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EFFECT OF *Apis mellifera* BEE VENOM AND GAMMA RADIATION ON BONE MARROW CELLS OF WISTAR RATS TREATED *in vivo*

E.A. Varanda[1,3], C.S. Takahashi[2,3], A.E.E. Soares[3] and S.A.J. Barreto[2]

ABSTRACT

To determine whether the venom of *Apis mellifera* can exert a radioprotective effect, by reducing the frequency of chromosome aberrations induced by radiation, five different experiments were performed on bone marrow cells of Wistar rats.

Animals weighing about 100 g were injected intraperitoneally with different venom concentrations (1.0 or 0.5 µl) 1 or 24 h before, or 30 min after being submitted to 3 or 4 Gy of gamma radiation, and sacrificed 24 h after the last treatment. For each experiment in addition to the group of animals submitted to combined treatment (venom + radiation) and to their control, there was also one group treated with radiation only and another treated with venom only. A decrease in the frequency of chromosome aberrations, and fragments in particular, as well as in the number of cells with aberrations was observed in the experiments in which venom was administered 24 h before irradiation, and the effect was more marked at the higher venom concentration (1 µl/100 g weight).

INTRODUCTION

The discovery that animals may be partially protected against the deleterious effects of ionizing radiation by the administration of certain chemical compounds has led

[1] Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas - UNESP, Araraquara, Rua Expedicionários do Brasil, 1621, 14801-902 Araraquara, SP, Brasil. Send correspondence to E.A.V.

[2] Departamento de Biologia, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto - USP, 14040-901 Ribeirão Preto, SP, Brasil.

[3] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto - USP, 14049-900 Ribeirão Preto, SP, Brasil.

to an area of research concerning radioprotectors. Roots and Okada (1972, 1975) employed several alcohols and sulfur compounds (SH) to study chemically mediated protection against radiation-induced DNA-single-strand breaks (SSBs) in mammalian cell cultures and concluded that 71% of SSBs were produced by indirect action, the OH radical being the primarily responsible factor. Several investigators (Chapman and Gillespie, 1981; Ward, 1981, 1985) have attempted a better definition of the mechanisms of interaction of the OH radicals with DNA and its constituents to elucidate the biochemical steps leading to the formation of single- and double-strand breaks and to directly and indirectly induced base damage in the DNA of mammals. Attempts have also been made to quantify the role of radicals in cell death and other adverse effects.

According to Prasad (1982), the efficacy of a radioprotective agent can be evaluated on the basis of delayed effects of radiation such as shortened survival time, development of cataracts, and induction of clastogenic and carcinogenic effects. Gupta and Uma Devi (1986) reported the protective effect of WR-2721 [ethylphosphorothioic S-2(3-aminopropylamino) acid] and of MPG (2-mercapto-propionyl-glycine) on mouse bone marrow chromosomes, when administered before exposure to gamma radiation. Shipman and Cole (1967), and Kanno *et al.* (1970) reported that the survival of mice injected with *Apis mellifera* venom 24 h before X-irradiation was increased and that the pathological changes induced by radiation in liver and bone marrow were reduced.

Some components of *Apis* venom have been tested separately for radioprotective activity, using animal survival as the parameter (Ginsberg *et al.*, 1968; Peck and O'Connor, 1974; Peck *et al.*, 1978). In addition to having a radioprotective effect, the venom of *A. mellifera* bees is considered to be a potentially valuable therapeutic system both in its natural whole state and as isolated or synthetically reproduced components (Peck and O'Connor, 1974). The objective of the present study was to evaluate the effect of *A. mellifera* venom associated with gamma radiation in terms of frequency of chromosome aberrations in bone marrow cells of Wistar rats (*Rattus novergicus*) treated *in vivo*.

Chromosome aberrations are manifestations of radiation - induced DNA damage. Radioprotection, defined in terms of decreased chromosome aberration frequencies has not been extensively studied even though many compounds have been tested, the parameter selected for analysis usually being survival of the cells or of the experimental animals.

Apis mellifera venom is colorless, transparent and water soluble. It has a specific weight of about 1.1313 and contains 0.3 to 0.4 mg solids/mg liquid venom (Haydak, 1951; Artemov, 1965).

MATERIALS AND METHODS

Venom collection

Fresh venom was used in all experiments. Bees were immobilized by freezing and their stings were removed together with the venom sac and the acid gland, which is responsible for venom production. The venom was squeezed out of the venom sac through the sting lancets by slight manual pressure and collected into graduated capillary tubes. The venom was then diluted in distilled water and injected into the animals.

