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Determination the levels of total bilirubin, total protein and liver histopathological changes in hepatotoxic rats and the role of Beta vulgaris roots ethanolic extract

Shams Kareem Mahmood
Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq
Corresponding author email: shamskreem299@gmail.com

Salma Jameel Askar
Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq
Email: d.salma@covm.uobaghdad.edu.iq

***Abstract*---**The present study was done to investigate the therapeutic efficiency of *Beta vulgaris* roots ethanolic extract versus N-acetylcysteine (NAC) in overcoming experimentally induced hapatotoxicity in rats by given double maximum therapeutic oral dose of Acetaminophen (N-acetyl-para-aminophenol (APAP). The experiment was performed by two parts: First part was involved the extraction of *Beta vulgaris* roots by using 90% ethanolic solvent and identification the main phytochemical constituents of *Beta vulgaris* roots ethanolic extract by GC-MS spectrometry analysis , while the second part of experiment was involved the determination and understand the therapeutic effect of *Beta vulgaris* roots ethanolic extract on hepatotoxicity induced by Acetaminophen which administered more than the recommended dose in rats. Three treated groups of male albino rats (12 rats each) and the dosing regimen was performed as follows: Each group was dosed (APAP) twice daily of (100 mg/kg) for 14 days as follows dosing regimen: Positive control group: Rats were daily administered Acetaminophen at dose (100 mg/kg) orally twice a day as (50 mg/kg) in the morning and (50 mg/kg) at the evening for 14 days followed by two weeks recovery. T1 group: Rats were daily administered Acetaminophen at dose (100 mg/kg) orally twice a day as (50 mg/kg) in the morning and (50 mg/kg) at the evening for 14 days followed by two weeks recovery, while the ethanolic extract of *Beta vulgaris* roots was given once daily at a dose (500 mg/kg) orally after 30 minutes post-APAP morning dose for 14 and 28 days. T2

group: Rats were daily administered Acetaminophen at dose (100 mg/kg) orally twice a day as (50 mg/kg) in the morning and (50 mg/kg) at the evening for 14 days followed by two weeks recovery, while the N-acetylcysteine (NAC) was given once daily at a dose (70 mg/kgRats were daily administered distilled water orally for 14 and 28 days. Blood was collected from animals after 14 and 28 days in order to study the parameters of Serum Total Bilirubin (STB) and Serum Total Protein as well as the examination of histopathological changes in liver sections. The results of GC-MS analysis showed the presence of 13 bioactive phytochemical constituents in *Beta vulgaris* roots ethanolic extract which include: Trichlooacetic acid undec-10-enyl ester , Hexadecanoic acid methyl ester , Hexadecanoic acid ethyl ester , 9-Octadecenoic acid (z)- methyl ester , Phytol , i-Propyl 9,12,15-octadecatrienoate , Octadecanoic acid 17-methyl ester, 7-Hexadecenoic acid methyl ester (z) , Diisooctyl adipate , Docosanoic acid (z)-methyl ester , dl-alpha-Tocopherol , Vitamin E and Squalene. The results of hepatotoxicity which was induced after 14 days post-APAP administration and even after 2 weeks of recovery revealed significant increase the levels of Serum Total Bilirubin at ($p<0.05$) and significant decrease the levels of Serum Total Protein at both periods in comparison with negative control group. The treatment of APAP-induced hepatotoxicity with ethanolic extract of *Beta vulgaris* roots caused a significant decline in Serum Total Bilirubin at ($p<0.05$) and significant increase the levels of Serum Total Protein at ($p<0.05$) in comparison with positive control group at both periods. Also the hepatotoxic effects of APAP was overcome by treatment with NAC by causing a significant decline in Serum Total Bilirubin and significant increase the levels of Serum Total Protein at ($p<0.05$) in comparison with positive control group in both periods. The results of histological sections of liver in positive control group after 14 days of treatments showed sever hepatitis with marked disarrangement of hepatic cords with sever atrophy , damage , dilation of the central vein , peri-vascular hepatic necrosis with infiltration of mononuclear cells (MNCs) while after 28 days of treatment the sections of liver in positive control group showed mild hepatitis with marked sinusoidal dilation , mild infiltration of (MNCs) & degenerated of hepatocytes. The histological sections of liver in (T1) groups that treated with acetaminophen and *Beta vulgaris* roots ethanolic extract after 14 & 28 days of treatments showed normal appearance of central vein, hepatocytes , sinusoids and kupffer cells. The sections of liver in (T2) group that treated with acetaminophen and NAC after 14 & 28 days of treatments showed mild hepatitis with marked mild sinusoidal congestion, mild infiltration of neutrophils, lymphocytes, apoptotic cells occur within normal hepatocytes and mild vacuolar degeneration of hepatocytes. It can be concluded that the administration of *Beta vulgaris* roots ethanolic extract in hepatotoxic rats has been exhibited hepatoprotective and therapeutic effects against Acetaminophen-induced hepatotoxicity by reducing the `harmful effects of hepatotoxicity.

