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Effects of *Geranium sanguineum* Ethanol Extract After i.p. Application in a Mouse Model of Ehrlich's Breast Cancer

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Geranium sanguineum has a strong anti-oxidant and antitumor activity documented *in vitro* but less examined *in vivo*. Recently, we obtained 80% ethanol extract from the plant roots and showed that it is a valuable antitumor agent in *in vitro* studies. The aim of the present study was to evaluate the effects of the above extract used i.p. in a mouse model of Ehrlich's breast carcinoma, to compare it with the effect of oxaliplatin and to look for a possible synergistic action of the two agents. The results showed that *G. sanguineum* and oxaliplatin have no synergistic action but act similarly when applied alone. This activity included directing the ascites cells to apoptosis and preventing metastases in the liver. The results presume the *G. sanguineum* ethanol extract to be a possible alternative/auxiliary chemotherapeutic to oxaliplatin for this type of tumor.

Key words: *Geranium sanguineum*, oxaliplatin, Erlich's carcinoma, pathomorphology

Introduction

Geranium sanguineum (blood geranium) is a perennial herbaceous plant of the *Geraniaceae* family, which rhizomes and leaves are widely used in folk medicine for the regulation of blood pressure, as an anti-septic, anti-inflammatory and hemostatic agent, in gastrointestinal diseases, and others [2]. Polyphenol-rich extracts have strong antioxidant and antitumor activity [3]. Recently, we obtained 80% ethanol extract (composed mainly of anthocyanidins and their derivatives) from the plant roots and showed its antitumor activity in a panel of tumor cells (unpublished results).

The aim of the present study was to evaluate the effects of the above extract used *i.p*. in a mouse model of Ehrlich's breast carcinoma, to compare it with the effect of oxaliplatin and to look for a possible synergistic action of the two agents.

Materials and Methods

G. sanguineum ethanol extract (GSE). Dried and crushed roots of the herb were treated with 80% ethanol in a ratio 1g : 10 mL for 3 hours, then the solid residue was re-extracted overnight. The collected filtrates were concentrated on a vacuum evaporator. Acetonitrile was added, evaporated, and the residue was treated with diisopropyl ether, filtered and dried *in vacuo*.

Animals and treatment. Male ICR mice (20, 20g b.w.), were randomly allocated to four groups (5 mice each), as follows:

Group 1 – inoculated with $1x10^6$ Ehrlich's cells i.p. to develop ascites tumor (positive controls);
Group 2 – like group 1 but treated daily i.p. with 30 mg/kg b.w. GSE;
Group 3 – like group 1 but treated i.p. with a single dose of 10 mg/kg b.w. oxaliplatin on the third day of the experiment;
Group 4 – like group 1 but treated i.p. with 15 mg/kg b.w. GSE (daily) and 10 mg/kg b.w. oxaliplatin on the third day of the experiment.

All the animals were fed and watered *ad libitum* during the testing period (10 days). The experiments were carried out in accordance with the national regulation Nr 20/01.11.2012 regarding laboratory animals and animal welfare. The animals from all groups were sacrificed by cervical dislocation on the 11^{th}day. Tissue samples from liver were extracted, stained with H&E and examined microscopically (Leica DM 5000B, Germany). Ascites' smears were stained with DiaPath May-Grü*ndwald*-Giemsa Fast Method and examined as above.

Results and Discussion

Ehrlich's carcinoma is a rapidly developing, poorly differentiated tumor in mice, similar to the most sensitive to chemotherapy human breast cancers. It is used to study the therapeutic potential of various substances [1,4] also in our laboratory [5]. We tested the possibilities of i.p. application of the GSE and oxaliplatin, which allows direct contact of the agent with the ascites tumor cells. The pilot study was conducted using the scheme of Elkhawaga et al. [1] with healthy animals at doses 15 or 30 mg/kg b.w.(daily) GSE and 10 mg/kg b.w. oxaliplatin (a single dose) as 0.2 ml aqueous solution. The experience did not show any deviations from the norm which let us approve the above test scheme.

Ascites smears showed a number of giant tumor cells, cells in different stages of mitosis and leukocyte effusions in positive controls and animals treated with both extract and oxaliplatin (**Fig. 1A** and **C**). In the animals treated only with extract or oxaliplatin respectively, the number of leukocytes was visibly lower and a substantial formation of apoptotic blebs in tumor cells was observed (**Fig. 1B** and **D**). Obviously, the antitumor agents applied separately tend to direct the tumor cells to apoptosis.

In the liver of positive controls (group 1) and in group 4, signs of acute serous hepatitis were noticed. Extended sinusoidal capillaries with effusions of inflammatory cells and metastatic tissue (in some animals) were also visible (**Fig. 2A**). In groups 2 and 3 a perivascular inflammation was present but no metastases were seen (**Fig. 2B** and **D**).

