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Antitumor Effect of *Coriolus versicolor* Queil Alcoholic Extract

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Abstract. *Coriolus versicolor* Queil is a mushroom traditionally used in Asian herbal remedies. In the present study, *C. versicolor* alcoholic extract was tested on Ehrlich Ascitic Carcinoma (EAC) model. The experiment was carried out on 30 white Swiss male mice, 10^6 EAC cells / animal i.p. Animals were divided in three equal groups; first group (*C. versicolor* + EAC group) received in the day 1, 4 and 7 - 50 ml sol./kg b.w. *C. versicolor* 1:10. The second group was the EAC group and the last group (Doxorubicin + EAC group) received i.p. Doxorubicin cloride 2.5 mg/ kg b.w. in day 6 of experiment. Other 30 animals were subject of administration of mushroom extract, control and Doxorubicine chloride alone in the same doses, at the same time. *C. versicolor* alcoholic extract provides the significant increase in white blood cells, and the platelets as compared to control. The survival rate was also higher in treated groups, and the extract was able to prevent in a significant manner the increasing in the body weight do to accumulation of the ascitic fluid. *C. versicolor* reduced, also, the ascitic volume and cellular concentration of the ascitic fluid, but not statistically significant. *C. versicolor* alcoholic extract revealed a considerable antitumoral activity, along with the safety profile; therefore *C. versicolor* is recommended for further studies, in other to find new remedies in complementary cancer therapy.

Keywords: Corioulus versicolor, Ehrlich Ascitic Carcinoma, mice, Doxorubicine, body weight.

INTRODUCTION

Coriolus versicolor Queil is a mushroom traditionally used in Asian herbal remedies. The most important compounds are polysaccharide K (PSK) and polysaccharide-peptide (PSP); they are being studied as possible complementary cancer treatments (Ergil *et al.*, 2002). Both polysaccharides have documented anticancer activity *in vitro, in vivo* and in human clinical trials, though PSK has been researched longer and has therefore undergone more thorough laboratory, animal and clinical testing. The mechanisms of biological response modification by PSK have not fully elucidated (Ergil *et al.*, 2002). Some studies suggest that PSK may act to increase leukocyte activation through up-regulation of key cytokines (Shinjiro *et al.*, 2009). Indeed, natural killer and lymphocyte-activated killer cell activation has been demonstrated *in vivo* and *in vitro,* (Ho *et al.*, 2004). An antimetastatic action of PSK has also been demonstrated throughout various mechanisms (Harhaji *et al.*, 2008). PSK has also been shown to cause differentiation of leukaemia cells *in vitro*, and this effect has been attributed to induction of differentiation cytokines (Jiménez-Medina *et al.*, 2008). Interestingly, studies

have also shown that PSK may actually inhibit carcinogenesis by inhibiting the action of various carcinogens on vulnerable cell lines. This action of PSK may play a role in preventing second malignancies due to the carcinogenic effects of radiotherapy and cytotoxic chemotherapy (Mitomi *et al.*, 1992).

MATERIALS AND METHODS

Plant materials: The mushroom was harvested in Valenii de Munte surrounding area, in September 2009.

*Preparation of tincture***:** 10g of powdered vegetal product were extracted with 100g of ethanol 70° at room temperature, as described in Romanian Pharmacopoeia Xth Edition.

To prevent toxic effect of the alcohol, often more toxic than plant compounds dissolved in it, alcoholic solution was maintained in a water bath until 3/4 of the content evaporates, than filed with sterile saline solution to 0.5 ml for each animal. The aqueous solution was administrated i.p. within few minutes in order to prevent the bacterial and fungus contamination.

The animals were caged in groups of 10 per cage, at controlled temperature of 21-22ºC, humidity (40-60%) and reversed 12/12h light/dark cycle (light off at 10 a.m.). Standard lab chow, provided by National Institute for Research and Development "Cantacuzino" Bucharest (Batch no. 2 / 26.03.2010), and water were freely available. The animal tests and experiments were allowed by the Bioethical Board of the Faculty of Veterinary Medicine Cluj-Napoca.

The experiment was carried out on 30 white Swiss male mice, 36.69 ± 3.86 g body weight; each animal received 10^6 Ehrlich Ascitic Carcinoma (EAC) cells intra peritoneal, in the day 0 of the experiment. The experiment last for 14 days, in the Department of Pathophysiology, University of Agricultural Science and Veterinary Medicine Cluj-Napoca. Ehrlich ascitic carcinoma (EAC) was a generous gift from the Oncology Institute "I. Chiricuta" Cluj Napoca. Body weight was measured at the beginning, and at the end of experiment.

