WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 6.647
Volume 6, Issue 12, 109-126 Research Article ISSN 2278 – 4357

THE PROTECTIVE EFFECT OF BEETROOT AND CARROT JUICES ON DOXORUBICIN-INDUCED CARDIAC INJURY AND ASSOCIATED INFLAMMATORY RESPONSE

Shimaa El-Masry and Amira Abd El-Rhman*

Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education A in Shams University.

Article Received on
08 October 2017,
Revised on 28 October 2017,
Accepted on 18 Nov. 2017
DOI: 10.20959/wjpps201712-10597

***Corresponding Author**
Dr. Amira Abd El-Rhman
Department of Biochemistry and Nutrition, Faculty of Women for Arts, Science and Education A in Shams University.

ABSTRACT

Doxorubicin (Dox), has an importance as antitumor antibiotic, but it's using is limited by the development of cardiomyopathy. Free radicals are involved in acute doxorubicin-induced toxicity and inflammatory condition in cardiac tissue. The study aimed to determine the protective effect of beetroot and/or carrot juices in acute Dox induced cardiac injury in adult male rats. Animals were randomly separated into 5 groups, G1control received normal saline I.P, G2 received single dose of Dox 20 mg/kg I.P, the other three groups also received doxorubicin 20 mg/kg I.P as single dose after four weeks cumulative doses of beetroot juice (10 mg/kg), carrot juice (10 mg/kg) and mixture of equal volumes of two juices respectively. Serum CK-MB, LDH, C-RP, Homocysteine and cardiac troponin-I were examined by ELIZA. Heart tissue histopathology, DNA damage of cardiac cell and oxidative stress and inflammatory markers were determined in all rats after 48hrs from doxorubicin injection. Doxorubicin induced cardiac injury was evidenced by a significant elevation in serum markers of heart function (CK-MB, LDH, Homocysteine and cardiac troponin-I), oxidative stress and inflammatory markers (MDA, CAT, IL-6 and CRP). Also cardiac cell's DNA damage was indicated by significant changes in comet assay parameters. All these results was confirmed by heart tissue histopathology. While, administration of beetroot and carrot juices protect heart tissue from damage by oxidative and inflammatory condition. Moreover mixing of two juices was more effective.

KEYWORDS: Doxorubicin, beetroot, carrot, oxidative stress, inflammatory response, DNA damage, CK-MB, LDH, C-RP, Homocysteine and cardiac troponin-I.

1. INTRODUCTION

Doxorubicin (Dox) is a potent anthracycline antibiotic that has been used as anti-cancer for many years. It used in treatment tumors such as hepatoblastoma., bone sarcomas, lymphoma, Wilm's tumor, soft tissue, neuroblastoma and leukemia (***El-Sayed et al., 2011).*** There is evidence suggest that oxidative stress with increased generation of Reactive Oxygen Species (ROS) mediates Dox-induced cardiotoxicity. Production of ROS after Dox treatment may results due to lipid peroxidation, with increasing malondialdehyde (MDA) levels. Dox injection also have been implicated in the incidence of cardiotoxic effects causing reduction of antioxidant enzyme levels (***Xin et al., 2011)***.

Beta vulgaris rubra, also known as red beetroot, is a potential herb used in cardiovascular conditions, the juice of beetroot is also used as a natural therapy to expel bladder and kidney stones (***Sharma et al., 2011)***. It was also reported that *Beta vulgaris* extracts possess antihypertensive, hypoglycemic, antioxidant (***Ninfali and Angelino, 2013)***, anti-inflammatory (***Jan and Sharma, 2011)*** and hepatoprotective activities (***El Gamal et al., 2014)***. Beetroot is among the highest nitrate-accumulating vegetables (***Hobbs et al., 2012)***. Nitrate ($NO3^{-}$) and nitrite ($NO2^{-}$), present in beetroot and in other food sources, were related to cardiovascular benefits (***El-Rai et al., 2016)***. The limited NO bioavailability is the main mechanism involved in endothelial dysfunction, which is crucial for the development of CVD (***Cai and Harrison, 2000)***. Beetroot is known to be a powerful antioxidant. Betalain pigments (betacyanins and betaxanthins) have specifically been shown to possess various antioxidant functions. Beets also contain small amounts of calcium, Vitamin A, Vitamin C, potassium, magnesium, copper, folate, phosphorus and iron (***Wettasinghe et al., 2002)***.

Carrot *(Daucus carota L.)* is a root vegetable from Apiaceae family. Carrots were used as a food after a long term use for medical purposes (***Simon, 2000)***. It is rich with flavonoids, carotenoids, polyacetylenes, minerals and vitamins which are of great nutritional and health benefits and act as, anticarcinogens, antioxidants and immune-enhancers (***Nicolle et al., 2003)***. Other benefits of carrots had been reported including, anti-inflammatory, cholesterol lowering, Anti-diabetic, anti-hypertensive, hepatoprotective and wound healing benefits (***Silva Dias, 2014)***. Reduction of oxidative DNA damage was observed following oral intake of carrot juice (***Olshansky et al., 2005).***

Therefore, the present study was conducted to evaluate the protective effects of beetroot and/or carrot juices on cardiac injury and associated inflammatory response induced by doxorubicin in rats.

2. MATERIALS AND METHODS

2.1. Plant materials

Beetroot and carrot were purchased from the local market, El-Salheyia, Cairo, Egypt. The juices were prepared from chopped beetroots and carrot by household juice extractor. The mixture of two juices was prepared by mixing equal volumes of two juices.

2.2. Chemicals

Doxorubicin drug was obtained from El-Ezaby Pharmacy, Cairo, Egypt.

