Original Article

Hepatoprotective Effect of Beetroot Juice on Liver Injury in Male Sprague–Dawley Rats

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Abstract

Background: The ability of plant extract to improve injury in the liver has gained interest in recent times. This could be due to the side effects and expense of modern medicines that are used to manage hepatic diseases; hence beetroot juice as a potential hepatoprotective agent was evaluated. **Materials and Methods:** Thirty Sprague–Dawley rats were assigned by weight into six groups average body weight (160.20 ± 2.54 g). Group I: rats plus distilled water (Normal control) 2 ml/kg; Group II: rats received olive oil (2 ml/kg); Group III: rats received carbon tetrachloride (CCl_4) suspended in olive oil (2 ml/kg) and 250 mg/kg of beetroot extract. Group IV: rats received CCl_4 suspended in olive oil (2 ml/kg) and 500 mg/kg of beetroot extract. Group V: rats received olive oil (2 ml/kg) and 100 mg/kg of silymarin; Group VI: rats received (2 ml/kg) CCl_4 suspended in olive oil. Liver injury was induced by oral administration of CCl_4 using gastric gavage at 2 ml/kg every 48 h for 14 days, followed by treatment with beetroot extract and silymarin. Animals were euthanized by decapitation, blood and liver tissue harvested for biochemical and histopathological evaluations. **Results:** Alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase levels were significantly ($P < 0.05$) increased in the groups treated with extract and silymarin when compared to the animals administered only CCl_4, whereas malondialdehyde level was significantly ($P < 0.05$) increased in the group administered only CCl_4 when compared to other groups. Histopathologically, the animals treated with 500 mg/kg of extract showed a normal hepatocyte and mild portal congestion. **Conclusion:** Beetroot juice has potential hepatoprotective effects on the liver in a dose-dependent manner.

Keywords: Beetroot juice, carbon tetrachloride, hepatoprotective, liver injury

Introduction

The well-documented health benefits of a diet high in fruit and vegetables have led to a growing interest in functional foods and their application in health and disease.[1] Herbal medicine is gaining popularity in developing countries as it has been estimated that 80% of the world population still depend mainly on traditional medicine and traditional treatment involving the use of plant extract.[2] Nigeria is richly endowed with indigenous plants which are used in herbal medicine to cure diseases and heal injuries.[3] Some of the plants are used as food or medicine. These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, antidiabetic, and antimicrobial functions.[3]

Cultivars of *Beta vulgaris* are grown throughout Europe and North America and in some part of West Africa. Beetroots are grown mainly in Jos, Plateau State of Northern Nigeria. This could be probably because beets develop best under cool conditions.[4] Beets are grown primarily for the enlarged bulbous root, which forms near or just above the soil surface. The plant is naturally a biennial, producing a rosette of leaves and a bulbous root one year, and a seed stalks the following year. Beets and their relatives are grown throughout the world for human and stock food. Sugar beets and Chard are among the more familiar types.[5]

Beetroot is a rich source of phytochemical compounds that includes ascorbic acid, carotenoids, phenolic acids, and flavonoids.[6-8] Beetroot is also one of the few vegetables that contain a group of highly bioactive pigments known as betalains.[9,10] A number of investigations have reported betalains

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to have anti-inflammatory and antioxidant capabilities *in vitro* and a variety of *in vivo* animal models.[10-12] This has sparked interest in a possible role for beetroot in clinical pathologies characterized by oxidative stress and chronic inflammation such as liver disease,[1,10] arthritis,[13] and cancer.[14-17] Beetroot's effect on the vascular endothelium of the blood vessels increases the blood flow, and this is largely attributed to its high inorganic nitrate, betaine and NO contents.[18] Nitrate is not the only constituent of beetroot proposed to have beneficial effects in health and disease.

