RESEARCH PAPER | Biochemistry | Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

Biological and Biochemical Efficiency of lyophilized Cranberry Extract on Regulation of Antioxidant Defense System in Nonalcoholic Steatohepatitic Rats

KEYWORDS: Nonalcoholic steatohepatitis, cranberry extract , Lipid parameters , Antioxidant enzymes, DNA fragmentation.

Rasha Hamed Mahmoud

Biochemistry Department, King Abdulaziz University ,Jeddah ,Saudi Arabia.
Biochemistry and Nutrition Department, Women College, Ain Shams University Cairo, Egypt

ABSTRACT *Cranberries are small, dark red fruits that are widely consumed as juice. Nonalcoholic steatohepatitis (NASH) is a condition that may progress to end-stage liver disease. Rats were classified into four groups as follows: normal control group, NASH rats fed high fat diet, nonalcoholic steatohepatitic rats fed high fat diet and received lyophilized powdered cranberry suspended in 0.5% CMC , group fed basal diet and received lyophilized powdered cranberry suspended in 0.5% carboxymethylcellulose CMC. The model of NASH rats elicited significant increase in serum lipid parameters: total cholesterol , total lipids, tricylglycerols and phospholipids , liver antioxidant enzyme activities with concomitant significant elevations in liver enzymes and glucose-6-phosphate dehydrogenase, in association with a reduction in reduced glutathione, glutathione peroxidase , serum total protein and direct and total bilirubin. Administration of cranberry to NASH rats produced significant increases in tested antioxidant enzyme activities, G6PD and serum total protein, direct and total bilirubin concomitant with significant decreases in the levels of serum lipids as well as liver enzymes AST, ALT, ALP, GST and ɣ-GT. In addition, it was noted that NASH rats exhibited a degree of DNA fragmentation; however, oral administration of cranberry extract partially inhibited the DNA fragmentation.*

Introduction

The risk of toxic liver damage has markedly increased in recent years due to the exposure to environmental toxins, pesticides and chemotherapeutics. Many compounds, including useful drugs, can cause liver cell damage through their metabolic conversion to highly reactive substances and the generation of free radicals **(White et al., 2010)** .

Nonalcoholic steatohepatitis (NASH) is an increasingly recognized condition that may progress to end-stage liver disease. It is characterized by fat infiltration of the liver, inflammation, hepatocellular damage (ballooning) and fibrosis, with NASH patients at higher risk of developing cirrhosis, terminal liver failure, and hepatocellular carcinoma **(Neuschwander et al., 2003).** The mechanisms leading to NASH remain unclear, but the "two-hit" mechanism is often used as a simple description for its etiology . The "first-hit" resulting in liver steatosis is caused by obesity, insulin resistance and excess lipid accumulation. Then, reactive oxygen/nitrogen species (ROS/RNS)-mediated "second hits" result in oxidative and nitrative modification to lipids and proteins, exacerbated liver injury and NASH (Bedogni et al., 2005). Detoxifying agents and plant-derived natural products, such as flavonoids, terpenoids and steroids , essential fatty acids for regeneration of hepatocyte membrane structure, hypocholesterinemic agents, antioxidants and anti-inflammatory substances have received considerable attention in recent years in the pharmacotherapy of liver damages due to their diverse pharmacological and biochemical activities **(White et al., 2010).**

Cranberries are small, dark red fruits that are widely consumed as juice. It contains significant amount of phenolic compounds which have antioxidant properties and other health benefits **(Kahlon & Smith , 2007)**. Studies have shown that supplementations with berries were effective in reducing oxidative stress associated with aging. Further, cranberries have been reported to possess anti-inflammatory and anti-mutagenic properties and provide cardio-protection **(Bagchi et al., 2004).**

Cranberry juice has long been consumed for the prevention of urinary tract infections **(Foo et al., 2000)** .These and other health benefits, including reduced risks of cancer and cardiovascular disease, are due to the presence of various polyphenolic compounds, including anthocyanins, flavonols, and procyanidins, and the synergistic effects among them (**Heinonen et al., 2007**). Considerable in vitro evidence has shown the antioxidant potential of cranberry phenolic compounds **(Narwojsz & Borowska,2010).**

Thus, the aim of the present study is to evaluate the antioxidant and antiatherogenic efficiency of cranberry extract in nonalcoholic steatohepatitic rats. The protective effect of cranberry extract was assessed by evaluating the enzymatic and nonenzymatic antioxidants and DNA fragmentation, histopathological examination, along with liver function tests.

Materials and methods

Animals

This study was carried out on healthy adult male Albino rats (Sprague-Dawley) strain weighing 60 ± 5 g, supplied from the breeding unit of the Egyptian Organization for Biological Products and Vaccines (Helwan, Egypt). Animals were maintained on a natural light/dark cycle and given food and tap water ad libitum.

Materials

Cranberry (V. macrocarpon) capsules extracts were purchased from GNC products (GNC Nature's Fingerprint-Cranberry 100 Capsules, USA). Each capsule contained 500 mg of lyophilized powdered fruits. Carboxymethylcellulose was purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA).