Animal treatment

Rats of the *Rattus novergicus* species, Wistar strain, weighing about 100 g were injected intraperitoneally with 1 or 0.5 µl of *A. mellifera* venom, diluted in 0.5 ml distilled water. The radiation dose was 3 or 4 Gy. A 60-cobalt gamma radiation source (Department of Genetics, Faculty of Medicine of Ribeirão Preto, USP) with a dose rate of approximately 1.3 Gy/h at distance of one meter from its center was used. For whole body irradiation, the animals were immobilized in perforated plastic tubes and placed 30 cm from the center of the source (dose rate 14.4 Gy/h).

Five experiments were performed. In experiments 1 and 2, the animals respectively received 1.0 and 0.5 µl venom, 24 h before irradiation (3 Gy). In experiment 3, venom (1 µl) was administered 24 h before irradiation (4 Gy). In experiment 4, venom (1 µl) was administered 1 h before irradiation (3 Gy), and in experiment 5, the animals were first irradiated (3 Gy) and then injected with venom 30 min later. In each experiment, in addition to the groups receiving combined venom + radiation treatment at different times and different radiation exposures, there was also a group treated with venom only, a group exposed to radiation only, and an untreated control group.

All animals were injected intraperitoneally with 0.5 ml 0.16% colchicine (MERCK) per 100 g body weight 1.5 h before sacrifice, which occurred 24 h after the radiation treatment.

Metaphase bone marrow cells were obtained by the technique of Ford and Hamerton (1956). Slides were coded and examined. The chromosomes of each metaphase analysed were drawn schematically, with careful observation of structural alterations such as gaps, chromatid and chromosome breaks, translocations, fragments, rings, dicentrics and triradial figures.

RESULTS

Of the five experiments performed, only those in which venom (0.5 or 1.0 µl/100 g weight) was used 24 h before irradiation (3 or 4 Gy) showed a reduction of number of cells with chromosome aberrations, as well as a reduction in number of aberrations, and fragments in particular.

Tables I and II respectively show the effect of 1.0 and 0.5 µl venom administered 24 h before irradiation with 3 Gy. The venom had a dose-dependent effect, i.e., a more marked reduction in number of cells with chromosome aberrations, as well as in the number of aberrations induced, was observed with the larger amount of venom.

Table I - Frequencies and distribution of different types of chromosome aberrations in bone marrow cells of 10 Wistar rats per treatment. 24 hours before irradiation (3 Gy) the animals received I.P. 1 µl honey bee venom/100 g body weight. 50 cells/animal were scored.

Treatment	No. of cells analyzed	Breaks G	C	IC	F	D	R	T*	Total no. of aberrations	No. of cells with aberrations (%)
Control	500	6	3	0	0	2	1		12	12 (2.4)
Venom	500	6	3	0	5	1	0		15	12 (2.4)
3 Gy	500	18	18	8	150	17	18,5*		234	112 (22.4)
Venom + 3 Gy	472	7	8	4	39	4	6		68	45 (9.53)

Abbreviations: G, gaps; C, chromatid-type aberrations; IC, isochromatid-type aberrations; F, fragments; D, dicentric chromosomes; R, rings; T*, triradial figures.

Angular scale analysis of variance (Sokal and Rohlf, 1969) showed that the main significant effect was due to radiation (Exp. 1: $F_{1.35} = 54.418$, $p < 0.1\%$; Exp. 2: $F_{1.20} = 89.423$, $p < 0.1\%$) and that the effect of the venom in the venom + radiation combination was also significant for $\alpha = 5\%$ (Exp. 1: $F_{1.35} = 54.403$, $2.5\% < p < 5\%$; Exp. 2: $F_{1.20} = 7.217$, $1\% < p < 2.5\%$).

Although the animal treatment schedule used in experiment 3 was similar to that of experiment 1, exposure to radiation was 4 Gy (Table III). It can be seen that, with the increase in radiation dose and the consequent increase in the frequency of chromosome aberrations, the effect of venom in the venom + radiation interaction was also statistically significant ($F_{1.20} = 5.67$, $2.5\% < p < 5\%$), resulting in a reduction of aberrations and of the number of cells with aberrations.

