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Dual effect of Algerian propolis on lung cancer: antitumor and chemopreventive effects involving antioxidant activity

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The purpose of our study was to divulge the antiproliferative effect of an ethanolic extract of Algerian propolis (EEP) in the human lung adenocarcinoma cell line (A549) and reveal the chemopreventive role against benzo(a)pyrene-induced lung carcinogenesis in albino Wistar rats. Cytotoxicity of EEP was evaluated using the MTT assay and cell adhesion in A549 cells. Moreover, rats were given 25 mg/kg of propolis for 5 days before induction of experimental lung cancer by a single intraperitoneal dose of 200 mg/kg benzo(a)pyrene. Body weight, lung weight, lipid peroxidation, marker enzymes, and enzymatic and non-enzymatic antioxidants were estimated. The EEP demonstrated an inhibitory effect on proliferation of A549 at 24 and 72 hours in a dose-dependent manner and blocked adhesion of the cells by fibrinogen. Moreover, EEP reduced the oxidative stress generated by benzo(a)pyrene. The pre-treatment showed that enzymatic and non-enzymatic antioxidants increased and lipid peroxidation decreased. A histological analysis further supported these findings and showed a decrease in the number of side effects. These results are particularly important for both clinical applications of propolis and the possibility for its use as a potential chemotherapeutic agent.

Keywords: Algerian propolis/effects. Benzo(a)pyrene. Chemoprevention. Lung cancer. Oxidative stress.

INTRODUCTION

Lung cancer is a type of malignant tumor of lung tissue (Yang *et al.*, 2016) and is considered to be one of the most significant diseases in respiratory medicine (Silva *et al.*, 2007). Lung cancer is the leading cause of cancer-related death in men and women worldwide, with about 1.8 million new cases each year (Siegel, Miller, Jemal, 2015). Several epidemiological studies have shown that exposure to polycyclic aromatic hydrocarbons (PAHs) can increase the risk of multiple cancers, such as those of the lung. One of these PAHs is benzo(a)pyrene (B(a)P), which has been widely studied because of its ability to induce carcinogenicity and mutagenicity in humans and animals (Anandakumar *et al.*, 2009; Kasala *et al.*, 2015). However, it must be metabolically activated to the B(a)P-7,8-dihydrodiol-9,10-epoxide metabolite (BPDE)

before exerting its toxic effects. BPDE is considered the ultimate and most carcinogenic derivative of B(a)P metabolism, as it reacts with DNA as well as the redox cycling of B(a)P-quinone and produces reactive oxygen species (ROS) (Briede *et al.*, 2004).

Therefore, it is necessary to develop more effective curative or preventive therapies with few side effects to significantly reduce lung cancer mortality. Several studies have reported the efficacies of different natural products for treating lung cancer, such as use of propolis, as their therapeutic potential to treat several diseases is well shown (Banskota, Tezuka, Kadota, 2001). The chemical composition of propolis or bee glue is very complex and varies according to geographical origin, the bee species, (Kurek-Górecka *et al.*, 2014), and the trees and plants in the ecosystem that influence the biological activities of the bees (Piccinelli *et al.*, 2013). Despite differences in origin and chemical composition, different types of propolis have a variety of biological activities, including antioxidant power. It has been suggested that the therapeutic activities of propolis depend mainly on the presence of polyphenols,

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specifically flavonoids (Campos *et al.*, 2015; Mouhoubi-Tafinine, Ouchemoukh, Tamendjari, 2016). At least 38 different flavonoids have been reported in propolis.

Algeria's distinctive climate, fauna, and flora contribute to its great biodiversity (Soltani *et al.*, 2017). According to some studies, Algerian propolis has a wide range of biological properties, including antitumor (Benguedouar *et al.*, 2016), bactericidal (Soltani *et al.*, 2017), renoprotective (Boutabet *et al.*, 2011), and antioxidant activities (Piccinelli *et al.*, 2013; Mouhoubi-Tafinine, Ouchemoukh, Tamendjari, 2016) and it contains several anti-cancer phenolic components (Boutabet *et al.*, 2011).

