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Protective Effect of Beet Root and Rosemary against Hepatic Oxidative Stress Injury in Rats

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Abstract: Beet roots and rosemary are rich in bioactive food components has therapeutic properties in hepatic injury. The present study aimed to evaluate the effect of beet roots and rosemary on reduction and management of hepatic injury symptoms. This study was conducted on thirty albino male rats, weighting 135 ± 5 g and randomly classified into six groups (6rats each). The first group kept as normal control fed standard diet only. The other four groups injected with injected subcutaneously by CIS to induce hepatic injury and reclassified into positive control, 10% beets, 10% rosemary and mixture treated groups. The treated groups with beet, rosemary and mixture showed a no significant difference in final weight, weight gain ,feed intake ,FER hemoglobin, packed cell volume ,RBCs ,ALT, AST and ALP enzymes activity, total protein, albumin, globulin in serum , A/G ratio and liver GSH,GPX ,MDA , glycogen ,cholesterol , total lipid and triglyceride, except rosemary group which showed a significant decrease in serum albumin and beet group showed a significant decreased in liver GSH but they showed significant increase in serum bilirubin when comparing with control (-ve) group. On the other side The treated groups with beet, and rosemary and mixture showed significant decrease in ALT, AST & alkaline phosphatase enzymes activity in serum bilirubin, A/G ratio, MDA ,cholesterol and total lipid in liver but showed significant increase in final weight, weight gain ,FER, hemoglobin, packed cell volume ,RBCs ,serum albumin , total protein ,globulin and liver GSH , GPX ,glycogen and triglyceride except rosemary group showed a no significant difference in serum albumin and also beet rat group which showed no significant difference in liver GSH when compared with control (+ve) rats group. It is recommended to consume beet, and rosemary and mixture of them to maintain the efficacy of the liver.

Keywords: Liver functions, glutathione, malondialdehyde, antioxidant enzymes, rats

Introduction

The beetroot (*Beta vulgaris L.*) has been used for centuries as a traditional natural coloring agent (Govind *et al.*, 2013). Beetroot contains red pigments (betacyanins) and yellow pigments (betaxanthins), known collectively as betalains. Medicinally, beetroot is employed as a popular folk remedy to stimulate the immune system and for the treatment of liver and kidney diseases. It is also employed as a special diet in the treatment of cancer (Craig, 2017 and Jiratanan, and Liu, 2014).

Rosemary (*Rosmarinus officinalis* Linn.) is a common household plant grown in many parts of the world. It is used for flavoring feed, as a beverage, and in cosmetics as well as in folk medicine for its choleric, hepatoprotective and antitumorigenic activity. Rosemary and its constituents (carnosol, carnosic acid, ursolic acid, rosmarinic acid, caffeic acid) have been intensively studied during the last 10 years. Different effects of this spice important from the point of view of cancer prevention were observed (Al-Sereiti *et al.*, 2016 and Slamenova *et al.*, 2012). Rosemary contains polyphenolics substances that are useful for stimulating the

immune system, increasing circulation, and improving digestion. Rosemary also contains anti-inflammatory compounds that may make it useful for reducing the severity of asthma attacks. In addition, rosemary has been shown to increase the blood flow to the head and brain, improving concentration. Among these polyphenolics, carnosic acid (a phenolic diterpene compound), and carnosol are the most potent antioxidant constituents which about 90% of antioxidant activity (Altinier *et al.*, 2017). This work aimed to study the effect of beet root and rosemary consumption on experimental rats induced hepatic injury by CISplatin.

Materials and Methods

Materials

Beet root (*Beta vulgaris L.*) and **rosemary** (*Rosmarinus officinalis Linn.*) were obtained from local market. Then dried in dry freezer and crushed into powder then added to standards diet.

CISplatin solution[®] (Platinol AQ) was obtained from Sigma Chemical Co. (St Louis, Mo, USA).

Experimental rats: thirty rats were purchased from Helwan Farm of Laboratory Animals.

