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Original Article

Cranberry Extract Enhance antioxidant Potential in Ehrlich's Ascites Carcinoma–Bearing Female Albino Mice

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Abstract

The present study was to evaluate anti-cancer effects of cranberry extract (75 and 150mg/kg.b.w), 5-Flourourasil (20mg/kg b.w. i.p) and their combinatorial formulation in female mice induced by Ehrlich ascites cells for 21 consecutive days prior. Ten days after intraperitoneal inoculation of tumor EAC cells in mice, cranberry extract was administrated at (75 and 150mg/kg.b.w) daily for 21 consecutive days. On the 22th day, the mice were sacrificed for the estimation of tumor growth (tumor volume), and biochemical parameters (glucose, insulin, 17β-estradiol, progesterone and follicular stimulating hormone (FSH) alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), lipid peroxides (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C, nitric oxide (NO) and tumor necroses factor-α (TNF-α). The results of this study also showed that administration of cranberry extract, 5-Flourourasil both individually and in combination for 21 days to the carcinoma induced mice demonstrated a significant ($P<0.01$) decrease in tumor volume and a significant ($P<0.01$) improvement in biochemical parameters and life span as compared to the EAC control mice than either agent alone. On the other hand, the results clearly suggest that the combination of cranberry extract and 5-Flourourasil produced higher antioxidant activities on experimental EAC control as well as 5-Flourourasil mice than their individual influences.

Key words: Cranberry extract, 5-Flourourasil, breast cancer, Ehrlich ascites cells and antioxidants.

INTRODUCTION

Cancer is an unnatural cell growth, where they can loss their natural function and spread through of the blood, at all the body. Breast cancer is the more commonly diagnosed in industrialized countries and has the highest death toll [1]. Oxidative stress is involved in the process development of cancer and tumors; due to that ROS can damage the macromolecules as lipids which react with metals (as free iron and copper) and produce aldehydes and synthesize malondialdehyde inducing mutations [2] or cause breaks in the double chain, produce modifications in guanine and thymine bases, and sister chromatid exchanges [3]. Humans have evolved with antioxidant systems to protect against free radicals and ROS. These systems include some antioxidants produced in the body (endogenous) and others obtained from the diet exogenous) [4]. The first include (a) enzymatic defenses, such as glutathione peroxidase, catalase, and superoxide dismutase, which metabolize superoxide, hydrogen peroxide, and lipid peroxides, thus preventing most of the formation of the toxic ROS [2]. Plants vegetables and spices used in folk and traditional medicine have gained wide acceptance as one of the main sources of prophylactic and chemopreventive drug discovery and development [5, 6]. It is widely accepted that a diet rich in fruits and plants are rich sources of different kinds of antioxidants, phenolic compounds are the most studied and have been recognized to possess a wide range of properties including antioxidant, antibacterial, anti-inflammatory, hepatoprotective and anticarcinogenic actions [5]. Many of the biological functions of flavonoids, phenolic, catechins, curcumin, resveratrol and genistein compounds

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have been attributed to their free radical scavenging, metal ion chelating and antioxidant activities [6, 7]. Several medicinal plants have been implicated in the mechanisms of chemoprevention which refers to the use chemical substances of natural origin or synthetic to reverse, retard or delay the multistage carcinogenesis process [6]. One of such plants, Cranberry ranks high among fruit in both antioxidant quality and quantity [8)] because of its substantial flavonoid content and a wealth of phenolic acids. Cranberry extracts rich in these compounds reportedly inhibit oxidative processes including oxidation of low-density lipoproteins [9, 10], oxidative damage to at neurons during simulated ischemia [11], and oxidative and inflammatory damage to the vascular endothelium [12]. 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 [13]. 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 [13–16]. Not surprisingly, plants such as cranberry extract contain high levels of unsaturated fatty acids and poly-phenols [9, 13], which are excellent scavengers of reactive and represent a promising anti-tumor effects. In vivo tests have been conducted with foods to determine for example, its hepatoprotective [8], hypolipidemic, hypoglycemic and antioxidant activity [7]. The present study aimed to evaluate the possible antitumor effect cranberry extract and 5-Flourourasil in the form of combinatorial formulation against Ehrlich ascites carcinoma (EAC) in female albino mice.

