

How to Cite:

Mahmood, S. K., & Askar, S. J. (2022). Study the hepatoprotective effects and oxidant-antioxidant status of *Beta vulgaris* roots ethanolic extract in hepatotoxic rats induced by acetaminophen. *International Journal of Health Sciences*, 6(S5), 1385–1403. <https://doi.org/10.53730/ijhs.v6nS5.8975>

Study the hepatoprotective effects and oxidant-antioxidant status of *Beta vulgaris* roots ethanolic extract in hepatotoxic rats induced by acetaminophen

Shams Kareem Mahmood

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq
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 hepatotoxicity in rats by given double maximum therapeutic dose of Acetaminophen (N-acetyl-para-aminophenol (APAP) or paracetamol®) orally. The experiment was performed by two parts: First part was involved the extraction of *Beta vulgaris* roots by using 90% ethanolic solvent and detection the main phytochemical constituents of *Beta vulgaris* roots ethanolic extract and their concentrations% by using spectrophotometry 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 B.W.) 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/kg) orally after 30 minutes post-APAP morning dose for 14 and 28 days of treatment. Negative control group: Rats 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 liver functions parameters which included [serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase (ALP) and total serum bilirubin (TSB) as well as the oxidant and antioxidant status parameters, serum reduced glutathione (GSH) and serum malondialdehyde (MDA). The results showed the presence of the following phytochemical components: Total phenols 114.25%, total flavonoids 52.33%, total tannins 2.14%, total alkaloid 6.22%, total saponins 0.74%, total terpenoids 3.55%, total glycosides 4.11% and p-coumaric acid 21.59 (ppm) in ethanolic extract of *Beta vulgaris* roots. The results of hepatotoxicity which was induced after 14 days post-APAP administration and even after 2 weeks of recovery revealed significant increase at ($p < 0.05$) levels of serum AST, serum ALT, serum ALP and TSB, respectively at both periods of experiments 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 ALT, serum ALP and TSB, while serum AST showed normal value 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 ALT, serum ALP and TSB with normal serum AST in comparison with positive control group at both treatment periods. The hepatoprotective effects of both ethanolic extract of *Beta vulgaris* roots and NAC after APAP administration was quite clearly by the results of N-acetylcysteine treatment caused a significant decline in values of serum MDA and a significant increase in serum GSH in comparison with positive control group at both treatment periods. It can be concluded that the administration of *Beta vulgaris* ethanolic extract in Acetaminophen inducing hepatotoxicity rats has been exhibited hepatoprotective effects against Acetaminophen-induced hepatotoxicity by reducing the oxidative stress by acting as antioxidant agent and reduced the harmful effects of hepatotoxicity.

Keywords---*Beta vulgaris*, Roots extract, Acetaminophen, NAC.

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). It is harmful substance that glutathione safely reduces it to mercaptate and cysteine molecules that are not poisonous which are then eliminated by the kidneys. An overdose reduction the stores of glutathione, and once they are still fewer than 30% of normal, 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. For this purpose different extracts of different plants have been use. *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, and hemostatic and renal protective properties and is a potential medicinal plant used in cardiovascular conditions (13). Beetroot is known as a powerful antioxidant (14). The phytochemical studies of leaves extracts have revealed containing of various constituents such as sterols, triterpenoids, tannins , flavonoids , alkaloids , glycosides and saponins (15 & 12) . In recent years , beetroot has become the naturally food to boost the energy in athletes (16 & 17). Also the leaves of *Beta vulgaris* were recommended by the Father of Medicine “Hippocrates” for faster healing of wounds (18).

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 °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. The resulted extract was later suspended in distilled water and evaluated for treating hepatotoxicity (19).

Spectrophotometry Phytochemical Analysis:**Total Phenolic Content:**

The total amount of phenolic compound was determined in the ethanolic extract with a standard Folin- Ciocalteu reagent. The reaction mixture contained 100 µl of the extract and 500 µl of the Folin- Ciocalteu reagent (Merk, Germany) and 1.5 ml of 20% sodium carbonate. The sample was then mixed on a vortex mixer and diluted with distilled water to the final volume of 10 ml. After 2 h reaction, the absorbance at 765 nm was determined and used to estimate the phenolic content using the calibration curve made with gallic acid (Sigma-Aldrich, Germany). The total amount of phenolic compound was expressed in mg gallic acid equivalent (GAE) per g dry weight using a UV-visible spectrophotometer Shimadzu UV-1600, Japan (20).

Total Flavonoid Content:

The total flavonoid content was determined by the aluminum chloride colorimetric method. In brief, 50 µl of extract (1 mg/ml ethanol) were made up to 1 ml with methanol, mixed with 4 ml of distilled water and then 0.3 ml of 5% NaNO₂ solution, 0.3 ml of 10% AlCl₃ solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then 2 ml of 1 mol/L NaOH solution were added and the final volume of the mixture was brought to 10 ml with double-distilled water. The mixture was allowed to stand for 15 min and absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve and the result was expressed as mg rutin equivalent per g dry weight (21).

Total Tannins Content:

Two gm of the extract was mixed with water and ethanol (20 : 80) which heated on water bath. The mixture was filtered and ferric chloride was added to the

filtrate. A dark-green solution indicates the presence of tannins. One ml of extract was added to two ml of sodium chloride (2%), filtered and mixed with five ml of 1% gelatin solution. A precipitate indicates the presence of tannin and the measurement absorption at 540 nm (22).