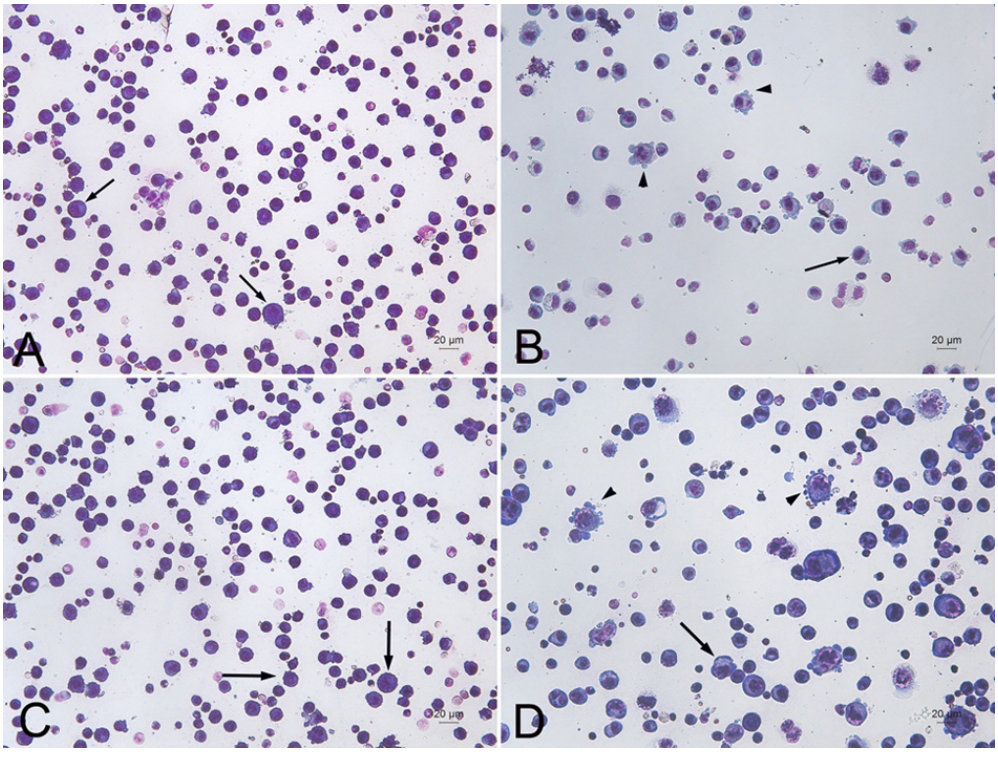

Fig. 1. Ascites form of Ehrlich's carcinoma. A – non-treated with anti-tumor agents positive control; B – mice treated only with GSE; C – mice treated with both GSE and oxaliplatin; D – treated only with oxaliplatin. Giant carcinoma cells (arrows). Only in groups 2 and 3 (treated with the extract or oxaliplatin respectively) – formation of apoptotic blebs (arrowheads). May-Gründwald-Giemsa staining, 200X

According to the above results it could be concluded that *G. sanguineum* ethanol extract has not a preventive effect on the development of Ehrlich's carcinoma in mice and has not a synergistic action with oxaliplatin. However, the extract applied alone has a very similar activity to that of oxaliplatin also applied alone: it prevents metastasizing of tumor cells and directs them to apoptosis in the ascites fluid. Those findings presume that *G. sanguineum* ethanol extract deserves to be studied as a possible alternative/ auxiliary chemotherapeutic to oxaliplatin for this type of tumor.

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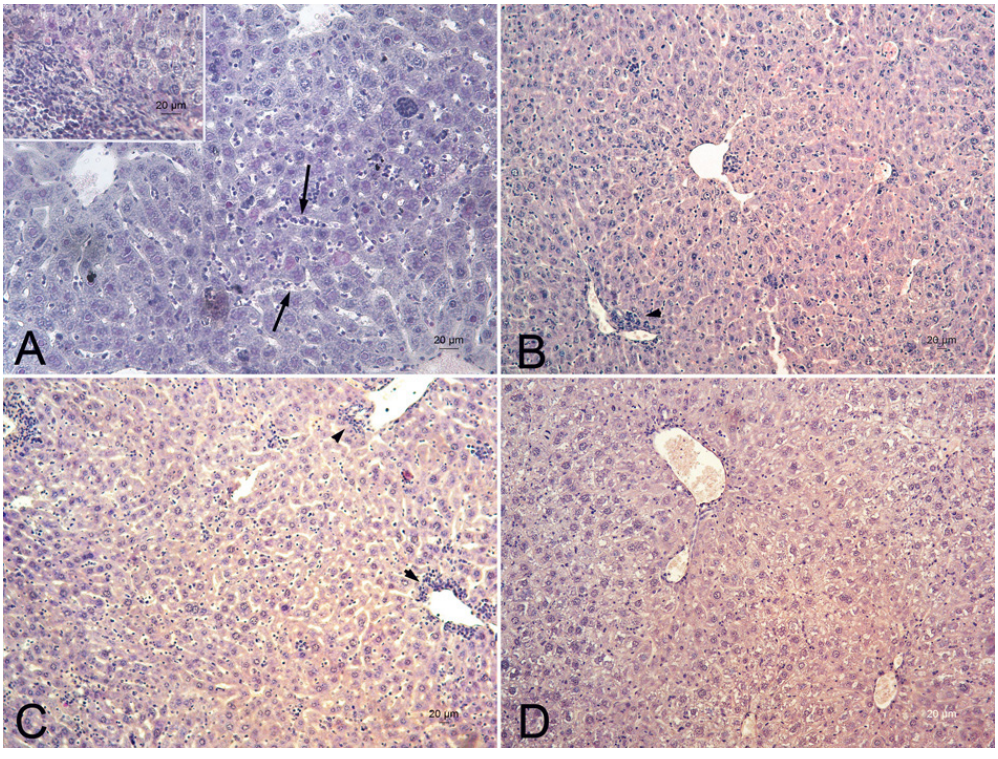

Fig. 2. Liver. A – non-treated with anti-tumor agents control; B – mice treated with GSE; C – mice, treated with both extract and oxaliplatin; D – mice, treated only with oxaliplatin. In controls – signs of acute serous hepatitis; extended sinusoidal capillaries with effusions of inflammatory cells (arrows); inclusion – metastatic tissue.Treated animals – no metastases but perivascular inflammation (arrowheads). H&E staining, 200X

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