Biochemical effects of cranberry extract in experimentally induced myocardial necrosis in rats

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ABSTRACT

The present study was designed to evaluate the cardioprotective effect of cranberry extract (75 and 150mg/kg.b.w.) against nicotine-induced heart toxicity in rats. Oral administration of nicotine (2.5mg/kg.b.w.) led to significant increase in plasma transaminases (L-alanine and L-aspartate), lactate dehydrogenase (LDH), creatine Kinase (CK), and TBARS as well as plasma total- and direct bilirubin, triglyceride, total cholesterol, and LDL-cholesterol. Also, treatment of rats with nicotine led to significant decrease in heart and plasma GSH, superoxide dismutase (SOD), catalase (CAT) as well as heart transaminases (L-alanine and L-aspartate) and plasma HDL. The obtained result revealed that cranberry extract (75 and 150 mg/kg. b.w.) prevents heart damage through increasing of GSH, SOD and CAT activities and decrease significantly TBARs level. These results suggest that, cranberry may be effective in the protection of heart toxicity by its radical scavenging effect and antioxidant activity.

Keywords: Nicotine, heart toxicity, cranberry, Antioxidant enzymes, lipid profile, GSH.

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1. INTRODUCTION

Nicotine is a naturally occurring alkaloid found in the nightshade family plants (*Solanaceae*), predominantly in tobacco plant (*Nicotianatabacum*) (Wu et al., 2002). Nicotine has many effects such as on heart rate, brain excitation, and blood pressure (Shivij et al., 2006). Wu et al. (2002), reported that, nicotine induced a wide range of biological effects and is a major risk factor in the development of chronic obstructive lung diseases, cardio- vascular disorders and lung cancer. Morever nicotine through smoking, induced an inflammatory response in the lung and plays a role in pathogenesis of obstructive pulmonary diseases (Carpagnano et al., 2003; Hackett et al., 2003). Apoptosis is strongly induced in alveolar epithelium exposed to smoking (Piipari et al., 2000). Cranberry is the best source of flavonols among 30 flavonol-containing plant foods studied (Aherne and O'Brien 2002), and the flavonol content of cranberry is almost twice as high as 12 other commonly consumed fruit juices, including pomegranate and grape (Mullen et al., 2007). Quercetin is the most abundant flavonol in cranberry, and it varies from 11 to 25 mg/100 g, primarily as the 3-o-galactoside (Vvedenskaya et al. 2004). Cranberry is also the best source of quercetin (Manach et al. 2004). Myricetin is the second most abundant flavonol, followed by kaempferol (Vvedenskaya et al. 2004). These compounds are yellow in color, and there are 20 different flavonol glycosides in cranberry, as confirmed by another article (Vvedenskaya and Vorsa 2004). Accordingly, the purpose of the present study was to investigate the effect of cranberry in rat's model of nicotine induced heart toxicity.

2. MATERIAL AND METHODS

2.1.Dose of Cranberry:

Cranberry extract was purchased from Virgin Extracts (TM), Chinese. Cranberry was given to female rats with 1/150 LD50 (75mg/kg. b.w.) and 1/75 LD50 (150mg/kg b.w.) daily for 4 weeks by oral gastric gavage tube. Nicotine 99% was purchased from Sigma Aldrich, USA.

2.2. Animals:

A 60 albino rats weighing around 180±10gms were divided into 6 groups, 10 rats in each. They were acclimatized to animal house conditions. Animals were provided with standard diet and water ad-libtum. Animals were kept under constant environmental condition and observed daily throughout the experimental work.

2.3. Experimental design:

The animals were divided into 5 groups consisting of 8 animals, two controls and three treatment groups: Group (1): Control negative (0.9% saline, 3ml/kg.b.w., orally). Group (2): Positive control (nicotine 2.5mg/kg.b.w. suspended in 1ml 0.9% saline was given I.P and day after day for 28 days)(Liu et al., 2003). Group (3): Nicotine 2.5mg/kg.b.w. (I.P and day after day for 28 days)+ cranberry (75mg/kg.b.w.) daily for 28 days, orally daily dose. Group (4): Nicotine 2.5mg/kg.b.w. (I.P and day after day for 28 days)+ cranberry (150mg/kg. b.w.) daily for 28 days, orally daily dose. Group (5): Nicotine 2.5mg/kg.b.w.(I.P and day after day for 28 days)+ vitamin C (1g/kg. b.w.) daily for 28 days, orally daily dose (Luo et al., 1994).

