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The Effect of Vitamin B17 on Cardiomyopathy against Ehrlich Tumor Development in Female Mice

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ABSTRACT

The transplantable tumor model of Ehrlich ascites tumor (EAC), which is a cancer simulation, is frequently used to study the antineoplastic effects of amygdalin (VB17). This study aims to find out how cardiac toxicity and oxidative stress triggered by EAC could be countered with VB17 in female mice. Twenty-five female mice were included and divided into 5 groups namely, the control group (Gp1), EAC (Gp2), VB17 control group (Gp3), EAC+VB17 (Gp4), EAC+VB17+SOR IP (Gp5). All groups underwent cardiac marker assessment in addition to oxidative stress marker MDA determination and the evaluation of the anti-oxidative stress marker of SOD. By comparison with the naïve control group, the EAC positive control group had a significantly higher level of Troponin, serum lactate dehydrogenase (LDH), CK-MB, CPK, and MDA content. On the other hand, the EAC group had significantly lower levels of cardiac SOD than the control group. Furthermore, better improvement in cardiac toxicity and oxidative stress was displayed by the cotreated (VB17+SOR) group 5 than by the (EAC + VB17) group 4. This led to the conclusion that VB17 conferred cardiac protective and antioxidant effects against EAC. This finding necessitates further research into the benefits of VB17 as adjuvant agents in the prevention and treatment of cardiac toxicity.

Introduction

Cardiomyopathy caused by cancer causes cardiac dysfunction, which is characterized by cardiac atrophy, metabolic remodeling, fibrosis, and changes in cardiac ultrastructure, similar to congestive heart failure [1]. Ehrlich carcinoma is one of the most prevalent experimental cancers, and it is extremely important for modeling purposes. Ehrlich ascites carcinoma (EAC) is a type of cancer that develops spontaneously as murine

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mammary adenocarcinoma, is originally hyperdiploid, and can transplant intraperitoneally passages on all mouse strains [2].

Chemotherapy and radiation are the basis of the majority of current cancer treatments, which work by triggering apoptosis to destroy cancer cells; however, this mechanism can also have an impact significantly on patient morbidity and mortality [3]. While clinical innovation has improved, the increase in the usage of complementary and alternative medication has recently [4].

Many types of natural products contain antitumor chemical compounds like VB17. The latter is one of the most commonly used alternative drugs in the treatment of tumors in the last 40 years [5]. Recently, some advances had been made on the antitumor mechanism of amygdalin by confirming that amygdalin can induce apoptosis in human leukemia cells [6].

Accordingly, this study aims to evaluate to find out how cardiac toxicity and oxidative stress triggered by EAC could be countered with VB17 in female mice.

2. Material and Methods

2.1 Chemical and reagent.

B17 and Sorafenib were purchased from (BAYER AG Company, Germany). Ehrlich ascites carcinoma (EAC) was conquered from the Cancer Biology Unit (CBU), Al-Kaser Al-Eini, Egypt which was maintained and propagated by serial transplantation (i.p) in an aseptic environment. All reagent kits were of analytical grade and purchased from a local supplier (Biomed, Egypt).

2.2 Experimental animals.

The experiment was implemented on 25 female mice weigh up to 17– 21 g. Mice were continued under standardized conditions. All mice were adjusted to the place for two weeks before the experiment starting. The Mice were kept in a precise temperature environment with a 24 h cycle. The animals were fed with a normal water *ad libtium* and diet throughout the days of the experiment. The trial method was consented to by Confined Ethics Committee and Animals Research, Tanta University (2020).

2.3 Experimental design.

The 25 female mice were permitted to become adapted to the experimental conditions for two weeks before they were disseminated indiscriminately in five groups that restricted 5 animals each. Thus, the control group (Gp1) consisted of mice that were not treated at all. The EAC group (Gp2) consisted of mice that carried EAC cells (300 µl of $2x10^6$ cells /mouse) for 14 days. The VB17 group (Gp3) was administered 300 mg/kg over the experiment period. The EAC+B17 group (Gp4) was administered daily by oral gavage 300 VB17 mg/kg over 14 days. The EAC+VB17+SOR IP group (Gp5) consisted of mice that received VB17 treatment at the beginning of EAC induction for 14 days and Sorafenib (30 mg/ kg mouse).

2.4 Blood collection.

After the exploratory period, mice were euthanized with sodium pentobarbital (intraperitoneal mixture) then, at that point blood tests were gathered from the inferior vena cava in non-heparinized glass tubes for assessment of biochemical parameters.