Animals were divided in three equal experimental groups; first group (*C. versicolor* + EAC group) received i.p. in the day 1, 4 and 7 - 50 ml sol. /kg b.w. *C. versicolor* 1:10 plant to extract in ethylic alcohol 70°. The second group, (EAC group) received 0.5 ml alcohol 70°, i.p (after evaporation in water bath in the same way like the mushroom extract) and the last group (Doxorubicin + EAC group) received i.p. Doxorubicin chloride 2.5 mg/ kg b.w. (Adriablastina 10 mg – Phizer) in day 6 of experiment.

To assess the biological effects of substances in study, other 30 animals were subject of administration of mushroom extract, control and Doxorubicine chloride alone in the same doses, at the same time.

In the end, the blood was harvested from the retro orbitary sinus under diethyl ether anesthesia and the euthanasia was made by prolonged ether narcosis. Total amount of ascitic fluid was measured, and viable EAC cells concentration was counted in a Burker camera (liquid diluted 1:200). Cell viability was assessed by tripan blue staining (Olinescu, 1992).

Blood hematology was investigated with Abacus Junior Vet, automatic analyzer Diatron Messtechnik, Budapest, Hungary. Blood chemistry (urea and creatinin) was measured using screen point semiautomatic analyzer, STAT – FAX 1904 Plus, Global Medical Instrumentation, Inc. 6511 Bunker Lake Blvd. Ramsey Minnesota, 55303 USA, by using special determination kits in the Department of Pathophysiology, University of Agricultural Science and Veterinary Medicine Cluj-Napoca.

Statistics - the data were expressed as the mean and standard deviation. T Student multiple range test from Excel Windows Software was used to assess the differences among groups. Differences at $p<0.05$ and $p<0.01$ were considered significant and respectively distinct significant.

RESULTS AND DISCUSSION

No significant changes in red blood cells has been found among experimental groups, the total red blood cells count, hemoglobin, hematocrit and red cells indices were within normal limit (Tab. 1, 2).

Tab. 1.

The hematological profile (mean ± S.D.)

	RBC 10^{12}/l	HGB g/dl	HCT %
EAC	6.48±1.21	9.46±1.95	28.73±5.63
EAC + Doxorubicin	7.04±0.90	11.75±1.60	35.99±4.67
EAC + *C. versicolor*	5.87±0.50	9.85±0.99	28.30±2.83
C. versicolor	7.38±0.67	11.76±0.76	35.36±2.94
Doxorubicin	7.33±0.98	11.26±1.29	34.35±5.26
Control	6.67±1.40	10.26±2.22	31.74±6.05

Normal values: RBC 7-12.5 10^{12}/l HGB 10.2-18 g/dl HCT 36-49 % (Uray, 1992)

Tab. 2.

The Red Cell Indices (mean ± S.D.)

	MCV fl	MCH pg	MCHC g/dl	RDWs fl
EAC	44.20±3.35	14.56±0.83	33.00±1.61	42.06±5.26
EAC + Doxorubicin	51.00±1.82	16.72±0.48	32.70±0.36	37.30±1.92
EAC + *C. versicolor*	48.28±1.70	16.84±1.30	34.84±2.04	34.94±1.54
C. versicolor	48.00±3.39	16.00±1.22	33.32±0.87	35.66±5.63
Doxorubicin	46.80±3.08	15.31±1.32	32.66±1.13	39.06±3.60*
Control	47.78±2.11	15.34±0.59	32.13±1.025	34.03±4.03

Normal values: MCV 53.6-56 fl, MCH 48.1-50 pg, MCHC 31.3-33.2 g/dl (Uray, 1992)

Doxorubicin, as expected induce a significant leucopenia as compared to control group, and interestingly was responsible for reducing the total white blood cell count (WBC) in EAC injected groups (EAC inoculation was related to elevated WBC concentration).

Coriolus versicolor alcoholic extract provides significant increase in WBC as compared to control mainly at granulocytes (GRA) suggesting a neutrophilia (Tab. 3). Some peritoneal local reaction do to i.p. injection of plant extracts was excluded by gross evaluation and cytology of peritoneal fluid made at 1, 2, 6, and 24 hours after injection, in preliminary studies (data no shown).

Tab. 3.

The leukogram (mean ± S.D.)

	WBC 10^9/l	LYM 10^9/l	MID 10^9/l	GRA 10^9/l
EAC	62.32±45.82	9.15±6.61	2.58±3.58	50.56±44.44
EAC + Doxorubicin	15.01±9.46	8.93±3.014	0.34±0.39	5.64±6.37
EAC + *C. versicolor*	59.19±37.43	7.92±4.11	3.84±5.02	48.01±30.14
C. versicolor	7.50±2.27*	5.78±1.79	0.26±0.13*	1.47±0.47**
Doxorubicin	2.48±1.42**	1.74±0.94***	0.11±0.10	0.64±0.49
Control	4.87±1.83	4.29±1.69	0.11±0.07	0.45±0.26

*= statistically significant at p<0.05 as compared to Control group
**= statistically distinct significant at p<0.01 as compared to Control group
***= highly statistically significant at p<0.001 as compared to Control group
Normal values WBC 6-15 10^9/l (Uray, 1992)

C. versicolor induces a significant increasing of the platelets (in concentration, and average volume), but no significant changes among other groups (Tab. 4).