Experimental Design

In this experiment , the high fat diet containing 35% fat (31.6% saturated fat and 3.2% unsaturated fat) , 57% of the metabolizable energy. Animals randomly enrolled into four groups of eight animals each and treated as following:

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

Group (1) (Control) fed basal diet and received carboxymethylcellulose (CMC; 0.5%) (1 ml/200 g body weight/day) orally for 30 consecutive days [9] .

Group (2) (NASH) nonalcoholic steatohepatitic rats fed high fat diet

Group (3) (NASH+CRAN) nonalcoholic steatohepatitic rats fed high fat diet and received cranberry extract suspended in 0.5% CMC (100 mg/kg; orally once daily for 30 consecutive days. The amount of food consumed was recorded every day and the animals were weighed weekly.

Group (4) (CRAN) fed basal diet and received CRAN lyophilized powder alone suspended in 0.5% CMC (100 mg/kg; orally once daily for 30 consecutive days

At the end of experimental period (30 days), the final body weight for each rat was recorded. Animals were fasted overnight then scarified under ether anesthesia and blood samples were collected from hepatic portal vein in centrifuge tubes. The serum was separated by allowing blood samples left for 15 minutes at temperature of 25°C then centrifuged at 4000 r.p.m for 20 minutes, then kept in plastic vials at −20°C until analysis. Rat liver was immediately removed, rinsed with ice cold saline, and blotted dry, weighed separately and the relative weight were calculated.

Biochemical Analysis

Serum was analyzed for the following biochemical parameters: total cholesterol , triacylglycerol, total lipids and phospholipids. (ALT) alanine aminotransferase and Aspartate aminotransferase (AST),alkaline phosphatase (ALP) , Serum albumin, total and direct bilirubin and glucose-6-phosphate dehydrogenase (G6PD) was assigned by commercial kits. All the kits were provided by Greiner Diagnostic GmbH (Greiner Bio-One GmbH, Brunel Way, Business Park, UK), and purchased from Indomedix Egypt Company

Determination of Liver Enzymatic and Non-enzymatic Antioxidant Status

One portion of liver (about 2 g) was used to prepare 10% homogenate in 1.15% KCl and 5% homogenate in 3% sulfosalicylic acid, centrifuged at 4000 r.p.m at 4°C for 20 min. and the supernatants were used to obtain the cytosolic fraction which was used for the assay of glutathione peroxidase (GPX) **(Arthur et al., 1985)**. gamma glutamyl transferase (ɣ-GT) (Szasz , 1969), glutathione-S-transferase (GST) and reduced glutathione (GSH) **(Habig et al., 1974)**.

DNA Fragmentation Analysis

Liver tissues were homogenized and incubated in 100 mM Tris-HCl (pH 8.0), 25 mM EDTA, 0.5 % SDS, and 0.1 µg/mL proteinase K at 60 °C for 3 h. DNA was extracted with phenol/chloroform (1:1) and chloroform/isoamyl alcohol (1:24). The extracted DNA was precipitated and digested in 10 mM Tris–HCl (pH 5.0) containing 1 mM EDTA and 10 µg RNase for 1 h at 37 °C. Five µg of DNA per sample were electrophoretically separated on 1.5 % agarose gel containing 0.5 µg/mL ethidium bromide. The DNA pattern was examined by with an ultraviolet transilluminator.

Assessment of Steatohepatitis

Fresh liver tissue samples were kept in 10% formalin solution and paraffin blocks were subsequently prepared. Paraffin-embedded liver sections were stained with hematoxylin-eosin and Masson-trichrome. All specimens were evaluated blindly by an expert pathologist, using the scoring system proposed ,steatosis (0-3), lobular inflammation (0-3) and ballooning degeneration (0-2). Fibrosis was minimal in all samples and was therefore not scored **(Kleiner et al, 2005)**.

Histological Examinations

Formalin-fixed and paraffin-embedded liver tissues were processed routinely for hematoxylin and eosin staining. Liver histology was examined under a light microscope and then graded according to the magnitude of steatosis, inflammation, and ballooning degeneration of hepatocytes as described before (2,20). Briefly, the degree of steatosis was graded 0–3 based on the average percent of fat-accumulated hepatocytes per field at ×200 magnification under H&E staining (grading: 0 = <5%, 1 = 5-25%, 2 = 26-50%, 3 = 51-75%, 4 = >75%). Inflammation was evaluated by the number of inflammatory cells counted in 10 random fields at ×200 magnification. The mean of these numbers was calculated and regarded as inflammatory cells/mm^2. Hepatocellular ballooning degeneration was evaluated as either negative (absent) or positive (present).

Statistical Analysis

The data were statistically analyzed by SPSS version 15.0 statistical packages. The results were expressed as means ± S.D, statistical differences between groups were performed using t-test. Differences considered significantly when $p< 0.05$.

Results

Weight gains and relative liver weight are shown in Table 1. Body weights were gradually increased in all groups; there was a significant increase in NASH group when compared to other experimental groups. On the other hand, relative liver weight was significantly increased in high fat diet groups NASH and NASH+CRAN than in Control group.

