International Journal of Phytomedicine 8 (2016) 217-227
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Cranberry extract as a supplemented food in treatment of oxidative stress and breast cancer induced by N-methyl-N-nitrosourea in Female Virgin Rats

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Abstract

Breast cancer is the most common cancer and a major cause of death in women. The present study was designed to evaluate the antioxidant and anticancer potential of cranberry extract against N-methyl-N-nitrosourea (MNU) induced mammary carcinoma in rats. The tumor was induced in Female virgin rats of age 50 days by single dose of MNU (50mg/kg.b.w i.p.). After 85 days; all rats developed at least one tumor. Animals were treated with cranberry extract (400 and 600 mg/kg.b.w.orally) and tamoxifen (2mg/kg.b.w. i.p) for 4 weeks (from day 86 to day 113). MNU treatment resulted in a significant decrease ($p < 0.05$) in blood hemoglobin (Hb), red blood cells (RBC), platelets (PLTs) as well as blood, liver and breast catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). However, MNU treatment resulted in a significant increase in White blood cells (WBC) as well as plasma, liver and mammary tissue gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), hexosamine, sialic acid and thiobarbituric acid reactive substances (TBARs). Upon administration of the cranberry extract, the levels of WBC, GGT, LDH, hexosamine, sialic acid, TBARs, Hb, RBC, PLTs, CAT, GPx and SOD were significantly normalized. Histopathological changes also confirmed the formation of tumor tubules and neovascularization after the MNU treatment. Cranberry extract administration significantly reduces the growth of MNU-induced mammary tumors, and therefore has strong potential as a useful therapeutic regimen for inhibiting breast cancer development. Comparing the beneficial effect of cranberry extract with that of MNU-induced breast cancer, cranberry extract showed antitumor and antioxidant activity indicated by the measured biochemical parameters and the histopathological examination of mammary tissue. The results of the present study indicate that cranberry extract possesses strong anticancer effects through its role in modulating glycoprotein components and the levels of oxidative stress biomarkers. Cranberry exerted a stronger anticancer effect at the dosage of 600 mg/kg body weight than at dosage 400 mg/kg body weight.

Keywords: MNU, tamoxifen, cranberry extract, breast cancer, liver, mammary tissue.

Introduction

Breast cancer is the most common cancer and a major cause of death in women [1]. It is a highly heterogeneous disease represented by tumors that have a diverse natural history, complex histology and a variable response to therapy [2]. The use of specific chemicals to prevent the development or retard the progression of carcinogenesis, a technique known as chemoprevention, offers a promising strategy for cancer prevention [3, 4].

Tamoxifen, a synthetic non-steroidal antagonist of estrogen receptor in breast tissue, used in the treatment and prevention of all stages of hormone dependent breast cancer in patients with early stage breast cancer as well as those with metastatic breast cancer. It reduces level of estrogen by competition with estrogen for binding to its receptor in breast tissue [5]. Many cancer chemotherapeutic agents exert their anticancer properties by inducing apoptosis and oxidative stress through mechanisms that involve mitochondria and nitric

oxide (NO) [6]. Tamoxifen leads to oxidative liver damage and it has been elucidated to be a hepatocarcinogen in rodents, many cases of tamoxifen-induced hepatotoxicity have been reported including toxic hepatitis, massive hepatic steatosis, and multifocal

hepatic fatty in filtration, hepatic necrosis, hepatic cirrhosis and even hepatic cell carcinoma [7].

A new group of phytochemicals that has been attracting much attention from both the general public and health professionals is "proanthocyanidins" which are poly-phenols and more specifically are polymers of favenols. Their main dietary sources are to be found in cranberry, grapes, cocoa and apples [8, 9]. Proanthocyanidins are famed for their potent antioxidant capacity and free radical scavenging properties. There is substantial evidence that proanthocyanidins intake from grapes or cocoa have anticarcinogenic and anti-inflammatory properties and increase the antioxidant status among hypercholesterolemic, hyperlipidemic, hemodialysis patients and smoker [9]. One of such plants rich in proanthocyanidins [10], Cranberry ranks high among fruit in both antioxidant quality and quantity [11] because of its substantial flavonoid content and a wealth of phenolic acids. Cranberry extracts are rich in these compounds, they reportedly inhibit oxidative processes including oxidation of low-density lipoproteins [12, 13], oxidative damage to at neurons during simulated ischemia [14], and oxidative and inflammatory damage to the vascular endothelium [15]. The antioxidant properties of the phenolic compounds in cranberry fruit may contribute to the observed antitumor activity. Plant-derived fractions are rich sources of phenolic compounds [16].Phenolics are known to have potential to prevent tumor and have been used in aromatherapy for obese middle-aged women. Flavonoids extracted from plants may have antioxidant activity that could mitigate tumor-related complications, including atherosclerosis and some cancers [17, 18]. Not surprisingly, plants such as cranberry extract contain high levels of unsaturated fatty acids and poly-phenols [10, 17], which are excellent antioxidant and antitumor agent [19, 20]. *In vivo* tests have been conducted with foods to determine for example, its antitumor[19], hypolipidemic, hypoglycemic and antioxidant activity [20]. As a continuation of interested research program in pharmaceutical importance of cranberry extract [19-21], we report here, a facile route to evaluate the antitumor and antioxidant effects of cranberry extract against MNU induced breast cancer in female rats.

Materials and methods

MNU was purchased from Sigma, USA and dissolved in 0.9% NaCl containing 0.05% acetic acid (pH 5). Tamoxifen was purchased from Fresenius Oncology Ltd., India. Cranberry extract was purchased from Virgin Extracts (TM), Chinese.

Rats

This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of October 6 University. Female virgin rats of age 50 days were purchased from National Cancer Institute, Cairo University, Egypt. They were individually housed in cages in an air-conditioned room with a temperature of 22 ± 2ºC, a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet *adlibitum*.

Experimental design

The animals were divided into 5 groups consisting of 8 animals, two controls groups and three treatment groups:

Group (1); 8 rats: Negative control rats received 1ml 0.9% NaCl, i.p.

Group (2); 32 rats: Rats were given single dose of MNU (50mg/kg.b.w.i.p.) (22).

After 85 days, group 2 was divided into 4 subgroups, 8 in each.

Subgroup (1): positive control (breast cancer bearing rats)

Subgroup (2): Animals were treated with tamoxifen (2 mg/kg/ day, I.P.) for 28 days (from day 86 to day 113).

