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The Protective Role of Royal Jelly Against Mutagenic Effect of Adriamycin and Gamma Radiation Separately and in Combination

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Abstract: The present study investigated the protective role of royal jelly against mutagenic effect of adriamycin and/or cobalt gamma radiation in rats. The pretreatment of royal jelly for ten days before adriamycin treatment or/and gamma exposure showed a decrease in their mutagenic effect. Royal jelly administration decreased the different types of chromosomal aberrations induced by adriamycin without significant levels in 2nd, 4th, 7th and 14th days after adriamycin treatment, except the total structural aberrations with gaps in 7th day decreased significantly at $p<0.05$. Also the royal jelly pretreatment induced decreases of DNA fragmentation induced by adriamycin with a highly significant level ($p<0.001$) in 4th day after adriamycin treatment and this significant decrease continued for two weeks after adriamycin treatment. The result showed that there was no significant decrease in the number of aberrations in 2nd and 4th days after irradiation, while the total number of structural aberrations decreased significantly at $p<0.01$ in 7th day and at $p<0.05$ in 14th days after gamma exposure. Also, the royal jelly pretreatment induced a significant decrease of the DNA fragmentation in 2nd day after gamma exposure at a level $p<0.01$ and this significant increase reached to $p<0.001$ level in 4th, 7th and 14th after exposure. The statistical analysis showed that the royal jelly pretreatment decreased the numbers of all aberrations induced in combined treatment (adriamycin plus gamma radiation) group without any significant level, except the total number of structural aberrations in 2nd day where it decreased at $p<0.05$. The royal jelly pretreatment induced a decrease of DNA fragmentation without significant level in 2nd and 4th days after combined treatment and this decrease reached to the significant level at $p<0.001$ in 7th day and at $p<0.05$ in 14th day.

Key words: Royal jelly, adriamycin, gamma radiation, chromosomal aberrations, DNA fragmentation

INTRODUCTION

The cancer is the most serious disease that causes death all over the world; it is also known as malignant tumors and neoplasms. It is defined as an abnormal growth of cells which tend to proliferate in an uncontrolled way (Grandis and Sok, 2004).

Adriamycin is widely used as an antineoplastic drug in the treatment of various tumors. It is used for the treatment of solid tumors such as those arising in the breast, bile ducts, liver, esoohagus, soft tissue sarcomas and non-Hodgkin's lumphoma (Prahalathan *et al*., 2006). Also, cobalt gamma radiation is a type of ionizing radiation which has been used to treat human malignancies. Gamma radiation therapy was used either alone or in combination with surgery and/or chemotherapeutic modalities (Eriksson, 2006).

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The intracellular effect of adriamycin includes free radical formation (Wagner *et al*., 2005), inhibition of DNA topoisomerase II (Gruber *et al*., 2005) and nucleotide intercalation (Jain *et al*., 2005). The DNA-damaging action of adriamycin correlated well with its antiproliferative effect on the cells. This action is appearing as DNA fragmentation, chromosomal aberrations and sister chromatid exchanges.

The radiotherapy treatment of human cancer is often limited by its several side effects; one of its most serious effects is the formation of the secondary tumors (Orta *et al*., 2006). Gamma radiation induced the increasing of intracellular reactive oxygen species in un-irradiated cells as well as the irradiated cells. The formation of reactive oxygen species may lead to the induction of genomic instability and neoplastic transformation (Goldberg and Lehnert, 2002; Prevost *et al*., 2004).

The DNA-damaging action of adriamycin or/and gamma is appearing as numerical aberrations (Islam *et al*., 1993; Touil *et al*., 2000) and structural aberrations, especially chromatid gaps and breaks (Klimova *et al*., 1990a, b; Ponnaiya *et al*., 1997; Hone *et al*., 2006), sister chromatid exchange (Ratain and Rowley, 1992; Osmak and Horvat, 1992), micronuclei formation (Antoshchina *et al*., 2005) and formation of small DNA fragments (Fram *et al*., 1983; Campa *et al*., 2004, 2005).

Many of the natural and artificial substances are used to reduce the mutagenic effect of chemotherapeutic drugs like adriamycin and radiotherapy like cobalt gamma radiation. These substances have antitumor effect beside their protective effect, so they increase the efficiency of the therapy without any side effects on the normal cells (Swellam *et al*., 2003). Royal jelly, one of honey products, stimulated cell survival, cell growth and cell differentiation and it also had a cytotoxic effect on the carcinoma cells (Salazar-Olivo and Paz-Gonzalez, 2005). Royal jelly has an anti-tumor effect (Bincoletto *et al*., 2005) and antimetastatic effect (Kimura *et al*., 2003).

The aim of this study is to investigate the protective role of royal jelly against chromosomal aberrations and DNA fragmentation induced in rat after adriamycin treatment or/and cobalt gamma exposure.

MATERIALS AND METHODS

Experimental Animals

This study was carried out between January 2005 and January 2006; 150 adult male Wistar rats weighting 150-200 g were used in this study. The animals were provided by the animal house of National Research Centre (NRC), Dokki, Cairo, Egypt. Food and water were given *ad libitum*. The animals were classified into seven groups (Table 1).

Anticancer Drug

Adriamycin (doxorubicin hydrochloride, Pharmacia Co.) is an anthracycline antibiotic. Adriamycin 50 mg vial was dissolved in 25 mL double distilled water to get a concentration of 2 mg mL^{-1}.

Irradiation

A cobalt-60 source of gamma radiation was used. The animals were irradiated in Eastern Regional Radioisotope Center for The Arab Countries, Dokki, Cairo, Egypt. The animals were irradiated once with a dose of 200 rad (2 Gy).

Royal Jelly

Pure Royal jelly capsules were used in this study (Pharco Pharmaceuticals Co., Egypt). Each capsule (1000 mg) was dissolved in 10 mL double distilled water to get a concentration of 100 mg mL^{-1}.

Table 1: The classification of animal groups under investigation

Group	Time of sacrifice after last treatment	No. of animal	Treatment
1	-ve control	6	No treatment
2	2nd day	6	Adriamycin[(2)] (5 mg kg^{-1}) (Galli *et al*., 2001)
	4th day	6	
	7th day	6	
	14th day	6	
3	2nd day	6	Royal jelly + Adriamycin (Adriamycin following 10 days of treatment with royal jelly)
	4th day	6	
	7th day	6	
	14th day	6	
4	2nd day	6	^{60}Co gamma radiation[(3)] (200 rad)
	4th day	6	
	7th day	6	
	14th day	6	
5	2nd day	6	Royal jelly + ^{60}Co gamma radiation (radiation following 10 days of treatment with royal jelly)
	4th day	6	
	7th day	6	
	14th day	6	
6	2nd day	6	Adriamycin + ^{60}Co gamma radiation (radiation following 20 hours of treatment with adriamycin)
	4th day	6	
	7th day	6	
	14th day	6	
7	2nd day	6	Royal jelly + Adriamycin + ^{60}Co gamma radiation (radiation following 20 hours of treatment with adriamycin preceded by 10 days of treatment with royal jelly)
	4th day	6	
	7th day	6	
	14th day	6	