The liver as a vital organ in the body is primarily responsible for the metabolism of endogenous and exogenous agents. It plays an important role in drug elimination and detoxification and liver damage may be caused by xenobiotics, alcohol consumption, malnutrition, infection, anemia, and medications.[19] Hepatotoxic agents can react with the basic cellular components and consequently induce almost all types of liver lesions. Chemical toxins (including acetaminophen, carbon tetrachloride (CCl_4), galactosamine, and thioacetamide) are often used as the model substances causing experimental hepatocyte injury in both *in vivo* and *in vitro* conditions.[20,21] CCl_4 treatment results in centrilobular steatosis, inflammation, apoptosis, and necrosis.[22-24] If the damage exceeds the repair capacity of the liver, it will progress to fibrosis and cirrhosis.[22,23]

A number of studies have shown that plant extracts having antioxidant activity protect against CCl_4 hepatotoxicity by inhibiting lipid peroxidation and enhancing antioxidant enzyme activity.[25] This study is to determine the hepatoprotective effect of aqueous extract of *Beta vulgaris* on Sprague–Dawley rats.

Materials and Methods

Plant material and extraction

Beetroot (*Beta vulgaris*) was obtained from a local vendor in a vegetable market on Airport Road, Benin City, Nigeria. The plant was identified by a Taxonomist at the Department of Plant Biology and Biotechnology at the University of Benin and a voucher number UBH_B 374 was deposited in the Herbarium. The beetroots were rinsed with clean water, peeled, and blended. The juice was extracted from the beetroot using a muslin cloth and distilled water and the shaft discarded. The juice obtained was stored in an airtight container (10 kg of beetroot yielded approximately 3 L of juice after extraction) and allowed to settle for 20–30 min. The juice obtained was freeze dried [Armfield vacuum freeze dryer Model FT 33, England] and ready for use. The freeze-dried sample is composed of a 10% beetroot juice containing 9808.0 mg GAE/100 ml polyphenols and 8334.0 mg QE/100 ml flavonoids.[26] The freeze-dried sample was stored at 0°C until ready for use.

Phytochemical screening

The freeze-dried beetroot was screened for the presence of saponins (foam test), alkaloids (Dragendorff's reagent), tannins (gelatin test), phenols (ferric chloride test), flavonoids (lead acetate test), and phytosterols (Keller–Killani and the Salkowski test).[27,28] A 10 g freeze-dried beetroot sample was dissolved in 100 ml distilled water, methanol, ethanol, and chloroform, respectively. Thereafter, they were homogenized to a uniform consistency with an Ultra-Turrax homogenizer (USA). The filtrate obtained from the filtered extract was used for the screening.

Animals

Thirty healthy Sprague–Dawley rats, male and female of average body weight (160.20 ± 2.54 g) were grouped according to sex and weight into six groups of five animals each. They were obtained from the animal house in the Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were housed in spacious plastic cages with animal beds in the animal house of the Department of Medical Biochemistry, University of Benin, Nigeria. The animals were acclimatized for two weeks and maintained under standard conditions of temperature (23°C ± 2°C) and (12 h light/dark cycle). The rats were fed standard pelleted feed (Top Feeds; Premier Feed Mills Co. Ltd., Ibadan, Nigeria) for two weeks. Before the study, food and water were given *ad libitum*. Approval for the study was obtained after a review of the protocol by the Ethical Committee of the College of Medical Sciences, University of Benin, Benin City, Nigeria, with number CMS/REC/2016/002.

Hepatotoxicity

Thirty healthy Sprague–Dawley male rats were assigned by weight into six groups (five rats per group, average body weight [160.20 ± 2.54 g]). The six groups are composed as follows: Group I: Healthy rats that received 2 ml/kg distilled water (normal control); Group II: Healthy rats that received 2 ml/kg olive oil (normal plus, olive oil); Group III: Healthy rats which received 2 ml/kg CCl_4 in olive oil (negative control); Group IV: Healthy rats that received 2 ml/kg CCl_4 in olive oil and 250 mg/kg body weight of beetroot extract/day. Group V: Healthy rats which received 2 ml/kg CCl_4 in olive oil and 500 mg/kg body weight of beetroot extract/day. Group VI: Healthy rats that received 100 mg/kg body weight of silymarin/day and 2 ml/kg CCl_4 in olive oil (positive control). Liver injury was induced by the oral administration of 40% concentration of CCl_4 in olive oil (GOYA Extra Virgin Olive oil, Manufactured by Goya En Espana, S. A. U. Sevilla, Spain) which served as a vehicle at a dose of 2 ml/kg body weight every 48 h (between 8.00 am and 9.00 am) for 14 days using gastric gavage which was modified.[29] Animals were fasted overnight and euthanized by decapitation. Blood was collected for biochemical evaluation, while the liver was dissected, freed of adherent tissues, and weighed.

