FLAVONOIDS FROM SUGAR BEET LEAVES AS HEPATOPROTECTIVE AGENT

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ABSTRACT

Objective: This work was designed to investigate the activity of *Beta vulgaris* (*B. vulgaris*) extract against hepatotoxicity induced by (carbon tetrachloride) CCl_4 in male rats.

Methods: Hepatoprotective study was performed on rats, divided into different groups; control healthy rats, the group received *B. vulgaris* extract, intoxicated rats by CCl_4, CCl_4 group treated with alcoholic leaves extract, and CCl_4 intoxicated rats treated with silymarin. The evaluation was done through measuring liver function indices and oxidative stress markers.

Results: The activities of Alanine Transferase (ALT), Aspartate Transferase (AST), Alkaline Phosphatase (ALP), and gamma-glutamyl transferase (GGT) increased by 187.07, 52.37, 50.58, and 94.59% respectively in CCl_4 group from control. Supplementation of beet extract decreased this elevation to 10.83, 26.43, 17.07 and 37.21% for the previous parameters respectively. The values obtained of the enzymes activity return nearly to that of control values, also a histopathological investigation of liver confirmed the results obtained.

Conclusion: Beet showed a remarkable anti-hepatotoxic activity against CCl_4 induced hepatic damage

Keywords: *B. vulgaris*, Hepatoprotective, Flavonoids, Liver function, Antioxidant enzyme, Histopathology

INTRODUCTION

Sugar beet, *Beta vulgaris* L. is native to the coasts of Mediterranean, extensively cultivated in Europe, America and Many parts of India. In Egypt, sugar beet was cultivated on 1982 in an area of 16943 feddan increased to 235259 at 2008 with a mean production of 50700 tons sugar [1]. Aqueous and ethanolic extracts of beet roots have been reported to possess free radical scavenging activity, reducing the radical cations and phase II enzyme-inducing activities in murine hepatoma cell *in vitro* study [2]. There are some reports indicating the potential hepatoprotective, antioxidant and anti-inflammatory activity of *B. vulgaris*. Duke [3] and Agrawal [4] in their investigation dealing with hepatoprotective activity against CCl_4-induced hepatic injury in rats, found that ethanolic extract of *B. vulgaris* given at doses of 1000, 2000, and 4000 mg/kg b. wt not only prevented the rise in serum level of ALT and ALP but also improved serum lipid profile in a dose-dependent manner. Ethanolic extract effects were attributed to phenolic amides. Murakami *et al.* [5] isolated hypoglycemic saponins from the roots of sugar beet. They succeeded in isolating nine triterpenes aminoglycosides called beta vulgaroside with a unique substituent acetal type which was found to show potent hypoglycemic activity.

Ranju [6] showed the effect of ethanol extract of *B. vulgaris* on CCl_4 induced hepatotoxicity in rats. Rats induced with CCl_4 showed a significant reduction in levels of protein and a significant increase in the levels of bilirubin and also the activities of AST, ALT and ALP. Treatment with an alcoholic extract of beet for a period of 10 d significantly restores the levels of the liver function similar to that of silymarin [7]. Histological profile of the animals treated with ethanol extract exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, the absence of necrosis and lesser fatty infiltration [6].

The aim of the present work is to evaluate the role of leaves extract of sugar beet plant as a hepatoprotective agent. Sugar beet leaves is considered as an agriculture waste, so if it has this effect will be an added value to the plant.

MATERIALS AND METHODS

Chemicals and reagents

The assay kits used for biochemical measurements of AST (EC 2.6.1.1.), ALT (EC 2.6.1.2), ALP (EC 3.1.3.1), GGT (EC 2.3.2.2), Reduced Glutathione (GSH), Malondialdehyde (MDA), Nitric oxide (NO), total protein, albumin, and bilirubin were purchased from Biodiagnostic Company, Dokki, Giza, Egypt. All other chemicals used were of analytical reagent grade.

Plant materials and extractions

Seeds of sugar beet were obtained from the Department of Sugar Crops Ministry of Agricultural Giza, Egypt. The seeds were sown directly on 25 October 2011 in rows 60 cm apart and one meter in between plants in the Experimental Farm, of Agriculture Faculty Cairo University, Giza. All seeds germinated after 7 to 10 d from sowing. The leaves were collected, cleaned, oven dried, grounded and then kept at room temperature until extracted. The leaves were extracted with increasing polarity of solvent as follows, pet ether, chloroform, ethyl acetate and methanol. All these extracts were subjected to analysis for polyphenol content and free radical scavenging activity.