Methods

Twenty four rats were weight 135 ± 5 g purchased from Helwan Farm of Laboratory Animals. The animals were kept under observation for five days before experiment and fed on standard diet and water ad libitum. The standard diet comprised of casein (200g/kg), corn starch (497g/kg), sucrose (100g/kg), cellulose (30 g/kg), corn oil (50g/kg), mineral mixture (100g/kg), vitamin mixture (20g/kg) and DL-methionine (3g/kg) according to Nelson, (2000). The rats were randomly classified into four groups (6 rats each). The control (-ve) fed on standard diet all over the period of the experiment. The other six groups injected with injected intraperitoneally into rats with LD50 of CIS in a dose of 12 mg/kg body weight according to Atssahin *et al.*, (2006) and reclassified into the control (+ve), treated with beet, rosemary and mixture groups fed on standard diet only or with beet, rosemary and mixture of them for four weeks and after one day of that, the rats were sacrificed.

During the study, the feed intake was calculated daily and the body weight gain was recorded daily. Part of blood from sacrificed rats was heparinized for estimation of hemoglobin and packed cell volume (Drabkin, 1949 and Mc Inory, 1954). The rest part of blood was left to coagulate then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum aminotransferase (ALT&AST), alkaline phosphates enzymes activity, total protein and albumin, were estimated according to Reitman and Frankel, (1957), Kind and King, (1954), Weichselbaum, (1946) and Bartholomev and Delany ,(1966) respectively. Serum globulin (G) value was determined by subtracting the albumin from the total proteins according to Coles, (1974). A/G ratio was calculated using albumin and globulin values for each individual sample. Livers of rats were rapidly removed and parts of them perfuse with 50 to 100 of ice cold 0.9%NaCL solution for estimation of glutathione (GSH), glutathione peroxidase (GPX), malondialdehyde (MDA), glycogen, cholesterol, total lipids and triglyceride according to Beuchamp and Fridovich, (1971), Beuther *et al.*, (1987), Habig *et al.*, (1974), Rerup and Lundquist, (1967), Abell *et al.*, (1952), Folch *et al.*, (1957) and Young and Pestaner, (1975), respectively.

Statistical analysis

Collected data were subjected to analysis according to SPSS program according to **Armitage and Berry (1987)**.

Results and Discussion

Effect of beets and rosemary on nutritional pattern of rats administrated CIS

The statistical data in Table (1) presented that, control (+ve) group showed a significant decrease in body weight gain and feed efficiency ratio while beet, rosemary and a mixture treated groups showed non-significant difference in weight gain ,feed intake and FER compared with control (-ve) group. Beets, rosemary and mixture rats groups showed a significant increase in weight gain and FER compared with control (+ve) group. These results were in agreement with those obtained by **Govind et al., (2013)** who found that, beets are teeming with folate and manganese and a good source of vitamin C, potassium and fiber. Beets are an excellent source of vitamins A and C, and the minerals potassium and magnesium, calcium, iron and copper beets are loaded with antioxidant phytonutrients like lycopene, anthocyanins and betaine. Betanin is, the extract of beetroot, used as a non-toxic, safe, natural feed colorant, and also be a potent cancer chemopreventive agent. **Schwartz et al., (2012) and Lichtenthaler and Marx, (2015)**. Rosemary is a good source of the minerals iron and calcium, as well as dietary fiber. Fresh has 25% more manganese and a 40% less calcium and iron, Rosemary is also contained many nutrients, including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids and amino acids **Al-Sereiti et al., (2016)**.

Table (1): Mean values ± SD of body weight gain, feed intake and feed efficiency ratio (FER) of control and CIS treated rats groups

Variables	Control (-ve)	CIS			
		Control (+ve)	Beet	Rosemary	Mixture
Weight gain (g)	115.77± 8.11 ^a	66.89± 6.11 ^b	106.33± 8.03 ^a	105.77± 9.17 ^a	108.14± 10.15 ^a
Feed intake (g/w)	15.32± 2.14 ^a	13.55± 2.55 ^a	15.40± 2.33 ^a	15.71± 2.71 ^a	15.81± 2.14 ^a
FER	0.125± 0.01 ^a	0.082± 0.03 ^b	0.115± 0.04 ^a	0.112± 0.05 ^a	0.113± 0.01 ^a

Mean values in each raw having different superscript letters are significantly different at p≤0.05.