2.3. Animals

Forty eight adult male albino rats "Sprague Dawely" weighed 250-300 g, kept in well-ventilated animal house of the Medical Research Center, Faculty of Medicine, Ain Shams University. Rats had been kept in the room for 1 week prior to the beginning of the experiment for acclimatization. They had access to 12h cycle of light/dark and provided with standard diet prepared by ***AIN (1993)*** and tap water *ad libitum.*

2.4. Experimental design

Rats were divided into five groups 8 rats / group except for group (2) contain 16 rats because of elevated mortality caused by doxorubicin. Group 1: (control group) rats were injected intraperitonealy (i.p) by normal saline. Group 2: (Dox. group) rats were injected i.p by 20 mg/kg Dox as a single dose according to ***Hadi, et al., (2012)*** Group 3: (Dox.+ BJ) received beetroot juice (10 ml/kg body weight/day) according to ***Rabeh (2015)*** orally using oral gavage for four weeks followed by doxorubicin injection (20 mg/kg). Group 4: (Dox.+CJ) received carrot juice (10 ml/kg body weight/day) according to ***Potter et al., (2011)*** orally using oral gavage for four weeks followed by doxorubicin injection (20 mg/kg). Group 5: (Dox.+BJ+CJ) received mixture of equal volumes of beetroot and carrot juices orally using oral gavage for four weeks followed by intraperitonial injection of Dox (20 mg/kg) as single dose.

2.5. Sample collection

At the end of experimental period (4weeks), all animals were sacrificed, blood samples were collected from hepatic portal vein. After blood clotting, serum was separated by centrifugation at 3000 r.p.m. for 10 min at 4°C and kept for biochemical analysis.

Hearts were removed, washed with saline solution, dried and weighed for its relative weight. Then heart tissue samples were taken for histopathological examination, determination of DNA damage in heart cell by comet assay and biochemical estimations.

2.6. Determination of Antioxidant Content in Beetroot and carrot juices

Total phenolic content was determined according to the Folin-Ciocalteu procedure and expressed as mg of gallic acid equivalent (GAE) per g of sample. Total flavonoid content was determined according to **Zilic *et al.*, *(2012)*** and expressed as mg of catechin equivalent (CE) per g of sample. Determination of radical DPPH scavenging activity (Antioxidant activity) was determined using the stable 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) according to ***Hwang and Do Thi., (2014).***

2.7. Determination of DNA damage in heart tissue

0.5 g of crushed samples were transferred to 1 ml ice-cold PBS., stirred for 5 min then filtered. Cell suspension was mixed with low-melting agarose (0.8% in PBS). 100 µl of this mixture was spread on pre-coated slides. The coated slides were immersed in lyses buffer (0.045 M TBE, pH 8.4, containing 2.5% SDS) for 15 min. The slides were placed in electrophoresis chamber containing the same TBE buffer, but devoid of SDS. The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40x increase for the count and measure the size of the comet. For visualization of DNA damage, observations are made of EtBr-stained DNA using a 40x objective on a fluorescent microscope according to ***Singh et al., (1988).***

2.8. Determination of oxidative stress and inflammatory biomarkers

Cardiac Catalase Activity was estimated by using Colorimetric/Fluorometric Assay Kit No.: #K773-100. Lipid Peroxidation byproduct, (MDA) was estimated in heart tissue using Colorimetric/Fluorometric Assay Kit No.: # K739-100. Interleukin-1α (IL-1α) was estimated as a pro-inflammatory marker using ELISA Kit No.: ERIL1A. Serum C - reactive protein [CRP] was estimated by using ELISA Kit No.: 557825.

2.9. Determination of serum heart function biomarkers

Estimation of serum Creatine Kinase MB (CK-MB) was performed by using ELISA Kit No.: ER0841, serum L-Lactate dehydrogenase (L-LDH) by using ELISA Kit No. : CSB-E11324r, cardiac troponin-I in serum using ELISA Kit No. KT-478 and serum Homocysteine (Hcy) using ELISA Kit No.: CSB-E13376r.

2.10. Histopathological Examination of heart

Cardiac tissues were immersed in formalin solution (10%), dehydrated in alcohol and embedded in paraffin. Sections of 5 µm were obtained, deparaffinized then stained with hematoxylin and eosin (H and E) then examined by standard light microscopy (***Bancroft et al., 1996).***

2.11. Statistical analysis

Results were expressed as mean ± Standard deviation (S.D.) of the mean. Differences among means were tested for statistical significance by one-way analysis of variance using SPSS package version 19. Statistical significance was considered when $P < 0.05$.

2. RESULTS

Table (1) shows the content of total phenols, total flavonoids and the antioxidant capacity of red beetroot and carrot. The chemical analysis revealed that red beetroot had higher amount of total phenols and total flavonoids compared with carrot. The DPPH which measure the relative antioxidant abilities of the two samples to scavenge free radicals generated in the assay system demonstrated that beetroot had higher antioxidant activity compared with carrot.

The results in table (2) indicated that heart weight and relative heart weight were significantly ($p<0.05$) decreased in doxorubicin treated rats that recorded (0.625±0.046) and (0.282±0.001) compared to control that recorded (0.712±0.099) and (0.380± 0.001) respectively. Administration of beetroot or (beetroot + carrot) juices before doxorubicin injection caused significant increase in heart weight and relative heart weight when and their recorded values were (0.750±0.092), (0.367±0.008), (0.737±0.074), (0.355±0.001) respectively. On the other hand, carrot juice administration before doxorubicin injection showed non-significant change at ($p<0.05$) on the heart weight and relative heart weight although its means were noticeably higher than in the doxorubicin group. Doxorubicin injection induced 30% mortality in rats,

this was markedly decreased to 10% in rats administered carrot juice before injection and abolished in rats treated with beetroot or (beetroot + carrot) juices before injection.

