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STUDY ON THE EFFECTS OF GRAPE SEED EXTRACT IN EHRLICH ASCITIC CARCINOMA

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Abstract: The seed extract (GSE) of Burgund Mare de Recas grape variety was evaluated for antitumor activity against Ehrlich ascites carcinoma(EAC)-bearing Swiss albino mice. The antioxidant activity of GSE was also estimated in EAC-bearing mice treated with doxorubicin 2x0.1 mg/mouse. The composition in polyphenols of the GSE, expressed as gallic acid, consisted of 11.477mg/l. The anti-tumor activity of GSE was assessed by measuring the tumor volume and by counting the tumor cells. As oxidative stress parameters the total thiobarbituric reactive reactive substances (TBARS) in the plasma were measured. The results indicate that GSE does not dot possess antitumor activity against Ehrlich ascitic carcinoma cells, does not interfere with the antitumor effects of doxorubicin in EAC-bearing mice and decreases the lipid peroxidation induced by doxorubicin treatment.

INTRODUCTION

Cancer is one of the most frequent neoplastic diseases in human population and one of the most frequent causes of death. There are a lot of pathological factors, including reactive oxygen species (ROS) involved in the process of cancer initiation, promotion and progression (1). First, an oxidative stress can induce DNA damages that lead to genomic instability and possibly stimulate cancer progression (2). Second, elevated ROS levels are responsible for constant activation of transcription factors and the progression of the disease(3). For these reasons, the search for antioxidants as cancer chemopreventive agents is a continued process. Another aspect of antioxidant administration in cancer patients is that these could affect antineoplastic efficacy or the development of side effects of anticancer drugs (4). A number of studies indicate that plant derived natural products, such as polyphenols, possess diverse pharmacological properties, among which antioxidant activity (5,6). The aim of the present study was to evaluate the antitumor and the antioxidant activity of the seed extract of Burgund Mare de Recas grape variety against Ehrlich ascitic carcinoma cells.

MATERIALS AND METHODS

Materials

Doxorubicin: the dose used in the experiment was 2x0.1 mg/mouse (7).

Grape seed extract: the powder of the seeds of Burgund Mare de Recas grape variety was provided by Department of Viticulture, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca. The grape seed extract (GSE) was prepared in the

Laboratory of Tumor Biology, "I.Chiricuta" Cluj-Napoca, stored at 4°C in light-tight containers until used and diluted with 0.5% carboxymethylcelullose when used. The composition in polyphenols of the GSE, expressed as gallic acid equivalents, consisted of 11.477mg/l.

Animals and treatment

Male Swiss albino mice, with an average body weight of 20± 2g were used in the experiment. The animals were housed at a room temperature of 23±2 ^{0} C, with a 12/12 hours light/dark cycle, with food and water *ad libitum.* They were allowed to acclimate for 1 week prior to experimental procedures. Mice were inoculated with $1x10^{6}$ EAC in the day 0 of the experiment (8).

Tumor cells

Ehrlich ascites carcinoma (EAC) cells were obtained from the Laboratory of Experimental Pathology, "I.Chiricuta" Oncological Institute Cluj-Napoca. The EAC cells were maintained in vivo in Swiss albino mice, by intraperitoneal (i.p.) transplantation of $1x10^{6}$ cells/mouse every 10 days (9).

Experimental protocol

Male Swiss albino mice were divided into 5 groups of 10 animals (n=10) each, as follows:

Group I (controls): EAC-bearing mice

Group II: EAC-bearing mice which were treated with doxorubicin (dox) i.p. the days +1 and +4 of the experiment

Group III: EAC bearing mice which were given GSE i.p. in the days 0, +1 and +4 of the experiment

Group IV: EAC-bearing mice treated with doxorubicin i.p. in the days +1 and +4 and with GSE i.p. in the days 0, +1 and +4 of the experiment

Group V: EAC bearing mice which were given only the vehicle (carboxymethylcelullose 0.5%) i.p. in the days 0, +1 and +4 of the experiment

On the day +11 all mice were killed by cervical dislocation; blood samples were collected and the plasma quickly separated and frozen at -80^{O}C until used. The anti-tumor activity of GSE was measured in EAC bearing mice with respect to the following parameters(10):

tumor volume. The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume of the fluid was measured by taking it in a graduated centrifuge tube.

tumor cell count. The ascitic fluid was diluted 1:10-1:15 and the cells were counted in a Burker counting chamber. The non-viable cells, as estimated by staining with trypan blue, were not counted.