(1): Royal jelly was given by gavage; (2): Adriamycin was given by intraperitoneal injection; (3): Whole body radiation

Chromosome Aberration Study

Chromosome Preparation

The chromosomes were prepared for microscopic examination according to Yosida *et al*. (1971). The animals were injected intraperitoneally with 0.5% colchicine (15 mg kg^{-1}). After 2 h, the animals were sacrificed by cervical dislocation. The femurs were removed and cleaned from muscles. Bone-marrow cells were aspirated from the femur using saline solution (0.9% NaCl). The bone-marrow cells were centrifuged at 1200 rpm for 10 min. The cell pellet was resuspended in 5 mL of warmed hypotonic solution (0.075 M KCl) and incubated for 30 min at 37°C. The cells were centrifuged at 1200 rpm for 10 min. The pellet was resuspended in 5 mL of the fixative (3:1 methanol/glacial acetic acid) and centrifuged at 1200 rpm for 10 min. The cells were fixed at least three times before dropping onto slides, dipped in 70% ethyl alcohol and dried over a hot plate.

Slides Staining

The slides were stained with 10% Giemsa solution (Giemsa stain/Sorenson's buffer) for 30 min and washed in Sorenson's buffer for 1 min. Then, the slides were immersed in distilled water and air dried.

Scoring

For chromosomal aberration, 50 well spread metaphases were examined for each animal. Both structural aberrations (deletions, breaks, gaps and fragments) and numerical aberrations (polyploidy and aneuploidy) were recorded.

DNA Fragmentation

DNA fragmentation was quantified by diphenylamine (DPA) method according to Gibb *et al*. (1997) with little modifications. About 0.2 g of rat liver was homogenized in 5 mL of saline solution

(0.9% NaCl). Two milliliter of the liver homogenization were centrifuged at 1500 rpm for 10 min at 4°C. The cell pellet was resuspended in 1600 µL of 0.01 M PBS and then 1400 µL of ice cold lysis buffer (5 mM Tris + 20 mM EDTA + 0.5% Triton X-100, pH 8.0) were added to the suspension and incubated on ice for 15 min to isolate DNA. The suspension was centrifuged at 13000 rpm for 20 min at 4°C.

After centrifugation, the supernatant which contains the fragmented DNA was transferred to a new tube. The pellet which contains the intact DNA was resuspended in 3 mL of TE buffer (10 mM Tris + 1 mM EDTA). Three milliliter of 10% Trichloroacetic Acid (TCA) were added to both fragmented and intact DNA and incubated at room temperature for 10 min.

The TCA precipitations were centrifuged at 2000 rpm for 15 min at 4°C. After discarding the supernatants, DNA hydrolyte by resuspended the pellets in 1400 µL of 5% TCA and then boiled at 100°C for 15 min and allowed to cool at room temperature. The suspensions were centrifuged at 1500 rpm for 15 min at 4°C and 1 mL of supernatants was transferred to new glass tubes. Two milliliter of diphenylamine (DPA) were added to the supernatants and then incubated overnight at 30°C. Blank was prepared by adding 1 mL of 5% TCA to 2 mL of DPA.

The amounts of both fragmented and intact DNA were determined by spectrophotometer at 600 nm. The fragmentation of DNA was calculated according to the equation:

$$\text{DNA fragmentation} = \frac{\text{OD of fragmented DNA}}{\text{OD of fragmented DNA} + \text{OD of intact DNA}} \times 100$$

Statistical Analysis

Chi-square test was used for statistical analysis of the results for the chromosomal aberrations, while the t-test of the difference between means was used for statistical analysis of results for DNA fragmentation (Othman, 2000).

RESULTS

Chromosomal Aberrations

The structural chromosomal aberrations investigated in this study included gaps (Fig. 2), deletions (Fig. 3), breaks (Fig. 4, 5) and fragments (Fig. 6), while the numerical abnormalities included the polyploid (Fig. 7) and aneuploid cells (Fig. 8, 9). Figure 1 shows a metaphase spread of rat bone marrow cell showing normal chromosomes.

From Table 2, it was observed that the total numbers of structural and numerical chromosomal aberrations induced in rat bone-marrow cells after treatment with adriamycin increased with significantly levels in all subgroups. The statistical comparison between the subgroups treated with adriamycin alone and those treated with adriamycin after royal jelly pretreatment showed that the different types of aberrations decreased without significant level, except the total structural aberrations with gap in 7th day subgroup that decreased significantly at $p<0.05$.

Table 3 showed that the total numbers of different structural and numerical chromosomal aberrations induced in rat bone-marrow cells after exposure to gamma radiation increased significantly at $p<0.001$ in all subgroups. Regarding to the effect of royal jelly pretreatment, it was shown that there was no significant decrease in the number of aberrations in 2nd and 4th days after irradiation, while the number of the breaks in 7th day and the number of the deletions in 14th day decreased significantly at $p<0.05$. The total number of structural aberrations decreased significantly at $p<0.01$ in 7th day and at $p<0.05$ in 14th day after gamma irradiation.

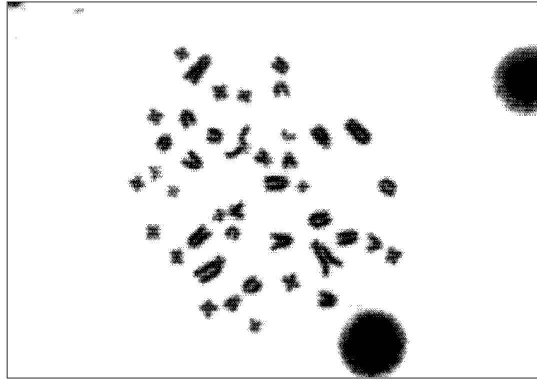

Fig. 1: Metaphase spread of rat bone marrow cell showing normal chromosomes. (Giemsa stain, X 1000)

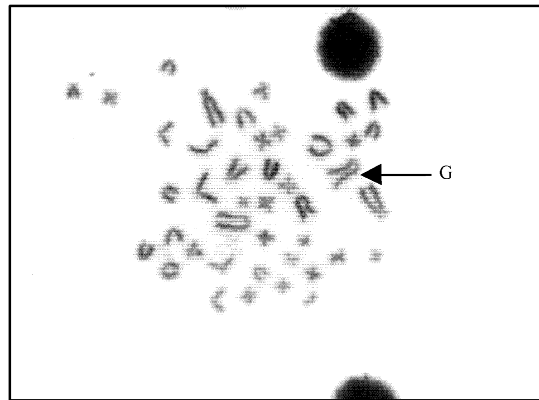

Fig. 2: Metaphase spread of rat bone marrow cell showing a chromatid gap (G). (Giemsa stain, X 1000)

