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ORIGINAL ARTICLE

Inhibition of the Growth of Tumors Induced Ehrlich Ascites by Pre-Treatment with Pomegranate and Beetroot Juice in Mice

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ABSTRACT

The present study was to investigate the antioxidant and antitumor effects of three natural juice (pomegranate, beetroot, and both of them pomegranate and beetroot) injected with Ehrlich ascites carcinoma (EAC) - bearing female mice. The results show that pomegranate and beetroot juice extract is notable sources of phenolic acids and flavonoids. The biological experimental were distributed into six mice groups, the control negative group, and the resultant mice were injected with EAC. The mice were divided into a control EAC positive, group (3) EAC and injected with cisplatin, in addition, the EAC (4, 5, and 6) groups were separately taken orally with pomegranate, beetroot, and both pomegranate and beetroot. The total blood count, hepatic lipid peroxidation index, thiobarbituric acid-reactive substances (TBARS), catalase enzyme activity (CAT), and glutathione peroxidase (GPx) levels were evaluated, as well as liver sections were histopathological examined. In mice harboring EAC, there was a reduction in CAT and GPx activity and an increase in TBARS content. Pomegranate, beetroot, and both pomegranate and beetroot juice treatments improved the complete blood count, the negative changes in the liver enzymes, and the histopathological changes in liver sections. These improvements included increased antioxidant activities, decreased lipid peroxidation, inhibition of EAC cell proliferation, and induction of apoptosis. From the results, it could be concluded that the pomegranate and beetroot had the highest contained phenolic and flavonoid content. As a result, it can prevent the biochemical and histological alterations brought on by EAC without having any negative side effects.

Keywords: Pomegranate, Beetroot juice, Ehrlich Ascites carcinoma, Cisplatian, Cancer

INTRODUCTION

The term "cancer" refers to a group of illnesses brought on by the unchecked growth of cancerous cells. There have been an unbearable number of deaths as a result of this global health burden. According to conservative estimates, cancer accounts for roughly 13% of yearly deaths worldwide[1].

Due to their lack of specificity and limitations in rural areas, conventional cancer medicines have a number of adverse effects[2]. Additionally, the robust resistance of malignant cells to cytotoxic and anti-cancer medications has posed a new challenge, resulting in poor management outcomes and unpredictable resistance to anti-cancer medications. As a result, it's important to turn back to homemade remedies and the usage of fruits and vegetables as cancer treatmentsr[3].

One of the most prevalent cancers, Ehrlich ascites carcinoma (EAC), is highly transplantable, does not relapse, and proliferates quickly. These tumors gradually multiply more until they eventually take the ascites form. Due to its undifferentiated nature and quick proliferation rate, EAC resembles human cancers, which are the most susceptible to treatment. At this stage, the utilization of natural sources as an alternative cancer therapy is believed to have considerable value for cancer control and the eradication of cancer-causing programs since the ideal medicine has been concentrated on but has been shown to be inefficient or only partially beneficial for normal cells[4].

Pepe et al.[5] examined how pomegranate (*Punica granatum* L.) juice extract affected intestinal epithelial 6-cells (IEC-6 cells), both under inflammatory settings and after receiving 5-fluorouracil therapy (5-FU). According to the analysis of pomegranate juice extract in IEC-6, pro-inflammatory variables such as the release of cytokines, the expression of cyclooxygenase-2 and inducible nitric oxide synthase, and the production of nitrotyrosine are all significantly inhibited. Additionally, pomegranate reduced the expression of adhesion proteins and oxidative stress. Pomegranate also reduced inflammatory and oxidative stress markers as well as apoptosis in IEC-6 that had been treated with 5-FU. These findings point to pomegranate's potential utility as an adjuvant in the management of intestinal inflammatory and oxidative stress states, which also happen during chemotherapy-induced mucositis.

