Original Article

A Comparative of Nutritional Impacts of Pomegranate and Beetroot on Female Mice Bearing Ehrlich Ascites Carcinoma

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Abstract

The present research was pointed to the protective impact of anthocyanin and betalain dye of pomegranate juice (P), beetroot juice (BR), and their mixture (PB) versus Ehrlich-ascites-carcinoma (EAC)-bearing female mice. Female mice were orally administrated with pomegranate, beetroot juices and their mixture (1 ml/ day), for sequential 15 days then, injected (i.p) by EAC. Cisplatin drug was injected two times as a comparative chemical drug. The results manifested that P BR and PB juices as anthocyanin and betalain dye sources significantly enhanced biological evaluation. Juice mixture induced significant decrement of the liver functions. Pomegranate and mixture juices have significantly decrement of undesirable blood lipids, versus increment benefit HDL and have major power to decrement of tumor cell count. Hence, the current study confirmed that these plant foods which have red dye can significantly protect the cells from EAC and improve the biochemical analysis and biological evaluation of the experimental animals.

Keywords: Anthocyanin, Betalains, Blood lipids, Cisplatin, Tumor cell count

Introduction

It is estimated that by the year 2025 there will be approximately 20 million patients with cancer [1-4]. Therefore, attention is focused on preventive procedures as a definitive cancer management strategy [5, 6]. Current estimates point out that two-thirds of cancer-related deaths can be prohibited via way of lifestyle changes, particularly through nutritional means [6, 7].

Cancer side effects for the body and psychosocial stats can represent the most important and dangerous part of a cancer patient's life. So, nutritional factors especially fruits and vegetables to be taken very seriously by increased care as a preventative and lower considerable side effect of cancer chemical drugs [8].

A lot of research has detected that regularly eating a rich plant's diet on the top fruits and vegetables is closely linked to lowering the risk of cancer by containing them on an abundance of phytochemicals that have strong anti-cancer properties [9-11].

Many new studies confirmed that pomegranate fruit is considered a strong nutritional anticancer factor [12, 13]. Pomegranate fruit (*Punica granatum L.*) has been discovered to extend therapeutic activity such as antioxidants, anti-carcinogens, anti-inflammatory, antimicrobials, and anti-parasites [14-17]. Scientific evidence references that phytochemicals (phenolic compounds, anthocyanins, tannins, and others) in plants have therapeutic actions by their antioxidant characteristic [18]. Pomegranate fruit has polyphenols as antioxidants which possess a wide range of impact towards various kinds of free radicals as compared to different known antioxidants actions such as ascorbic acid, vitamin E, and β-carotene [19]. Anthocyanin in pomegranate fruit is one type of polyphenols that have high significant antioxidant activity compared to the famous antioxidants in green tea and red wine [20].

Beetroot (*Beta vulgaris* L.) is a known vegetable that contains a large number of carbohydrates, fat, minerals, vitamins, and phytochemical ingredients with bioactive properties [21]. The phytochemical compounds of plants

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involve carotenoids, flavonoids, polyphenols, saponins, the water-soluble pigments like betalain, which interact in the cells to produce bioactive actions [22, 23]. The antioxidant power of the beetroot plant makes it at the pinnacle of ten vegetables because it has a high percentage of these phytochemicals [24, 25].

Several previous researchers have elucidated the anticancer activities of pomegranate and beetroot plant ingredients in a chain of human cancer cells [26-30].

Despite the development in the field of pharmaceutical industries, but it has a lot of cumulative interference on human health that leads to death in the long term for its use. As there is a great need to raise the immunity system against diseases, especially cancers, the idea was for this research. And because of what it contains pomegranate and beetroot plants and their mixture as natural foods, popular, accessible to everyone and its actions as protective chemical activities at the top anthocyanins and betalain, it was used against carcinogen of Ehrlich ascites carcinoma (EAC) as a tumor model in female mice.

Materials and Methods

Materials

Animals

Female CD-1 mice (8 weeks of age) were obtained from Taif University KSA. Public Health Guide for the care and use of laboratory animals was applied by the guidelines of Ethics. Mice laboratory conditions were adapted by leaving a week before the experiment [31]. Ehrlich Ascites Carcinoma (EAC) was obtained from National Cancer Institute-Cairo, Egypt, and transplanted in mice as the ascitic form. Preparing the solution of phosphate buffer saline (PBS) at 2 $\times 10^6$ viable cells/ml was performed to induce tumor cell and injected intraperitoneally (i.p.) at dose 0.25×10^6 tumor cells/mice suspended in 0.1 ml [32].