2.5 Cardiac biomarkers assessment.

Serum Troponin (cTn) was detected by the method of Han et al. [7]. Serum LDH and CPK levels were measured by a kinetic method of Babson et al. [8] and Szasz et al., [9] respectively. CK-MB activity in serum was determined by the method described by Vaidya et al. [10].

2.6 Determination of cardiac malondialdehyde (MDA) and cardiac superoxide dismutase (SOD) contents.

The tissue homogenate MDA content (total MDA) was determined by Ohkawa et al. [11]. MDA results were expressed in nm/g tissue. SOD homogenate activity was determined as described by Beyer [12]. This method works with xanthine and xanthine oxidase to produce superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitro phenol-s-phenyl tetrazolium chloride) to form a red formazan dye. SOD activity is then calculated by the degree of inhibition of this reaction.

2.7 Statistical Analysis

The statistical software program Prism (GraphPad.Prism.v5.01) was working to bearing statistical analysis. Results were examined via one-way analysis of variance (ANOVA) tailed by the Least Significant Difference (LSD) tests to match between different groups. Data were symbolized as the mean±SEM. *P* values less than 0.05 were considered significant.

3. Results

3.1 Effect of VB17/SOR on cardiac markers

As shown in **Figure 1 (A, B, C)**, by comparison with the control group and the B17 group, the EAC group bared significantly ($P<0.001$) higher levels of Troponin, LDH, and CPK. By contrast, The EAC+B17+SOR had a significantly ($P<0.001$) lower level of these markers than the EAC+B17 group.

3.2 1 Effect of VB17/SOR on cardiac oxidative stress marker MDA.

As can be seen in **Table 1**, there was a significant ($P\leq0.05$) increase in MDA levels in the heart of EAC mice (Gp2) as compared to the other groups. This level was significantly decreased following treatment with B17 (Gp4) as compared to the EAC untreated group (G2). Also, no significant difference was noticed among the B17 group and control group.

3.2.2 Effect of VB17/SOR on cardiac SOD.

There was a significant ($P\leq0.05$) decrease in SOD levels in the heart of EAC mice (Gp2) as compared to all groups as shown in Table 3. This level was significantly improved following treatment with B17 (Gp4), However, the Addition of SOR to treatment enhɑnced the results of SOD upregulation from those mice treated only by B17. Also, no significant difference was noticed among the B17 group and untreated group as shown in **Table 2**.

4. Discussion

Ehrlich carcinomas may be inherently malignant, hyperdiploid, highly transplantable, do not regress, proliferate rapidly, have short survival rates, lack tumor-specific transplantation antigens, and can be transplanted for cancer [2]. The purpose of this study was to observe how cardiac toxicity and oxidative stress triggered by EAC could be refuted with VB17 in female mice. In this publication, a new mouse mo del is used to assess how EAC cancer affects cardiac function.

The results showed that cardiac function was indeed compromised by tumor formation. More

specifically, Troponin, LDH, CK-Mb, and CPK levels were significantly higher in the EAC group than in the control group. LDH plays a major role in tumor onset and metabolism [13]. In the present study, excessive levels of LDH were observed in mice carrying EAC, which could have been due to cardiac toxicity, or rapid tumor cell metabolism. According to our study, the results are comparable to those of Alkhatib et al. [14] and Mookerjee et al. [15], who found that subcutaneous EAC is associated with elevated levels of cardiac enzymes. Despite the reduction in cardiac enzymes with B17 treatment, the EAC+VB17+SOR group had significantly lower LDH, CK, and CK-MB levels than the EAC+B17 group. Thus, cardiotoxicity from EAC could be significantly attenuated by VB17. The findings of this study were consistent with those reported by Gebreel et al., who observed that cardiac toxicity could be induced by EAC [16].

Oxidative mechanisms play an impo rtant role in the emergence, develop ment, and progression of cancer [17]. In contrast to normal cells, cancer cells contain a higher level of reactive oxygen species (ROS), which leads to increased oxi dative stress that damages cell components [18]. Our study found that MDA level increased in the EAC group, and lipid peroxidation content decreased by administration of VB17, however, the concomitant administration of SOR decreased the MDA level more than B17 alone. Cancer cells elicit free radicals to be shaped in excess, destructive lipids, and theoretically generating lipid peroxidation [19], which may be the cause for the notable upsurge in the levels of cardiac MDA persuaded by the EAC group in this study. These findings are also consistent with the results of Aldubayan [20] who revealed that the EAC cells injected into mice showed a significant decrease in TAC and a significant increase in the level of malondialdehyde in the heart tissues. In addition, the results of this study are in good agreement with the results of Joukar et al. [21], who showed that MDA levels in the liver, kidneys, and testes are increased due to the formation of EAC tumors.