Effect of beets and Rosemary on hemoglobin, packed cell volume and RBCs pattern of rats administrated CIS

In Table (2), the control (+ve) group showed a significant decrease in hemoglobin, packed cell volume and RBCs but beets, rosemary and mixture rat groups showed non-significant difference in compared with control (-ve) group. The beets and rosemary and mixture treated groups showed a significant increase in hemoglobin, packed cell volume and RBCs compared with control (+ve) group. Administration of CIS reduces hemoglobin concentration in the blood accompanied with erythrocytosis with an increase in the packed cell volume levels and lymphocytosis with neutropenia **Lee et al., (2015)**. Beets show hepatoprotective properties by acting as an antioxidant and probably are mild iron chelators **Lu et al., (2009)**. The nitrates found naturally in beets protect blood vessels and keep them healthy. Beet containing betaine, betanin, flavonoids, and other polyphenols has a beneficial effect on liver injury caused by ischemia-reperfusion (**László et al., 2014**).

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Long-term daily intake of rosemary has an antithrombotic effect, which is probably due to inhibition of platelets and stimulation of endothelial cells. Flavonoid content of rosemary may be responsible for the antithrombotic effect. Flavone-8-acetic acid prolonged bleeding time. The mechanism of the antithrombotic effect may involve suppression of platelet reactivity and stimulation of the vascular endothelium (Mruk *et al.*, 2018 and Aki *et al.*, 2008).

Table (2): The Mean values \pm SD of hemoglobin (HB), packed cell volume (PCV) and RBCs parameters in control and CIS treated rats groups

Variables	Control (-ve)	CIS			
		Control (+ve)	Beet	Rosemary	Mixture
HB (g/dl)	14.34 \pm 1.99 ^a	10.41 \pm 1.33 ^b	13.99 \pm 1.31 ^a	13.89 \pm 1.01 ^a	14.11 \pm 1.77 ^a
PCV	40.11 \pm 4.77 ^a	30.17 \pm 3.33 ^b	35.01 \pm 4.17 ^a	36.55 \pm 4.11 ^a	38.71 \pm 4.22 ^a
RBCs (x10 ⁶ / μ l)	5.11 \pm 0.99 ^a	2.29 \pm 0.82 ^{b c}	4.37 \pm 1.03 ^a	4.92 \pm 1.11 ^a	5.21 \pm 1.31 ^a

Mean values in each row having different superscript letters are significantly different at $p \leq 0.05$.

Effect of beets and rosemary on liver function pattern of rats administrated CIS

Table (3) showed that, control (+ve) group showed a significant increase in serum ALT, AST and alkaline phosphatase enzymes activity while the beets, rosemary and mixture groups showed non-significant difference in compared with control (-ve) group. The beet, rosemary and mixture treated groups showed significant decrease in ALT, AST and alkaline phosphatase enzymes activity compared with control (+ve) group. It is known that, the enzymatic activity depends upon the extent of inflammatory or necrobiotic damage to the hepatocytes, and residual parenchymal mass. Accordingly, subnormal, normal or differently increased values of ALT & AST are found. With the progressive loss of liver parenchyma, the transaminase values fall to the normal range, and occasionally even to subnormal levels (Amodio *et al.*, 2011). Table beet ingestion can increase formaldehyde concentration which is the marker of increased biological methylation / demethylation and simultaneous positive change in the antioxidant parameters. The additive effect of endogenous transmethylation helped to improve liver function during hepatic ischemia reperfusion injury (László *et al.*, 2014). Rosemary constituents are composed of vast numbers of polyphenolics, including carnosic acid, carnosol, rosemarinic acid, ursolic acid (Ramirez *et al.*, 2016).

Table (3): The Mean values \pm SD of serum amino transferase (ALT and AST), and alkaline phosphatase enzymes (Alk-phos), of control and CIS treated rats groups

Variables	Control (-ve)	CIS			
		Control (+ve)	Beet	Rosemary	Mixture
ALT (μ /ml)	31.13 \pm 4.51 ^b	55.71 \pm 8.14 ^a	38.71 \pm 4.89 ^b	34.81 \pm 4.01 ^b	35.71 \pm 3.11 ^b
AST (μ /ml)	60.13 \pm 6.10 ^b	119.31 \pm 13.11 ^a	70.33 \pm 8.40 ^b	65.91 \pm 7.11 ^b	66.77 \pm 8.4 ^b
Alk-hos (μ /ml)	42.71 \pm 5.02 ^b	60.78 \pm 7.01 ^a	45.41 \pm 4.31 ^b	43.33 \pm 5.11 ^b	44.77 \pm 5.06 ^b

Mean values in each row having different superscript letters are significantly different at $p \leq 0.05$.

