Antimutagenic Activity of Egyptian Propolis and Bee Pollen Water Extracts Against Cisplatin-Induced Chromosomal Abnormalities in Bone Marrow Cells of Mice

Abdella EM[1], Tohamy A[2], Ahmad RR[1]

Abstract

Background: Bee-collected pollen and propolis are apicultural products which are recognized as a well balanced food. These beehive products are composed of nutritionally valuable substances and contain considerable amounts of polyphenol substances which may have several useful pharmacological properties.

Methods: The protective activity of Bee-collected pollen (BPE) and water-soluble derivative of propolis(WSDPE) aqueous extracts was studied on cisplatin(CDDP) induced genotoxicity in male albino mice(Mus mascullus).

Results: The treatment of mice with Bee-collected pollen and propolis extracts at doses 140 and 8.4 mg/kg body weight/day, respectively for 14 days synergistically with the intraperitoneal administration of cisplatin at dose of 2.8 mg/kg b.wt exhibited significant chemoprotective activity. Genotoxicity and cytotoxicity was evaluated by the bone marrow chromosomal aberration assay and mitotic index, respectively. WSDPE and BPE, alone did not significantly induce chromosomal aberrations confirming their non-mutagenic effects. While, the animals in groups five and six (G5 and G6), that were injected i.p. with CDDP alone for one week and then for the next 14 days these animals were given WSDPE and BPE in synergistic with i.p. injection of CDDP, exhibited a significant decrease in cytogenetic damages induced by CDDP in bone marrow cells. The anti-cytotoxicity effects of WSDPE and BPE were also evident, as observed by significant increase in mitotic index, when compared to positive control group (G2).

Conclusion: Thus, results of the present investigation revealed that WSDP and BPE have chemoprotective potential against CDDP induced genotoxicity in bone marrow cells of male albino mice. Also, the present investigation indicated that the chemoprotective frequency of BPE was much greater than WSDPE.

Keywords: bee pollen, propolis, antimutagenic, bone marrow, mice

1. Faculty of Science, Zoology Department, Beni-Suef University, Egypt
2. Faculty of Science, Zoology Department, Helwan University, Egypt

Corresponding Author:
Ehab M Abdella MD
Associate Professor of Genetics and Biotechnology
Tel: (#30)20164006605
E-mail: ehababdella@hotmail.com

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Introduction

Cisplatin[cis-diammine-dichloro-platinum(II)] (CDDP) is a potent antineoplastic agent used for the treatment of a wide range of malignant solid tumors including testicular, ovarian, breast, lung, bladder, head and neck cancer [1,2,3,4]. Nevertheless, the majority of antineoplastic drugs, besides their generic growth property, display genotoxic and cytotoxic effects which in turn contribute to growth inhibition. These toxic effects may lead to initiation of unrelated tumors [5]. This drug has severe toxic effects that interfere with its therapeutic efficacy, namely bone marrow toxicity, neurotoxicity, nephrotoxicity, hepatotoxicity and show the impairment of bone formation years after cessation of chemotherapy [6, 7, 8]. Also, the oxidative stress is one of the most important mechanisms involved in CDDP-induced toxicity [9].

Recently, a considerable emphasis is being laid down on the use of dietary constituents as chemoprotective measure for control of neoplastic and genetic diseases. Bee-collected pollen and propolis are apicultural products which are recognized as a well balanced food [10]. These beehive products are composed of nutritionally valuable substances and contain considerable amounts of polyphenol substances which may have

several useful pharmacological properties, such as antibiotic, anti-neoplastic, anti-inflammatory, anti-diarrhoeatic and antioxidant [11, 12].

Honeybee-collected pollen is a mixture of flower pollen collected by honeybees from a variety of plants and is the insect's primary food source. Pollen grains, which are flowers' male reproductive cells, contain concentrations of phytochemicals and nutrients. Bee pollen is rich in carotenoids, flavonoids and phytosterols. The exact profile varies depending on the plant sources and growing conditions; however, beta-carotene, beta-sitosterol, isorhamnetin, kaempferol, lycopene, quercetin and rutin are consistently reported [13, 11].

