RADIOPROTECTIVE EFFECT OF A WATER-SOLUBLE DERIVATIVE OF PROPOLIS IN MICE

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Summary: *Radioprotection by a water-soluble derivative of propolis (WSDP) was investigated in the whole - body irradiated (WBI) CBA mice by γ source. Protective effect was evaluated by colony forming efficacy, survival of mice and the levels of intracellular glutathione. Given perorally (po) 50 mg/kg to mice for 20 consecutive days WSDP prolonged the survival time of WBI mice with 5 - 9 Gy. The number of either endogenous or exogenous colony forming units in the spleen (CFUs) was greater in WSDP - treated mice as compared to control. Using the exogenous CFUs assay, it was found that CFUs from the spleen of mice treated with WSDP were more resistant to irradiation than the CFUs from the spleen of normal mice; the difference between surviving fraction of spleen CFUs from WSDP - treated and untreated mice was significant (<0.05 and <0.01). The radioprotective effect of propolis seems to be at least partially mediated by its effect on production of glutathione in hematopoietic tissue.*

Keywords: *Colony forming units (CFUs), Hematopoiesis, Mice, Propolis, Radioprotection*

Introduction

Propolis is a resinous material collected by bees from various plants[1]. It is used in the beehive as a disinfectant and as a glue. Chemical analysis of propolis have revealed that it contains a variety of flavonoids, phenols, alcohols, terpenes, sterols, vitamins, amino acids, etc[2]. Healing properties of propolis have been known in folk medicine from antiquity. Recently, the interest in propolis as a harmless medicine has increased. 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 antitumor effect both "in vitro" and "in vivo"[17-22]. Antioxidative, immunostimulative and regenerative properties of propolis have also been recorded[23-27]. Since the antioxidative activity of propolis could account for the improved protection to radiotherapy, radiologic protecting effect of WSDP was investigated in this research.

Materials and Methods

Mice

We used CBA mice bred at our conventional animal facility. They were three months old, approximately 20 g body weight at the initiation of the experiment, and were maintained at 20 °C and at 12L: 12 D photoperiod with free access to food and water.

All studies were carried out according to the guidelines in force in the Republic of Croatia (Law on the Welfare of the Animals; Narodne Novine No. 19, 1999) and in compliance to the Guide for the

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Propolis

A water-soluble derivative of propolis (WSDP) was prepared from the crude resin obtained from the bee-hives kept in the vicinity of Zagreb, Croatia. The method of preparation was described previously[28]. Briefly, crude propolis was dissolved in 96% ethanol with constant stirring overnight at room temperature. Ethanol was evaporated in vacuum and semisolid ethanol extract was slowly poured into the stirring solution of 8% solution of L - lysine at room temperature. After two hours, the solution was filtered and vacuum - evaporated to dryness. The resulting WSDP was bright yellow, crystalline powder. The WSDP was stored at room temperature until use.

Mice were given WSDP *per os (po)* via gastral tube. The WSDP was given daily for 20 and 40 days respectively, and the daily dose contained 50 mg/kg body weight.