2.4. Blood samples:

Blood samples were collected from the heart at the end of experimental period in dry, clean, and screw capped tubes, Also, plasma was separated by centrifugation at 2500r.p.m for 15 minutes. Serum was separated by automatic pipette and received in dry sterile samples tube and kept in a deep freeze at -20 oc until used for subsequent biochemical analysis.

2.5. Tissue specimen (heart tissue):

At the end of the experiment, rats of each group were sacrificed by cervical decapitation. The abdomen and chest were opened and the heart specimen was quickly removed and opened gently using a scrapper, cleaned by rinsing with ice-cold isotonic saline to remove any blood cells, clots, then blotted between 2 filter papers and quickly stored in a deep freezer at (-20 °C) for subsequent biochemical estimation of plasma and heart transaminases (L-alanine and L-aspartate) (Reitman and Frankel, 1957), lactate dehydrogenase (LDH) (Buhl and Jackson, 1978), creatine Kinase (CK) (Stein W., 1988), GSH (Chanarin, 1989), superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Sinha 1972) and TBARS (Nichans and Samulelson, 1968) as well as plasma total- and direct bilirubin (Fevery, et al., 1976), albumin (Doumas, et al., 1971)., total protein (Weichselbaum, 1946), triglyceride (Fossati and Prencipe 1982), total cholesterol (Allain et al., 1974), HDL-(Burnstein et al.,1970) and LDL-cholesterol (Falholt et al., 1973).

2.6. Statistical analysis:

The obtained data were statistically analyzed and using the statistical package for social science for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity.

3. RESULTS

Table 1 showed that I.P. administration of nicotine (2.5 mg/kg. b.w) resulted in a significant increase in plasma ALT, AST, LDH and CK compared to the normal control group ($p< 0.01$). Supplementation

of cranberry extract at 75 and 150mg/k.g. b.w. resulted in a significant decrease in plasma ALT, AST, ALP and LDH compared to the group that received nicotine ($p< 0.05$). Table 2 showed that I.P. administration of nicotine (2.5 mg/kg.b.w) resulted in a significant decrease in heart ALT, AST, LDH and CK compared to the normal control group ($p< 0.01$). Supplementation of cranberry extract at 75 and 150 mg/k.g. b.w. resulted in a significant increase in heart ALT, AST, ALP and LDH compared to the group that received nicotine ($p< 0.05$). Table 3 showed that I.P. administration of nicotine (2.5 mg/kg. b.w) resulted in a significant increase in plasma total – and direct bilirubin ($p< 0.05$) as well as non-significant decrease of plasma albumin when compared to the normal control group. Also, oral administration of nicotine showed non-significant change in plasma total protein. Supplementation of cranberry extract at 75 and 150mg/k.g. b.wk , resulted in a significant decrease in plasma total – and direct bilirubin ($p< 0.05$) as well as non-significant increase in plasma albumin and total protein compared to the group that received nicotine ($p< 0.05$). Table 4 showed that I.P. administration of nicotine (2.5 mg/kg.b.w) resulted in a significant increase in plasma total cholesterol (TC), triglycerides (TG) and LDL-C as well as a significant decrease in plasma HDL-C compared to the normal control group ($p< 0.01$). Supplementation of cranberry extract at 75 and 150mg/k.g.b.w , resulted in a significant decrease in plasma total cholesterol (TC), triglycerides (TG) and LDL-C as well as a significant increase in plasma HDL-C compared to the group that received nicotine ($p< 0.05$). Table 5 showed that I.P. administration of nicotine (2.5mg/kg.b.w) resulted in a significant decrease in blood reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) as well as a significant increase in plasma TBARs compared to the normal control group ($p< 0.01$). Supplementation of cranberry extract at 75 and 150mg/k.g.b.w. resulted in a significant increase in blood GSH, SOD and CAT as well as a significant decrease in plasma TBARs compared to the group that received nicotine ($p< 0.05$). Table 6 showed that I.P. administration of nicotine (2.5mg/kg.b.w) resulted in a significant decrease in heart reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) as well as a significant increase in heart TBARs compared to the normal control group ($p< 0.01$). Supplementation of cranberry extract at 75 and 150mg/k.g. b.w. resulted in a significant increase in heart GSH, SOD and CAT as well as a significant decrease in heart TBARs compared to the group that received nicotine ($p< 0.05$).