MATERIALS AND METHODS

Chemicals: A-5-fluorouracil was from Merck Ltd., Germany. All the other reagents used were of analytical grade and were obtained commercially.

Dose of Cranberry: **Cranberry** extract was purchased it from Virgin Extracts (TM), Chinese.

Cranberry was given to female mice with 1/150 LD_{50}(75mg/kg.b.w.) and 1/75 LD_{50} (150mg/kg.b.w.) daily for 3weeks (24 hours after inoculation of ascetic fluid) by oral gastric gavage tube.

Mice: This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of October 6 University. Adult mice weighing around 25 ± 2gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were individually housed in cages in an air-conditioned room with a temperature of 22 ± 2^{o}C, 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 *ad-libitum*.

Experimental design: EAC cells were obtained from Cancer institution, Cairo.The cells maintained in vivo in Swiss albino mice by intraperitoneal transplantation and later tumor cells were injected intraperitoneally ($2x10^6$ cells per mouse) to animals of all groups except the first group [17].

5- Experimental design:

The animals were divided into 7 groups consisting of 8 animals, two controls groups and five treatment groups:

Group (1): Control negative nontumor bearing mice (NTB).

Group (2): EAC control (tumor bearing mice (TB))

Group (3): EAC (tumor bearing mice (TB)) + 75mg/kg.b.w. daily for 3 weeks after subcutaneous implantation of EAC.

Group (4): EAC (tumor bearing mice (TB)) + 150mg/kg.b.w. daily for 3 weeks after subcutaneous implantation of EAC.

Group (5): EAC (tumor bearing mice (TB)) + 5-fluorouracil (20mg/kg) was given by intraperitoneal injection on alternate days for 3 weeks after subcutaneous implantation of EAC.

Group (6): EAC (tumor bearing mice (TB)) + 75mg/kg.b.w. daily for 3 weeks after subcutaneous implantation of EAC + 5-fluorouracil (20mg/kg) was given by intraperitoneal injection on alternate days for 3 weeks after subcutaneous implantation of EAC.

Group (7): EAC (tumor bearing mice (TB)) + 150mg/kg.b.w. daily for 3 weeks after subcutaneous implantation of EAC + 5-fluorouracil (20mg/kg) was given by intraperitoneal injection on alternate days for 3 weeks after subcutaneous implantation of EAC.

On 31th day, after 24h of dose, 8 mice from each group were dissected and the ascites fluid was collected from peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube. The tumor weight was measured by taking the weight of mice before and after collection of ascites fluid from peritoneal cavity [20, 21]. At the end of the study, all mice were sacrificed blood was collected, centrifuged, and plasma was used freshly for estimation of plasma glucose [22]. The plasma insulin, progesterone, 17-β estradiol and Follicle Stimulating Hormone (FSH) concentration were measured using ELISA kits (Shibayagi Co. Japan) [23-26], respectively, as well as transaminases (L-

alanine and L-aspartate) [27], alkaline phosphatase (ALP) [28]. Also, lactate dehydrogenase (LDH) [29], TBARS, Nitric Oxide (NOx), tumor necroses factor-α (TNF-α) and GSH levels in blood and hepatic were done by the methods described by Buhl and Jackson [30], Miranda and Espey [31] Beyaert and Fiers [32], Koster, et al., [33] and Chanarin [34], respectively. Blood and liver Superoxide dismutase (SOD) and catalase (CAT) activities were carried out Paglia and Valentine [35], Sinha [36], respectively. Plasma triglyceride, total cholesterol and HDL- cholesterol were determined using commercially available kits (Asan and Youngdong Pharmaceutical Co., Korea) [37-39]. Plasma LDL-cholesterol level was calculated from Falholt et al [40] formula (LDL-cholesterol = total cholesterol – triglycerides/5 – HDL-cholesterol).

Statistical analysis: All the grouped data were statistically evaluated with SPSS/11 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. *P* values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for eight separate determinations.