Spleen hematopoietic colonies

The formation of exogenous hematopoietic colonies in the spleen (CFUs) was induced by the method described elsewhere[29-30]. Briefly, 21 or 41 days after the initiation of the experiment, mice were intravenously *(iv)* injected with 50 IU of heparin (Sigma Chemical Company, Deisenhofen). Within 10-15 minutes, groups of mice were anesthetized, exsanguinated from axillary blood vessels and the number of white blood cells (WBC) was determined using a hemacytometer. Spleens were removed, passed through nylon mesh and syringed in and out through #20 gauge needle. The resultant cells suspension was washed twice by centrifugation in Hanks balanced salt solution. Bone marrow from each mouse was washed out from the shaft of femur and resuspended by syringing through #20 gauge needle. The cell viability of both bone marrow and spleen cell suspensions were determined by Trypan Blue exclusion assay and were found to be above 90%. The recipient mice were whole - body irradiated (WBI) with 9 Gy using a ^{60}Co γ ray source. After irradiation each recipient group was *iv* injected with either bone marrow, or spleen or whole blood from either normal or WSDP treated mice at concentrations $5x10^4$, $5x10^5$, and $5x10^6$ cells, respectively. Endogenous spleen colony assay was performed as follows: 24 hours after the last treatment with WSDP, mice were submitted to WBI irradiation with single doses of γ-rays ranging from 4-9 Gy using a ^{60}Co γ ray source. Mice in these experiments drank acidified (pH 2.0) water containing 1g/l of antibiotic Geomycin™ ("Pliva" Pharmaceutical Company, Zagreb). Endogenous or exogenous hematopoietic activity in mice was determined 9 days following the irradiation; mice were killed their spleens removed and placed in Bouin solution for 24 hours. The hematopoietic colonies on the surface of the spleen were counted under a dissecting microscope. For histological examinations, spleens were fixed as described above, mounted using standard histological procedures and stained with hematoxylin - eosin. The 5 mm slides were examined under the light microscope, and the presence of different types of hematopoietic colonies was determined using criteria described elsewhere[29].

Blood cell counts

From the initiation of the experiment every seven days apart, WSDP - treated mice were bled from a tail vein and the number of erythrocytes, leukocytes, and thrombocytes was determined in a hemacytometer by standard laboratory procedure. Blood smears were prepared and stained by May Grünwald - Giemsa method.

Glutathione assay

Total glutathione (GSH) content was determined by the method described by Tietze[31]. Briefly, bone marrow cell suspension, was incubated for 1 hour at 25 °C in reaction mixture containing 0.1 M sodium phosphate, 0.005 M EDTA buffer, pH 7.5. After centrifugation at 13,000 rpm for 45 min supernatants

Table 1. Survival of normal and WSDP - treated CBA mice subjected to whole - body irradiation

WBI (Gy)	Survival of mice (days)				
	Normal mice		WSDP[a]		P (Mann - Whitney U test)
	Mean ± SD	Range	Mean ± SD	Range	
5	10.21 > 100[b]		>100[b]		<0.01
6	12.2 ± 2.0	8-14	14.7 ± 1.7	12 - 17	<0.05
7	9.4 ± 1.1	8-13	11.8 ± 1.1	11 - 13	<0.05
8	8.2 ± 1.2	5-11	10.2 ± 0.9	9 - 12	<0.05
9	8.3 ± 0.9	5-10	8.1 ± 0.8	7 - 10	NS[c]

[a] *50 mg / kg WSDP was given po 20 days before WBI.*
[b] *Individual values.*
[c] *Not significant.*
Groups were comprised of 7 - 21 mice each.

were assayed for GSH activity at 412 nm. Total GSH is expressed as micromoles of the tripeptide per mg of cell protein (μ M GSH/mg protein).

Results

To test whether WSDP alters sensitivity of mice to the lethal effects of WBI, it was given *po* and on either 21st or 41st day after initiation of the experiment, groups of mice were irradiated with γ rays ranging from 5 to 9 Gy and checked for either survival or endogenous CFUs formation.

Results in Table 1 show the mean survival times of normal and WSDP-treated mice. Marked difference in the survival time between groups was observed; the WSDP-treated mice submitted to irradiation were less sensitive to the deleterious effects of the WBI.

Similar effects of WBI on survival were observed in mice treated with WSDP for 40 days. These results suggest that the WSDP treatment induced resistance to WBI might be the consequence of the influence of WSDP on hematopoiesis.

In mice treated with WSDP for 20 days and then exposed to 6 Gy WBI, the reduction of spleen weight and cellularity of spleen and bone marrow was less pronounced than in control (Table 2). This was determined 1-9 days following WBI. Thus, many more cells were destroyed by WBI in normal mice than in WSDP treated mice.

Spleens of the WSDP-treated and WBI mice contained more than twice as many endogenous hematopoietic colonies as the spleens of normal mice (Table 3).