Propolis is a resinous substance collected by honeybees (Apis mellifera) from exudates and buds of plants and mixed with secreted beeswax. People have used propolis as a folk medicine from ancient times. Even though propolis has diverse physiologic functions such as antioxidant, anticarcinogenic, antimicrobial and anti-inflammatory effects, [14, 15, 16]. Such effects have been associated with the presence of phenolic compounds, such as flavonoids and aromatic acids [17, 15].

The antioxidant activity of flavonoids present in bee collected pollen or propolis has been shown to be capable of scavenging free radicals. The radical scavenging activity of phenolic compounds is assigned to the hydrogen-donating ability of compounds [18]. Antioxidants intercept the free radical chain oxidation by donating hydrogen from the phenolic hydroxyl groups, thereby forming stable end products, which does not initiate or propagate further oxidation [19, 20].

Development and utilization of more effective antioxidants of natural origin are desired. Naturally occurring polyphenols are expected to help reducing the risk of alkylating agents and various life-threatening diseases, including cancer and cardiovascular diseases, due to their antioxidant activity. The purpose of the present study was to evaluate and compare the effectiveness of the water extracts of honeybee-collected pollen (BPE) and water-soluble derivative of propolis (WSDP) from Beni-Suef, Egypt, as in vivo antimutagenic agents against cisplatin-induced chromosomal abnormalities in bone marrow cells of mice (Mus musculus).

Materials and Methods

Chemicals

Cisplatin [cis-diammine-dichloroplatinum (II)] (CDDP) was purchased from MERCK in a form of ampoules; each contains 25 mg of CDDP in 25 ml sterile saline solution. All other chemicals were obtained from Sigma (St. Louis, MO, USA).

Experimental animals

The experimental animals used in this work were random bred adult males of laboratory mice Mus musculus (20-30 gm in weight). Animals were obtained from Ophthalmology research institute. All animals were housed in plastic cages with wired covers and kept under normal laboratory conditions for the different periods of time used. The animals were not treated with antibiotics, vitamins or insecticides and fed a standard commercials diet (ATMID Company, Egypt) and drunk tap water.

Extract preparations

The honeybee collected pollen and propolis were provided by local beekeepers in Beni Suef, Egypt. These samples were harvested in September 2006. Bee collected pollen was obtained as yellow pellets contain mixture of pollen from the anthers of flowers of the plants growing in the surroundings of the beehives, while propolis was derived in the form of yellow-brown powder of water-soluble derivative of propolis (WSDP).

Bee pollen and propolis extracts prepared according to the methods of [21, 22]. The powder of bee pollen (280 mg) was suspended in 10 ml of distilled water and mixed vigorously. This suspension was kept stand overnight in dark and centrifuged at 10000 rpm in a cooling centrifuge for 45 minutes at 10°C. The supernatant fraction was collected and filtrated. The filtrate kept in freeze condition at -10°C until use. On the other hand, propolis extract was prepared under sterile conditions by dissolving the WSDP powder in 15 ml distilled water and mixed vigorously for 10 minutes. Finally, this suspension was centrifuged at 1000 rpm for 10 minutes in room temperature. The supernatant was collected and kept under freeze condition until use.

Doses and organization of experimental groups

The single dose (2.8 mg/kg b.wt) of CDDP used in The present study was selected with reference to the dose range that has been used in previously published reprints dealing with the cytotoxicity and genotoxicity of CDDP [23], while bee pollen (BPE) and propolis (WSDPE) water extracts concentrations used in the present study were 140 and 8.4 mg/kg b.wt, respectively [22, 24].

Mice were divided into 6 groups (5 animals each). The animals of group one (G1) served as a negative control group received 0.9% of NaCl solution by intraperitoneally injection (i.p.) twice/week for three

weeks. The animals of group two (G2) received i.p. injection of CDDP (2.8 mg/kg b.wt.) twice/week for three weeks. In group three (G3) 8.4 mg/kg b.wt of WSDPE was given to the animals through oral intubation once/day for 14 days consecutively. The animals of the group four (G4) received oral administration of BPE (140 mg/kg b.wt) once/day for 14 days. The animals of the groups five and six (G5 and G6) were injected i.p. with CDDP alone for one week and then for the next 14 days these animals were given WSDPE and BPE through oral intubation in synergistic with i.p. injection of CDDP.