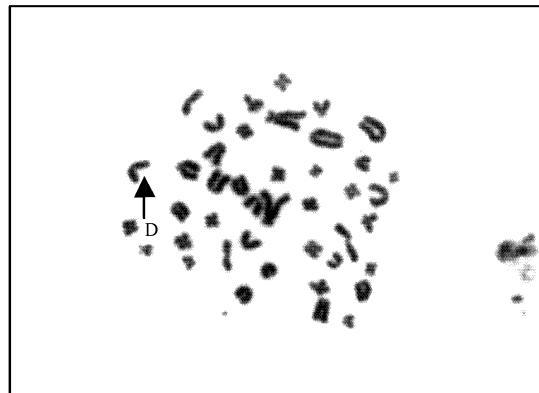

Fig. 3: Metaphase spread of rat bone marrow cell showing deletion (D). (Giemsa stain, X 1000)

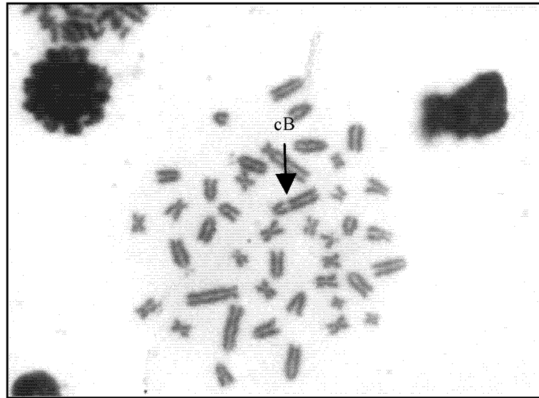

Fig. 4: Metaphase spread of rat bone marrow cell showing a chromosome break (cB). (Giemsa stain, X 1000)

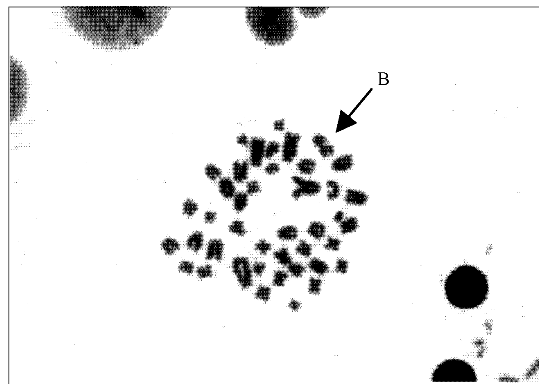

Fig. 5: Metaphase spread of rat bone marrow cell showing a chromatid break (B). (Giemsa stain, X 1000)

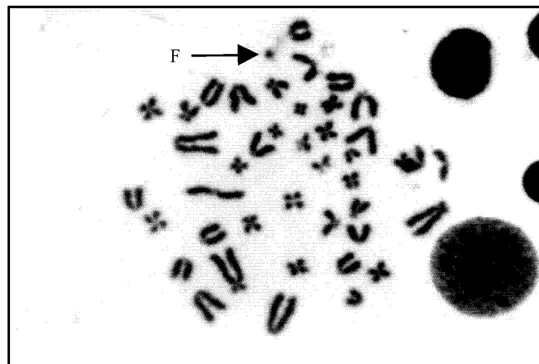

Fig. 6: Metaphase spread of rat bone marrow cell showing a fragment (F). (Giemsa stain, X 1000)

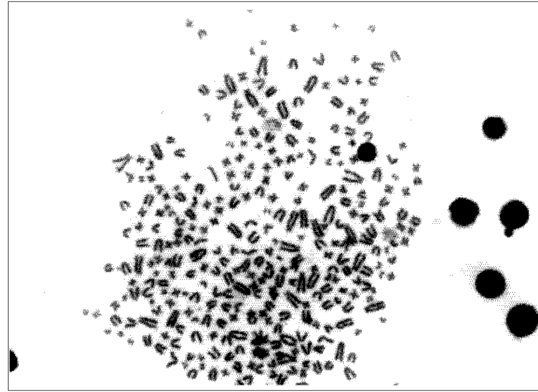

Fig. 7: Metaphase spread of rat bone marrow cell showing a polyploid cell (Giemsa stain, X 1000)

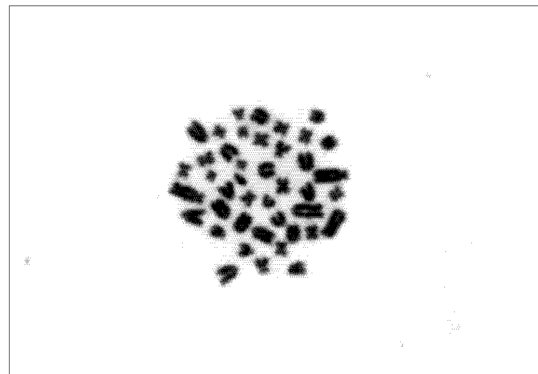

Fig. 8: Metaphase spread of rat bone marrow cell showing an aneuploid cell (2n-1) (Giemsa stain, X 1000)

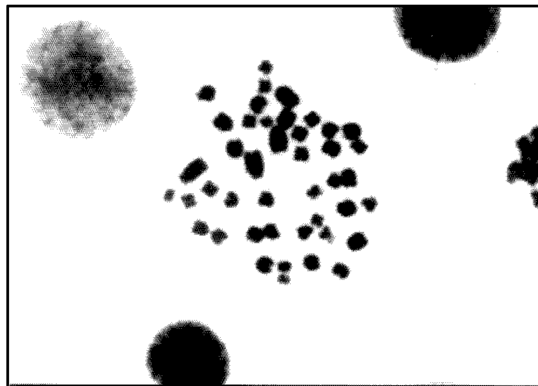

Fig. 9: Metaphase spread of rat bone marrow cell showing an aneuploid cell (2n+1) (Giemsa stain, X 1000)

Table 2: The effect of royal jelly on chromosomal aberrations induced by adriamycin in rat bone-marrow cells

Time of sacrifice after last treatment	Groups	No. of animals	No. of examined cells	Structural aberrations						Numerical aberrations		
				Del.	Gap	Br.	Frag.	Total with gap	Total without gap	Poly.	Aneu.	Total
	Control	6	300	1	7	2	1	11	4	2	1	3
2nd day	ADR	6	300	21c	27c	14b	15c	77c	50c	12b	27c	39c
	ADR + R.j.	6	300	15	26	13	9	63	37	6	25	31
4th day	ADR	6	300	18c	29c	12b	10b	69c	40c	9a	24c	33c
	ADR + R.j.	6	300	12	24	11	7	54	30	5	15	20
7th day	ADR	6	300	15c	25b	9a	10b	59c	34c	4	15c	19c
	ADR + R.j.	6	300	8	18	9	6	41d	23	3	11	14
14th day	ADR	6	300	9a	16	4	3	32c	16b	2	9a	11a
	ADR + R.j.	6	300	4	14	3	1	22	8	3	7	10

ADR: Adriamycin; R.j: Royal jelly; Del: Deletion; Br: Break; Frag: Fragment; Poly: Polyploidy; Aneu: Aneuploidy. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$, a, b and c: The significant levels of the comparison between the control and treated groups. d: $p<0.05$, d: The significant level of the comparison between adriamycin and adriamycin plus royal jelly groups

