Synergistic Antitumor Effect of Polyphenolic Components of Water Soluble Derivative of Propolis against Ehrlich Ascites Tumour

Nada ORŠOLIĆ,∗,* Ivan KOSALEC,† and Ivan BASIĆ∗

∗Department of Animal Physiology, Faculty of Science, University of Zagreb; Rooseveltov trg 6, HR-10 000 Zagreb, Croatia; and †Institute of Microbiology, Faculty of Pharmacy and Biochemistry, University of Zagreb; Schrottova 39, HR-10 000 Zagreb, Croatia. Received October 14, 2004; accepted January 21, 2005

Effect of two preparation (Croatian and Brazilian) of water-soluble derivative of propolis (WSDP), caffeic acid, quercetin, chrysin, naringenin (components present in WSDP) on the development of Ehrlich ascites tumour (EAT) was evaluated. Test components (50 mg/kg) were given perorally or intraperitoneally 2 h prior the intraperitoneal injection of EAT (2×10⁶ cells). It was observed that all test compounds effectively inhibited tumour growth and the proliferation of EAT. The volume of ascitic fluid induced by EAT cells and total number of cells present in the peritoneal cavity was markedly reduced in EAT-bearing mice treated with test components. In treated mice the number of polymorphonuclear (PMN) cells in the peritoneal cavity was increased while the number of macrophages was decreased. The macrophage spreading activity revealed that WSDP and all test compounds affected the functional state of macrophages increasing their tumorcidal activity; the effect of WSDP was most pronounced indicating synergistic effect of components present in WSDP. Antitumour activity of WSDP may be the result of different specific mechanism(s) of flavonoids present as compared to individual flavonoid given alone. It is likely that the part of antitumor efficacy of test components against EAT cells was the results of increased activity of macrophages.

Key words propolis; polyphenolic compound; Ehrlich ascites tumour (EAT) tumor

Malignant diseases are responsible for the death of about one fifth of the population. The target of much research has been on the discovery of natural and synthetic compounds that can be used in the prevention and/or treatment of cancer. Many plants and animal extracts have shown various biological activities like immunopotentiating and antitumour activities. Honey bee propolis and its components (caffeic acid, caffeic acid phenethyl ester, artepilin C, quercetin, naringenin, resveratrol, galangin, genistein and other) are of the most promising as antitumour agent.1−8

Propolis (bee glue) is the generic name for the resinous substance collected by honey bees from various plant sources and used by bees to seal holes in their honeycombs, smooth out the internal walls, and protect the entrance against intruders.11 It is rich in biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, vitamins, amino acids, etc.23 Healing properties of propolis are known in folk medicine from antiquity. Recently, the interest for propolis as a harmless medicine is increasing. There have been many attempts to validate biological effects of propolis and elucidate its composition.3−8 It was shown that propolis and its constituents have strong antimicrobial effect, acting on viruses,9−11 bacteria,12−14 and fungi.15,16 It was also demonstrated that propolis and some of its active substances have a pronounced cytostatic, anticarcinogenic and antitumour effect both in “in vitro” and “in vivo” tumor models.6−8,17−22 Immunomodulatory effects of propolis has also been recorded,23−26 It has been suggested that the therapeutic activities of propolis depend mainly on the presence of flavonoids.28 Flavonoids have also been reported to induce the immune system,29,30 and to act as strong oxygen radical scavengers. Dietary intake of antioxidant has been associated with a diminished risk of cancer at various anatomical sites.31 This led us to compare how the routes (oral and/or systemic) of administration of different polyphenolic compounds deriving from propolis and of propolis itself influence on Ehrlich ascites tumour growth. Thus, we analyzed not only tumor growth but also parameters of immunomodulatory response of animals inoculated with test compounds, determining the effect of them on polymorphonuclear (PMN) and mononuclear (MN) cells, mainly macrophages.

