Original Article

Genotoxic, Antigenotoxic, and Antioxidative Potentials of Thai Bee Products

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Abstract

Introduction: Bee products are becoming increasingly used as nutritional supplements. They have been reported to have antibacterial and antitumor properties.

Objective: We aimed to compare the efficacies on antioxidative and antigenotoxic potentials among these products from Chiangmai, northern Thailand, including their genotoxicity.

Methods: The genotoxicity and antigenotoxicity were assessed using *in vitro* sister chromatid exchange assay in human lymphocytes. Chemical compositions and antioxidative activities were investigated using standard chemical methods.

Results: Our results revealed that the lipid extracts tended to have the least genotoxicity compared to their defatted and crude extracts. The lipid extract of propolis at a nongenotoxic dose had the highest antigenotoxic activities, followed by the defatted and crude extracts of propolis. All extracts of bee pollen and royal jelly had much lower potency. The lipid extract of propolis had the highest antioxidant activities.

Conclusions: In summary, the lipid extract of propolis is the best promising candidate as a genoprotectant.

Keywords: Antigenotoxicity, Bee pollen, Genotoxicity, Propolis, Royal jelly

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Introduction

Cancer is a multistage process. An induction of genetic damage is one of significant risk factors for cancer. Cancer burden on people's health and economic status made us interested in domestic natural products that could be used as food supplements with antigenotoxic properties. Bee's products, especially propolis, bee pollen, and royal jelly, are commonly used as traditional medicine for antibacterial, anti-inflammation, antitumor, etc.[1] Propolis is a resinous product derived from a mixture of bee saliva, pollen, beeswax, and trees' bark cracks. It consists of various functional, active compounds such as phenolic acids, flavonoids, terpenes, alkaloids, fatty acids, amino acids, and proteins. Bee pollen is a bee's product with high nutritional value, consisting of pollen, nectar, and bee secretion. It is used as a food supplement and traditional medicine to treat gastric ulcers and prostatitis.[2] Royal jelly, a thick substance secreted by worker bees to feed their queens and young larvae, is used to promote long life and vitality.[2] It contains nutritious substances such as vitamins, amino acids, special fatty acids, and polyphenols.

However, pharmacological activities of these products depend on their chemical compositions, which vary from one to other areas. Ayres et al. (2007) stated that propolis from various regions in Brazil had different parasite reduction activities in macrophage cells.[3] Since these bee's products are increasingly used for health benefits, more studies are needed to validate their activities from each source. Moreover, their genotoxicity should be assessed to assure their safe use in terms of their ability to induce DNA damage. In addition, their antigenotoxicity should be studied to explore whether their benefits may arise from their ability to protect DNA damages from a potent genotoxic agent.

This study aimed to compare the genotoxic, antigenotoxic, and antioxidative potentials among propolis (PP), bee pollen (BP) and royal jelly (RJ) extracts from Chiang Mai, Thailand. Our previous studies have reported those activities in crude extract (CE), lipid extract (LE) and defatted extract (DE) of bee pollen,[4] and in CE of royal jelly.[5,6] In this study, we continued our investigation on those activities of propolis extracts (CE, DE, LE) and other royal jelly extracts (DE and LE). Therefore, the extracts being assessed in our study were PP_{CE}, PP_{DE}, PP_{LE}, RJ_{DE}, and RJ_{LE}. The genotoxic and antigenotoxic activities were assessed using *in vitro* sister chromatid exchange (SCE) assay in human lymphocytes. SCE is the reciprocal interchange of DNA between chromatids. Measurement of SCE frequency is a sensitive and well-established method for detecting DNA damage.[7] Antigenotoxic activities of all extracts were tested against doxorubicin (DXR). DXR is a potent genotoxic chemotherapeutic agent,[8] which induces oxidative DNA damage and acts as a DNA topoisomerase II inhibitor.[9] Their chemical compositions, antioxidative activities, and contents of total flavonoid, total phenolic, quercetin, rutin, ferulic acid, and gallic acid were investigated.

Methods

The experiment was approved by the Human Ethics Committee of Thammasat University (MTU-EC-DS-2-067/56 and MTU-EC-DS-2-001/60). Blood donations were taken from donors who are our students or officers in the faculty, aged 25-35 years. The donors were not exposed to any radiation or drugs. The standard venipuncture procedure was used to obtain 10 ml of a whole blood sample.