Table 3: The effect of royal jelly on chromosomal aberrations induced by cobalt gamma radiation in rat bone-marrow cells

Time of sacrifice after last treatment	Groups	No. of animals	No. of examined cells	Structural aberrations						Numerical aberrations		
				Del.	Gap	Br.	Frag.	Total with gap	Total without gap	Poly.	Aneu.	Total
	Control	6	300	1	7	2	1	11	4	2	1	3
2nd day	RAD	6	300	30c	33c	23c	19c	105c	72c	13b	29c	42c
	RAD + R.j.	6	300	25	31	19	15	90	59	10	24	34
4th day	RAD	6	300	23c	32c	17c	13b	85c	53c	11a	25c	36c
	RAD + R.j.	6	300	19	29	15	10	73	44	9	22	31
7th day	RAD	6	300	22c	29c	16c	13b	80c	51c	7	21c	28c
	RAD + R.j.	6	300	16	22	6 d	7	51e	29e	5	18	23
14th day	RAD	6	300	12b	26c	5	6	49c	23c	2	16c	18c
	RAD + R.j.	6	300	3 d	21	4	2	30 d	9 d	4	11	15

RAD: Radiation; R.j: Royal jelly; Del: Deletion; Br: Break; Frag: Fragment; Poly: Polyploidy; Aneu: Aneuploidy. a: $p<0.05$; b: $p<0.01$; c: $p<0.001$, a, b and c: The significant levels of the comparison between the control and treated groups, d: $p< 0.05$; e: $p<0.01$, d and e: The significant levels of the comparison between ^{60}Co gamma radiation and radiation plus royal jelly groups

Table 4: The effect of royal jelly on chromosomal aberrations induced by combined treatment in rat bone-marrow cells

Time of sacrifice after last treatment	Groups	No. of animals	No. of examined cells	Structural aberrations						Numerical aberrations		
				Del.	Gap	Br.	Frag.	Total with gap	Total without gap	Poly.	Aneu.	Total
	Control	6	300	1	7	2	1	11	4	2	1	3
2nd day	Com	6	300	33c	52c	28c	20c	133c	81c	30c	33c	63c
	Com + R.j.	6	300	28	42	20	18	108d	66	29	30	59
4th day	Com	6	300	24c	47c	18c	15c	104c	57c	13b	30c	43c
	Com + R.j.	6	300	17	38	17	14	86	48	10	25	35
7th day	Com	6	300	23c	42c	16c	14c	95c	53c	8	24c	32c
	Com + R.j.	6	300	18	37	11	10	76	39	6	20	26
14th day	Com	6	300	13b	33c	9a	10b	65c	32c	6	17c	23c
	Com + R.j.	6	300	10	29	8	4	51	22	5	15	20

Com:combined treatment; R.j: Royal jelly; Del: deletion; Br: Break; Frag: Fragment; Poly: Polyploidy; Aneu:Aneuploidy, a: $p<0.05$; b: $p<0.01$; c: $p<0.001$, a, b and c: The significant levels of the comparison between the control and treated groups. d: $p<0.05$ d: The significant level of the comparison between combined treatment and combined treatment plus royal jelly groups

The total number of structural and numerical chromosomal aberrations increased after combined treatment with a highly significant level at $p<0.001$ in all subgroups (Table 4), except the total number of structural aberrations without gap in 14th day increased significantly at $p<0.01$. The statistical

comparison between the subgroups treated with combined treatment and the subgroups treated with combined treatment plus royal jelly showed that the numbers of all aberrations decreased without any significant level, except the total number of structural aberrations in 2nd day, it decreased with a low significant level ($p<0.05$).

DNA Fragmentation

Table 5 shows the effect of adriamycin with or without royal jelly on DNA fragmentation. The statistical comparison between the negative control group and adiamycin-treated subgroups showed the significant increase of the means of DNA fragmentation in all days at $p<0.001$. The statistical comparison between the subgroups treated with adriamycin alone and those treated with adriamycin after royal jelly pretreatment showed a high significant decrease at $p<0.001$ in the means of DNA fragmentation in 4th, 7th and 14th days after adriamycin treatment but the significant level was absent in 2nd day.

The statistical comparison between the negative control and the subgroups exposed to gamma radiation showed the high significant increase of the means of DNA fragmentation in all days at $p<0.001$. The statistical comparison between the subgroups exposed to gamma radiation alone and those exposed to gamma radiation after royal jelly pretreatment showed the significant reduction in the means of DNA fragmentation in all days. In 2nd day after irradiation, the significant level of the reduction was at $p<0.01$, while in 4th, 7th and 14th days, the significant level was at $p<0.001$ (Table 6).

The high significant increase of the means of DNA fragmentation in all days was noted in the statistical comparison between the negative control and the subgroups after combined treatment

Table 5: The effect of royal jelly on the DNA fragmentation induced by adriamycin in rat liver cells

Time of sacrifice after last treatment	Group	No. of animals	DNA fragmentation percent Range	Mean±SD
	Control	6	2.426-3.864	2.9897±0.445
2nd day	ADR	6	13.250-21.24	17.3750±2.38c
	ADR + R.j.	6	10.722-17.746	14.4020±2.54
4th day	ADR	6	14.215-17.949	16.3870±1.224c
	ADR + R.j.	6	8.160-12.77	10.6390±1.83f
7th day	ADR	6	11.609-17.768	15.4920±1.93c
	ADR + R.j.	6	8.827-11.537	10.5180±0.95f
14th day	ADR	6	12.630-15.262	13.9180±0.954c
	ADR + R.j.	6	8.151-11.267	9.2480±1.128f

ADR: Adriamycin; R.j: Royal jelly; SD: Standard Deviation, c: $p<0.001$,c: The significant level of the comparison between the control and treated groups. f:. $p<0.001$, f: The significant level of the comparison between adriamycin and adriamycin plus royal jelly groups

Table 6: The effect of royal jelly on DNA fragmentation induced by cobalt gamma radiation in rat liver cells

Time of sacrifice after last treatment	Group	No. of animals	DNA fragmentation percent Range	Mean±SD
	Control	6	2.426-3.864	2.9897±0.445
2nd day	RAD	6	19.520-28.55	22.0200±1.708c
	RAD + R.j.	6	11.240-19.92	16.2030± 2.832e
4th day	RAD	6	18.761-25.471	21.0500±2.112c
	RAD + R.j.	6	13.495-17.849	15.4800±1.57f
7th day	RAD	6	18.314-22.53	20.0200±1.398c
	RAD + R.j.	6	10.111-15.013	12.8800±1.842f
14th day	RAD	6	16.412-19.91	18.2100±1.245c
	RAD + R.j.	6	8.386-13.967	12.2980±1.836f

RAD: radiation; R.j: Royal jelly; SD: Standard Deviation, c: $p<0.001$,c: The significant level of the comparison between the control and treated groups. e:. $p<0.01$; f: $p<0.001$, e and f: The significant level of the comparison between ^{60}Co gamma radiation and ^{60}Co gamma plus royal jelly groups