Pomegranate (*Punica granatum* L.) extract's anti-cancer properties were examined by Uddandrao et al.[6] in Ehrlich-Ascites-carcinoma (EAC)-carrying Swiss albino mice. Pomegranate significantly reduced tumor size, viable cell count, tumor weight, and prolonged life in mice suffering EAC. In mice given pomegranate treatment, hematological parameters like RBC, hemoglobin, and lymphocyte count returned to normal levels. When administered to experimental animals at a dose of 400 mg/kg BW, the extract significantly decreased the degree of lipid peroxidation, markedly elevated the levels of antioxidant enzymes in the liver, and significantly reversed the histological abnormalities. Pomegranate and beetroot significantly reduced the levels of harmful blood lipids, increased the beneficial HDL, and significantly reduced the number of tumor cells in the blood[7]. Thus, the present investigation demonstrated that pomegranate was equivalent to 5-fluorouracil in its ability to prevent tumor development in ascitic animals. The presence of the numerous phytoconstituents in pomegranates may be the cause of their anticancer effects

Red beetroot extract significantly inhibited the growth of tumors in mouse skin and lung bioassays, according to an examination of its in-vivo anti-tumor promoting potential. Therefore, the red beetroot extract's cytotoxic activity in the established estrogen receptor-positive human breast cancer cells (MCF-7) and in the androgen-independent human prostate cancer cells (PC-3) revealed a powerful anticancer potential. In cells treated with MCF-7, betanin/is betanin extract exhibited anticancer action[8].

Flavonoids and polyphenols, two active substances found in fruit and vegetables, have been shown to have anti-cancer, anti-inflammatory, anti-proliferative, neuroprotective, and hepatoprotective activities. The betalains, or red (betacyanins) and yellow (betaxanthins) pigments, are present in red beetroot (Beta vulgaris). A large variety of beneficial biological benefits, including chemopreventive, anticarcinogenic, anti-tumorogenic, antiangiogenic, and proapoptotic actions, are held by betanin, which accounts for 75–95 percent of all betacyanins[9].

Phytochemicals are abundant in the nutrient-dense fruit known as the pomegranate (*Punica granatum* L.)[10]. These substances have drawn growing interest as potential treatments for a number of oxidative stress-related disorders. In order to reduce cardiotoxicity, pomegranate polyphenols may be a suitable option[11].

Betalains, water-soluble pigments with heterocyclic nitrogen atoms in their structure, are abundant in beetroot (*Beta vulgaris* L.). The class of betalains includes yellow betaxanthins and purple-red betacyanins[12]. The largest concentration of betalains, known as betalin, is found in beetroot. The peel of this vegetable also contains phenolic acids, such as p-coumaric acid and ferulic acid[13],

in high amounts. All of these substances have considerable antioxidant activity, and in vivo studies using mice, models have demonstrated that they can prevent the growth of skin and lung cancer. It is hypothesized that the presence of betalains was the cause of this advantageous qualities[14].

This study's objective was to assess the potential anticancer efficacy of pomegranate and beetroot extracts against Ehrlich Ascites Carcinoma (EAC) tumours in mice and compare those results to treatment with cisplatin.

MATERIALS AND METHODS

Materials

Preparation of plant materials: Pomegranates and beetroot were brought from the local market. Beetroot and pomegranate pulp seeds were directly squeezed and filtered by an electric mixer to produce fresh juices.

The ascitic type of Ehrlich Ascites Carcinoma (EAC) was implanted into mice by the National Cancer Institute in Cairo, Egypt. To generate tumor cells, phosphate buffer saline (PBS) solution was prepared at a concentration of 2 $x10^6$ viable cells/ml and given intraperitoneally (i.p.) at a dosage of 0.25 $x10^6$ tumor cells/mice suspended in 0.1 ml[15]. Chemicals and vegetation All of the chemicals used in the study, as well as cisplatin (cis-diamminedichloroplatinum) and other kits for measuring various biological experimental parameters, were bought from Sigma-Aldrich Corp., USA.