Tab. 4.

The platelets (mean ± S.D.)

	PLT 10^9/l	PCT %	MPV fl	PDWs fl
EAC	648.80±205.39	0.40±0.21	6.20±0.38	6.70±0.60
EAC + Doxorubicin	498.50±110.03	0.32±0.08	6.38±0.31	6.40±0.24
EAC + *C. versicolor*	897.00±360.37	0.63±0.26	7.00±0.42	7.69±0.80
C. versicolor	695.40±149.94**	0.42±0.11**	6.02±0.32	5.98±0.67
Doxorubicin	643.70±190.93	0.38±0.11*	5.91±0.17	5.88±0.42
Control	389.33±181.25	0.23±0.11	5.97±0.19	5.84±0.43

*= statistically significant at p<0.05 as compared to Control group
**= statistically distinct significant at p<0.01 as compared to Control group
***= highly statistically significant at p<0.001 as compared to Control group
Normal values PLT 160-410 10^9/l (Uray, 1992)

In EAC group only 8 from 10 animals survived in the end of the study, in the other two experimental groups all animals survived to the end; development of EAC was expectedly followed by a significant increasing in body weight. Doxorubicin revealed a strong protective effect; in treated animals, the body weight was slightly decreased. Even no so effective like Doxorubicin, *Coriolus versicolor* alcoholic extract provides a relevant protective effect (Fig. 1).

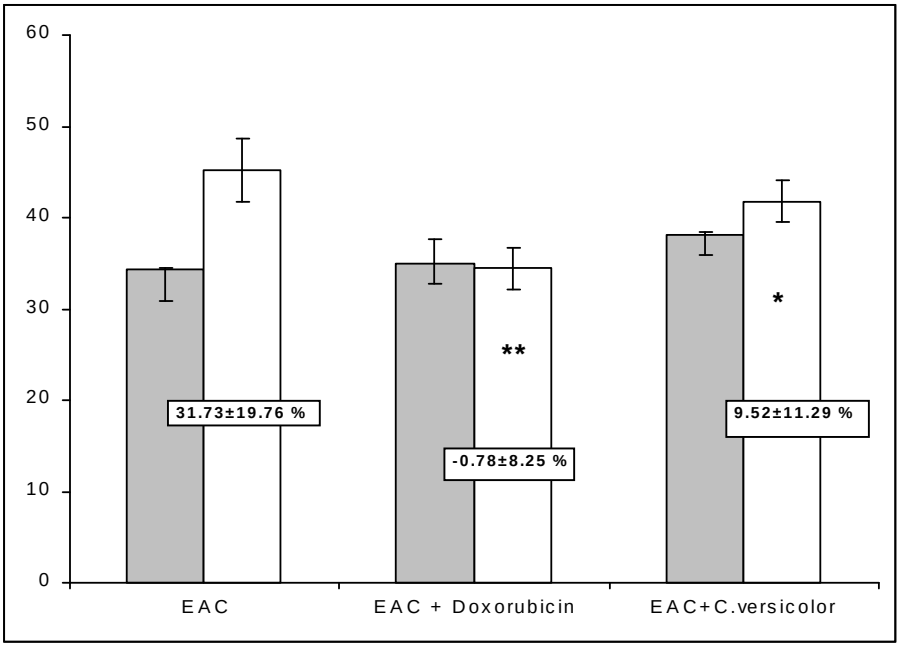

Fig. 1. The variation on body weight among experimental groups (mean ± S.D.) (g)
*= statistically significant at $p<0.05$ as compared to Control group
**= statistically distinct significant at $p<0.01$ as compared to Control group

Both ascitic volume and cellular concentration of the ascitic fluid was highly decreased in Doxorubicin treated groups, *C. versicolor* provides some protection, but not statistically significant (Fig. 2, 3). However, this cytostatic effect should not be excluded, at higher dosage. Important both substances provide cytostatic effects, and no citotoxic ones, because tripan blue staining revealed almost 100% viable cells in ascitic fluid. Plasma biochemistry, gross evaluation and histopathological exam reveal no significant lesion for *Coriolus versicolor* alcoholic extract.