Table 7: The effect of royal jelly on DNA fragmentation induced by combined treatment in rat liver cells

Time of sacrifice after last treatment	Group	No. of animals	DNA fragmentation percent Range	Mean±SD
	Control	6	2.426-3.864	2.9897±0.445
2nd day	Com	6	20.746-27.722	24.4030±2.545c
	Com + R.j.	6	20.198-27.748	22.7240±2.76
4th day	Com	6	18.237-27.85	22.7430±4.072c
	Com + R.j.	6	17.774-23.316	19.7130±1.879
7th day	Com	6	19.734-25.466	22.5310±2.326c
	Com + R.j.	6	14.418-20.508	18.0320±2.091e
14th day	Com	6	16.200-28.683	21.5840±3.849c
	Com + R.j.	6	14.046-21.283	17.2090±2.537d

Comb.: combined treatment; R.j: Royal jelly; SD: standard deviation,c: p<0.001, c: The significant level of the comparison between control groups and treated groups, d:. p<0.05; e: p<0.01, d and e: The significant level of the comparison between combined treatment and combined treatment plus royal jelly groups

($p<0.001$). While the comparison between the subgroups after combined treatment and after combined treatment plus royal jelly showed a decrease without any significant level in 2nd and 4th days after treatment. The significant decrease at $p<0.01$ appeared in 7th day, while it was at $p<0.05$ in 14th day after treatment (Table 7).

DISCUSSION

Chemotherapy and radiotherapy are widely used for cancer treatment. The basic action mode of these both therapies is making lesions in the malignant cells led to the apoptosis. But they are also possibly related to the induction of carcinogenesis and mutagenesis in the normal cells. Some natural and artificial substances are used to reduce the toxic effect of chemotherapy and radiotherapy; these substances are known as antimutagenic agents or protective agents.

The present study was designed to investigate the protective role of royal jelly against mutagenicity induced in rats after treatment with adriamycin or/and exposure to cobalt gamma radiation. The chromosomal aberrations; numerical and structural aberrations and DNA fragmentation are the parameters used in this study.

Adriamycin and gamma radiation induced DNA double strand breaks, this action is correlated with their antiproliferative effect on the cells (Nishio *et al*., 1982). DNA double strand breaks can induce cell cycle arrest, induction of apoptosis or mitotic cell death caused by loss of genomic material (Olive, 1998). These different modes determine the therapeutic success of cancer treatment but, adversely, also side effects are possibly caused in normal tissue (Rothkamm and Löbrich, 2002). The non- or misrepair of DNA double-strand breaks lead to chromosomal aberrations especially the chromatid aberrations (Klimova *et al*., 1990a). DNA double strand breaks induced the cell cycle arrest by activation of p53 (Jackson and Pereira-Smith, 2006). The adriamycin-induced p53 activation arrested the cell cycle at G_2-phase or S-phase (Minemoto *et al*., 2001) whereas exposure to gamma radiation induced the cell cycle arrest at G_2/M checkpoint (Yuan *et al*., 2003). The activation of p53 is followed by the formation of DNA fragments (Wang *et al*., 2004). DNA fragmentation is used to detect the apoptosis (Zhan *et al*., 2001).

The present results showed that the separated and combined treatment with adriamycin and/or cobalt gamma radiation induced the significant increase in the frequencies of chromosomal aberrations, structural and numerical, in all treated groups when compared with those in negative controls.

The cytogenetic measurement of adriamycin mutagenic effect showed induction of the structural chromosomal aberrations in the previous studies, especially the chromatid aberrations (Klimova *et al*., 1990a, b; Khlusova *et al*., 1992). Regarding the numerical chromosomal aberrations, our findings agreed with result of Aly *et al*. (1999). They recorded a significant increase of trisomy chromosomes 7 and 17 in blood cultures after exposure to adriamycin.

Chromosomal aberrations are also considered to be highly sensitive biological indicators for the effects of ionizing radiation (Varanda and Takahashi, 1993). Ponnaiya *et al*. (1997) reported an increase in the chromatid-type gaps and breaks in human epithelial cells after exposure to gamma radiation. The numerical chromosomal aberrations were also induced by gamma ionized radiation (Devi and Satyamitra, 2005), especially an increase in the number of aneuploid cells. Newcomb *et al*. (1989) recorded tumor production in mice treated with ionized gamma radiation characterized by trisomy of chromosome 15, while, Kang *et al*. (2006) recorded the increase in the copy number of chromosomes 4 and 5 and loss in the copy number of chromosomes 11, 16 and 19.

The activation of p53 is followed by the formation of DNA fragments (Wang *et al*., 2004). DNA fragmentation is used to detect the apoptosis (Zhan *et al*., 2001). Adriamycin-induced DNA fragmentation was reported in rat cells by Jang *et al*. (2004) and Kim *et al*. (2005) and in mouse cells by Dominguez-Rodriguez *et al*. (2001). Also in this study, DNA fragmentation was increased in all days of experiment with a highly significant level. The effect of ^{60}Co gamma radiation on the formation of DNA fragments was similar to the adriamycin effect. This result agreed with the results of Liu and Zhang (2003), who measured the DNA fragmentation in mouse bone-marrow cells after irradiated with ^{60}Co gamma radiation and Campa *et al*. (2004), who reported the formation of DNA fragments in Chinese hamster V79.

The combined therapy has a genotoxic effect more than either treatment given alone (Jagetia and Nayak, 2000; Dutta *et al*., 2005; Bergs *et al*., 2006). The strong mutagenic effect of combined treatment showed in this study agreed with the results of Lichter and Lawrence (1995), Bilban-Jakopin and Bilban (2001) and Lègal *et al*. (2002).

Royal jelly, a substance produced by bee worker, used as a treatment for many diseases (Leigh, 1999) like reduction of total lipids and cholesterol level in the serum and liver, improvement of the highly coagulant status of blood (Shen *et al*., 1995; Vittek, 1995) and suppression of the development of atopic dermatitis-like skin lesions (Taniguchi *et al*., 2003) and it has anti-allergic effect (Oka *et al*., 2001; Okamoto *et al*., 2003), anti-fatigue effect (Kamakura *et al*., 2001) and immunomodulatory effect (Sver *et al*., 1996). Royal jelly also has anti-tumor effect (Tamura *et al*., 1987; Bincoletto *et al*., 2005; Kimura *et al*., 2003; Salazar-Olivo and Paz-Gonzalez, 2005).

In the present study, the pretreatment of royal jelly for ten days before adriamycin treatment decreased the percentages of total structural and numerical chromosomal aberrations induced by adriamycin in all days of experiment. Also the protective effect of royal jelly against genotoxicity of adriamycin appeared in the study of DNA fragmentation. When the royal jelly was administrated for ten days before adriamycin treatment, DNA fragmentation decreased with a highly significant level ($p<0.001$) which appeared after four days from the last treatment and continued for two weeks.