Chemicals and Plants

Cisplatin (cis-diamminedichloroplatinum) and other kits used in the analysis were purchased from Sigma–Aldrich, Co., USA. The plant materials pomegranate and beetroot were purchased from the local market. Fresh juices of pomegranate pulp seeds and beetroot were obtained after compressed and filtered directly by the electric mixer.

Experimental Design

Mice were divided into six groups nine mice per group. **Gr.1:** served as a negative control (healthy mice) injected by PBS (10 mg/mice i.p.). **Gr.2:6** mice were injected (i.p) by EAC at dose 0.25×10^6 cells/mice after treated with oral administration of juices for 15 days. **Gr. 2:** served as EAC positive control group. **Gr. 3:** served as a reference of chemically treated drug group by injected Cisplatin (10 mg/mice i.p.) two times from 5-7 days of injection of the EAC. All mice were feed by oral administration of pomegranate and beetroot juices before 15 days of injected EAC as nutritional treatment as following: **Gr. 4**: was take a single oral administration of pure pomegranate juice (1ml/mice) on an empty stomach daily. **Gr. 5:** was take a single oral administration of beetroot juice on an empty stomach daily. **Gr. 6:** was take a mixture of pomegranate and beetroots juice as a single oral administration (1ml/mice) on an empty.

At the end of the experiment period, mice were fasted overnight then weighed and blood samples were drawn from the optic vein of the mice's eye and sacrificed by cephalic separation of the mice spinal cord. Biochemical analysis was performed by clear serum after the Eppendorf tube centrifuged at 3000 rpm. for 15 minutes.

Biological Evaluation

The animal diet was restricted daily as feed intake then body weight was recorded weekly. The biological evaluation was calculated by determination of body weight gain % (BWG %) feed efficiency ratio (FER) according to the method of Herawati *et al.* [33]. The liver of all mice groups was removed, washed with saline solution, dried with filter paper, and weighted according to the method of Drury and Wallington to calculate a relative liver weight [34].

Biochemical Analysis

Separated mice serum was taken to determine liver function as total protein and albumin according to Yatzidis then globulin calculated by formula= TP- Albumin [35]. Liver enzymes as 'Aspartate aminotransferase (AST), alanine aminotransferase (ALT) according to Anyanwu *et al*. [36], and alkaline phosphatase (ALP) according to Young *et al.* [37]. The serum blood lipid concentration was estimated as total cholesterol and HDL and LDL and VLDL were calculated by the method of Alsoodeeri *et al*. [38], triglyceride according to Fossati *et al.* [39].

Tumor Cell Count

The ascetic fluid was withdrawn from EAC-bearing mice after sacrificed by using a 10ml plastic syringe containing 5 mL of cold saline. EAC cells were separated individually from the ascetic fluid by centrifugation at 300 xg for 2 minutes and carefully transfer the supernatant fluid into a tube. PBS was used in the cells washing twice to eliminate the blood cells. Then, saline solution was re-suspended in a fixed volume for the washed packed cells. The tumor cell suspensions were prepared and enumerated using trypan blue dye exclusion and hemocytometer [40].

Statistical Analysis

All data results found in tables are expressed as means ± standard deviation (S.D.), P values <0.05 and 0.001 were considered significant which analyzed by one-way analysis of variance (ANOVA) using LSD test. The SPSS computer

Program (v.16) were statistically analyzed by using methods of Armitage and Berry [41].

Results and Discussion

Biological Evaluation

Results in **Table 1** show the effect of P, BR juices and their mixture on body weight gain (**BWG%)**, feed intake (**FI)**, feed efficiency ratio (**FER),** and the relative liver weight of mice injected with EAC, and CIS compared to the normal healthy mice. A relatively significant increment was observed in the body weight gain at ($P < 0.05$) in the P+ EAC and PB+ EAC groups (7.33±0.87 and 6.16±1.41, respectively). Feed efficiency ratio (FER) demonstrated a highly significant rise in the P juice group (1.98±0.53) as compared to the normal group. No significant change in the feed intake was found in any of the P, BR, and PB mix juice groups, also no significant increase in relative liver weight was recorded in oral administrated groups as compared to the normal group.

Table 1. Biological evaluation and relative liver weight of normal, EAC, CIS, and oral administrated mice groups.