Comet assay is a sensitive method for the detection of DNA damage. Dox injection cause damage in cardiomyocyte DNA the percent of damage (Tailed DNA%) reached (36.812±0.60) in Dox group compared to control (4.962±0.64). Moreover Dox injection led to increase the levels of comet parameters (% tail DNA, tail length and tail moment) and the percentages of increase were (208.27%, 194.51% and 399.67%) compared to the control group table (3). Beetroot and /or carrot juices pretreatment significantly reduced the Dox-induced cardiomyocyte DNA damage as shown from % of tailed DNA (12.775±0.28), (14.850±0.23) and (10.475±0.75) respectively when compared with (36.812±0.60) in Dox group. Also it was noticed that there was a significant improvement in comet parameters in groups pretreated with BJ and/or CJ when compared with Dox group. The results tabulated in table (3) were confirmed by figure (1) that showed appearance and percentages of damaged DNA (tailed DNA) in all groups.

The data in Table (4) represent the effect of beetroot and/or carrot juices on Dox-induced changes in cardiac oxidative status and inflammation markers. The results demonstrated that Dox injection extremely increased malondialdehyde level in heart tissue as a marker for lipid peroxidation comparing to control group, the elevation of MDA was from (115.257±0.090) to (234.122±0.084) ($P<0.05$) and the percentage of increment in MDA was (203.13%). Pretreatment with beetroot and/or carrot juices causes a significant reduction MDA level, with a percentages (160.73%, 167.95% and 158.25%) respectively compared to (203.13%) with doxorubicin alone. There was a marked reduction in catalase CAT activity Dox-injected rats (9.088±0.089) compared to control (14.685±0.035). And the percentage of decrement was (61.88%) when compared to control. While pretreatment with beetroot and/or carrot juices resulted in a significant increase in cardiac CAT enzyme activity the percentages of change in CAT activity were (87.44%, 83.74% and 88.11%) respectively compared to (61.88%) with doxorubicin alone. These results indicate that beetroot and carrot down regulate the level of cardiac MDA and up regulate the cardiac CAT activity so they have antioxidant activity.

From the results in table (4) it was also noticed that Dox injection produced a significant increase in the cardiac inflammatory biomarker IL-1α compared to control rats and the percentage of increment was 212.62% compared to control. However, the change in IL-1α

levels were significantly decreased in beetroot and/or carrot treated groups and mean values reached (6.885±0.057), (6.495±0.037) and (6.278±0.033) respectively compared to Dox group mean value (11.231±0.039). Serum C-reactive protein a marker of acute phase of inflammation showed a marked increase in Dox injected group (805.58±0.12) compared to control (398.38±0.07). While the pretreatment with BJ, CJ and BJ+CJ modulate this increment to reach (428.09±0.04), (433.57±0.06) and (405.28±0.04) respectively. These results indicate that beetroot and carrot down regulate levels of the cardiac IL-1α and serum CRP so they have anti-inflammatory activity. Moreover, we can notice that the antioxidant and anti-inflammatory effects of beetroot preceded the effects of carrot while their combination together was the best.

Parameters of heart function are listed in table (5), included serum (LDH, CK-MB, Troponin-1 and Homocysteine). From the results we can observe that Dox injection caused a significant increase of serum LDH level (2.321±0.030) to (4.627±0.056) with 199.35% of rise compared to baseline value ($P< 0.05$). While the pretreatment with BJ, CJ and BJ+CJ modulated the increased serum LDH levels induced by Dox with a rise percentage 138.38%, 149.41% and 133.69% respectively. We can observe a significant increase of serum CK-MB level from (11.493±0.048) in control to (17.992 ±0.040) in Dox injected group with a percentage of rise 156.54% compared to baseline value. While, pretreatment with BJ, CJ and BJ+CJ reduced the increased CK-MB level. In present study we noticed a significant increase in the group injected with doxorubicin from (0.286±0.026) to (0.608±0.036), as shown in table (5). While the oral administration of beetroot and/or carrot juices significantly reduced the level of serum troponin- I $p<0.05$ to approach the normal level especially in group treated with (BJ+CJ) the mean values were (0.311±0.023), (0.372±0.017) and (0.292±0.024) respectively. Serum homocysteine level recorded a significant increase in Dox injected group when compared to control the percentage of increase was 283.02% compared to baseline value ($P<0.05$). While pretreatment with beetroot and/or carrot juices improved this increment of serum homocysteine significantly the percentages of raise were (187.27%, 157.85% and 136.15%) compared to the percentage of Dox alone 283.02%. From the results of heart function parameters we can notice that pretreatment with two juices together had the best effect on enhancement of heart function.

Table (1): Total phenols, total flavonoids and antioxidant activity content of red beetroot and carrot.

Parameters / Samples	Total Phenols (GAE mg/100g)	Total Flavonoids (CE mg/100g)	DPPH (AAE mg/100g)
Red Beetroot	87.9	132.9	78.00
Carrot	78.72	18.55	29.92

GAE: Gallic acid equivalent, CE: Catchin equivalent, AAE: Ascorbic acid equivalent.

Table (2): Effect of beetroot and/or carrot juices on doxorubicin-induced changes in relative body weight, heart weight and percent of mortality compared with control group.

Parameters / Groups	Heart weight (g)	Relative heart weight (%)	Percent of mortality (%)
Control	b 0.712±0.099	b 0.380±0.001	0
Doxorubicin	a 0.625±0.046	a 0.282±0.001	30
Dox.+ B.J.	b 0.750±0.092	b 0.367±0.008	0
Dox. +C.J.	a 0.675±0.111	a 0.337±0.001	10
Dox.+B.J.+C.J.	b 0.737±0.074	b 0.355±0.001	0

Values are represented as mean ± SD, There was no significant difference between means have the same letter in the same column (P<0.05).

Table (3): Effect of beetroot and/or carrot juices on doxorubicin-induced changes in DNA damage markers (comet assay) in heart tissue compared with control group.