The protective effect of royal jelly appeared clear when it combined with cobalt gamma radiation (^{60}Co). The total number of structural chromosomal aberrations decreased significantly after royal jelly pretreatment when it was compared with those in groups exposed to cobalt gamma radiation (^{60}Co) alone. The numerical chromosomal aberrations also decreased in the royal jelly pretreatment group but did not reach any significant level. On the other hands, the royal jelly pretreatment induced a significant decrease in DNA fragmentation

The royal jelly had a slightly protective effect against the genotoxicity of adriamycin/^{60}Co gamma radiation combined treatment and its effect was less than that in separated treatment groups. There was no significant difference between the total numbers of chromosomal aberrations induced by combined treatment with and without royal jelly pre-treatment. On the other hands, the significant levels appeared between DNA fragmentation with and without royal jelly pretreatment was $p<0.01$ after one week and $p<0.05$ after two weeks from the last treatment. The slightly protective effect of royal jelly with the combined treatment may be due to the increase of the cell sensitivity to ionizing radiation by the chemotherapeutic drug, adriamycin, beside its direct effect on dividing cells (Lichter and Lawrence, 1995).

CONCLUSION

The protective effect of royal jelly may be due to its component vitamins, antioxidant vitamins A, E and C, vitamin D and vitamin B complex (Leigh, 1999). These vitamins themselves had anticancer effect (Pour and Lawson, 1984; Liu *et al*., 2000; Klaassen and Braakhuis, 2002; Giovannucci *et al*., 2006; Zou *et al*., 2006) and protective effects against the genotoxicity of chemotherapy and radiotherapy (Parchure *et al*., 1984; Chen and Pan, 1988; Sarma and Kesavan, 1993; Antunes and Takahashi, 1998, 1999; Tavares *et al*., 1998; Konopacka *et al*., 2002; Gülkaç *et al*., 2004; Kocak *et al*., 2004). Royal jelly also contained protein fractions (Salazar-Olivo and Paz-Gonzalez, 2005) that they had also anticancer effects.

REFERENCES

Aly, M.S., O.E. Othman and S.M. El Nahas, 1999. Specific numerical chromosomal aberrations induced by adriamycin. Environ. Mol. Mutag., 33 (2): 161-166.

Antoshchina, M.M., N.I. Riabchenko, V.A. Nasonova, E.V. Fesenko and I.I. Pelevina, 2005. The genome instability in the descendants of the Chinese hamster of the cells, irradiated by the low dose and by various intensities of gamma-radiation. Radiats Biol. Radioecol., 45 (3): 291-293.

Antunes, L.M. and C.S. Takahashi, 1998. Effects of high doses of vitamins C and E against doxorubicin-induced chromosomal damage in Wistar rat bone marrow cells. Mutat. Res., 419 (1-3): 137-143.

Antunes, L.M. and C.S. Takahashi, 1999. Protection and induction of chromosomal damage by vitamin C in human lymphocyte cultures. Teratog Carcinog Mutag., 19 (1): 53-59.

Bergs, J.W., N.A. Franken, T. Ten Cate, C. Van Bree and J. Haveman, 2006. Effects of cisplatin and gamma-irradiation on cell survival, the induction of chromosomal aberrations and apoptosis in SW-1573 cells. Mutat. Res., 594 (1-2): 148-154.

Bilban-Jakopin, C. and M. Bilban, 2001. Genotoxic effects of radiotherapy and chemotherapy on circulating lymphocytes in patients with Hodgkin's disease. Mutat. Res., 497 (1-2): 81-88.

Bincoletto, C., S. Eberlin, C.A. Figueiredo, M.B. Luengo and M.L. Queiroz, 2005. Effects produced by royal jelly on haematopoiesis: Relation with host resistance against Ehrlich ascites tumour challenge. Int. Immunopharmacol., 5 (4): 679-688.

Campa, A., G. Esposito, M. Belli, G. Simone and M.A. Tabocchini, 2004. DNA fragmentation in V79 cells irradiated with light ions as measured by pulsed-field gel electrophoresis. II. Simulation with a generalized broken stick model. Int. J. Radiat Biol., 80 (3): 229-238.

Campa, A., F. Ballarini, M. Belli, R. Cherubini, V. Dini, G. Esposito, W. Friedland, S. Gerardi, S. Molinelli, A. Ottolenghi, H. Paretzke, G. Simone and M.A. Tabocchini, 2005. DNA DSB induced in human cells by charged particles and gamma rays: Experimental results and theoretical approaches. Int. J. Radiat Biol., 81 (11): 841-854.

Chen, G. and Q.C. Pan, 1988. Potentiation of the antitumor activity of cisplatin in mice by 3-aminobenzamide and nicotinamide. Cancer Chemother. Pharmacol., 22 (4): 303-307.

Devi, P.U. and M. Satyamitra, 2005. Tracing radiation induced genomic instability *in vivo* in the haemopoietic cells from fetus to adult mouse. Br. J. Radiol., 78 (934): 928-933.

Dominguez-Rodriguez, J.R., P.C. Gomez-Contreras, G. Hernandez-Flores, J.M. Lerma-Diaz, A. Carranco, R. Cervantes-Munguia, S. Orbach-Arbouys and A. Bravo-Cuella, 2001. *In vivo* inhibition by antioxidants of adriamycin-induced apoptosis in murine peritoneal macrophages. Anticancer Res., 21 (3B): 1869-1872.

Dutta, A., A. Chakraborty, A. Saha, S. Ray and A. Chatterjee, 2005. Interaction of radiation and bleomycin-induced lesions and influence of glutathione level on the interaction. Mutagenesis, 20 (5): 329-335.

Eriksson, D., 2006. Experimental radioimmunotherapy and effector mechanism. Ph.D Thesis, Diagnostic Radiology and Radiation Physics, Umeå University, Sweden. Web site: http://www.diva-portal.org/diva/getDocument?urn_nbn_

Fram, R.J., E.M. Egan and D.W. Kufe, 1983. Accumulation of leukemic cell DNA strand breaks with adriamycin and cytosine arabinoside. Leuk Res., 7 (2): 243-249.

Galli, L.G., R.A. Volpini, R.S. Costa, C.G. da Silva and T.M.Coimbra, 2001. Tubular cell lesion, albuminuria and renal albumin handling in rats treated with adriamycin. Ren Fail., 23 (5): 693-703.

Gibb, R.K., D.D. Taylor, T. Wan, D.M. O'Connor, D.L. Doering and C. Gerçel-Taylor, 1997. Apoptosis as a measure of chemosensitivity to cisplatin and taxol therapy in ovarian cancer cell lines. Gynecol. Oncol., 65 (1): 13-22.

Giovannucci, E., Y. Liu, E.B. Rimm, B.W. Hollis, C.S. Fuchs, M.J. Stampfer and W.C. Willett, 2006. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J. Natl. Cancer Inst., 98 (7): 451-459.

Goldberg, Z. and B.E. Lehnert, 2002. Radiation-induced effects in unirradiated cells: A review and implications in cancer. Int. J. Oncol., 21 (2): 337-349.

Grandis, J.R. and J.C. Sok, 2004. Signaling through the epidermal growth factor receptor during the development of malignancy. Pharmacol. Ther., 102 (1): 37-46.