Table 1. Effect of oral administration of Cranberry extract on body weight gain and relative liver weight in nonalcoholic steatohepatitic rats

Parameters	G1 (control)	G2 (NASH)	G3 (NASH+CRAN)	G4 (CRAN)
Body weight gain (g)	110.13±5.9	162.75±10.7	148.3±10.2	137.75±6.2
Relative weight of liver	3.54±0.47	3.96±0.22	3.49±0.16	3.19±0.16

The results about the effect of cranberry on serum lipid profiles are shown in Table 2. Feeding rats with high fat diet for 4-weeks resulted in a significant elevation of serum total cholesterol (111.65 %;), total lipids (16.8%), triacylglycerol (54.1%) and serum phospholipids (97.75%). Concurrent administration of cranberry resulted in a significant decrease in the levels of serum total lipids, total cholesterol, triacylglycerol and phospholipids compared to NASH group. It was noticed that no significant difference between the value of total cholesterol and triacylglycerol in CRAN group when compared to control one.

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

Table 2. Effect of oral administration of Cranberry extract on serum total cholesterol, total lipids, triacylglycerols and phospholipids in nonalcoholic steatohepatitic rats

Groups Parameters	G1 (control)	G2 (NASH)	G3 (NASH+CRAN)	G4 (CRAN)
Total Cholesterol mg/dl	104.11±3.86	220.35±5.25	175.44±7.30	100.48±3.20
Total lipids mg/dl	618.75±8.68	723.25±15.52	635.5±9.89	549.5±15.87
Triacylglycerol mg/dl	65.49±1.87	100.93±2.45	85.32±3.13	67.99±1.7
Phospholipids mg/dl	124.25±5.06	245.71±17.29	190.45±5.20	139.1±8.32

Results obtained in Table (3) summarized that, feeding rats high fat diet resulted in Nonalcoholic fatty liver disease which was manifested by significant increase in serum AST, ALT, ALP and G6PD activities by 83.17% , 95.32%, 45.5% and 112.35% respectively when compared to normal control rats, thus indicating liver damage . It was noticed that, oral administration of cranberry to NASH rats significantly reduced the elevated levels of ALT, AST, and ALP activities along with more increasing activity of G6PD.

Table 3. Effect of oral administration of Cranberry extract on serum concentrations of AST, ALT and ALP of nonalcoholic steatohepatitic rats

Groups Parameters	G1 (control)	G2 (NASH)	G3 (NASH+CRAN)	G4 (CRAN)
Aspartate aminotransferase (AST) (U/L)	107.00±8.91	196.00±9.72	154.0±8.6	106.6±9.98
Alanine aminotransferase (ALT) (U/L)	72.70±3.37	142.00±3.33	119.6±3.4	75.3±2.98
Alkaline phosphatase (ALP) (U/L)	199.97±4.48	291.09±4.80	231.9±4.1	179.8±3.78
G6PD (mol/mg protein/min)	21.85 ± 2.29	46.4 ± 1.48	53.50 ±4.84	29.12 ±4.04

Table (4) showed that feeding rats high fat diet causes a reduction in total albumin level by 28.4% compared to normal controls ($p<0.05$) this change was increased by 18 % following oral administration of cranberry. On the other hand, the levels of direct and total bilirubin significantly reduced in NASH rats by 43.6% and 44.4% respectively indicating liver injury. Oral administration of cranberry elicited dramatic increase in direct and total bilirubin levels by 15.9% and 14.8% when compared to pretreated NASH rats. Meanwhile, administration of cranberry to control rats did not cause any effect.

Table 4. Effect of oral administration of Cranberry extract on serum concentrations of Albumin , Direct and total bilirubin of nonalcoholic steatohepatitic rats

Groups Parameters	G1 (control)	G2 (NASH)	G3 (NASH+CRAN)	G4 (CRAN)
Total Albumin (g/dl)	6.82±0.10	4.88±0.43	5.77±0.49	6.91±0.46
Direct bilirubin (mg/dl)	0.94.01	0.53±0.02	0.79±0.09	0.92±0.03
Total bilirubin (mg/dl)	0.81±0.01	0.45±0.01	0.69±0.01	0.84±0.03

High fat diet resulted in liver injury manifested by significant decreases in the activities of the liver antioxidant enzymes: GSH and GPX activities by 36.78% and 46.4% respectively, compared to normal controls. Oral administration of Cranberry extract elicited dramatic increase in these enzymes. On the other hand, there was an increased activity of ɣ-GT and GST followed feeding high fat diet in NASH rat group (45.5% and 62.9 % respectively) when compared to normal control rats. Cranberry supplementation to NASH rats decreased the levels of ɣ-GT and GST activities compared to NASH rat group. The levels of reduction of ɣ-GT and GST activities were 19.9% and 30.6% respectively when compared to NASH rats (Table 5).