Parameters / Groups	Tailed (%)	Untailed (%)	Tail DNA (%)	Tail Length (µM)	Tail Moment (Units)
Control	a 4.962±0.64	d 95.03±0.64	a 1.655±0.033	a 1.622±0.036	a 2.728 ±0.006
Doxorubicin	d 36.812±0.60	a 63.187±0.60	c 3.447±0.036	c 3.155±0.028	d 10.903±0.003
Dox.+ B.J.	b, c 12.775±0.28	b, c 87.225±0.28	b 1.896±0.0311	b 1.803±0.030	b 6.206±0.003
Dox. +C.J.	c 14.850±0.23	b 85.150±0.23	b 1.822±0.027	b 1.741±0.035	c 7.891±0.003
Dox.+B.J.+C.J.	b 10.475±0.75	c 89.525±0.75	a 1.582±0.028	a 1.676±0.040	b 5.148±0.004

Values are represented as mean ± SD, There was no significant difference between means have the same letter in the same column (P<0.05).

Table (4): Effect of beetroot and/or carrot juices on doxorubicin-induced changes on oxidative stress and inflammation markers in heart tissue and serum compared with control group.

Parameters / Groups	MDA nmol/mg	CAT mU/mg	IL-1 α pg/mg	Serum CRP ng/ml
Control	a 115.257±0.090	c 14.685±0.035	a 5.282±0.045	a 398.38±0.07
Doxorubicin	d 234.122±0.084	a 9.088±0.089	c 11.231±0.039	c 805.58±0.12
Dox.+ B.J.	b 185.255±0.117	b 12.841±0.042	b 6.885±0.057	b 428.09±0.04
Dox. +C.J.	c 193.581±0.113	b 12.298±0.018	b 6.495±0.037	b 433.57±0.06
Dox.+B.J.+C.J.	b 182.398±0.010	b 12.940±0.036	a,b 6.278±0.033	a 405.28±0.04

Values are represented as mean ± SD, There was no significant difference between means have the same letter in the same column (P<0.05).

Table (5): Effect of beetroot and/or carrot juices on doxorubicin-induced changes in serum markers for heart function compared with control group.

Parameters / Groups	LDH mU/ml	CKMB pg/ml	TROP-I ng/mL	HOMOCYST nmol/ml
Control	a 2.321±0.030	a 11.493±0.048	a 0.286±0.026	a 159.26±0.041
Doxorubicin	c 4.627±0.056	c 17.992±0.040	c 0.608±0.036	d 450.74±0.410
Dox.+ B.J.	b 3.212±0.038	b 13.353±0.045	a, b 0.311±0.023	c 298.26±0.058
Dox. +C.J.	b 3.468±0.045	b 13.606±0.033	b 0.372±0.017	b 251.40±0.057
Dox.+B.J.+C.J.	b 3.103±0.031	b 13.041±0.040	a 0.292±0.024	b 216.84±0.084

Values are represented as mean ± SD, There was no significant difference between means have the same letter in the same column (P<0.05).

Histopathological examination of heart tissue

The histopathological examination revealed that cardiac tissues of control group rats showed normal cardiac myocytes figure (2), meanwhile, heart of rats injected with doxorubicin showed dilatation and congestion of myocardial blood vessel, mononuclear cells infiltration between cardiac myocytes and focal necrosis of cardiac myocytes figure (3). Meanwhile, pretreatment with beetroot juice significantly maintained the normal histological appearance

of myocardium except congestion of myocardial blood vessel in some sections figure(4). Also, some examined sections from carrot juice pretreated rats showed congestion of myocardial blood vessels whereas, other sections from this group revealed no histopathological changes figure (5). The best histopathological results were clearly showed in rats which pretreated with both beetroot and carrot juices together, this was indicated in figure (6) which revealed no histopathological changes.

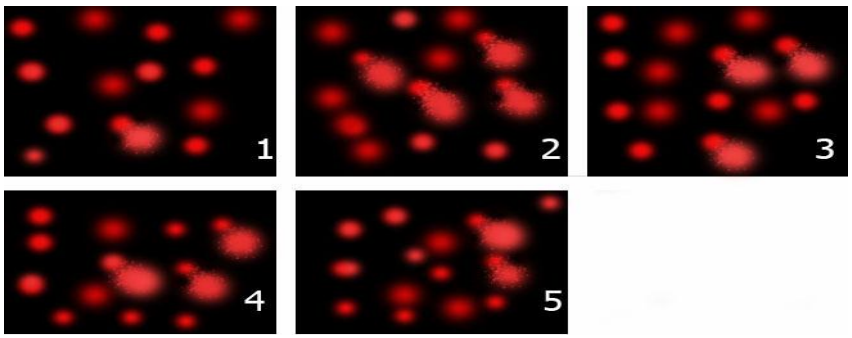

Fig. (1): The comets tails lengths were measured from the middle of the nucleus to the end of the tail with 40x increase for the count and measure the size of the comet.

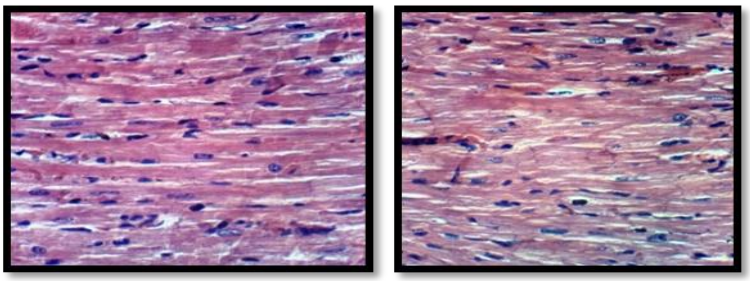

Fig. (2): Heart of rat from group 1 showing normal cardiac myocytes (H & E X 400).