Determination of serum markers in liver damage

The serum from each group was used to determine the levels of alanine aminotransferase (ALT: Randox, UK. Cat. No AL 100), aspartate aminotransferase (AST: Randox, UK. Cat. No AS 101), alkaline phosphatase (ALP: Teco Diagnostics, USA. A506),

and total protein (Randox, UK. Cat. No TP 245) using commercial kits according to the manufacturer's direction.

Measurement of lipid peroxidation in liver tissue

The extent of lipid peroxidation was determined by measuring thiobarbituric acid (TBA) reactive substances, in terms of malondialdehyde (MDA) formation.[30] To 0.6 ml of the liver homogenate sample in test tubes, 0.3 ml of trichloroacetic acid (TCA)-TBA-HCl mixture was added and incubated in laboratory water bath (SurgiFriend Medicals, England) at boiling temperature for 15 min. The content of each test tube was allowed to cool and then centrifuged for 10 min to remove flocculent precipitates (Centrifuge Model 80-2; Uniscope SM 801 A at 2130 ×g, Max speed: 4000 r/min, CHINA). The absorbance of each supernatant was read at 532 nm against a blank which contained 0.3 ml of TCA-TBA-HCl solution dissolved in 0.6 ml of distilled water. The concentration of MDA was determined.

Histopathological examination

The liver harvested was trimmed of any adherent tissue and placed in sterile containers, containing 10% buffered formalin. The tissues were cut so that they can enter the cassettes and the areas of interest exposed for processing. The processing was done by removing the tissue from buffered formalin and dehydrated by passing it through graded concentration of alcohol starting from the lowest (70%, 90%, 95%, and absolute). The alcohol in the tissues was cleared with xylene and the latter was replaced with paraffin wax by impregnation. Staining was done with hematoxylin and eosin, microtome was used to section at 5 μ and trimmed 10 microns. The processed tissues on the slides were analyzed microscopically.

Statistical analysis

Biochemical data were expressed as mean ± standard error of the mean. The difference between the groups was tested using ANOVA. Duncan's multiple range test was used to test for significant difference among the means ($P < 0.05$).

Results

Biochemical assessment

The phytochemical screening of beetroot revealed the presence of phenols, alkaloids, tannins, flavonoids, saponins, phytosterols, and glycosides. The phytochemical determination of beetroot as assessed is contained in Table 1. The weight of liver was significantly ($P < 0.05$) reduced in the group of animals administered only olive oil when compared to other groups [Table 2]. The ALT level was significantly ($P < 0.05$) increased in the group of animals administered 250 mg of beetroot extract and 100 mg/kg body weight of silymarin when compared to the animals that were treated with only CCl_4 [Figure 1]. There was a significant ($P < 0.05$) increase in AST level on administering 250 and 500 mg/kg body weight of extract when compared to the group that was treated with only CCl_4 [Figure 1]. The level of ALP was significantly ($P < 0.05$) reduced at 250 mg/kg of extract, while at 100 mg/kg of silymarin it was significantly ($P < 0.05$) increased when compared to negative control [Figure 1].

Table 1: Phytochemical screening of beetroot extract

Phytochemicals	Aqueous	Methanol	Ethanol	Chloroform
Phenols	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	++	++	++	+
Phytosterols	+	+	+	+
Alkaloids	+	+	+	+
Glycosides	+	+	+	+

The data showed that beetroot is richest in saponins (aqueous, methanol, and ethanol medium) as its major constituent of dry matter. +: Positive or present

Table 2: Effect of beetroot extract on relative liver weight in Sprague-Dawley rats

Groups	Relative liver weight (g)
Group 1 (normal rats + distilled water)	3.46±0.14^b
Group II (normal rats + 2 ml/kg olive oil)	2.64±0.88^a
Group III (normal rats + 2 ml/kg CCl_4)	3.69±0.12^b
Group IV (normal rats + 250 mg extract + 2 ml/kg CCl_4)	3.71±0.24^b
Group V (normal rats + 500 mg extract + 2 ml/kg CCl_4)	3.61±0.27^b
Group VI (normal rats + 100 mg Silymarin + 2 ml/kg CCl_4)	3.89±0.08^b

Values are liver weights in (g) and are expressed as mean±SEM (n=5). Data that share different superscripts are significantly different (P<0.05). CCl_4: Carbon tetrachloride, SEM: Standard error of mean