MATERIALS AND METHODS

Water-Soluble Derivative of Propolis (WSDP) Treatment A water-soluble derivative of propolis (WSDP) was prepared by the method described elsewhere.32 Briefly, Croatian propolis from beehives kept at the outskirts of Zagreb, Croatia or Brazilian propolis (CONAP, Belo Horizonte, Minas Gerais, Brazil) was extracted with 96% ethanol, which was filtered and evaporated to dryness in vacuum evaporator. The resultant resinous product was added to a stirred solution of 8% L-lysine (Sigma Chemie, Deisenhofen, Germany) and freeze-dried to yield WSDP, a yellow-brown powder. WSDP was stored under sterile conditions at −20 °C. Before use WSDP was dissolved in distilled water. Nikolov el al.32 demonstrated that WSDP contains: caffeic acid 6.7%, γ,γ'-dimethylallyl ferulate 1.2%, isopentyl-2-enyl-caffeate 7.4%, pentenyl caffeate 2.2%, γ,γ'-dimethylallyl caffeate 8.5%, pinobanksin 2.3%, pinocembrin 9.2%, pinobanksin-3-acetate 13.6%, benzyl caffeate 0.4%, galangin 7.8%, β-phenyl ethyl caffeate 1.2%, total flavonoids 32.9%, total esters of phenyl acid 20.9%.

WSDP was refrigerated under sterile conditions. Before use WSDP was dissolved in distilled water and mice were given WSDP per os (p.o.) via gastric tube or intraperitoneally (i.p.). The WSDP was given daily for 7 d, and the daily dose contained 50 mg kg−1 body weight.

Polyphenolic Compounds For experiments using polyphenolic compounds we used Caffeic acid (CA)−3,4-di-
hydroxycinnamic acid (Aldrich-chemie, Milwaukee, WI, U.S.A.), Quercetin dihydrohydrate (QU) (Fluka, BioChemica, Switzerland), Chrysin and Naringenin (Sigma, Germany).

All polyphenolic compounds were dissolved in ethanol and further dilutions were made in water. The final concentration of ethanol was less than or equal to 0.1%. Ethanol (0.1%) was used in the control group. No difference between water as control and 0.1% of ethanol in water was observed in preliminary experiments.

**Mice** Animal studies were carried out according to the guidelines in force in R. Croatia (Law on the Welfare of Animals, N. N. # 19, 1999) and in compliance to the Guide for the Care and Use of Laboratory Animals, DHHS Publ. # (NIH) 86—123. Male albino mice of the Swiss strains, weighing from 20—25 g from our conventional mouse colony were used. In any experiment, mice were of the same sex and were approximately 2 months old at the initiation of each study. The animals were kept not more then five to a cage and were maintained on a pellet diet and water ad libitum. Experimental groups comprised of 7 or 15 mice each.

**Tumor** Cells of Ehrlich ascites tumor were used and maintained in male Swiss albino mice through serial intraperitoneal inoculation at 7- or 9-d intervals in an ascitis form. After harvesting and preparation of cells, their total number and viability were determined by counting in Bürker–Türk chamber using trypan blue dye. The desired concentration of tumor cells (2×10⁶ cells per 0.5 ml) was obtained by dilution with saline (0.9% sodium chloride solution). Viability of tumor cells was always higher than 90%. Below this percentage, the cells were discarded and the entire procedure was repeated.

**Count of the Total Number of Cells Present in the Peritoneal Cavity** The total number of cells present in the peritoneal cavity was determined by counting in Bürker–Türk chamber.

**Differential Count of the Cells Present in the Peritoneal Cavity** The cells in the peritoneal cavity of mice were harvested and stained with May Grünwald and water solution of Giemsa (1 part Giemsa: 2 part water) and later differentiated into MN, PMN and tumor cells. To differentiate MN from PMN, Giemsa (1 part Giemsa: 2 part water) and later differentiated cells were fixed with 2.5% glutaraldehyde. Then, the cells were stained with 5% solution of Giemsa and examined under microscope where the percentage of spread cells were determined under 40× magnification. Spread cells were those that presented cytoplasmic elongation, while the non-spread cells were rounded, see Fig. 4. Wet adherent cells were photographed at ×400 magnification with a phase-contrast microscope.

**Experimental Procedure** Seven groups of 15 mice were formed for p.o. treatment and seven groups of 7 mice for i.p. treatment, as follows in Table 1.