Table 5. Effect of oral administration of Cranberry extract on hepatic concentrations of GSH , GPX, ɣ-GT and GST activities nonalcoholic steatohepatitic rats

Groups Parameters	G1 (control)	G2 (NASH)	G3 (NASH+CRAN)	G4(CRAN)
(GSH) (µmol/dL)	40.21±0.12	25.42±0.25	35.9 ±0.25	43.85±0.6
(GPX) (nmol/ml)	93.2 ± 9.4	49.9 ± 3.4	71.8±0.6	100.8±2.6
(ɣ-GT) (nmol/mg protein)	98.1 ±7.1	142.8±10.6	114.3 ± 4.3	92.6±8.3
(GST) (nmol/mg protein/min)	234.3±12.4	381.8 ±9.68	264.7 ± 19.2	225.5 ± 17.3

Liver DNA

NASH rats showed a degree of hepatic DNA fragmentation which was nearly abolished in Cranberry treated rats (Fig.1)

Histopathological Analyses

The histological studies of the liver samples from the control and the test groups were performed to investigate the occurrence of steatohepatitis. Liver samples of the control animals showed no evidences of steatosis, inflammation or

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

fibrosis (Fig. 2A), the NASH group showed grade.Two liver steatosis, inflammation and ballooning degeneration. Furthermore, no incidence of fibrosis was recorded among the control and the CRAN-fed rats (Fig. 2, B and 2D).

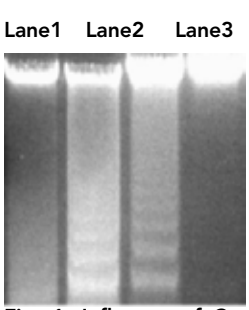

Fig. 1. Influence of Cranberry extract administration on NASH hepatic DNA fragmentation

Lanes 1 : no DNA fragmentation in normal control. Lane 2 depicts strong DNA fragmentation in NASH rats. Lane 3 depicts weak DNA fragmentation in NASH +CRAN rats. Lane 4 depicts no DNA fragmentation in rats administered CRAN only

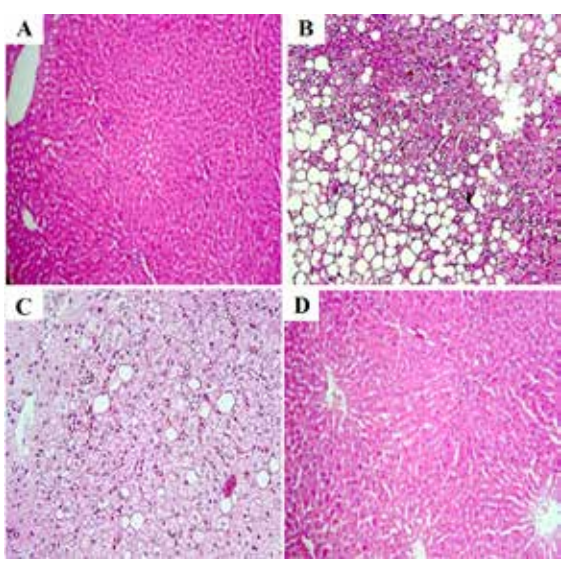

Fig. 2. Histological evaluation of rats' livers.

Photographs of tissues after Hematoxylin-eosin (A-D). Original magnifications ×100. (A) Normal liver histology of a rat fed the standard diet. (B) (NASH group) showing ballooning degeneration, pericentral macrovesicular steatosis and lobular inflammation. (C) group NASH+CRAN showing slight ballooning degeneration and steatosis. (D) group CRAN normal architecture with neither steatosis nor inflammation.

Discussion

This study examined the serum lipid profile, ameliorative and antioxidative effects of cranberry extract in rats fed atherogenic diet. Cranberry is known to be a good source of antioxidants, which have health benefits such as anti-adhension activity, antiviral, and anticancer properties **(Howell et al., 2005)**.

Feeding rats with high fat diet leads to induction of nonalcoholic steatohepatitis. In the case of high dietary intake of fats, animal studies have shown that fatty liver is the result of increased delivery of fatty acids through the portal circulation together with a 25% higher de novo lipogenesis **(Mastrocola et al., 2003)**.

After 30 days of feeding high fat diet treatment, the animals showed typical signs of nonalcoholic steatohepatitis with increased markers of liver damage. Plasma ALT and AST activities were elevated and bilirubin level (both total and free bilirubin) in plasma decreased, and the ratio of liver weight / body weight increased.

Weight gains and relative liver weight are shown in Table 1. Body weights were gradually increased in all groups; there was a significant increase in NASH group when compared to other experimental groups. On the other hand, relative liver weight was significantly increased in high fat diet groups NASH and NASH+CRAN than in Control group. NASH is observed in a subset of patients with nonalcoholic fatty liver disease (NAFLD), defined as fat accumulation in the liver exceeding 10% by weight **(Vuppalanchi & Chalasani , 2009**). Although still poorly understood, NASH pathogenesis is widely recognized as a two-hit model in which the first hit is an initial metabolic disturbance that increases inflow of free fatty acids and de novo lipogenesis, leading to steatosis; the "second hit" includes oxidative stress, decreased hepatic ATP production, and induction of proinflammatory cytokines, which triggers necroinflammation leading to the progression of steatohepatitis **(Day & James ,1998)**.