Frozen propolis and royal jelly were purchased from an apiary with HACCP certification from SGS Thailand, located in Chiang Mai, northern Thailand. Most resinous propolis collected by *Apis mellifera* bees were mainly from *Mangifera indica* buds and barks. The royal jelly derived from *Apis mellifera* worker bee secretions.

Analysis of Chemical Compositions

The lipid content was extracted from 22.0 g of PP and 68.0 g of RJ by diethyl ether (300 ml) using Soxhlet apparatus for 24 h and was analyzed, similar to our previously described protocol.[10] The protein content was investigated using the Kjeldahl method.[11] Ash, moisture, and fiber contents were quantified using the AOAC method.[12] The carbohydrate content was calculated from 100-(protein+lipid+fiber+moisture+ash). Total flavonoid content was determined using aluminum chloride colorimetric assay.[13] Total phenolic content was quantified using Folin-Ciocalteu's reagent.[14] Quercetin, rutin, ferulic acid, and gallic acid were analyzed using

quantitative high-performance liquid chromatography.[15] Antioxidant capacity was assessed using ABTS assay.[16]

Sister Chromatid Exchange Assay in Human Lymphocytes

Preparation of samples: The crude extracts and the lipid fractions were dissolved in dimethyl sulfoxide (DMSO) separately for an hour and centrifuged. The supernatant was then mixed with RPMI for another hour and was kept as the stock solution of crude extract or lipid extract. For the defatted fraction, the defatted extract was prepared by dissolving in RPMI medium. Ten-fold serial dilutions of each extract in RPMI medium were freshly prepared before initiating cell culture.

Genotoxic studies: Human lymphocytes were cultured (1×10^6 cells/ml) for 24 h in culture medium containing RPMI1640 (Hyclone, USA), fetal bovine serum albumin (Hyclone, USA), autologous plasma, penicillin-streptomycin (Seromed, Germany), L-glutamine (Hyclone, USA), and phytohemagglutinin (Seromed, Germany) using standard blood culture conditions as previously described.[17] The lymphocyte cultures were then centrifuged for packed cells, and the supernatant medium was saved for reuse after treatments. The remaining lymphocytes were treated with 0.005, 0.05, 0.5, 5 mg/mL PP_{CE}; 0.005, 0.05, 0.5, 5 mg/mL PP_{DE}; 0.0025, 0.025, 0.25, 2.5 mg/mL PP_{LE}; 0.0005, 0.005, 0.05, 0.5, 5 mg/mL RJ_{DE}; 0.00025, 0.0025, 0.025, 0.25, 2.5 mg/mL RJ_{LE}; for 3 h. DXR at 0.1 µg/mL was used as a positive control. Finally, they were centrifuged for packed cells. The cells were rinsed twice and continued to culture at 37°C in the dark using the previously saved medium. Bromodeoxyuridine (BrdU) solution (Sigma-Aldrich, USA) was added to the medium for the final concentration at 5 mM, and the cell culture was continued at 37°C in the dark. The cell harvest was performed at 72 h after initiation. Fluorescent plus Giemsa technique was used as a staining procedure, and only cells having second metaphase staining (MII phase) were analyzed for SCE frequencies.

Antigenotoxic studies: Human lymphocytes were cultured (1×10^6 cells/ml) for 24 h. After that, the supernatant was saved. The remaining lymphocyte were treated with 0.005, 0.05, 0.5, 5 mg/mL PP_{CE}; 0.005, 0.05, 0.5, 5 mg/mL PP_{DE}; 0.0025, 0.025, 0.25, 2.5 mg/mL PP_{LE}; as well as 0.0125, 0.125, 1.25, 12.5, 125 mg/mL RJ_{DE}, 0.00025, 0.0025, 0.025, 0.25, 2.5 mg/mL RJ_{LE}; in plain RPMI 1640 medium for 2 h at 37°C. Then the supernatant was discarded. The cells were further treated with DXR solution (0.1 µg/mL) for 2 h at 37°C. After that, the DXR solution was discarded. The cells were rinsed twice and continued to culture at 37°C in the dark using the previously saved medium. BrdU solution was added to the culture medium at the final concentration of 5 mM. Then, they were harvested and stained at 77 h after initiation because of cell cycle delay resulting from the treatments.

SCE frequencies were investigated in two to three independent experiments for each genotoxic and antigenotoxic studies. Twenty-five cells per dose per experiment showing MII phase staining pattern were scored from coded slides.