Groups	BWG	FI (g)	FER	Liver
Normal mice	3.33±0.88	3.43±0.52	0.93±0.51	5.61±0.60
EAC	4.83±0.75	3.58±0.31	1.36±0.28	4.60±0.29
EAC+CIS	3.66±0.49	3.75±0.18	0.98±0.34	4.60±0.88
P+ EAC	7.33±0.87*	3.68±0.21	1.98±0.53*	5.32±0.63
BR+ EAC	5.16±0.48	3.78±0.17	1.36±0.31	6.53±1.02
PB+ EAC	6.16±1.41*	3.80±0.08	1.61±0.86	6.07±0.85

Data in the table recorded as mean ± SD of nine mice. * Significant P-value < (0.05) **Significant P-value <0.001compared to positive control (EAC) with LSD post-test. EAC: Ehrlich Ascitic Carcinoma, CIS: cisplatin, P: Pomegranate, BR: Beet Root, PB: Pomegranate+ Beet Root.

Biochemical Analysis

Data in **Table 2** illustrated results of liver enzymes as aspartate aminotransferase (AST) (U/L), alanine aminotransferase (ALT) (U/L), and alkaline phosphatase (ALP) (U/L) of all groups compared to the EAC control group. The levels of AST, ALT, and ALP recorded a significant increment at ($P < 0.001$) in the CIS group (35.00±3.74,37.83±1.16 and 332.27±3.93 U/L, respectively) as compared to the EAC control group.

On the other hand, daily oral administration juice with P group induced a significant reduction at ($P < 0.001$) in the level of liver enzymes (AST, ALT, and ALP) (15.33±2.25, 11.50±1.04 and 170.79±1.79 U/L, respectively), furthermore ALT and ALP revealed to a significant decrement ($P < 0.001$) of PB juice group (12.67±0.81 and 166.69±2.95 U/L, respectively, also, BR group demonstrated a significant diminish at ($P < 0.001$) with ALP enzyme (194.96±3.63 U/L) as compared to the EAC control group. It was noted that the best results were with the oral administration of P and PB juice.

Table 2. Liver enzymes (AST, ALT, and ALP) of normal, EAC, CIS, and oral administrated mice groups.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal mice	12.16±2.31	6.00±1.41	141.18±2.18
EAC	19.83±2.78	19.50±1.64	271.48±2.98
EAC+CIS	35.00±3.74**	37.83±1.16**	332.27±3.93**
P+ EAC	15.33±2.25**	11.50±1.04**	170.79±1.79**
BR+ EAC	20.50±1.97	20.00±1.78	194.96±3.63**
PB+ EAC	20.33±2.65	12.67±0.81**	166.69±2.95**

Data in the table recorded as mean ± SD of nine mice. * Significant P-value < (0.05) **Significant P-value <0.001compared to positive control (EAC) with LSD post-test. EAC: Ehrlich Ascitic Carcinoma, CIS: cisplatin, P: Pomegranate, BR: Beet Root, PB: Pomegranate+ Beet Root.

Results found in **Table 3** showed total protein (TP), albumin, and globulin as liver functions of all groups compared to the EAC control group. The levels of TP and albumin recorded a significant increment at ($P < 0.001$) and ($P < 0.05$) in all of daily oral administration juices groups (6.59±0.26, 6.50±0.11, 7.07±0.26, 3.36±0.19, 3.31±0.15 and 3.51±0.22 g/dL, respectively) as compared to the EAC control group. It is also, noticed that daily oral administration of juices led to a noticeable rise of TP and albumin, especially when compared to CIS which leads to a marked decrease of the two function results. On the other hand, the globulin level recorded a non-significant increment in PB mixture juice as compared to the EAC group.

Table 3. Liver functions as total protein (TP), albumin, and globulin of normal, EAC, CIS, and oral administrated mice groups.

Groups	TP (g/dL)	Albumin (g/dL)	globulin (g/dL)
Normal	7.66±0.44	3.82±0.19	3.84±0.23
EAC	6.11±0.75	2.80±0.19	3.31±0.60

EAC+CIS	5.78±0.49	2.63±0.19	3.15±0.45
P+EAC	6.59±0.26*	3.36±0.19**	3.23±0.18
BR+EAC	6.50±0.11*	3.31±0.15**	3.19±0.14
PB+EAC	7.07±0.26**	3.51±0.22**	3.56±0.19

Data in the table recorded as mean ± SD of nine mice. *Significant P-value < (0.05) **Significant P-value <0.001compared to positive control (EAC) with LSD post-test. EAC: Ehrlich Ascitic Carcinoma, CIS: cisplatin, P: Pomegranate, BR: Beet Root, PB: Pomegranate+ Beet Root.

In **Table 4** the results review the total cholesterol (TC) (mg/dL), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL) of all groups compared to the EAC control group.

The levels of TC, TG, LDL, and VLDL recorded a high significant raise at ($P < 0.001$) in the CIS group (144.21±5.89, 106±3.91, 97.08±6.12, and 21.38±1.07mg/dL, respectively). In contrast, the HDL level had a significant decrease ($P < 0.001$) in the CIS group (25.75±2.97 mg/dL) as compared to the EAC control group.