RESULTS

Administration of cranberry extract at 75 and 150mg/kg. and injection of 5-fluorouracil on tumour volume and weight to mice resulted in a significant decrease in tumour volume and weigh compared to the group that received subcutaneous implantation of EAC (table 1). The decrease in tumour volume and weight in group of mice which supplemented POS and 5-fluorouracil in combination (Group 7) more pronounced than their supplemented each one individual (Groups 3-6). Subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant decrease in plasma glucose and insulin compared to the normal control group (table 2) ($p< 0.01$). Administration of cranberry extract at 75 and 150mg/kg. and intraperitoneal administration of 5-fluorouracil to mice resulted in a significant increase in plasma glucose and insulin compared to the group that received subcutaneous implantation of EAC ($p< 0.05$). The increase in plasma glucose and insulin was a significant in the group that was treated with cranberry + 5-fluorouracil compared to the groups that received cranberry and 5-fluorouracil individual ($p< 0.05$). Subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant decrease in plasma glucose, insulin, estrogen, progesterone and follicular stimulating hormone (FSH) compared to the normal control group (table 2) ($p< 0.01$). Administration of cranberry extract at 75 and 150mg/kg. and intraperitoneal administration of 5-fluorouracil to mice resulted in a significant increase in plasma glucose, insulin, 17β-estradiol, progesterone and follicular stimulating hormone (FSH) compared to the group that received subcutaneous implantation of EAC ($p< 0.05$). The increase in plasma glucose and insulin was a significant in the group that was treated with cranberry + 5-fluorouracil compared to the groups that received cranberry and 5-fluorouracil individual ($p< 0.05$).

Tables 3-5 showed that subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant increase in plasma AST, ALT, ALP, LDH and TBARs as well as decrease in blood GSH, SOD and CAT compared to the normal control group ($p< 0.01$). Administration of cranberry extract at 75 and 150mg/kg. and intraperitoneal administration of 5-fluorouracil to mice resulted in a significant decrease in plasma AST, ALT, ALP, LDH and TBARs as well as decrease in blood GSH, SOD and CAT compared to the group that received subcutaneous implantation of EAC ($p< 0.05$). The effect of cranberry + 5-fluorouracil in combination is more pronounced than when cranberry and 5-fluorouracil supplemented individually ($p< 0.05$).

Tables 6 showed that subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant decrease in plasma cholesterol (TC), triglycerides (TG), HDL-C and LDL-C compared to the normal control group ($p< 0.01$). Administration of cranberry extract at 75 and 150mg/kg. and intraperitoneal administration of 5-fluorouracil to mice resulted in a significant increase in plasma TC, TG, HDL-C and LDL-C compared to the group that received subcutaneous implantation of EAC ($p< 0.01$). The effect of cranberry + 5-fluorouracil in combination is more pronounced than when cranberry and 5-fluorouracil supplemented individually ($p< 0.01$). Subcutaneous implantation of EAC into the right thigh of the lower limb of mice resulted in a significant increase in plasma nitrous oxide (NO) and tumor necroses factor-α (TNF-α) compared to the normal control group ($p<0.01$). Administration of cranberry extract at 75 and 150mg/kg. and intraperitoneal administration of 5-fluorouracil to mice resulted in a significant decrease in plasma nitrous oxide (NO) and tumor necroses factor-α (TNF-α) compared to the group that received subcutaneous implantation of EAC ($p< 0.01$) (table 7). The effect of cranberry + 5-fluorouracil in combination is more pronounced than when cranberry and 5-fluorouracil supplemented individually ($p< 0.01$).

Table 1: Effect of cranberry, 5-fluorouracil and there combination on tumor weight

No.	Groups	**Tumor Volume (ml)**	**Tumor weight (gm)**
(I)	Normal (Non-tumor bearing mice (NTB)	0.0 ± 0.0	0.0 ± 0.00
(II)	EAC control (tumor bearing mice (TB)	1.66± 0.11*	1.47± 0.25*
(III)	EAC + Cranberry (CB) 75mg/kg.b.w	1.43± 0.31$^{@}$	1.1± 0.09$^{@}$
(IV)	EAC + Cranberry 150mg/kg.b.w	1.31± 0.14$^{@}$	1.03± 0.16$^{@}$
(V)	EAC + 5-Fluorourcil 20mg/kg.b.w.	1.29± 0.20$^{@}$	0.95± 0.22$^{@}$
(VI)	EAC + CB 75mg + 20mg 5-Fluorourcil	1.20± 0.09$^{@}$	0.92 ± 0.08$^{@}$
(VII)	EAC + CB150mg + 20mg 5-Fluorourcil	1.05± 0.07$^{@}$	0.64± 0.10$^{@}$