Cytotoxicity was assessed from the mitotic index and proliferation index. Mitotic index (M.I.) was the total number of mitotic cells/1,000 cells. Proliferative index (P.I.) was (MI + 2MII + 3MIII)/100 cells; MI, MII, and MIII are the number of mitotic cells in the first cell cycle, second cell cycle, and the third cell cycle, respectively.

Statistical Analysis

Raw data obtained from SCE assays were transformed to stabilize the variance using the procedures of Whorton et al. (1984)[18] as follows: Transformed SCE (SCE_T) = $\sqrt{SCE}$.

Dunnett's t-test was performed to analyze the differences between the mean of the treated groups and of the control group using the transformed data.

Results

Chemical Composition Profiles and Antioxidant Activities

The chemical contents of the lipid and defatted extracts of the propolis and royal jelly are shown in Table 1. Chemical compositions of crude Thai brown propolis (pH 6) were 72.7 g% (w/w) lipid with 13.5 g% protein, 8.8 g% carbohydrate, 1.9 g% fiber, and no ash. The calculated energy was 7.4 cal/g. The antioxidant activities of the PP_{CE}, PP_{DE}, PP_{LE} are 392.7 ± 1.9, 419.0 ± 1.9, 445.3 ± 1.9, respectively. Those of the BP_{DE}, BP_{LE} are 240.3 ± 1.0, 95.7 ± 1.9, respectively. PP_{LE} had

the highest antioxidant activities: ~1.1-fold higher than PP_{CE} and PP_{DE}.

Cytotoxic and Genotoxic Studies of Thai Propolis and Royal Jelly Extracts

PP_{CE} (0.005-5 mg/mL), PP_{DE} (0.005-5 mg/mL) and PP_{LE} (0.0025-2.5 mg/mL) had no observable cytotoxicity since regular mitotic cells were shown (Table 2). No statistically significant differences in the mitotic index and proliferation index among PP_{CE}, PP_{DE}, PP_{LE}, and 2%V/V DMSO solvent control were observed, compared to the RPMI negative control and the 0.1 μg/mL DXR positive control (Table 2). However, PP_{CE} (0.005-5 mg/mL) significantly induced dose-dependent SCE increment (1.3-1.6-fold), as shown in Figure 1. All PP_{DE} (0.005-5 mg/mL) treated cells increased in SCEs 1.4-fold without dose-dependency. It is notable that PP_{LE} (0.0025-0.25 mg/mL) did not significantly increase SCE, but at the highest dose of 2.5 mg/mL induced 1.2-fold increase.

RJ_{DE} (0.0005-5 mg/mL), and RJ_{LE} (0.0025-2.5 mg/mL) had no cytotoxicity (Table 3). Significantly increase in SCEs were only observed at high doses of RJ_{DE} and RJ_{LE}; 0.5 and 5 mg/mL RJ_{DE} exhibited a 1.4-fold increase in SCEs, and 2.5 mg/mL RJ_{LE} significantly increased SCEs by 1.2-fold compared to the negative control ($P < .05$) (Figure 1).

Antigenotoxic Studies of Thai Propolis and Royal Jelly Extracts Against DXR

The 0.1 μg/mL DXR alone significantly increased SCE levels by 2.1-fold, compared to the negative control (Figure 2). PP_{CE} pretreatments (0.005-5 mg/mL) followed by the 0.1 μg/mL DXR treatment did not significantly decrease the DXR-induced SCEs. Remarkably, all PP_{DE} and PP_{LE} pretreatments significantly decreased DXR-induced SCE ($P < .05$), without dose-dependency. PP_{DE} pretreatment at 0.5 mg/mL maximally decreased DXR-induced SCE levels by 0.5-fold, which returned to normal levels as the negative control (Figure 2: 3b), Other doses of PP_{DE} significantly decreased SCE levels by 0.6-0.7-fold. PP_{LE} pretreatment at 0.025 mg/mL effectively and significantly decreased SCE levels by 0.4-fold, which returned to the normal level (Figure 3c). Other doses at 0.0025, 0.25, and 2.5 mg/mL PP_{LE} pretreatments significantly decreased SCEs by 0.6-0.7-fold. There were no statistically significant differences in the mitotic index and proliferation index among PP_{CE}, PP_{LE}, PP_{DE}, and 2%V/V DMSO solvent control treatments, compared to the negative and positive controls (Table 4).