A total of 54 female CD-1 mice (8 weeks old) were bought from the National Organization for Drug and Control Research in Giza, Egypt, and the rodents were given the Pell et al [16]-recommended baseline diet.

Methods

Preparation of crude aqueous extract from pomegranate and beetroot: With distilled water, beetroot and pomegranate were cleaned. The edible pulp (arils) of the pomegranate was meticulously separated after it had been peeled. The beetroot was being chopped up at the same time. In an electric blender, the pomegranate and beetroot were extracted for 5–10 seconds. After that, the extract was centrifuged for 10 minutes at 3000 rpm. The pomegranate and beetroot supernatants from the centrifugation stage were collected, filtered, and extracted twice with ethanol at a 70 percent concentration. According to Muyenga et al.[17], the filtrate was then concentrated and kept in a refrigerator until additional testing.

Estimation of antioxidant activity: Total phenolic content was determined as mg Gallic acid Equiv/g dry weight using the Folin-Ciocalteu reagent and technique developed by Qawasmeh et al. 1[8]

Eghdami and Sadeghi[19] evaluated the total flavonoid concentration and computed it as milligrams of quercetin equivalent per gram of dry weight.

Biological experimental: Nine experimental mice were allocated into six groups at random and fed a baseline diet for seven days. The first group, known as the control negative mice group (1), received an intraperitoneal injection of phosphate buffer saline (PBS)(10 mg/mice). They were then given a baseline diet for four weeks.

EAC administered an intraperitoneal injection of 0.25x 10^6 cells/mice to the five groups' 45 mice in order to develop cancer. The five groups were separated again, with the second receiving only the basal diet and the third the positive EAC control group. The third group of EAC received chemotherapy treatment by receiving two injections of cisplatin (10 mg/mice i.p.) starting 5-7 days after the EAC injection and being fed just a baseline diet for four weeks. Pure pomegranate juice (1ml/mice) was administered orally/day administration for the fourth EAC group on an empty stomach each day. On an empty stomach, the fifth EAC group received a daily oral dose of pure beetroot juice (1ml/mice). On an empty stomach, the sixth EAC group received an oral daily dosage of a pure blend of pomegranate and beetroot juice (1ml/mice). Before 15 days of EAC injection, pomegranate and beet juice were given orally to all animals as nutritional therapy..

Mice were starved for the end of the experiment, weighed, and had blood samples taken from their optic veins before being murdered via cephalic severance of their spinal cords. Clear serum was used for biochemical analysis after the Eppendorf tube had been spun at 3000 rpm for 15 minutes.

Tumor cell count: After the mice had been slaughtered, the ascetic fluid was removed using a 10 ml plastic syringe containing 5 ml of cold saline. Centrifugation at 300 xg for 2 minutes was used to separate each EAC cell from the ascetic fluid, and the supernatant fluid was carefully transferred into a tube. To get rid of the blood cells, PBS was used twice in the cell washing process. For the washed packed cells, saline solution was then re-dissolved in a predetermined volume. The tumor cell suspensions were made, and they were counted by hemocytomete and pan-blue dye exclusion[20].

Complete blood count: Whole blood samples were used to assay the total count of peripheral blood leukocytes (PBL). An automated CBC device (VetScan HM2™ Hematology System, Abaxis®, CA, and USA) was used to count all leukocytes in peripheral blood[21].

Biochemical analysis: Lipid peroxidation was estimated as a thiobarbituric acid-reactive (TBARS) substance in the liver using a spectrophotometer at 532 nm according to Yagi[22].

Glutathione peroxidase (GPx) enzyme activity was estimated at 412nm according to Chiu et al.[23].

Determination of catalase enzyme activity (CAT) in liver regions was assayed by the method of Aebi[24].

Histopathological examination of liver: The liver was kept in formaldehyde to be used for examination and immersed in neutral buffered formalin for one day and cut into 4-5 µm-thick with hematoxylin and eosin (H&E) by using the method Manhong et al.[25].