Total Alkaloid Content:

Bromocresol green solution (BCG) was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2M sodium phosphate (71.6 gm Na₂HPO₄ in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 gm citric acid in 1 L distilled water). Atropine standard solution was made by dissolving 1 mg of pure Atropine (AR-grade procured from Sigma Company) in 10 ml distilled water. The extract was dissolved in 2N HCL and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform. Accurately measured aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of Atropine standard solution was transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform. The absorbance of the complex in chloroform was measured at spectrum of 470 nm against the blank prepared as above but without Atropine (23 & 24).

Total Terpenoid Content:

Take (1.5 gm) of extract and add (7 ml) mixture of methanol and acetonitrile which put on shaking system for 30 min , then leave the sample in dark place for 24 hour. Put the sample in centrifuge (6000 rpm) and take (5 ml) of it and add (1.5 ml) chloroform + (0.5 ml) concentrated H₂SO₄ and mix well for 1 min (if heating occur must cool the tube by putting it on snow for not exceed 5 min ,then add methanol to complete the volume to 10 ml. Prepare standard solution of (Linalool) in several concentrations and read them on spectrophotometer at 538 nm (25).

Total Glycoside Content:

Determination of glycosides were quantitatively determined according to (26). For determination of glycosides, a 10% extract which mixed with 80% methanol and left for 24 h in room temperature. After that take 10 ml of extract and add 5 ml of freshly prepared Baljet's reagent (95 mL of 1% picric acid + 5 ml of 10% NaOH). After an hour, the mixture was diluted with 20 ml distilled water and the absorbance was measured at 495 nm. For preparation of the standard curve, 10 ml of different concentrations (12.5-100 mg/L) of securidaside were prepared. Total glycosides from triple replicates were expressed as a percent (%).

Total Saponins Content:

The saponin content of the samples was determined by double extraction gravimetric method described by (27). A measured weight (5g) of the extract sample was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated in water bath for 90 minutes at 55°C; it was then filtered through Whatman filter paper (No 42). The residue was extracted with 50 ml of 20% ethanol and both extract were poured together and the combined extract was reduced to about 40 ml at 90°C and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Re extraction by partitioning was done repeatedly until the aqueous layer become clear in colour. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre- weighed evaporation dish. It was dried at 60°C in the oven and reweighed after cooling in a dessicator. The process was repeated two more times to get an average. Saponin content was determined by difference and calculated as a percentage of the original sample thus:

Percentage (%) of saponins = $(W2 - W1 / \text{Wt. of sample}) \times 100$

W1 = Weight of evaporating dish

W2 = Weight of evaporating dish + sample

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 rat B.W. (19).

Preparation of Acetaminophen Solution:

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 (28), who represented 1/20 of the reported oral LD50 in the rat (29). To prepare adjustable concentration for such a dose, a tablet of paracetamol 500mg 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 (30). To prepare such dose for administration in rats, a sachet contained 600mg 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 0.2ml/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 (28).
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 (19).
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 (30).

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 °C) until analysis for:

- Serum aspartateaminotransferase (AST).
- Serum alanineaminotransferase (ALT).
- Serum alkaline phosphatase (ALP).
- Serum Reduced Glutathione (GSH).
- Serum Malondialdehyde (MDA).

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 (31).

Results

Spectrophotometry Quantitative Phytochemical Analysis:

The *Beta vulgaris* roots were extracted by using 90% ethanolic solvent and the result of crude alcoholic extract after full dryness was dark-red. As illustrated in table (3.1) the phytochemical analysis of ethanolic extract of *Beta vulgaris* roots contain total phenolic (133.5 mg Gallic acid /100 g), total flavonoids (52.33 mg Rutin /100 g), total tannin (2.14 mg /100 g), total alkaloid (6.22 mg /100 g), total saponins (0.74 mg/100 g), total steroids (1.25 mg/100g), total terpenoid

(3.55 mg/100 g), total glycoside (4.11 mg/100 g) and P-Coumaric acid (21.59 ppm).

Table 3.1: Quantitative Phytochemical Analysis of *Beta vulgaris* Roots Ethanolic Extract:

No	Phytochemical constituents	identification	%
1	Total Phenolic content (mg Gallic acid/100g)	+	114.25
2	Total Flavonoids content (mg Rutin/100g)	+	52.33
3	Total Tannins content	+	2.14
4	Total Alkaloids content	+	6.22
5	Total Saponins content	+	0.74
6	Total Terpenoids content	+	3.55
7	Total Glycosides content	+	4.11
8	Total Coumarins content (ppm)	+	21.59

Serum Aspartate aminotransferase (AST) (U/L):

Table (3.2) listed the results of serum AST activities that showed significant increase ($P \leq 0.05$) in positive control group in comparison with other induced treated groups and negative control one. Interestingly, T1 and T2 groups recorded significant less activity in the levels of AST in comparison with APAP positive control group.

Table (3.2): Measurement of Serum Aspartate aminotransferase (U/L) of APAP induced hepatotoxicity in rats orally for 14 days with/without treatment with *Beta vulgaris* ethanolic extract or N-acetylcysteine for 28 days versus negative control group.