Both RJ_{DE} pretreatments (0.0125-125 mg/mL) and RJ_{LE} pretreatments (0.00025-2.5 mg/mL) did not decrease SCE level compared to the DXR-treated cells alone (Figure 2). The treatment of DXR alone significantly increased the SCE level by 2.2-fold above that of the negative control ($P < .05$). Therefore, the RJ_{DE} and RJ_{LE} could not protect cells from DXR-induced genotoxicity. There was no significant difference in the mitotic index and proliferation index induced by RJ_{DE} and RJ_{LE} pretreatments compared to the DXR treatment alone (Table 5).

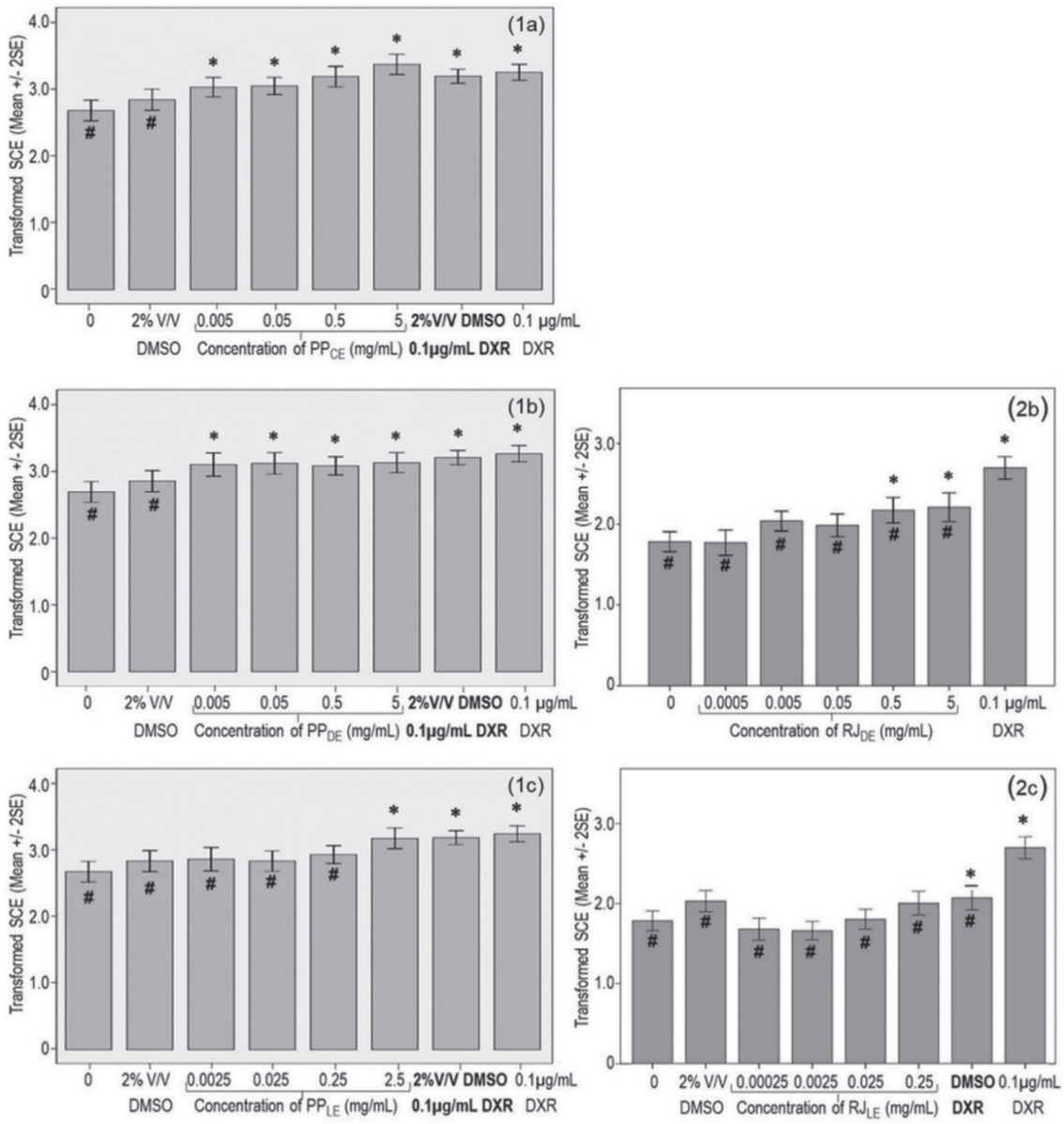

Figure 1 SCE$_T$ induced by propolis (1) and royal jelly (2) extracts in genotoxic studies (n = 3).
(a) crude extract, (b) defatted extract, (c) lipid extract.
*$P < 0.05$ significantly different from the RPMI-treated negative control. # $P < 0.05$ significantly different from the DXR-treated positive control.