Results showed that feeding rats with high fat diet for 4-weeks resulted in a significant elevation of serum total cholesterol, total lipids, triacylglycerol and serum phospholipids. Concurrent administration of cranberry resulted in a significant decrease in the levels of serum total lipids, total cholesterol, triacylglycerol and phospholipids compared to NASH group. Lipid metabolism (fatty acid oxidation and lipogenesis) involves several interdependent and "cross-regulated" pathways. It is closely connected to the metabolism of carbohydrates that may be converted to fats. The liver takes up nonesterified fatty acids from the blood in proportion to their concentration, because there is no evidence that uptake of fatty acids is regulated **(Tamura & Shimomura, 2005)**.

Fatty acids are synthesized mainly in liver from acetyl-CoA, althought most tissues can assemble triacylglycerols from acyl-CoAs and glycerol-3-phosphate. Fatty acids are converted back to acetyl- CoA by β-oxidation and used as fuel, and the synthesis and breakdown isregulated to meet the cellular energy needs. The traditional perception of adipose tissue as a mere storage place for fatty acids has been replaced over the past years **(Hajer et al., 2009).** In addition to its role in insulating and cushioning the body and storing free fatty acids (FFA) after food intake and releasing FFAs during the fasting, the adipose tissue produces a wide range of hormones and cytokines involved in glucose metabolism, lipid metabolism, and feeding behaviour as well as in inflammation, coagulation, and blood pressure **(Trayhurn & Beattie, 2001).**

It was noticed that no significant difference between the value of total cholesterol and triacylglycerol in CRAN group when compared to Control one. Polyphenols in cranberry might reduce the cardiovascular disease risk factor such as LDL oxidation, platelet aggregation, and high blood pressure. Previous studies have shown that the proanthocyanidins found in cranberry powder inhibit low-density lipoprotein oxidation and have an overall positive effect on lipid metabolism and cholesterol **(Howell et al., 2005).**

It was reported that plasma LDL-cholesterol and LDL oxida-

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

tion were reduced by cranberry extract fortification. However, R**uel et al. (2006)** reported that total and LDL-cholesterol were not changed but HDL-cholesterol was increased by cranberry juice consumption in men. On the other hand, dysregulation of triglycerides and free fatty acids, resulting in lipid and inflammatory stress, which favors the progression of NASH, were markedly reduced in rats in which orally supplemented with cranberry extract.

In the current study, similar results was observed that serum total cholesterol and triacylglycerols levels were not significantly different in rats that administered cranberry when compared to control rats .

In this study it was observed that, Nonalcoholic fatty liver disease was manifested by significant increase in serum AST, ALT, ALP and G6PD when compared to normal control rats, thus indicating liver damage . On the other hand, oral administration of cranberry to NASH rats significantly reduced the elevated levels of ALT, AST, and ALP activities along with more increasing activity of G6PD.

Vozarova et al., (2002) mentioned that the elevated activities of AST, ALT and ALP enzymes were signs of impaired liver functions in the elevation of liver enzymes occurred due to their release from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage.

Glucose-6-phospahe dehydrogenase G6PD is an enzyme that catalyzes the first step in the hexose monophosphate pathway, produces ribose, which is incorporated into nucleotides and NADPH, the major cytoplasmic reducing compound. NADPH is a substrate for phase I and II detoxification enzymes. G6PD is elevated in response to external stimuli like toxic agents and oxidative stress. The activity of G6PD is up regulated by carcinogens and oxidative stress. Cranberry administration to NASH rats showed elevated G6PD activity, indicating that increased amounts of NADPH are required for detoxification process **(Frederiks et al., 2003).**

Serum total Albumin is present in blood plasma abundantly (60%) and structurally well characterized. In the present study it was observed decreased levels of serum albumin which is an evidence of existence of NASH when compared to normal animals. Studies have shown that cellular proteins may be affected by free radical accumulation leading to the formation of carbonyl derivatives. The carbonyl derivatives of proteins may result from oxidative modification of amino acid side chains and reactive oxygen-mediated peptide cleavage .Further, **Bala et al., (2006)** have reported that the primary target of the oxygen-radical attack, promoted by ethanol, is represented on cellular proteins. Serum albumin level was increased following oral administration of cranberry. In a human intervention study with the dried cranberry juice, serum albumin level was significantly increased by dried cranberry consumption (**Serviddio et al., 2008).**