Seven mice of each group were sacrificed in ether chamber on the 14th day after tumor cell inoculation. After disinfec-
tion of external abdominal region, each animal was inoculated with 3 ml of saline solution and after gentle agitation of abdominal well, the solution containing peritoneal cells was removed for cellular evaluation. The following variables were analyzed: the total number of cells, differential count of the cells present in the peritoneal cavity, and determination of functional activity of macrophages. The remaining animals, i.e., 8 animals of each group for the survival analysis were used.

**Survival Analysis** For the survival analysis CBA mice were given test components p.o. at doses of 50 mg kg⁻¹ for 7 d starting 2 h prior tumor inoculation. Endpoint of experiments was determined by spontaneous death of animals. Results are expressed as percent of mean survival time of treated animals over mean survival time of the control group (treated vs. control, T/C %). The percentage of increased lifespan (ILS%) was calculated according the formula: ILS%=(T–C)/C×100 where T represents mean survival time of treated animals; C represents mean survival time of the control group. By NCI criteria, T/C exceeding 125% and ILS exceeding 25% indicate that the drug has significant antitumour activity.

**Statistics** Results are expressed as means±S.D. obtained from 2 experiments. Statistical significance was evaluated using the Student’s t test.

**RESULTS**

**Determination of Total Number of Cells in the Peritoneal Cavity** The effect of Croatian and Brazilian WSDP and its polyphenolic compounds on volume of ascitic fluid induced by EAT cells, total number of cells and cell viability in mice-bearing Ehrlich ascites tumour were examined by counting total cells in peritoneal cavity in Bürker–Türk chamber using trypan blue. The volumes of ascites fluid and total number of EAT cells were significantly reduced in

Table 1. Experimental Design

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tumor control (EAT)⁰</th>
<th>Tumor control (EAT)⁰</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test components⁰ were given to mice orally.</td>
<td>EAT + 50 mg kg⁻¹ of Brazilian WSDP</td>
<td>EAT + 50 mg kg⁻¹ of Brazilian WSDP</td>
</tr>
<tr>
<td>EAT + 50 mg kg⁻¹ of Croatian WSDP</td>
<td>EAT + 50 mg kg⁻¹ of Croatian WSDP</td>
<td></td>
</tr>
<tr>
<td>EAT + 50 mg kg⁻¹ of caffeic acid</td>
<td>EAT + 50 mg kg⁻¹ of caffeic acid</td>
<td></td>
</tr>
<tr>
<td>EAT + 50 mg kg⁻¹ of chrysin</td>
<td>EAT + 50 mg kg⁻¹ of chrysin</td>
<td></td>
</tr>
<tr>
<td>EAT + 50 mg kg⁻¹ of naringenin</td>
<td>EAT + 50 mg kg⁻¹ of naringenin</td>
<td></td>
</tr>
<tr>
<td>EAT + 50 mg kg⁻¹ of quercetin</td>
<td>EAT + 50 mg kg⁻¹ of quercetin</td>
<td></td>
</tr>
</tbody>
</table>

a) 2×10⁶ tumor cells/mouse injected i.p.; groups comprised of 7 or 15 mice each.

b) The test components were given daily for 7 d starting 2 h prior tumor inoculation, and the daily dose contained 50 mg kg⁻¹ body weight.
mice-bearing EAT protected with WSDP or its polyphenolic compounds (Table 2, Fig. 1). Among six individual test components, Croatian WSDP was the most potent, inhibiting proliferation of EAT cell for 79%. Brazilian WSDP and naringenin were intermediate in their inhibitory ability. Systemic treatment with all components (without Croatian WSDP and naringenin) was more effective than perorally treatment in reduction of total number of cells for 1—25%.

The effect of test components on the survival rate of mice-bearing EAT cells is shown in Table 3. The results demonstrate that ILS for WSDP was 27% or 32.4% in p.o. treated group. Polyphenolic compounds inhibited tumor growth and increased life span (ILS) of mice for 7 to 27%. Survival rate of mice-bearing EAT after i.p. treatment with test components were not done.

**Determination of the Percentage of the Mononuclear, Polymorfonuclear Cells and Tumour Cells in Peritoneal Cavity** Figure 2 and Table 4 show the effects of two preparations of WSDP or its polyphenolic compounds on the percentage of MN, PMN and tumour cells. All tumour-bearing groups treated with test components revealed significantly higher percentage of PMN and less percentage of MN cells comparing to control. This effect was more pronounced in mice treated with test components intraperitoneally.