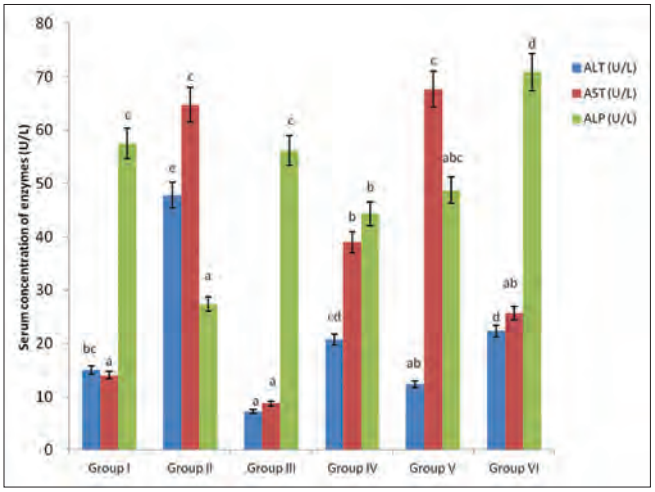

Figure 1: Effect of oral administration of different doses of beetroot extract and silymarin on serum liver enzymes in Sprague–Dawley rats. Serum concentrations of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase in the liver were expressed as mean ± standard error of the mean. Values with different superscripts are significantly different when $P < 0.05$. Group 1 (normal rats + distilled water); Group II (normal rats + olive oil); Group III (normal rats + CCl_4); Group IV (normal rats + 250 mg extract + CCl_4); Group V (normal rats + 500 mg extract + CCl_4); Group VI (normal rats + 100 mg silymarin + CCl_4)

The concentration of total protein was significantly ($P < 0.05$) reduced in the group of animals administered 500 mg of beetroot extract when compared to the other groups [Figure 2]. Nevertheless, the level of MDA was significantly ($P < 0.05$) increased in the group of animals that were administered only CCl_4 when compared to the tested doses of extract and silymarin [Figure 3].

Histopathological analysis

The histological examination of the liver of rats treated with aqueous extract of beetroot showed a well-formed normal hepatocyte and a mild portal congestion at 500 mg/kg body weight compared to the controls (Plates 1, 2, and 3) [Figure 4].

Discussion

To ascertain whether the freeze-dried beetroot juice extract would ameliorate liver damage, rats were treated with 250 and 500 mg of the beetroot extract and 100 mg silymarin. CCl_4 intoxication is used for modeling liver injury in rats. Hepatotoxicity of CCl_4 is the result of cytochrome P-450 dependent reductive dehalogenation to form a highly reactive trichloromethyl free radical CCl_3.[31] CCl_4-induced damage is characterized by hepatocyte membrane damage, caused by lipid peroxidation, increased plasma levels of hepatic enzymes, fatty degeneration (steatosis, i.e. accumulation of triglycerides in the liver), reduced β-oxidation of fatty acids, and necrosis.[32,33]

MDA is a reactive aldehyde that could be cytotoxic.[22] Since it is formed as a by-product of lipid peroxidation, it can also be used as an indicator of the amount of lipid peroxidation. The free radicals produced by the metabolism of CCl_4 attack polyunsaturated fatty acids in the cellular membrane, initiates lipid peroxidation, and ultimately resulting in a loss in membrane integrity.[22,23] In this study, we found that the organ weight ratio were significantly ($P < 0.05$) increased in negative, positive, and treated groups when compared to the control groups; this may be due to the highly reactive trichloromethyl free radical generated from CCl_4 which had predilection for liver membrane, thereby eliciting inflammatory processes.