PP$_{CE}$ = crude extract of propolis
PP$_{DE}$ = defatted extract of propolis
PP$_{LE}$ = lipid extract of propolis
RJ$_{DE}$ = defatted extract of royal jelly
RJ$_{LE}$ = lipid extract of royal jelly
DXR = doxorubicin

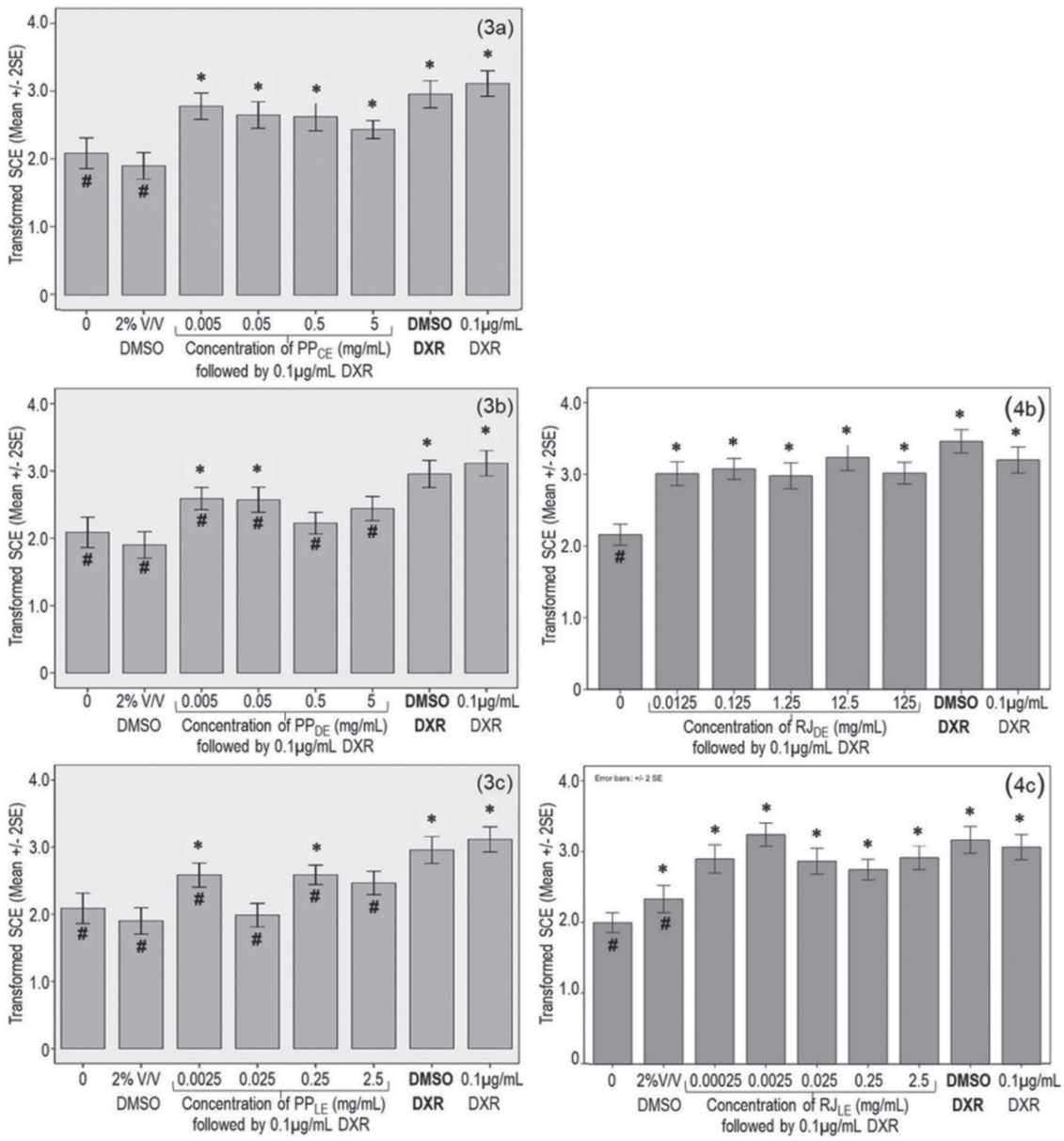

Figure 2 SCE_T levels induced by propolis (3) and royal jelly (4) extracts, followed by 0.1 µg/mL DXR in antigenotoxic studies (n = 3).