Table II - Frequencies and distribution of different types of chromosome aberrations in bone marrow cells of Wistar rats. 24 hours before irradiation (3 Gy) the animals received I.P. 0.5 µl honey bee venom/100 g body weight. 100 cells/animal were scored.

Treatment	No. of cells analyzed	G	Breaks		F	D	R	Total no. of aberrations	No. of cells with aberrations (%)
			C	IC			T*/T**		
Control	600	2	0	0	2	0	0	4	3 (0.5)
Venom	600	5	4	1	3	0	0	13	12 (2.0)
3 Gy	600	16	17	9	154	7	9*/6**	218	114 (19.0)
Venom + 3 Gy	525	11	14	1	109	11	5*/7**	158	75 (14.29)

Abbreviations: G, gaps; C, chromatid-type aberrations; IC, isochromatid-type aberrations; F, fragments; D, dicentric chromosomes; R, rings; T*, translocations; T**, triradial figures.

Table III - Frequencies and distribution of different types of chromosome aberrations in bone marrow cells of Wistar rats. 24 hours before irradiation (4 Gy) the animals received I.P. 1 µl honey bee venom/100 g body weight. 100 cells/animal were scored or 600 cells/treatment.

Treatment	G	Breaks		F	D	R	Total no. of aberrations	No. of cells with aberrations (%)
		C	IC			T*/T**		
Control	18	0	0	7	0	0	25	23 (3.83)
Venom	13	0	0	9	0	0	22	17 (2.83)
4 Gy	40	105	0	946	17	11*/15**	1134	301 (50.16)
Venom + 4 Gy	25	55	0	797	1	1,12*/2**	893	205 (34.16)

Abbreviations: G, gaps; C, chromatid-type aberrations; IC, isochromatid-type aberrations; F, fragments; D, dicentric chromosomes; R, rings; T*, translocations; T**, triradial figures.

To determine the significance of the differences between treatments (C = control, V = venom, R = radiation, VR = venom + radiation), the data were analyzed statistically by the Tukey test for multiple comparisons. The results showed that the mean

proportion of cells with chromosome aberrations was similar for C and V, and significantly different from that obtained for R and VR, which were significantly different from one another.

The results of the experiment in which the animals were treated with venom 1 h before radiation (Experiment 4) are shown in Table IV. The four treatments differed from one another ($F_{3.36} = 171.134$, $p < 0.1\%$) and, as was the case for the previous experiment, the main significant effect was due to radiation ($F_{1.16} = 511.106$, $p < 0.1\%$), whereas the effects of venom in the venom + radiation combination was not significant ($F_{1.36} = 1.766$, $10\% < p < 25\%$).

Table IV - Frequencies and distribution of different types of chromosome aberrations in bone marrow cells of 10 Wistar rats per treatment. Honey bee venom (1 µl/100 g body weight) was injected 1 hour before irradiation (3 Gy). 50 cells/animal were scored or 500 cells/treatment.

Treatment	G	Breaks		F	D	R	Total no. of aberrations	No. of cells with aberrations (%)
		C	IC			T*/T**		
Control	1	0	1	0	0	0	2	3 (0.6)
Venom	2	4	0	1	1	0	8	8 (1.6)
3 Gy	10	52	8	223	6	2,14*/1**	316	151 (30.2)
Venom + 3 Gy	10	42	4	221	6	1,10/5**	299	145 (29.0)

Abbreviations: G, gaps; C, chromatid-type aberrations; IC, isochromatid-type aberrations; F, fragments; D, dicentric chromosomes; R, rings; T*, translocations; T**, triradial figures.

Analysis of the data obtained in experiment 5, in which the animals were initially treated with radiation and then with venom (Table V), showed that, in terms of decrease in the number of induced chromosomal aberrations, they were similar to those obtained in experiment 4 (Table IV). In this case also, the main, significant effect was due to radiation ($F_{1.53} = 130.46$, $p < 0.1\%$), but the effect of venom in the venom + radiation interaction was not significant ($F_{1.53} = 0.043$, $p > 0.75\%$). When the data obtained in experiments 4 and 5 were analyzed by the multiple comparisons test (Tukey test), no significant differences were detected between the control group and the venom treated group, or between the irradiated group and the animals subjected to venom + radiation. However, significant differences were detected in the following comparisons: control X radiation, control X venom + radiation.

The mean proportions of cells with aberrations obtained in the five experiments are summarized in Table VI.