As SOD catalyzes the dismutation of superoxide anion (O_2^-) to H_2O_2 and O_2 [22], our results confirmed that the Addition of SOR to treatment enhanced the results of SOD upregulation from those mice treated only by B17. These findings are in agreement with other studies, with different used treatment regimens especially the combination therapy significantly increase ROS levels which leads to oxidative damage to cellular structures and decreases antioxidant enzymes SOD and CAT activity in tumor tissue compared with the EAC group [23], [24], [25].

To sum up, the EAC model can induce oxidative stress in mice hearts. VB17 appears to reduce the inflammatory process, probably due to its antioxidant activity, which may in part explain the mechanism for ameliorating other chronic inflammatory conditions such as inflammation and cancer. Hence, the scavenging process has been improved. Our results may help to better understand the mechanism of heart injury during EAC and provide new targets for evaluating the effect of VB17 and SOR as a dual treatment. It has been determined that more research is needed to investigate the benefits of VB17 and

SOR coadministration in the prevention and treatment of cardiotoxicity.

References

[1] S. Mishra et al., "Subcutaneous Ehrlich ascites carcinoma mice model for studying cancer-induced cardiomyopathy," Sci. Rep., vol. 8, no. 1, pp. 1–11, 2018.

[2] H. M. Nasser, S. A. El-Naggar, M. E.-S. R. El-Sayed Rizk, A. Elmetwalli, and A. F. Salama, "Effect of Sorafenib on Liver Biochemistry Prior to Vitamin B17 Coadministration in Ehrlich Ascites Carcinoma Mice Model: Preliminary Phase Study," Biochem. Lett., vol. 17, no. 1, pp. 40–49, 2021.

[3] N. P. Staff, A. Grisold, W. Grisold, and A. J. Windebank, "Chemotherapy- induced peripheral neuropathy: a current review," Ann. Neurol., vol. 81, no. 6, pp. 772–781, 2017.

[4] S. B. Johnson, H. S. Park, C. P. Gross, and B. Y. James, "Complementary medicine, refusal of conventional cancer therapy, and survival among patients with curable cancers," JAMA Oncol., vol. 4, no. 10, pp. 1375–1381, 2018.

[5] E. Jaszczak-Wilke, Ż. Polkowska, M. Koprowski, K. Owsianik, A. E. Mitchell, and P. Bałczewski, "Amygdalin: Toxicity, Anticancer Activity and Analytical Procedures for Its Determination in Plant Seeds," Molecules, vol. 26, no. 8, p. 2253, 2021.

[6] M. Qadir and K. Fatima, "Review on pharmacological activity of amygdalin," Arch. Cancer Res., vol. 5, no. 04, p. 160, 2017.

[7] X. Han, S. Li, Z. Peng, A. M. Othman, and R. Leblanc, "Recent development of cardiac troponin I detection," ACS sensors, vol. 1, no. 2, pp. 106–114, 2016.

[8] A. L. Babson and S. R. Babson, "Kinetic colorimetric measurement of serum lactate dehydrogenase activity," Clin. Chem., vol. 19, no. 7, pp. 766–769, 1973.

[9] G. Szasz, W. Gruber, and E. Bernt, "Creatine kinase in serum: 1. Determination of optimum reaction conditions.," Clin. Chem., vol. 22, no. 5, pp. 650–656, 1976.

[10] H. C. Vaidya, Y. Maynard, D. N. Dietzler, and J. H. Ladenson, "Direct measurement of creatine kinase-MB activity in serum after extraction with a monoclonal antibody specific to the MB isoenzyme.," Clin. Chem., vol. 32, no. 4, pp. 657–663, 1986.

[11] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," Anal. Biochem., vol. 95, no. 2, pp. 351–358, 1979.

[12] W. F. Beyer Jr and I. Fridovich, "Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions," Anal. Biochem., vol. 161, no. 2, pp. 559–566, 1987.

[13] A. L. Marzan, C. Nedeva, and S. Mathivanan, "Extracellular vesicles in metabolism and metabolic diseases," Subcell Biochem, vol. 97, pp. 393–410, 2021.