5-Flourourasil was given i.p. as a daily dose of 20mg/kg b.w. It was given to all groups except the normal and control one (NTB). The test cranberry was orally given daily for 4weeks at 75 and 150mg/kg.b.w. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. $^{@}$ Significantly different from control group at $p< 0.05$.

Table 2: Level of plasma glucose, insulin estrogen, progesterone and follicular stimulating hormone (FSH) in normal and experimental groups of mice

No.	Groups	**Glucose (mg/dL)**	**Insulin (uIU/ml)**	**Estrogen (pg/ml)**	**Progesterone (ng/ml)**	**FSH (ng/ml)**
(I)	Normal (Non-tumor bearing mice (NTB)	93.43 ± 4.66	3.01 ± 0.44	23.65 ± 2.77	16.46 ± 1.85	7.62 ± 1.46$^{@}$
(II)	EAC control (tumor bearing mice (TB)	168.33 ± 7.81*	1.03 ±0.08*	11.12 ± 0.78*	7.56 ±1.18*	4.60 ± 0.88$^{@}$
(III)	EAC + Cranberry (CB) 75mg/kg.b.w	117.9 ± 16.43$^{@}$	2.07 ± 0.65$^{@}$	18.58 ±3.87$^{@}$	9.86 ± 1.25$^{@}$	6.62 ± 0.92$^{@}$
(IV)	EAC + Cranberry 150mg/kg.b.w	106 ± 13.40$^{@}$	2.34 ± 0.20$^{@}$	20.66 ± 2.35$^{@}$	12.68 ± 1.08$^{@}$	7.8 ±1.73$^{@}$
(V)	EAC + 5-Fluorourcil 20mg/kg.b.w.	92.5 ± 11.70$^{@}$	3.09 ± 0.65$^{@}$	16.42 ± 3.08$^{@}$	10.38 ± 1.44$^{@}$	8.11 ±1.28$^{@}$
(VI)	EAC + CB 75mg + 20mg 5-Fluorourcil	86.6 ± 4.52$^{@}$	2.09 ± 0.57$^{@}$	21.78 ± 2.75$^{@}$	13.19 ± 1.80$^{@}$	6.85 ± .87$^{@}$
(VII)	EAC + CB150mg + 20mg 5-Fluorourcil	76.96 ± 5.29$^{@}$	2.50 ± 0.30$^{@}$	22.96 ± 3.14$^{@}$	13.91 ± 1.23$^{@}$	9.55 ±1.44$^{@}$

5-Flourourasil was given i.p. as a daily dose of 20mg/kg b.w. It was given to all groups except the normal and control one (NTB). The test cranberry was orally given daily for 4weeks at 75 and 150mg/kg.b.w. Blood samples were collected. Values are given as mean ± SD for groups of eight animals each.* significantly different from normal group at $p< 0.01$. $^{@}$ Significantly different from control group at $p< 0.05$.

Table 3: Level of plasma alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) in normal and experimental groups of mice