The possibilities that may account for the elevated number of spleen colonies in WSDP - treated mice subjected to the WBI include: a) the presence of more CFUs at the time of irradiation, b) increased

Table 2. Effect of whole - body irradiation on the spleen weight and spleen and bone marrow cellularity in normal and WSDP - treated CBA mice

Days after 6 Gy WBI[b]	Spleen				Bone marrow	
	Weight (mg)[a]		Cellularity (x10⁶)[a]		Cellularity (x10⁶)[a]	
	Normal mice	WSDP - treated mice[c]	Normal mice	WSDP - treated mice[c]	Normal mice	WSDP - treated mice[c]
1	35.1 ± 5.4	89.2 ± 3.4	19.8 ± 3.2	91.0 ± 5.4	2.8 ± 0.9	4.4 ± 1.7
4	28.4 ± 3.1	46.1 ± 3.1	0.9 ± 0.7	37.2 ± 2.7	1.4 ± 0.3	3.1 ± 1.6
9	23.2 ± 1.9	63.3 ± 3.6	8.7 ± 1.9	51.3 ± 0.4	1.5 ± 0.4	5.2 ± 1.9

[a] *Mean ± SD*
[b] *Whole - body irradiation was applied 24 hours following last WSDP treatment.*
[c] *50 mg / kg WSDP was given po for 20 consecutive days.*
Groups contained 6 - 7 mice each.

Table 3. Endogenous CFUs in normal and WSDP - treated CBA mice exposed to different doses of whole - body irradiation

Doses of WBI (Gy)[b]	Number of spleen colonies[a]		
	Normal mice	WSDP - treated mice	P (Mann - Whitney U testi)
6	4.5 ± 1.7	11.3 ± 2.0	<0.05
7	2.4 ± 0.9	6.7 ± 1.9	<0.05
8	1.2 ± 0.9	4.6 ± 1.5	<0.01
9	0	3.2 ± 1.1	

[a] *Mean ± SD.*
[b] *Whole - body irradiation was given 24 hours after administration of WSDP during 20 days period. Groups were comprised of 10 mice each.*

trapping of CFUs in the spleen of treated mice, and c) increased radioresistance of CFUs. To test whether WSDP causes an increase in the number of CFUs in hematopoietic tissue of treated animals, mice receiving WSDP throughout either 20 or 40 consecutive days were killed and their whole blood, spleen, or bone marrow cell suspensions injected iv into 9 Gy WBI syngeneic recipients. Table 4 shows that the number of CFUs originating from different sources of hematopoietic tissue of WSDP- treated mice increased more than twofold as compared to the control. However when the amount of CFUs of the spleen was calculated on the basis of cellularity and CFUs setting efficiency (*f* value) the number of CFUs in the WSDP increased by 7 and 10 fold, respectively (Table 4). Since the WSDP treated mice possess more CFUs than normal mice, it is likely that more of them will be spared from irradiation.

Treatment with WSDP did not affect erythrocytes count during experimental period (not shown). To determine whether increased trapping and/or more fertile microenvironment participated in higher production of CFUs in WSDP-treated WBI mice, $2.5x10^5$ bone marrow cells from normal mice were injected into 9 Gy WBI normal or 20 days WSDP - treated WBI mice. Injected cells produced an equal number of CFUs in the spleen of both normal and WSDP - treated recipients (Table 5) disputing thus against this possibility.

To study whether WSDP exhibits changes in radioresistance of CFUs in treated mice, animals receiving WSDP for 20 days were exposed to WBI with .3 Gy or 6 Gy of γ rays. Within 2 hours following WBI mice were killed and the cell suspensions of their spleens injected into normal 9 Gy WBI syngeneic recipients. Spleen cells from normal mice WBI with either 3 or 6 Gy were also assayed for the presence of CFUs. Table 6 shows that CFUs deriving from spleen of WSDP mice were more resistant to irradiation than CFUs of normal mice.