Estimation of oxidative stress parameters

As oxidative stress parameters in plasma we determined the level of lipid peroxides (11), which result from interaction of free radicals with unsaturated lipids, and which are bound to lipoproteins, and the level of free malonaldehyde (12), which is a product of decomposition of oxidized lipids in plasma. The amount of oxidation products was expressed as thiobarbituric reactive substances (TBARS), which represents the sum of lipid peroxides and free malonaldehyde, as nmol/ml plasma.

RESULTS AND DISCUTIONS

The tumor volume and the total EAC cells in the 5 study groups are presented in Tab.I.

Tab.I. The tumor volume and the total EAC cells in controls and in treated animals

	Tumor volume (ml)	EAC cells $x10^6$
Group I controls	2.67±1.39	350.35±234.84
Group II doxorubicin	2.16±1.40	26.68±17.87*
Group III GSE	1.64±0.67	164.40±114.48
Group IV doxorubicin+GSE	1.55±0.33	76.25±37.75
Group V vehicle	2.83±0.88	424.20±102.72

* $p<0.05$

In the present study, doxorubicin demonstrates a marked antitumor effect in mice inoculated with Ehrlich tumor cells. To et al. (13) also reported the antitumor properties of the anthracycline against Ehrlich carcinoma cells. The results are not surprisingly, as doxorubicin rank among the most effective anticancer drugs ever developed, possessing significant activity against solid and hematologic malignancies (14). Regarding the antitumor effects of polyphenols, studies in the literature show various results, depending on the experimental model and the source of polyphenols used. It was reported that the nonhydroxylated core structure of the flavones, flavone, induce programmed cell death and growth inhibiton in cancer cell lines (15). Nyska et al. (16) also reported that NAO, a mixture of mainly aromatic polyphenols and flavonoids, but not epigallocatechin-3-gallate, was effective in slowing the spontaneous prostatic carcinogenic process in some rodent models of carcinogenesis. It was suggested that resveratrol, a polyphenolic compound, has antitumor activity in cell culture, but weak antileukemic effect in mice injected with leukemic cells (17). In our *in vivo* model, even it was noted a tendency of reduction of tumor cell number for GSE alone, the difference was not significant, leading us to the conclusion that GSE doesn't demonstrate antitumor activity.

The mechanisms of action of doxorubicin are multiple. First of all, it forms a covalent complex with DNA-topoisomerase, thus preventing the activation of this enzyme. Secondly, doxorubicin intercalates in the DNA bone and leads to single or double strand breaks. Thirdly, hepatic metabolism of the drug leads to production of oxygen reactive species, which, in turn, damage DNA and cause cell death (14). Indeed, in our study, as shown in Fig.1, the levels of plasma lipid peroxidation were significantly higher in EAC-bearing mice treated with doxorubicin than in those untreated. On the contrary, plasma levels of lipid peroxidation products in EAC-bearing mice did not differ in GSE or vehicle treated animals as compared to controls (Fig. 2).

Fig.1. Plasma TBARS in doxorubicin treated mice as compared to controls

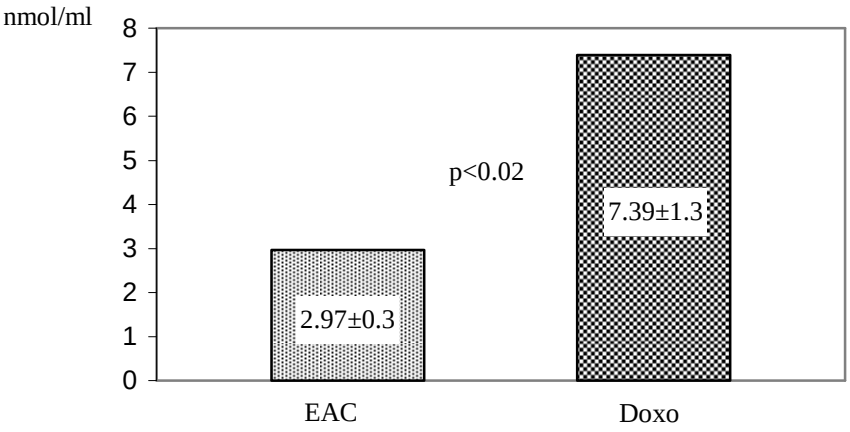
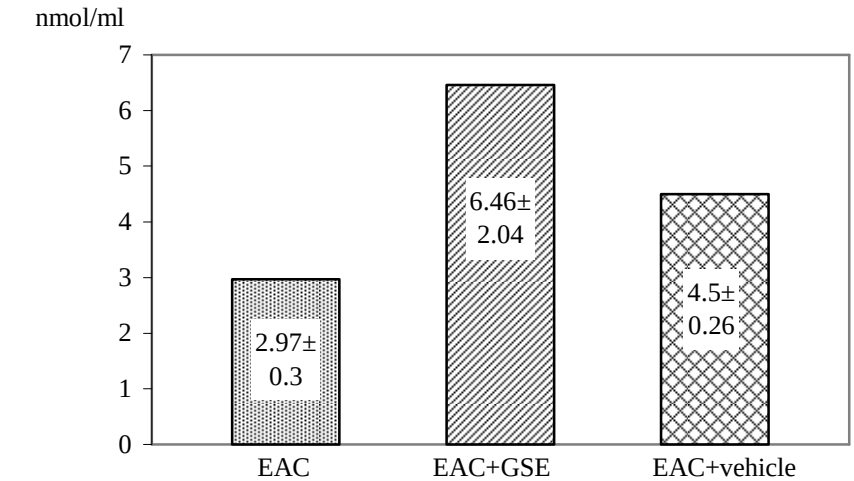