(a) crude extract, (b) defatted extract, and (c) lipid extract.

* $P < 0.05$ significantly different from the RPMI-treated negative control. # $P < 0.05$ significantly different from the DXR-treated positive control.

PP_{CE} = crude extract of propolis
PP_{DE} = defatted extract of propolis
PP_{LE} = lipid extract of propolis
RJ_{DE} = defatted extract of royal jelly
RJ_{LE} = lipid extract of royal jelly
DXR = doxorubicin

Table 1 Chemical contents

Chemical contents	Thai PP		Thai RJ		Thai BP[4]	
	DE	LE	DE	LE	DE	LE
Chemical content (g%)	52.0	72.7	22.3	2.9	84.7	11.3
Total phenolic content (mg GAE*/g)	6.7 ± 0.1	4.7 ± 0.1	0.9 ± 0.5	1.4 ± 0.7	9.2 ± 0.2	1.1 ± 0.1
Gallic acid (µg/g)	35.1 ± 0.1	50.8 ± 0.1	ND	ND	195.6 ± 0.1	111.5 ± 0.1
Ferulic acid (µg/g)	ND	20.7 ± 0.0	5.3 ± 0.1	96.8 ± 0.1	9.2 ± 0.1	131.3 ± 0.1
Total flavonoid content (RE**/g)	35.7 ± 3.2	8.1 ± 5.0	0.0 ± 0.1	1.9 ± 0.1	11.9 ± 0.8	4.5 ± 5.8
Quercetin (µg/g)	42.2 ± 0.6	13.5 ± 6.3	ND	ND	ND	ND
Rutin (µg/g)	24.1 ± 0.8	12.0 ± 4.0	ND	ND	ND	ND

*GAE: Gallic acid equivalent, **RE: Rutin equivalent, ND = not detected

Table 2 M.I. and P.I. induced by Thai propolis in genotoxic studies (n = 3)

Propolis Extracts	Concentration (mg/mL)	M.I. ± S.E.	P.I. ± S.E.
CE	0.005	10.9 ± 0.1	2.2 ± 0.0
	0.05	12.5 ± 0.7	2.4 ± 0.2
	0.5	12.0 ± 0.4	2.5 ± 0.6
	5.0	11.1 ± 1.4	2.1 ± 0.3
DE	0.005	11.8 ± 0.2	2.5 ± 0.0
	0.05	10.8 ± 1.2	2.2 ± 0.4
	0.5	12.3 ± 0.3	2.7 ± 0.2
	5.0	11.1 ± 0.9	2.4 ± 0.1
LE	0.0025	11.4 ± 0.4	2.3 ± 0.1
	0.025	11.8 ± 0.5	2.2 ± 0.0
	0.25	11.2 ± 0.3	2.4 ± 0.0
	2.5	11.6 ± 0.9	2.2 ± 0.2
Control	0	10.1 ± 1.0	2.1 ± 0.1
	2%V/V DMSO	11.1 ± 0.2	2.2 ± 0.0
	0.1 µg/mL DXR	12.1 ± 1.1	2.3 ± 0.2

Table 3 M.I. and P.I. induced by Thai royal jelly in genotoxic studies (n = 3)

Royal Jelly Extracts	Concentration (mg/mL)	M.I. ± S.E.	P.I. ± S.E.
DE	0.0005	17.5 ± 0.7	2.9 ± 0.5
	0.005	20.0 ± 4.2	2.9 ± 0.6
	0.05	20.0 ± 5.6	3.1 ± 0.9
	0.5	15.0 ± 0.0	2.3 ± 0.0
	5.0	17.5 ± 6.3	2.9 ± 1.3
LE	0.00025	23.5 ± 6.3	3.6 ± 1.2
	0.0025	16.5 ± 4.9	2.3 ± 0.6
	0.025	17.5 ± 6.3	2.6 ± 0.9
	0.25	25.0 ± 2.8	4.0 ± 0.7
	2.5	22.5 ± 2.1	3.3 ± 0.4
Control	0	19.0 ± 2.0	2.7 ± 0.3
	2%V/V DMSO	18.5 ± 0.7	2.4 ± 0.0
	0.1 μg/mL DXR	12.0 ± 4.2	1.6 ± 0.4

Table 4 M.I. and P.I. induced by propolis and DXR in antigenotoxic studies (n = 3)