For isolation of active substances, air-dried leaves of sugar beet (1 kg) were extracted with 70% ethanol several times. The combined alcoholic extracts were concentrated under reduced pressure at 45 °C to yield 52g residue. The crude alcoholic extract was dissolved in hot water, left overnight, filtered and was successively partitioned with chloroform then n-butanol. The butanol part was evaporated till solvent-free to give (4.5g). The butanol part was fractionated by loading on polyamide column using 80% methanol, in fractions of 50 ml, then monitored on TLC. Similar fractions were combined, then evaporated. Four main substances were obtained, detected by UV light 366 nm. Each substance was then passed through small sephadex LH_{20} column, eluted with 50% methanol, then fractionated by TLC plates to check its purity and crystallized from MeOH.

Determination of polyphenolic content

Total soluble phenolics were determined using Folin-Ciocalteu reagent by the method of Slinkard and Singleton [8]. The amount of total phenolic compounds is calculated as mg of gallic acid equivalents from the calibration curve of gallic acid. The phenolic acids found in the alcoholic extract of leaves of sugar beet were determined by HPLC adopting the method described by Leonardis *et al.* [9]. The HPLC system used a HP 1100 chromatograph (Agilent

Technologies, Palo Alto, CA, USA) equipped with an auto-sampler, quaternary pump and a diode array detector. The measurements were integrated by Chemstation chromatographic software interfaced to a personal computer. The analytical column was ZORBAX Eclipse XDB C18 column (15 cm x 4.6 mm I.D., 5 µm, USA).

Free radical scavenging activity by DPPH

The model of scavenging the stable DPPH radical is widely used to evaluate antioxidant activity in a relatively short time compared to other methods. The method adopted was that of Shimada *et al.* [10].

In vivo **study**

Animals

Adult male healthy Wistar strain albino rats weighing 150-200g were obtained from the Animal House, National Research Centre, Dokki, Egypt. Animals were fed a standard diet (control diet; consists of vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95% pure) 10.5%, and starch 54.3%), water *ad libitum*. Animals were acclimatized to the laboratory condition for one week before starting the experiment. Temperature through the housing was adjusted to 24 °C with relative humidity 65 ± 5% and 12/12 hr of light/dark cycles. The experimental work on rats was performed with the approval of the Animal Care & Experimental Committee, National Research Centre, Giza, Egypt. Approval No 134 2010.

Acute oral toxicity experiment

Fifteen rats of 200± 10g were subdivided into three subgroups (5 rat each) and received one oral dose of 500, 1000, and 1500 mg/of leaves extracts as mg/kg b.w. No. of dead animals were counted along 15 d. The mortality rate and LC_{50} were monitored.

Hepatoprotective experimental design

Healthy male rats were divided into five groups, eight/rats each. Group 1 served as normal control rats. Group 2 was received beet leave's alcoholic extract (500 mg/kg b. wt) administered orally. Group 3 was CCl_4 treated rats.0.5 ml CCl_4/kg b. wt. diluted to 1: 9 (v/v) in olive oil which was injected intraperitoneally [4]. Group 4 was injected with CCl_4 and treated with beet leaves extract (500 mg/kg b. wt) administered orally at the same time and for the same duration [11]. Group 5 was injected with CCl_4 and treated with legalon drug (silymarin) as reference herbal drug, orally at dose 100 mg/kg b. wt [12]. The administration regime was twice per week for six consecutive weeks.

Blood samples

Animals were sacrificed under anesthesia and blood sample were withdrawn from the retro-orbital venous plexus in clean and dry test tubes. Blood left 10 min to clot and centrifuged at 3000 rpm for 20 min at 4 °C. The supernatant serum was collected and stored at–80 °C for further determination of different biological parameters.

Liver homogenate

The liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissue was then kept at-80 °C until analysis. At experiment time, a portion of liver was weighed and homogenized with saline (0.9% NaCl) (1: 9 w/v) using glass homogenizer at 4 °C. The homogenates were centrifuged at 3000 rpm for 10 min at 4 °C and the clear supernatant was used for further determination of liver function enzymes, oxidative stress markers and hepatic total protein and bilirubin.