Gruber, B.M., E.L. Anuszewska, I. Bubko, A. Gozdzik, W. Priebe and I. Fokt, 2005. Relationship between topoisomerase II-DNA cleavable complexes, apoptosis and cytotoxic activity of anthracyclines in human cervix carcinoma cells. Anticancer Res., 25 (3B): 2193-2198.

Gülkaç, M.D., G. Akpinar, H. Üstün and A.Ö. Kanli, 2004. Effects of vitamin A on doxorubicin-induced chromosomal aberrations in bone marrow cells of rats. Mutagenesis, 19 (3): 231-236.

Hone, P., D. Lloyd, M. Szluinska and A. Edwards, 2006. Chromatid damage in human lymphocytes is not affected by 50 HZ electromagnetic fields. Radiat. Prot. Dosimetry. [Epub ahead of print]

Islam, M.Q., I. Kopf, A. Levan, S. Granberg, L.G. Friberg and G. Levan, 1993. Cytogenetic findings in 111 ovarian cancer patients: Therapy-related chromosome aberrations and heterochromatic variants. Cancer Genet. Cytogenet., 65 (1): 35-46.

Jackson, J.G. and O.M. Pereira-Smith, 2006. Primary compensatory role for RB family members at cell cycle gene promoters that are deacetylated and downregulated in doxorubicin-induced senescence of breast cancer cells. Mol. Cell Biol., 26 (7): 2501-2510.

Jagetia, G.C. and V. Nayak, 2000. Effect of doxorubicin on cell survival and micronuclei formation in HeLa cells exposed to different doses of gamma-radiation. Strahlenther. Onkol., 176 (9): 422-428.

Jain, M., S.K. Barthwal, R. Barthwal and C. Govil, 2005. Restrained molecular dynamics studies on complex of adriamycin with DNA hexamer sequence d-CGATCG. Arch. Biochem. Biophys., 439 (1): 12-24.

Jang, Y.M., S. Kendaiah, B. Drew, T. Phillips, C. Selman, D. Julian and C. Leeuwenburgh, 2004. Doxorubicin treatment *in vivo* activates caspase-12 mediated cardiac apoptosis in both male and female rats. FEBS. Lett., 577 (3): 483-490.

Kamakura, M., N. Mitani, T. Fukuda and M. Fukushima, 2001. Antifatigue effect of fresh royal jelly in mice. J. Nutr. Sci. Vitaminol., 47 (6): 394-401.

Kang, H.M., J.J. Jang, C. Langford, S.H. Shin, S.Y. Park and Y.J. Chung, 2006. DNA copy number alterations and expression of relevant genes in mouse thymic lymphomas induced by gamma-irradiation and N-methyl-N-nitrosourea. Cancer Genet. Cytogenet., 166 (1): 27-35.

Khlusova, M.Iu., V.V. Novitskii and E.D. Gol'dberg, 1992. The cytogenetic effects of antitumor preparations with different mechanisms of action. Eksp. Klin. Farmakol., 55 (2): 49-52.

Kim, C., N. Kim, H. Joo, J.B. Youm, W.S. Park, D.V. Cuong, Y.S. Park, E. Kim, C.K. Min and J. Han, 2005. Modulation by melatonin of the cardiotoxic and antitumor activities of adriamycin. J. Cardiovasc. Pharmacol., 46 (2): 200-210.

Kimura, Y., T. Takaku and H. Okuda, 2003. Antitumor and antimetastatic actions by royal jelly in Lewis lung carcinoma-bearing mice. J. Tradit. Med., 20 (5): 195-200.

Klaassen, I. and B.J. Braakhuis, 2002. Anticancer activity and mechanism of action of retinoids in oral and pharyngeal cancer. Oral. Oncol., 38 (6): 532-542.

Klimova, M.I., V.V. Novitskii and E.D. Gol'dberg, 1990a. Early and late effects of the cytogenetic action of doxorubicin. Antibiot Khimioter., 35 (1): 30-32.

Klimova, M.I., I.V. Bogdashin, V.V. Novitskii and E.D. Gol'dberg, 1990b. Sensitivity of bone marrow cells damaged by antitumor drugs to cytotoxic action of effectors of a normal spleen. Biull. Eksp Biol. Med., 109 (1): 60-62.

Kocak, N., H. Ustun, M.D. Gulkac, A.O. Kanli, A. Borazan and A. Yilmaz, 2004. Effects of 10alpha, 25-dihydroxyvitamin D3 on doxorubicin-induced chromosomal aberrations in rat bone marrow cells. Acta Oncol., 43 (2): 204-208.

Konopacka, M., O. Palyvoda and J. Rzeszowska-Wolny, 2002. Inhibitory effect of ascorbic acid post-treatment on radiation-induced chromosomal damage in human lymphocytes *in vitro*. Teratog. Carcinog. Mutag., 22 (6): 443-450.

Lègal, J., R. De Crevoisier, E. Lartigau, K. Morsli, J. Dossou, N. Chavaudra, N. Sanfilippo, J. Bourhis, F. Eschwège and C. Parmentier, 2002. Chromosomal aberrations induced by chemotherapy and radiotherapy in lymphocytes from patients with breast carcinoma. Int. J. Radiat. Oncol. Biol. Phys., 52 (5): 1186-1195.

Leigh, B.C., 1999. Review of bee products: Honey, pollen, propolis and royal jelly. Nutr. Sci. News, 4 (8): 366-368.

Lichter, A.S. and T.S. Lawrence, 1995. Recent advances in radiation oncology. N Engl. J. Med., 332 (6): 371-379.

Liu, B., K. Wu and D. Zhao, 2000. Inhibition of human gastric carcinoma cell growth by vitamin E succinate. Wei Sheng Yan Jiu, 29 (3): 172-174.

Liu, F.Q. and J.R. Zhang, 2003. Protective effect of coenzyme I on mouse hematopoietic system against radiation. Di Yi Jun Yi Da Xue Xue Bao., 23 (4): 349-351.

Minemoto, Y., J. Gannon, M. Masutani, H. Nakagama, T. Sasagawa, M. Noue, Y. Masamune and K. Yamashita, 2001. Characterization of adriamycin-induced G_2 arrest and its abrogation by caffeine in FL-amnion cells with or without p53. Exp. Cell Res., 262 (1): 37-48.

Newcomb, E.W., M. Corominas, W. Bayona and A. Pellicer, 1989. Multistage carcinogenesis in murine thymocytes: Involvement of oncogenes, chromosomal imbalances and T cell growth factor receptor. Anticancer Res., 9 (5): 1407-1415.

Nishio, A.F. DeFeo, C.C. Cheng and E.M. Uyeki, 1982. Sister-chromatid exchange and chromosomal aberrations by DHAQ and related anthraquinone derivatives in Chinese hamster ovary cells. Mutat. Res., 101 (1): 77-86.

Oka, H., Y. Emori, N. Kobayashi, Y. Hayashi and K. Nomoto, 2001. Suppression of allergic reactions by royal jelly in association with the restoration of macrophage function and the improvement of Th1/Th2 cell responses. Int. Immunopharmacol., 1 (3): 521-532.