On the other hand, the levels of direct and total bilirubin significantly reduced in NASH rats indicating liver injury. This may due decreased conjugation and decreased secretion from the liver or blockage of bile ducts **(Mahmoud & Elnour ,2013)** . Oral administration of cranberry elicited dramatic increase in direct and total bilirubin levels as compared to pretreated NASH rats. High fat diet resulted in liver injury manifested by significant decreases in the activities of the liver antioxidant enzymes: GSH and GPX activities compared to normal controls. A decrease in antioxidant defenses is also a major factor promoting oxidative stress in NASH cases. Decreases in antioxidant factors including glutathione, and glutathione S-transferase correlate with the severity of liver disease .Lower mitochondrial GSH has been detected in many models of NASH **(Llacuna et al., 2011)**. The reduction of mitochondrial GSH could be due, at least in part, to its reduced importation into mitochondria as a result of increased levels of cholesterol within the inner mitochondrial membrane **(Caballero et al., 2010)**.Depletion in hepatic GSH content may be caused by the decrease in synthesis of S-adenosylmethionine (SAMe), the major methyl donor in liver and precursor to GSH . Superoxide anion is dismutated by mitochondrial manganese superoxide dismutase into hydrogen peroxide, which is detoxified into water by the mitochondrial glutathione peroxidase (GPx).Importantly, GPx needs an adequate amount of reduced glutathione (GSH) within the mitochondrial matrix to detoxify H2O2, so that the depletion of mitochondrial GSH below a crucial level can lead to, or favor, mitochondrial dysfunction and cell death **(Schafer & Buettner , 2001).**

Oral administration of Cranberry extract elicited dramatic increase in these enzymes. With a reduction in the levels of ɣ-GT and GST activities compared to NASH rat group. **Deyhim et al. (2007)**. reported that cranberry juice increased plasma antioxidant capacity and reduced malondialdehyde concentrations. **Vattem et al. (2005)** also reported that cranberry phenolics decreased MDA formation in oxidatively stressed porcine muscle and it suggested that exogenously treated phenolic phytochemicals could be reducing the oxidative stress. Including the results from the current study, several trials have shown beneficial effect of cranberry consumption on protein and lipid oxidation. These consensus outcomes may explain the antioxidative effect of cranberry powder and juice against oxidative damage.

Phenolic extracts obtained from berries (blackberries, red raspberries, sweet cherries, blueberries, and strawberries) were shown to inhibit the oxidation of low-density lipoproteins and of liposomes. Berries have also shown a remarkably high scavenging activity toward chemically generated ROS .Historically, cranberry juice has been consumed to prevent urinary tract infections. These and other health benefits, including reduced risks of cancer and cardiovascular disease, are believed to be due to the presence of various polyphenolic compounds, including anthocyanins, flavonols, and procyanidins, and the synergistic effects among them . Considerable in vitro evidence has shown the antioxidant potential of cranberry phenolic compounds **(Khanal et al., 2010).**

Cranberry is rich in phenolic phytochemicals such as phenolic acids, flavonoids and ellagic acid. These phytochemicals act as antioxidants, and showed health benefits including reduction of oxidative damage that can cause cancer, heart disease, and other degenerative diseases . C**hu and Liu (2005)** reported that phytochemicals in cranberries could inhibit LDL oxidation and induce expression of LDL receptors. **Mckay and Blumberg (2007**) also reported that cranberry reduced the risk of cardiovascular disease (CVD) by inhibition of LDL oxidation and platelet aggregation, and reducing blood pressure via other anti-thrombotic and anti-inflammatory mechanisms.

The data obtained confirmed the free radical-scavenging and antioxidant properties of cranberry extracts. Still, cranberry phenolic compounds have been shown to have free radical-scavenging properties against superoxide radical (O2−), hydrogen peroxide (H2O2), hydroxyl radicals (•OH),

Volume : 4 | Issue : 9 | September 2014 | ISSN - 2249-555X

and singlet oxygen (1O2), and they can also inhibit lipid peroxidation, as well as protein and lipid oxidation in liposomes **(Seeram & Heber, 2007)**.

Mitochondria are a principal source of cellular ROS as the result of inefficiencies in the flow of electrons along the electron transport chain (ETC) . In the physiological state, most reactive incompletely reduced forms of oxygen, such as superoxide, are detoxified into water, keeping the steady state of oxidants at a relatively low rate (less than 1% of total oxygen consumed by mitochondria, by a variety of antioxidant defenses and repair enzymes **(Brand , 2010)**.

Impairment of oxidative phosphorylation, such as reduced hepatic ATP synthesis and increased ROS production, has been reported in patients with NASH **(Hensley et al., 2000)** . These biochemical changes are associated with ultrastructural abnormalities in liver mitochondria that appear scarce in number, swollen, and rounded, with loss of cristae and presence of paracristalline inclusions . A report by **Teodoro et al. (2006)** showed that in spite of the increase in the volume density of mitochondria in cells of the cirrhotic liver (by 28%), the concentration of internal mitochondrial membranes and the total length of the internal membrane were reduced.