Biochemical studies

MDA as a product of polyunsaturated fatty acid oxidation was determined by the method of Buege and Aust [13]. Its concentration

was calculated using the extinction coefficient value 1.56 x $10^5 M^{-1}$ cm^{-1} and was read at 535 nm. NO was estimated by the method of Nishikimi *et al.* [14] where the increase in NADH oxidation was measured at 560 nm using its molar extinction coefficient 6.22 x $10^3 M^{-1} cm^{-1}$. NO was assayed by the method of Moshage *et al.* [15]. The assay is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. Hepatic aspartate and ALT were measured by the method of Gella *et al.* [16] where the transfer of an amino group from aspartate or alanine formed oxaloacetate or pyruvate, respectively and the developed color was measured at 520 nm. Hepatic alkaline phosphatase was catalyzed in an alkaline medium; the phosphate group transferred from 4-nitrophosphate to 2-amino-2-methyl-prozanol CAMP) and liberated 4-nitrophenol. The developed color was measured at 510 nm [17].

Hepatic GGT was estimated by the method of Szasz [18] where the GGT enzyme reacted with L-γglutamyl 3-carboxy–p-nitroanilide and glycyl-glycine to give L-γglutamyl-glycyl-glycine and 5-amino 0-2-nitrobenzoate. The decrease in absorbance was read at 450 nm at 1 min intervals for 3 min. Total protein was assayed by the method of Bradford [19] where coomassie Brilliant Blue dye reacted with Bradford reagent and gave a complex blue read at 595 nm.

Histopathological examination

Small pieces of liver tissues in each group were collected in 10% neutral buffered formalin. The tissues were processed and embedded in paraffin wax. Sections of 5µm thickness were cut and stained with eosin. The sections were examined microscopically for the evaluation of histopathological changes [20].

Statistical analysis

Analysis of data was carried out by one-way analysis of variance (ANOVA) Costat computer software program accompanied with the least significant difference between means at P ≤0.05.

RESULTS AND DISCUSSION

Polyphenolic content

A major and great part of phytochemicals which is constantly synthesized by plants for development of drugs and for the defensive purpose are polyphenolic in nature. These polyphenols comprise antioxidant character that is produced as a defensive mechanism to prevent tissues destruction caused by highly reactive chemical species, which are formed from various biochemical reactions [7]. It is also considered as a major class of natural antioxidants. In the present study, polyphenols were determined using Folin-Ciocalteu method.

Table 1 compiles the polyphenolic content in different beet leaves extracts which reveals that alcohol extract possesses the higher polyphenolic content.

Table 1: Total polyphenolic in sugar beet leaves extracts

Extract	Phenolic content ug/gallic acid
Pet. ether	0.66 ± 0.12[b]
Chloroform	1.31± 0.15[a]
Ethyl acetate	0.59 ± 0.06[b]
Methanol	1.63± 0.17[a]

Data are means ± SD., Values are not sharing superscripts letters differ significantly at *p*<0.05.

Phenolic acid content

Phenolic acids were determined using HPLC, gallic acid was the highest, amounted to 344 ug/ml followed by ferulic 89.7 while a small amount of other acids were found (table 2).

Table 2: HPLC determination of phenolic acids in *B. vulgaris* leaves alcoholic extract

Catechin	Gallic	Coffeic	Vaneltic	Coumaric	Ferutic	Cinnanic
4.2	344	3.79	1.25	0.46	39.7	3.54

Isolation and structure elucidation

Alcohol extract (52g) was dissolved in hot water, filtered then successively extracted with chloroform (which was discarded) then with butanol to give 5.4g. The butanol fraction was subjected to fractionation with paper chromatography on Whatman 3MM with butanol-acetic acid-water 4: 2: 1 (v/v) as eluant. Four major bands were detected under UV light with the following R_f values, 1.9, 2.5, 3.3 and 3.4. All the bands were cut off, eluted with 70% alcohol, evaporated and were subjected to Sephadex LH_{20} column, eluted with 50% MeOH. The pure compounds (fig. 1) were subjected to H-^{1}NMR, which reveals the following identification:

Compound 1

Compound I was obtained as a yellow amorphous powder, with R_f value 0.34 (BAW 4: 2:1), and appeared as a yellow spot on PC and purple under UV light, which showed bright yellow color when fumed with ammonia vapours. ^{1}H NMR (500 MHz, MeOD) of compound 1 indicated with presence of quercetin moiety appeared at 6.18 (1H, d, j= 2.0 Hz, H-6), 6.39 (1H, d, J=2.0 Hz, H-8), 6.88 (1H, d, J= 8.3 Hz, H-5'), 7.62 (1H, dd, J=8.3; 2.1Hz, H-6'), 7.74 (1H, d, J=2.1Hz, H-2'). Identification of the compound was further confirmed by co-chromatography with the authentic sample of the aglycone quercetin as well as comparing the spectral data with those reported for quercetin ($C_{15}H_{10}O_7$).

Compound 2

Compound 2 was obtained as a brownish yellow amorphous powder, with R_f value: 0.19 (BAW 4: 2: 1), and appeared as a yellow spot on PCwith purple color under UV light, and also showed bright yellow color when fumed with ammonia vapours. Complete acid hydrolysis yielded quercetin aglycone and galactose as sugar moiety in which both of them were co-chromatographed with authentic samples. ^{1}H NMR (Me OD, 500 MHz): 6.12 (1H, d, J= 1.9 Hz, H-6), 6.30 (1H, d, J = 1.9 Hz, H-8), 6.85 (1H, d, J= 8.0 Hz, H-50), 7.57 (1H, dd, J= 2.0, 7.5Hz, H-60), 7.82 (1H, d, J = 2.0 Hz, H-20), 5.04 (1H, d, J=7.6 Hz, H-100), 3.82 (1H, m, H-200), 3.54 (1H, m, H-300), 3.85 (1H, d, J=2.0Hz, H-400), 3.45 (1H, m, H-500), 3.58 (1H, dd, J=11.0 and 7.0 Hz, H-60a), 3.65 (1H, dd, J= 11.0 and 4.0 Hz, H-60 b).

Compound 3

Compound 3 was isolated as a yellow amorphous powder, with R_f value 2.5 (BAW 4: 2: 1), it has a purple color under UV light turning to bright yellow when fumed with ammonia vapours. Complete acid hydrolysis yielded quercetin aglycone and glucose as sugar moiety in which both of them were co-chromatographed with authentic samples.. ^{1}H-NMR (500MHz, MeOD) spectrum indicated the presence of quercetin moiety appeared at δ 7.70 (1H, d, H-2`, J= 1.9 Hz), 7.58 (1H, dd, H-6', J=8.5Hz, 1.9Hz), 6.86 (1H, d, H-5', J= 8.5 Hz), 6.36 (1H, d, H-8, J= 2.2Hz), 6.17 (1H, d, H-6, J= 2.2Hz), 5.22 (1H, d, H-1", J=7.3 Hz), 3.85-3.30 (6H, sugar protons). Quercetin 3-O-glucoside is the 3-glucoside of the flavonol quercetin, a type of polyphenols. Polyphenols are plant secondary metabolites with a molecular formula of $C_{21}H_{20}O_{12}$.

Compound 4

Compound 4: Needles form MeOH, appeared on PC as a violet blue spot under UV light with $R_f$value0.34 (BAW). ^{1}H NMR (500MHz, MeOD): displayed a singlet at δ 7.06 (2H, s, H-2, H-6) assigned for two equivalent m-coupled aromatic protons.
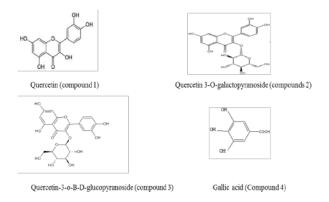

Fig. 1: chemical structure of the four isolated compounds from alcoholic leaves extract of sugar beet

Free radical scavenging activity (DPPH)

To evaluate the antioxidant activity of the different extracts of beet leaves, radical DPPH is widely used to assess this activity in a relatively short time. Substances capable of donating electrons/hydrogen atoms are able to convert DPPH radical into their non-radical form. So, positive DPPH test suggests that the samples were free radical scavengers [21].