The occurrence that the intracellular content of glutathione (GSH) in nontumorigenic V79 cell line[33] compared to HeLa cell line was elevated when

Table 4. Number of CFUs in the whole blood cells ($5x10^6$) bone marrow cells ($5x10^4$) or spleen cells ($5x10^5$) of normal or mice treated with WSDP

Days	20			40		
Tissue	Whole blood[a]	Bone marrow	Spleen	Whole blood	Bone marrow	Spleen
Normal	14.7 ± 39.0	8.6 ± 2.8	4.1 ± 1.9 (0.8 ± 0.04)[b]	12.7 ± 1.2	9.4 ± 2.7	2.0 ± 1.5 (0.5 ± 0.03)
WSDP treated	36.0 ± 3.2	18.1 ± 3.4	11.6 ± 4.4 (5.8 ± 0.6)	33.5 ± 3.5	23.6 ± 3.0	12.2 ± 1.1 (5.2 ± 0.4)

a Mean ± SD
b Calculated total number of CFUs in the spleen on the basis of cellularity and CFUs setting efficiency in the spleen of whole - body irradiated mice (f value) of 17 % [32].
Groups were comprised of 7-8 mice each.

Table 5. Number of CFUs in 9 Gy -irradiated normal or WSDP - treated CBA mice produced by bone marrow cells from normal donors.

Mice	No of spleen colonies with injection of	
	$2.5x10^4$ cells	10^5 cells
Normal	5.7 ± 1.3 [a]	17.03 ± 1.8
WSDP treated	6.2 ± 1.1	15.8 ± 1.5

[a] *Mean ± SD*
Groups were comprised of 7 mice each.

treated with WSDP (Orsolic in press) suggested that it could be likely that treatment of mice with WSDP might have a protective effect on mice against dreadful effects of WBI due to elevation of GSH levels in their bone marrow. To confirm this, mice were treated with WSDP for 20 consecutive days and 24 hours following treatment submitted to WBI with either 3 Gy or 6 Gy of γ rays. The intracellular level of GSH of their bone marrow was determined 24 hours either before or after the WBI. Table 7 shows that neither 3 Gy nor 6 Gy WBI compromised the level of GSH in the WSDP treated mice.

Discussion

The WSDP, given orally throughout a period of 20 consecutive days increased the mean survival time of CBA mice submitted to WBI with γ rays (Table 1). Even mice receiving a dose of irradiation such as 8 Gy survived significantly (<0.05) longer than the untreated control; the onset of death, however, was postponed in WSDP-treated groups as compared to the control. WSDP treatment induced extensive proliferation of nucleated cells in the spleen and bone marrow, which are mainly macrophages[21] and hematopoietic cells (Table 3 and 4). Stimulated hematopoietic activity in WSDP-treated mice, as evidenced by the increased number of cells capable of producing hematopoietic colonies in the spleen of lethally irradiated recipients (Table 3 and 4), has also been evidenced in animals treated with other biological response modifiers such as *C. parvum*[34,35]. Such activity however, was insufficient to protect *C. parvum* treated mice from death caused by irradiation. Multiple *po* treatments with WSDP, although highly stimulative in elevating the cellularity of the spleen and bone marrow of treated mice did not produce higher protection of these cells from irradiation caused destruction (Table 2); approximately similar proportion of cells compared to the control were killed. However in mice treated with equal irradiation dose, WSDP - treatment saved almost three times more CFUs cells in their spleen as compared to the control (Table 3). This factor seems to be one of the pillars for radioprotective activity of WSDP, as shown in Table 6, where the surviving fraction of CFUs was twice as big as the surviving fraction of normal spleen CFUs. These findings suggest a different mode of activity of WSDP as a modifier of

Table 6. Fractions of spleen from normal or WSDP - treated mice surviving irradiation with 3 or 6 Gy of γ rays.