Effect of beets and rosemary on serum bilirubin, total protein, albumin, globulin and albumin/globulin ratio (A/G) pattern of rats administrated CIS

Table (4) showed that, control (+ve) group showed a significant increase in serum bilirubin and A/G ratio and significant decrease in serum total protein, albumin and globulin in compared with control (-ve) group. Beets, rosemary and mixture treated groups showed significant increase in serum bilirubin and non-significant difference in serum total protein, albumin, globulin and A/G ratio except rosemary group which showed significant decrease in albumin comparing with control (-ve) group. The beets, rosemary and mixture rat groups showed significant decrease in bilirubin and A/G ratio and significant increase in total protein, globulin and albumin except rosemary group which showed no significant difference in albumin when compared with control (+ve) group. The protein and amino-acid metabolism of liver is characterized by production and breakdown of proteins, production and breakdown of amino acids as well as regulation of their concentrations in the blood, and detoxification of ammonium via the synthesis of urea (excretory form) and glutamine with simultaneous regulation of the acid-base balance. The breakdown of branched-chain amino acids occurs exclusively in the muscular system by way of deamination (Gjoen *et al.*, 2016). The hypoproteinemia is mostly associated with a reduction in albumin synthesis as found in liver disease or with excessive loss. The alteration in serum albumin is found more commonly in chronic liver disease. Therapeutic or prophylactic administration of an antioxidant leads to an increase in the level of albumin in the serum (Yokogawa *et al.*, 2006). Beet is a potent methyl donor in transmethylation help in liver protection. The cellular mechanisms of apoptosis and cell proliferation are affected by formaldehyde that plays a key role in protection against oxidative stress Kahlon *et al.*, (2012). Rosemary leaves contain carnosic acid which has antimicrobial activity, can inhibit lipid absorption in humans and is a free radical scavenger, due to its phenolic skeleton Ninomiya *et al.*, (2014) and Moreno *et al.*, (2016).

Table (4): The Mean values ± SD of serum bilirubin, total protein, albumin, globulin and albumin/globulin ratio (A/G) of control and CIS treated rats groups

Variables	Control (-ve)	CIS			
		Control (+ve)	Beet	Rosemary	Mixture
Bilirubin (mg/dl)	0.29±0.01 ^c	1.12±0.12 ^a	0.37±0.03 ^b	0.34±0.04 ^{b*}	0.30±0.01 ^{b*}
T. protein (g/dl)	7.55±1.51 ^a	4.01±0.91 ^b	6.31±1.31 ^a	6.77±1.21 ^a	7.21±1.49 ^a
Albumin (g/dl)	3.71±0.81 ^a	2.99±0.91 ^b	3.64±0.81 ^a	2.98±0.95 ^{b*}	3.61±0.95 ^a
Globulin (g/dl)	3.84±0.87 ^a	1.02±0.97 ^b	3.97±0.65 ^a	3.89±0.55 ^a	3.80±0.45 ^a
A/G ratio	0.96±0.01 ^b	2.93±0.33 ^a	0.92±0.04 ^b	0.77±0.05 ^b	0.95±0.04 ^b

Mean values in each raw having different superscript letters are significantly different at p≤0.05.

Effect of beets and rosemary on serum of serum antioxidant pattern of rats administrated CIS