Fig.2.Plasma TBARS in controls, GSE and vehicle treated mice (p>0.05)



One problem preventing more widespread of anthracyclines use has been the development of side effects. Experimental evidence supports oxidant stress as an important trigger and/or mediator of doxorubicin-induced cardiotoxicity (18), hence the attempts which were made to attenuate the oxidative stress by the mean of antioxidants(19). In this respect, a number of studies demonstrated the antioxidant effects of plant polyphenols in animal models (5,6). The results of the present study indicate that in EAC-bearing Swiss albino mice treated with doxorubicin, GSE lowered significantly the plasma levels of lipid peroxidation products, as shown in Fig.3.

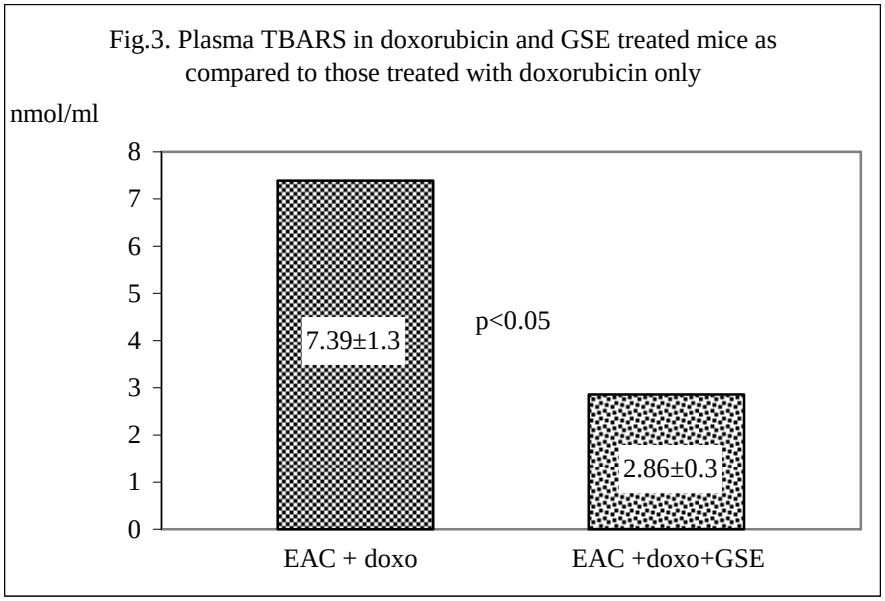


Fig.3. Plasma TBARS in doxorubicin and GSE treated mice as compared to those treated with doxorubicin only

A problem raised by the use of antioxidant in cancer patients is that if generation of ROS by a cancer chemoterapeutic agent or a free radical intermediate of the drug plays a role in its cytotoxicity, antioxidants may interfere with the drug's neoplastic activity (4). Our results (Tab.I)suggest that GSE reduces the oxidative effects of doxorubicin, without impairing its antitumor activity.

CONCLUSIONS

GSE (Burgund Mare de Recas) does not demonstrate antitumor activity against Ehrlich ascitic carcinoma cells

GSE decreases the lipid peroxidation induced by doxorubicin treatment

GSE does not interfere with the antitumor effects of doxorubicin in EAC-bearing mice

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