No.	Groups	**ALT (U/L)**	**AST (U/L)**	**ALP (U/L)**	**LDH (U/L)**
(I)	Normal (Non-tumor bearing mice (NTB)	35.33 ± 5.07	38.65 ± 2.52	96.85 ± 7.10	138.5 ± 11.39
(II)	EAC control (tumor bearing mice (TB)	82.08 ± 6.38*	78.1 ±4.87*	208.5 ± 10.16*	334 ± 16.04*
(III)	EAC + Cranberry (CB) 75mg/kg.b.w	56.36 ± 6.24$^{@}$	54.98 ± 5.35$^{@}$	148.00 ± 8.66$^{@}$	246.00 ± 8.73$^{@}$
(IV)	EAC + Cranberry 150mg/kg.b.w	43.51 ± 5.86$^{@}$	44.9 ± 4.85$^{@}$	123.22 ± 15.46$^{@}$	213.00 ± 16.29$^{@}$
(V)	EAC + 5-Fluorourcil 20mg/kg.b.w.	50.98 ± 4.52$^{@}$	47.66 ± 4.28$^{@}$	157.33 ± 14.7$^{@}$	275.16 ± 13.44$^{@}$
(VI)	EAC + CB 75mg + 20mg 5-Fluorourcil	37.58 ± 3.65$^{@}$	34.59 ± 3.89$^{@}$	83.7 ± 4.80$^{@}$	179.4 ± 18.84$^{@}$
(VII)	EAC + CB150mg + 20mg 5-Fluorourcil	28.88 ± 3.26$^{@}$	30.8 ± 2.96$^{@}$	70.66 ± 4.24$^{@}$	142.16 ± 11.20$^{@}$

5-Flourourasil was given i.p. as a daily dose of 20mg/kg b.w. It was given to all groups except the normal and control one (NTB). The test cranberry was orally given daily for 4weeks at 75 and 150mg/kg. b.w. Blood samples were collected. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. $^{@}$ Significantly different from control group at $p< 0.05$.

Table 4: Level of blood reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and plasma thiobarbituric acid reactive substances (TBARs) in normal and experimental groups of mice

No.	Groups	**GSH (mg%)**	**SOD (U/ml)**	**CAT (U/ml)**	**TBARs (µmol/ml)**
(I)	Normal (Non-tumor bearing mice (NTB)	32.57 ± 2.85	239.46 ± 11.67	46.21 ± 4.97	15.79 ± 1.66
(II)	EAC control (tumor bearing mice (TB)	15.35 ± 1.64*	147.50 ±15.28*	19.27 ± 2.06*	34.88 ± 3.75
(III)	EAC + Cranberry (CB) 75mg/kg.b.w	22.18 ± 2.25$^{@}$	222.85 ± 18.48$^{@}$	35.41 ± 3.00$^{@}$	17.73 ± 2.95
(IV)	EAC + Cranberry 150mg/kg.b.w	28.20 ± 1.96$^{@}$	242.6 ± 13.75$^{@}$	43.34 ± 3.58$^{@}$	13.18 ± 1.55
(V)	EAC + 5-Fluorourcil 20mg/kg.b.w.	21.91 ± 3.48$^{@}$	189.82 ±15.90$^{@}$	26.21 ± 4.05$^{@}$	21.81 ± 2.89
(VI)	EAC + CB 75mg + 20mg 5-Fluorourcil	31.29 ± 2.65	212.8 ± 21.45	42.74 ± 3.81	16.01 ± 1.63
(VII)	EAC + CB150mg + 20mg 5-Fluorourcil	36.06 ± 3.00	236.38 ± 20.66	53.7 ± 4.80	12.22 ± 1.15

5-Flourourasil was given i.p. as a daily dose of 20mg/kg b.w. It was given to all groups except the normal and control one (NTB). The test cranberry was orally given daily for 4 weeks at 75 and 150mg/kg.b.w. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. $^{@}$ Significantly different from control group at $p< 0.05$.

Table 5: Level of liver reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and thiobarbituric acid reactive substances (TBARs) in normal and experimental groups of mice

No.	Groups	**GSH (mg/g tissues)**	**SOD (U/gm tissue)**	**CAT (Umol H_2O_2 consume/mg tissue)**	**TBARs (µmole/g tissue) × 10^{-6}**
(I)	Normal (Non-tumor bearing mice (NTB)	2.89 ± 1.25	39.89 ± 4.00	35.72 ± 2.15	3.08 ± 0.76
(II)	EAC control (tumor bearing mice (TB)	0.98 ± 0.64*	18.24 ±2.64*	20.44 ± 2.85*	4.94 ± 0.44
(III)	EAC + Cranberry (CB) 75mg/kg.b.w	2.11 ± 0.22$^{@}$	33.62 ± 3.52$^{@}$	30.56 ± 2.55$^{@}$	3.26 ± 0.27
(IV)	EAC + Cranberry 150mg/kg.b.w	2.58 ± 0.16$^{@}$	41.34 ± 6.38$^{@}$	34.72 ± 3.07$^{@}$	2.66 ± 0.58
(V)	EAC + 5-Fluorourcil 20mg/kg.b.w.	1.52 ± 0.13$^{@}$	25.11 ±2.95$^{@}$	33.08 ± 3.12$^{@}$	3.79 ± 0.47
(VI)	EAC + CB 75mg + 20mg 5-Fluorourcil	2.31 ± 0.46	36.68 ± 3.87	33.24 ± 2.64	3.09 ± 0.98
(VII)	EAC + CB150mg + 20mg 5-Fluorourcil	2.67 ± 0.32	44.93 ± 4.18	39.60 ± 2.50	2.42 ± 0.65

