

**The Modulatory Effect of  
Iraqi Propolis Extract on  
Mitomycin-C Induced Microneucleus  
Formation in Albino Male Mice**

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**Summary:**

**Background:**

Propolis (bee glue) is a resinous hive product. It consists of exudate from plants mixed with beeswax and used by bees as glue in general-purpose as sealer and draught-exclude for beehives. Propolis, used in folk medicine, has attracted researchers attention to elucidate its therapeutic properties, as antioxidant and anticancer. Therefore, the study aimed to investigate the role of propolis in modulating effects of mitomycin C; MMC.

**Methods:**

Ethanol solutions of propolis were prepared and administered to albino male mice (*Mus musculus*) through three types of experiments. In the first, the propolis was tested alone, while in the second and third experiments, propolis was given orally before and after MMC (pre- and post-treatments, respectively). All experiments were paralleled by negative and positive controls. The investigated parameters were total count of leucocytes, mitotic index, micronucleus formation and chromosomal aberrations.

**Results:**

In the first experiment, the results indicated that 10 mg/kg/day of propolis enhanced the parameters investigated, and a significant increase was observed in the total count of leucocytes and mitotic index. Also, there was no significant difference in the rate of micronucleus formation and chromosomal aberrations as compared to negative controls. In the second (pre-treatment) and third (post-treatment) experiments, a similar picture was drawn. However, the dose 10 mg/kg/day was more effective in this respect.

**Conclusion:**

The results indicate that propolis may act upon the immunological system and has the potential to inhibit the genotoxic effects of MMC. A further study will be needed to determine the effects of compounds isolated from propolis and evaluate the synergistic effects on MI and MN.

**Keywords:**

Propolis, Micronucleus, Mitotic Index, Chromosomal aberrations, Mitomycin C.

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**Introduction:**

Honeybee Propolis is a hive product containing chiefly beeswax and plant-derived

substances such as resin and volatile compounds (1). Folkloric medicine, as well as natural products researches have revealed that propolis is a unique medicinal products with

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several therapeutic potentials, for instances antimicrobial, immune modulator and free radical scavenging (2,3,4,5,6). The latter potential has augmented the view that propolis may serve as anti-mutagenic and anti-carcinogenic agent (7,8). In agreement with this scope, chemical analysis of propolis revealed several compounds such as, caffeic acid (CA), caffeic acid phenethyl ester (CAPE), artemisinin, quercetin, luteolin and other are the most promising anti-mutagenic agents (9,10,11,12). To shed light on these potentials, MN, chromosomal aberrations, MI and total count of leucocytes were investigated in mice model (*Mus musculus*) to determine the effects of propolis on these parameters, especially after its interactions genotoxic drug mitomycin C; MMC.

#### **Materials and Methods:**

Iraqi propolis samples were collected from an Apiary located in Al-Tarmiya (a region 60 km north-east Baghdad) in different seasons and stored at 4°C. For the purpose of extraction, one gram of propolis was cut into small pieces, and extracted at room temperature with 50 ml of 70% ethanol using ultrasonic bath (Decon FS 300, England) for 90 minutes. Then, the alcoholic extract was evaporated at 50°C until dryness.

Albino male mice (*Mus Musculus*) were the tested animals, which were at the age 6-8 weeks at the beginning of experiments. They had free excess to water and food (*ad libitum*) during experiments. The total number of animals was 70 mice.

Two oral doses of propolis were investigated (5 and 10 mg/kg/day). These doses were evaluated to test their effect in the animals for the investigated parameters through three types of experiments. In the first, the animals were dosed a daily single dose of propolis for seven days. For this experiment, negative and positive controls (treated with phosphate buffer saline and mitomycin C; MMC, respectively). In the second, propolis was given for six days, followed by MMC 1 day seven (pre-treatment). In the third, MMC was given in first day, while propolis was given in the next six days. The latter two experiments were paralleled with two controls, in which propolis replaced with phosphate buffer saline. In all experiments, the animals were dissected in day eight for cytoegenetic evaluations.

In day eight, the animals were injected intraperitoneally with 0.25 ml colcemid solution (1 mg/ml). after 1 h the animal was dissected and the bone marrow was obtained and collected in test tubes from animals using phosphate buffer saline. The tubes were centrifuged at 2000 rpm for 5 minutes and the supernatant was discarded. The pellet was resuspended using 5 ml of hypotonic solution (0.075 M KCl) and the tubes were incubated at 37°C for a further 30 min with a gently shaking every 5 min. The tubes were centrifuged at 2000 rpm for 5 min and the supernatant was discarded, and the pellet was resuspended using 5 ml of fresh fixative (three parts of absolute methanol to one part of glacial acetic acid) and incubates for 30 min at 4°C. The tubes were centrifuged at 2000 rpm for 5 min and the supernatant was discarded. This procedure was repeated three times. The pellet was resuspended and 0.5-1 ml of fresh, cold fixative solution was added to the tubes. Then 3-4 drops of the cell suspension were dropped on to a cold wet glass slide and placed on a hot plate to dry, and then stained with 5% Giemsa for 6 min for mitotic index (MI) and chromosomal aberration examination. MI was determined as follows:

MI (%) = (Number of mitotic cells / Total number of cells) \* 100

For micronucleus assessments, the slides were immersed in 5N HCl for 20 min at room temperature. After washing with tap water, the slides were kept in Schiff solution (250 mg basic fuchsin was melted in boiling water and cooled till 50°C), after that 250 mg sodium-bisulphate was added and mixed till the color was changed to red wine color) for 90 min in the dark. The slides were passed through the sodium-bisulphate for 2 min three times. The slides were washed with tap water thrice, 30 min each time, stained for 40 min in 5% fast green solution (250 mg fast green is weighted and completed to 50 ml with 95% ethanol) and washed with tap water to remove extra stain. The slides were randomly scored to a single observer. From each slide, about 1000 cells were examined under 400 magnifications and when cells with micronucleus (MN) were located they were examined under 1000 magnification. Nuclear blebings (MN-like structure connected with the main nucleus with a bridge) were not considered, and only micronuclei equal to or smaller than one-fifth of the main nucleus was assumed to have

resulted from chromosome breakage were considered. Multi micronucleated cells were also considered in the evaluation of MN frequency (13). Micronucleus formation was determined as follows:

MN (%) = (Number of micronucleated cells / Total number of cells) \* 100

Total leukocyte counts were performed using the conventional method of blood cell counting. The blood obtained from the tail of animals and cells were stained by the Giemsa method (14).

#### **Statistical Analysis:**

Data were statistically analyzed using SPSS statistical software. Level of significant was assessed by using the Analysis Variance

(ANOVA) test. The level of significance was shown using the least significant difference (LSD) test. Values are given as mean  $\pm$  standard deviation. "P" values < 0.05 were considered statistically significant.

#### **Results:**

Both disease (5 and 10 mg/kg/day) of propolis elevated the leucocyte counts (6.5 and  $7.0 \times 10^3$  cell/cu.mm.blood) when compared with negative controls ( $5.2 \times 10^3$  cell/cu.mm.blood). However, the difference was significant ( $P < 0.05$ ) when the comparison was made with positive controls (Table 1).

**Table 1: The effect of propolis on some cytogenic parameters in albino male mice**

	Mean $\pm$ Standard Deviation			
	Negative Controls	Propolis (5 mg/kg/day)	Propolis (10 mg/kg/day)	Positive Controls
<b>Total leucocyte count * <math>10^3</math> (cells/cu.mm.blood)</b>	5.2 $\pm$ 1.0	6.5 $\pm$ 0.8	7.0 $\pm$ 1.5	4.6 $\pm$ 0.5
<b>Mitotic Index (%)</b>	9.0 $\pm$ 0.8	10.0 $\pm$ 0.7	12.0 $\pm$ 1.2	6.0 $\pm$ 0.7
<b>Micronucleus Cells (%)</b>	0.0	0.0	0.0	12.0 $\pm$ 0.8
<b>Chromosomal Aberrations (%)</b>	0.0	0.0	0.0	6.0 $\pm$ 0.5

Similar increase in the mitotic index were observed for both doses of propolis (10 and 12%, respectively), as compared to negative and positive controls (9 and 6%, respectively). No chromosomal aberration or micronucleus formation was observed in the animals of negative controls or propolis-treated animals. In contrast, animals treated with MMC showed a high frequency of chromosomal aberration (6%) and MN (12%). The dose 10 mg/kg/day

was remarkable in this regard, and a significant differences was revealed as compared to both controls (Table 1).

The forthcoming evaluations in the two interactions (pre- and post-treatment) with the genotoxic drug MMC showed much more enhancements of the total leucocyte counts and MI of both doses of propolis, although the dose 10 mg/kg was more effective than the dose 5 mg/kg/day (Table 2 and 3).

