85^{ab}	876.3 ±111^{ab}
E. vulgare 500 mg	13.8 ±0.79^{a}	41.4 ±2.4^{a}	8.04 ±1.02^{a}	6.53 ±1.47^{b}	919.3 ±67.3^{a}

The effect of different concentration from *Echium vulgare* on lipid profile in the rats:

The serum total lipid values of rats fed on different diets are presented in Table (6). The results showed that the serum total lipids in negative control were 0.65 g/dl. Also, the results in the same table showed a significant increase in serum total lipids in the positive control group that were 1.42 g/dl which were more than those in the negative control. It could be noticed that the lowest values of total serum lipids were found in the group fed orally on *Echium vulgare* extract at 250 and 500 mg/kg, which were 0.78 and 0.73 g/dl, less than the amounts in the negative and positive control.

The results shown in the same table illustrated that the serum triglycerides were the lowest in the negative control being 112.3 mg/dl. It could be noticed that, the significant increase in the positive control group was 245.7 mg/dl, which was less than the other groups during the experimental period. The data present in the same table also showed that the rats fed orally on *Echium vulgare* extract at 250 and 500 mg/kg had the lowest values of triglycerides; 141 and 118.7 mg/dl, respectively. These results are similar to those reported by [54] who found a high fat induced hepatic steatosis with significant increases in the serum levels of free fatty acids, triglyceride, total cholesterol, and insulin.

The total serum cholesterol of hyperlipidemic rats fed on the different diets during the experimental period is reported in Table (6). The data showed that the serum total cholesterol in the negative control was 86.3 mg/dl. It could be observed that the highest significant values of serum total cholesterol in the positive control was 196.3 mg/dl, which was more than that in the negative control.

The cholesterol in the rats fed orally on *Echium vulgare* extract at 250 and 500 mg/kg was 127 and 110.3 mg/dl, respectively; lower than that in the positive control. The results of the high density lipoprotein presented in Table (6) showed a significant increase in the positive control. The high density lipoprotein in rats fed on basal diet was 57.3 mg/dl at the end of the feeding period, which was 53.7 mg/dl in the negative control.

The high density lipoprotein was decreased in the groups fed orally on *Echium vulgare* extract at 250 and 500 mg/kg to 44 and 40 mg/dl, respectively; which was lower than that in the positive and negative controls. The results in the same table showed that the low density lipoprotein cholesterol level was lowered in the negative control to 25 mg/dl compared with the positive control group which was 131.7 mg/dl. The low density lipoprotein cholesterol decreased after feeding orally on *Echium vulgare* extract at 250 and 500 mg/kg, to 79.67 and 60.3 mg/dl, respectively that was lower than the positive control. These results are similar to the several studies which have demonstrated that the uniquely high level of poly phenols in *Echium vulgare* may play an important role in contributing to the health benefits such as lowering LDL cholesterol level, and reducing the risk of cardiovascular diseases [55, 56]. [57] reported the reduction in the accumulation of cholesterol in the aorta tissue and the reduction in the oxidation of LDL cholesterol.

Table 6. The effect of different concentration from *Echium vulgare* on lipid profile

Groups	T. Lipid (g/dl)	Triglycerides (mg/dl)	T. cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Negative control	0.65 ±0.03^{b}	112.3 ±6.1^{b}	86.3 ±1.1^{d}	53.7 ±10.0^{b}	25.0 ±5.56^{c}
Positive control	1.42 ±0.17^{a}	245.7 ±27.9^{a}	196.3 ±6.5^{a}	57.3 ±17.2^{a}	131.7 ±20.2^{a}
Echium Vulgare 250 mg	0.78 ±0.13^{b}	141.0 ±30.0^{b}	127.0 ±7.0^{b}	44.0 ±5.3^{c}	79.67 ±10.0^{b}
Echium vulgare 500 mg	0.73 ± 0.06^{b}	118.7 ±9.07^{b}	110.3 ± 3.5^{c}	40.0 ±3.0^{d}	60.3 ± 6.03 ^{b}

The effect of different concentration of *Echium vulgare* on the liver function:

The serum alanine (ALT) and aspartate (AST) transaminoferase values of rats fed on different diets under investigation during the experimental period are summarized in Table (7). The results showed that, alanine transaminoferase (ALT) activity in negative control group was 36.6 U/L, and also the same table showed a significant increase in serum alanine (ALT) transaminoferase activity in the positive control group which elevated gradually to reach its maximum level of 109U/L compared with the negative control.

It could be observed that in the groups which fed orally on *Echium vulgare* extract at 250 and 500 mg/kg, alanine transaminoferase reduced to 71 and 53 U/L. Moreover, the

aspartate transaminoferase (AST) was paralleled with alanine transaminoferase (ALT). These results agree with [58] who reported that alanine transaminoferase and aspartate transaminoferase activities in the serum were significantly stimulated by feeding on hypercholesterolemia diet.

Table 7. The effect of different concentrations of *Echium vulgare* on the liver function in the rats

Groups	ALT (U/L)	AST (U/L)
Negative Control	36.6 ±7.6^{d}	21.6 ±1.5^{c}
Positive Control	109 ±4.4^{a}	50.0 ±4.4^{a}
Echium vulgare 250 mg	71.0 ±2.6^{b}	34.0 ±4.6^{b}
Echium vulgare 500 mg	53.0 ±7.2^{c}	25.0 ±2.0^{c}

Histopathological experimental of Heart:

The results in Table (8) and fig (1) showed that the cross section in the heart wall showed normal orientation in the cardiac muscle with multinucleated fibers. The endocardium and epicardium appeared normal, covered with endothelium, and the purkinje appeared in the deep area of endocardium of the negative control. It was observed that the hyaline degeneration (+) "hyalinization" and granularition (+) in the wall of the heart "myocardium" appeared in the positive control. The cardic muscle showed a loss of structure, and was homogenous with pyknotic nucleus.