Statistical analysis: In the tables, all data findings are shown as means with standard deviation (S.D.). One-way analysis of variance (ANOVA) was used to examine the data, and the LSD test was used to determine if P values between 0.05 and 0.001 were significant. The Armitage and Berry techniques were used to conduct a statistical analysis of the SPSS computer program (v.16).

RESULTS AND DISCUSSION

Phenolic and flavonoids in pomegranate and beetroot juice extract: The secondary metabolites of plants known as phenolic compounds, which include flavonoids and phenolic acids, are crucial and have a wide range of structural and functional characteristics [27].

Table (1) indicated that the highest phenolic content in pomegranate and beetroot juice extract were 10.46 and 18.36 mg/g of Gallic acid, respectively. Meanwhile, flavonoid content had contained 6.79 and 9.78 mg/g of Quercetin, respectively. Thus, the consumption of fresh pomegranate and beetroot juice was caused by an increase in the varieties of rich bioactive compounds which are an important source of desirable antioxidant properties for health promotion[28].

Pomegranates (*Punica granatum*) have been utilized extensively in traditional Eastern medicine across a wide range of nations and cultures and are a substantial source of bioactive chemicals. Pomegranate pulp possesses a significantly anti-atherogenic effect against atherosclerotic illnesses in people and animals, and pomegranate peel and pulp have a substantial antioxidant capacity[29]. In addition, Platosz et al.[30] determined the phenolic acids and flavonoids in red beetroot juice. The results revealed that beetroot was a rich source of phenolic acids and flavonoids.

Table (1): Total flavonoids and phenolic content from pomegranate and beetroot extract

Sample	Total flavonoid mg/g Quercetin	Total phenolic mg/g Gallic acid
Pomegranate extract	7.69±0.45	10.46±0.83
Beetroot extract	9.78±0.62	18.36±0.97

Data in table recorded as mean ± SD (n=3)

Tumor cell count of EAC and treated with pomegranate and beetroot in mice groups: To estimate the anti-tumor effects of pomegranate and beetroot and both of them against EAC cells and changes in tumor cell viability of different treated groups are reported in Figure (1). The results observed that the tumor cell viability was not detected in the control negative group, whilst the control positive group injected with EAC was the highest (14.87x 106 cell/mice) in tumor cell viability.

The EAC-bearing mice group was treated with cisplatin as chemotherapy showed a significantly low tumor cell viability inoculation compared to the EAC control positive group. Regarding cell viability cisplatin displayed a significant decrease in EAC cells reaching 72.60 %, compared to the EAC control group.

The EAC-bearing mice group was treated with pomegranate juice as a natural treatment showed that the tumor cell viability was decreased to 76.66%compared to the EAC control positive group. Whilst, The EAC-bearing mice group was treated with beetroot juice as a natural treatment showed that the tumor cells viability was decreased to 71.89% followed by the EAC-bearing mice group was treated with pomegranate plus beetroot juice was 71.84% compared to the EAC control positive group. The discovery that flavonoids and phenolic display a wide range of pharmacological and biological activities, often scavenge free radicals, and play a crucial role in the prevention and treatment of cancer led to these discoveries. It is generally known that beets and pomegranates are abundant producers of these phenolic and flavonoid compounds, both of which have antioxidant properties. Cancer patients showed a link between the development of malignant cells and endogenous antioxidant systems because of the endogenous antioxidant system's diminished activity[31]. Additionally, Beck et al.[32] investigated propolis' capacity to prevent the development and spread of Ehrlich ascites carcinoma (EAC) cells in mice. The findings of the EAC cells showed the treated group's volume, total cell count, viable percentage, percentage of dead cells, mean survival time (MST), life span (ILS) %, and treated vs positive control (T/C) percentage all decreased.