Table 1: Activity of alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and Creatine Kinase (CK) in plasma of normal and experimental groups of rats.

Group Number	Dose (mg/kg)	No. of animals/group	No. of dead animals	(Z)	(d)	(Z.d)
1	3000	10	0	0.5	3000	1500
2	6000	10	1	1.5	3000	4500
3	9000	10	2	4.5	3000	13500
4	12000	10	7	8.0	3000	24000
5	15000	10	9	8.5	3000	25550
6	18000	10	10	0	00	00

* Significantly different from normal group at/K 0.0J, ® Significantly different from control group at/K 0,05,. a: significant from normal control; b: significant from Nicotine (2.5 mg/kg.b.w) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mgAg b.w.).

Table 2: Activity of alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and Creatine Kinase (CK) in plasma of normal and experimental groups of rats.

Groups	ALT (U/L)	AST (U/L)	LDH (U/L)	CK (mU/mL)
Normal saline 0.9%	19.24 ± 2.55	31.9 ± 3.44	104.65 ± 6.40	201.84 ± 13.60
Control Nicotine (2.5 mg/kg.b.w)	56.7 ± 3.25*a	79.68 ± 4.80*a	185.6 ±8.70*a	413.89 ±19.18*a
Cranberry extract (75 mg/kg.b.w.)	21.79 ± 5.00@b	34.04 ± 7.67@b	142.32 ± 11.20@b	240.72 ± 15.84@b
Cranberry extract (150 mg/kg b.w.)	24.28 ± 4.76@b	30.2 ± 6.35@b	118.34 ± 6.37@abc	205.63 ± 11.30@b
Vitamin C (1 g/kg,b.w)	22.9 ± 3.10@b	32.65 ± 2.76@b	132.09±8.59@abcd	213.45 ± 12.47@b

* Significantly different from normal group at $p< 0.01$, @ Significantly different from control group at $p< 0.05$. . a: significant from normal control; b: significant from Nicotine (2.5 mg/kg.b.w) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.).

Table 3: Activity of alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) and creatinine kinase (CK) in heart tissue of normal and experimental groups of rats.

Groups	ALT (U/g)	AST (U/g)	LDH (U/g)	CK (mU/g)
Normal Saline 0.9%	24.58 ± 1.49	63.3 ± 4.35	52.01 ± 4.18	24.41 ± 2.15
Control Nicotine (2.5 mg/kg.b.w)	12.45 ± 2.11*a	41.2 ± 3.86*a	30.08 ±3.90*a	10.35 ±1.66*a
Cranberry extract (75 mg/kg.b.w.)	20.73 ± 3.60@b	59.78± 4.30@b	49.43 ± 3.40@b	18.51 ± 1.58@b
Cranberry extract (150 mg/kg b.w.)	26.1± 2.86@bc	64.53 ± 5.40@b	56.74± 4.75@bc	23.48± 2.18@b
Vitamin C (1 g/kg,b.w)	21.65± 2.50@bd	58.29 ± 3.77@b	50.32± 3.76@b	19.37± 2.26@bd

* Significantly different from normal group at $p< 0.01$, @ Significantly different from control group at $p< 0.05$. . a: significant from normal control; b: significant from Nicotine (2.5 mg/kg.b.w) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.).

Table 4: Activity of total- and direct bilirubin, albumin and total protein in plasma of normal and experimental groups of rats.

Groups	Total bilirubin (mg/dL)	Direct bilirubin (mg/dl)	Albumin (g/dL)	Total protein (g/dL)
Normal saline 0.9%	0.63± 0.034	0.23± 0.027	3.29± 0.24	6.84± 0.97
Control Nicotine (2.5 mg/kg.b.w)	0.91± 0.073@a	0.33± 0.034@a	3.00±0.45	7.02±0.86
Cranberry extract 75 mg/kg.b.w.	0.68± 0.076@b	0.28± 0.022@b	3.35± 0.35	7.09± 0.69
Cranberry extract 150 mg/kg b.w.	0.64± 0.055@b	0.25± 0.015@b	3.59± 0.42	7.3± 0.44
Vitamin C (1 g/kg,b.w)	0.68± 0.076@b	0.29± 0.036@b	3.48± 0.64	7.37± 0.88

Table 5: Level of plasma total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C of normal and experimental groups of rats.

Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Normal saline 0.9%	187.74 ± 12.65	98.61± 6.40	36.11 ± 4.11	86.47± 6.75
Control Nicotine (2.5 mg/kg.b.w)	214.5 ± 11.80^{*a}	154.5±11.66^{*a}	26.80 ± 2.46^{*a}	121.2± 10.60^{*a}
Cranberry extract (75 mg/kg.b.w.)	178.45 ± 9.86$^{@b}$	93.11±7.50$^{@b}$	30.80 ± 2.87 $^{@b}$	112.6± 13.00$^{@b}$
Cranberry extract (150 mg/kg b.w.)	174.58 ± 21.45$^{@b}$	97.64± 8.09$^{@b}$	35.26 ± 4.00$^{@b}$	91.3± 8.07$^{@b}$
Vitamin C (1 g/kg,b.w)	179.48 ±15.47$^{@b}$	87.23±6.58$^{@b}$	33.10 ± 3.78$^{@b}$	112.54± 11.90$^{@b}$

Table 6: Level of blood reduced glutathione (GSH) and activities of superoxide dismutase (SOD), catalase (CAT) and Thiobarbaturic acid reactive substances (TBARs) in normal and experimental groups of rats.

Groups	GSH (mg %)	SOD (U/mL)	CAT (U/mL)	TBARs (mmol/dL)
Normal saline 0.9%	269.72± 15.47	40.42 ± 2.99	33.83 ± 3.50	3.5 ± 0.44
Control Nicotine (2.5 mg/kg.b.w)	161.5± 11.75^{*a}	21.71±2.86^{*a}	17.2 ± 4.33^{*a}	6.2 ± 0.59^{*a}
Cranberry extract (75 mg/kg.b.w.)	199.5± 16.09$^{@ab}$	31.17± 3.77$^{@ab}$	23.2 ± 2.80$^{@ab}$	4.2 ± 0.65$^{@ab}$
Cranberry extract (150 mg/kg b.w.)	265.6 ± 22.65$^{@b}$	40.13 ± 2.79$^{@b}$	33.68± 4.26$^{@bc}$	3.7 ± 0.55$^{@b}$
Vitamin C (1 g/kg,b.w)	243.6 ± 10.90$^{@abcd}$	35.25 ± 3.76$^{@b}$	31.7± 2.87$^{@bc}$	4.2 ± 0.68$^{@ab}$

* Significantly different from normal group at $p< 0.01$, @ Significantly different from control group at $p< 0.05$.
. a: significant from normal control; b: significant from Nicotine (2.5 mg/kg.b.w) supplement group; c: significant from cranberry extract (75 mg/kg.b.w.); d: significant from cranberry extract (150 mg/kg b.w.).

4. DISCUSSION

Nicotine the major component of cigarette smoke plays an important role in the development of lung complications. Early-stage disease can be treated with curative intent although the risk for relapse is notoriously high. Unfortunately, the majority of lung cancer patients present at an advanced stage. Despite an initial response to treatment, most of these late stage patients will eventually progress on standard therapy and die from their disease. Despite the complex nature of lung cancer biology, its molecular underpinnings are becoming increasingly clear (Salgia et al., 2011). Nicotine is considered a prototype polycyclic aromatic hydrocarbon (PAH), classic DNA damaging agent and carcinogen. Antioxidants are the first source of protection of the body against free radicals and other oxidants, being the compounds that the attack and the formation of radical species within cells. The group of antioxidants inside the organism is known as the total antioxidant state (TAS) (Teixeira et al., 2013). The antioxidant protection of human cells includes enzyme mediated and non-enzymatic defense mechanisms. Superoxide dismutase (SOD), catalase (CAT) and glutathione-peroxidase (Gpx) are the most important antioxidant enzymes. SOD catalyses' the reaction of superoxide anion to hydrogen peroxide (H2O2); in turn, CAT converts H_2O_2 into water and oxygen. The