Donors[a]	Surviving fraction following			
	3Gy		6Gy	
Normal mice	0.1848 ± 0.055[b]	<0.01[c]	0.1304 ± 0.019	<0.05
WSDP treated mice[d]	0.3577 ± 0.065		0.1846 ± 0.021	

a Mice were whole - body irradiated with 3 or 6 Gy of γ rays and within 2 hours thereafter killed for the spleen cell preparation. Different number of spleen cells were injected into mice irradiated with 9 Gy of γ rays and the number of spleen colonies determined 8 days later.
b Mean ± SD
c Mann - Witney U test
d 50 mg/kg WSDP was given po for 20 consecutive days before WBI. Groups were comprised of 10 mice each

Table 7. Effect of whole - body irradiation on the intracellular GSH content of bone marrow cells of normal and WSDP - treated mice

Dose of WBI (Gy)	GSH content (μmol/mg)					
	Normal mice			WSDP treated mice [a]		
	Before [b]WBI	P[c]	After [d]WBI	Before WBI	P	After WBI
3	21.4 ± 2.4	<0.01	9.2 ± 1.1	39.6 ± 2.9	NS[e]	34.3 ± 3.1
6	22.3 ± 1.9	<0.01	2.4 ± 0.8	42.1 ± 3.3	NS	29.2 ± 4.2

a 50 mg / kg WSDP was given po for 20 consecutive days.
b WBI was applied on day 21 after treatment with WSDP; sampling was performed 12 hours after last treatment.
c Mann - Witney U test; results are means ± SD from two independent experiments (bone marrow cell suspensions derived from 3 mice).
d Sampling performed 24 hours after irradiation.
e Not significant.

hematopoietic activity than those described for *C. parvum* or BCG, where proliferative activity of hematopoietic CFUs was strongly stimulated and onset of death of animals subjected to irradiation delayed, but the sensitivity of CFUs to radiation was increased and survival time of irradiated animals shortened[34,36].

It has been shown that tumor cell glutathione cycle is a rate - limiting factor of their survival after treatment with different cytostatic agents possessing antitumor property[37]. Inhibition of the pathways of glutathione synthesis makes tumor cells more susceptible to the action of different antitumor agents; glutathione deficiency sensitizes cells to the disadvantageous effect of radiation[38,39]. It was demonstrated that the tumor cell resistance to various antitumor agents was partially associated with an overproduction of glutathione synthesis in those cells, and that its production could be reversed by treatment with selective inhibitors of glutathione synthesis[40]. Our findings showing that the glutathione content of tumorigenic cell line was higher than in nontransformed cell line are in accordance with the findings mentioned above. The increase in glutathione in V79 cell line, but not in HeLa cell line after the treatment with WSDP suggests that the elevation of glutathione level in "normal" cell line may also be the mode of resistance of hematopoietic cells to the effect of irradiation. Results in Table 7 showed that WSDP protected more of those cells possibly due to elevation of glutathione synthesis in their bone marrow which was not compromised by either 3 Gy or 6 Gy WBI.

Data presented in this paper show that mice treated with WSDP are more resistant to WBI ranging from 5 - 8 Gy. More CFUs in their hematopoietic tissues as shown by either endogenous or exogenous spleen colony assay, indicate that WSDP given continuously *po* to mice did not adversely affect hematopoiesis. In contrast, it is likely that it increases the ability of hematopoietic tissue to synthesize glutathione in bone marrow compartment (Table 7), making treated mice more resistant to the deleterious damages of irradiation.

These studies encourage further investigations on the mode of the radioprotective action of WSDP and its use not only in research concerning hematopoiesis, but also in studies related to its use in combined therapy with radiation and/or cytotoxic drugs.

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References

1. Ghisalberti, E., Propolis: a review, Bee World, 60, 59-84, 1979.
2. Walker, P., Crane E., Constituents of propolis, Apidologie, 18, 327-334, 1987.
3. Stojko, A., Scheller, S., Biological properties and clinical application of propolis, VIII. Arzneim.-Forsch./Drug Res. 28, 35-37, 1987.
4. Scheller, S., Stojko, A., Biological properties and clinical application of propolis, VI. Arzneim.-Forsch./Drug Res. 27, 2138-2140, 1977.