Preparation of the mice bone marrow cell system

Bone marrow cell preparations for the analysis of chromosomal aberrations and mitotic index were produced by the colchicine–hypotonic technique.

After completion of the treatment period, animals in each group were scarified at sampling time of 24 hours post-injection with NaCl, CDDP, propolis extract or bee pollen extract, by cervical dislocation. Colchicine was given at the dose of 4 mg/Kg b.w. intraperitoneally at 22 h prior to sacrificing the animals. The bone marrow smears of animals in each group were prepared according to [25] protocol. Slides were stained with Giemsa and 50 well spread metaphase plates/animal were analyzed for chromosomal aberrations including structural chromosomal aberrations (chromatid breakage {include break and deletion}, chromatid gap, centromeric attenuation, centric fusion and end to end association) and numerical chromosomal aberrations (polyploidy and Endomitosis) and Incidence of aberrant cells for each group. The mitotic index was obtained by counting the number of mitotic cells in 1000 cells/animal. While the percentage of suppressed aberrant cells was calculated according to [26] as follows: 100 – (% of aberrant cells in CDDP+extract treated groups (G5 or G6)/% of aberrant cells in positive control (CDDP treated) group) x 100.

Statistical analysis

Statistical analysis for the difference in the mean number of chromosomal aberrations and mitotic index between groups was carried out using student-*T*-test ($P < 0.05$ was considered significant).

Results

According to the cytogenetic results illustrated in tables 1 and 2, seven structural and numerical chromosomal aberrations were determined in the control and the experimental groups. The results obtained in the first phase of cell cycle (24 hr sampling time), revealed that cisplatin (CDDP) when given at a single dose of 2.8 mg/kg b.wt, twice/week for three weeks (G2) induced a high frequency of chromosomal aberrations in bone marrow cells of mice when compared with the control (G1) group [Tables1and2]. In the CDDP-treated groups the most frequent chromosomal aberration was chromatid breakage. The mitotic index was significantly decreased ($P < 0.05$) 37.75, over control, indicating bone marrow cytotoxicity [Table 2].

When the propolis extract (WSDPE) treated group (G3) was compared with the control group (G1) in terms of mean total number of structural chromosomal aberrations, percentage of incidence of aberrant cells and number of aberrations/cell, G3 displayed significant increase ($P < 0.05$), whereas the mean total number of numerical chromosomal aberrations was significantly decreased ($P < 0.05$). The WSDPE was not cytotoxic at this given dose (8.4 mg/kg b.wt), as there was no significant change in mitotic index over G1 [Table2]. However, the aqueous bee pollen extract (BPE) treated group (G4) was compared with the control group (G1) in terms of mean total number of numerical chromosomal aberrations, percentage of incidence of aberrant cells and number of aberrations/cell displayed no significant differences ($P < 0.05$) confirming its non-mutagenicity [Tables 1 and 2]. The BPE was also not found to be cytotoxic at the given dose (140 mg/kg b.wt), as there was no significant changes in mitotic index over G1 [Table 2].

Moreover, in the WSDPE or BPE and CDDP treated groups (G5 and G6, respectively) there was a significant decrease in rates of clastogenetic changes compared with the CDDP treated group [Table1and2]. All types of chromosomal aberrations induced by CDDP including breaks, gaps, end to end association, centric fusion, centromeric attenuation, and other multiple damages were found to be reduced by WSDPE and BPE but still significantly higher than negative control group (G1). Also, the mitotic index was found to be increased significantly ($P < 0.05$), indicating its anti-cytotoxicity towards CDDP [Table 2]. The percentages of aberrant cells which were found to be 50.00 ± 4.147 in CDDP treated animals, were reduced to 34.80 ± 3.382 and 30.80 ± 1.743 ($P < 0.05$) by WSDPE and BPE, respectively [Table 2]. Also a significant decrease in the number of aberrations per cell was observed in G5 and G6 over the CDDP treated group (G2). The calculated suppressive effect was 30.40% and 38.40%, by WSDPE and BPE, respectively[Table2].