Complete blood count of normal, EAC, CIS, and oral administrated mice groups: Complete blood count was determined in mice carcinoma groups and compared with EAC control positive mice and the results are reported in Table (2).and Fig (2) White blood cells (WBCs), red blood cells (RBCs), hemoglobin (Hb), platelets, lymphocytes, Monocytes, and Granulocytes were 6.83 x10^3µl, 7.30 x10^3µl, 14.17 g/dl, 6.48x10^5µl, 41.82% 0.8%, and 40.68%, respectively, in the control group healthy mice. Whereas, the results from positive control EA carcinoma found 9.97x10^3µl, 6.18 x10^6µl, 11.97 g/dl, 4.73x10^5µl, 47.35%,1.55%, and 49.67% respectively. The results in the group EA carcinoma treated with cisplatin as chemotherapy showed the improvement in the complete blood count to 7.03 x10^3µl, 6,65 x10^3µl, 11.98 g/dl, 5.12 x10^5µl, 37.65% 1.35%, and 40.87%, respectively. Three successive doses of metformin were administered to mice that had been infected with Ehrlich ascites carcinoma (EAC). In contrast to cisplatin, the standard anti-neoplastic treatment, to determine its eligibility as a potential safe choice against EAC cells for further combination therapy. The EAC cells treated with metformin or cisplatin were found to be in a quiescent condition after cell cycle analysis. Furthermore, damage to EAC cells is caused by cisplatin or metformin by an increase in ROS levels (ROS). As a result, in the future, cisplatin and metformin may be effective treatments for the EAC cells' aggressiveness [33].

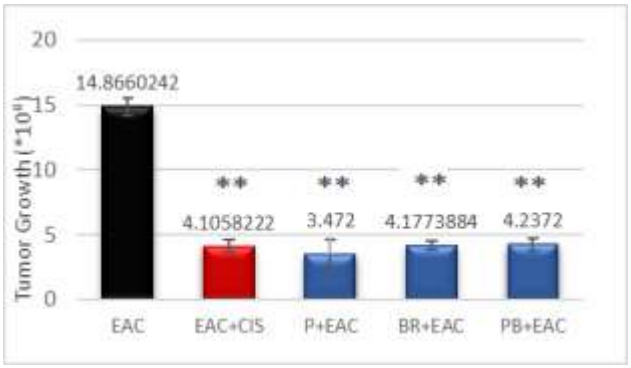

Fig. 1: Tumor cell count of normal, EAC and treated mice groups

Figure1: Group (1) negative control has not been detected, Group (2) positive control Ehrlich Ascitic Carcinoma (EAC),Group (3): EAC plus(CIS) cisplatin, Group (4): Treated with pomegranate (P+EAC), Group (5): Treated with beetroot(BR+EAC), Group (6): Treated with pomegranate+ Beetroot (PB+EAC).

The different mice groups injected with EA which induces carcinoma cells and treated with pomegranate, beetroot, and both of them as a natural therapy, the results showed an improvement in the complete blood count in all mice groups. Moreover, the results from mice group treated with cisplatin as chemotherapy was parallel to taken orally beetroot juice, the results found as 7.0 x10^3µl, 7.23 x10^6µl, 12.42 g/dl, 5.22 x10^5µl, 35.68%, 0.70%, and 44.10 %, respectively, followed by pomegranate juice, the results was 7.67 x10^3µl, 7.18 x10^6µl, 13.20 g/dl, 5.86 x10^5µl, 40.2%, 1.08%, and 45.25 %, and the results of both juice from pomegranate and beetroot was 7.93 x10^3µl, 7.18 x10^6µl, 12.45 g/dl, 4.96 x10^5µl, 41.38%, 0.85%, and 47.47 %, respectively. These results from the mice group EA carcinoma treated with cisplatin were parallel to mice group EA carcinoma treated with natural juice, maybe due to the pomegranate and beetroot had contained high amounts from phenolic and flavonoids content which scavenging the free radical in the blood.