5. Scheller, S., Wilczok, T., Imielski, S., Krol, W., Gabrys, J., Shani, J., Free radical scavenging by ethanol extract of propolis, Int. J. Radiat, Biol. 57, 461-465, 1990.

6. Bankova, V., Christov, R., Stoev, G., Popov, S., Determination of phenolics from propolis by capillary gas chromatography, J. Chromatogr, 607, 150-153, 1992.

7. Bankova, V., Christov, R., Popov, S., Volatile constituents of propolis, Z. Naturforsch. 49, 6-10, 1994.

8. Marcucci, M. C., Propolis: chemical composition, biological properties and therapeutic activity, Apidologie, 26, 83-99, 1995.

9. Amoros, M., Sauvager, F., Girre, L., In vitro anti viral activity of propolis, Apidologie, 23, 231-245, 1992.

10. Debiaggi, M., Tateo, F., Pagani, L., Effects of propolis flavonoids on virus infectivity and replication, Microbiologica, 13, 207-213, 1990.

11. Amoros, M., Lurton, E., Boustie, J., Comparison of the anti-herpex simplex virus activities of propolis and 3-methylbut-2-enyl caffeate, J. Nat. Prod. 57, 644-647, 1994.

12. Brumfitt, W., Hamillton - Miller J.T.M., Franklin, I., Antibiotic activity of natural products: 1. Propolis, Microbios, 62, 19-22, 1990.

13. Grange, J.M., Davey, R.W., Antibacterial properties of propolis (bee glue), J. R. Soc. Med. 83, 159-160, 1990.

14. Focht, J., Hansen S. H., Nielsen, J. V., Bactericidal effect of propolis in vitro against agents causing upper respiratory tract infections, Arzneim.-Forsch./Drug Res, 43, 921-924, 1993.

15. La Torre, A., Guccione, M., Imbroglini, G., Indagine preliminary sull'azione di preparati a base di propoli nei confronti di *Botrytis cinerea* Pers della fragola, Apicoltura, 6, 169-177, 1990.

16. Lori, G.A., Accion fungicida del propoleos en la dermatomicosis bovina, Ind. Apic., 1, 38-43, 1990.

17. Ban, J., Popovic, S., Maysinger, D., Cytostatic effect of propolis in vitro. Acta Pharm. Jugosl, 33, 245-255, 1983.

18. Scheller, S., Krol, W., Swiacik, J., Owczarek S., Gabrys J., Shani J., Antitumoral property of ethanolic extract of propolis in mice bearing Ehrlich carcinoma, as compared to bleomycin, Z. Naturforsch, 44c, 1063-1065, 1989.

19. Chiao, C., Carothers, A. M., Grunberger, D., Solomon, G., Preston, A. G., Barrett C. J., Apoptosis and altered redox state induced by caffeic acid phenethyl ester (CAPE) in transformed rat fibroblast cells, Cancer Res, 55, 3576-3583, 1995.

20. Rao, C. V., Desai, D., Rivenson, A., Simi, B., Amin, S., Reddy, S. B., Chemoprevention of colon carcinogenesis by phenylethyl - 3 – methylcaffeate, Cancer Res, 55, 2310-2315, 1995.

21. Orsolic N., Basic, I., Immunomodulation by water-soluble derivative of propolis (WSDP) a factor of antitumor reactivity, J. Ethnopharmacology, 84, 265-273, 2003.

22. Basic, I., Orsolic, N., Tadic, Z., Macedo Fereire Alcici, N., Brbot Saranovic, A., Bendelja, K., Krsnik, B., Rabatic, S., Antimetastatic effect of propolis, caffeic acid phenethyl ester and caffeic acid on mammary carcinoma of CBA mouse, 17th International Cancer Congress, Rio de Jeneiro, Brazil, (ed.) M. Moraes, R. Brentani and R. Bevilaqua, pp 63-75, 1998.

23. Manolova, N., Maximova, V., Manolova, Z., Stoilova, I., Korczak, E., Denis, A., Immunobiological effect of propolis, I. Effect on cellular immunity, Acta Microbiol. Bulg., 21, 76-81, 1987.