***Keywords*---**Beta vulgaris, roots extract, GC-MS.

Introduction

Drug induced hepatotoxicity is an important condition. It has become the leading cause of liver failure which accounting for 20-40 % of cases for liver transplantation in US (1). These drugs affect liver cells by different mechanisms like oxidative stress, fatty acid peroxidation, fat accumulation, antibody mediated cyto-toxicity and apoptosis (2). Paracetamol known as acetaminophen or N-acetyl – para- aminophenol (APAP) is one of the most commonly used oral analgesics and antipyretics described in the 1960 in the USA (3). It has an excellent safety profile when administered in therapeutic doses but excessive use causes paracetamol poisoning and liver damages (4). In the United States and the United Kingdom, paracetamol is the most common cause of acute liver failure (5 & 6). The toxic dose of paracetamol is highly variable, in general the recommended maximum daily dose for healthy adults is 4 grams (7 & 8). In the first 24 hours following overdose, usually 7g per day, patients have few or nonspecific symptoms like abdominal pain or nausea, yellowish skin, blood clotting problems, and confusion occurs (9). Paracetamol poisoning is the most commonly cause of acute liver failure which results not from paracetamol itself but from its metabolite N acetyl–*p*-benzoquinone imine (NAPQI) which decreases the liver's glutathione and directly damages hepatocytes (10). Metabolism essentially happens across glucuronidation and sulfuration, both of these pathways occur in the liver. These pathways get saturated in an overdose, more acetaminophen is changed by Cytochrome P450 to N-acetyl –p-benzoquinone imine (NAPQI). The NAPQI level rises and binds to macromolecules in the liver and then causes hepatic necrosis (11). Many medicinal plants have been evaluated for their protective effect against drug induced toxicities. *Beta vulgaris* is the plant belongs to Amaranthaceae family. The leaves are tonic , diuretic , anti-inflammatory which are useful in diseases of spleen and liver (12) while the roots of *Beta vulgaris* have long been used in traditional Arab medicine to treat a variety of diseases. The therapeutic uses of *Beta vulgaris* include antitumor , carminative , emmenagogue , hemostatic, renal protective and is a potential medicinal plant used in cardiovascular conditions (13). The phytochemical analysis of leaves extract have revealed presence of various constituents such as sterols , triterpenoids , tannins , flavonoids , alkaloids , glycosides and saponins (12 & 14). In recent years , beetroot has become the naturaly food to boost the energy in athletes (15 & 16).

Materials and Methods

Animals

This study was performed under the guidelines supervision of Ethical Committee for lab. Animals work in the College of Veterinary Medicine, University of Baghdad. Forty eight Swiss albino male rats about three months of age with body weight ranged between 200-230 gm were used to perform the experiment of the present study. Rats were housed in plastic cages of 20×50 ×75 cm dimensions, placed in a special housing room belongs to the Department of Physiology,

Biochemistry and Pharmacology / College of Veterinary Medicine /University of Baghdad for two weeks for acclimatization. Standard rodent diet (Commercial feed pellets) and tap water were freely available. Housing condition were maintained at 20-25 ^{o}C in air-conditioned room, the air of the room was changed continuously by using ventilation vacuum, while the light/ dark cycle was 12/12 h. in housing place.

Plant Material

The fresh *Beta vulgaris* roots were collected from a local market in Baghdad, Iraq.They were cleaned from soils and dust and washed with tap water. The plant roots were identified and classified by Ministry of Agriculture / State Board for seed Testing and certification (S.B.S.T.C.) in Abu-Graib, Baghdad , Iraq.

Extraction of *Beta vulgaris* Roots

Beta vulgaris 1 kg cut into small pieces were exhaustively macerated by soaking in 90% (1.5 L.) of 90% ethanol and the process repeated for three successive days. The obtained alcoholic extract was then concentrated under reduced pressure using rotatory evaporator till complete drying (17).

Gas Chromatograph - Mass Spectrometer (GC-MS) Analysis

Instrument and Chromatographic Conditions

This process was conducted at Ibn Al-Bitar Center, Ministry of Industry & Minerals, Iraq. The gas chromatography–mass spectrometry (GC– MS) coupled to mass spectrometry detector, Agelint Technology (7820A), USA which was performed with its instruments that include : The analytical column, Agelint HP-5ms Ultra Ineit (30 m length × 250 µm inner diameter × 0.25 µm film thickness). Helium 99.99% was used as carrier gas, and the flow rate was 1 ml/min with an injection volume of 1.0 µl and with pressure 11.933 psi. The GC Inlet Line Temperature is 250 ^{0}C , while Aux heaters Temperature is 300 ^{0}C. The Injector Temperature is 250 ^{0}C Scan Range : m/z 25-1000 and the Injection Type is Splitless. The The GC oven temperature program gradient was as follows: Ram 1 is 60 ^{0}C hold for 3 min initially, Ram 2 is 60 ^{0}C to 180^{0}C increased 7 ^{0}C/ min, Ram 3 is 180 to 280 ^{0}C increased 8 ^{0}C/min ,while Ram 4 is 280 ^{0}C hold to 3 min. GC was used in conjunction with the Thermo TSQ 800 triple quadrupole MS, figure (2.1).