[14] M. H. Alkhatib, H. M. Alkreathy, K. S. Balamash, and F. Abdu, "Antitumor activity of doxorubicine-loaded nanoemulsion against Ehrlich ascites carcinoma-bearing mice," Trop. J. Pharm. Res., vol. 15, no. 5, pp. 937–943, 2016.

[15] A. Mookerjee et al., "A novel copper complex induces ROS generation in doxorubicin resistant Ehrlich ascitis carcinoma cells and increases activity of antioxidant enzymes in vital organs in vivo,"

BMC Cancer, vol. 6, no. 1, pp. 1–11, 2006.
[16] D. T. Gebreel, A. M. Elatrash, F. A. Atta, E. Tousson, and S. Allam, “Roles of Chitosan Nanoparticles on Ehrlich Ascites Carcinoma Induced Changes in Some Blood Parameters,” Asian Oncol. Res. J., pp. 71–77, 2020.
[17] R. A. de Boer, W. C. Meijers, P. van der Meer, and D. J. van Veldhuisen, “Cancer and heart disease: associations and relations,” Eur. J. Heart Fail., vol. 21, no. 12, pp. 1515–1525, 2019.
[18] N. M. Abdel-Hamid et al., " Oxidative stress predominates apoptosis during experimental hepatocellular carcinoma. Journal of Contemporary Medical Sciences, vol. 3, no. 12, pp. 295-299.2017.
[19] G. Y. Sun et al., “Docosahexaenoic Acid (DHA) Supplementation Alters Phospholipid Species and Lipid Peroxidation Products in Adult Mouse Brain, Heart, and Plasma,” NeuroMolecular Med., vol. 23, no. 1, pp. 118–129, 2021.
[20] M. A. Aldubayan, “Evaluation of the cardiac protection conferred by proanthocyanidins in grape seeds against development of ehrlich solid tumors in mice,” Biomed Res. Int., vol. 2020, 2020.
[21] S. Joukar, B. Shahouzehi, H. Najafipour, A. Gholamhoseinian, and F. Joukar, “Ameliorative effect of black tea on nicotine induced cardiovascular pathogenesis in rat,” EXCLI J., vol. 11, p. 309, 2012.
[22] H. Zhao, R. Zhang, X. Yan, and K. Fan, “Superoxide dismutase nanozymes: an emerging star for anti-oxidation,” J. Mater. Chem. B, 2021.
[23] A. Nabil et al., “Zinc oxide nanoparticle synergizes sorafenib anticancer efficacy with minimizing its cytotoxicity,” Oxid. Med. Cell. Longev., vol. 2020, 2020.
[24] H. Takano, Y. Zou, H. Hasegawa, H. Akazawa, T. Nagai, and I. Komuro, “Oxidative stress-induced signal transduction pathways in cardiac myocytes: involvement of ROS in heart diseases,” Antioxidants Redox Signal., vol. 5, no. 6, pp. 789–794, 2003.
[25] D. K. Das and N. Maulik, “Resveratrol in cardioprotection: a therapeutic promise of alternative medicine,” Mol. Interv., vol. 6, no. 1, p. 36, 2006.

Table 1: Effect of VB17/SOR on cardiac oxidative stress marker MDA

Group	Fold change mean	SEM
Control (untreated) (Gp1)	3.02[d]	0.07
EAC (Gp2)	16.81[a]	0.16
B17 (Gp3)	3.26[d]	0.10
EAC+ B17 (Gp4)	13.67[b]	0.14
EAC+B17+Sorafenib IP (Gp5)	5.95[c]	0.13

Means within the same column carrying different superscript letters [a (the highest value, d (the lowest value)] are significantly different (P≤ 0.05).

Table 2: Effect of VB17/SOR on cardiac SOD

Group	Fold change mean	SEM
Control (untreated) (Gp1)	26.82[d]	0.17
EAC (Gp2)	16.75[a]	0.38
B17 (Gp3)	26.61[d]	0.30
EAC + B17 (Gp4)	20.50[b]	0.30
EAC + B17 + Sorafenib IP (Gp5)	23.70[c]	0.21

Means within the same column carrying different superscript letters [a (the highest value, d (the lowest value)] are significantly different (P≤ 0.05).