5-Flourourasil was given i.p. as a daily dose of 20mg/kg b.w. It was given to all groups except the normal and control one (NTB). The test cranberry was orally given daily for 4 weeks at 75 and 150mg/kg.b.w. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. $^{@}$ Significantly different from control group at $p< 0.05$.

Table 6: Level of plasma total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C of normal and experimental groups of mice

No.	Groups	**TC (mg/dl)**	**TG (mg/dl)**	**HDL-C (mg/dl)**	**LDL-C (mg/dl)**
(I)	Normal (Non-tumor bearing mice (NTB)	149.88 ± 8.76	113.6 ± 5.84	35.16 ± 3.25	91.85 ± 6.39
(II)	EAC control (tumor bearing mice (TB)	239.8 ± 15.48*	211.38 ±12.95*	30.38 ± 3.26*	166.77 ± 11.75*
(III)	EAC + Cranberry (CB) 75mg/kg.b.w	203.13 ± 18.90@	182.01 ±17.34@	32.5 ± 4.87 @	133.5 ± 9.43@
(IV)	EAC + Cranberry 150mg/kg.b.w	189.08 ± 11.25@	152.81 ± 8.11@	36.5 ± 3.88@	121.5 ± 14.63@
(V)	EAC + 5-Fluorourcil 20mg/kg.b.w.	149.78 ±7.50@	119.41 ±16.04@	35.8 ± 2.95@	90.91 ± 9.32@
(VI)	EAC + CB 75mg + 20mg 5-Fluorourcil	159.18 ± 16.40@	131.4 ± 11.59@	37.6 ± 5.00@	90.10 ± 8.69@
(VII)	EAC + CB150mg + 20mg 5-Fluorourcil	153.95 ± 13.80@	115.2 ± 5.09@	37.3 ± 4.74@	93.62 ± 5.11@

5-Flourourasil was given i.p. as a daily dose of 20mg/kg b.w. It was given to all groups except the normal and control one (NTB). The test cranberry was orally given daily for 4weeks at 75 and 150mg/kg.b.w. Values are given as mean ± SD for groups of eight animals each. LDL-C (mg/dl) = TC-HDL-[TG / 5]. *Significantly different from normal group at $p< 0.01$. @ Significantly different from control group at $p< 0.05$.

Table 7: Level of plasma nitrous oxide (NO) and tumor necroses factor-α (TNF-α) of normal and experimental groups of mice

No.	Groups	**NO (umol/ml)**	**TNF-α (U/ml)**
(I)	Normal (Non-tumor bearing mice (NTB)	29.54± 2.11	255.50 ± 19.84
(II)	EAC control (tumor bearing mice (TB)	17.70± 1.58*	313.09±17.64*
(III)	EAC + Cranberry (CB) 75mg/kg.b.w	24.44± 2.67@	160.02± 8.00@
(IV)	EAC + Cranberry 150mg/kg.b.w	33.22± 3.09@	107.72± 8.52@
(V)	EAC + 5-Fluorourcil 20mg/kg.b.w.	25.60± 2.55@	175.18± 5.44@
(VI)	EAC + CB 75mg + 20mg 5-Fluorourcil	31.47± 4.26@	130.72± 13.17@
(VII)	EAC + CB150mg + 20mg 5-Fluorourcil	35.61± 3.15@	87.55 ± 7.45@

5-Flourourasil was given i.p. as a daily dose of 20mg/kg b.w. It was given to all groups except the normal and control one (NTB). The test cranberry was orally given daily for 4weeks at 75 and 150mg/kg. b.w. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$. @ Significantly different from control group at $p< 0.05$.