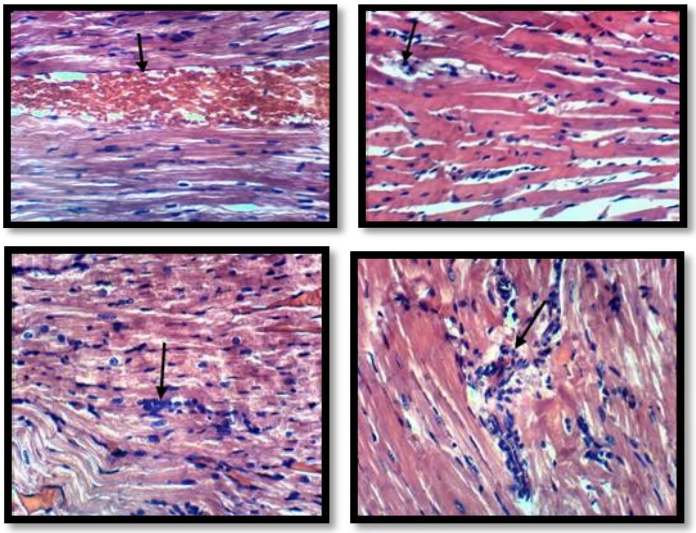

Fig. (3): Heart of rat from group 2 showing dilatation and congestion of myocardial blood vessel, mononuclear cells infiltration between cardiac myocytes and focal necrosis of cardiac myocytes (H & E X 400).

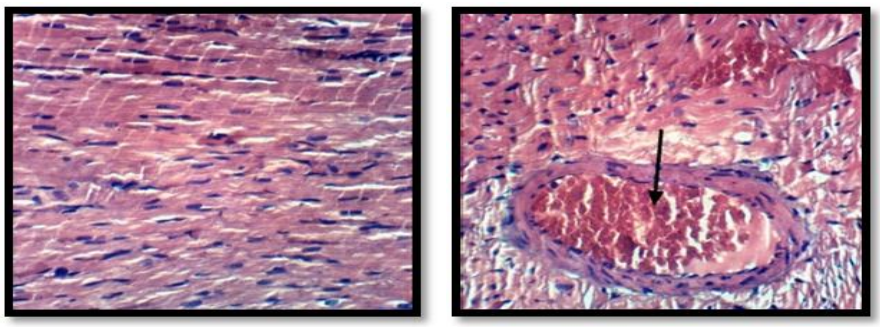

Fig. (4): Heart of rat from group 3 showing reduced Dox-induced degenerative changes in myocardial blood vessels and maintained normal histological appearance of myocardium (H & E X 400).

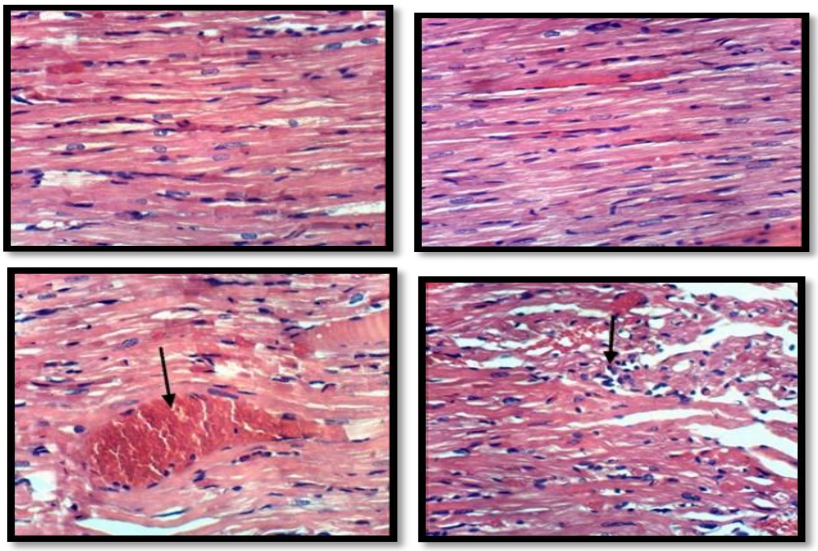

Fig. (5): Heart of rat from group 4 showing reduction in congestion of myocardial blood vessels and fewer mononuclear cells infiltration between cardiac myocytes (H & E X 400).

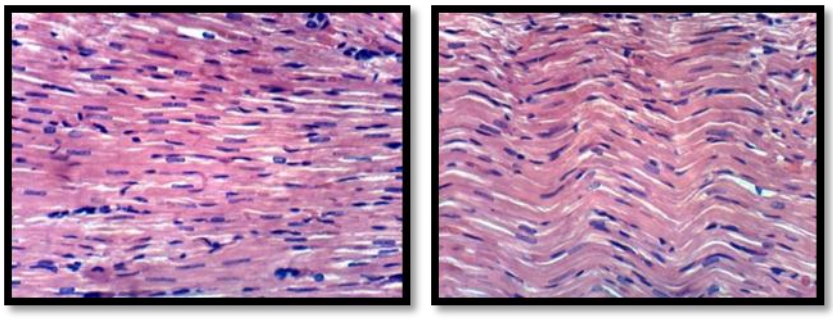

Fig. (6): Heart of rat from group 5 showing no histopathological changes and maintained normal histological appearance of myocardium (H & E X 400).

3. DISCUSSION

From the results in table (1) it is clear that samples of red beetroot and carrot have efficient amounts of total phenols and total flavonoids, beetroots have higher amount of total phenols

and total flavonoids followed by carrot. The DPPH assay, that measure the antioxidant abilities of beetroot and carrot to scavenge free radicals showed that red beetroots have good antioxidant properties and these results were in agreement with ***Váli et al. (2007)*** who mentioned that beetroots contain important bioactive agents (betains and polyphenols), which have a wide range of physiologic effects. ***Pal et al., (2010)*** reported the presence of flavonoids, carbohydrate, betains and anthocyanin pigments in, *Beta vulgaris* (beetroot). In the phytochemical studies the antioxidant activity determined by the DPPH method exhibited 40% and 78% activity in methanol extracts of carrot and beetroot respectively (***Shyamala and Jamuna, 2010).*** So, the results from chemical analysis suggest that red beetroot and carrot can be exploited for their antioxidant components and used for value addition in food formulations.