Figure (2.1): GC-MS Spectrometry

Identification of Phytochemical Constituents By GC-MS

The mass spectrum of each separated phytochemical on specific retention time was compared with the mass spectrum stored in the database of the National Institute Standard and Technology (NIST14). The name of each phytochemical with its relative quantification of the compounds present in each sample which was obtained from the relative area of the peaks in the chromatograms and the nature of each compound were recorded and tabulated. Pharmacological activity for each identified compound was obtained with an exhaustive search in scientific publications. The identification of (13) components as the principal phytochemical components were recorded and tabulated with their nature and pharmacological activities.

Preparation of *Beta vulgaris* Roots Ethanolic Extract Solution

Solution of *Beta vulgaris* roots ethanolic extract was prepared by dissolving 5000 mg (5 g.) from dried extract with distilled water and completed the volume to (20 ml) to get a concentration of (250 mg/ml) which was given orally to each rat at a dose volume of 0.2 ml/100g (17).

Acetaminophen Solution: 2.6 Preparation of

The selected dose of the paracetamol that has been used for inducing hepatotoxicity in rats in the current study was (100 mg/kg B.W). This dose was chosen from the reported human hepatotoxic dose according to (18), who represented 1/20 of the reported oral LD50 in the rat (19). To prepare adjustable concentration for such a dose, a tablet of paracetamol 500 mg was dissolved in 20 ml distilled water to prepare a concentration of 25 mg / ml. The selected dose was equally divided into 2 doses at a dose volume of 0.2 ml/100g B.W in the morning and 0.2 ml/100g B.W at the evening.

Preparation N-acetylcysteine (NAC) Solution

The selected dose of NAC that has been used in this study was 70 mg/kg B.W according to (20). To prepare such dose for administration in rats, a sachet contained 600 mg of NAC was dissolved in 17.14 ml distilled water to prepare concentration of NAC 35 mg/ml that was given at a dose volume of 0.2 ml/100g B.W.

Experimental Design

In the present study , 48 male albino rats were randomly and equally divided into 4 groups (12 rats each) as follows:

1. Negative control group: Rats were daily administered distilled water for 14 and 28 days.
2. Positive control group: Rats were daily administered acetaminophen at dose (100 mg/kg) orally twice a day as (50 mg/kg) in the morning and (50 mg/kg) at the evening for 14 days followed by two weeks recovery (18).

3. T1 group: Rats were daily administered (APAP) at dose (100 mg/kg) orally twice a day as (50 mg/kg) in the morning and (50 mg/kg) at the evening for 14 days followed by two weeks recovery and *Beta vulgaris* ethanolic extract was given at dose (500 mg/kg) orally) once daily after 30 minutes post acetaminophen morning dose which continued for 28 days (17).
4. T2 group: Rats were daily administered (APAP) at dose (100 mg/kg) orally twice a day as (50 mg/kg) in the morning and (50 mg/kg) at the evening for 14 days followed by two weeks recovery and reference N-Acetylcysteine was given at dose (70 mg/kg) orally) once daily after 30 minutes post acetaminophen morning dose which continued for 28 days (20).

Parameters Studied

After the end of 14 and 28 days of treatments of each group , the rats blood samples were collected via the heart puncture. Blood was kept in gel tubes and serum was isolated after centrifugation at a speed of 3000 (rpm) for 15 minutes , which were stored at (- 20 ^{o}C) until analysis for:

- Serum Total Bilirubin (STB).
- Serum Total protein (STP)
- Liver histopathological sections were examined from each group at the 14 and 28 days of treatments.

Statistical Analysis

The Statistical Analysis System – SAS (2012) program was used to detect the effect of difference factors in study parameters. Least significant differences – LSD test (Analysis of Variation- ANOVA) and T- test was used to significant compare between means (21).

Results

Identification Of Phytochemical Constituents By GC-MS Analysis

The analysis of ethanolic extract of *Beta vulgaris* roots by Chromatogram GC-MS showed the presence of (13) bioactive phytochemical compounds which are listed in (Fig. 3.1 and Table 3.1). The compounds that were identified include: Trichlooacetic acid undec-10-enyl ester , Hexadecanoic acid methyl ester , Hexadecanoic acid ethyl ester , 9-Octadecenoic acid (z)- methyl ester , Phytol , i-Propyl 9,12,15-octadecatrienoate , Octadecanoic acid 17-methyl ester, 7-Hexadecenoic acid methyl ester (z) , Diisooctyl adipate , Docosanoic acid (z)-methyl ester , dl-alpha-Tocopherol , Vitamin E and Squalene.