Subgroup (2): Animals were treated with cranberry extract at 400 mg/kg/ day, orally. for 28 days (from day 86 to day 113) (19).

Subgroup (4): Animals were treated with cranberry extract at 600 mg/kg/ day, orally. for 28 days (from day 86 to day 113) (19).

On day 114, at the end of the study, all rats were sacrificed, blood was collected, one part of blood was collected for hematological parameters such as hemoglobin (Hb), red blood cells (RBC), leucocyte (WBC) and platelet count (PLT) were determined as described by Jain [23]. Also, the other part centrifuged, and plasma was used freshly for estimation of plasma gamma glutamyl transferase [24] and lactate dehydrogenase (LDH) [25]. The levels of hexosamine and sialic acid in plasma, liver and mammary gland were estimated by the methods of Niebes, and Wagner respectively [26, 27].

blood, liver and mammary tissue catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and plasma thiobarbituric acid reactive substances (TBARs) levels were estimated by the methods of Sinha [28], Paglia and Valentine [29], Suttle [30] and Nichans and Samulelson [31], respectively. Protein was estimated by the method of Lowry *et al*[32].

Measurement of antioxidant enzymes activity

Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities were determined using commercially available assay kits (Bio Diagnostics Inc., River Falls, WI, USA). Briefly, tissues were weighed and homogenized with appropriate buffers (provided by the kits). The homogenates were then determined following the procedures provided by the respective manufacturers. The Superoxide Dismutase Assay Kit utilizes a tetrazolium salt for detection of superoxide radicals generated by red formazan dye reduction produced (29). One unit (U) of SOD activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The Glutathione Peroxidase Assay Kit measures GPx activity indirectly by a coupled reaction with GR [33]. Oxidized glutathione, produced upon reduction of hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a

decrease in absorbance at 340 nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the A340 is directly proportional to the GPx activity. One unit (U) of GPx activity is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to $NADP^+$ per minute at 25 ºC. The specific activities of the various enzymes in the rat tissues are expressed in U/µg of the protein with the protein content determined as stated above. The Catalase Assay Kit utilizes the peroxidative function of CAT for determination of enzyme activity [34]. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The generated formaldehyde is assayed spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole as the chromogen. One unit (U) of CAT activity is defined as the amount of enzyme that will cause the formation of 1.0 nmol of formaldehyde per minute at 25 ºC

Histological assessment:_Female rats were sacrificed and breast tissues from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with Hematoxylin and Eosin (H&E) for light microscopic analyses according to the method of Bancroft and Steven [35]. The slides were coded and were examined by a histopathologist who was ignorant about the treated groups, after which photographs were taken.

Statistical analysis

All data were expressed as mean ± SD. All analyses utilized SPSS 13.0 statistical package for Windows (SPSS, 13.0 software, Inc., Chicago, IL, 2009) [36]. A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value <0.05 was accepted as statistically significant.

Table.1 showed that MNU treatment showed significant changes compared to normal control group. MNU treatment showed significant reduction in the blood levels of RBC, PLTs and Hb indicating a tendency of anemia, whereas concomitant increase in WBC was observed indicating the diseased state. (P<0.05) compared to normal control group. Treatment with cranberry extract (400 and 600 mg/kg.b.w.); all hematological parameters which were altered by induction of mammary cancer were considerably restored.

Table 2 showed that administration of MNU (50mg/kg body weight) resulted in a significant increase in plasma and liver GGT and LDH compared to the normal control group (p< 0.05). Supplementation of cranberry extract at 400 and 600 mg/k.g.b.w. resulted in a significant decrease in plasma and liver GGT and LDH compared to the group that received MNU (p< 0.05).

Table 3 showed that administration of MNU (50mg/Kg.b.w.) resulted in a significant increase in plasma, liver and mammary tissue glycoprotein (hexosamine and sialic acid) compared to the normal control group (p< 0.05). Supplementation of cranberry extract at 400 and 600 mg/k.g.b.w. resulted in a significant decrease in plasma, liver and mammary tissue glycoprotein (hexosamine and sialic acid) compared to the group that received MNU (p< 0.05) (table 3).

Tables 4-6 showed that administration of MNU (50mg/kg.b.w) resulted in a significant decrease in blood, liver and breast catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) as well as a significant increase in plasma, liver and breast TBARs compared to the normal control group (p< 0.05). Supplementation of cranberry extract at 400 and 600 mg/k.g.b.w. resulted in a significant increase in blood, liver and breast CAT, SOD and GPx as well as a significant decrease in plasma, liver and breast TBARs compared to the group that received MNU (p< 0.05).

Results

Table 1: Effect of cranberry and Tamoxifen on hematological parameters in MNU induce breast cancer in rats

No.	Groups	Hb% (g/dL)	RBCs (10^6/µL)	WBCs ($x10^3$ cells/ mm^3)	PLT 10^3 / mm^3
(I)	Normal group	13.45 ± 1.03	5.260 ± 1.44	6.300 ± 0.64	595.25 ± 58.04
(II)	Positive control (MNU, 50mg/kg.b.w.)	10.73 ± 1.17^{*a}	3.690 ± 0.95^{*a}	13.250 ± 2.32^{*a}	382.46 ± 74.92^{*a}
(III)	Positive control + Tamoxifen (2mg/kg. b.w.)	9.40 ± 0.87^{*ab}	3.540± 0.89^{*ab}	8.300 ± 1.09 ^{*ab}	400.4 ± 6.90^{*ab}
(IV)	Positive control + 400mg/kg.w.b. Cranberry	12.29 ±0.51^{*bc}	4.240± 1.00^{*bc}	7.250 ± 1.92^{*abc}	490.60 ± 61.49^{*bc}
(V)	Positive control + 600mg/kg.w.b. Cranberry	12.60 ± 1.19^{*bc}	4.380 ± 0.97^{*abc}	6.200 ±1.40^{*bcd}	520.85 ± 86.20^{*bc}

Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.); d: significant from cranberry extract (400mg/kg. b.w.). * Values are statistically significant at *P*<0.05.