DISCUSSION

The present article aimed to study the antitumor activity of Administration of cranberry extract at 75 and 150mg/kg. in EAC bearing mice as well as compare its activity with 5-Flourourasil, a standard antitumor drug. Our results showed that cranberry when combined with 5-Flourourasil or individual were able to significantly decrease the tumor volume and weigh as compared to that of the EAC control group. Cancer is a pathological state involving uncontrolled proliferation of tumor cells. Reduced volume and weight of tumor indicated a decrease in abnormal cell divisions, *i.e.* tumor proliferation (39, 40). In this study, we observed and reported that cranberry can revert or inhibit EAC induced tumor [41], which may be due to free radical scavenging property of extract in the presence of antioxidant phytochemicals [5-9]. The present work showed that EAC implantation caused fall of blood glucose and insulin in EAC control mice. Hypoglycemia was proportional to the number of tumor cells inoculated into the host. One reason for hypoglycemia could be an augmented consumption of glucose by the cells of the tumor [42, 43]. Indeed, hypoglycemia was most expressed in mice with large tumors, i.e., with the highest tumor volume and weight due to transport of glucose through the membrane of tumor [44]. Facilitated transport of glucose is attributed to the changes of the membrane of tumor cells [45] and increase of insulin-like (glucose-lowering) substances level in the tumor cells, or produces an insulin-like (glucose-lowering) principle itself. Several authors have described higher concentration of insulin-like substances in the plasma of mice with some tumors [46, 47]. However, we have found a decrease of insulin activity in the plasma of EAC control group. Supplementation of cranberry and 5-Flourourasil resulted to increase glucose and insulin levels when compare to EAC control group. According to the

presented results cranberry containing antioxidant phytochemicals [8-12] inhibit EAC induced tumor which my led to decrease the rate of glucose and insulin transport to the tumor cells.

Liver is considered to be the main organ of drug detoxifying organ, some liver marker enzyme levels were measured from serum. AST, ALT, ALP, LDH, NO, TNF-α and TBARs levels were increased in EAC controlled mice, whereas GSH, SOD and CAT levels were decreased. In the present study, subcutaneous implantation of EAC into the mice resulted in a significant decrease in blood GSH, SOD and CAT as well as plasma TC, TG, HDL-C and LDL-C with a significant increase in plasma TBARs compared to the normal control group. These results were in agreement with Raju and Arockiasamy [48] who reported that the consumption of free amino acid for building the proteins of rapidly dividing tumor cells might result in the disturbance of the enzyme activity in the liver [49]. On treatment with cranberry altered liver enzyme level was restored as that of the normal group. Alterations of cholesterol metabolism, including increased cholesterol synthesis and accumulation of cholesterol esters in tumor tissues associated with a decrease of high density lipoprotein cholesterol in serum, were previously observed in different models of neoplastic cell proliferation including haematological malignancies. A number of studies had indicated that reactive oxygen species (ROS) are involved in a variety of different cellular processes ranging from apoptosis and necrosis to cell proliferation and carcinogenesis. Flavonoids and tannins are well known polyphenolic natural antioxidants. The flavonoids present in cranberry extract are thought to be the cause of their antitumor and anti-inflammatory effects [8-12]. Flavonoids have a chemopreventive role in cancer by means of their effect in signal transduction in cell proliferation and angiogenesis [50]. This important property may be responsible for its antitumor activity against EAC *in vivo*. Antioxidant activity of cranberry extract against different reactive oxygen and nitrogen species has already been established by the present authors [8, 9].

The present work showed that EAC implantation caused fall of plasma sex hormones; estrogen, progesterone and FSH when compare with normal control mice. EAC bearing mice associated with increase receptor population [51] and altered estrogen, FSH and progesterone levels were brought back to normal by cranberry and 5-Flourourasil treatment.

Therefore, from the present study it can be concluded that cranberry showed promising antitumor potential in Ehrlich ascites carcinoma bearing albino mice which can be attributed to its flavonoids content. This could serve as a stepping stone towards the discovery of newer safe and effective antitumor agents.

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