Groups	Mean \pm SE	
	14 Days	28 Days
Negative control (Distilled water)	122.33 \pm 4.02 B a	125.50 \pm 3.82 B a
Positive control: (APAP 100mg/kg)	142.00 \pm 1.26 A b	161.83 \pm 1.27 A a
T1 (APAP + <i>Beta vulgaris</i> ethanolic extract) (100mg/kg + 500 mg/kg)	125.00 \pm 1.93 B a	121.83 \pm 2.78 B a
T2 (APAP + NAC) (100mg/kg + 70 mg/kg)	125.83 \pm 2.04 B a	124.00 \pm 1.71 B a
LSD value	7.473*	7.650*

-Different capital letters mean significant differences * ($P \leq 0.05$) between groups.

-Different small letters mean significant differences ($P \leq 0.05$) within groups.

-The data expressed as M \pm SE

Serum Alanine aminotransferase (ALT) activity (U/L):

Table (3.3) showed the result of serum ALT activities in which APAP positive control group recorded a significant increase in the ALT ($P \leq 0.05$) in comparison with other induced treated groups and negative control one. Both T1 and T2 groups that treated with either *Beta vulgaris* ethanolic extract and NAC showed significantly less ALT level than positive group, but they are still significantly ($P \leq 0.05$) increase in comparison with negative control group.

Table (3.3): Measurement of Serum Alanine aminotransferase (U/L) of APAP induced hepatotoxicity in rats orally for 14 days with/without treatment with *Beta vulgaris* ethanolic extract or N-acetylcysteine for 28 days versus negative control group.

Groups	Mean \pm SE	
	14 Days	28 Days
Negative control (Distilled water)	70.50 \pm 2.84 C a	71.50 \pm 2.91 C a
Positive control: (APAP 100mg/kg)	138.00 \pm 2.47 A a	146.33 \pm 2.89 A a
T1 (APAP + <i>Beta vulgaris</i> ethanolic extract) (100mg/kg + 500 mg/kg)	110.00 \pm 4.14 B a	106.16 \pm 3.54 B a
T2 (APAP + NAC) (100mg/kg + 70 mg/kg)	109.66 \pm 3.45 B a	102.50 \pm 1.78 B a
LSD value	9.712*	8.426*

-Different capital letters mean significant differences * ($P \leq 0.05$) between groups.

-Different small letters mean significant differences ($P \leq 0.05$) within groups.

-The data expressed as M \pm SE

Serum Alkaline Phosphatase (ALP) activity (U/L):

The result of ALP activity listed in the table (3.4) showed significant increase in ALP levels in positive control group ($P \leq 0.05$) as compared with T1 or T2, as well as negative control one. Both groups T1 and T2 showed less significant levels of ALP in comparison with APAP positive control without significant differences between them at induced toxic period (14 days), while there were significant ($P \leq 0.05$) differences at (28 days) of treatment.

Table (3.4): Measurement of Serum Alkaline Phosphatase (ALP) activity (U/L) of APAP induced hepatotoxicity in rats orally for 14 days with/without treatment with *Beta vulgaris* ethanolic extract or N-acetylcysteine for 28 days versus negative control group.

Groups	Mean \pm SE	
	14 Days	28 Days
Negative control	80.67 \pm 1.58	77.33 \pm 2.17

(Distilled water)	C a	D a
Positive control: (APAP 100mg/kg)	140.00 ± 1.81 A b	157.00 ± 1.59 A a
T1 (APAP + <i>Beta vulgaris</i> ethanolic extract) (100mg/kg + 500 mg/kg)	103.33 ± 5.01 B a	101.67 ± 5.40 C a
T2 (APAP + NAC) (100mg/kg + 70 mg/kg)	102.33 ± 2.51 B a	112.00 ± 2.11 B a
LSD value	8.988*	9.431*

-Different capital letters mean significant differences * ($P \leq 0.05$) between groups.

-Different small letters mean significant differences ($P \leq 0.05$) within groups.

-The data expressed as $M \pm SE$

Serum Reduced Glutathione ($\mu\text{mol/L}$): 3.5

The results of Serum Reduced Glutathione (GSH) levels listed in the table (3.5) showed a significant decrease ($p \leq 0.05$) in APAP positive control group than other induced treated groups and negative control group, while in T1 group recorded significantly ($p \leq 0.05$) higher level in serum GSH which showed nearly normal level of negative control group in comparison with positive control and T2 treated groups.

Table (3.5) Measurement of Serum Reduced Glutathione ($\mu\text{mol/L}$) of APAP induced hepatotoxicity in rats orally for 14 days with/without treatment with *Beta vulgaris* ethanolic extract or N-acetylcysteine for 14 and 28 days.

Groups	Mean \pm SE	
	14 Days	28 Days
Negative control (Distilled water)	4.09 \pm 0.08 A a	4.08 \pm 0.08 A a
Positive control: (APAP 100mg/kg)	2.11 \pm 0.07 C a	2.13 \pm 0.04 C a
T1 (APAP + <i>Beta vulgaris</i> ethanolic extract) (100mg/kg + 500 mg/kg)	4.16 \pm 0.12 A a	4.11 \pm 0.12 A a
T2 (APAP + NAC) (100mg/kg + 70 mg/kg)	3.01 \pm 0.24 B a	3.15 \pm 0.23 B a
LSD value	0.438*	0.414*

-Different capital letters mean significant differences * ($P \leq 0.05$) between groups.