Figure 3.1: GC-MS Chromatogram of *Beta vulgaris* Roots Ethanolic Extract

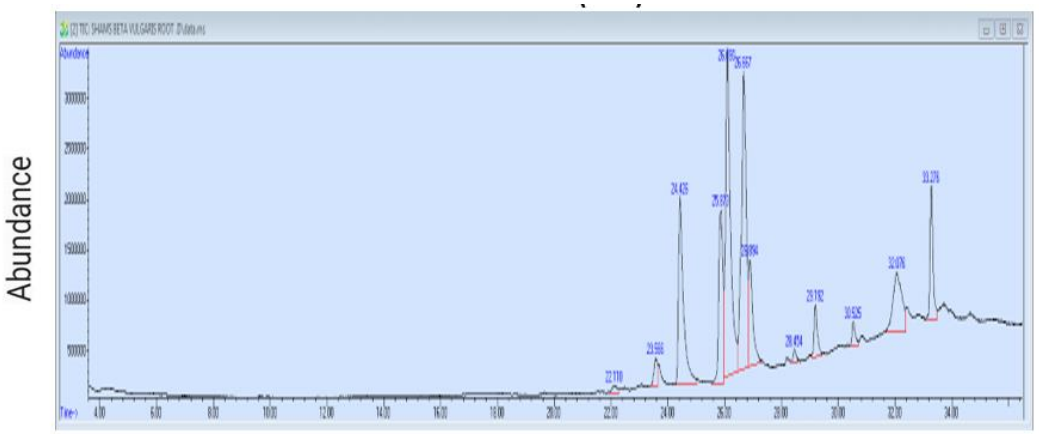

Table 3. 1: GC–MS Spectral Analysis of *Beta vulgaris* Roots Ethanolic Extract

No	Chemical Name	Chemical Nature	Peak Area (%)	Pharmacological Activities
1	Trichlooacetic acid undec-10-enyl ester	Kerolytic agent	0.56	Toxic agent , used for topical medication of warts and treat acne.
2	Hexadecanoic acid methyl ester	Fatty acid	1.52	Antioxidant & antifungal (Pinto et al., 2017).
3	Hexadecanoic acid ethyl ester	Fatty acid	14.21	Antioxidant (Ahmad et al., 2015 and Luo et al., 2017).
4	9-Octadecenoic acid (z)-methyl ester	fatty acid (oleic acid)	11.15	Antioxidant which preventing heart diseases , reducing cholesterol , inflammation and preventing cancer (Pinto et al., 2017).
5	Phytol	Diterpene alcohol	25.14	Antioxidant which preventing heart diseases , reducing cholesterol , inflammation and preventing cancer (Pinto et al., 2017).
6	i-Propyl 9,12,15-octadecatrienoate	Fatty acid	22.19	AntimicrobiaI Analgesic, antipyretic, anticonvulsant and antiseptic (Rahman et al., 2014).
7	Octadecanoic acid 17-methyl ester	Fatty acid	6.52	Antioxidant
8	9-Hexadecenoic acid	Fatty acid (oleic	0.56	Antioxidant (Pinto et al.,

	methyl ester (z)	acid		2017)
9	Diisooctyl adipate	Diester of isopropyl alcohol	2.74	Increase hepatic activities of peroxisome-associated enzymes (catalase & carnitine acetyl transferase) Liebert, 1984
10	Docosanoic acid (z)-methyl ester	Fatty acid	1.20	a protective barrier against the environment in order to maintain good skin quality. In skincare, behenic acid has lubricant, emollient, and soothing properties
11	dl-alpha-Tocopherol	Lipid-soluble vitamin	8.21	Antioxidant , vitamin E source , protect vitamin A and carotene in oils (Bartolini *et al.*, 2021).
12	Vitamin E	Lipid-soluble vitamin	8.21	Antioxidant , treat Vit. E deficiency that occur in diseases such as severe liver disease , termination lipid peroxidation and it is a major free radical scavenging antioxidant (Niki and Abe ,2019).
13	Squalene	Triterpene, polyunsaturated hydrocarbon	5.99	Antioxidant (Oxygen Scavenger) , antibacterial, antitumor, anticancer, immunostimulant and lipoxygenase inhibitor (Amarowicz, 2009)

Serum Total Bilirubin (STB) Concentration (mg/dl)

The results of (STB) levels listed in the table (4.2.4) showed a significant increase ($p \leq 0.05$) in positive control group than other induced treated groups and negative control one while the treatment with *Beta vulgaris* ethanolic extract or NAC recorded a significantly ($p \leq 0.05$) decrease in these levels in comparison with positive control group and significantly higher in treated groups in both treatment periods. However, no significant differences were recorded between periods in all experimental groups.

Table (4.2.4) The Effects of *Beta vulgaris* Ethanolic Extract on Serum Total Bilirubin Levels (mg/dl) in Hepatotoxic Rats induced By Acetaminophen

Groups	Mean ± SE	
	14 Days	28 Days
Negative control (Distilled water)	1.38 ± 0.16 D a	1.46 ± 0.18 D a
Positive control:	4.41 ± 0.24	4.51 ± 0.21

(APAP 100mg/kg)	A a	A a
T1 (APAP + *Beta vulgaris* ethanolic extract) (100mg/kg + 500 mg/kg)	2.41 ± 0.14 C a	2.58 ± 0.15 C a
T2 (APAP + NAC) (100mg/kg + 70 mg/kg)	3.08 ± 0.17 B a	3.25 ± 0.12 B a
LSD value	0.554*	0.504*

-Different capital letters mean significant differences * (P≤ 0.05) between groups.
-Different small letters mean significant difference (P≤ 0.05) within groups.
-The data expressed as M ± SE

Serum Total Protein (mg/dl)

Rats in positive control group showed a significant reduction (p ≤ 0.05) in the levels of total protein as compared with negative control group. Treatment with alcoholic extract of *Beta vulgaris* for a period of 14 and 28 days significantly serum total protein similar to that of NAC, table (3.3).