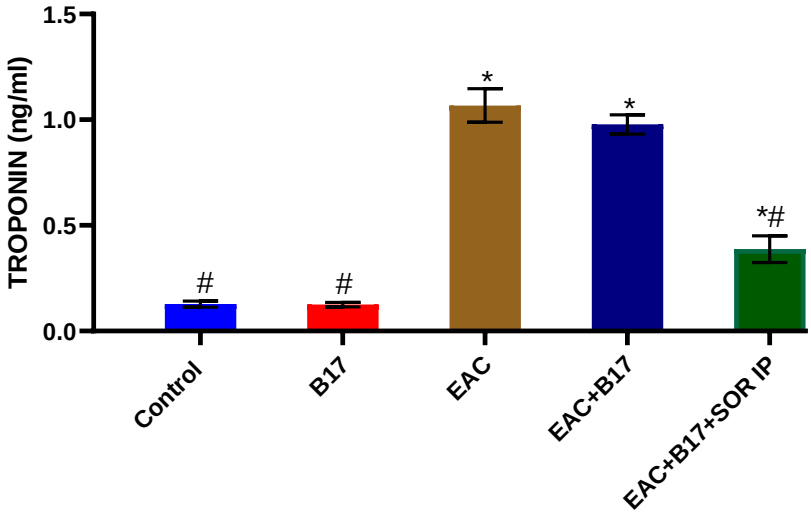

Figure 1A: Effect of VB17/SOR on Troponin activity; (*) significant difference compared to control group. (#) highly significant difference compared to EAC group.

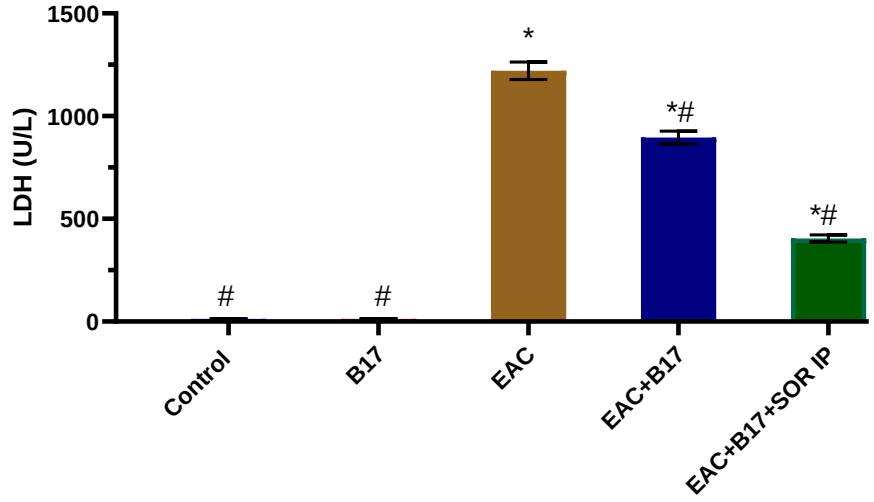

Figure 1B: Effect of VB17/SOR on LDH activity; (*) significant difference compared to control group. (#) highly significant difference compared to EAC group.

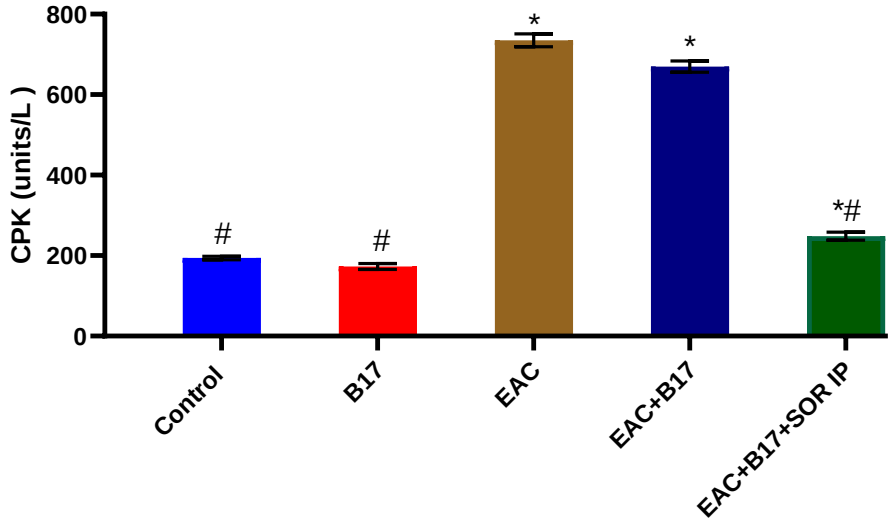

Figure 1C: Effect of VB17/SOR on CPK activity; (*) significant difference compared to control group. (#) highly significant difference compared to EAC group.