Table 3 represents the concentration response of DPPH radical scavenging activity of beet leaves extracts. Methanol extract and ascorbic acid had scavenging effect with increasing concentrations, in a concentration-dependent manner. The data in table 3 proved that methanol extract of sugar beet leaves was capable of scavenging DPPH radical in concentration dependent manner. Other extracts produced positive results with higher concentrations only (pet. ether & ethyl acetate).

Ascorbic acid was used as a reference for radical scavengers. The IC_{50} was found to be 2.59µg/ml for ascorbic acid, while in methanol extract it was 6.55µg/ml. In this regard, the antioxidant activity could not be attributed only to the major compounds but also the minor ones may play a significant role in the antioxidant activity and hence synergistic effects were reported [7]. The alcoholic extract of leaves proved to have the highest total polyphenolic content with higher scavenging activity.

Table 3: Radical scavenging activities (% DPPH inhibition)

Sugar beet leaves Extract	Sample concentration µg/ml					IC_{50}
	1.25	2.5	5.0	7.5	10	µg/ml
Methanol	2.80±1.34[a]	15.23±2.32[a]	15.23±2.32[a]	36.61±1.63[a]	65.0± 2.03[a]	6.55
Ethyl acatate	0.00	0.00	0.00	0.80±.010[c]	2.45± 0.21[c]	0.00
Chloroform	1.26± 0.39[b]	5.15± 0.65[b]	5.15± 0.55[b]	6.39± 1.08[b]	8.21± 1.24[b]	0.00
Pet-ether	0.00	0.00	0.00	0.00	1.89± 0.21[c]	0.00
Ascorbic acid	6.16± 0.67[a]	11.86±1.64[a]	27.39±1.82[a]	57.61±1.35[a]	89.15±3.82[a]	2.59

Data are means ± SD of eight rats. Unshared letters between brackets are significant value between groups

Hepatoprotective effect of sugar beet leaves extract

Six parameters were assayed AST, ALT, ALP, GGT, tissue protein, albumin and bilirubin to explore the status of these parameters in different rat groups. Control rats when administered the alcoholic beet leaves extract, no significant changes in the values of the six parameters were observed. This means that beet leaves extract is safe enough and did not alter these parameters. The diseased or intoxicated rats (CCl_4 group) show drastic changes in correspondence to each parameter when compared with control. As shown in table 4 CCl_4 developed severe hepatic damage, evidenced by marked elevation of the parameters studied when compared with control.

Table 4: Ameliorative effect of alcoholic extract of sugar beet leaves on liver enzymes

Treatment	ALT u/ml	%of change	AST ul/ml	% of change	AIP	%of change	GGT	%of change
Control	43.76± 1.11ᵃ	0.00	39.39± 1.74ᵃ	0.00	78.93± 6.23ᵃ	0.0	258±0.26ᵃ	0.0
CCl₄	125.62± 8.51ᶜ	187.07	60.20± 1.79ᶜ	52.37	118.85± 3.73ᵈ	50.58	5.02± 0.46ᵈ	94.57
beet	45.60± 0.66sᵇ	4.20	42.79±0.97ᵃᵇ	8.63	83.82±2.46ᵃᵇᶜ	9.40	3.64 ± 0.26ᵃᵇᶜ	17.8
CCl₄₊beet	48.50± 8.63ᵃᵇ	10.83	49.80± 3.60ᵃᵈ	26.43	92.40± 0.77ᶜ	17.07	3.54± 0.31ᶜ	37.2
CCl₄+silymarin	3.67 ± 0.63ᵈ		0.845 ±0.24ᵈ		312.81± 12.8ᵈ		24.78± 6.97ᵈ	

Each value is a mean of 8 rats±S. D. Statistical analysis is carried out by one-way analysis of variance (ANOVA) followed by Duncan's test, using SPSS (SPSS 17.0 for windows). values are not sharing superscripts letters (a, b, c, d) differ significantly at $p<0.05$. Percentage of change = (treatment-control)/control) x 100.

Co-administration of *B. vulgaris* leaves alcoholic extract (500μg/ ml) to the intoxicated group, showed significant improvement in the levels of all the severely altered biochemical parameters when compared with the intoxicated group. The last groups, which include the treatment with silymarin shows a percentage of amelioration effect in the liver enzymes, however, more slowly than the beet extract. The activities of ALT, AST, ALP, and GGT increased by 187.07, 52.37, 50.58, and 94.59% respectively from control. Supplementation of beet extract decreased this elevation to 10.83, 26.43, 17.07 and 37.21% for the previous parameters respectively.