Epidemiological and preclinical studies have suggested that the polyphenols and flavonoids in propolis possess direct antitumor and chemopreventive effects. This has accentuated cancer prevention strategies in which propolis was used as a dietary supplement (Seydi *et al.*, 2016). The anticarcinogenic activity of the flavonoids in propolis can suppress tumor growth by inhibiting angiogenesis and inducing apoptosis of tumor cells (Szliszka *et al.*, 2011). The protective effect of propolis against several types of cancer, including lung cancer, may occur by directly inhibiting tumor cell growth and inducing apoptosis (Valente *et al.*, 2011) or indirectly by scavenging ROS and increasing antioxidant enzyme activities (Oršolić *et al.*, 2013).

In the present study, we investigated the anti-tumor effect of Algerian propolis on a human lung adenocarcinoma cell line and its chemopreventive effect in lung cancer induced experimentally by B(a)P for the first time.

MATERIAL AND METHODS

Material and major reagents

The A549 human lung adenocarcinoma cell line was obtained from Professor José Luis (UMR_S 911 INSERM, Marseille, France). RPMI, fetal bovine serum (FBS), DPPH, and B(a)P were purchased from Sigma-Aldrich (St. Louis, MO, USA). Crude propolis was collected from a protected area in Algeria and supplied by a honeybee keeper in the Kaous-Jijel region (Algerian eastern Mediterranean coast) in 2014. The propolis was stored at 4 °C in airtight/dark containers until analysis.

Preparation of the propolis extract

The raw propolis was cut into small pieces and added to 95% ethanol for 2 weeks (1 g in 9 volumes of ethanol)

with agitation. After filtration, the filtrate was evaporated at 80 °C in a rotary evaporator (Evaporator E100; Heidolph Instruments, Schwabach, Germany). The ethanolic extract of propolis (EEP) was resuspended in 70% methanol and allowed to steep for 1 night before a second evaporation. The extract was stored in clean, dark, airtight bottles at 4–6 °C until analysis (Alyane *et al.*, 2008).

Cell culture

The A549 cells were cultured in RPMI supplemented with 10% FBS containing growth factors, 1% antibiotics penicillin and streptomycin, and 1% glutamine. The cells were incubated in 5% CO₂ and 95% humidity in a 37 °C incubator. The A549 cells were seeded in 25-cm² culture flasks and grown for 1-2 days before use.

Cytotoxicity of the propolis extract

The number of viable A549 cells after treatment with the EEP was evaluated by the MTT (3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay as described previously (Mosmann, 1983). In brief, A549 cells (1 × 10⁴ cells/well) were seeded in 24-well microtiter plates in 50 µL of RPMI. After a 24 h incubation (time required for attachment), the medium was renewed with or without different concentrations of the EEP (1, 5, 10, 20, 40, 80, and 100 µg/mL). The wells were washed with PBS after 24 and 72 h, and then 100 µL/well of the MTT solution (5 mg/mL) was added. After a 3 h incubation with MTT at 37°C, the MTT crystals were solubilized by adding 100 µLDMSO to each well. Absorbance was measured at 550 nm. The percentage cell viability was calculated as follows:

Cell viability (%) = mean optical density (OD) of treated cells/mean OD of control cells × 100%.

The median inhibitory concentration (IC₅₀) values of the EEP were determined.

Cell adhesion test

Adhesion assays were performed as described previously (Delamarre *et al.*, 2009). Briefly, 24-well plates were coated with purified extracellular matrix (ECM) protein solutions of fibrinogen (Fg), collagen type I (Coll I), and polylysine (PL) for 2 h at 37 °C and blocked with 0.5% PBS/bovine serum albumin. Cells previously preincubated with different concentrations of the EEP (10, 20, and 40 µg/mL) for 30 min at 37 °C were added

to wells and allowed to adhere to the substrata for 2 h at 37 °C. After washing, the adherent cells were fixed in 1% glutaraldehyde, stained for 30 min with 0.1% crystal violet, and lysed with 1% sodium dodecyl sulfate. Cell adhesion was quantified by measuring absorbance at 600 nm with a microtiter plate reader.

DPPH free radical scavenging activity

The free radical-scavenging activity of the test compounds (EEP and quercetin) was investigated according to the method of Brand-Williams, Cuvelier, Berset (1995) and the resulting decrease in DPPH absorption at 517 nm was measured after 30 min.