-Different small letters mean significant difference ($P \leq 0.05$) within groups.

-The data expressed as $M \pm SE$

Serum Malondialdehyde ($\mu\text{mol/L}$):

Table (3.6) showed the result of serum (MDA) levels in which APAP positive control group recorded a significantly higher increase ($P \leq 0.05$) in comparison with other induced treated groups (T1 and T2) and negative control. Both T1 and T2 groups showed significantly lesser levels than APAP positive control group.

Table (3.6) Measurement of serum Malondialdehyde ($\mu\text{mol/L}$) of APAP induced hepatotoxicity in rats orally for 14 days with/without treatment with *Beta vulgaris* ethanolic extract or N-acetylcysteine for 14 and 28 days.

Groups	Mean \pm SE	
	14 Days	28 Days
Negative control (Distilled water)	5.45 \pm 0.19 BC a	5.58 \pm 0.14 B a
Positive control: (APAP 100mg/kg)	12.38 \pm 0.17 A a	14.38 \pm 0.94 A a
T1 (APAP + <i>Beta vulgaris</i> ethanolic extract) (100mg/kg + 500 mg/kg)	5.35 \pm 0.16 B a	5.61 \pm 0.13 B a
T2 (APAP + NAC) (100mg/kg + 70 mg/kg)	6.50 \pm 0.14 B a	6.10 \pm 0.16 B a
LSD value	1.135*	1.450*

-Different capital letters mean significant differences * ($P \leq 0.05$) between groups.

-Different small letters mean significant difference ($P \leq 0.05$) within groups.

- The data expressed as $M \pm SE$

Discussions

The results of ethanolic extract of *Beta vulgaris* roots and quantitative phytochemical analysis are similar to previous studies of (32 & 33), who found that the main components of red beet root extract are polyphenols, alkaloids, tannins and flavonoids. These results are also in agreement with previous studies (34 & 35). Phenolic compounds have been identified as antioxidant agents that act as free radical oxidation terminators (36) while flavonoids are natural polyphenolic molecules which include flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones which have number of nutritional functions and have been described as biological response modifiers, which act as antioxidants and some have anti-inflammatory properties. The presence of the secondary metabolites in red beet root has contributed to its medicinal value as well as physiological activity (37) 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 (38) while alkaloids contribute to plant species fitness of survival and have pharmacological effects and are used as medication and recreational drugs (39). In addition, the ethanolic extract of red beet root contains a number of flavonoids compounds such as myricetin, neringenin, kaempferol and apigenin as reported by (40).

Moreover, (41) found the presence of five phenolic acids (ferulic, vanillic, syringic, ellagic, and caffeic), three flavonoids (quercetin, kampferol, and myricetin) for roots of red beet by using Liquid chromatography–mass spectrometry which act as antioxidation, antiinflammation and inhibition of tumor proliferation.

For evaluation of the hepatic injury, the rise of hepatic enzymes (AST, ALT, and ALP) is commonly used as a marker for hepatocellular necrosis. So, the serum levels of these enzymes are used as quantitative markers of the type and the degree of injury in hepatic cells (42). Also previous studies indicated the role of free radicals which produce from the oxidative stress are the main factors to liver injury (43 & 44).

In the present study, the results of enzymes (AST, ALT and ALP) were clearly indicated the hepatotoxic effect of the APAP at the dose used since there were a significant increase in all of these hepatic enzymes in APAP group at both periods (14 and 28 days). Elevated liver enzymes (AST, ALT and ALP) in positive control group with liver damage and cell necrosis were also reported in other studies which attributed to the accumulation of APAP toxic metabolite, namely NAPQI in liver tissues causing oxidative stress effects (45). 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 (46). This mechanism leads to the initiation of programmed cell death (apoptosis), which leads to hepatic necrosis and dysfunction in the form of elevation of AST, ALT and ALP (47).

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 levels of these serum enzymes which indicated the recovery of hepatotoxic effect nearly better than that recorded by NAC treatment at both periods (induced and 28 days) of treatments. Another study indicated that NAC could decrease AST, ALT and ALP levels and reduce hepatocellular injury due to its antioxidant effect (48), while the results of study by (49) also reported the reduced effects of NAC on the levels of serum AST, ALT and ALP.

In the present study the treatment of rats with Beetroots ethanolic extract along with paracetamol results in reducing the serum levels of AST, ALT and ALP significantly nearly to normal levels in rats. On the other hand, in human that taking beetroot juice showed decrease in the serum levels of these enzymes (50). Beetroot mechanisms of action may be attributed to its antioxidant and anti-inflammatory properties and by enhances energy production through nitrate (51). 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 (52).

The reduction of AST and ALT activities by the extracts of Beetroot is an indication of repair of hepatic tissue damage induced by paracetamol and

increases the hepatoprotective activity against the toxic effect of paracetamol. Our findings can be attributed to the effect of Beetroot ethanolic extract which may cause stabilized cell membrane and protect the liver against the deleterious agents and free radical mediated toxic damages to the liver cells, this is may be reflected in the reduction of liver enzymes levels. On the other hand, the extract helps the liver to maintain its normal function by accelerating the regeneration capacity of its cells. The highly hepatoprotective effects of Beetroot ethanolic extract may be attributed to its active ingredients such as nitrate, betalains, phenolics and ascorbic acid , for example betalains are important in inflammation and oxidative stress beside phenolics and ascorbic acid (53).