Table 2: Effect of cranberry and Tamoxifen on plasma and liver gamma glutamyl transferase and lactate dehydrogenase in MNU induce breast cancer in rats

No.	Groups	Gamma glutamyl transferase		Lactate dehydrogenase	
		Plasma	Liver	Plasma	Liver
(I)	Normal group	1.48± 0.19	5.06± 0.41	0.43± 0.06	2.45± 0.29
(II)	Positive control (MNU, 50mg/kg.b.w.)	3.67± 0.28^{*a}	8.37± 0.55^{*a}	2.17± 0.27^{*a}	6.18± 0.62^{*a}
(III)	Positive control + Tamoxifen (2mg/kg. b.w.)	6.42± 1.38 ^{*ab}	12.70± 1.77^{*ab}	3.44± 0.82^{*ab}	10.20± 2.57^{*ab}
(IV)	Positive control + 400mg/kg.w.b. Cranberry	3.17±0.79 ^{*abc}	6.34±0.87^{*abc}	1.57±0.40^{*abc}	4.40±0.76^{*abc}
(V)	Positive control + 600mg/kg.w.b. Cranberry	2.75±0.77^{*abc}	5.76±1.08^{*abcd}	0.65±0.49* ^{abcd}	3.08±1.14^{*abcd}

Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.).; d: significant from cranberry extract (400mg/kg. b.w.). * Values are statistically significant at $P<0.05$. Liver Gamma glutamyl transferase is expressed as µmoles of p-nitroaniline liberated per minute/ mg of protein; liver lactate dehydrogenase isexpressed as µmoles of pyruvate liberated per minute/ mg of protein; plasma Gamma glutamyl transferase lactate dehydrogenase are expressed as (IU/L).

Table 3: Effect of cranberry and Tamoxifen on plasma, liver and mammary tissue glycoprotein in MNU induce breast cancer in rats

No.	Groups	Hexosamine			Sialic acid		
		Plasma	Liver	Mammary tissue	Plasma	Liver tissue	Mammary
(I)	Normal group	39.27 ± 3.91	3.87 ± 0.25	0.73 ± 0.09	63.56 ± 6.32	3.48 ± 0.27	0.35 ± 0.04
(II)	Positive control (MNU, 50mg/kg.b.w.)	61.53± 7.02^{*a}	9.46 ± 1.07^{*a}	1.86 ± 0.15^{*a}	157.32 ± 9.50^{*a}	7.82 ± 0.64^{*a}	1.07 ± 0.13^{*a}
	Positive control + Tamoxifen (2mg/kg. b.w.)	57.39 ± 3.20^{*ab}	7.35 ± 0.37^{*ab}	1.30 ± 0.14^{*ab}	122.61 ± 7.19^{*ab}	5.27 ± 0.57^{*ab}	0.73 ± 0.10^{*ab}
(IV)	Positive control + 400mg/kg.w.b. Cranberry	43.14 ±3.25^{*abc}	5.86 ±0.60^{*abc}	1.07 ±0.07^{*abc}	71.87 ±6.88^{*abc}	4.08 ±0.34^{*abc}	0.63 ±0.12^{*abc}
(V)	Positive control + 600mg/kg.w.b. Cranberry	34.27 ±2.73^{*abc}	4.18 ± 0.59^{*abcd}	0.85 ± 0.10^{*abcd}	65.49 ±4.78^{*abcd}	3.65 ±0.36^{*abcd}	0.43 ±0.08^{*abcd}

Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.).; d: significant from cranberry extract (400mg/kg. b.w.). * Values are statistically significant at $P<0.05$. Liver and mammary tissues hexosamine and sialic acid are expressed as mg/g tissue and plasma hexosamine and sialic acid are expressed as mg/dL.

Table 4: Levels of blood catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and plasma thiobarbituric acid reactive substances (TBARs) in MNU induce breast cancer in rats

No.	Groups	CAT (U/L)	GPx (U/ml)	SOD (U/ml)	TBARs (nmol/ml)
(I)	Normal group	314.77 ± 9.41	52.27 ± 2.97	26.50 ± 4.05	3.09 ± 0.76
(II)	Positive control (MNU, 50mg/kg.b.w.)	293.38 ± 1021^{*a}	43.80 ± 4.12^{*a}	19.36 ± 2.30^{*a}	4.58 ± 0.56^{*a}
(III)	Positive control + Tamoxifen (2mg/kg. b.w.)	228.56 ± 11.13^{*ab}	35.73 ± 3.92^{*ab}	10.80 ± 2.11^{*ab}	8.41 ± 1.56^{*ab}
(IV)	Positive control + 400mg/kg.w.b. Cranberry	297.33 ±8.06^{*abc}	49.08 ±2.44^{*abc}	23.39 ±2.13^{*abc}	3.84 ±0.41^{*abc}
(V)	Positive control + 600mg/kg.w.b. Cranberry	300.74 ±18.81^{*abcd}	55.20 ±3.11^{*abc}	26.66 ±3.38^{*abc}	3.13 ±0.61^{*abcd}

Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.) ; d: significant from cranberry extract (400mg/kg. b.w.). * Values are statistically significant at $P<0.05$.

Table 5: Levels of liver catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and plasma thiobarbituric acid reactive substances (TBARs) in MNU induce breast cancer in rats

No.	Groups	CAT (U/g protein)	GPx (U/g protein)	SOD (U/mg protein)	TBARs (nmol/g protein)
(I)	Normal group	46.37 ± 3.78	20.37 ± 3.94	65.83 ± 8.91	6.86 ± 1.53
(II)	Positive control (MNU, 50mg/kg.b.w.)	36.58 ± 4.41^{*a}	14.83 ± 2.04^{*a}	43.36 ± 6.09^{a}	16.04 ± 3.02^{*a}
(III)	Positive control + Tamoxifen (2mg/kg. b.w.)	29.80 ± 2.44^{*ab}	11.56 ± 1.93^{*ab}	33.66 ± 3.00^{*ab}	22.83 ± 3.15^{*ab}
(IV)	Positive control + 400mg/kg.w.b. Cranberry	40.25 ±3.42^{*bc}	17.05 ±1.71^{*abc}	51.28 ±4.88^{*abc}	7.52 ±1.20^{*abc}
(V)	Positive control + 600mg/kg.w.b. Cranberry	48.45 ±2.16^{*cd}	19.55 ±1.51^{*abcd}	57.05 ±2.72^{*abcd}	6.68 ±2.20^{*abcd}