Animals

A total of 24 male albino Wistar rats (weight, 100-200g) were used in this study. The animals were acclimated to polystyrene cages and had free access to food and water. They were randomly divided into three equal groups of eight rats each, and their weight change was followed throughout the treatment period:

Group 1 served as a control and received a single injection of olive oil (B(a)P vehicle); group 2 received, a single dose of B(a)P (200 mg/kg/dissolved in the same vehicle) intraperitoneally (i.p.); and group 3 received the EEP (25 mg/kg/day) by gavage for 5 days prior to B(a)P administration. After 5 days, they were injected i.p. with B(a)P (200 mg/kg).

The animals were killed by cervical dislocation after 20 weeks of treatment. The lungs of each rat were collected, washed in 9‰ NaCl, weighed, and subjected to macroscopic examination for any signs of inflammation or malignancy. Part of the lung was stored at -20 °C until analysis of cellular oxidative stress and the other part was immersed in ethanol-acetic acid-formalin fixative saline (EAFS) for histopathological processing. Blood was collected from the retro-orbital sinus, centrifuged at 3,300 rpm for 10 min, and the serum was collected and frozen for biochemical analyses.

Cytosolic fraction from lung tissue

A 1 g portion of pulmonary tissue was homogenized in 3 mL of phosphate buffer (0.1 M, pH 7.4) containing 1.17% KCl. The homogenate was centrifuged at 2,000 rpm for 15 min at 4 °C to remove cell debris and then at 12,000 rpm for 30 min. The clear supernatant was used for the biochemical assays (Iqbal *et al.*, 2003).

Assessment of oxidative stress markers

Malondialdehyde (MDA) was assayed using thiobarbituric acid according to the method described by Ohkawa, Ohishi, Yagi (1979). Total glutathione (GSH) was assayed using the method of Ellman (1959). Superoxide dismutase (SOD) was assessed according to the method of Beauchamp and Fridovich (1971). Glutathione S-transferase activity (GST) was measured using the method of Habig, Pabst, Jakoby (1974) which uses chlorodinitrobenzene as the substrate, and catalase (CAT) activity was assayed by the method of Sinha (1972) based on the disappearance of H₂O₂ in the presence of the enzyme source.

Serum samples and marker enzymes

Serum was used to determine the activities of γ -glutamyl transpeptidase (γ -GT) and lactic dehydrogenase (LDH) employing a kit adapted to an auto analyzer (ECLIA Cobas 411).

Assessment of protein content

Proteins were measured at all stages according to the Bradford method (Bradford, 1976).

Histopathological study

After fixing the tissues for 48 h in EAFS (Harrison, 1984), the lung tissue samples were processed routinely and embedded in paraffin wax to form a paraffin block. Sections of 5 μ m in thickness were cut and stained with hematoxylin followed by 1% eosin (H&E) and examined under a light microscope.

Statistical analysis

Student's *t*-test was used to assess the statistical significance of the treated groups vs. the control group. P-values < 0.05 were considered significant.

RESULTS

Effect of propolis on A549 cell viability

After treating the A549 cell lines with different concentrations of the EEP, their morphology was abnormal compared to that of the untreated control (data not shown). The MTT assay revealed that A549 cell growth was inhibited after 24 and 72 h of treatment with the EEP in a dose-dependent manner. The percentage cell viability

after treatment with the EEP is shown in Figure 1. The IC_{50} values 24 and 72 h after treatment for the mean of three independent experiments were 69.94 and 14.32 $\mu\text{g}/\text{mL}$, respectively.

Propolis extract affects adhesion of lung tumor cells

We investigated the possible anti-adhesive activity of the EEP in A549 cells. We performed cell adhesion

assays using different purified ECM proteins, such as Fg, Coll I, and PL. As shown in Figure 2, the EEP blocked adhesion of A549 cells to Fg, while no effect was observed for Coll I or PL. This inhibitory action of the EEP on Fg was dose-dependent.