MtDNA is extremely sensitive to oxidative damage because of its proximity to the inner membrane (the main cellular source of ROS), the absence of protective histones, and incomplete repair mechanisms in mitochondria .Therefore, oxidative damage to mtDNA by ROS may lead to DNA strand breaks and the occurrence of somatic mtDNA mutations . The accumulation of mtDNA mutations may result in dysfunction of the respiratory chain, leading to increased ROS production in mitochondria and subsequent accumulation of more mtDNA mutations. Genetic susceptibilities, in particular related to mitochondrial dysfunction, could explain, at least in some patients, a faster progression of the liver disease. Indeed, genetic defects in mitochondrial DNA (mtDNA) cause impairment of mitochondrial function and, therefore, decrease their capacity to oxidize fatty acids and increases the production of reactive oxygen species (ROS) that in turn trigger lipid peroxidation, cytokine release, and cell death **(Wallace & Fan, 2010)**.

The observed decrease in the antioxidant enzyme activities can be explained on the basis of their exhaustion in combating the previously observed oxidative stress. Our results indicated that CRAN mitigated the decrease of the activity of the antioxidant enzymes. CRAN rich in these compounds were reported to inhibit oxidative processes in other tissues . Specifically, most of CRAN's protective properties may be attributed to its proanthocyanidins content. They have been shown to scavenge 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and superoxide effectively and improve the ferric- reducing antioxidant power in plasma **(Chang et al., 2007)**.

Conclusion

The data obtained from this study, indicate that oral administration of Cranberry extract (which has potent free radical scavenging and antioxidant properties) to NASH rats, at least partially, alleviates liver injury by preventing lipid peroxidation enzyme system, reducing serum lipid levels inhibiting DNA **fragmentation**, and increasing antioxidant enzyme activities. Thus indicating that cranberry had different mechanisms of antioxidant action and lipid improving effects, helps Therefore, cranberry administration seems to be a highly promising agent for protecting hepatic tissue against oxidative damage and in preventing hepatic injury and dysfunction.