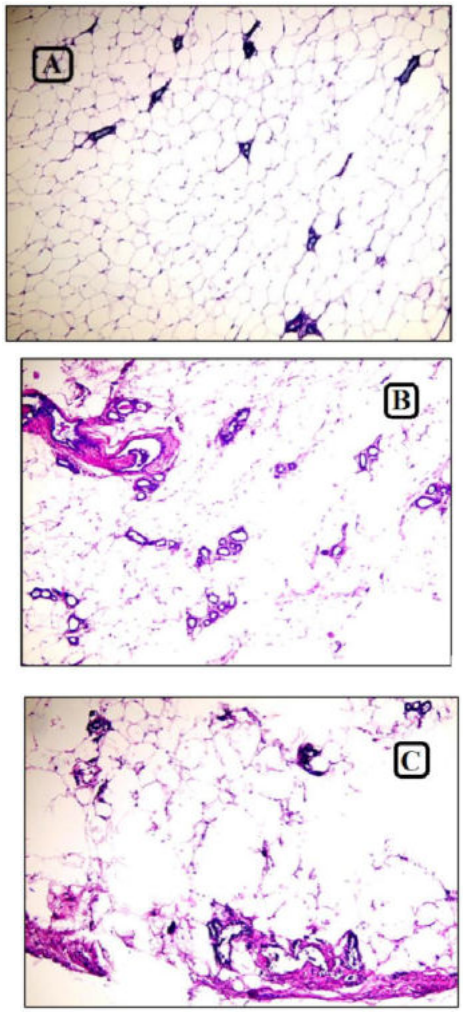
Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.) ; d: significant from cranberry extract (400mg/kg. b.w.). * Values are statistically significant at $P<0.05$.

Table 6: Levels of breast catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) and plasma thiobarbituric acid reactive substances (TBARs) in MNU induce breast cancer in rats

No.	Groups	CAT (U/g protein)	GPx (U/mg protein)	SOD (U/mg protein)	TBARs (nmol/g protein)
(I)	Normal group	608.44 ± 28.82	1.37 ± 0.28	3.15 ± 0.58	3.07 ± 0.26
(II)	Positive control (MNU, 50mg/kg.b.w.)	528.74 ± 17.63^{*a}	0.85 ±0.1 ^{*a}	1.44 ± 0.40^{*a}	4.25 ±0.22^{*a}
(III)	Positive control + Tamoxifen (2mg/kg. b.w.)	468.25 ± 15.85^{*ab}	0.28 ± 0.05^{*ab}	0.98 ± 0.3^{*ab}	5.11 ± 0.28^{*ab}
(IV)	Positive control + 400mg/kg.w.b. Cranberry	570.16 ±20.07^{*abc}	0.93 ±0.1^{*abc}	2.48 ±0.36^{*bc}	3.56 ±0.71^{*bc}
(V)	Positive control + 600mg/kg.w.b. Cranberry	666.09 ±18.44^{*bcd}	1.44 ±0.07^{*bcd}	3.89 ±0.24^{*abcd}	2.37 ± 0.24^{*bc}

Values are given as mean ± SD for groups of eight animals each. a: significant from normal control; b: significant from MNU (50mg/kg.b.w) supplement group; c: significant from tamoxifen (2mg/kg. b.w.) ; d: significant from cranberry extract (400mg/kg. b.w.). * Values are statistically significant at $P<0.05$.

Histology of normal mammary gland (Figure 1A) shows the presence of lobules (L) with numerous acini and clear basement membrane (BM). Figure 1B of mammary tissue of treated MNU (50mg/kg.b.w.) group clearly shows marked proliferation of stroma (SP) resulted due to stromal reaction with carcinogen. Figure 1C of mammary tissue treated MNU (50mg/kg.b.w.) + tamoxifen (2mg/kg.b.w.) showed moderate decreased of glandular elements. Figure 1D of mammary tissue treated MNU + cranberry extract (400mg/kg.b.w.) showed mild decrease of glandular elements. Figure 1E of mammary tissue treated MNU + cranberry extract (600mg/kg.b.w.) showed moderate decrease of glandular elements.



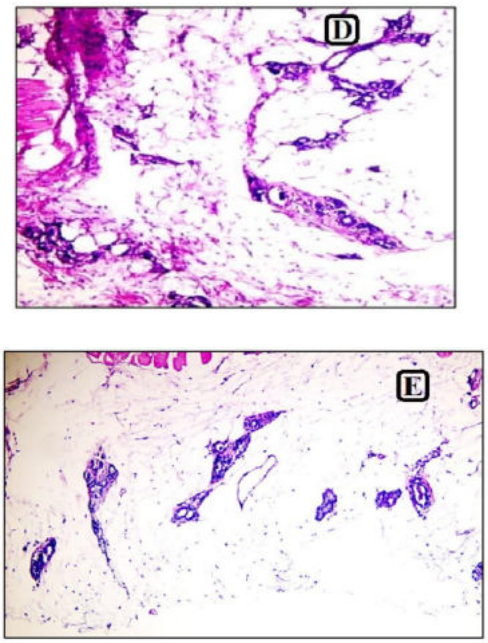


Figure (1A): Negative control of rat mammary tissue as control with few glandular elements. Figure (1B): Positive control (MNU, 50mg) rat mammary tissue with mammary glandular proliferation. Figure (1C): MNU (50mg.) + tamoxifen (2mg), the mammary tissue showed moderate decreased of glandular elements. Figure (1D): MNU (50mg) + cranberry (400mg), the breast tissue showed mild decrease of glandular elements. Figure (1E): MNU (50mg.) + cranberry (600mg.), the mammary tissue showed moderate decrease of glandular elements.

Discussion

Animal models are particularly useful for the study of human mammary carcinogenesis. Since the rat mammary gland shows a high susceptibility to develop neoplasms which closely mimic human breast cancer [37]. The carcinogen N-methyl-N-nitrosourea (MNU) induces hormone dependent mammary tumors in rats. This model has previously been used to develop breast cancer [38].

Breast cancer is one of the main life-threatening diseases [39]. Although different anticancer drugs are present in the market, their serious adverse effects still need to identify potent anticancer molecules from natural origin. Herbal medicine has been regarded as one of the most visible fields for cancer chemoprevention and it constitutes the main source of effective new anticancer agents [40, 41]. In the present research, *in vivo* anticancer and antioxidant activity of cranberry extract against MNU induced mammary cancer

in female rats was reported for the first time. The induction of mammary tumours in Sprague-Dawley rats has also been well reported [42, 43]. Its effects on Wistar rats were examined by da Silva Franchi et al [44], while Akanni et al.,[45] reported its effects on the hematology of Wistar rats. Results of the present study, showed that oral administration of cranberry can normalize the levels of hematological parameters, which may be due to the presence of antioxidant phytochemicals [46-50]. These results were in agreement with previous studies which concluded that administration cranberry extract provided normalization in hematological parameters in leucopenia rats (21).