Table 5 represents the data obtained from the effect of beet leaves extract on total protein, albumin, and bilirubin through the five groups of animals. The data obtained revealed that no significant change in total protein was observed when healthy rats were treated with beet extract meaning safety of an alcoholic extract of leaves. On the other hand, CCl₄ induced liver damage by decreasing total proteins compared to control.

Table 5: Effect of beet extract on total protein, albumin, and bilirubin

Control	T. P g/dl	Albumin %	Bilirubin %
Control	7.12 ± 0.15ᵇ	5.03± 0.23ᶜ	0.91 ± 0.07ᵇ
beet extract	7.29± 0.39ᵇ	5.01 ± 0.33ᶜ	0.92 ± 0.04ᵇ
CCl₄	6.14± 0.31ᵃ	4.26 ± 0.11ᵃ	2.12 ± 0.14ᵃ
CCl₄+beet extract	7.31± 0.55ᵇ	5.02 ± 0.09ᶜ	1.00 ± 0.06ᵇ
CCl₄+Silymarin	43.66 ± 0.52ᵈ	5.02 ± 0.09ᵈ	2.88± 0.13ᵈ

Each value is a mean of 8 rats±S. D. Statistical analysis is carried out by one-way analysis of variance (ANOVA) followed by Duncan's test, using SPSS (SPSS 17.0 for windows). ᵃ,ᵇ,ᶜ,ᵈ values are not sharing superscripts letters (a, b, c, d) differ significantly at $p<0.05$.

Toxicated animal group by CCl₄ caused significant changes in the levels of total protein; albumin and bilirubin amounted to 6.14, 4.26 and 2.12 mg/dl respectively. These parameters were restored to nearly values in control when treated with an alcoholic extract of *B. vulgaris* leaves, the change in total protein concentration might be due to the imbalances between the rate of protein synthesis and the rate of its degradation in the liver.

The same pattern was obtained with albumin. When diseased rats were treated with beet extract, total protein and albumin restored to that levels of healthy control rats. Dealing with bilirubin, diseased rats possessed high fig. than that of control, which returned to nearly that of control meaning that alcoholic extract of *B. vulgaris* leaves has the ability to protect and treat intoxicated liver. In this concern, Kim *et al.* [7] reported the occurrence of two flavones glycosides from *B. vulgaris,* vitexin 7-O-β-D glucopyranoside, and vitexin 2-O-β-D-glucopyrcenoside. Their hepatoprotective was assessed by measuring their effects on the release of ALT from the primary cultures of rat hepatocyte injured by CCl₄. In addition, the flavones c-glycoside vitexin is known to possess an inhibitory activity on TNFα [22].

Hayes [23] reported that one of the most indicators for liver damage and function is the increase in the activities of transaminases, in the serum. According to [24], the development of toxic liver injury follows a two-staged course; the first phase characterized by initiation of the injury and may involve direct interaction with a toxicant, which may exhibit dose dependency. In contrast, the second phase is characterized by the progress of the injury in a an independent toxicant fashion that is dominated by secondary events, which is holding true with the results in the present investigation.

Effect of beet leaves extract on hepatic antioxidant enzymes

Sugar beet leaves alcoholic extract induce higher improvement in comparison to the standard drug acetylcysteine. In addition, beet leaves extract showed a significant increase in glutathione concentration with the percentage of improvement amounted to 118.55% compared to acetylcysteine hepatoprotective reference drug which recorded 44%. On the other hand, a significant decrease was detected in both MDA and NO levels of post treated intoxicated rats with beet. The improvement reached 78.8 and 61.2 for MDA and NO for diseased rats treated with beet extract (table 6).