**Determination of the Percentage of Macrophage Harvested in the Peritoneal Cavity** The results obtained for the variable percentage of spreading of macrophages harvested in peritoneal cavity are shown in Figs. 3 and 4. The treatment with test components yielded an increase in the functional activity of macrophages in mice bearing-EAT compared with control group. It should also be pointed out that for the tumour-bearing group treated with test components, there was an increase in the median values of spreading percentage accompanying with an increase in the test components cytotoxicity to EAT cells and in their effect on induction of apoptosis in tumour cells (Fig. 2). Similar data were given in mice treated i.p. with preparations of WSDP and its polyphenolic compounds.

**DISCUSSION**

The analysis of the total number of cells present in the peritoneal cavity revealed that all the experimental groups inoculated with tumour cells in the presence of test components, exhibited a significant reduction of tumour size as well as the total number of cells in peritoneal cavity (Table 2, Fig. 1). Moreover, the survival rates of EAT-bearing mice were increased after treatment with test components (Table 3). These data suggest that test components might interfere with the growth of Ehrlich ascites tumour cells directly during early phase of treatment leading to a considerable elimination of these cells. Treatment with test components yielded an increase in the percentage of PMN cells when compared with

### Table 2. Effect of WSDP and Its Polyphenolic Compounds on Viability and Tumor Volume in Mice-Bearing Ehrlich Ascites Tumor after Perorally or Intraperitoneally Treatment

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Test components were given p.o.</th>
<th>Test components were given i.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Volume of ascitic fluid (ml)</td>
<td>Total number of cells (N × 10⁶)</td>
</tr>
<tr>
<td>Control</td>
<td>7.1</td>
<td>882.49 ± 63.70</td>
</tr>
<tr>
<td>Cr. WSDP</td>
<td>2.65</td>
<td>186.49 ± 55.9*</td>
</tr>
<tr>
<td>Br. WSDP</td>
<td>4.75</td>
<td>285.82 ± 87.13*</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>5.5</td>
<td>436.62 ± 86.5*</td>
</tr>
<tr>
<td>Naringenin</td>
<td>4.05</td>
<td>233.62 ± 56.9*</td>
</tr>
<tr>
<td>Chrysin</td>
<td>7.15</td>
<td>773.00 ± 48.75</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5.8</td>
<td>472.47 ± 86.0*</td>
</tr>
</tbody>
</table>

*a* The test components were given p.o. or i.p. daily for 7 d starting 2 h prior tumor inoculation, and the daily dose contained 50 mg kg⁻¹ body weight. *b* Mice (n=7) of each groups were sacrificed on the 14th day after i.p. tumor cell inoculation (2×10⁶/tumor cells/mouse). The value was significantly different (*p*<0.001) from the corresponding value of untreated animals analyzed by Student’s t test.

### Table 3. Antitumor Activity WSDP and Its Polyphenolic Components on Ehrlich Ascites Tumor

<table>
<thead>
<tr>
<th>Group</th>
<th>Range of survival time (d)</th>
<th>Mean of survival time (d)</th>
<th>ILS%</th>
<th>T/C%</th>
<th>Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11—14</td>
<td>12.5</td>
<td>32.36</td>
<td>132.36</td>
<td>+</td>
</tr>
<tr>
<td>Croatian WSDP</td>
<td>12—26</td>
<td>15.8</td>
<td>32.36</td>
<td>132.36</td>
<td>+</td>
</tr>
<tr>
<td>Brazilian WSDP</td>
<td>12—26</td>
<td>14.36</td>
<td>26.4</td>
<td>126.4</td>
<td>+</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>12—17</td>
<td>14.75</td>
<td>14.75</td>
<td>114.75</td>
<td>—</td>
</tr>
<tr>
<td>Naringenin</td>
<td>13—19</td>
<td>15.83</td>
<td>26.66</td>
<td>126.66</td>
<td>+</td>
</tr>
<tr>
<td>Chrysin</td>
<td>12—17</td>
<td>16.54</td>
<td>14.88</td>
<td>114.88</td>
<td>—</td>
</tr>
<tr>
<td>Quercetin</td>
<td>11—17</td>
<td>13.3</td>
<td>6.66</td>
<td>106.66</td>
<td>—</td>
</tr>
</tbody>
</table>