PP extracts	Extract conc. (mg/ml)	DXR (mg/ml)	M.I. ± S.E.	P.I. ± S.E.
CE	0.005	0.1	17.4 ± 1.9	2.4 ± 0.4
	0.05	0.1	18.2 ± 2.6	2.3 ± 0.1
	0.5	0.1	19.9 ± 0.8	2.3 ± 0.1
	5.0	0.1	24.1 ± 6.3	3.1 ± 0.9
DE	0.005	0.1	20.8 ± 2.5	2.9 ± 0.2
	0.05	0.1	22.8 ± 6.6	3.3 ± 1.0
	0.5	0.1	19.9 ± 6.0	2.5 ± 0.7
	5	0.1	23.2 ± 0.6	3.3 ± 0.0
LE	0.0025	0.1	13.9 ± 6.0	1.9 ± 0.8
	0.025	0.1	15.2 ± 4.4	2.4 ± 0.6
	0.25	0.1	20.0 ± 4.3	1.5 ± 0.2
	2.5	0.1	10.4 ± 0.4	1.6 ± 0.3
Control	0	0	20.8 ± 0.1	3.3 ± 0.0
	2%V/V DMSO	0	26.0 ± 1.2	4.1 ± 0.4
	2%V/V DMSO	0.1	14.1 ± 4.4	1.6 ± 0.3
	0	0.1	15.0 ± 3.4	2.1 ± 0.4

Table 5 M.I. and P.I. induced by royal jelly and DXR in antigenotoxic studies (n = 3)

RJ extracts	Extract conc. (mg/ml)	DXR (mg/ml)	M.I. ± S.E.	P.I. ± S.E.
DE	0.0125	0.1	17.0 ± 2.8	2.9 ± 4.2
	0.125	0.1	25.5 ± 4.9	3.6 ± 6.0
	1.25	0.1	22.0 ± 2.8	3.2 ± 4.2
	12.5	0.1	18.0 ± 1.4	2.5 ± 1.4
	125	0.1	19.5 ± 0.7	3.1 ± 0.7
LE	0.00025	0.1	20.0 ± 1.4	2.6 ± 2.1
	0.0025	0.1	19.0 ± 1.4	2.6 ± 2.1
	0.025	0.1	18.5 ± 3.5	2.9 ± 4.9
	0.25	0.1	21.0 ± 2.8	3.4 ± 4.2
	2.5	0.1	12.5 ± 2.1	2.1 ± 3.5
Control	0	0	19.5 ± 3.5	3.7 ± 2.8
	2%V/V DMSO	0	19.5 ± 0.7	3.1 ± 5.6
	2%V/V DMSO	0.1	15.0 ± 0.0	1.9 ± 5.6
	0	0.1	19.5 ± 3.5	2.9 ± 3.5

Discussion

As earlier reports,[4,5] chemical compositions of crude Thai royal jelly (pH 4) were 2.5 g% (w/w) lipid with 2.2 g% protein, 32 g% carbohydrate, 0.2 g% fiber, and no ash. Those of crude Thai bee pollen (pH 6.5) were 11 g% (w/w) lipid with 26 g% protein, 55 g% carbohydrate, 1.7 g% fiber, and no ash. The antioxidant activities of the BP_{CE}, RJ_{CE}, RJ_{DE}, RJ_{LE} are 157.0 ± 3.0, 17.9 ± 1.9, 14.6 ± 3.8, 21.2 ± 1.9, respectively. Among bee products, propolis had the highest lipid content and total flavonoid content, followed by bee pollen,[4] and royal jelly,[5] respectively. Concerning antioxidant activities, propolis extracts had the highest values than other products, especially the PP_{LE}. It is approximately 4.6-fold higher than BP_{LE} and 21.0-fold higher than RJ_{LE}.

Thai propolis and royal jelly extracts tend to have low risk of cytotoxicity. PP_{CE} and PP_{DE} showed some genotoxicity. In contrast, low doses of PP_{LE} (0.0025-0.25 mg/mL) showed the same level of SCEs induction as the negative control that were significantly different from the positive control. The low risk of genotoxicity was also found in low doses of royal jelly (0.0005-0.05 mg/mL RJ_{DE}, and 0.00025-0.25 mg/mL RJ_{LE}). In our previous studies,[5] RJ_{CE} (0.005-0.5 mg/mL) did not show genotoxicity, but at the highest dose of 5 mg/mL increased SCEs by 1.4-fold. We have also reported the genotoxicity of Thai BP.[4] BP_{CE} only at 0.005 and 0.5 mg/mL exhibited a 1.2-fold increase in SCEs. BP_{DE} (0.005-5 mg/mL) induced 1.2 to 1.3-fold increase in SCEs. BP_{LE} at low doses (0.0025-0.25 mg/mL) did not increase SCEs, but only at the highest dose of 2.5 mg/mL increased 1.2-fold SCEs. These results suggested that the diethyl ether lipid extracts from all bee products tended to have the lowest genotoxicity level, compared to their crude extracts and defatted extracts.