When the types of chromosome aberrations and the frequencies of their induction in the five experiments are analyzed (Table I, II, III, IV and V), it can be seen that the response pattern in terms of decrease in the number of induced chromosome aberrations is the same when only the number of cells with aberrations is considered.

Table V - Frequencies and distribution of different types of chromosome aberrations in bone marrow cells of 15 rats per each treatment. The animals were irradiated with 3 Gy of gamma radiation and received I.P. 1 µl of honey bee venom/100 g body weight 30 minutes later. About 50 cells/animal were scored.

Treatment	No. of cells analyzed	G	Breaks		F	D	R	Total no. of aberrations	No. of cells with aberrations (%)
			C	IC			T*/T**		
Control	804	28	1	1	28	0	1	59	43 (5.34)
Venom	778	32	4	1	7	0	0,2*	46	34 (4.37)
3 Gy	961[a]	22	23	2	489	8	8,8*/3**	563	203 (21.12)
3 Gy + Venom	1006	55	13	1	429	11	3,10*/3**	525	220 (21.87)

[a]14 animals.

Abbreviations: G, gaps; C, chromatid-type aberrations; IC, isochromatid-type aberrations; F, fragments; D, dicentric chromosomes; R, rings; T*, translocations; T**, triradial figures.

DISCUSSION

The venom of *A. mellifera* is known to be a potent anti-inflammatory agent (Lorenzetti *et al.*, 1972; Banks *et al.*, 1976; De Vera, 1978; Short and Jackson, 1978; Von Bredow *et al.*, 1978; Guyton, 1978) in addition to having other pharmacological effects on animals (Fredholm and Haeger, 1969; Vick and Shipman, 1972; Banks *et al.*, 1976; Paterson and Valentine, 1982). Venom components have been reviewed by Dotimas and Hider (1987).

In our study venom had no clastogenic effect when injected into the rats, whereas exposure to 3 or 4 Gy of τ-rays increased the frequency of chromosome aberrations. When the treatments were combined the results varied according to the pretreatment time and concentration of the venom in relation to irradiation.

Table VI - Mean proportions of cells (%) with at least one chromosome aberration obtained in experiments carried out on Wistar rats submitted to treatment with *Apis* venom and gamma radiation.

Situation experimental	Treatment			
	Control	Venom	Radiation	Venom + Radiation
a	2.40 ± 0.70	2.40 ± 0.70	22.40 ± 1.90	9.0 ± 1.40
b	0.50 ± 0.29	2.00 ± 0.57	19.00 ± 1.60	14.29 ± 1.53
c	4.00 ± 0.80	2.83 ± 0.67	50.16 ± 2.04	34.17 ± 1.93
d	0.60 ± 0.34	1.60 ± 0.56	30.20 ± 2.05	29.00 ± 2.03
e	5.80 ± 0.82	4.88 ± 0.77	21.12 ± 1.32	21.87 ± 1.30

a - Animals treated with venom (1 µl/100 g body weight), 24 h before irradiation (3 Gy).

b - Animals treated with venom (0.5 µl/100 g body weight), 24 h before irradiation (3 Gy).

c - Animals treated with venom (1 µl/100 body weight), 24 h before irradiation (4 Gy).

d - Animals treated with venom (1 µl/100 body weight), 1 h before irradiation (3 Gy).

e - Animals irradiated (3 Gy) and then treated with venom (1 µl/100 g body weight).

We used fresh Africanized bee venom removed manually immediately before intraperitoneal administration to the animals. Since preliminary tests had shown that a dose of 2.0 µl/100 g weight was lethal, we decided to use concentrations of 0.5 and 1.0 µl/100 g weight, which were well tolerated.

Shipman and Cole (1967), in their studies of the effect of radioprotection with bee venom on mouse survival, used extremely high doses of crystallized venom (≅ 1 µg/g weight administered intraperitoneally and ≅ 5 µg/g weight administered subcutaneously). According to Ginsberg *et al.* (1968), both whole venom and melittin (one of its major components) are highly toxic when injected intravenously or intraperitoneally).

Though on one hand our data disagree with those of Shipman and Cole (1967) in terms of the venom concentration used, on the other they are similar in terms of time of treatment with venom before irradiation, i.e., for the radioprotector effect to be detected, a certain time was necessary for the venom to interact with the animal. The same authors (Shipman and Cole, 1967) proposed three possible mechanisms of action for the venom: 1) through the production of a type of physiological stress in the animal; 2) through its antibacterial property; 3) by promoting changes in the hematopoietic system.