A single dose of CCl_4 leads to centrizonal necrosis and steatosis,[34] while prolonged administration leads to liver failure, cirrhosis, and hepatocellular carcinoma.[35] Overproduction of trichloromethyl-free radicals is considered the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell apoptosis and necrosis.[23,36] The evidence accumulating presently suggests that free radicals can oxidatively damage mitochondria, regulate gene expression, thereby contributing to the development of fibrosis and can perpetuate chronic inflammatory processes.[37] In chronic hepatocyte injury, mainly in cirrhosis, ALT is more commonly elevated than AST, however, as fibrosis progresses, ALT activities typically decline, and the ratio of AST to ALT gradually increases, so that by the time cirrhosis is present, AST is often higher than ALT.[38,39] Increases in serum AST, ALT, and ALP levels by CCl_4 have been attributed to hepatic structural damage because these enzymes are normally localized to the cytoplasm and are released into the circulation after cellular damage has occurred.[40] Cellular membranes that are weak allow sufficient leakage of calcium into the cytosol which disrupts intracellular calcium homeostasis. High calcium levels in the cytosol activate calcium-dependent proteases and phospholipases that can increase the breakdown of membranes.

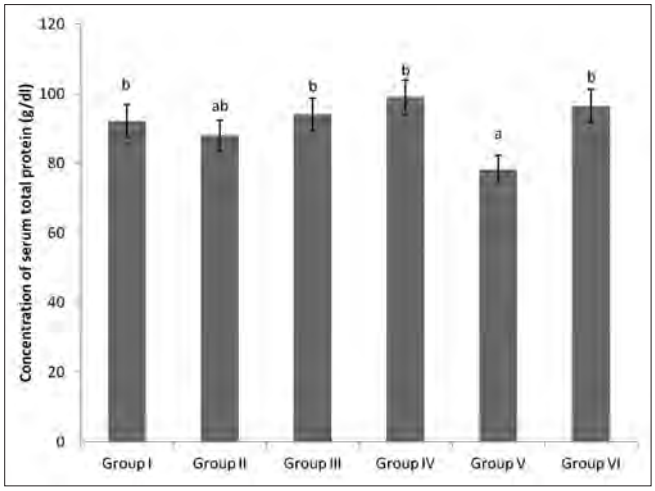

Figure 2: Effect of oral administration of different doses of beetroot extract and silymarin on serum protein in Sprague–Dawley rats. Serum concentration of protein was expressed as mean ± standard error of the mean. Values with different superscripts are significantly different when $P < 0.05$. Group 1 (normal rats + distilled water); Group II (normal rats + olive oil); Group III (normal rats + CCl_4); Group IV (normal rats + 250 mg extract + CCl_4); Group V (normal rats + 500 mg extract + CCl_4); Group VI (normal rats + 100 mg silymarin + CCl_4)

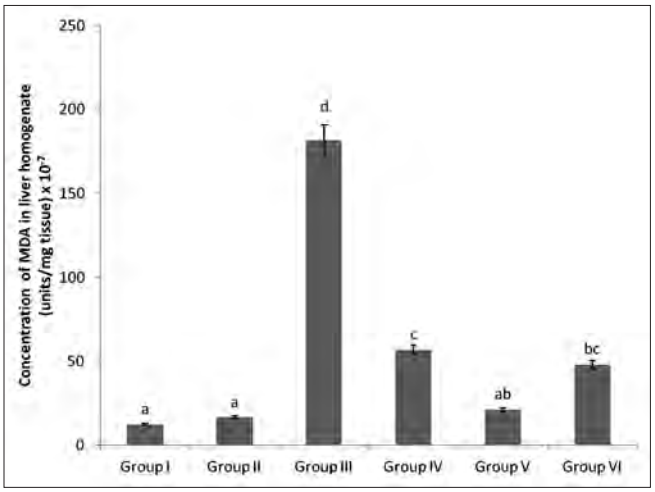

Figure 3: Effect of oral administration of different doses of beetroot extract and silymarin on malondialdehyde concentration in the liver homogenate of Sprague–Dawley rats. Homogenate concentration of in the liver was expressed as mean ± standard error of the mean. Values with different superscripts are significantly different when $P < 0.05$. Group 1 (normal rats + distilled water); Group II (normal rats + olive oil); Group III (normal rats + CCl_4); Group IV (normal rats + 250 mg extract + CCl_4); Group V (normal rats + 500 mg extract + CCl_4); Group VI (normal rats + 100 mg silymarin + CCl_4)