affinity of CAT for H_2O_2 is relatively low, therefore, some H_2O_2 remains in the cell. GSH-px is capable of detoxifying the remaining H_2O_2 (Arrigoni & De Tullio, 2002). Cranberry extract contained significant levels of vitamin C, total phenols, catechins and anthocyanins with associated antioxidant activity (Cao and prior 1999). The cranberry flavonoids are members of three groups; anthocyanins, flavonols, and proanthocyanins. The A and the B ring attach by the three carbon bridge on the C ring. The numbers on the rings represent position where functional groups attach. Anthocyanins Anthocyanins are very abundant in edible berries. They are responsible for the pigment of the berry and serve as a natural antioxidant. Anthocyanins vary in the number and position of -OH groups, sugar groups, and other functional groups. The present study showed that oral administration of nicotine (2.5 mg/kg.b.w) resulted in a significant increase in plasma ALT, AST, LDH, CK, TBARs total cholesterol, triglycerides and LDL-cholesterol as well as significant decrease in heart ALT, AST, LDH, CK, SOD, GSH, CAT and HDL-cholesterol compared to the normal control group (tables 1-6). Studies have shown that cranberry containing anthocyanins are effective at reducing oxidative stress induced by nicotine. Anthocyanins also act as an anti-inflammatory agent and help with platelet aggregation. These properties protect the heart by maintaining good blood flow (Zafra-Stone et al., 2007). Huang et al., (2009), demonstrate that inhibition of transcriptional response of the factor NF-kB could affect the synthesis of NO. The significant amelioration in antioxidant parameters (SOD, CAT and GSH) could be attributed to proanthocyanidins contents of cranberry extract. The availability of the phenolic hydrogen as hydrogen donating radical scavengers and singlet oxygen quenchers predicts their antioxidant activity (Bagchi et al., 2000). Huang et al., 2009, demonstrate that cranberry extract led to a significant reduction in NF-κβ transcriptional activation and TNF-α expression. Proanthocyanidins and flavonoids from cranberry and other Vaccinium berries functioning by blocking the expression of MMPs involved in remodelling the extracellular matrix (Pupa et al., 2002). In addition, the administration of cranberry extract inhibits NF- κB transcription activity (Huang et al., 2009). Cranberry extract received the highest ORAC value and exhibited superior antioxidant properties compared to vitamin C, E, and A. (Bagchi et al., 2004). Through this research it is thought that a combination of berry extracts exhibits the highest antioxidant capacity. This suggests that a combination of berry extracts, rather than one individual berry extract is optimal for reducing oxidative stress to the body. Another research study examined the anti-atherosclerotic activity of anthocyanins when supplemented in hamsters. Hypercholesterolemic hamsters were fed 10 mg per 1 gram of b.w of a berry extract including fruit extracts from wild blueberry, billberry, cranberry, elderberry, raspberry seeds, and strawberry for 12 weeks. Flavonols are the most common flavonoid found in foods. Flavonols are located mainly in the leaves and the outer parts of plants and can be found in both fruits and vegetables (Manach et al., 2004). One of the main representatives of flavonols is quercetin. Quercetin has two hydroxyl groups attached to the B ring. Compounds with dihydroxyl groups on the B ring have been shown to be more stable and exihibit antioxidant activity (Terao, Piskula, & Qing, 1994). Quercetin is found in cranberries and has been shown to be a very potent anti-oxidant (Manach et al., 2004). This suggests that a diet rich in flavonols may help reduce deaths related to CHD (Hertog et al., 1993). Proanthocyanins have the highest degree of polymerization of all the flavonoids that are present in cranberries. Proanthocyanins have been thought to have up to 50 or more sub-units attached to their flavonoid ring structure. This increased degree 20 of polymerization has been linked to a greater ability to inhibit LDL oxidation (Cunningham, et al., 2001).

From the obtained results, it could be concluded that cranberry extract was an effective in protection against heart toxicity induced by nicotine in rats since cranberry extract was able to ameliorate plasma oxidative stress biomarkers as well as enzymatic and non-enzymatic antioxidant defense system in heart tissue.

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