PP_{DE} and PP_{LE} pretreatments significantly decreased DXR-induced SCE without dose-dependency, whereas RJ_{DE} and RJ_{LE} could not protect cells from DXR-induced genotoxicity. However, in our previous study,[5] RJ_{CE} pretreatment at 0.005 and 0.5 mg/mL significantly decreased the DXR-induced SCEs by 0.8-fold ($P < .05$). The discrepancies may result from 1) depletion of the active compound during the extraction process, or 2) the active compound needs to be in the crude extract form allowing the interaction between substances in DE and LE, or 3) only the crude form has the antigenotoxic property rather than separated forms.

Comparing the antigenotoxicity efficacies among propolis, bee pollen, and royal jelly extracts

against DXR from this study and the previous studies[4,6]: our data indicated that the propolis extracts exhibited the highest antigenotoxicity, followed by the bee pollen and royal jelly extracts, respectively. PP_{LE} pretreatments demonstrated the most effectiveness, followed by PP_{DE}, whereas the rest showed relatively weak potency. At the proper doses, both PP_{LE} and PP_{DE} could return the genotoxic damage to the normal level, whereas no other extracts could. In addition, PP_{LE} at 0.0025-0.25 mg/mL had no genotoxicity on human lymphocytes, whereas PP_{DE} (0.005-5 mg/mL) were genotoxic. Therefore, PP_{LE} demonstrated as the most promising chemopreventive compound without genotoxicity nor cytotoxicity.

Our results showed that the propolis contained mainly lipids (72 g%) with 13.5 g% proteins and 8.8 g% carbohydrates. Its lipid extract had high gallic and ferulic acid levels, whereas the defatted extract had high quercetin and rutin levels. The ethereal extract with Soxhlet apparatus had a relatively high yield of lipid content (72 g%), compared to other extracts reported earlier. Besides different topographic origins, the variation in lipid content might be related to different solvent extraction protocols, e.g., 70% ethanolic extract of Thai propolis from Phayao, Thailand (18 g%),[19] 80% ethanolic extract of the Brazilian red propolis (65 g%),[20] and 60% ethanolic extract of Kashmir propolis (33 g%).[21] The diethyl ether extraction using the Soxhlet apparatus possibly extracted more trapped lipids and included more nonpolar lipids e.g., triglyceride, wax, and long chain fatty acids than the alcohol extraction. Notably, propolis's ethereal lipid extract exhibited the most effective genoprotectant against DXR with the highest antioxidative activities. Furthermore, no genotoxicity was shown at its effective dose. The defatted extract from propolis exhibited antigenotoxicity against DXR, but it was genotoxic. Therefore, we recommend lipid extract of propolis for safe use. Besides, this extract also had high antioxidant activities and was enriched with gallic and ferulic acids. Gallic and ferulic acids are potent free radical scavenger phenolic compounds. Gallic acid showed a neuroprotective effect on 6-hydroxydopamine-induced apoptosis in human dopaminergic cells, SH-SY5Y.[22] Ferulic acid protected DNA damage induced by hydrogen peroxide or ultraviolet light.[23] Accordingly, the lipid extract of Thai propolis exhibited the most promising candidate as genoprotectant with high antioxidative activities.

In summary, we assessed antioxidative and antigenotoxic activities of PP_{CE}, PP_{DE}, PP_{LE}, RJ_{DE}, and RJ_{LE} in this study. We compared the results with our previous studies[4-6] to get a full view of their potencies among the bee products from Chiangmai, Thailand. Although the results from the previous studies of $BP_{CE, LE, DE}$[4] and RJ_{CE}[5,6] may not seem appropriate to be compared with this study, in fact all these experiments were contributed in the same period with the same protocols. Therefore, the results could be compared without discrepancies in the materials and methods. Our results revealed that propolis's lipid extract is the most promising candidate for genotoxic prevention with a high level of antioxidative activities. Further study on *in vivo* antigenotoxicity and investigation of principal active substances are required to confirm their effectiveness. Moreover, further *in vivo* studies would suggest the appropriate doses that can be consumed in order to reach the antigenotoxic and antioxidative properties.

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