*a* Tumor cells (2×10⁶) were injected to mice i.p.; group comprises 8 mice each. *b* The test components were given p.o. daily for 7 d starting 2 h prior tumor inoculation, and the daily dose contained 50 mg kg⁻¹ body weight. *c* ILS% (increased life span %)=(T−C)/C×100; T: mean survival days of treated group; C: mean survival days of control group. *d* T/C, treated vs control. *e* +: ILS% ≥25; -: ILS% <25.
April 2005 697

Table 4. Percentage of the Cells Present in the Peritoneal Cavity of the Animals on the 14th Day of Ehrlich Ascites Tumor Growth after Perorally or Intraperitoneally Treatment with WSDP and Its Polyphenolic Compounds

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Test components were given p.o.</th>
<th>Test components were given i.p.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lymphocytes X ± S.D.</td>
<td>Neutrophils X ± S.D.</td>
</tr>
<tr>
<td>Control</td>
<td>0.75±0.88</td>
<td>9.00±6.69</td>
</tr>
<tr>
<td>Cr. WSDP</td>
<td>1.50±2.13</td>
<td>31.00±21.13</td>
</tr>
<tr>
<td>Br. WSDP</td>
<td>1.50±1.19</td>
<td>19.25±6.62**</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>1.40±1.78</td>
<td>23.00±16.30</td>
</tr>
<tr>
<td>Chrysine</td>
<td>0.50±1.21</td>
<td>18.50±17.56</td>
</tr>
<tr>
<td>Naringenin</td>
<td>1.37±2.08</td>
<td>30.37±15.87**</td>
</tr>
<tr>
<td>Quercetin</td>
<td>2.00±2.72</td>
<td>32.62±15.04**</td>
</tr>
</tbody>
</table>

a) The test components were given p.o. or i.p. daily for 7 d starting 2 h prior tumor inoculation, and the daily dose contained 50 mg kg⁻¹ body weight. b) Mice (n=7) of each groups were sacrificed on the 14th day after i.p. tumor cell inoculation (2×10⁶/tumor cells/mouse). The value was significantly different (*p<0.05; **p<0.01; ***p<0.001) from the corresponding value of untreated animals analyzed by Student’s t test.

Fig. 2. The Cells Obtained from Ascites Fluid in the Peritoneal Cavity of the Animals on the 14th Day of Ehrlich Ascites Tumor Growth after p.o. or i.p. Treatment with WSDP and Its Polyphenolic Compounds

The test components were given p.o. or i.p. daily for 7 d starting 2 h prior tumor inoculation, and the daily dose contained 50 mg kg⁻¹ body weight. Cells were stained with May Grünwald and water solution of Giemsa (1 part Giemsa: 2 part water) and later differentiated into MN, PMN and tumor cells. Treatment with WSDP and its polyphenolic compounds increased the functional activity of macrophages to tumor cells and induced the apoptotic process in them (a, b, e).
control group. According to Freire, PMN cells can lead to the elimination of cells that are strange to the host by oxidative and non-oxidative mechanisms. However, in spite of noticing a quantitative increase of PMN cells in test component treated groups, a marked decrease in the percentage of tumour cells were not seen. This suggests that PMN cells alone are not capable of inhibiting tumour growth. The number of MN cells was decreased to the presence of test components. Most of the MN cells in the peritoneal cavity were macrophages that are considered the major cells involved in tumour rejection. However, in spite of the non-occurrence of an increase in the number of MN cells, a significant increase was observed in the activity of peritoneal macrophages in test component-treated groups. It should also be pointed out that for the tumor-bearing group treated with test components, there was an increase in the median values of spreading percentage accompanying the increase in the test components cytotoxicity on EAT cells and in their effect.

Fig. 3. The Percentage of Macrophage Spreading in the Peritoneal Cavity of the Animals on the 14th Day of Ehrlich Ascites Tumor Growth after Perorally Treatment with WSDP and Its Polyphenolic Compounds

The test components were given p.o. daily for 7 d starting 2 h prior tumor inoculation, and the daily dose contained 50 mg kg$^{-1}$ body weight. Mice (n=7) of each groups were sacrificed on the 14th day after i.p. tumor cell inoculation (2×10$^6$/tumor cells/mouse). The value was significantly different ($^*$p<0.05; $^{**}$p<0.01; $^{***}$p<0.001) from the corresponding value of untreated animals analyzed by Student’s t test.