Tumor markers are most useful for monitoring response to therapy and early detection of cancer. The result of this study showed elevated plasma and liver GGT and LDH activity in the tumor-bearing female rats. Other study showed that serum ALP concentration increased significantly in cancer patients with metastasis [51]. In our rats, there was evidence of metastasis, suggesting that the increased GGT and LDH may in fact be due to the primary tumor. Liver metastasis [52] and heptotoxicity [53] also can be determined by changes in serum GGT and LDH. Plasma and liver LDH concentrations often increased significantly in MNU treated female rats (54). According to Perumal et al. (55), the elevated activity of LDH may be due to overproduction by tumor cells, or it may be due to the release of isoenzymes from destroyed tissues. Our results are also consistent with the above reports. The significant high ($P < 0.05$) plasma and liver LDH concentrations observed in this study were similar to those in human cancer patients with endometrial adenocarcinoma, ovarian cyst adenocarcinomas and breast cancers. However, it was suggested that the plasma LDH concentration is nonspecific for the diagnosis of metastasis [51]. Some studies showed correlations between serum GGT concentration and malignant neoplasm such as cancers of the digestive, respiratory, female genital, lymphoid and hematopoietic organs [56], Our findings showed that the serum GGT concentrations were significantly different in normal and tumor-bearing rats suggesting that this serum parameter is not a good biomarker for rat mammary gland tumors. These results were hand in hand with other studies[57] that reported that serum GGT significant increase in women with breast malignant neoplasm.

It is quite well known that flavanones, a cranberry flavonoid act as antioxidant molecules [58], which can scavenge the excess free radicals in biological system. Since cranberry has shown antioxidant and free radical scavenging activity [59], the present study primarily ameliorating the effect of cranberry' polyphenols on free radicals accumulation and oxidative damage in the liver of MNU treated rat is studied. Oral administration of cranberry extract significantly inverse the MNU induced peroxidative damage in liver which is evidenced from the lowered levels of GGT and LDH. This may be due to the antioxidative effect of polyphenols [60]. An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reaction can produce free radicals, which start chain reactions that damage cells.

Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result are often reducing agents such as thiols, ascorbic acid or polyphenols [61].

Breast cancer is the third most common malignancy affecting female population, and approximately 19% of cancer mortality was reported worldwide [62]. Glycoproteins; e.g. hexosamine and sialic acid are common components of cell surfaces and are also commonly found as constituents of lysosomes and among the products secreted/exposed by the cell [63]. The cell surface glycoproteins have been shown to play important roles in tumourogenesis (64). Elevation of glycoprotein contents are useful indicators of carcinogenic process and these changes alter the rigidity of cell membranes [65]. Abnormal increase in the levels of plasma glycoprotein components have been related to the changes in hepatic cells during neoplastic transformation. Sialic acids are widely distributed in nature as terminal sugars in glycoproteins or glycolipids, impart a net negative charge to cell surface and are reported to be important in cell-to-cell and cell-to matrix interactions [66]. It was previously demonstrated that neoplastic transformation leads to elevated plasma sialic acid concentration [67] through the shedding or secreting of sialic acid from the tumor cell surfaces [68]. In the present study increased levels of glycoproteins in plasma, liver and breast tissues of cancer bearing animals were observed.

Flavonoids and other phenolic compounds are well known natural antioxidants. The flavonoids present in cranberry extract are thought to be the cause of their antitumor and anti-inflammatory effects [46-50]. Flavonoids have a chemopreventive role in cancer by means of their effect in signal transduction in cell proliferation and angiogenesis (21). This important property may be responsible for its antitumor and antioxidant activity against MNU induced breast cancer. Antioxidant and antitumor activity of cranberry extract against different carcinogenic agents has already been established by the present authors (21).

CAT, GPx and SOD are inducible enzymes important in the detoxication of many different xenobiotics in mammals. The antioxidant enzymes achieve detoxication by catalyzing the conjugation of reduced glutathione to various electrophilic substrates [69]. It serves as a marker for hepatotoxicity in rodent system, and also plays an important role in carcinogen detoxification [70]. Consequently, inhibition of antioxidant enzymes activity might potentiate the deleterious effects of many environmental toxicants and carcinogens. Antioxidant enzymes are also engaged in the intracellular transport of variety of hormones, endogenous metabolites, and drugs, by virtue of their capacity to bind these substances [71]. The decreased activity of antioxidant enzymes in group II cancer bearing animals may be due to the excessive utilization of this enzyme in conjugation process and also may be due to the enhancement of the covalent binding of MNU metabolites to cellular DNA and results in an increase in the degree

of cell damage leading to neoplastic growth. Results of the present study, showed that administration of MNU resulted in significant decrease in CAT, SOD and GPx activity as well as significant increase in TBARs level.

Also, in this study it was found that tamoxifen significantly reduced the blood, hepatic and mammary tissue antioxidant enzymes CAT, SOD and GPx accompained with significant elevation of TBARS level; a product of lipid peroxidation, compared to normal control group [72-73]. The activities of intracellular antioxidant enzymes decreased with the increase of lipid peroxidation levels [74], which were concomitant with the results achieved from this study. More over depletion of the hepatic reduced glutathione GSH results in the reduction of GPx activity as glutathione peroxidase utilizes GSH for H_2O_2 detoxification into water and organic peroxides (R-OOH) and this would eventually result in H_2O_2 accumulation which in turn leads to exhaustation of antioxidant superoxide dismutase (SOD) and catalase (CAT) enzymes [75].

Superoxide dismutase, catalase and glutathione peroxidase constitute the major enzymatic antioxidant defenses which convert active oxygen molecules in to non-toxic compounds [76]. Superoxide dismutase is a ubiquitous enzyme with an essential function in protecting aerobic cells against oxidative stress. It is primarily mitochondrial enzyme usually found in the plasma membrane [77].Catalase is a tetrameric heme protein that undergoes alternative divalent oxidation and reduction at its active site in the presence of hydrogen peroxide [78]. As a substrate for the antioxidant enzyme glutathione peroxidase, reduced glutathione protects cellular constituents from the damaging effects of peroxides formed in metabolism and other reactive oxygen species reaction [79]. Glutathione peroxidase catalyzes the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction product of the hydroperoxide [80]. Cranberry extract offers protection against oxidative damage due to the ability of enhanced antioxidant activity [81]. Antioxidant and hepatoprotective effects of cranberry extract might be associated with the structure-antioxidant relationship of its active constituents such as vitamin C, vitamin E and polyphenols (anthocyanins).