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 (54). The phenolic compounds 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 (34).

Glutathione (GSH) is well known low molecular weight tripeptide is perhaps the most famous natural endogenous antioxidant (55). Antioxidant might act as a scavenger of electrophilic and oxidant species either in a direct way or through enzymatic catalysis: (1) it directly quenches reactive hydroxyl free radicals, other oxygen-centered free radicals and radical centers on DNA as well as on other biomolecules such as methylglyoxal and 4-hydroxynonenal and (2) GSH is the cosubstrate of glutathione peroxidase, permitting the reduction of peroxides (hydrogen and lipid peroxides) and producing oxidized/disulfide glutathione (GSSG). In turn, GSSG is reduced to 2 GSH by using NADPH reducing equivalents and glutathione disulfide reductase, catalysis. Electrophilic endogenous compounds and xenobiotics (drugs, pollutants, and their phase I metabolites) are conjugated with GSH through activation by glutathione-S-transferases (56). The resulting conjugates are substrates of GGT, which initiates the mercapturic acid pathway and facilitates toxic elimination (55).

In the present study the decreased levels of reduced glutathione (GSH) in positive control group nearly to the half at both periods indicating a clear depletion of glutathione antioxidant concentration. In NAC group the level showed a significant rise in glutathione concentration in comparison with APAP group but not as that of the control one while in *Beta vulgaris* roots ethanolic extract treated group the increased in glutathione concentration reaches the same levels as that in control at both periods.

At therapeutic doses, about 90% of APAP is eliminated via sulfuration or glucuronidation pathways and another 5% is metabolized by cytochrome P450

2E1 (CYP2E1) to NAPQI. The NAPQI subsequently binds to glutathione (GSH) to produce mercuric acid and cysteine conjugates before being eliminated from the body. Different studies demonstrated that overdose of APAP may result in the depletion of GSH and cause NAPQI-induced hepatic cell injury (57). The production of ROS including hydrogen peroxide, hydroxyl radicals, and superoxide anions can be enhanced by NAPQI. Lipid peroxidation, DNA, and protein oxidation, as well as a decrease in radical-scavenging enzymes of GPx and superoxide dismutase (SOD) have also been reported in APAP-induced liver injury (58).

The depletion of GSH by NAPQI is an important component of APAP-induced oxidative stress followed by liver injury, while the malondialdehyde (MDA) level is an index of oxidative stress, was significantly increased (59). In study of the effect of NAC on glutathione was found that NAC has many different pharmacological effects, predominantly based on increased mitochondrial and cytosolic production of GSH, one of the principal free radical scavenging agents in humans. In paracetamol toxicity, GSH depletion reduces the level of covalent binding of the toxic metabolite, making NAC an effective antidote (60). In the present study the effects of *Beta vulgaris* roots ethanolic extract on glutathione revealed the prevent GSH depletion by scavenging reactive oxygen species. Therefore, it inhibits the oxidative damage of cellular macromolecules.

The interesting results in the present study in the turnover of GSH serum level to the normal one at both periods in *Beta vulgaris* roots ethanolic extract treated group indicating complete improvement in oxidant and antioxidant status. Lipid peroxidation (LPO) is frequent even today invoked as a mechanism of cell death during APAP hepatotoxicity. Lipid peroxidation is a free radical reaction process that is initiated by hydroxyl radical formation from hydrogen peroxide (Fe²⁺-dependent Fenton reaction) and the generation of lipid radicals leading to the destruction of polyunsaturated fatty acids in lipid membranes (61). Lipid peroxidation can cause a rapid catastrophic breakdown of the membrane potential and ion gradients leading to cell necrosis. Early studies on LPO in the APAP mouse model used ethane and pentane exhalation and hepatic malondialdehyde as indicators of LPO, (62). Malondialdehyde the final stage product by lipid peroxidation and indicative of cell membrane damage as a result of oxidative of polyunsaturated fatty acid in the membrane, is usually used as a bio-indicator of oxidative stress (61).

In the recent study the APAP positive control group showed that there was a significantly higher increase in the levels of MDA at the induced period (14 days) more than two times that continue at recovery period which indicative of the high lipid peroxidation induced by APAP in the liver cellular membrane tissue, while treatment with NAC reduced the level of MDA in comparison with APAP but still higher than control and T1 treated group which showed that the levels of MDA reduced nearly to the normal at induced period, these indicative that *Beta vulgaris* roots ethanolic extract treatment has more antioxidant effect than NAC treatment and could combat the damage induced by APAP.

N-acetylcysteine (NAC) reported to exert its effect on MDA insult contributing to the maintenance of the membrane integrity of hepatocytes serving as markers of

liver damage , this compound was also able to markedly reduce the production of MDA, an indicator of LPO and to return the intracellular levels of GSH to the control levels (63).

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 decrease the excessive increasing of AST, ALT and ALP levels by increasing and maintaining the integrity of the hepatocytes cell membrane by its activity as antioxidant agent. 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.

Acknowledgment

The authors would like to thank Assist. Prof. Dr. Hassan Alghetaa, Head of Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq for assistance in completing this work.