REFERENCE

Arthur, J.R. and Boyne, R. 1985. Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. Life Science, 36, 1569-1575. | Bagchi, D., Sen, C.K., Bagchi, M., Atalay, M. 2004 Anti-angiogenic, antioxidant, and anti-carcinogenic properties of a novel anthocyanin-rich berry extract formula. Biochemistry,69, 75–80. | Bala, I., Bhardwaj, V., Hariharan, S., Kumar, M. 2006.Analytical methods for assay of ellagic acid and its solubility studies. J Pharm Biomed Anal , 40,206-10. | Bedogni, G., Miglioli, L., Masutti, F., Tiribelli, C., Marchesini, G., Bellentani, S. 2005. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos Nutrition and Liver Study. Hepatology, 42,44–52. | Brand, M. D. 2010.The sites and topology of mitochondrial superoxide production. Exp. Gerontol. 45,466–472. | Caballero, F., Fernández, A., Matías, N., Martínez, L., Fucho, R., Elena, M., Caballeria, J., Morales, A., Fernández-Checa, J. C., García-Ruiz, C.2010. Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial S-adenosyl-L-methionine and glutathione. J. Biol. Chem. 285,18528–18536. | Chang, W.T., Shao, Z.H., Yin, J.J., Mehendale, S., Wang, C.Z., Qin, Y., Li, J., Chen, W.J., Chien, C.T., Becker, L.B., Vanden-Hoek, T.L., Yuan, C.S., 2007. Comparative effects of flavonoids on oxidant scavenging and ischemia-reperfusion injury in cardiomyocytes. Eur. J. Pharmacol. 566, 58–66. | Chu, Y.F. and Liu, R.H .2005. Cranberries inhibit LDL oxidation and induce LDL receptor expression in hepatocytes. Life Sci 77,1892- 1901. | Day, C.P., James, O.F.1998. Steatohepatitis: a tale of two "hits"? Gastroenterology | ,114,842–5. | Deyhim, F., Patil, B.S., Villarreal, A., Lopez, E., Garcia, K., Rios, R., Garcia, C., Gonzales, C. and Mandadi, K. 2007. Cranberry juice increases antioxidant status without affecting cholesterol homeostasis in orchidectomized rats. J Med Food. 10,49-53. | Foo, L.Y., Lu, Y., Howell, A.B., Vorsa, N., 2000. A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated Escherichia coli. J. Nat. Prod. 63, 1225–1228. | Frederiks, W.M., Bosch, K.S., De Jong, J.S., Van, N. 2003. Post-translational regulation of Glucose-6-phosphate dehydrogenase activity in preneoplastic lesions in rat liver. J. Histochem.Cytochem, 51, 105-112. | Habig, W.H., Pabst, M.J., Jakoby, W.B. 1974. Glutathione-S-transferase: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130-7139. | Hajer, G., Haeften, T., Visseren, F.2008. Adipose tissue dysfunction in obesity, diabetes, and vascular disease. Eur Heart J .29,2959-2971. | Heinonen, M. 2007. Antioxidant activity and antimicrobial effect of berry phenolics—A Finnish perspective. Molecular Nutrition & Food Research, 51, 684–691. | Hensley, K., Robinson, K. A., Gabbita, S. P., Salsman, S., Floyd, R. A.2000. Reactive oxygen species, cell signaling, and cell injury. Free Radic. Biol. Med. 28:1456–1462. | Howell, A.B., Reed, J.D., Krueger, C.G., Winterbottom, R., Cunningham, D.G. and Leahy, M. 2005. A-type cranberry proanthocyanidins and uropathogenic bacterial anti-adhesion activity. Phytochemistry. 66, 2281-2291. | Kahlon, T. S., and Smith, G. E. 2007. In vitro binding of bile acids by blueberries (Vaccinium spp.), plums (Prunus spp.), prunes (Prunus spp.), strawberries (Fragaria ananassa), cherries (Malpighia punicifolia), cranberries (Vaccinium macrocarpon) and apples (Malus sylvestris). Food Chemistry. 100, 1182–1187. | Khanal, R. C., Rogers, T. J.,Wilkes, S. E., Howard, L. R., & Prior, R. L. 2010. Effects of dietary consumption of cranberry powder on metabolic parameters in growing rats fed high fructose diets. Food & Function. 1, 116–123. | Kleiner, D.E., Brunt, E.M., Van Natta, M., Behling, C., Contos, M.J., Cummings, O.W., Ferrell, L.D., Liu, Y.C., Torbenson, M.S., Unalp-Arida, A., Yeh, M., McCullough, A.J., Sanyal, A.J.2005. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. ,41(6):1313-21. | Llacuna, L., Fernández, A., Montfort, C. V., Matías, N., Martínez, L., Caballero, F., Rimola, A., Elena, M., Morales, A., Fernández-Checa, J. C., García-Ruiz, C. 2011.Targeting cholesterol at different levels in the mevalonate pathway protects fatty liver against ischemia–reperfusion injury. J. Hepatol. 54,1002–1010. | Mahmoud, R.H, Elnour , W.A. 2013.Comparative evaluation of the efficacy of ginger and orlistat on obesity management, pancreatic lipase and liver peroxisomal catalase enzyme in male albino rats. European Review for Medical and Pharmacological Sciences. 17, 75-83 | Mastrocola, R., Aragno, M., Betteto, S., Brignardello, E., Catalano, M. G., Danni, O., Boccuzzi, G.2003. Pro-oxidant effect of dehydroepiandrosterone in rats is mediated by PPAR activation. Life Sci. 73,289–299. | Mckay, D.L. & Blumberg, J.B. 2007. Cranberries (Vaccinium macrocarpon) and cardiovascular disease risk factor. Nutr Rev. 65:490-502. | Narwojsz, A., and Borowska, E. J. 2010. Cranberry and strawberry juices—Influence of method production on antioxidants content and antioxidative capacity. Polish Journal of Natural Sciences, 25, 209–214. | Neuschwander-Tetri, B., Caldwell, S.2003. Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. Hepatology 37,1202–1219. | Ruel, G., Pomerleau, S., Couture, P., Lemieux, S., Lamarche, B. & Couillard, C. 2006. Favourable impact of low-calorie cranberry juice consumption on plasma HDL-cholesterol concentrations in men. Br J Nutr. 96,357-364. | Schafer, F. Q., Buettner, G. R.2001. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic. Biol. Med. 30:1191–1212. | Seeram, N. P., & Heber, D. 2007. Impact of berry phytochemicals on human health: Effects beyond antioxidant. ACS Symposium Series, 956, 326–336. | Serviddio, G., Sastre, J., Bellanti, F., Viña, J., Vendemiale, G., Altomare, E. 2008. Mitochondrial involvement in non-alcoholic steatohepatitis. Mol. Aspects Med. 29, 22–35. | Szasz ,G .1969. Kinetic determination of serum gamma glutamyle transferase. Clin. Chem . 15, 124-126 | Tamura, S., Shimomura, I. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. J. Clin. Invest. 115:1139–1142; 2005. | Teodoro, J., Rolo, A. P., Oliveira, P. J., Palmeira, C. M. 2006.Decreased ANT content in Zucker fatty rats: relevance for altered hepatic mitochondrial bioenergetics in steatosis. FEBS Lett. 580,2153–2157. | Trayhurn, P., Beattie, J.H.2001. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc Nutr Soc .60,329-339 | Vattem, D.A., Randhir, R. , Shetty, K. 2005. Cranberry phenolicsmediated antioxidant enzyme response in oxidatively stressed porcine muscle. Process Biochem. 40,2225-2238. | Vozarova, B., Stefan, N., Lindsay, S., Saremi, A., Pratley, E., Bogardus, C. and Tataranni, A. 2002. High alanine aminotransferase is masociated with decreased hepatic insulin sensitivity and predicts the development of type2 diabetes . Diabetes. 51, 1889-1 895. | Vuppalanchi, R., Chalasani, N.2009. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management. Hepatology 49,306–317. | Wallace, D. C., Fan, W. 2010.Energetics, epigenetics, mitochondrial genetics. Mitochondrion 10,12–31. | White, B. L., Howard, L. R., & Prior, R. L. 2010. Polyphenolic composition and antioxidant capacity of extruded cranberry pomace. Journal of Agricultural and Food Chemistry, 58, 4037–4042. |