Okamoto, I., Y. Taniguchi, T. Kunikata, K. Kohno, K. Iwaki, M. Ikeda and M. Kurimoto, 2003. Major royal jelly protein 3 modulates immune responses *in vitro* and *in vivo*. Life Sci., 73 (16): 2029-2045.

Olive, P.L., 1998. The role of DNA single- and double-strand breaks in cell killing by ionizing radiation. Radiat. Res., 150 (5): 42-51.

Orta, L., U. Suprun, A. Goldfarb, I. Bleiweiss and S. Jaffer, 2006. Radiation-associated extraskeletal osteosarcoma of the chest wall. Arch. Pathol. Lab. Med., 130 (2): 198-200.

Osmak, M. and D. Horvat, 1992. Chromosomal analysis of Chinese hamster V79 cells exposed to multiple gamma-ray fractions: Induction of adaptive response to mitomycin C. Mutat. Res., 282 (4): 259-263.

Othman, O.E., 2000. Cytogenetic effect of the anticancer drug epirubicin on Chinese hamster cell line *in vitro*. Mutat. Res., 468 (2): 109-115.

Parchure, M., R.Y. Ambaye and S.V. Gokhale, 1984. Combination of anticancer agents with folic acid in the treatment of murine leukaemia P388. Chemotherapy, 30 (2): 119-124.

Ponnaiya, B., M.N. Cornforth and R.L. Ullrich, 1997. Induction of chromosomal instability in human mammary cells by neutrons and gamma rays. Radiat. Res., 147 (3): 288-294.

Pour, P.M. and T. Lawson, 1984. Modification of pancreatic carcinogenesis in the hamster model. XV. Preventive effect of nicotinamide. J. Natl. Cancer Inst., 73 (3): 767-770.

Prahalathan, C., E. Selvakumar and P. Varalakshmi, 2006. Lipoic acid modulates adriamycin-induced testicular toxicity. Reprod. Toxicol., 21 (1): 54-59.

Prevost, J.B., A. Bossi, R. Sciot and M. Debiec-Rychter, 2004. Post-irradiation sarcoma after external beam radiation therapy for localized adenocarcinoma of the prostate. Tumori, 90 (6): 618-621.

Ratain, M.J. and J.D. Rowley, 1992. Therapy-related acute myeloid leukemia secondary to inhibitors of topoisomerase II: From the bedside to the target genes. Ann. Oncol., 3 (2): 107-111.

Rothkamm, K. and M. Löbrich, 2002. Misrepair of radiation-induced DNA double-strand breaks and its relevance for tumorigenesis and cancer treatment (review). Int. J. Oncol., 21 (2): 433-440.

Salazar-Olivo, L.A. and V. Paz-Gonzalez, 2005. Screening of biological activities present in honeybee (*Apis mellifera*) royal jelly. Toxicol. *In Vitro*, 19 (5): 645-651.

Sarma, L. and P.C. Kesavan, 1993. Protective effects of vitamins C and E against gamma-ray-induced chromosomal damage in mouse. Int. J. Radiat. Biol., 63 (6): 759-764.

Shen, X., R. Lu and G. He, 1995. Effects of lyophilized royal jelly on experimental hyperlipidemia and thrombosis. Zhonghua Yu Fang Yi Xue Za Zhi, 29 (1): 27-29.

Sver, L., N. Orsolic, Z. Tadic, B. Njari, I. Valpotic and I. Basic, 1996. A royal jelly as a new potential immunomodulator in rats and mice. Comp. Immunol. Microbiol. Infect. Dis., 19 (1): 31-38.

Swellam, T., N. Miyanaga, M. Onozawa, K. Hattori, K. Kawai, T. Shimazui and H. Akaza, 2003. Antineoplastic activity of honey in an experimental bladder cancer implantation model: *In vivo* and *in vitro* studies. Int. J. Urol., 10 (4): 213-219.

Tamura, T., A. Fujii and N. Kuboyama, 1987. Antitumor effect of royal jelly. Nippon Yakurigakur Zasshi, 89 (2): 73-80.

Taniguchi, Y., K. Kohno, S. Inoue, S. Koya-Miyata, I. Okamoto, N. Arai, K. Iwaki, M. Ikeda and M. Kurimoto, 2003. Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. Int. Immunopharmacol., 3 (9): 1313-1324.

Tavares, D.C., A.O. Cecchi, L.M. Antunes and C.S. Takahashi, 1998. Protective effects of the amino acid glutamine and of ascorbic acid against chromosomal damage induced by doxorubicin in mammalian cells. Teratog. Carcinog. Mutag., 18 (4): 153-161.

Touil, N., A. Elhajouji, H. Thierens and M. Kirsch-Volders, 2000. Analysis of chromosome loss and chromosome segregation in cytokinesis-blocked human lymphocytes: Non-disjunction is the prevalent mistake in chromosome segregation produced by low dose exposure to ionizing radiation. Mutagenesis, 15 (1): 1-7.

Varanda, E.A. and S.C. Takahashi, 1993. Effect of pretreatment with venom of *Apis mellifera* bees on the yield of gamma rays induced chromosome aberrations in human blood lymphocytes. Brazil J. Genet., 16 (3): 551-559.

Vittek, J., 1995. Effect of royal jelly on serum lipids in experimental animals and humans with atherosclerosis. Experientia, 51 (9-10): 927-935.

Wagner, B.A., C.B. Evig, K.J. Reszka, G.R. Buettner and C.P. Burns, 2005. Doxorubicin increases intracellular hydrogen peroxide in PC3 prostate cancer cells. Arch. Biochem. Biophys., 440 (2): 181-190.

Wang, S., E.A. Konorev, S. Kotamraju, J. Joseph, S. Kalivendi and B. Kalyanaraman, 2004. Doxorubicin induces apoptosis in normal and tumor cells via distinctly different mechanisms. Intermediacy of H(2)O(2)- and p53-dependent pathways. J. Biol. Chem., 279 (24): 25535-25543.

Yosida, T.H., K. Tsuchiya and K. Moriwaki, 1971. Frequency of chromosome polymorphism in Rattus rattus collected in Japan. Chromosoma, 33 (1): 30-40.

Yuan, L., W.M. Yu and C.K. Qu, 2003. DNA damage-induced G_2/M checkpoint in SV40 large T antigen-immortalized embryonic fibroblast cells requires SHP-2 tyrosine phosphatase. J. Biol. Chem., 278 (44): 42812-42820.

Zhan, M., D. Yu, A. Lang, L. Li and R.E. Pollock, 2001. Wild type p53 sensitizes soft tissue sarcoma cells to doxorubicin by down-regulating multidrug resistance-1 expression. Cancer, 92 (6): 1556-1566.

Zou, W., P. Yue, N. Lin, M. He, Z. Zhou, S. Lonial, F.R. Khuri, B. Wang and S.Y. Sun, 2006. Vitamin C inactivates the proteasome inhibitor PS-341 in human cancer cells. Clin. Cancer Res., 12 (1): 273-280.

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