24. Neychev, H., Dimov, V., Vuleva, V., Shirova, L., Slavcheva, E., Gegova, G., Manolova, N., Bankova, V., Immunomodulatory action of propolis, II. Effect of water-soluble fraction on influenza infection in mice, Acta Microbiol. Bulg., 23, 58-62, 1988.

25. Dimov, V., Ivanovska, N., Bankova, V., Popov, S., Immunodulatory action of propoolis, IV. Prophylactic activity against gram-negative infections and adjuvant effect of the water-soluble derivate, Vaccine, 10, 817-823, 1992.

26. Dimov, V., Ivanovska, N., Manolova, N., Bankova, V., Nikolov, N., Popov, S., Immunodulatory action of propolis, Influence on anti-infectious protection and macrophage function, Apidologie, 22, 155-162, 1991.

27. Krol, W., Czuba, Z., Scheller, S., Gabrys, J., Grabiec, S., Shani, J., Anti-oxidant property of ethanolic extract of propolis (EEP) as evaluated by inhibiting the chemiluminescence oxidation of luminol, Biochem, Int. 21, 593-597, 1990.

28. Nikolov, N., Marekov, N., Bankova, V., Popov S., Ignatova, R., Vladimirova, I., Method for the preparation of water-soluble derivative of propolis, Bul. Pat. Appl., 79903/28, 05, 1987.

29. Curry, J. L., Trentin, J. J., Hemopoietic spleen colony studies, I. Growth and differantiation. Develop, Biol. 15, 395-399, 1967.

30. Milas, L., Basic, I., In: Proceedings of Symposium on Malignant Tumours of the Head and Neck, I. Oupar (ed.), pp. 37-45, Yugoslav Academy of Sciences and Arts, Zagreb, 1972.

31. Tietze F., Enzymatic method for quantitative determination of nanogram amounts of total and oxidised glutathione: Applications to mammalian blood and other tissues, Analyt. Biochem, 27, 502-522, 1969.

32. Siminovitch, L., McCulloch, E. A., Till, J. E., The distribution of colony-forming cells among apleen colonies, J.Cell.Comp.Physiol, 62, 327-331, 1963.

33. Bradley, M. O., Bhuyan, B., Francis, M. C., Mutagenesis by chemical agents in V79 Chinese hamster cells: a review and analisys of the literature, A reportof the gene - tox program. Mutant. Res. 87, 81-85, 1981.

34. Maruyama, V., Magura, C., Feoba, J., *Corynebacterium parvum* –induced, diosensitivity and cyclic changes of hematopoietic spleen colony-forming units. J. Natl. Cancer Inst. 59, 173-178, 1977.

35. Gordon, M.Y., Aguado, M., Blackett, N. M., Effect of BCG and *Corynebacterium parvum* on the hematopoietc precusor cell in contineusly irradiated mice: possible mechanisms of action in immunotherapy, Europ. J. Cancer 13, 229-233, 1977.

36. Basic, I., Milas, L., Effect of whole body irradiation on mice treated with Corynebacterium parvum, Europ. J. Cancer 13, 901-908, 1979.

37. Meister A., Glutathione, ascorbate, and cellular protection, Cancer Res. 54, 1969-1975, 1994.

38. Dethmers, J.K., Meister, A., Glutathione exports by human lymphoid cells: depletion of glutathione by inhibition of its synthesis decreases export and increases sensitivity to irradiation. Proc. Natl. Acad. Sci. U.S.A. 78, 7492-7497, 1981.

39. Meister, A., Glutathione deficiency produced by inhibition of its synthesis and its reversal; application in research and therapy, Pharmacol. Ther. 51, 155-159, 1991.

40. Hamilton, T., O'Dwyer, P., Yong, R., Phase I trial of buthionine sulfoximine (BSO) plus melphalan (L - PAM) in patients with advanced cancer. Proc. A. Meet. Am. Soc. Clin. Oncol. 9, A281, 1990.