Finally, histopathological examination showed marked degree of mammary tissue proliferation in MNU-treated rats (Figure 1B). Comparing the beneficial effect of cranberry extract with that of MNU-induced breast cancer, cranberry showed antitumor activity indicated by the measured biochemical parameters and the histopathological examination of mammary tissue. In addition, group of rats continuously treated with cranberry extract with MNU injection showing mild decrease of glandular elements (fig. 1D&E).

In this study, the most novel and relevant finding was that cranberry extract supplementation was accompanied by the alleviation of mammary tissue proliferation in this model. Antitumor and antioxidant effect of cranberry extract against breast cancer induced by MNU has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind.

In conclusion, the present study showed that cranberry extract has a powerful antitumor and hepatoprotective activity against MNU induced breast cancer and liver toxicity. These effects could be due to membrane protective action of cranberry by scavenging the free radicals and its antioxidant action. This could serve as a stepping stone towards the discovery of newer safe anticancer and antioxidant agents.

Acknowledgement

Thanks to Ass. Prof. Dr.Amal Haridy, Faculty of Medicine, Cairo University, Egypt, for her involvement in histopathological examination.

References

[1]. Cameron D and Bell R. Optimizing treatment for patients with breast cancer. Seminar in Oncol 2004; 31: 4-5.

[2]. Schultz LB and Weber BL. Recent advances in breast cancer biology. Curr Opin Oncol 1999; 11: 429-434.

[3]. Alexandrov VA and Bespalov VG. Preclinical and clinical study of cancer chemopreventive agents. St. Petersburg: Esculap 1997; 32 (in Russian).

[4]. Kelloff GJ. Perspectives on cancer chemoprevention research and drug development. Adv Cancer Res 2000; 78: 199-334.

[5]. Fisher B, Costantino JP and Wickerham DL. Tamoxifen for prevention of breast cancer: current status of the national surgical adjuvant breast and bowel project P-1 study. Journal of the national cancer institute 2005; 97:1652-1662.

[6]. Loo SA, Lesoon-Wood LA and Cooney RV. Effects of tamoxifen on nitric oxide synthesis and neoplastic transformation in C3H 10T1/2 fibroblasts. Cancer Lett 1998; 122: 67–75.

[7]. Elefsiniotis I, Pantazis K, Ilias A, Pallis L, Mariolis A, Glynou I, Kada H and Moulakakis A. Tamoxifen induced hepatotoxicity in breast cancer patients with preexisting liver steatosis: the role of glucose intolerance. Eur J

Gastroenterol Hepatol 2004; 16:593–598.
[8]. Manach C, Scalbert A, Morand C, Rémésy C and Jiménez L. Polyphenols: food sources and bioavailability. The American Journal of Clinical Nutrition 2004;79:727-747.
[9]. Santos-Buelga C and Scalbert A. Proanthocyanidins and tannin-like compounds: nature, occurrence, dietary intake and effects on nutrition and health. Journal of the Science of Food and Agriculture 2000;80:1094-1117.
[10]. Porter, ML., Krueger, CG., Wiebe, DA., Cunningham, DG. and Reed, JD. Cranberry proanthocyanidins associate with low-densitylipoprotein and inhibit in vitro Cu2 1 -induced oxidation. J Sci Food Agric., 2001; 81: 1306–13.
[11]. Vinson, JA., Su, X., Zubik, L. and Bose P. Phenol antioxidant quantity and quality in foods: Fruits.J. Agric. Food Chem., 2001;49:5315–21.
[12]. Yan, X., Murphy, B.T., Hammond, G.B., Vinson, J.A. and Neto CC. Antioxidant activities and antitumor screening of extracts from cranberry fruit (Vaccinium macrocarpon). J Agric Food Chem., 2002; 50: 5844–9.
[13]. Neto, CC., Sweeney-Nixon, MI., Lamoureaux, TL., Solomon, F., Kondo, M. and MacKinnon, SL.Cranberry phenolics: Effects onoxidative processes, neuron cell death and tumor cell growth. In Shahidi F, Ho C-T, editors. Symposium Series No. 909:Phenolic Compounds in Foods and Natural Health Products Columbus, OH: ACS Books; 2005; 271 –282.
[14]. Youdim, KA., McDonald, J., Kalt, W. and Joseph, JA. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. J Nutr Biochem., 2002;13: 282–8.
[15]. Ebrahimzadeh, MA., Nabavi, SF. and Nabavi, SM. Essential oil composition and antioxidant activity of Pterocarya fraxinifolia. Pak JBiol Sci., 2009; 12: 957–963.
[16]. da Silva, NA., da Silva, JK., Andrade, EH., Carreira, LM., Sousa, PJ. and Maia, JG. Essential oil composition and antioxidant capacity of Lippia schomburgkiana. Nat Prod Commun 2009; 4:1281 –1286.
[17]. De´corde,´ K., Agne, A., Lacan, D., Ramos, J., Fouret, G., Ventura E., Feillet-Coudray, C., Cristol, JP. and Rouanet, JM. Preventive effect of melon extract rich in superoxide scavenging activity onabdominal and liver fat and adipokine imbalance in high-fat-fedhamsters. J Agric Food Chem.,2009;22: 6461 –6467.
[18]. Han, SH., Yang, BS. and Kim, HJ. Effectiveness of aromatherapy massage on abdominal obesity among middle aged women. J KoreanAcad Nurss. 2003; 33:839–846.
[19]. Abdel-Maksoud, HA, Hussein, MA, Mohammed, RR. and Zayed, HT. Cranberry extract Enhance antioxidant Potential in Ehrlich's Ascites Carcinoma–Bearing Female Albino Mice. World Journal of Pharmaceutical Sciences. 2015; 3(3): 484-491.
[20]. Abdel-Maksoud, HA., Hussein, MA. and Barakat, MA. Cranberry Extract as a Functional Food in Treatment of Myocardial Toxicity Induced by nicotine in Rats. International Journal of Pharma Sciences. 2015; 4: 1174-1180.
[21]. Hussein MA and Boshra SA. Anti-leukopenic and Antioxidant Effects of Cranberry Extract in Benzene and fluorouracil induced leukopenia in Rats. IJARNP, 2015; inpress.
[22]. Gotoh T, Yamada K, Ito A, Yin H, Kataoka T and Dohi K. Chemoprevention of N-nitroso-N-methylurea-induced rat mammary cancer by miso and tamoxifen, alone and in combination. Jpn J Cancer Res 1998; 89: 487-495.
[23]. Jain WC: Schalm's Veterinary Hematology, ed 4, Lea and Febiger, Philadelphia. 1986, pp. 69 - 71.
[24]. Rosalki SB, Rau D: Serum gamma glutamyl transpeptidase activity in alcoholism. Clin Chim Acta 1972; 39: 41-47.
[25]. Buhl SN, Jackson KY. Optimal conditions and comparison of lactate dehydrogenase catalysis of the lactate to pyruvate to lactate reactions in human serum at 25, 30 and 37 ^{0}C. Clin. Chem. 1978; 2415: 828.
[26]. Wagner WD: More sensitive assay discriminating galactosamine and glucosamine in mixtures. Anal biochem 1979; 94:394-397.
[27]. Niebes P: Determination of enzyme and degradation products of GAG metabolism in the serum of healthy and various subjects. Clin Chim Acta 1972; 42: 399-408.
[28]. Sinha, AK. Colorimetric assay of catalase. J.AnalBiochem. 1972; 47 (2): 389-94.
[29]. Paglia D, Valentine W. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med 1967; 70:158.
[30]. Suttle N. Copper deficiency in ruminants; recent developments. The Veterinary Record. 1986; 119: 519-22.
[31]. Nichans WH, Samulelson B. Formation of malondialdehyde from phospholipid arachidonateduring microsomal lipid peroxidation. *Eur J Biochem*1968; 6: 126-30.
[32]. Lowry OH, Rosebrough NJ, Farr AL, Randall R: Protein measurement with Folin's phenol reagent. J Biol Chem 1952; 193: 265-275.
[33]. Horn HD, Burns FH. Assay of glutathione reductase activity. In: Bergmeyer HV (Ed.), Methods of Enzymatic Analysis. Academic Press, New York, USA, 1 978; pp. 1 42-1 46.
[34]. Sedlak J, Lindsay RH1968. Estimation of total protein bound and nonprotein sulfhydryl group in tissue with Ellman's reagents. Analyst Biochem 1968; 25: 192–205.
[35]. Bancroft GD, Steven A. In. Theory and practice of histological technique 4th Ed. London; Churchill Livingstone. 1983; Pp. 99 – 112.
[36]. Abo-Allam, R.M., 2003. Data Statistical Analysis using SPSS Program. 1 Ed., Publication for 1stUniversities, Cairo.
[37]. Rajarshi R, Mitali G, Kartick S and Malay C. Vanadium, a versatile biochemical effector in chemical rat mammary carcinogenesis. Nutrition and Cancer 2005; 51(2): 184–196.