It could be observed that in the groups fed orally on *Echium vulgare* extract at 250 and 500 mg/kg no histopathological changes occurred, those changes were just observed in the positive control.

Table 8. Histopathological changes in heart of rats fed on different diets

Groups	Hyaline degeneration	Granularity
Negative Control	-	-
Positive Control	+	+
Echium vulgare 250 mg	-	-
Echium vulgare 500 mg	-	-

(+) changes (-) No changes

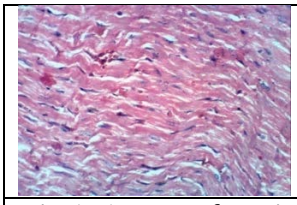

Fig. (1.1). Heart of rats in the negative control, untreated group showing normal cardiac myocytes (Hand E X 400).

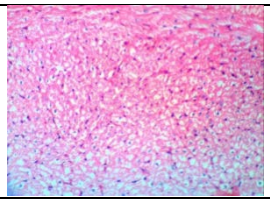

Fig. (1.2). Heart of rats in the positive control showing granularity of the sarcoplasm of Myocytes (Hand E X 400).

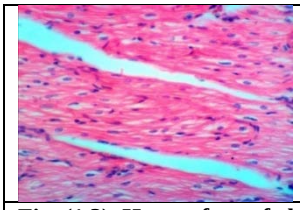

Fig. (1.3). Heart of rats fed orally on *Echium vulgare* extract at 250 mg/kg which showed no detected histopathological changes (Hand E X400).

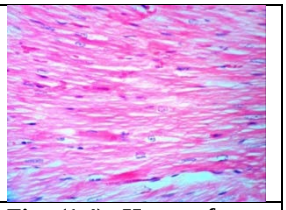

Fig. (1.4). Heart of rats fed orally on *Echium vulgare* extract at 500 mg/kg which showed no detected histopathological changes (Hand E X 400).

Fig. 1. Histopathological changes in heart of rats fed on different diets

Histopathological experimental of liver:

The results in Table (9) and fig (2) illustrated that the cross section in the liver showed the central vein surrounded by the hepatic cords consisting of hepatocytes contains the central nucleus. The liver contained the vacular degeneration (-), hydrobic degeneration (-) with lymphocytic infiltration. Large fat globules accumulated in the hepatcytes that showed fatty changes (-), Infiltration of inflammatory (-) and Thickening bile duct (-) of the negative control. The hepatocyte showed multiple changes including vacular (+), hydrobic (+) degeneration with lymphocytic in filtration. Large fat globules accumulated in the hepatcytes that showed fatty changes (+), Infiltration of inflammatory (+) and Thickening bile duct (+) of the positive control.

It could be observed that in the groups that have been fed orally on *Echium vulgare* extract at 250 and 500 mg/kg, no histopathological changes were observed except for the positive control.

Table 9. Histopathological changes in liver of rats fed on different diets

Groups	Hydrobic degeneration	Vacuolar degeneration	Fatty changes	Infiltration of inflammatory	Thickening bile duct
Negative Control	-	-	-	-	-
Positive Control	+	+	+	+	+
Echium vulgare 250 mg	-	-	-	-	-
Echium vulgare 500 mg	-	-	-	-	-

(+) changes (-) No changes

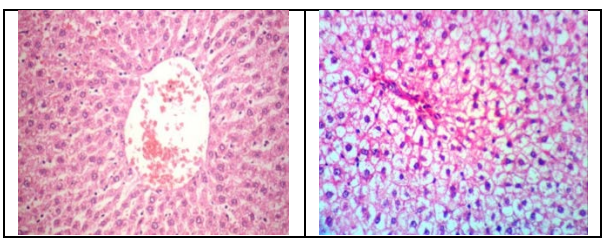

Fig. (2.1). Liver of rats from negative control group showing the normal histopathological structure of hepaticlobule (Hand E X 400).	**Fig. (2.2). Liver of rats from positive control group showing vacuolar degeneration of hepatocytes (Hand E X 400).**
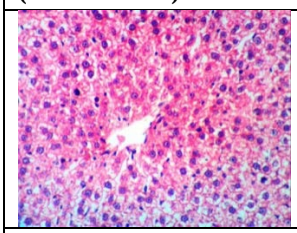	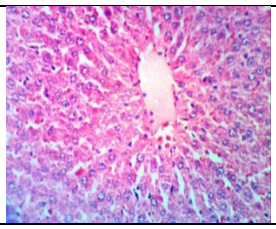
Fig. (2.3). Liver of rat from fed orally on *Echium vulgare* extract at 250 mg/kg group showed slight vascuolation of hepatocytes (Hand E X 400).	**Fig. (2.4). Liver of rat from fed orally on *Echium vulgare* extract at 500 mg/kg group showed no detected histopatholoical changes (Hand E X 400).**

Fig. 2. Histopathological changes in liver of rats fed on different diets

CONCLUSIONS

From the results, it could be concluded that in the rats fed on basal diet plus 1% cholesterol and fed orally of 250 and 500 mg/kg body weight of *Echium vulgare* extract, the risk of hyperlipidima was lower compared to the positive control fed on basal diet plus 1% cholesterol and the negative control fed on basal diet. Therefore, it could be recommended that the *Echium vulgare* extract is a good source of polyphenol as a natural antioxidant, and therefore beneficial for health.

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