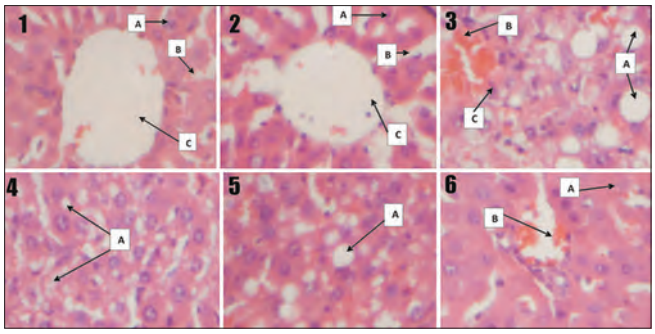

Figure 4: Photomicrograph of sections of the rat liver after 14 days of treatment with silymarin, 250 mg and 500 mg of extract (plates 4, 5, and 6, respectively) compared to control (normal, olive oil and CCl_4; Plates 1, 2, and 3, respectively). Plates 1 and 2: A-hepatocyte, B-sinusoids, C-central vein. Plate 3: A-macrovesicular steatosis, B-moderate vascular congestion, C-mild periportal infiltrates of inflammatory cells. Plate 4: A-patchy macrovesicular steatosis (100 mg silymarin). Plate 5: A-patchy macrovesicular steatosis (250 mg of extract). Plate 6: A-normal hepatocyte and B-mild portal congestion (500 mg of extract). Sections were stained with H and E ×400

This ultimately increases intracellular calcium, which activates endonucleases that can cause chromosomal damage and also contribute to cell death.[22] In this study, the level of AST was significantly ($P < 0.05$) reduced in the group administered only CCl_4 when compared to the groups treated with beetroot juice extract and silymarin. This reduction is probably not unconnected with the necrosis and steatosis processes that may have occurred in the liver, however, on the administration of the tested doses of extract and silymarin, there was an upregulation of the enzyme activities. Similarly, the leakage of ALT from a compromised liver that has undergone chemical assault will have its concentration highly elevated in the serum. In end-stage cirrhosis, the levels of ALT and AST enzymes are generally not elevated and may be low due to massive tissues destruction.[41] Interestingly, this study showed that the activity of ALT was significantly ($P < 0.05$) reduced in the group of rats that had only CCl_4. However, the group of animals treated with 250 mg/kg body weight of beetroot extract/day for 14 days showed a significant ($P < 0.05$) increase in ALT when compared to the negative control. It was also observed that at 500 mg of extract and 100 mg/kg body weight of silymarin, there was upregulation of ALT concentration. It is proposed that these increases may be due to the extract's ability to regenerate liver tissue by interrupting necrosis or fibrosis processes, thereby enhancing the production of new cells. The observed elevation of ALP in the groups treated with CCl_4 may be due to the stimulation of its production from other sources other than the liver. Total protein is a quantitative measurement of the concentration of all proteins present in serum which excludes clotting factors. It suggests the presence of an autoimmune component to chronic liver disease. Total protein could be elevated in a liver that has been assaulted with CCl_4. An increase in protein breakdown with an overall fall in whole-body protein turnover has been reported in patients with alcoholic cirrhosis.[42] When there is an elevated total proteins, it is an indication of increased catabolism of other proteins, in addition to collagen. This catabolism that is enhanced may be due to the extreme centrilobular necrosis of the liver tissue during hepatic fibrosis.[43] This study showed that at 500 mg/kg body weight of beetroot extract/day, there was a significant ($P < 0.05$) reduction in the serum total protein when compared to the group of animals that were treated with 250 mg of extract and silymarin. This healing effect of the tested dose (500 mg) on the liver may be that the extract was able to prevent lipid peroxidation and the accumulation of MDA which enhances collagen synthesis in hepatic fibrosis.

The elevation of MDA level in rats treated with CCl_4 is attributed to enhanced lipid peroxidation, leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals.[44] In this study, the level of MDA was significantly ($P < 0.05$) reduced in the group of rats administered beetroot extract or silymarin when compared to the negative control. The overwhelming significant ($P < 0.05$) reduction in MDA on administering 500 mg/kg body weight of beetroot to the rats may be attributed in part to its antioxidant and free radical scavenging abilities. This is evidenced in our histopathological evaluation which revealed that at a dose of 500 mg/kg body weight, there was a normal hepatocyte when compared to the 250 mg of extract and positive control (silymarin-treated group).