Radical scavenging activity of propolis

The results of the DPPH scavenging assay compared with those of a reference antioxidant (quercetin) are

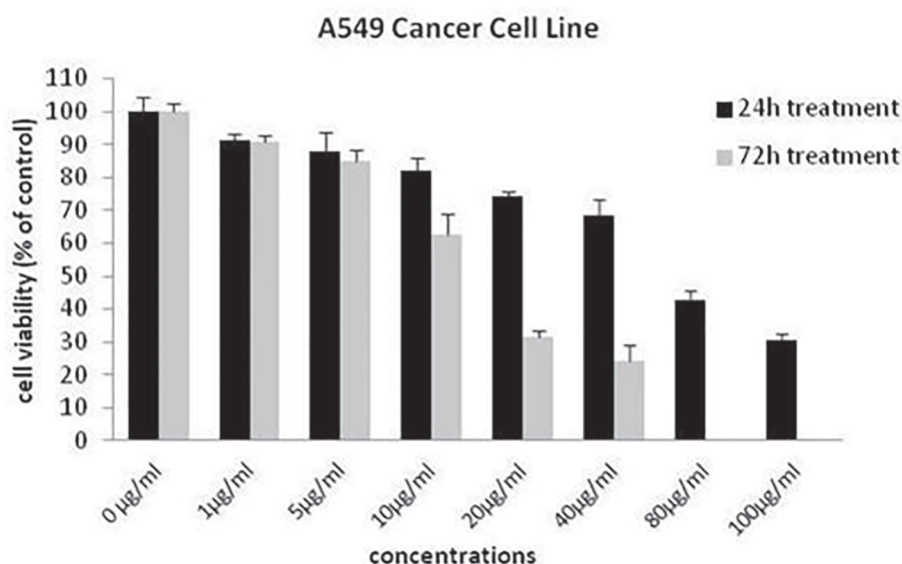


FIGURE 1 - Concentration effectiveness of the Algerian propolis extract on viability of lung carcinoma A549 cells 24 and 72 h after treatment. Values are mean $\pm$ standard error of three independent experiments.

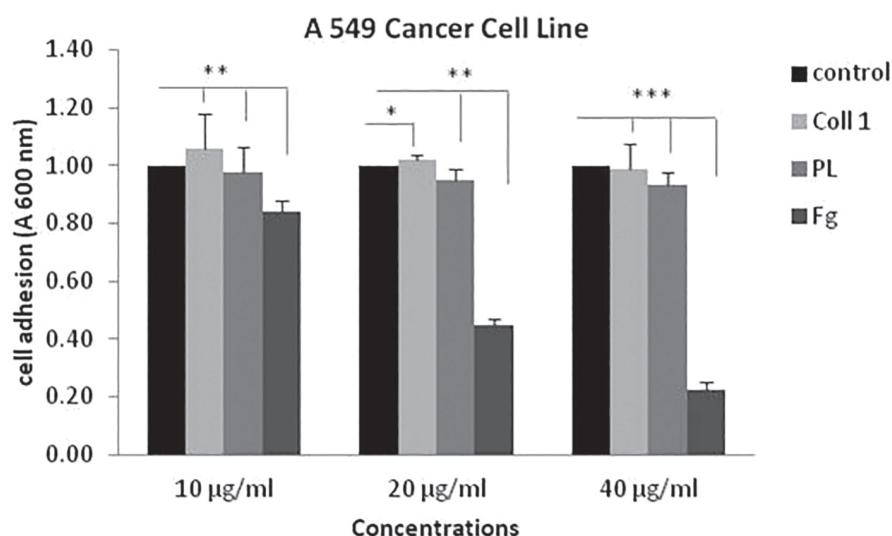


FIGURE 2 - Inhibition of human lung adenocarcinoma cell line (A549) adhesion by the Algerian propolis extract. A 549 cells were pre-incubated with different concentrations of the propolis extract and added to wells coated with 50 $\mu\text{g}/\text{mL}$ fibrinogen (Fg), 10 $\mu\text{g}/\text{mL}$ collagen I (Coll I), and 10 $\mu\text{g}/\text{mL}$ poly-L-lysine (PL). Values are mean $\pm$ standard error of three independent experiments. p-value < 0.05: significant (*), p < 0.01: very significant (**), p < 0.001: highly significant (***), NS (not significant).

demonstrated in Figure 3. The EEP reduced the DPPH free radical in a concentration-dependent manner. Strong antioxidant activity was detected at EEP concentrations of 30, 75, 150, 200, and 300 $\mu\text{g/mL}$, with maximum reactive rates of 41.74, 70.67, 75.35, 85.52, and 90.73% respectively. The IC_{50} values for antioxidant activity of the EEP and quercetin were 43.9 and 54.9 $\mu\text{g/mL}$, respectively.