Fig. 4. Macrophage Spreading Obtained from Ascites Fluid in the Peritoneal Cavity of the Animals on the 14th Day of Ehrlich Ascites Tumor Growth from Control Group (a, b) or after p.o. or i.p. treatment with WSDP and Its Polyphenolic Compounds (c—f)

The test components were given p.o. or i.p. daily for 7 d starting 2 h prior tumor inoculation, and the daily dose contained 50 mg kg$^{-1}$ body weight. Macrophages were stained with a 5% solution of Giemsa.
on induction of apoptosis in tumor cells (Fig. 2). The increase of macrophage activity, possibly due to the test components, might have been responsible for the slower growth of tumour cells. It is well known that MN cells, mainly macrophages, are the major cells involved in tumour rejection. The variable macrophage spreading revealed that the treatment with test components affects the functional state of macrophages. Figure 4 shows macrophage spreading with an increase in size and content large cytoplasmic vacuoles. The best results were achieved with preparations of WSDP (Croatian and Brazilian). It is likely that the antitumor activity of WSDP is the result of synergistic activities of its polyphenolic compounds. Thus, the antitumor effect observed might be due to an increase of peritoneal macrophage activity and not to an increase in macrophage number. Reason of the decrease of the percentage of macrophages in test component-treated groups might be due to an increase of phagocytic activity of macrophages. The other possible mechanisms of antitumour influence of test components, as we described previously, include immunomodulatory activity of these products their cytotoxic activity to tumour cells, their capability of inducing changes in the cellular level of glutathione, and their capability to induce apoptosis and/or necrosis. Test components may have direct and/or indirect action on tumour cells by stimulating the host cells, mainly macrophages. Such stimulation might induce production and release of several cytokines such as IL-1, IL-6, IL-8, TNF-α and NO. Some of these cytokines have direct cytotoxic effect on tumour cells while others act on other cells and activate them: natural killer cells-NK and cytotoxic T lymphocytes. In addition, these cytokines might stimulate production of C-reactive protein and complement factor C3 that would act as opsonins on tumor cells. The combination of these effects might impede tumour growth and lead to elimination of tumour cells. It is likely that for antitumor effect close contact of test components (especially flavonoids) and tumor cells are more important for cytotoxic effect to tumor cells and induction of apoptosis. Since the antitumor effect of WSDP in concentration of 50 mg/kg (mixture of different flavonoids) inhibited proliferation of Ehrlich ascites tumor cells better than individual compounds given alone in the same concentration we presumed that combination of flavonoids in WSDP could have synergistic effect. It was shown that the combination of different flavonoids added to culture of human breast cell line (MDA-MB-453 cells) were more effective in inhibition of proliferation of tumor cells than individual flavonoid alone. In addition, when the citrus flavonoids were combined individually with quercetin, they suppressed the proliferation of the cells at much lower concentration than either compound given alone. Furthermore, if the combination of flavonoids was given to rats induced with mammary tumors by 7,12-dimethylbenz(a)anthracene (DMBA) it was shown that combination of flavonoids was the most effective at delaying the onset of tumors as well as in reducing tumor burden comparing to the individual treatments. Our observation is in agreement with these findings, implying that more different mechanisms are included in inhibition of proliferation of EAT cells with combination of flavonoid (WSDP) treatments as compared to mechanism(s) involved with action of an individual compound. These findings and the results of the influence of WSDP on formation of metastasis published recently suggest that flavonoids present in WSDP exert synergistic effect to the proliferation of tumor cells.

In conclusion, we have shown that WSDP and/or its polyphenolic compounds are effective at reducing tumour size as the total number of cells in peritoneal cavity in mice-bearing EAT. The increase in the animals’ survival time and the important macrophage stimulation after treatment with test components are significant results which certainly deserve further studies. It is also important to emphasize that p.o. route of administration influences the tumor growth, giving priority to oral use of tested compounds in further investigation either in experimental or clinical trials.

REFERENCES

1148 (1997).