References

1. Lee, W.M. (2003). Drug induced hepatotoxicity. Eng J. M., 349 :474-85.
2. Akshirsagar, Y., Vetal Ashok, P. and Bhosle, Dingawale. (2008). Drug Induced Hepatotoxicity. Int. J. of Pharmacol. Comprehensive Review, 7:1-9.
3. Yoon, E. ,Babar, A. Choudhary, M. Kutner, M. and Pysopoulos, N. (2016). Acetaminophen induced hepatotoxicity : a comprehensive update. J Clin Gastroenterol Hepatol.,4 (2): 131-142.
4. Woolley, D. and Woolley, A. (2017). Practical toxicology: evaluation, prediction and risk, third ed., CRC press, pp.330.
5. Ryder , SD. and Beckingham , IJ. (2001) . Other causes of parenchymal liver disease. Bmj. 322 (7281): 290-292.
6. Ferri , FF. (2016). Ferri's Clinical Advisor 20175 Books in 1.First ed., Elsevier Health Sciences. Rhode Island, (10): 125-129.
7. Freifeld ,CC. Brownstein , JS. Menone, CM. Bao, W. Filice, R. Kass-Hout ,T. and Dasgupta, N. (2014). Digital drug safety surveillance: monitoring pharmaceutical products in twitter. Drug saf., 37 (5): 343-350.
8. Twycross , AM. Parker, R. McKeever , S. and Wiseman, T. (2017). An integrative review of interventions to support parents when managing their child's pain at home. Pain ManagNurs.,19 (2): 139-156.

9. Podolsky, DK. Camilleri , M. Fitz , JG. Kalloo , AN. Shanahan , F. and Wang,TC. (2016).Yamada's Textbook of Gastroenterology. 6th ed. John Wiley and Sons, Texas, 1537-3070
10. Prout , J. Jones , T. and Martin, D. (2014). Advanced Training in Anaesthesia. OUP Oxford. 166.
11. More, S.S. , Nugent, J., Vartak, A.P., Nye, S. M. and Vince, R. (2017). Hepatoprotective effect of glutathione in a murine model of Acetaminophen-induced liver toxicity. Chemical research in toxicology, 30(3): 777-784.
12. Jain, N. and Singhai, AK. (2012). Protective role of *Beta vulgaris* leaves extract and fractions on ethanol mediated hepatotoxicity. ACTA Poloniae Pharma Drug Res. 69:945-50.
13. V`ali , E. L. Stefanovits-B`anyai, K. and Szentmih`alyi et al., (2007). "Liver protecting effects of table beet (*Beta vulgaris* var. *rubra*) during ischemia-reperfusion," Nutrition, 23(2): 172-178 .
14. Winkler, B. W. C. , Schroecksadel , K., Schennach, H. and Fuchs, D. (2005). "In vitro effects of beet root juice on stimulated and unstimulated peripheral blood mononuclear cells," The American Journal of Biochemistry and Biotechnology, 1: 180-185
15. Makkar, HPS. and Berker, k. (1996). Nutritional value and anti-nutritional components of whole and ethanol extracted *Moringa oleifera*. Anim Feed Sci Technol. 63:211-28.
16. Ormsbee, M. J. Lox, J. and Arciero, P. J. (2013). "Beetroot juice and exercise performance," Nutrition and Dietary Supplements, 5: 27-35
17. Ormsbee, M. J. Bach, C. W. and Baur, D. A. (2014). "Pre-exercise nutrition: the role of macronutrients, modified starches and supplements on metabolism and endurance performance," Nutrients, 6(5):1782-1808
18. Singh, A. Garg,V. K. Sharma, P. K. and Gupta, S. (2011) . "Wound healing activity of ethanolic extract of *Beta vulgaris*," Pharmacology online, 1: 1031-1038
19. El - Gamal , A. A. ; El - Mansour , S . and Rafatullah, S. (2014). Beetroot (*Beta vulgaris* L.) Extract Ameliorates Gentamicin-Induced Nephrotoxicity Associated Oxidative Stress , Inflammation, and Apoptosis in Rodent Model. Hindawi Publishing Corporation Mediators of Inflammation. 2014: 1-12
20. Laouini, S. E. and Ouahrani M. R., (2017). PHYTOCHEMICAL SCREENING, IN VITRO ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF RUMEX VESICARIUS L. EXTRACT. St. Cerc. St. CICBIA., 18 (4): 367 - 376
21. Habibatni, S. , Zohra, A.F. , Khalida, H. and Ali, N. A. (2017). In-vitro antioxidant , Xanthine oxidase – inhibitory and in-vivo Anti-inflammatory , analgesic , antipyretic activity of *Onopordum acanthium*. International Journal of Phytomedicine , 9: 92-100.
22. Abdelkader, M. , Ahcen, B., Rachid, D. and Hakim, H. (2014). Phytochemical Study and Biological Activity of Sage (*Salvia officinalis* L.). International Journal of Bioengineering and Life Sciences, 8(11): 1253-1257.
23. Trease, G.E. and Evans, W. C. (2002). Pharmacognosy, 15th ed. Philadelphia: WB Saunders, Elsevier Science Limited, 336.
24. Ajanal, M., Gundkalle1, M. B. and Nayak, S. U. (2012). Estimation of total alkaloid in *Chitrakadivati* by UV-Spectrophotometer . Ancient Science of Life, 31 (4): 198-201