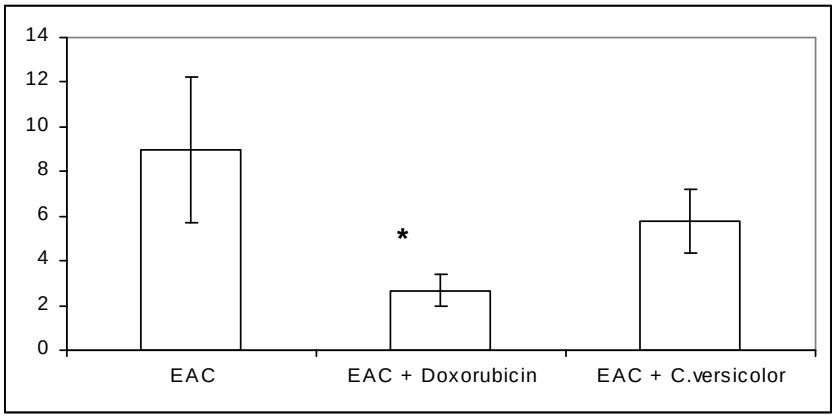

Fig. 2. The ascitic volume (mean ± S.D.) (ml)
*= statistically significant at $p<0.05$ as compared to Control group

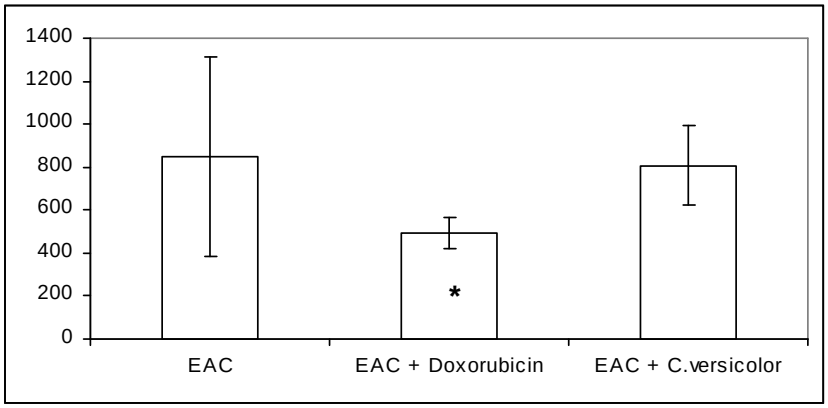

Fig. 3. The cellular concentration within ascitic fluid (mean ± S.D.) (10^6/ml)
*= statistically distinct significant at $p<0.05$ as compared to Control group

Several clinical and experimental assays have reported the anti-tumor properties of *C. versicolor* and its synergistic effect in combined therapies identified a new cytostatic anti-tumor activity, and immunostimulatory effects of *C. versicolor* (Mitomi *et al.*, 1992).

The combined administration of *C. versicolor* with IL-3 increased the haematological recovery of myelosuppressed mice. The peripheral blood leukocyte count during the recovery stage was significantly increased when these cytokines were administered with *C. versicolor* compared to when the cytokines were used individually. Moreover, the phase at which *C. versicolor* has effects on haematopoietic cells seems to be at a more immature level than with IL-3. The combined administration of *C. versicolor* and the above cytokines may improve myelosuppression after chemotherapy in patients with malignancy (Kohgo *et al.*, 1994).

The cytostatic activity varied according to the histological origin of the tumor cell lines in study, the inhibition was higher in melanoma cell lines Ando-2 (human) and B16 (mice) (Jiménez-Medina *et al.*, 2008).

In vitro treatment of the cells with the methanol extract reduced melanoma cell viability in a dose-dependent manner; the proliferation of B16 cells was arrested in the G(0)/G(1) phase of the cell cycle, followed by both apoptotic and secondary necrotic cell death. *In vivo* methanol extract treatment inhibited tumor growth in C57BL/6 mice inoculated with syngeneic B16 tumor cells. Moreover, peritoneal macrophages collected 21 days after tumour implantation from methanol extract-treated animals exerted stronger tumoristatic activity than macrophages from control melanoma-bearing mice (Harhaji *et al.*,2008).

C. versicolor alcoholic extracts exert pronounced anti-tumor activity, both directly through antiproliferative and cytostatic effects on tumor cells and indirectly through promotion of macrophage, and generally cellular antitumor activity

CONCLUSIONS

C. versicolor alcoholic extract revealed certain antitumoral activity on Ehrlich Ascitic Carcinoma model, proved not only by reduction of tumor cells proliferation, but hematological data suggest significant immunostimulatory effects. No side effect toxicity has been found. *C. versicolor* alcoholic extract are not yet ascertained, its powerful antitumoral activity, along with the safety profile, recommend alcoholic extract of *C. versicolor* for further studies, in other to find new remedies in complementary cancer therapy.

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