In the EAC mice group, the total WBC count was elevated hemoglobin and the RBC count was reduced. These results are in agreement with those of other studies (34). This may be because of myelopathic conditions or iron deficiency or hemolytic. This could be due to high levels of antioxidants in kiwifruit that can improve iron bioavailability, and indicate an important function in the immune system[32,35]. Moreover, these finding shows that treatments have an antitumor effect against EAC cells when compared to the side effects of CIS. Despite the fact that cisplatin had better result in the treatment, lacks selectivity, for tumor tissues, which leads to severe side effects[36].

Table (2): Complete blood cell count of normal, EAC, CIS, and oral administrated mice groups.

Groups	WBCs (10^3 / ul)	RBCs (10^6 / ul)	Hb g/dl	Platelets (10^5 / ul)	Lymphocytes (%)	Monocytes (%)	Granulocytes **(%)**
Healthy mice	6.83 ±0.95	7.30 ±0.26	14.17 ±0.32	6.48 ±1.04	41.82 ±2.81	0.85 ±0.24	40.68 ±1.31
EAC	9.97 ±1.79	6.18 ±0.88	11.97 ±0.90	4.73 ±0.67	47.35 ±3.79	1.55 ±0.13	49.67 ±1.27
EAC+CIS	7.03 ±1.61**	6.65 ±0.39	11.98 ±0.44	5.12 ±1.06	37.65 ±2.87**	1.35 ±0.15*	40.87 ±3.49**
P+ EAC	7.67 ±2.14*	7.18 ±0.31**	13.20 ±0.40**	5.86 ±0.63*	40.20 ±2.44**	1.08 ±0.14**	45.2 ±2.26**
BR+ EAC	7.00 ±2.41**	7.23 ±0.43**	12.42 ±0.59	5.22 ±0.58	35.68 ±2.87**	0.70 ±0.14**	44.10 ±2.63**
PB+ EAC	7.93 ±1.71*	7.18 ±0.67**	12.45 ±0.92	4.96 ±0.57*	41.38 ±1.90*	0.85 ±0.10**	47.47 ±3.75

Data in 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.

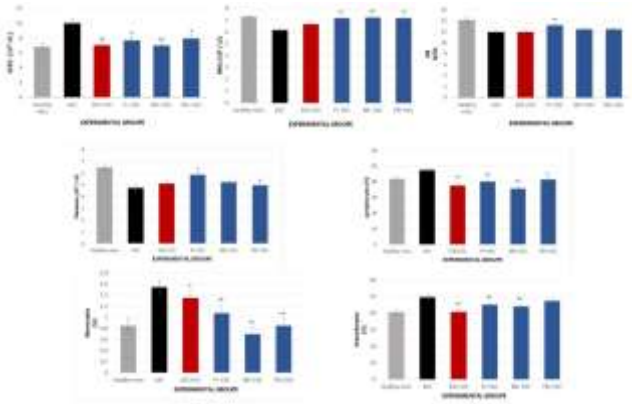

Fig. 2: Complete blood cell count of normal, EAC, CIS, and oral administrated mice groups.

Antioxidant activity enzymes from liver: As shown in Table (3) and Fig (3) marked elevation in Thiobarbitutic acid-reactive substances (TBARS) levels (54.80 nmol/g tissue) and significant inhibition of glutathione peroxidase enzyme (GPx) content (20.08 U/mg protein), and catalase enzyme (CAT) activities (1.58 U/mg protein) were observed in the liver of mice EA carcinoma cells as a positive group than negative control was 27.32 nmol/g tissue, 31.56 and 3.13U/mg protein, respectively. Overproduction of MDA in the liver may be due to lipid peroxidation, causing hepatic injury and apoptosis[37].