Table (3.3): The Effects of *Beta vulgaris* Ethanolic Extract on Serum Total Protein (mg/dl) Levels (mg/dl) in Hepatotoxic Rats induced By Acetaminophen

Groups	Mean ± SE	
	14 Days	28 Days
Negative control (Distilled water)	76.77± 1.72 A a	76.83± 1.81 A a
Positive control: (APAP 100mg/kg)	31.67± 0.95 B a	31.50 ± 1.41 B a
T1 (APAP + *Beta vulgaris* ethanolic extract) (100mg/kg + 500 mg/kg)	72.33± 1.87 A a	73.16± 2.71 A a
T2 (APAP + NAC) (100mg/kg + 70 mg/kg)	72.66± 2.24 A a	74.00± 2.76 A a
LSD value	5.204*	6.646*

-Different capital letters mean significant differences * (P≤ 0.05) between groups.
-Different small letters mean significant differences (P≤ 0.05) within groups.
-The data expressed as M ± SE

Histopathological Examination of Liver Sections

The liver tissue slides were examined microscopically for changes that may occur. The histological sections of liver in positive control group that given acetaminophen after 14 days of treatments showed sever hepatitis with marked disarrangement of hepatic cords with sever atrophy , damage & dilation of the central vein & peri-vascular hepatic necrosis with infiltration of mononuclear cells

(MNCs) figure (3.4.1), while after 28 days of treatment the sections of liver in positive control group showed shows mild hepatitis with marked sinusoidal dilation , mild infiltration of (MNCs) degenerated of hepatocytes , figure (3.4.2). The histological sections of liver in (T1) group that treated with acetaminophen and *Beta vulgaris* roots ethanolic extract after 14 days of treatments showed normal appearance of central vein, hepatocytes , sinusoids and kupffer cells , figure (3.4.3) while the section of liver in (T1) group after 28 days of treatment also showed normal appearance of central vein , hepatocytes , sinusoids and dilation of central vein with vacuolar degeneration of hepatocytes, figure (3.4.4). The sections of liver in (T2) group that treated with acetaminophen and NAC after 14 days of treatment showed mild hepatitis with marked mild sinusoidal congestion and mild infiltration of neutrophils , lymphocytes and apoptotic cells occur within normal hepatocytes and mild vacuolar degeneration of hepatocytes , figure (3.4.5) while the sections of liver in (T2) group after 28 days of treatment showed sinusoidal congestion , mild infiltration of lymphocyte , macrophages with vacular degeneration of hepatocytes , figure (3.4.6).

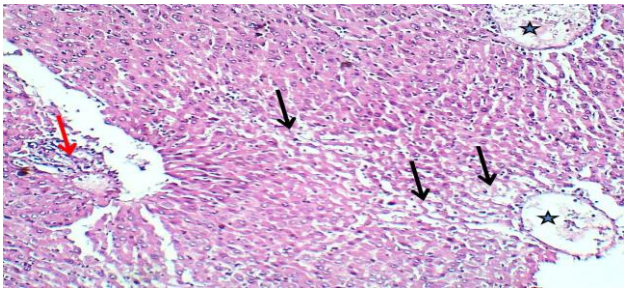

Figure (3. 4. 1) : Section of liver in positive control group that given Acetaminophen after 14 days shows: Sever hepatitis with marked disarrangement of hepatic cords , sever atrophy ( ) , damage , dilation of the central vein ( ) & perivascular hepatic necrosis with infiltration of MNCs ( ). H&E stain.40x.

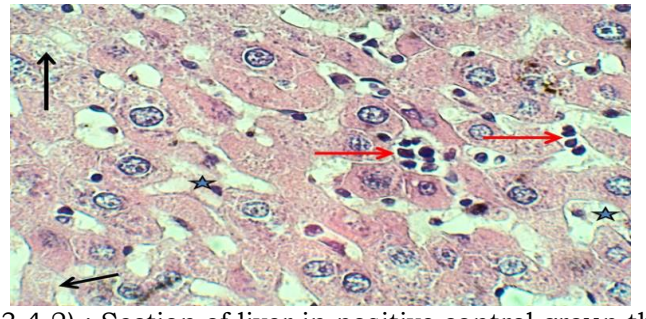

Figure (3.4.2) : Section of liver in positive control group that given Acetaminophen after 28 days shows: Mild hepatitis with marked sinusoidal dilation ( ),mild infiltration of MNCs ( ) & degenerated of hepatocytes ( ). H&E stain.40x.