**Table 2: The effect of propolis – MMC interaction (pre-treatment) on some cytogenic parameters in albino male mice**

Parameters	Mean $\pm$ Standard Deviation		
	Negative Controls	Propolis (5 mg/kg/day)	Propolis (10 mg/kg/day)
<b>Total eucocyte Count * <math>10^3</math> (cells/cu.mm.blood)</b>	6.7 $\pm$ 0.6	5.2 $\pm$ 0.3	7.4 $\pm$ 0.7
<b>Mitotic Index (%)</b>	8.0 $\pm$ 1.0	9.0 $\pm$ 0.8	10.0 $\pm$ 1.4
<b>Micronucleus Cells (%)</b>	0.0	6.0 $\pm$ 0.6	4.0 $\pm$ 0.5
<b>Chromosomal Aberrations (%)</b>	0.0	3.0 $\pm$ 0.8	2.0 $\pm$ 0.5

**Table 3: The effect of propolis – MMC interaction (post-treatment) on some cytogenic parameters in albino male mice**

Parameters	Mean $\pm$ Standard Deviation		
	Negative Controls	Propolis (5 mg/kg/day)	Propolis (10 mg/kg/day)
Total eucocyte Count * 10 <sup>3</sup> (cells/cu.mm.blood)	5.4 $\pm$ 1.0	5.0 $\pm$ 0.8	5.4 $\pm$ 0.6
Mitotic Index (%)	7.7 $\pm$ 0.7	7.0 $\pm$ 0.9	8.0 $\pm$ 0.5
Micronucleus Cells (%)	0.0	7.0 $\pm$ 1.2	5.0 $\pm$ 0.6
Chromosomal Aberrations (%)	0.0	4.0 $\pm$ 1.3	2.0 $\pm$ 0.8

This indicates that propolis may be an anti-genotoxic agent. Therefore, it is possible to suggest that propolis can protect the body cells from the cytotoxic and carcinogenic effects of MMC, whatever the treatment was before or after MMC.

#### **Discussion:**

The use of natural products has been one of the most successful strategies for the discovery of new medicines (15); 78% of new antibiotics and 61% of new antitumor drugs were natural products or derived from natural products (16). According to Harvey (15), the access to biodiversity is fundamental to expanding the range of natural products to be used in the search for new drugs. In this context, propolis a relatively unexplored natural product could be a valuable resource for exploration of new bioactive compounds because of the high chemodiversity of this natural substance (17, 18, 19). The use of bee propolis by man dates back to at least the year 300 B.C.; it was already used in ancient Egypt for embalming the dead (20). Antiseptic, antimycotic, bacteriostatic, anti-inflammatory and anesthetic has all been attributed to this substance (3, 6, 21). Recently, researchers demonstrated that propolis have antioxidant properties and reduce proliferative activity of many cancer cell lines (22), increase protein, gamma globulins and regenerative process of different tissues (23,24,25). However, the increase counts of leukocyte in this study may be reflect that propolis stimulates protein biosynthesis and it enhanced mitosis of cells and that it stimulated the body's immune response. It has been demonstrated that propolis increases the ratio of CD4+/CD8+ cells, which are the main producer of cytokines, and an increase in their ratio is in

favor of immune enhancement (26,27,28). Also, our data indicated that mitomycin C enhance MN and chromosomal aberrations rate with decrease of leukocyte counts. This decrease in leukocyte counts may be lead to inhibition of immune system. In living creatures, which are exposed to a mutagen factor, the probability of defects is increased, and the rate of MN could increase due to this increase (29) with severe inhibition to the immune system (30). The carcinogenic substances pass into target cells and also systematically lead to decreased cell metabolism, producing a relative immune deficiency (31). Many studies reveal that mitomycin C has genotoxic properties in bacteria, in mammalian cells in vitro, in *Drosophila melanogaster* and in mammals in vivo, inducing DNA cross-linking, mutations, chromosomal aberrations, micronuclei and sister chromatin exchanges (32,33). Under normal circumstances the incidence rate of MN is very low, usually 0.2% (34). However, in our study, the results showed that the mean value of the MN rate of treated 5 and 10 mg/kg/day of propolis (propolis only) and negative control was (0.0%). This is lower than in the positive control. Interestingly, interaction between propolis with MMC (before and after treatment) caused decrease in MN, as compared with positive control, this findings is agreement with chromosomal aberrations rate. The decreasing MN and chromosomal aberrations rates in leucocytes indicate that propolis may be an anticarcinogenic and also antigenotoxic agent. Interaction of propolis in vivo with MMC caused confrontational changes in the oxidation activity of MMC. However, propolis is a natural source of anti-oxidants, which protect cells from oxidation. The index of

oxidation is dependent on the phenolic compounds and to a lesser extent, the unsaturated long chain fatty acids (22,35). On the other hand, galangin, an abundant activity and suppressing the genotoxicity of many chemical products, has been proposed as an agent to be used in the chemoprevention of cancer (36,37). To sum up, the decrease of MN indicates that propolis is an anticarcinogenic agent. In contrast the rise of MI showed that propolis activates immune

cells. This indicates that propolis acts upon the immunological system and inhibit the genotoxicity effect of mitomycin-C. A further study will be needed to determine the effects of compounds isolated from propolis and evaluate the synergistic effects on MI and MN.

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