The thiobarbitutic acid-reactive substances level (TBARS) in different mice groups treated with cisplatin and natural juice as pomegranate, beetroot and both of them were determined. The results observed that very close between cisplatin and natural juice were reduced TBARS levels than positive control due to the pomegranate, and beetroot had contained high amounts of antioxidant compounds. These results were confirmed by Uddandrao et al. (38-39) reported that TBARS, the end product of lipid peroxidation LPO increased in carcinomatous tissue than in non-diseased organs. As a result, the TBARS levels were greater in the EAC control liver tissues than in healthy liver tissues. Elevated LPO caused tissue damage and failure of the endogenous antioxidant defense systems to prevent an excess of ROS, according to an increased level of TBARS in EAC control animals. A rise in the level of TBARS revealed that pomegranate treatment enhanced hepatic LPO. Its pro-oxidant action was shown by the pomegranate's covert stimulation of ROS production in tumor-bearing mice. As a result, using natural products is seen to be one of the best ways to cure cancer because it has been shown to have fewer adverse effects. Antioxidant-rich diets were employed as a cancer preventive strategy[40].

GPx and CAT as antioxidants in the liver were determined in different carcinoma mice and the results are reported in Table (3).and Fig (3) The results indicated that the group mice injected with Ehrlich ascites carcinoma and cisplatin was near to the results from control negative healthy mice by 31.56 and 3.13 U/mg protein for CAT and GPx, respectively. Whilst, the mice taken orally separately with pomegranate and both of them pomegranate and beetroot and injected with Ehrlich ascites carcinoma, the results were equal, whereas, the mice taken orally with beetroot and injected with Ehrlich ascites carcinoma beetroot was the lowest results 16.01 and 1.64U/mg protein for GPx and CAT, respectively. By scavenging ROS, regulating cellular redox state, and serving as a cofactor for antioxidant enzymes, the endogenous antioxidant system known as GPx plays a significant role in safeguarding cells. It is particularly abundant in the liver [41]. On the other hand, the free radical scavenging system, CAT, and SOD are supposed to function as a buffer against the hydrogen peroxide and superoxide's potentially harmful reactivity[20].

Table (3): Determination of thiobarbituric acid-reactive substances level (TBARS), Determination of catalase enzyme activity (CAT) and Determination of glutathione peroxidase enzyme activity (GPx)

Groups	TBARS(nmol/g tissue)	CAT(U/mg protein)	GPx(U/mg protein)
Normal	27.32±1.20	31.56±1.00	3.13±0.13
EAC	54.80±2.92	20.08±1.31	1.58±0.12
EAC+CIS	73.18±4.20**	12.64±0.94**	1.01±0.06**
P+ EAC	45.10±1.99**	22.40±2.03**	2.40±0.34**
BR+ EAC	56.69±1.66	16.01±0.82**	1.64±0.04
PB+ EAC	45.20±1.39**	22.27±1.19**	2.25±0.06**

Data in 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.

Histological analysis in mice carcinoma liver: Fig (4) showed the Histopathological of mice liver sections. The negative control liver section group appeared normal kupffer cells, blood sinusoids, hepatocytes, and nuclei (photo A). These results were reported by Pittman[42] who discovered that hepatocytes in the control group of mice had polygonal shapes with eosinophilic granular cytoplasm and vesicular basophilic nuclei, as well as radially organized cords that extended from a central vein to the periphery of the hepatic lobules.

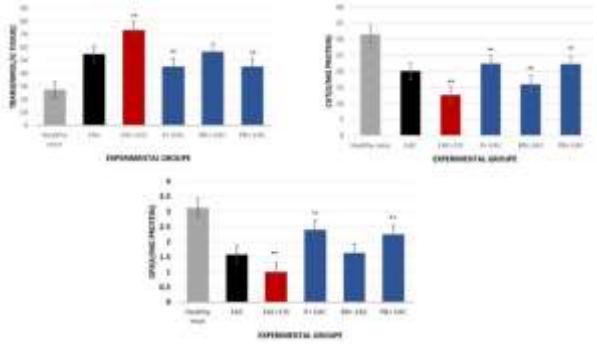

Fig 3 Determination of thiobarbituric acid-reactive substances level (TBARS), Determination of catalase enzyme activity (CAT) and Determination of glutathione peroxidase enzyme activity (GPx)