Dox is a known effective chemotherapeutic agent used against different types of cancers. However, it had a limited use due to its toxic effect on cardiac muscles. (***Kontny et al., 2013).*** Our results have shown Dox injected rats possess increased mortality, 30% of the animals died. Cardiomyopathy make the most important contribution to the mortality. An observed reduction in heart/body weight ratio indicated a severe cardiac performance dysfunction (***El-Sayed et al., 2011)***. While decreasing the percentage of mortality in group that consumed carrot juice to 10% confirms the cardio-protective effects of this juice (***Potter at al., 2011).*** Moreover disappearing of mortality (0%) in groups consumed beetroot juice and beetroot with carrot juices confirms the better effect of beetroot if alone or compound with carrot juice (***Hobbs et al., 2012).***

The oxidative damage of cardiomyocyte DNA may lead to of cardiotoxicity and death) ***Clifford et al., 2015).*** In the present study, the Comet parameters (tail DNA, tail extent moment and tail length) in cardiac cell were disturbed in Dox group rats compared with control rats. While in groups that pretreated with beetroot juice and /or carrot juice comet parameters showed significant enhancements when compared with Dox group. Our results agreed with the results of ***Lee et al., (2009)*** who examined the effect of red beetroot on oxidative damage of DNA and they found an effective reduction in lymphocytes damaged DNA manifested as a significant decrease in Comet parameters ($p<0.05$). Moreover, ***Rabeh, (2015)*** confirmed that vitamin C and E were strong antioxidants used to reduce the cardiotoxicity (***Ayaz et al., 2004).*** Our results were in agreement with studies showing relation between antioxidant effects of plants and cardiac injury***) Erboga et al., 2016).*** In

previous reports it was confirmed that beetroot has beet pigments known as betalains these pigment are of higher antioxidant power (***Lee et al., 2005)***. These results go hand in hand with our results in determination of antioxidant (DPPH) that showed a potent antioxidant activity, which further supports its radical scavenging activity. The observed antioxidant and cardio protective effect is considered to be related to the phyto-constituents of beetroot) ***frank et al., 2005).*** They also exert anti-carcinogenic activities, reduce inflammatory insult, and modulate immune response) ***Silva Dias, 2014).*** The oxidative stress parameters and inflammatory markers tested in our study (MDA, CAT. IL1α and CRP) were affected by Dox-injection that associated with production of ROS. Dox produce free radical by two different ways, the first is the formation of a semiquinone free radical which gives superoxide radicals ***(Singal et al., 2000)*** and the second way produces Fe^{2+} Dox complex ***(DeBeer et al., 2001)*** that can reduce oxygen to H2O2 and other active species ***(Schinella et al., 2002).*** In our study, elevated MDA levels was observed after Dox treatment but the pretreatment with beetroot and carrot juices either single or compound together attenuated lipid peroxidation evoked by doxorubicin in heart of rats and lead to decreased MDA level. The increased MDA levels can be explain the fact that, hydroxyl radicals (OH•) greatly enhances the NADH-dependent microsomal lipid peroxidation and thus lead to oxidative damage to cell membranes. MDA was significantly reduced by the effect of BJ and CJ this can be explained by that antioxidant in these juices allows free radicals to attract a hydrogen atom from the antioxidant rather than attacking from polyunsaturated fatty acids, this will break the free radical reactions ***(Hadi et al., 2012)***. ***Erboga et al., (2016)*** indicated the reduced activity of CAT in Dox group. A study by ***Subburaman et al., (2014)*** examined the naringen protective effect of against Dox-induced cardiotoxicity, Dox injection reduced CAT activity. Similar reduction in CAT activity was observed in the study by ***Alpsoy et al., (2013)*** in which Dox was administered at a dose of 30 mg/kg. In line with previous reports, Dox was associated with reduced levels of CAT, while administration of BJ, CJ were associated increased levels of CAT. Moreover their combination together had the best effect. Elevation of cardiac IL-1α in Dox injected rats is an indicator of cellular inflammation since IL-1α is produced mainly by activated macrophages, as well as neutrophils, epithelial cells and endothelial cells. Serum CRP level increased significantly in Dox group that reflect the inflammatory condition occur by Dox. While this inflammation improved in other groups due to pretreatment with natural juices this results agreed with ***Potter et al., (2011)*** who noted that the decrease in the C-reactive protein, as an index for inflammation may have been related to drinking carrot juice which is a good source of b-carotenes and α-carotenes.

Heart tissue were very sensitive to ROS induced damage this may due to its fewer antioxidant defenses compared to other organs this explain the increase of LDH and CK concentration in the serum of animals injected with doxorubicin. The LDH and CK mechanism for release seems to be from oxidative damage of Dox to cardiac tissue and the subsequent release of its contents into circulation ***(Raskovic et al., (2011).*** While normalization of CK-MB and LDH elevated levels in groups consumed beetroot juice and carrot juice confirms the cardio-protective effects of these juices. These results are on the same line with the previous studies reported by ***Nagi and Mansour, (2000)*** that demonstrate a significant increase in serum enzymes, LDH and CK after Dox injection due cardiac damage by doxorubicin which as a result of of nucleic acid inhibition and protein synthesis (***Hadi et al., 2012)***.