The data in table (5) demonstrated that, control (+ve) group showed significant decrease in the values of liver GSH and GPX were and significant increase in the values of liver MDA compared with control (-ve) group. On the other side, the beet, rosemary and mixture treated groups showed non-significant difference in liver GSH,GPX and MDA except beet treated group showed a significant decreased in GSH compared with control (-ve) group. The beet, rosemary and mixture treated groups showed significant increase in the values of GSH and GPX, while was and significantly decrease in the values of liver MDA except beets group showed no significant difference in GSH in comparison with control (+ve) group. It is well known that cisplatin is metabolized by mixed function oxidase system in the endoplasmic reticulum of the liver. Hepatic lipid peroxidation was significantly correlated with GPX activity and related to an increase in free radical production. Glutathione reduces both hydrogen peroxide and organic peroxides, in reactions catalyzed by glutathione peroxidase and glutathioneS-transferase **Cabre et al., (2010) and Szymonik et al., (2013)**. The consumption of table beet has numerous beneficial physiologic effects, some of which may be appropriate for the protection of the liver of presurgical patients. Because beet contains a great number of different components, it may be able to modulate various cellular pathways. The beneficial medical effect is due to bioactive components, such as betaine, betanin, betaxanthins, flavonoids, polyphenols, vitamins (thiamine, riboflavine, pyridoxine, ascorbic acid), folic acid, and biotin, and soluble fiber and pectin. Mainly betanin, flavonoids as quercetin, and other polyphenols have extremely effective antioxidant properties (**Craig, 2017**). The antioxidant and pro-oxidant properties of active rosemary constituents are powerful inhibitors of lipid peroxidation in microsomal and liposomal systems, and good scavengers of peroxy radicals generated by pulse radiolysis. Four diterpenoids, carnosic acid, carnosol, rosmanol, and epirosmanol, isolated as antioxidants from the leaves of rosemary. These diterpenoids protected biological systems against oxidative stresses **Keith, (2015)**.

Table (5): The Mean values ± SD of some liver glutathione (GSH), glutathione peroxidase (GPX) and malondialdehyde (MDA) in control and CIS treated rats groups

Variables	Control (-ve)	CIS			
		Control (+ve)	Beet	Rosemary	Mixture
GSH (mg/g)	4.56± 0.91 ^a	2.01± 0.51 ^b	3.76± 0.41 ^{bc}	3.91± 0.29 ^a	4.24± 0.28 ^a
GPX (µg /mg)	55.34 ± 9.41 ^a	39.17± 6.71 ^b	49.71± 6.91 ^a	50.71± 7.35 ^a	54.31± 7.14 ^a
MDA (nmol/g)	39.46± 5.71 ^b	89.31± 10.33 ^a	45.22± 7.11 ^b	43.13± 8.29 ^b	40.18± 7.10 ^b

Mean values in each row having different superscript letters are significantly different at p≤0.05.

Effect of beets and rosemary on serum of some liver glycogen, cholesterol, total lipids and triglyceride pattern of rats administrated CIS

The data in Table (6) demonstrated that, control (+ve) group showed significant decrease in the values of liver glycogen and triglyceride and significant increase in the values of liver cholesterol and total lipid when compared with control (-ve) group. On the other side, the beet, rosemary and mixture groups showed non-significant difference in the liver glycogen ,cholesterol , total lipid and triglyceride compared with control (-ve) group. Treated with beet, rosemary and mixture groups showed significant increase in the values of glycogen and triglyceride and significant decrease in the values of liver cholesterol and total lipid in comparison with control (+ve) group. In liver cirrhosis, the glycogen content of the liver and glycogenolysis are reduced to 20-30% of the normal level. The clearly decreased glycogenolysis is compensated by increased gluconeogenesis (three or four times above normal). Absorption of glucose in the liver is reduced, while the release of glucose is unaffected. These operate through a reduction in de novo cholesterol synthesis, an increase in cholesterol elimination in bile secretions, and an inhibition of LDL oxidation **Keith, (2015)**. Red beet contains natural red pigments such as betalein, which considered with potential antioxidative properties. Red beet showed antioxidant ability against LD L oxidation and ROS production in human mononuclear cells **Sugihara et al., (2009)**. The most important constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid and carnosic acid, which have antioxidant effects and therapeutic potential in the treatment or prevention inflammatory diseases, hepatotoxicity, atherosclerosis **Al-Sereiti et al., (2016) and Mruk et al., (2018)**

Table (6): The Mean values ± SD of some liver glycogen, cholesterol, total lipids and triglycerides in control and CIS treated rats groups