Table 6: Effect of *B. vulgaris* on hepatic antioxidant hepatic antioxidant enzymes levels

Parameters	Normal control	Normal control treated with *B. vulgaris*	CCl₄	CCl₄ treated with *B. vulgaris*	CCl₄ Acetyl systeine drugs
GSH	2.00±0.08ᵇᶜ	2.28±0.26ᵇᶜ	1.28±0.13ᵈ	3.65±0.26ᵃ	2.10±0.52ᵇᶜ
MDA	2.30±0.18ᵇ	2.2±0.12ᵇᶜ	3.1±0.56ᵃ	1.80±0.14ᵇᶜ	2.00±0.18ᵇᶜ
NO	4.93±0.05ᵇ	4.43±0.49ᵇᶜ	7.21±0.46ᵃ	4.35±0.13ᵇᶜ	4.65±0.31ᵇ

Data are means±SD of eight rats in each group., Data are expressed as μg/mg protein for glutathione, nitric oxide and MDA., Total protein is expressed as mg/gram of liver tissue., Unshared letters between groups are the significance value at p ≤ 0.05.

The chemical part of this investigation revealed the occurrence of higher polyphenolic content and higher radical scavenging activity for the alcoholic extract. The fractionation of this extract indicates the occurrence of phenolic acids from which Gallic acid was higher in concentration. In addition, three flavonoids were isolated and identified. These flavonoids were inclined on quercetin as aglycone or its glycosidic nature with two different sugar moiety.

Among the many attributes of quercetin are its antioxidant, anti-inflammation and anti-apoptosis qualities [25-29]. Furthermore, there is a reason to believe that quercetin can shield the liver from damage brought about by hepatotoxins [30, 31]. Once quercetin is absorbed in the gastrointestinal tract, it is proceeded by phase II enzymes in the epithelial cells of the stomach and intestine. The combined metabolites are then processed further in the liver and kidney [32]. The β-ring catechol structure undergoes methylation at the 3` or 4` hydroxyl site by catechol–O-methyl transferase to from isorhamnetin and tamarixetin respectively [33]. Quercetin metabolites even were seen to build up in tissues shortly after quercetin–rich vegetables are eaten. *In vitro* studies have shown that quercetin metabolites that are produced in enterocytes and the liver function as antioxidants by raising the resistance of low-density lipoprotein (LDL) cholesterol to oxidation [34].
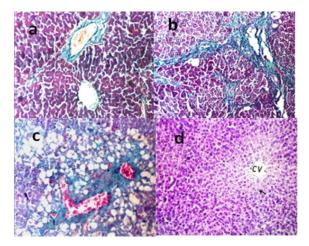

Fig. 2: Photomicrograph of masson's trichrome stained liver section (100x) of control (a), CCl$_4$ (b), silymarin (c) and beet (d) treated group

Histopathological studies

Liver section of healthy control rats shows hepatic lobular architecture. The hepatocytes were within normal limits and arranged in thin plates. The cells were separated by narrow blood sinusoids lined by endothelial cells. A portal tract was extended with no fibrous tissue or lymphocytes deposition (fig. 2a). Liver injured with CCl$_4$ revealed portal loss of hepatic labular architecture. Ballooning of hepatocytes, deformed cord arrangement and disturbed sinnsoids were seen (fig. 2b). The hepatocytes showed a marked degree of hydropic and stertotic changes as well as massive hepatic necrosis. Portal tract was extended with a marked number of chronic inflammatory cell and Porto central fibrosis (fig. 2b). Treatment intoxicated liver with CCl$_4$ treated with silymarin shows fibrosis, inflammations, and infiltrations. Small arrows indicate, hepatocytes, swelling, foamy appearance and a stage of steorosis (fig. 2c). On the other hand treatment of injured liver with sugar be*et* alcoholic extract after CCl$_4$ toxication showed marked improvement in hepatocytes architecture (fig. 2d).

In this regard Boots *et al.* [28] reported on the health effect of quercetin from antioxidant to nutraceutical activity and their findings go parallel to the results obtained. In the same aspect Nabavi*et al.* [29] ascertained the results reported from Boots *et al.* [28] and our present results which indicate the protective effect of quercetin which normalize the oxidative stress in rats.

It is interesting that *B. vulgaris* leaves are considered as a waste product to the industry of sugar and hence can be added value to the production of sugar beet in Egypt.

CONCLUSION

Based on our results, we suggest that beet leaves extract showed a remarkable anti-hepatotoxic activity against carbon tetrachloride induced hepatic damage, by ameliorating all liver enzymes, antioxidant enzymes which confirmed by histopathological examination.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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