Serum troponin I results of the current were coincided with the work of ***Hadi et al., (2012)*** who reported marked macrophage accumulation with dysfunction of cardiac endothelium. Serum homocysteine showed a marked increase in Dox injected rats. Hardening of arteries due to the accumulation of fatty plaques may occur due to high levels of homocysteine. Vegetables as beetroots and carrots contain vitamins among them is pyridoxine (B6) which is necessary for carbohydrate, fat and protein metabolism. Pyridoxine and folate inhibits the of homocysteine formation and reduces the heart disease risk (***Silva Dias, 2014).***

Histological examination by light microscopy can prove doxorubicin toxicity, but it can only reveal gross cellular changes. By using electronic microscopy more common ultra-structural changes such as sarcotubular edema and disorders of the fine mitochondrial structure can be found (***Raskovic et al., 2011).*** In our study the structural and functional changes, demonstrated microscopically in the heart tissue of doxorubicin intoxicated rats, showed dilatation and congestion of myocardial blood vessel, mononuclear cells infiltration between cardiac myocytes and focal necrosis of cardiac myocytes associated with mononuclear cells infiltration. These results go hand in hand with increased serum levels of troponin I, homocysteine and increased ROS condition that were observed in Dox group. While pretreatment with beetroot and /or carrot juices significantly reduced the Dox-induced histopathological changes in the cardiac tissue and maintained the normal histological appearance of myocardium this effect may be due to the highly antioxidant activities of these natural juices ***(Injac et al., 2009)***.

4. CONCLUSION

The present study revealed that beetroot and carrot juices administration are beneficial natural anti-inflammatory and antioxidant products that can attenuate the cardiac injury that caused by doxorubicin injection. Moreover; mixing together enhance the protective effect against DNA damage rather than using each of them alone.

5. Conflicts of interest

There are no conflicts of interest to declare.

6. REFERENCES

1. Alpsoy, S., Aktas, C., Uygur, R., et al., (2013): Antioxidant and anti-apoptotic effects of (Allium cepa) extract on doxorubicin-induced cardiotoxicity in rats. J Appl Toxicol, 33(3): 202-28.
2. Ayaz, S.A., Bhandari, U., Pillai, K.K., et al., (2004): Influence of DL α-lipoic acid and vitamin E against doxorubicin - induced biochemical and histological changes in the cardiac tissue of rat. Indian J Pharmacol, 37(5): 294-299.
3. Bancroft, J.D., Stevens, A. and Turner D.R., (1996): Theory and practice of histological techniques, 4th ed. Livingstone, New Yourk, Churchill.
4. Cai, H. and Harrison, D. G. (2000): Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circulation Research, 87(10): 840–844.
5. Clifford, T., Howatson, G., West, D.J. and Stevenson E. J. (2015): The Potential Benefits of Red Beetroot Supplementation in Health and Disease Nutrients, 7: 2801-2822.
6. De Beer E.L., Bottone, A.E. and Voes, E.E., (2001): Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment. Eur. J. Pharmacol., 415: 1-11.
7. El Gamal, A. A., AlSaid, M. S., Raish, M., Al-Sohaibani, M., Al-Massarani, S.M., Ahmad, A., Hefnawy, M., Al-Yahya, M., Basoudan, O.A. and Rafatullah, S., (2014): Beetroot (Beta vulgaris L.) Extract Ameliorates Gentamicin-Induced Nephrotoxicity Associated Oxidative Stress, Inflammation and Apoptosis in Rodent Model. Mediators of Inflammation, Volume 2014, Article ID 983952, 12 pages.
8. El-Rei, J., Cunha, A. R., Trindade, M. and Neves, M.F., (2016): Beneficial Effects of Dietary Nitrate on Endothelial Function and Blood Pressure Levels. International Journal of Hypertension, Volume 2016, Article ID 6791519, 6 pages.

9. Erboga, M., Donmez, Y. B., Sener, U., Erboga, Z.F., Aktas, C. and Kanter, M., (2016): Effect of Urtica Dioica against Doxorubicin-Induced Cardiotoxicity in Rats through Suppression of Histological Damage, Oxidative Stress and Lipid Peroxidation. Eur J Gen Med, 13(2): 139-144.
10. Frank, T., Stintzing, F. K., Carle, R., Bitsch, I., Quaas, G., Bitsch, R., Netzel, M., (2005): Urinary pharmacokinetics of betalains following consumption of red beet juice in healthy humans. Pharmacol. Res., 52: 290-297.
11. Hadi, N., Yousif, N.G., Al-amran, F.G., Huntei, N.K., Mohammad, B.I. and Ali, S.J., (2012): Vitamin E and telmisartan attenuates doxorubicin induced cardiac injury in rat through down regulation of inflammatory response. BMC Cardiovascular Disorders, (12): 63-69.
12. Hobbs, D.A., Kaffa, N., George, T. W., Methven, L. and Lovegrove, J.A., (2012): Blood pressure lowering effects of beetroot juice and novel beetroot enriched bread products in normotensive male subjects. British Journal of Nutrition, 8: 1-9.
13. Hwang, E.S. and Do Thi, N. (2014): Effects of Extraction and Processing Methods on Antioxidant Compound Contents and Radical Scavenging Activities of Laver (Porphyra tenera). Prev. Nutr. Food Sci., (19): 40-48.
14. Injac, R., Perse, M., Cerne, M., Potocnik, N., Radic, N., Govedarica, B., Djordjevic, A., Cerar, A. and Strukelj, B., (2009): Protective effects of fullerenol C60(OH)24 against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats with colorectal cancer. Biomaterials, 30: 1184-1196.
15. Jain, S. G. V. and Sharma, P. K., (2011): Anti-inflammatory activity of aqueous extract of Beta vulgaris L. Journal of Basic and Clinical Pharmacy, 2: 83-86.
16. Kontny, N.E., Würthwein, G., Joachim, B., Boddy, A.V., Krischke, M., et al., (2013): Population pharmacokinetics of doxorubicin: establishment of a NONMEM model for adults and children older than 3 years. Cancer Chemother Pharmacol, 71: 749-763.
17. Lee, C. H., Wettasinghe, M., Bolling, B. W., Ji, L. L. and Parkin, K. L., (2005): Betalains, phase II enzyme-inducing components from red beetroot (Beta vulgaris L.) extracts, Nutrition and Cancer, 53(1): 91-103.
18. Lee, J. H., Son, C. W., Kim, M. Y., Kim, M.H., Kim, H. R., Kwak, E.S., Kim, S. and Kim, M.R., (2009): Red beet (Beta vulgaris L.) leaf supplementation improves antioxidant status in C57BL/6J mice fed high fat high cholesterol diet. Nutrition Research and Practice, 3(2): 114-121.