Effect of propolis on body and lung weight

Table I presents the effect of the EEP on body and lung weight in the control and experimental groups of animals that were sacrificed at the end of the study. Rats treated with B(a)P (group 2) demonstrated a loss of body weight compared to that in the untreated control rats (group 1). In contrast, lung weight increased significantly ($p < 0.01$) compared to that of the control group. The EEP significantly ($p < 0.01$) increased final body weight and significantly ($p < 0.05$) reduced lung weight in group 3 compared with those in group 2. These data show an observable increase in the relative weights of the lungs in groups 3 and 2, respectively, compared with that in the control group.

Effect of propolis on serum marker enzymes

Table II presents the effect of the EEP on serum marker enzyme activities in the control and experimental groups. The activities of the marker enzymes LDH and γGT increased significantly ($p < 0.01$) in the lung cancer-bearing animals (group 2) compared with those in the control group. The EEP treatment (group 3) caused a significant ($P < 0.05$; $P < 0.001$) decrease in these enzymes activities compared with those in the cancer-bearing group.

Oxidative status

Five parameters were evaluated to determine the oxidative status of tissues. The assay results of cellular GSH and the enzymatic activities of SOD, CAT, and GST as well as MDA levels in lung tissues of the control and experimental groups are presented in Figure 4.

The rats injected with B(a)P showed a significant increase in MDA levels ($p < 0.001$) and a significant decrease in GSH ($p < 0.001$), SOD, CAT, and GST activities ($p < 0.01$) compared with those in the control

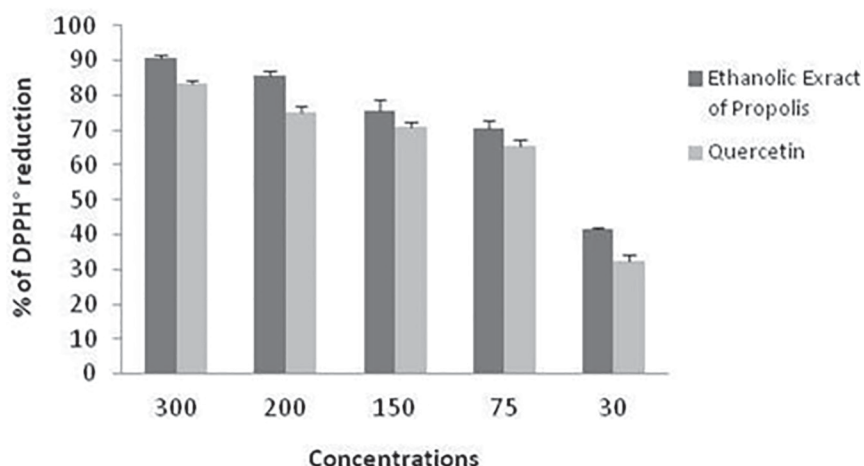


FIGURE 3 - Scavenging effect of the Algerian ethanolic extract of propolis (EEP) vs. quercetin against DPPH free radicals. Each value is expressed as mean $\pm$ standard deviation of three independent experiments.

TABLE I - Effect of administering the ethanolic extract of propolis on the body and lung weight and ratio of lung to body weight in rats

Parameters	Group 1	Group 2	Group 3
Number of rat examined	6	6	6
Body weight (g)	300 $\pm$ 10	204.53 $\pm$ 21.87 ^{**} , ¹	246.66 $\pm$ 15.27 ^{**} , ²
Lung weight (g)	1.15 $\pm$ 0.06	2.31 $\pm$ 0.2 ^{**} , ¹	1.58 $\pm$ 0.1 [*] , ²
The relative lung weight (%)	0.38	1.129	0.64

Each value is expressed as mean $\pm$ standard deviation ($n = 6/\text{group}$). P value < 0.05 : significant (*), $p < 0.01$: very significant (**), $p < 0.001$: highly significant (***), NS (not significant), as ¹group 2 vs. group 1. ²group 3 vs. group 2.