Table 1: *Protective effects of propolis and bee pollen aqueous extracts against cisplatin induced structural and numerical chromosomal aberrations in mouse bone marrow cells*

Groups	Treatment	Number of examined cells	Number of structural chromosomal aberrations [a]						Number of numerical chromosomal [a] aberrations		
			Chromatid breakage	Chromatid gap	Centromeric attenuation	Centric fusion	End to end association	TSA	Polyploidy	Endomitosis	TNA
G1	-ve control	250	18 3.6 ± 0.600	——	2 0.40 ± 0.244	1 0.20 ± 0.200	2 0.40 ± 0.244	23 4.60 ± 0.678	——	8 1.60 ± 0.244	8 1.60 ± 0.244
G2	CDDP-control	250	101 [b] 20.20 ± 1.462	14 [b] 2.80 ± 1.157	——	11 [b] 2.20 ± 1.067	19 [b] 3.80 ± 0.860	145 [b] 29.00 ± 3.420	——	23 [b] 4.60 ± 0.400	23 [b] 4.60 ± 0.400
G3	WSDPE	250	45 [b] 9.00 ± 0.447	——	——	2 0.40 ± 0.244	2 0.40 ± 0.244	49 [b] 9.80 ± 0.374	——	——	——
G4	BPE	250	32 [b] 6.40 ± 0.509	——	——	——	2 0.40 ± 0.244	34 [b] 6.80 ± 0.374	——	——	——
G5	CDDP + WSDPE	250	82 [c] 16.40 ± 2.039	7 [c] 1.40 ± 0.400	1 0.20 ± 0.200	3 [c] 0.60 ± 0.244	9 [c] 1.80 ± 0.374	102 [c] 20.40 ± 2.039	1 0.20 ± 0.200	1 [c] 0.20 ± 0.200	2 [c] 0.40 ± 0.244
G6	CDDP + BPE	250	55 [c] 11.00 ± 0.707	4 [c] 0.80 ± 0.200	3 [c] 0.60 ± 0.400	6 [c] 1.20 ± 0.489	11 [c] 2.20 ± 0.200	79 [c] 15.80 ± 0.489	3 [c] 0.60 ± 0.400	5 [c] 1.00 ± 0.447	8 [c] 1.60 ± 0.678

[a] Values represent mean ± SE of five animals.
[b] Significantly different from untreated control (G1) $P < 0.05$.
[c] Significantly different from positive control (G2) $P < 0.05$.
Chromatid Breakage: Total number of chromatid breaks + chromatid deletions.
TSA: Total structural aberration
TNA: Total numerical aberration.

Table 2: *Protective effects of propolis and bee pollen aqueous extracts against cisplatin induced cytotoxicity and genotoxicity in mouse bone marrow cells*

Groups	Mitotic index[a]	Number of cells[a] with one aberration	Number of cells[a] with more than one aberration	Incidence of [a] aberrant cells (%)	Number of [a] aberrations/cell	Suppression (%)
G1	83.29 ± 1.047	6.20 ± 0.800	____	12.40 ± 1.600	0.124 ± 0.016	
G2	37.75 ± 8.603 [b]	17.80 ± 0.969 [b]	6.40 ± 1.363 [b]	50.00 ± 4.147 [b]	0.672 ± 0.020 [b]	
G3	76.58 ± 1.393	7.60 ± 0.400 [b]	1.20 ± 0.200 [b]	17.50 ± 1.166 [b]	0.180 ± 0.010 [b]	
G4	85.65 ± 3.735	5.20 ± 0.734 [b]	0.60 ± 0.244 [b]	11.60 ± 0.979	0.136 ± 0.007	
G5	73.28 ± 1.124 [c]	13.60 ± 1.363 [c]	3.80 ± 0.374 [c]	34.80 ± 3.382 [c]	0.416 ± 0.042 [c]	30.40
G6	77.74 ± 1.660 [c]	14.00 ± 0.948 [c]	1.40 ± 0.400 [c]	30.80 ± 1.743 [c]	0.348 ± 0.013 [c]	38.40

[a] Values represent mean ± SE of five animals.
[b] Significantly different from untreated control (G1) $P < 0.05$.
[c] Significantly different from positive control (G2) $P < 0.05$.