The third mechanism, which proposes the induction of changes in the hematopoietic system, is consistent with results obtained by Hyre and Smith (1986) in *in*

vitro studies which showed that bee venom effects T and B lymphocyte function in BALB/c mice. According to Natarajan and Obe (1982), these two lymphocyte types share the ontogenic origin of stem located in bone marrow.

It is generally accepted that survival after a radiation dose equal to half the lethal dose mainly depends on the recovery of the hematopoietic system (Smith *et al.*, 1966a). Thus, if the venom has the ability to stimulate this system, this could be one of the possible mechanisms which could explain our results.

Radioprotection, due to stimulation of the hematopoietic system, has also been attributed to other compounds tested, such as attenuated bacterial endotoxin that can stimulate bone marrow. When the typhoid paratyphoid vaccine (TAB) was injected into mice 24 h before irradiation with X-rays, maximum protection was observed and this time was of fundamental importance for the radioprotective effect to occur (Smith *et al.*, 1966b). Similarly, Walden *et al.* (1988) observed radioprotection in mice submitted to treatment with leukotrienes (Lts) which are lipoxygenases derived from arachidonic acid. When the animals were treated with Lts before exposure to a sublethal dose of radiation, the number of stem cells of the hematopoietic system increased and this protection was found to be dependent on pretreatment time and concentration.

In our study, only the animals that received 0.5 or 1.0 µl/100 g weight 24 h before irradiation (3 or 4 Gy) responded with a significant decrease in the number of cells with chromosome aberrations as well as in the frequency of these aberrations, and fragments in particular, when compared to animals submitted to irradiation alone. This venom effect was dose dependent. In the trials in which the animals were treated with venom 1 h before irradiation and in the trials in which the venom was administered after irradiation, these differences were not observed, nor were any significant differences observed in the control groups or in the groups treated with venom alone.

The most frequent types of chromosome aberrations, especially in cells from irradiated animals, were fragments, although gaps, chromatid and chromosome breaks, dicentrics, triradial figures and rings were also observed.

Theoretically, the presence of chromatid breaks indicates that the damage occurred during the S phase or after DNA synthesis (G_2). When the break occurs before DNA replication (G_1), the cells observed during metaphase will present isochromatid damage. However, according to Cohen (1971), the question of chromatid versus isochromatid chromosome aberrations may be merely academic. For the evaluation of mutagenesis, the main factor is whether or not there was any damage.

Since bone marrow contains cells in all stages of the cell cycle (Heddle and Carrano, 1977), both chromosome and chromatid aberrations may be considered (Evans and Savage, 1963; Wolff and Luippold, 1964). A simple chromosome break at G_1 may lead to the formation of fragments that may present two chromatids after replication. Irradiation during S or G_2 may produce fragments with only one chromatid.

In our study, fragments were observed at a higher frequency, most of them being of the chromosome type, but a considerable number of chromatid breaks were also observed, suggesting heterogeneity of the cell population. Treatment with venom 24 h before irradiation led to a reduction of the frequency of cells with aberrations and of the number of such aberrations, especially fragments. Littlefield *et al.* (1988) obtained a modulation of chromosome aberrations, and dicentrics and fragments in particular, when they used various DMSO concentrations in human lymphocytes exposed to X-rays. Since we were dealing with a cell population at different stages of the cell cycle, many of the dicentrics that should have been observed, as shown in studies using ionizing radiation (Luchnik and Sevankaev, 1976; Hirai and Nakai, 1977; Prosser *et al.*, 1979; Littlefield *et al.*, 1988), may have been lost, with only the fragments being left. This is probably due to the fact that we sacrificed the animals 24 h after treatment with radiation. Thus, these results suggest that further experiments should be performed using different times of treatment with radiation in order to better characterize the radioprotector effect of *A. mellifera* venom on chromosome aberration formation.

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RESUMO

Estudos têm demonstrado que o veneno da abelha *Apis mellifera* possui uma ação protetora contra os efeitos das radiações ionizantes, com relação à sobrevivência do organismo. Para determinar se essa zootoxina pode exercer o efeito radioprotetor, reduzindo a freqüência de aberrações cromossômicas induzidas por radiação; foram realizados 5 experimentos diferentes, usando células da medula óssea de ratos Wistar.