Daily oral administration with PBmix juices induced alow significant diminishes at ($P < 0.001$) in all levels of lipid profile as compared to the EAC control group. Followed by juice which revealed a low significant reduction at ($P < 0.001$) in the level of lipid profile except HDL level showed reduced at ($P < 0.05$) (38.51±1.28 mg/dL). At the end of the juice group effect, BR juice occurred a low significant least in TG and VLDL levels (110.41±4.99 and 22.00±1.32 mg/dL) at ($P < 0.001$), then in other data lower at ($P < 0.05$) as compared to the EAC control group.

These results proved that plant juices and their mixture led to a raise of benefit lipids as HDL and lower bad lipids when compared to EAC or CIS as a treated chemical drug.

Table 4. Serum lipid profile as TC, TG, HDL, LDL, and VLDL of normal, EAC, CIS, and oral administrated mice groups.

Groups	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Normal	89.64±3.31	69.33±3.66	48.30±4.47	27.80±5.33	13.54±1.04
EAC	121.06±4.93	78.18±1.99	34.82±2.72	70.60±2.57	15.64±0.49
EAC+CIS	144.21±5.89**	106±3.91**	25.75±2.97**	97.08±6.12**	21.38±1.07**
P+EAC	102.17±6.61**	90.37±2.72**	38.51±1.28*	45.76±7.25**	17.89±0.56**
BR+EAC	116.93±8.62	110.41±4.99**	30.96±2.80*	63.97±7.42*	22.00±1.32**
PB+EAC	90.62±5.56**	87.25±2.81**	42.19±2.28**	30.93±4.70**	17.50±0.72**

Data in the table recorded as mean ± SD of nine mice. *Significant P-value < (0.05) **Significant P-value <0.001compared to positive control (EAC) with LSD post-test. EAC: Ehrlich Ascitic Carcinoma, CIS: cisplatin, P: Pomegranate, BR: Beet Root, PB: Pomegranate+ Beet Root.

EAC Cells – Cell Account

Table 5 illustrated the tumor cell count of mice injected with EAC i.p. 0.25×106 cells/mice, CIS as a treated chemical drug, P, BR, and PB as a natural juice. The treatment of CIS and all daily oral administration juices occurred a high significant decrement at ($P<0.001$) as compared to the EAC control group, P juice improved the count of tumor cells then BR and PB mixture juice.

Table 5. Tumor cell count of normal, EAC, CIS, and oral administrated mice groups

Groups	Tumor cell count (10^6 cell/mice)
EAC	14.87±1.57
EAC+CIS	4.11±1.10**
P+ EAC	3.47±2.66**
BR+ EAC	4.18±0.72**
PB+ EAC	4.24±1.10**

Data in the table recorded as mean ± SD of nine mice. * Significant P-value < (0.05) ** Significant P-value <0.001compared to positive control (EAC) with LSD post-test. EAC: Ehrlich Ascitic Carcinoma, CIS: cisplatin, P: Pomegranate, BR: Beet Root, PB: Pomegranate+ Beet Root.

Carcinogenesis is the process by which a normal cell is converted into a tumor cell. These have several stages started by oxidative stress and inflammation, which in turn leads to many anomalous genetic expressions. Chemical prevention of cancer with biologically active foods occurs through the rearrangement of these inappropriate genetic activities that occur with carcinogenesis [29]. Due to problems in current chemotherapy treatment systems, there are transmit to natural compounds that may be useful in preventing and treating cancer.

This study evaluated the protective impact of daily intake of pomegranate pill juice, beetroot juice, and its mixture as a source of anthocyanin and betalain dye of this natural food against the EAC tumor model and compared them with CIS as the chemotherapy-treated drug of cancer.

The results showed a significant increase in weight gain%, feed efficiency ratio with P and their mixture juices groups, as well as non-significant results in other groups. This means that pre-treatments improved feed efficiency ratio, and body weight gain. The weight gain increases in some groups may be due to the calories of pomegranate and

beetroot, as per Serving of pomegranate juice gives 105 calories and 26.44g carbohydrates that contain fructose and glucose in equal quantities [42], while 100g of beetroot gives 9.96g carbohydrates [43]. So, the intake of carbohydrates (fructose and glucose) of fruit opens the appetite hen raise the bodyweight which follows increase internal relative organs weight compared to body weight.