19. Nagi, M. N. and Mansour, M. A., (2000): Protective effect of thymoquinone against doxorubicin–induced cardiotoxicity in rats: a possible mechanism of protection Pharmacological Research, 41(3): 283-289.
20. Nicolle, C., Cardinault, N., Aprikian, O., Busserolles, J., Grolier, P., Rock, E., Demigné, C., Mazur, A., Scalbert, A., Amouroux, P. and Rémésy, C. (2003): Effect of Carrot Intake on Cholesterol Metabolism and on Antioxidant Status in Cholesterol-Fed Rat. European Journal of Nutrition, 42: 254-261.
21. Ninfali, P. and Angelino, D., (2013): Nutritional and functional potential of Beta vulgaris cicla and rubra. Fitoterapia, 89(1): 188-199.
22. Olshansky, S.J., Passaro, D.J., Hershow, R.C., Layden, J., Carnes, B.A., Brody, J., Hayflick, L., Butler, R.N., Allison, D.B. and Ludwig, D.S., (2005): A potential decline in life expectancy in the United States in the 21st century. N Engl J Med, 352: 1138-1145.
23. Pal, R., K. Girhepunje, H. Gevariya and N. Thirumoorthy, (2010): Hepatoprotective activity of Beta vulgaris against CCl 4 induced acute hepatotoxicity in rats. Archives of Applied Science Res., 2(1): 14-18.
24. Pool-Zobel, BLBA, Liegibel, UM, Treptow-van Lishaut S, Rechkemmer G, (1998): Mechanisms by which vegetable consumption reduces genetic damage in humans. Cancer Epidemiol Biomarkers Prev, 7: 891-899.
25. Potter, A. S., Foroudi, S., Stamatikos, A., Patil, B. S. and Deyhim, F., (2011): Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. Nutrition Journal, 10: 96-102.
26. Potter, A.S., Foroudi, S., Stamatikos, A., Patil, B.S. and Deyhim, F., (2011): Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. Nutrition Journal, (10): 96-101.
27. Rabeh, N.M., (2015): Effect of Red Beetroot (Beta vulgaris L.) And its Fresh Juice Against Carbon Tetrachloride Induced Hepatotoxicity in Rats. World Applied Sciences Journal, 33(6): 931-938.
28. Rašković, A., Stilinović, N., Kolarović, J., Vasović, V., Vukmirović, S. and Mikov, M., (2011): The Protective Effects of Silymarin against Doxorubicin-Induced Cardiotoxicity and Hepatotoxicity in Rats. Molecules, 16: 8601-8613.
29. Revees, G., Nielsen, F. H. and Fahey, G. C., (1993): AIN reformation of the AIN-76: Purified Diets for Laboratory Rodents: Final report of the American Institute of Nutrition Purified Ad Hoc Writing Committee on the reformation of the AIN-76 Arodent diet. American Istitute, J. Nutr.; 26: 1939-1951.

30. Schinell, G.R., Tournier, H.A., Prieto, J.M. and Rios, J.L., (2002). Antioxidant activity of anti-inflammatory plant extracts. Life Sci., 70: 1023-1033.
31. Sharma, N., Tanwer, B.S. and Vijayvergia, R., (2011): Study of medicinal plants in Aravali regions of Rajasthan for treatment of kidney stone and urinary tract troubles. International Journal of Pharm Tech Research, 3(1): 110-113.
32. Shyamala, B.N. and Jamuna, P., (2010): Nutritional Content and Antioxidant Properties of Pulp Waste from D carota and Beta vulgaris. Mal. J. Nutr., 16(3): 397-408.
33. Silva Dias, J. C., (2014): Nutritional and Health Benefits of Carrots and Their Seed Extracts. Food and Nutrition Sciences, 5: 2147-2156.
34. Simon, P.W., (2000): Domestication, Historical Development and Modern Breeding of Carrot. Plant Breeding Reviews, 19: 157-190.
35. Singal, P.K., Li, T., Kumar, D., Danelisen, I. and Iliskovic, N., (2000): Adriamycin induced heart failure: Mechanism and modulation. Mol. Cell. Biochem, 207: 77-85.
36. Singh, N. P., McCoy, M. T., Tice, R. R. and Schneider E. L., (1988): A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res., (175): 184-191.
37. Subburaman, S., Ganesan, K. and Ramachandran, M., (2014): Protective role of naringenin against doxorubicin-induced cardiotoxicity in a rat model: histopathology and mRNA expression profile studies. J Environ Pathol Toxicol Oncol, 33(4): 363-76.
38. Váli, L., E. Stefanovits-Bányai, K. Szentmihályi, H. Fébel, E. Sárdi, A. Lugasi, I. Kocsis and A. Blázovics, (2007): Liver-protecting effects of table beet (Beta vulgaris var. rubra) during ischemia-reperfusion. Nutr., 23(2): 172-8.
39. Wettasinghe, M., Bolling, B., Plhak, L., Xiao, H. and Parkin, K., (2002): phase II enzyme-inducing and antioxidant activities of beetroot (Beta vulgaris L.) extracts from phenotypes of different pigmentation. Journal of Agricultural and Food Chemistry, 50(23): 6704-6709.
40. Xin, Y., Zhang, S., Gu, L., et al., (2011): Electrocardiographic and biochemical evidence for the cardioprotective effect of antioxidants in acute doxorubicin-induced cardiotoxicity in the beagle dogs. Biol Pharm Bull, 34: 1523-6.
41. Zilic, S., A. Serpen, G. Akýllýoglu, M. Jankovic and V. Gökmen, (2012): Distributions of phenolic compounds, yellow pigments and oxidative enzymes in wheat grains and their relation to antioxidant capacity of bran and debranned flour. Journal of Cereal Science, (56): 652-658.