

# The Ability of Ethanolic Extract of Propolis (EEP) to Protect Mice against Gamma Irradiation

Stanislaw Scheller\*, Grazyna Gazda\*, Wojciech Krol\*, Zenon Czuba\*, Aleksander Zajusz\*\*, Janusz Gabrys<sup>+</sup>, and Jashovam Shani<sup>++</sup>

\*Department of Microbiology and <sup>+</sup>Department of Histology and Embryology, Silesian School of Medicine, Zabrze-Rokitnica, Poland; \*\*Institute of Oncology, Gliwice, Poland, and <sup>++</sup>School of Pharmacy, University of Southern California, Los Angeles, California 90033, U.S.A.

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Ethanolic extract of propolis (EEP) was tested as a protective agent against gamma irradiation in mice. The mice were exposed to 6 Gy gamma irradiation from a <sup>60</sup>Co source, and were treated intraperitoneally with EEP, administered before and after their irradiation. While the non-treated mice expired within 12 weeks, the mice that received a series of EEP treatments survived the irradiation, and their leucocyte count as well as their spleens' plaque-forming activity returned to normal. It is suggested that an antioxidant and a free radical scavenger in the EEP are responsible for the radiation protective effect of the extract of this natural product.

## Introduction

Propolis is a resinous substance produced by honey-bees, and used by them to wax their hives. Previous publications from this laboratory reported some features of propolis which are of medical interest and of its ethanol extract (EEP) and demonstrated their immunological properties in laboratory animals and in patients [1, 2]. In experiments carried out in mice it was shown that if the animals' immune system had been triggered by sheep red blood cells, EEP intensified the immunization process, as evaluated by increase in the number of their plaque-forming spleen cells [1]. In the clinic we have shown that in aged people with impaired immune system, EEP revitalized their immune system remarkably [2].

Recently we noticed a rise in the lytic capacity of some human cell lines preincubated with EEP (unpublished). We also noticed higher survival of mice with Ehrlich ascites carcinoma, pretreated with EEP [3]. Some of these properties of EEP were related to its antioxidant and scavenging abilities of free radicals [4, 5]. As oxidation products and radiation-initiated free radicals are major determinants in the viability of some immune mechanisms [6–8], people became alert to appearance of radiolysis products in

their bodies. Such products result in DNA fragmentation, chromosomal aberration, hyaluronic acid degradation, lipid peroxidation and diminished contents of proteins containing sulphhydryl groups, and may alter the immune mechanism. These changes can be blocked by antioxidants and free radical scavengers [9]. We wondered, therefore, whether EEP could prevent the effects of gamma irradiation on the immune system when administered shortly before or after irradiation of mice.

## Materials and Methods

About 250 male BALB/c mice, 28–30 gm each (12–16 weeks old), were used in this study which was performed in two phases. In the first phase, 41 mice were divided into 3 groups of 12–14 each, treated as described below and their survival monitored for 17 weeks. Their treatment included exposure of all mice to 6 Gy of whole-body gamma irradiation. The mice were kept in plastic holders attached to a <sup>60</sup>Co irradiation unit (Gammatron, Siemens, Germany), and were subjected to one of the following treatments: Group "A" received a series of 15 intraperitoneal (IP) EEP injections (two before, and 13 after their irradiation); group "B" received only three IP EEP injections (one before and two after their irradiation) and twelve injections of solvent, while group "C" received 13 IP solvent injections at the same frequency as groups "A" and "B". The schedule of injections is given in Fig. 1. Immediately

Reprint requests to Prof. Dr. S. Scheller, Institute of Microbiology, Silesian School of Medicine, 19 Karl Marksa Street, 41-808 Zabrze-Rokitnica, Poland.

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after irradiation, the mice were transferred to large cages and were kept three per cage throughout the study. Their survival was monitored weekly and presented graphically.