The biochemical data indicated that AST, ALT, and ALP were very high upon treatment with CIS. This result agrees with the result of Dalia and Nabila [44] they found increased with AST and ALT enzymes. Oral administration of pomegranate and beetroot juices and their mixture induced an improvement of ALT, AST, and ALP enzymes level, which indicate come down of the hepatotoxicity of EAC injection.

Amr and Aml [45] indicated that lower levels of AST, ALT, and ALP with pomegranate juice after elevated by N-nitrosodiethylamine (NDEA)-induced hepatocellular carcinoma may be due to the inhibition of lipid peroxide, and the enhancement of hepatic antioxidant defensive capacity as well as its anti-inflammatory activity by modifying of NF-κ β. which regulated inflammatory pathway. Prasetyastuti *et al.* [46] reported that supplementation with pomegranate juice significantly reduced the serum levels of ALT, AST, and ALP in rats Infected with cancer induced by CCl_4 (a carcinogenic compound), so it can improve liver damage. Also, the daily nutritional use of P, BR juices and their mixture raised the level of TP and albumin while the CIS group reduced when compared to the EAC group. This result shows that the improved liver functions by these fresh natural juices. This may be related to the anti-inflammatory and antioxidant possibility in pomegranate juice.

The lipids profile results outlined an increment in the TC, TG, LDL, and VLDL with the CIS treatment group, this agrees with Afrah *et al.* [47] who reported increase TC and decrease HDL with CIS as treatment of Ehrlich Ascites Carcinoma. The daily oral administration of P, BR, and PB juices results showed a decrement in blood lipids and increment of HDL as compared with EAC- bearing mice. Also, pomegranate reduces LDL cholesterol from the body and prevents atherosclerosis [48]. This action is explained by Qamar *et al*. [42] who indicated that pomegranate is rich in major antioxidants like anthocyanin and tannins that may help to block the buildup of cholesterol in arteries which in return protect heart damage. Also, Qamar *et al*. [42] pointed out that juice of pomegranate fruit may help to reduce the concentration of low-density lipoproteins from the body, and hence, it may protect the body from a stroke attack.

Regarding tumor cell count results, a significant decrease was observed with the CIS group as chemotherapy treatment and P, BR, and PB juices groups when compared to the EAC control group. This finding suggests that pomegranate and beetroots have an antitumor effect against EAC cells. These results agree with Sathibabu *et al*. [10] when they used pomegranate extract against EAC.

Interestingly, treatment with pomegranate decreased most of the pathological modifications stimulated using EAC cells in mice considering that it contains antioxidant compounds that had been cytotoxic in the direction of tumor cells [13]. This means that P, BR, and PB as protective action significantly inhibited tumor cell count with the same level of chemotherapy when reducing the toxicity resulting from it, and its ameliorative impacts on enzymes and functions of the liver and lipids by EAC injection.

The experiments in *vivo* revealed the little percent of dietary pigments that may prevent the formulation of tumors in mice [due to the pomegranate contains bioactive compounds (phenolics and flavonoids) and has a wealthy supply of two kinds of polyphenolic compounds: anthocyanins and hydrolyzable tannins, which account for 92% of the antioxidant action of the complete fruit. Anthocyanins from pomegranate fruit have a significantly higher antioxidant activity compared to other recognized antioxidants [20]. Eleonora *et al.* [49] reported that the anticancer efficacy of pomegranate can be viewed in a chemopreventive and/or chemotherapeutic process. All these therapeutic activities are associated with the presence of anthocyanins. So, Pomegranate juice can act as one of the best anti-cancer agents [27].

In beetroots, the polyphenols have chemopreventive features, as they have hydroxyl groups that provide their protons to reactive oxygen species (ROS) due to the deep red color of beetroot in the presence of pigments of betalain, these pigments replace functionally anthocyanins. The great advantages of betalains as dietary antioxidants refer to their bioavailability, and their superior stability compared to anthocyanins [29]. Gandia-Herrero *et al.* [50] suggest that betalains may play an important role in antioxidant activity against cancer. Studies with varied cell lines have shown the possibility of betalains in the chemoprevention of cancer.

The daily consumption of P, BR, and PB at one dose before injected by EAC, improved mice body weight, the levels of liver functions, serum lipid profile, and decreased tumor cell count. The current study recommends that daily consumption of pomegranate and Beetroot may encourage a preventive effect against tumor and carcinoma.

Conclusion

Pomegranate and beetroot juices brought back the biochemical analysis to near-normal levels. Also, raised desirable serum-HDL concentration, and degreased undesirable serum-LDL concentration, and significantly inhibited tumor cell count in EAC- bearing mice. In this regard, this research can confirm the role of pomegranate

and beetroot as anti-cancer foods against EAC- bearing mice and their side effects on other internal organs.

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