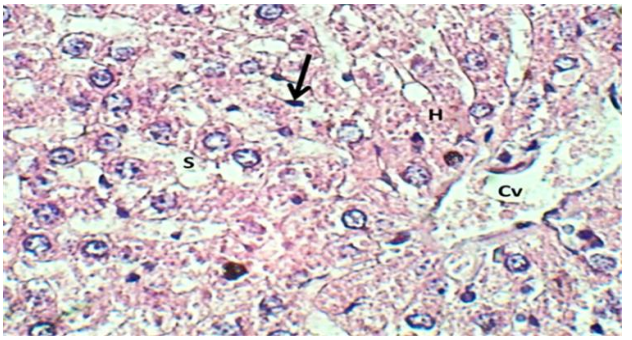

Figure (3.4.3) : Section of liver in T1 group that treated with Acetaminophen and *Beta vulgaris* roots ethanolic extract after 14 days shows: Normal appearance of central vein (Cv), hepatocyte (H), sinusoid (S) & kupffer cells ( ). H&E stain.40x

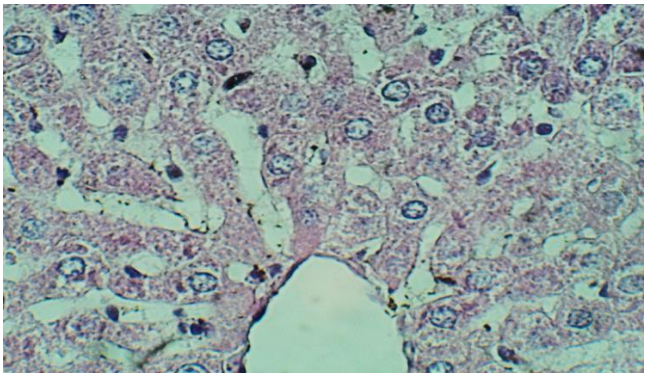

Figure (3.4.4) : Section of liver in T1 group that treated with Acetaminophen and *Beta vulgaris* roots ethanolic extract after 28 days shows: Normal appearance of central vein, hepatocytes & sinusoids. H&E stain.40x.

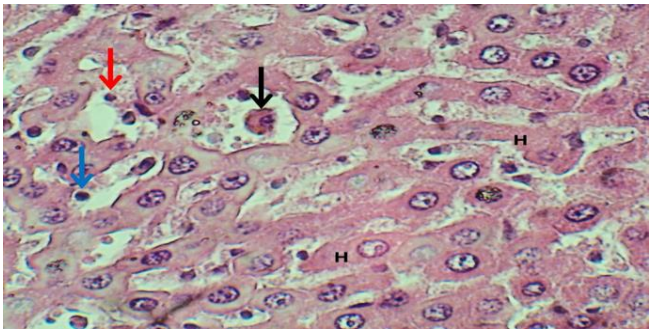

Figure (3.4.5) : Section of liver in T2 group that treated with Acetaminophen and NAC after14 days shows: Mild hepatitis with mild infiltration of neutrophils ( ) , lymphocytes ( ) , apoptotic cells ( ) within normal hepatocytes. H&E stain.40x.

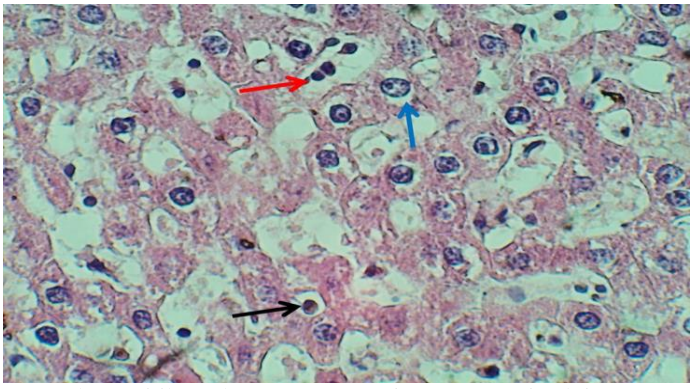

Figure (3.4.6) : Section of liver in T2 group that treated with Acetaminophen and NAC after 28 days shows: Mild infiltration of lymphocytes ( ) , macrophages ( ) and vacular degeneration ( ) . H&E stain.40x.

Discussions

The results of phytochemical analysis of *Beta vulgaris* roots ethanolic extract and their concentrations are similar to previous studies (22 and 23) , who found that the main components of red beet roots extract are polyphenols, alkaloids, tannins and flavonoids. These results are also in agreement with previous studies (24 and 25). Phenolic compounds have been identified as antioxidant agents that act as free radical oxidation terminators (26) while flavonoids are natural polyphenolic molecules which include flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones that have a number of nutritional functions and have been described as biological response modifiers. Most of them act as antioxidants and some have anti-inflammatory properties (27). In addition, the ethanolic extract of red beet roots contain a number of flavonoids compounds such as myricetin , neringenin , kaempferol and apigenin (24).

The presence of the secondary metabolites in red beet roots have contributed to its medicinal value as well as physiological activity (28) which they are used for therapeutic purposes to cure various diseases and to heal injuries. For instance flavonoids have been shown to have antioxidant which act as free radical scavenger and metal chelators (29) while alkaloids contribute to plant species fitness of survival and have pharmacological effects and are used as medication and recreational drugs (30). Moreover , (31) found presence of five phenolic acids (ferulic, vanillic, syringic, ellagic, and caffeic) and three flavonoids (quercetin, kampferol, and myricetin) in roots of *Beta vulgaris* by using Liquid chromatography–mass spectrometry which act as antioxidants , anti-inflammatory and inhibition of tumors proliferation.