Foram usados animais de 100 g nos quais injetou-se intraperitonealmente diferentes concentrações do veneno (1,0 ou 0,5 µl) 1 ou 24 horas antes ou 30 minutos após terem sido submetidos a 3 ou 4 Gy de radiação gama. O sacrifício dos animais foi sempre realizado 24 h após o último tratamento. Para cada experimento realizado, além do grupo de animais que foram submetidos à combinação de tratamentos (veneno + radiação), havia também um grupo tratado com radiação somente, outro tratado só com o veneno além do controle. Observou-se uma diminuição na freqüência de aberrações cromossômicas, principalmente de fragmentos, bem como no número de células com aberrações, nos experimentos onde o veneno foi administrado 24 h antes da irradiação, e esse efeito foi mais acentuado quando foi usada a maior concentração de veneno (1 µl/100 g de peso).

REFERENCES

Artemov, N.M. (1965). The bee venom as a produce of the apiculture. *Apimondia XX Int. Beekeep. Congress*, Bucharest, Romenia.

Banks, B.E.C., Rumjanek, F.D., Sinclair, N.M. and Vernon, C.A. (1976). Possible therapeutic use of a peptide from bee venom. *Bull. Inst. Pasteur* (Paris) *74*: 137.

Chapman, J.D. and Gillespie, C.J. (1981). Radiation-induced events and their time scale in mammalian cells. *Adv. Radiat. Biol. 9*: 143-198.

Cohen, M.M. (1971). Cytogenetic studies in animals. In: *Chemical Mutagens. Principles and Methods for their Detection* (Hollaender, A., ed.). Vol. 2. Plenum Press, New York, pp. 515-534.

De Vera, H. (1978). Effectiveness of bee venom on adjuvant induced arthritis in the rat. *Proc. N. Am. Apiother. Soc. 1*: 147.

Dotimas, E.M. and Hider, R.C. (1987). Honeybee venom. *Bee World. 68*: 57-70.

Evans, H.J. and Savage, J.R.K. (1963). The relation between DNA synthesis and chromosome structure as resolved by X-ray damage. *J. Cell. Biol. 18*: 525.

Ford, C.E. and Hamerton, J.L. (1956). A colchicine, hypotonic citrate squash sequence for mammalian chromosomes. *Stain Techn. 31*: 247-251.

Freedholm, B. and Haeger, M. (1969). Studies on the histamine releasing effect of bee venom fractions and compound 48/80 on skin and lung tissue of the rat. *Acta. Physiol. Scand. 76*: 288.

Ginsberg, N.J., Dauer, M. and Slotta, K.H. (1968). Melittin used as a protective agent against X-irradiation. *Nature 220*: 1334.

Gupta, R. and Uma Devi, P. (1986). Protection of mouse chromosomes against whole body gamma irradiation by sulfhydryl compounds. *Br. J. Radiol. 59*: 625-627.

Guyton, F.E. (1978). 16 years of treating arthritis with bee stings. *Proc. N. Am. Apiother. Soc. 1*: 62.

Haydak, M.H. (1951). Bee venom. *Iowa State Apiarist Report 33*: 105-126.

Heddle, J.A. and Carrano, A.V. (1977). The DNA content of micronuclei induced in mouse bone marrow by γ-irradiation: evidence that micronuclei arise from acentric chromosomal fragments. *Mutat. Res. 44*: 63-69.

Hirai, M. and Nakai, S. (1977). Dicentric yields induced by γ-radiation and chromosome arm number in primates. *Mutat. Res. 43*: 147-158.

Hyre, H.M. and Smith, R.A. (1986). Immunological effects of honey bee (*Apis mellifera*) venom using BALB/c mice. *Toxicon. 24*: 435.

Kamo, I., Ito, Y. and Okuyama, S. (1970). Radioprotection by bee venom. *Nippon Igaku Hoshasen Gakkai Zasshi 29*: 1494-1500 (Ja) Tohoku Univ., Sendai, Japan.

Littlefield, L.G., Joiner, E.E., Colyer, S.P., Sayer, A.M. and Frome, E.L. (1988). Modulation of radiation-induced chromosome aberrations by DMSO, an OH radical scavenger. 1: Dose-response studies in human lymphocytes exposed to 220 KV X-rays. *Int. J. Radiat. Biol. 53*: 875-890.