The positive control group showed kupffer cells hyperplasia, sinusoidal dilation and hydropic and swelling changes in the hepatocytes (photo B). Liver histopathological examination presented that (EAC) bearing mice lead to a defect in liver sections simulate, showed marked degeneration, necrosis and infiltration also, revascularization, or the formation of countless new blood capillaries, was evident in the surrounding tissue with little to no inflammatory reaction. This tumor had disorganized tissue architecture, a significant degree of cellular anaphase, pleomorphism, and anisocytosis, as well as nuclear vascularity, a typicality, hyperchromasia, and mitoses[43].

Whereas groups taken orally with pomegranate and beetroot juices then injection Ehrlich Ascites showed the nuclei appeared normal, meanwhile, the cell outlines are disappeared and the hepatocytes had slight coagulative necrosis (photo D and F). The liver section of the group taken orally pomegranate + beetroot Juice and injection Ehrlich Ascites showed normal Kupffer cells and nuclei, while, the hepatocytes strands lined with endothelial cells were penetrated by the blood sinusoids. On other hand, the hepatocytes with radial arrangement were clearly noticed (photo E). Moreover, (photo C) showed the histopathological of liver sections for Injection of Ehrlich Ascites and cisplatin, which appeared similar to normal control.

Uddandrao et al.[6] examined the Ehrlich-ascites-carcinoma (EAC)-bearing Swiss albino mice and the aqueous pomegranate (*Punica granatum* extract) fruit extract's (PGET) anticancer efficacy. PGET was given intraperitoneally to EAC-bearing mice at dosages of 100, 200, and 400 mg/kg body weight (BW) for 14 days

in a row. The mice were euthanized 24 hours after the last treatment and during an 18-hour fast. According to the findings, PGET was just as effective as 5-Fluorouracil at reducing tumor development in ascitic animals. The presence of the numerous phytoconstituents in *P. granatum* may be the cause of its anticancer activities.

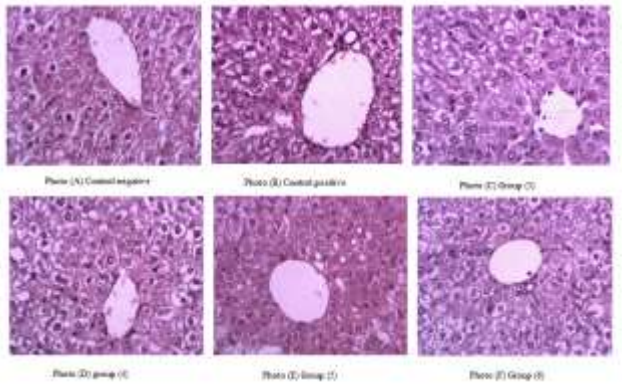

Fig. (4): Light micrograph of liver sections of (Photo A) normal control (NC), (Photo B) positive control (PC)Injection Ehrlich Ascites, (Photo C) Injection Ehrlich Ascites and cisplatin, (Photo D) Injection Ehrlich Ascites and taken orally pomegranate Juice, (Photo E) Injection Ehrlich Ascites and taken orally pomegranate and beetroot Juice, (Photo F) Injection Ehrlich Ascites and taken orally beetroot JuiceH&E×400

CONCLUSION

Therefore, the capacity of pomegranate and beetroot extract to offer defense against EAC-induced biochemical and histopathological alterations without negative side effects could be inferred.. In addition, these extracts reduced proliferation and induced apoptosis in EAC cells. Pomegranate extract is more effective than both them pomegranate and beetroot extract as an antioxidant which in its turn is more effective than beetroot extract.

Conflict of interest: None
Financial support: None
Ethics statement: This study is cleared by the ethical committee of the Department of Food Science and Nutrition, Faculty of Science, Taif University, Taif–Al-Haweiah, KSA. As well as the Department of Nutrition and Food Science, Faculty of Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

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