In the second phase, 214 mice were divided into 17 cages of 12–13 each: mice of 5 cages received the same EEP treatment as group “A” and were immunized IP with 0.4 ml of 10% sheep red blood cells suspended in PBS: 6 days, 10 days, 5 weeks, 12 weeks and 17 weeks after irradiation. Four days following each immunization, one group of mice was sacrificed. Spleens were excised, and the number of cells producing 19S antibodies (“plaque forming”) were counted, according to Jerne *et al.* [10], as modified by Mishell and Dutton [11]. Mice of four cages received the same EEP treatment as group “B”, immunized IP at 10 days, 5 weeks, 9 weeks and 17 weeks after their irradiation, sacrificed 4 days later and their spleens processed as described. Mice of eight cages were assigned for group “C”. Their immunization was performed at 10 days, 5 weeks, 8.5 weeks and 12 weeks after irradiation, and plaque counting was performed as described above. In group “C”, mice from two cages were pooled for each of the first three time intervals, and three cages for the fourth one (12 weeks;  $n = 5$ ). Immediately prior to immunization, 0.3 ml of blood was obtained by heart puncture (under light ether anesthesia) from each mouse for a leucocyte count. The four-day interval between immunization and sacrifice, as well as the dose of EEP administered, were determined in preliminary studies to be the optimal length of time and concentration for plaque formation [1].

Propolis was collected in the beehive of the University's farm, and was extracted by a series of solvents as described earlier [12]. The extract was filtered through filter-paper Whatman # 4, and evaporated until dry under vacuum. The dried residual powder (EEP) was kept at  $-20^{\circ}\text{C}$  in order to minimize bacterial contamination, and was dispersed immediately prior to use in 10% Tween 80 in saline to give an injectable preparation. EEP was administered IP, 20 mg/kg BW, in a fixed volume of about 0.3 ml, and the controls received 0.3 ml of the solvent.

Significance of the results was calculated by the student's “t” test in comparison with the extrapolated zero-time values for both parameters: 10,000 leucocytes per  $\text{mm}^3$  and 350/10<sup>6</sup> plaque-forming cells in the spleen cultures.

## Results and Discussion

The protective effect of EEP against a single 6 Gy dose of whole-body gamma irradiation is demonstrated in the survival curves of the mice (Fig. 1). While none of the irradiated, non-EEP-treated mice, survived the 12th week after their irradiation (group “C”) – half of the mice receiving three EEP injections (group “B”) reached the 17th week, and all but one mouse in group “A”, who were subjected to 15 EEP injections, survived the complete study period.

The regenerative effect of EEP on antibody formation of the plaque-forming cells is summarized in Fig 2. The number of these cells was reduced to about 20% of the healthy population in all three

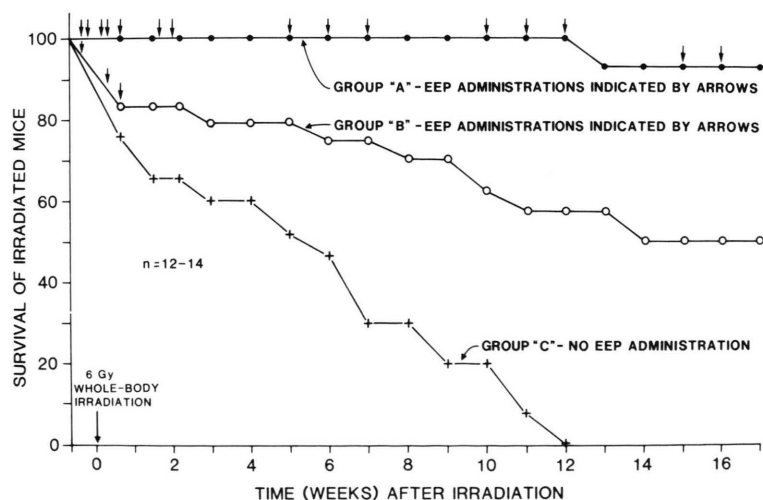


Fig. 1. Survival of BALB/c mice pre-exposed to 6 Gy whole-body gamma irradiation (“0” time), after being treated with 15 (group “A”) or 3 (group “B”) doses of EEP, as indicated by arrows ( $n = 12-14$ ).

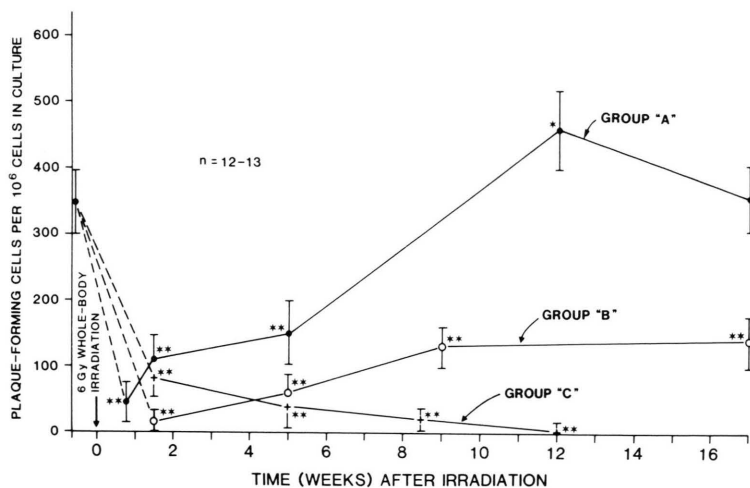


Fig. 2. Plaque formation in the spleens of mice pre-exposed to 6 Gy gamma irradiation ("0" time) and treated with EEP ( $n = 12-13$ ). \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