[38]. Gusterson BA and Williams JC. N-nitrosomethylurea-induced rat mammary tumours as models of human breast cancer. J R Soc Med 1981; 74: 56-59.

[39]. Angelopoulos N, Barbounis V, Livadas S, Kaltsas D and Tolis G. Effects of estrogen deprivation due to breast cancer treatment. Endocr Relat Cancer 2004; 11: 523-535.

[40]. Jo EH, Hong HD, Ahn NC, Jung JW, Yang SR and Park JS. Modulations of the Bcl-2/Bax family were involved in the chemopreventive effects of licorice root (Glycyrrhiza uralensis Fisch) in MCF-7 human breast cancer cell. J Agric Food Chem 2004; 52: 1715-1719.

[41]. Ivanova D, Gerova D, Chervenkov T and Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacol 2005; 96: 145-150.

[42]. Takahashi H, Uemura Y, Nakao I and Tsubura A. Induction of mammary carcinomas by the direct application of crystalline N-methyl-N-nitrisourea onto rat mammary gland. Cancer Lett. 1995;92:105-11.

[43]. Thompson HJ, McGinley JN, Rothhammer K and Singh M. Rapid induction of mammary intraductal proliferations, ductal carcinoma in situ and carcinomas by the injection of sexually immature female rats with 1-methyl-1-nitrosourea. Carcinogenesis.1995; 16:2407-411.

[44]. da Silva Franchi CA, Bacchi MM, Padovani CR and de Camargo JL. Thymic lymphomas in Wistar rats exposed to N-methyl-N-nitrosourea (MNU). Cancer Sci. 2003;94(3):240-43.

[45]. Akanni EO, Oloke JK and Fakunle EE. Haematological characterization in N-nitroso-N- ethylurea induced tumour bearing rats on oral administration of pleurotus pulmonarius and pleurotus ostreatus metabolites. IJRRAS. 2010; 3(3):343-48.

[46]. Vinson JA, Su X, Zubik L, Bose P. Phenol antioxidant quantity andquality in foods: Fruits. J. Agric. Food Chem., 2001;49:5315–21.

[47]. Yan X, Murphy BT, Hammond GB, Vinson JA, Neto CC. Antioxidantactivities and antitumor screening of extracts from cranberry fruit(Vacciniummacrocarpon). J Agric Food Chem., 2002;50:5844–9.

[48]. Porter ML, Krueger CG, Wiebe DA, Cunningham DG, Reed JD.Cranberry proanthocyanidins associate with low-density lipoproteinand inhibit in vitro Cu2 1-induced oxidation. J Sci Food Agric., 2001;81:1306–13.

[49]. Neto CC, Sweeney-Nixon MI, Lamoureaux TL, Solomon F, Kondo M,MacKinnon SL. Cranberry phenolics: Effects on oxidative processes,neuron cell death and tumorcell growth. In Shahidi F, Ho C-T, editors.Symposium Series No. 909: PhenolicCompounds in Foods and NaturalHealth Products Columbus, OH: ACS Books; 2005; 271–282.

[50]. Youdim KA, McDonald J, Kalt W, Joseph JA. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. J NutrBiochem., 2002;13:282–8.

[51]. Mishra S, Sharma DC and Sharma P. Studies of biochemical parameters in breast cancer with and without metastasis. Indian J. Clin. Biochem. 2004; 19: 71-75.

[52]. Wang JY, Xu BH, Tian LJ and Wang Y. Clinical characteristics and potential prognostic factors of breast cancer patients with liver metastases. Zhonghua Zhong Liu. Za. Zhi. 2006; 28: 612-616.