Variables	Control (-ve)	CIS			
		Control (+ve)	Beet	Rose- mary	Mixture
Glycogen (mg/100g)	5.44± 1.14 ^a	2.99± 0.33 ^{b**}	4.04± 1.22 ^a	4.55± 1.31 ^a	5.01± 1.55 ^a
Cholesterol (mg/dl)	3.77± 1.30 ^b	7.11± 2.14 ^a	4.71± 1.17 ^b	4.33± 1.31 ^b	4.53± 1.35 ^b
Total lipids (mg/dl)	37.63± 3.36 ^b	49.33± 4.11 ^a	38.54± 3.20 ^b	38.45± 4.18 ^b	39.44± 4.19 ^b
Triglyceride (mg/dl)	2.33± 0.41 ^a	1.77± 0.01 ^b	2.07± 0.22 ^a	2.11± 0.11 ^a	2.23± 0.44 ^a

Mean values in each raw having different superscript letters are significantly different at p≤0.05.

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التأثير الوقائي لجذور البنجر وحصالبان ضد الإصابة بالإجهاد

التأكسدي الكبدي في فئران

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الملخص: تحتوى كلا من جذور البنجر وإكليل الجبل على المكونات الغذائية النشطة بيولوجيا التي لها العديد من خصائص علاجية في الإصابة الكبدية. تهدف هذه الدراسة إلى تقييم تأثير كلا من جذور البنجر وإكليل الجبل على الحد من أعراض الإصابة الكبدية. وأجريت هذه الدراسة على 30 من الفئران يتراوح أوزانهم بين 125 ± 5 جرام وتم تقسيمهم إلى 6 مجموعات المجموعة الضابطة السالبة التي تغذت على الوجبة القياسية. وتم حقن المجموعات الأربعة الأخرى تحت الجلد بواسطة السيسيلاتين للحث على إصابة الكبدية ثم قسمت إلى المجموعة الموجبة والمجموعات المعالجة بـ 10% جذور البنجر و 10% من حصالبان والمخلوط منهما. وقد أسفرت النتائج المتحصل عليها فيما يلي ان المجموعة الضابطة الموجبة اظهرت انخفاض معنوي في الوزن النهائي و المكتسب و نسبة كفاءة الطعام و الهيموجلوبين و (PCV) وكرات الدم الحمراء و البروتين الكلي و الالبيومين و الجلوبيلين في السيرم ونسبة الالبيومين الى الجلوبيلين وايضا (GSH, GPX) و الجليكوجين و الجليسيريدات الثلاثية في الكبد، وارتفاع معنوي في MDA و الكولسترول والدهون الكلية في الكبد و (ALT, AST, ALP) و البيلروبين في السيرم بالمقارنة بالمجموعة الضابطة السالبة إما مجموعة حصالبان فقد أظهرت انخفاض معنوي في الالبيومين بينما مجموعة البنجر التي اظهرت انخفاض معنوي في GSH و ارتفاع معنوي في البيلروبين في السيرم بالمقارنة بالمجموعة الضابطة السالبة. وعلى الجانب الاخر ، فمجموعات البنجر وحصالبان والمخلوط منهما اظهرت انخفاض معنوي في ALT, AST and ALP enzymes activity في السيرم ونسبة الالبيومين الى الجلوبيلين و البيلروبين و MDA و الكولسترول والدهون الكلية في الكبد كما اظهرت ارتفاع معنوي في الوزن النهائي و المكتسب و نسبة كفاءة الطعام و الهيموجلوبين و (PCV) و كرات الدم الحمراء و البروتين الكلي و الالبيومين و الجلوبيلين في السيرم وايضا (GSH, GPX) و الجليكوجين و الجليسيريدات الثلاثية في الكبد ما عدا مجموعة حصالبان فقد اظهرت عدم تواجد اختلاف معنوي في الالبيومين بينما مجموعة البنجر اظهرت عدم تواجد اختلاف معنوي في GSH بالمقارنة بالمجموعة الضابطة الموجبة. كما أظهرت النتائج الباثولوجية لأنسجة الكبد وجود تغيرات مرضيه واضحة في المجموعة الضابطة الموجبة و انخفضت هذه التغيرات في باقي مجموعات الدراسة. وتوصى الدراسة بضرورة تناول البنجر وحصالبان للوقاية و المحافظة على كفاءة الكبد مما ينعكس على الصحة العامة.

الكلمات المفتاحية: وظائف الكبد، الجلوتاثيون، المالنوالدهيد، الانزيمات المضادة للأكسدة، الفئران.