Conclusion

The results obtained from this study suggest that oral administration of 250 or 500 mg/kg of beetroot extract can ameliorate rat liver injury through possibly the interruption of apoptosis machinery or secondary necrosis. Further studies will be required to isolate its active ingredients and determine the mechanism of action.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ninfali P, Angelino D. Nutritional and functional potential of *Beta vulgaris* cicla and rubra. Fitoterapia 2013;89:188-99.
2. World Health Organization. Promoting the Role of Traditional Medicine in Health Systems: A Strategy for the African Region 2001-2010. Harare: World Health Organization; 2000.
3. Okwu DE, Nnamdi FU. Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial activity. J Chem Pharm Res 2011;3:1-10.
4. Mitchell SC. Food idiosyncrasies: Beetroot and asparagus. Drug Metab Dispos 2001;29:539-43.
5. Berglund L, Brunstedt J, Nielsen KK, Chen Z, Mikkelsen JD, Marcker KA, *et al.* A proline-rich chitinase from *Beta vulgaris*. Plant

Mol Biol 1995;27:211-6.
6. Georgiev VG, Weber J, Kneschke EM, Denev PN, Bley T, Pavlov AI, *et al.* 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 Hum Nutr 2010;65:105-11.
7. Kujala TS, Vienola MS, Klika KD, Loponen JM, Pihlaja K. Betalain and phenolic compositions of four beetroot (*Beta vulgaris*) cultivars. Eur Food Res Technol 2002;214:505-10.
8. Wootton-Beard PC, Brandt K, Fell D, Warner S, Ryan L. Effects of a beetroot juice with high neobetanin content on the early-phase insulin response in healthy volunteers. J Nutr Sci 2014;3:e9.
9. Lee CH, Wettasinghe M, Bolling BW, Ji LL, Parkin KL. Betalains, phase II enzyme-inducing components from red beetroot (*Beta vulgaris* L.) extracts. Nutr Cancer 2005;53:91-103.
10. Vulić JJ, Ćebović, TN, Čanadanović-Brunet JM, Ćetković GS, Čanadanović VM, Djila SM, *et al. In vivo* and *in vitro* antioxidant effects of beetroot pomace extracts. J Funct Foods 2014;6:168-75.
11. Tesoriere L, Allegra M, Butera D, Livrea MA. Absorption, excretion, and distribution of dietary antioxidant betalains in LDLs: Potential health effects of betalains in humans. Am J Clin Nutr 2004;80:941-5.
12. Vidal PJ, López-Nicolás JM, Gandía-Herrero F, García-Carmona F. Inactivation of lipoxygenase and cyclooxygenase by natural betalains and semi-synthetic analogues. Food Chem 2014;154:246-54.
13. Pietrzkowski Z, Nemzer B, Spórna A, Stalica P, Tresher W, Keller R, *et al*. Influence of betalin-rich extracts on reduction of discomfort associated with osteoarthritis. New Med 2010;1:12-7.
14. Das S, Williams DS, Das A, Kukreja RC. Beet root juice promotes apoptosis in oncogenic MDA-MB-231 cells while protecting cardiomyocytes under doxorubicin treatment. J Exp Second Sci 2013;2:1-6.
15. Kapadia GJ, Azuine MA, Rao GS, Arai T, Iida A, Tokuda H, *et al.* Cytotoxic effect of the red beetroot (*Beta vulgaris* L.) extract compared to doxorubicin (Adriamycin) in the human prostate (PC-3) and breast (MCF-7) cancer cell lines. Anticancer Agents Med Chem 2011;11:280-4.
16. Kapadia GJ, Azuine MA, Sridhar R, Okuda Y, Tsuruta A, Ichiishi E, *et al.* Chemoprevention of DMBA-induced UV-B promoted, NOR-1-induced TPA promoted skin carcinogenesis, and DEN-induced phenobarbital promoted liver tumors in mice by extract of beetroot. Pharmacol Res 2003;47:141-8.
17. Kapadia GJ, Rao GS, Ramachandran C, Iida A, Suzuki N, Tokuda H, *et al.* Synergistic cytotoxicity of red beetroot (*Beta vulgaris* L.) extract with doxorubicin in human pancreatic, breast and prostate cancer cell lines. J Complement Integr Med 2013;10. pii:/j/jcim. 2013.10.issue-1/jcim-2013-0007/jcim-2013-0007.xml.
18. Ormsbee MJ, Lox J, Arciero PJ. Beetroot juice and exercise performance. J Int Soc Sports Nutr 2013;5:27-35.