The phenolics are ubiquitous secondary metabolites having wide therapeutic values like antioxidant, anti-carcinogenic and free radical scavenging activities. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups ,while flavonoids are a group of polyphenolic compounds that exhibit anti-hepatotoxic, anti-inflammatory activities. They inhibit enzymes such as aldose reductase and xanthine oxidase is capable of scavenging the ROS due to their

phenolics hydroxyl groups and potent antioxidants. The presence of flavonoids and phenolics that are reported in the *Beta vulgaris* ethanolic extract may reveal a positive correlation between phenolics content and antioxidant activity, suggesting phenolics and flavonoids might be the active phytochemicals in *Beta vulgaris* ethanolic extract (24).

Gas chromatography and mass spectrometry is an effective combination for phytochemical constituents analysis. The results of various phytochemicals that observed which listed and tabulated in recent study and chromatogram showed the presence of totally 13 phytochemicals which were identified in etanolic extract of *Beta vulgaris* rootsit. It is known that oleic acid is present in higher concentration and the remaining compounds in moderate amount only. and termination of lipid peroxidation (36 and 37) , Diisooctyl adipate, increase the hepatic activities of peroxisome-associated enzymes such as catalase & carnitine acetyl transferase (38) and Squalene which acting as antioxidant (oxygen scavenger) , antibacterial , antitumor , anticancer , immunostimulant and lipoxygenase inhibitor (39).

The most compounds that were identified by using GC-MS are fatty acids which act as antioxidants such as, Hexadecanoic acid methyl ester, Hexadecanoic acid ethyl ester, 9-Octadecenoic acid (z)- methyl ester, i-Propyl 9,12,15-octadecatrienoate, Octadecanoic acid 17-methyl ester, 9-Hexadecenoic acid methyl ester (z) and Docosanoic acid (z)-methyl ester. These active components were acting as antioxidants and antifungal as reported by (32 , 33 and 34) , antimicrobiaI , analgesic , antipyretic, anticonvulsant and antiseptic (35), while the phytol acting as antioxidant , preventing heart diseases , reducing cholesterol , anti - inflammation and preventing cancer (32) , dl-alpha-Tocopherol and Vitamin E (Lipid vitamins) acting as Antioxidant , treat vit. E deficiency that occur in diseases such as severe liver disease

Gas chromatography and mass spectrometry is an effective combination for phytochemical constituents analysis. The results of various phytochemicals that observed which listed and tabulated in recent study and chromatogram showed the presence of totally 13 phytochemicals which were identified in etanolic extract of *Beta vulgaris* rootsit. It is known that oleic acid is present in higher concentration and the remaining compounds in moderate amount only. The retention time starts at 22.110 and ends at 33.276. The most compounds that were identified by using GC-MS are fatty acids which act as antioxidants such as, Hexadecanoic acid methyl ester, Hexadecanoic acid ethyl ester, 9-Octadecenoic acid (z)- methyl ester, i-Propyl 9,12,15-octadecatrienoate, Octadecanoic acid 17-methyl ester, 9-Hexadecenoic acid methyl ester (z) and Docosanoic acid (z)-methyl ester. These active components were acting as antioxidants and antifungal as reported by (Pinto *et al.*, 2017, Ahmad *et al.*, 2015 and Luo *et al.*, 2017) , antimicrobiaI , analgesic , antipyretic, anticonvulsant and antiseptic (Rahman *et al.*, 2014), while the phytol acting as antioxidant , preventing heart diseases , reducing cholesterol , anti - inflammation and preventing cancer (Pinto *et al.*, 2017) , dl-alpha-Tocopherol and Vitamin E (Lipid vitamins) acting as Antioxidant , treat vit. E deficiency that occur in diseases such as severe liver disease and termination lipid peroxidation.

Bilirubin, the product of heme breakdown from red blood cells, is exclusively eliminated by the liver. Thus, circulating bilirubin is widely used as a diagnostic biomarker for liver function. In the setting of a clinical trial of a new drug candidate, elevations in serum bilirubin may also indicate severe liver injury with global hepatic dysfunction. Bilirubin is one of the main marker for liver function its increase was indicative of liver injury due to any insult both microbiological and chemical to the liver tissue (40). In the current study APAP treated group reported significant high level in the serum bilirubin at both periods, this indicated that there was liver damage due to paracetamol high dose and that the liver still not recovered after two weeks from the stoppage of the acetaminophen treatment, while best reduction in the levels recorded by *Beta vulgaris* ethanolic extract treatment which significantly different from that NAC group which caused statistical moderate reduction in comparison with APAP group and this was supported by the histopathological results in the liver sections at both periods (14 and 28 days) of treatments.