Lorenzetti, O.J., Portenberry, B. and Busby, E. (1972). Influence of bee venom in the adjuvant induced arthritic rat model. *Res. Commun. Chem. Path. Pharmac. 4*: 339.

Luchnik, N.V. and Sevankaev, A.V. (1976). Radiation-induced chromosomal aberrations in human lymphocytes. I - Dependence on the dose of gamma-rays and an anomaly at low doses. *Mutat. Res. 36*: 363-378.

Natarajan, T.A. and Obe, G. (1982). Mutagenicity testing with cultured mammalian cells: Cytogenetic Assays. In: *Mutagenicity New Horizons in Genetic Toxicology* (Heddle, J.A., ed.). Academic Press, New York, pp. 194.

Patterson, R. and Valentine, M. (1982). Anaphylaxis and related allergic emergencies, including reactions due to insect stings. *J. Am. Med. Ass. 248*: 2632.

Peck, M.L. and O'Connor, R. (1974). Procamine and other basic peptides in the venom of the honeybee (*Apis mellifera*). *J. Agric. Fd. Chem. 22*: 51.

Peck, M.L., O'Connor, R., Johnson, T.J., Isbell, A.F., Martell, A.E., McLenden, G., Neff, R.D. and Wright, D.A. (1978). Radioprotective potential and chelating properties of glycyl-histamine an analog of histamine terminal peptides found in bee venom. *Toxicon 16*: 690-694.

Prasad, K.N. (1982). Acute radiation syndromes. In: *Radiation Biology* (Pizzarello, D.J., ed.). CRC Press, Inc. Boca Raton, Florida, pp. 205.

Prosser, J.S., White, C.M. and Edwards, A.A. (1979). The effect of oxygen concentration on the X-ray induction of chromosome aberrations in human lymphocytes. *Mutat. Res. 61*: 287-295.

Roots, R. and Okada, S. (1972). Protection of DNA molecules of cultured mammalian cells from radiation-induced single-strand scissions by various alcohols and SH compounds. *Int. J. Radiat. Biol. 21*: 329-342.

Roots, R. and Okada, S. (1975). Estimation of life times and diffusion distance of radicals involved in X-ray-induced DNA strand breaks or killing of mammalian cells. *Radiat. Res. 64*: 306-320.

Shipman, W.H. and Cole, L.J. (1967). Increased radiation resistance of mice injected with bee venom one day prior to exposure. *Nature Lond. 215*: 311-312.

Short, T.R. and Jackson, R. (1978). Treatment of canine arthritis with bee venom. *Proc. N. Am. Apiother. Soc. 1*: 86.

Smith, W.W., Budd, R.A. and Cornfield, J. (1966a). Estimation of radiation dose-reduction factor for β-mercaptoethylamine by endogenous spleen colony counts. *Radiat. Res. 27*: 363-368.

Smith, W.W., Brecher, G., Budd, R.A. and Fred, S. (1966b). Effects of bacterial endotoxin on the occurrence of spleen colonies in irradiated mice. *Rad. Res. 27*: 369-374.

Sokal, R.R. and Rohlf, F.J. (1969). *The principles and practice of statistics in biological research* (Emerson, R., Kennedy, D., Park, R.B., Beadle, G.W. and Whitaker, D.M., ed.). W.H. Freeman and Company, San Francisco, pp. 1-776.

Vick, J.A. and Shipman, W.H. (1972). Effects of whole bee venom and its fractions (apamine and melittin) on plasma cortisol levels in the dog. *Toxicon 10*: 337.

Von Bredow, J., Bradford, C., Froehlich, H. and Vick, J. (1978). Treatment of equine arthritis with bee venom. *Proc. N. Am. Apiother. Soc. 1*: 141.

Walden, T.L. Jr., Patchen, M.L. and MacVittie, T.J. (1988). Leucotriene-induced radioprotection of hematopoietic stem cells in mice. *Radiat. Res. 113*: 388-395.

Ward, J.F. (1981). Symposium on radical processes in radiobiology and carcinogenesis. Some biochemical consequences of the spatial distribution of ionizing radiation-produced free radicals. *Radiat. Res. 86*: 185-195.

Ward, J.F. (1985). Biochemistry of DNA lesions. *Radiat. Res. 104*: S103-S111.

Wolff, S. and Luippold, H.E. (1964). Chromosome splitting as revealed by combined X-ray and labelling experiments. *Exp. Cell Res. 34*: 548.

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