[53]. Lynch ED, Gu R, Pierce C and Kil J. Combined oral delivery of ebselen and allopurinol reduces multiple cisplatin toxicities in rat breast and ovarian cancer models while enhancing anti-tumor activity. Anticancer Drugs, 2005; 16: 569-579.

[54]. Koukourakis MI, Kontomanolis E, Giatromanolaki A, Sivridis E and Liberis V. Serum and Tissue LDH Levels in Patients with Breast/Gynaecological Cancer and Benign Diseases. Gynecol. Obster. Invest. 2006; 67: 162-168.

[55]. Perumal SS, Shanthi P and Sachdanadam P. Therapeutic effect of tamoxifen and energy-modulating vitamins on carbohydrate metabolizing enzymes in breast cancer. Cancer Chemother. Pharmacol. 2005; 56: 105-114.

[56]. Strasak AM, Pfeiffer RM, Klenk J, Hilbe W, Oberaigner W, Gregory M, Concin H, Diem G, Pfeiffer KP, Ruttmann E and Ulmer H. Prospective study of the association of gamma-glutamyltransferase with cancer incidence in women. Int. J. Cancer, 2008; 123: 1902-1906.

[57]. Sani HA, Rahmat A, Ismail M, Rosli R and Endrini S. Potential anticancer effect of red spinach (Amaranthus gangeticus) extract. Asia Pac. J. Clin. Nutr. 2004; 13: 396-400.

[58]. Chanet A, Milenkovic D, Manach C, Mazur A and Morand C. Citrus flavanones: what is their role in cardiovascular protection, J. Agric. Food Chem. 2012; 60:8809–8822.

[59]. Kannampalli P, Sang HP and Kyong CK. Hesperidin a flavanoglycone protects against -irradiation induced hepatocellular damage and oxidative stress in Sprague Dawley rats, Eur. J. Pharmacol. 2008; 587: 273–280.

[60]. Sies H. Oxidative stress, oxidants and antioxidants, Exp. Physiol. 1997; 82: 291–295.

[61]. Veerappan RM, Senthil S, Rao MR and Ravikumar R. Redox status and lipid peroxidation in alcoholic hypertensive patients and alcoholic hypertensive patients with diabetes, Clin. Chem. Acta 2004; 340: 207–212.

[62]. Jensen ON, Estene J and Moller H. Renardcarin the European community and members state. Eur J Cancer 1990; 26: 1167-1256.

[63]. Bolt HM, Filser JG. Irreversible binding of chlorinated ethylenels to macromolecules. Environ Health Perspect 1977; 21: 107-112.

[64]. Hughes RC. Membrane glycoproteins. Butterworth, London, 1976. pp: 114-134, 152-170.

[65]. Selvam S and Nagini S. Administration of the plasticizer di(englhexyl)phthalate

alters glycoconjugate profile. Ind J Physiol Pharmocol 1995; 39: 252-254.
[66]. Schauer R. Sialic acids and their role as biological masks. Trends Biochem Sci 1985; 10: 357-360.
[67]. Wongkham S, Bhudhisawadi V, Chau-in S, Boonla C, Muisuk K, Kongkham S and Wongkham C. Clinical significance of serum total sialic acid in cholangiocarcinoma. Clin Chim Acta 2003; 327(1-2): 139-147.
[68]. Singhal A and Hakomori S. Molecular changes in carbohydrate antigens associated with cancer. Bioassays 1990; 12: 223-230.
[69]. King RS, Teitel CH, Shaddock JG, Casciano DA and Kadlubar FF. Detoxification of carcinogenic aromatic and heterocyclic amines by enzymatic reduction of the N-hydroxy derivative. Cancer Lett 1999; 143(2): 167-71.
[70]. Sato K. Glutathione s-transferase as markers of preneoplasia and neoplalsia. Adv Cancer Res 1989; 52: 205-255.
[71]. Oakley A. Glutathione transferases: a structural perspective. Drug Metab Rev 2011; 43(2): 138–51.
[72]. Suhel P, Heena T, Hasibur R, Basu Dev B, Mohammad A, Sheikh R. Catechin prevents tamoxifen-induced oxidative stress and biochemical perturbations in mice. Toxicology 2006; 225:109–118.
[73]. Hesham AE, Ahmed MM, Ayman AN, Ashraf BA. Amelioration of tamoxifen-induced liver injury in rats by grape seed extract, black seed extract and curcumin. Experimental biology.(2010);48,280-288.
[74]. Hesham AE. The effect of dimethyl dimethoxy biphenyl dicarboxylate (DDB) against tamoxifen-induced liver injury in rats: DDB use Is curative or protective. J Biochem Mol Biol 2005; 38: 300-306.
[75]. Diplock AT, Rice CA and Burdon RH. Is there a significant role for lipid peroxidation in the causation of malignancy and for antioxidants in cancer prevention. Cancer Res 1994; 54: 1952-1956.
[76]. Lee CP, Shih PH, Hsu CL and Yen GH. Hepatoprotection tea seed oil (Camellia oleifera Abel) against CCl4- induced oxidative damage in rats. Food and chem
toxicol 2007; 45: 888-893.
[77]. Deisseroth A and Dounce AL. Catalase: physical and chemical properties, mechanism of catalysis. Physiological role, Physiol. Rev. 1970; 50:319–375.
[78]. Deneke, SM. and Fanburg, BL. Regulation of cellular glutathione, Am. J. Physiol. Lung Cell Mol. Physiol. 1989; 257:163–173.
[79]. Eaton, JW. Catalase, glutathione peroxidase and hydrogen peroxidase, J. Clin. Lab. Med. 1993; 118:3–4.
[80]. Tirkey N, Pilkhwal S, Kuhad A and Chopra K. Hesperidin, a citrus bioflavonoid, and decreases the oxidative stress produced by carbon tetrachloride in rat liver and kidney, BMC Pharmacol. 2005; 5: 2-8.
[81]. Winker BS. Unequivocal evidence in support of non enzymatic redox coupling between glutathione/glutathione disulphide and ascorbic acid/dehydroascorbic acid, Biophys. Acta 1992; 117:287–290.
[82]. Choi SW, Benzic IFE, Collins AR and Hannigan RM. Vitamin C and E: acute interactive effects on biomarkers of antioxidant defence and oxidative stress, Mut. Res.2004; 551:109–117.