Discussion

Propolis and bee-collected pollen are apicultural products which are composed of nutritionally valuable substances and contain considerable amounts of polyphenol substances which may act as potent antioxidants. Development and utilization of more effective antioxidants of natural origin are desired. Naturally occurring polyphenols are expected to help reducing the risk of various life-threatening diseases, including cancer diseases, due to their antioxidant activity [27]. Also, Phenolic compounds are known to counteract oxidative stress in the human body by helping maintaining a balance between oxidant and antioxidant substances [28, 29].

Flavonoids and phenolic acids are major classes of polyphenolic compounds, whose structure-antioxidant activity relationships in aqueous or lipophilic systems have been extensively reported [30, 31]. In addition to antioxidant activity, many phenolic compounds have been shown to exert anticarcinogenic or antimutagenic activity to a greater or lesser extent [32, 33]. Their physiological and pharmacological activities may be derived from their antioxidant properties, which are related to their molecular structure [17]. Mechanisms of antioxidant action may include suppression of oxygen reactive species (ROS) formation, removal or inactivation of oxygen reactive species and up-regulation or protection of antioxidant defenses [34, 35].

Cisplatin (CDDP) is an inorganic platinum compound with a broad spectrum antineoplastic activity against different types of human tumors [36]. CDDP has been demonstrated to have the potential for initiating genetic events in non-tumor cells in human and in animal systems. Nevertheless, both clinical and experimental studies reported a dose-limiting nephrotoxicity which restricts cisplatin's optimal usefulness in cancer chemotherapy [37]. Bone marrow cytogenetic is a useful short-term technique, for elucidating the mechanism as well as to identify the substances for their clastogenic and anticlastogenic activity [38, 39].

The results of the present investigation revealed that, administration of CDDP at a single dose of 2.8 mg/kg b.wt, twice/week for three weeks induced cytogenotoxic effects. These results are consistent with those previously reported [3, 5, 7, 40]. In the present study, the administration of Bee pollen (BPE) and propolis (WSDPE) aqueous extracts (140 and 8.4 mg/kg b.wt, respectively), by gastric intubation synergistically with the intraperitoneal injection of CDDP for two weeks, effectively reduced incidence of chromosomal damages induced by CDDP in bone marrow cells and increase the frequency of the mitotic indices of bone marrow cells. These results revealed the protective efficiency of Egyptian bee pollen and propolis aqueous extract. This is consistent with those reported by [27, 41, 42, 43, 44].

The anti-mutagenic actions of bee propolis extract involve enhancement of the level of glutathione S-transferase (GST), inhibiting cytochrome P-450 activity and interaction with microsome-generated proximate mutagens to generate an inactive complex [45,46]. These effects were associated with inhibition of cell cycle progression, accelerating the detoxification of mutagens and carcinogens and induction of apoptosis [41, 47, 48]. Lotfy (2006) indicated that, Egyptian propolis is characterized by the presence of unusual esters of caffeic acid with C12- C16 fatty alcohols, mainly saturated. Flavonoid glycones and especially flavanones are typical components of propolis [49]. All such constituents of crude Egyptian propolis have increased its pharmaceutical demand and have rendered it an interesting subject of study.

However, the mechanism for protection of the bee pollen extract involves scavenging potentially toxic and mutagenic electrophiles and free radicals and modification of antioxidant pathways [50]. The recent investigations indicated that, bee pollen extract contains significant amounts of polyphenolic substances, mainly flavonoids [51, 52, 53]. Also several researchers found out that polyphenols are antioxidants with redox properties which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers [54, 55]. The polyphenols also have metal chelation properties [56]. These compounds league metals to it (chelation of metals) and react with free radicals and genotoxic substances or carcinogenics [57]. Epidemiologic studies have shown a correlation between an increased consumption of phenolic antioxidants and a reduced risk of cardiovascular disease and certain types of cancer [58].

According to the results obtained, bee pollen extract seems to have interesting biological properties than propolis. The protective effect of bee pollen and propolis extracts towards CDDP induced toxicity implies a good marker of its antimutagenic, activity. Further investigations are needed to elucidate the interaction of bee pollen and propolis constituents with genotoxic compounds at genetic level.

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Conflict of interests

The authors have no conflict of interests in this article.

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