19. Mroueh M, Saab Y, Rizkallah R. Hepatoprotective activity of *Centaurium erythraea* on acetaminophen-induced hepatotoxicity in rats. Phytother Res 2004;18:431-3.
20. Domenicali M, Caraceni P, Giannone F, Baldassarre M, Lucchetti G, Quarta C, *et al.* A novel model of CCl4-induced cirrhosis with ascites in the mouse. J Hepatol 2009;51:991-9.
21. Rousar T, Kucera O, Kriváková P, Lotková H, Kandár R, Muzáková V, *et al.* Evaluation of oxidative status in acetaminophen treated rat hepatocytes in culture. Physiol Res 2009;58:239-46.
22. Manibusan MK, Odin M, Eastmond DA. Postulated carbon tetrachloride mode of action: A review. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2007;25:185-209.
23. Weber LW, Boll M, Stampfl A. Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. Crit Rev Toxicol 2003;33:105-36.
24. Shi J, Aisaki K, Ikawa Y, Wake K. Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride. Am J Pathol 1998;153:515-25.
25. Shahjahan M, Sabitha KE, Jainu M, Shyamala Devi CS. Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats. Indian J Med Res 2004;120:194-8.
26. Olumese FE, Oboh HA. Antioxidant and antioxidant capacity of raw and processed Nigerian beetroot (*Beta vulgaris*). Niger J Basic Appl Sci 2016;24:35-40.
27. Harbone JB. Phytochemical methods. A guide to modern techniques of plant analyses. 3rd ed. New York: Chapman and Hall Int; 1998.
28. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd.; 1993. p. 191-289.
29. Hismiogullari SE, Hismiogullari AA, Sunay FB, Paksoy S, Can M, Aksit H, *et al*. The proctective effect of curcumin on carbon tetrachloride induced liver damage. Revue Med Vet 2014;165:194-200.
30. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302-10.
31. McCay PB, Lai EK, Poyer JL, DuBose CM, Janzen EG. Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. Observation of lipid radicals *in vivo* and *in vitro*. J Biol Chem 1984;259:2135-43.
32. Heimberg M, Weinstein I, Dishmon G, Dunkerley A. The action of carbon tetrachloride on the transport and metabolism of triglycerides and fatty acids by the isolated perfused rat liver and its relationship to the etiology of fatty liver. J Biol Chem 1962;237:3623-7.
33. Boll M, Weber LW, Becker E, Stampfl A. Mechanism of carbon tetrachloride-induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. Z Naturforsch C 2001;56:649-59.
34. Pierce RA, Glaug MR, Greco RS, Mackenzie JW, Boyd CD, Deak SB, *et al.* Increased procollagen mRNA levels in carbon tetrachloride-induced liver fibrosis in rats. J Biol Chem 1987;262:1652-8.
35. Pérez Tamayo R. Is cirrhosis of the liver experimentally produced by CCl4 and adequate model of human cirrhosis? Hepatology 1983;3:112-20.
36. Basu S. Carbon tetrachloride-induced lipid peroxidation: Eicosanoid formation and their regulation by antioxidant nutrients. Toxicology 2003;189:113-27.
37. Albano E. Alcohol, oxidative stress and free radical damage. Proc Nutr Soc 2006;65:278-90.
38. Williams AL, Hoofnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. Gastroenterology 1988;95:734-9.
39. Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1998;93:44-8.
40. Recknagel RO, Glende EA Jr., Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 1989;43:139-54.
41. Pincus MR, Tierno P, Dafour R. editors. Evaluation of liver function In: Henry's Clinical Diagnosis and Management of Laboratory Methods. 21st ed. Philadelphia, USA: Sanders Elsevier; 2007. p. 269.
42. Morrison WL, Bouchier IA, Gibson JN, Rennie MJ. Skeletal muscle and whole-body protein turnover in cirrhosis. Clin Sci (Lond) 1990;78:613-9.
43. George J, Chandrakasan G. Biochemical abnormalities during the progression of hepatic fibrosis induced by dimethylnitrosamine. Clin Biochem 2000;33:563-70.
44. Shenoy KA, Somayaji SN, Bairy KL. Hepatoprotective effect of *Ginkgo biloba* against CCl4-induced hepatic injury in rats. Indian J Pharm 2001;33:260-6.