Previous studies by **(**41) reported mild hyper bilirubinemia results from the biochemical changes after high dose APAP administration. Moreover, the formation of superoxide anion and nitric oxide react together to produce peroxynitrite, which again exhibits hydroxyl radical-like activity causing hepatotoxicity which is contributed by hepatic macrophages via different mechanisms. Generally, bilirubin conjugates with glucoronic acid in the liver and is later excreted through bile. The presence of higher proportion of bilirubin in serum indicates that the process of conjugation is hampered, reflecting loss of hepatic function (42) .

Effect of NAC on serum total bilirubin in the current study, also reported in another study in which their results revealed that bilirubin declines in patients receiving NAC. Other results showed a significant decrease in total and direct bilirubin levels following NAC administration (43). While other researchers found declines in bilirubin levels and hepatic enzymes by administration of NAC alone, which improving parameters related to hepatocyte necrosis and bile excretion including ALT and bilirubin (44).

In this study, a significant decrease in the levels of total protein was observed. The decreased levels of total protein are due to increased production of free radicals, which initiate lipid peroxidation that leads to cellular damage. In the present study, Beetroots ethanolic extract administration possesses significant effect on Acetaminophen induced hepatotoxicity. The significant increase in protein after treatment with Beetroots ethanolic extract indicated that the hepatoprotective effect of the extract may be due to the presence of its chemical contents, such as phenols and flavonoids (45).

Furthermore, Beetroots ethanolic extract seems to preserve the structural integrity of the hepatocellular membrane in T1 group which indicated by increase level of total protein as compared with Acetaminophen positive control group which occur as Beetroots posses strong antioxidant contents as phenols , flavonoids and free radical scavenging properties. Thus, enzymatic scavenging of ROS could be achieved through the complex but elaborate coordination among the enzymes involved. The Higher levels of antioxidant enzymes enable the plant

extract to act as a very good free radical scavenger and help to prevent diseases caused by oxidative damage **(**46).

Moreover, the results indicated that the hepatotoxicity was not recovered after two weeks of stopping APAP dosing , while the treatment with *Beta vulgaris* roots ethanolic extract caused significant reduction in the effects of hepatotoxicity which indicated the recovery of hepatotoxic effects nearly better than that recorded by NAC treatment at both periods (induced and 28 days) of treatments. The recent findings in rats that given *Beta vulgaris* roots ethanolic extract along with paracetamol which reducing the serum levels of these parameters and be significantly nearly to normal levels which may be attributed to Beetroots mechanisms of action by antioxidant and anti-inflammatory properties and by enhance the energy production through nitrate (47). The present results were in agreement with previous study that used beetroots which emphasized on its role as supportive antioxidant and protective effect on liver tissue through assayed these enzymes within Acetaminophen induced stress (48).

It was reported that a toxic dose of paracetamol leads to the consumption of the stored glutathione (GSH) and sulfate. This converts the excess levels of paracetamol to the CYP - 450 oxidase system, which will lead to the formation of a more reactive intermediate (NAPQI) that will make bonds to protein macromolecules intracellularly leading to hepatic cells injury (49). This mechanism leads to the initiation of programmed cell death (apoptosis), which leads to hepatic necrosis and dysfunction (50 and 51*).*

The histopathological studies support the hepatoprotective role of Beetroots ethanolic extract and its ability to suppress the oxidative stress caused by Acetamonophen , this may be due to presence of active phytochemical constituents in the plant roots extract and these may offer antioxidant activity such as phenolics, flavonoids, Vit. E, Vit. C, Carotene, Thiamine and etc. (24).Our findings were supported by the results of histopathology which indicates clearly the lesions in the liver of APAP group observed in different sections at both periods (14 and 28 days) while the partial recovery was observed in the liver section of NAC group and nearly complete recovery in *Beta vulgaris* ethanolic extract in (T1) group after 14 & 28 days of treatment.

From all of these data, we conclude that the superiority of *Beta vulgaris* roots ethanolic extract as antioxidative effect over NAC in reversing APAP hepatotoxic effects which might be attributed to the presence of major metabolic constituents in *Beta vulgaris* roots ethanolic extract making them more able to regenerate and maintain the integrity of the cell membrane , cell organelles and biological finished. Also, the ability of the plant extract to regenerate GSH and important vitamins (C and E) which contribute as antioxidative stress effects making *Beta vulgaris* roots ethanolic extract a better antioxidant than NAC and possibly better candidate for therapy of hepatotoxicity that induced by APAP or possibly another hepatotoxic agents.

Conclusions

From the data of our study, we could conclude that the *Beta vulgaris* roots ethanolic extract has hepatoprotective and therapeutic effects against hepatotoxicity induced by Acetaminophen, due to decline the levels of STB and STP which confirmed by the results of liver histological sections which regenerate liver tissue to the normal structures by its activity as antioxidant. Our findings also suggest that the *Beta vulgaris* roots ethanolic extract could be used as a new potential drug in protecting against hepatotoxicity which cause harmful effects.

Recommendations

According to our result, we can suggest that ethanolic extract of *Beta vulgaris* roots is a possible antidote candidate for APAP hepatotoxicity and can be used in the research and development of hepatoprotective drugs of natural products which are inexpensive with high safety profile .

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