25. Ghorai, N., Chakraborty, S., Gucchait, S. , Saha, S. K. and S. Biswas , S. (2017). Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. Protocol Exchange. 1-6.
26. Tofighi, Z. and Saeidi, N. G. (2016). Determination of cardiac glycosides and total phenols in different generations of *Securigera securidaca* suspension culture. Research Journal of Pharmacognosy, 3(2): 25-31.
27. Ezeabara, C. A., Okeke, C. U., Aziagba, B. O., Ilodibia, C.V. and Emeka, A.N. (2014). Determination of Saponin Content of Various Parts of Six *Citrus* species. International Research Journal of Pure & Applied Chemistry, 4(1): 137-143
28. Ye, H., Nelson, L.J., Del Moral, M.G., Martinez-Naves, E. and Cubero, F.J. (2018). Dissecting the molecular pathophysiology of drug-induced liver injury. World Journal of Gastroenterology, 24(13): 1373
29. McCuskey, R.S.(2006). Sinusoidal endothelial cells as an early target for hepatic toxicants. Clinical Hemorheology and Microcirculation, 34(1-2): 5-10
30. Green, J.L., Heard, K.J., Reynolds, K.M. and Albert, D. (2013). Oral and intravenous acetylcysteine for treatment of Acetaminophen toxicity: a systematic review and meta-analysis. Western Journal of Emergency Medicine, 14(3): 218.
31. SAS., (2018). Statistical Analysis System, User Guide. Statistical Version 9 , 6 th ed. SAS. Inst.Inc.Cary.N.C. USA.
32. Mroczek, A., Kapusta, I., Janda, B. and Janiszowska, W. (2012). Triterpene saponin content in the roots of red beet (*Beta vulgaris* L.) cultivars. J. Agr. Food Chem., 60: 12397-12402.
33. Mokhtari-Dehkordi, S., Hojjati, M., Rouhi, L., Rabiei, Z. and Alibabaei, Z. (2014). Effect of ethanolic extract of beet roots and leaves on motor coordination in male Wistar rats. J. Shahrekord Univ. Med. Sci., 16: 14-23.
34. Rose, M. H., Sudha, P.N. and Sudhakar, K. (2014). EFFECT OF ANTIOXIDANTS AND HEPATOPROTECTIVE ACTIVITIES OF METHANOL EXTRACT OF BEET ROOT (*BETA VULGARIS* L.) AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RAT MODELS.
35. Nahla, T.K., Wisam, S.U. and Tariq, N.M. (2018). Antioxidant Activities of Beetroot (*Beta vulgaris* L.) Extracts. Pakistan Journal of Nutrition, 17(10): 500-505
36. Lee, Y.R., Woo, K.S., Kim, K.J. and Jeong, H.S. (2007). Antioxidant activities of ethanolic extracts from germinated specialty rough rice. Food Sci. Biotechnol., 16: 765-770.
37. Mishra, A.K., Okoli, J.O., Ohaju-obodo and Eifidiyi, K. (2009). Inhibitory activity of India specie plant *Cinnamomum zeylanicum* extracts against *Alternaria solani* and *Curvularia lunata*, the pathogenic dematiaceous moulds. Ann. Clin. Microbiol. Antimicrob., 8(9): 1-7.
38. Roger, M.F. and Wink, M. (1998). Alkaloids: Bio-chemistry, ecology and medicinal applications. Plenum press, 2-3.
39. Pyo, Y.H., Lee, T.C., Logendra, L. and Rosen, R.T. (2004). Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris* subspecies *cycla*) extracts. Food Chem., 85: 19-26.
40. Ben Haj Koubaier, H., Snoussi, A., Essaidi, I., Chaabouni, M.M., Thonart, P. and Bouzouita, N. (2014). Betalain and phenolic compositions, an-tioxidant activity of tunisian red beet (*Beta vulgaris* L. *conditiva*) roots and stems extracts. Int. J. Food. Prop., 17(9): 1934-1945.