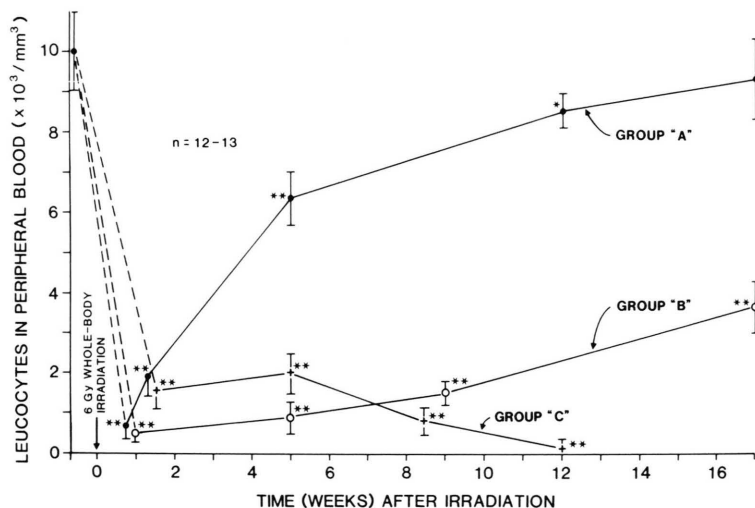


Fig. 3. Leucocyte count in peripheral blood of mice pre-exposed to 6 Gy gamma irradiation ("0" time) and treated with EEP ( $n = 12-13$ ). \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

groups, but while the survival of the non-EEP-treated mice continued to decline gradually until expiration, this number significantly increased in both EEP-treated groups: group "B" demonstrated a slow survival, but group "A" demonstrated a full recovery, and even significant hyperactivity was noticed (Fig. 2). A parallel situation was reflected in leucocyte count: after irradiation it decreased in all three groups to 10–20% of its initial levels with a continued drop in the untreated group ("C"), a very shallow increase in group "B" and full recovery in group "A" (Fig. 3).

From these results it emerges that EEP exerts a distinct radiation-protective effect on mice when administered immediately before and shortly after their exposure to sublethal doses of gamma irradiation. The survival of the EEP-treated groups was complete, while the mortality of the non-treated group was 50%. The formation of 19S antibodies, as evaluated by the spleens' ability to form plaques, and the leucocyte counts in the treated groups were all significantly higher than in the non-treated controls (group "C").

As the active antioxidant in the EEP has not yet

been isolated and its rate of elimination from the body has not yet been established – one can only conclude that its  $t_{1/2}$  of elimination in the Tween-80 solvent is longer than 24 h, as apparently some of it was present in the body and exerted the protective effect when the mice were irradiated. The better survival of group “A” as compared to group “B” is partially attributed to the apparent higher level of EEP-derived antioxidant in their circulation during irradiation.

The ability of EEP to stimulate production of plaque-forming cells was reported by us recently [1]. The plaque-formation capacity of the spleen cells tripled under EEP treatments from a basic level of 300–400/10<sup>6</sup> spleen cells. The complete recovery of the plaque-forming ability and of the leucocyte count

of the irradiated mice, as demonstrated in this study, indicate that there are some components in the EEP which demonstrate cell-regeneration properties: the recovery in the plaque-formation ability from 40/10<sup>6</sup> after irradiation to about 440/10<sup>6</sup> after EEP, and the increase of leucocyte count from 1000/mm<sup>3</sup> to about 9300/mm<sup>3</sup> are remarkable and highly significant. These findings are in line with our earlier reports that EEP restores the immune system in T-lymphocytes and granulocytes [2], probably due to its high content of flavonoids [13, 14]. The ability of EEP to activate enzymes *in vivo* [15] and *in vitro* [16] have also to be taken into consideration as a contributing mechanism. The complexed mechanism of the radiation-protective effect of EEP is currently being studied in our laboratory.

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