41. Ferah, I., Halici, Z., Bayir, Y., Demirci, E., Unal, B., and Cadirci, E. (2013). The role of infliximab on Paracetamol -induced hepatotoxicity in rats. *Immunopharmacology and immunotoxicology*, 35(3), 373-381.
42. Lozovoy, M.A., Simão, A.N. and Panis, C. (2011). "Oxidative stress is associated with liver damage, inflammatory status, and corticosteroid therapy in patients with systemic lupus erythematosus," *Lupus*, 20(12): 1250-1259 .
43. Ramadhan, S.J. and khudair, kh. K. (2019). Effect of Betaine on Hepatic and Renal Functions in Acrylamide Treated Rats. *The Iraqi Journal of Veterinary Medicine*, 43(1):138 - 147.
44. Qinna, N. A., and Ghanim, B. Y. (2019). Chemical induction of hepatic apoptosis in rodents. *Journal of Applied Toxicology*, 39(2), 178-190.
45. Allam, W., Kasem, S., Mahmoud, S., Abdallah, A., and Hilal, M. (2021). Experimental Study of the Possible Protective Effect of Alpha-Lipoic Acid on Paracetamol induced Oxidative Stress and Hepatic Toxicity in albino rats. *Ain Shams Journal of Forensic Medicine and Clinical Toxicology*, 36(1), 75-89
46. Lakshmi, T., Renukadevi, B. S., Senthilkumar, S., Haribalan, P., Parameshwari, R., Vijayaraghavan, R., and Rajeshkumar, S. (2018). Seed and bark extracts of *Acacia catechu* protects liver from Acetaminophen induced hepatotoxicity by modulating oxidative stress, antioxidant enzymes and liver function enzymes in Wistar rat model. *Biomedicine and Pharmacotherapy*, 108, 838-844.
47. Al-Asmari, A. K., Al-Said, M. S., Abbasmanthiri, R., Al-Buraidi, A., Ibrahim, K. E., and Rafatullah, S. (2020). Impact of date palm pollen (*Phoenix dactylifera*) treatment on Paracetamol -induced hepatorenal toxicity in rats. *Clinical Phytoscience*, 6(1), 1-12.
48. Fusai, G., Glantzounis, G. K., Hafez, T., Yang, W., Quaglia, A., Sheth, H., Davidson, B. R. (2005). N-acetylcysteine ameliorates the late phase of liver ischaemia/reperfusion injury in the rabbit with hepatic steatosis. *Clinical Science*, 109(5), 465-473.
49. Kakaei, F., Fasihi, M., Hashemzadeh, S., Zarrintan, S., Beheshtirouy, S., Asvadi-Kermani, T., Sanei, B. (2020). Effect of N-acetylcysteine on liver and kidney function tests after surgical bypass in obstructive jaundice: A randomized controlled trial. *Asian journal of surgery*, 43(1), 322-329.
50. Clifford, T., Bell, O., West, D.J., Howatson, G. and Stevenson, E.J. (2017). Antioxidant-rich beetroot juice does not adversely affect acute neuromuscular adaptation following eccentric exercise. *J Sports Sci.*, 35: 812-19.
51. Gallardo, E.J. and Coggan, A.R. (2018). What's in Your Beet Juice? Nitrate and nitrite content of beet juice products marketed to athletes. *Int J Sport Nutr Exerc Metab.*, 1-17.
52. Vulić, J.J., Čebović, T.N., Čanadanović-Brunet, J.M., Četković, G.S. and Šaponjac, V.T.T. (2014). *In-vivo* and *in-vitro* antioxidant effects of beetroot pomace extracts. *Journal of Functional Foods*, 6: 168-175.
53. Clifford, T., Howatson, G., West, D.J. and Stevenson, E.J. (2015). The potential benefits of red beetroot supplementation in health and disease. *Nutrients*, 7: 2801-22.
54. Georgiev, V.G., Weber, J., Kneschke, E.M., Denev, P.N. and Pavlov, A.I. (2010). Antioxidant activity and phenolic content of betalain extracts from intact plants and hairy root cultures of the red beetroot *Beta vulgaris* cv. Detroit dark red. *Plant Foods for Human Nutrition*, 65:105-111.

55. Gaucher, C., Boudier, A., Bonetti, J., Clarot, I., Leroy, P., and Parent, M. (2018). Glutathione: antioxidant properties dedicated to nanotechnologies. *Antioxidants*, 7(5), 62.
56. Sarentonglaga, B., Ashibe, S., Kato, T., Atchalalt, K., Fukumori, R., and Nagao, Y. (2021). The effects of glutathione ethyl ester in in vitro maturation on the developmental ability of oocytes derived from cattle with liver abnormalities. *Theriogenology*, 170, 85-90.
57. Jaeschke, H., and Ramachandran, A. (2020). Acetaminophen-induced apoptosis: Facts versus fiction. *Journal of Clinical and Translational Research*, 6(2), 36.
58. Jeong, T. B., Kim, J.-H., Kim, S. H., Lee, S., Son, S. W., Lim, Y., Kwak, J.-H. (2019). Comparison of toxic responses to Acetaminophen challenge in ICR mice originating from different sources. *Laboratory animal research*, 35(1), 1-7.
59. Tobwala, S., Khayyat, A., Fan, W., and Ercal, N. (2015). Comparative evaluation of N-acetylcysteine and N-acetylcysteineamide in Acetaminophen-induced hepatotoxicity in human hepatoma HepaRG cells. *Experimental Biology and Medicine*, 240(2), 261-272.
60. Vairetti, M., Di Pasqua, L. G., Cagna, M., Richelmi, P., Ferrigno, A., and Berardo, C. (2021). Changes in Glutathione Content in Liver Diseases: An Update. *Antioxidants*, 10(3), 364
61. Jaeschke, H., and Ramachandran, A. (2018). Oxidant stress and lipid peroxidation in Acetaminophen hepatotoxicity. *Reactive oxygen species (Apex, NC)*, 5(15), 145.
62. Yoshioka, H., Aoyagi, Y., Fukuishi, N., Gui, M.-Y., Jin, Y.-R., Li, X.-W., Hitotsuyanagi, Y. (2017). Suppressive effect of kamebakaurin on Acetaminophen-induced hepatotoxicity by inhibiting lipid peroxidation and inflammatory response in mice. *Pharmacological Reports*, 69(5), 903-907.
63. Acharya, M. and Lau-Cam, C. A. (2010). Comparison of the protective actions of Nacetylcysteine, hypotaurine and taurine against Acetaminophen induced hepatotoxicity in the rat. *Journal of Biomedical Science*, 17(1), 1-11.