TABLE II - Effect of propolis on serum marker enzyme activities in the control and experimental animals

Parameters	Group 1	Group 2	Group 3
LDH ($\mu\text{mol}/\text{min}/\text{mg}$ protein)	1.15 ± 0.016	$3.07 \pm 0.046^{**1}$	$1.51 \pm 0.076^{*2}$
GGT (nmoles/min/mg protein)	1.938 ± 0.102	$3.414 \pm 0.364^{***1}$	$2.574 \pm 0.225^{***2}$

Each value is expressed as mean $\pm$ standard deviation. (n = 6/group). p-value < 0.05: significant (*), p < 0.01: very significant (**), p < 0.001: highly significant (***), NS (not significant) as ¹group 2 vs. group 1. ²group 3 vs. group 2. GGT: γ -glutamyl transpeptidase. LDH: lactate dehydrogenase

group. MDA was significantly lower ($p < 0.05$) in group 3 compared with that in group 2, whereas SOD, CAT, and GSH activities increased significantly ($p < 0.01$). The enzymatic antioxidants (SOD, CAT, and GST) and non-enzymatic antioxidants (GSH) were enhanced after the EEP treatment compared with those in the carcinogen-induced lung cancer group 2, which was associated with a decrease in MDA level.

Histopathological study

A macroscopic examination of the lungs of the control rats showed an intact structure, compared with the lungs of rats treated with B(a)P alone. Lungs from rats treated with B(a)P were dark in color possibly due to necrosis and merged pulmonary lobules; however, the lungs treated with the EEP appeared less toxic (data not shown).

The lungs of the control rats showed normal histological structure under the microscope (Figure 5). However, the alveoli were destroyed and intraparenchymal lymphocyte infiltration was detected with formation of a follicle in the B(a)P group. However, administration of EEP significantly decreased intraparenchymal lymphocyte and macrophage infiltration compared with that in the B(a)P group.

DISCUSSION

One of the strategies to fight cancer is the use of natural products. The incidence of cancer is less common in people whose diet is based on fruits and vegetables. Results from several studies indicate that propolis, as a natural product, and its components has anti-proliferative and anti-neoplastic properties. In the present study, we showed that Algerian propolis decreased cell viability by exerting a cytotoxic effect on A549 human lung adenocarcinoma cells. The antiproliferative and cytotoxic effects of the EEP occurred in a dose-dependent manner. Our results are in accordance with those of Frión-Herrera *et al.* (2015), who showed that Brazilian propolis inhibits

the growth of A549 cells in a dose-dependent manner and that propolis shows selectivity toward tumor cells compared to normal cells. The IC_{50} at 72 h of the EEP was $14.32 \mu\text{g}/\text{mL}$, which is less than that of Brazilian propolis (Frión-Herrera *et al.*, 2015) and propolis from Thailand (Khacha-Ananda *et al.*, 2013) with IC_{50} values of 69.17 and $85.05 \mu\text{g}/\text{mL}$, respectively. These results indicate that the Algerian EEP was more toxic against cancer cells than many other propolis, and that inhibition of cancer cell growth may be related to flavonoid content depending on the geography and source of propolis which affect their composition.

Because angiogenesis is an essential process for cancer progression, we evaluated the capacity of propolis to exert anti-adhesive activity using purified ECM proteins, such as the integrin-dependent substratum. ECM proteins are a potential target for pharmacological agents in the treatment of tumor malignancies with the aim to control metastatic spread (Akalu, Cretu, Brooks, 2005). Taken together, our results show that EEP from Algeria notably blocked adhesion of A549 cells to Fg suggesting that the effect of our propolis may involve the integrin family of adhesion receptors and that all integrins are not likely affected because inhibition was not observed by Coll I thereby supporting the findings of Benguedouar *et al.* who showed that galangin, one of the most abundant flavonoids in our Algerian propolis, reduces the expression of many integrins that play a key role regulating both mitogenic signaling, cell adhesion, and cell migration. Galangin of Algerian propolis induces melanoma cell autophagy/apoptosis dose dependently by activating p38 mitogen activated protein kinase and inhibiting *in vivo* tumor growth and metastasis in a mice melanoma model (Benguedouar *et al.*, 2016). The same authors showed the importance of both caffeic acid and its derivatives, i.e., CAPE and (+)-chicoric acid methyl ester, present in Algerian propolis for their antioxidant and anticancer properties (Benguedouar *et al.*, 2016; Segueni *et al.*, 2011). Previous studies have shown that CAPE effectively suppresses transforming growth factor (TGF)- β -enhanced cell motility and TGF- β -induced Akt

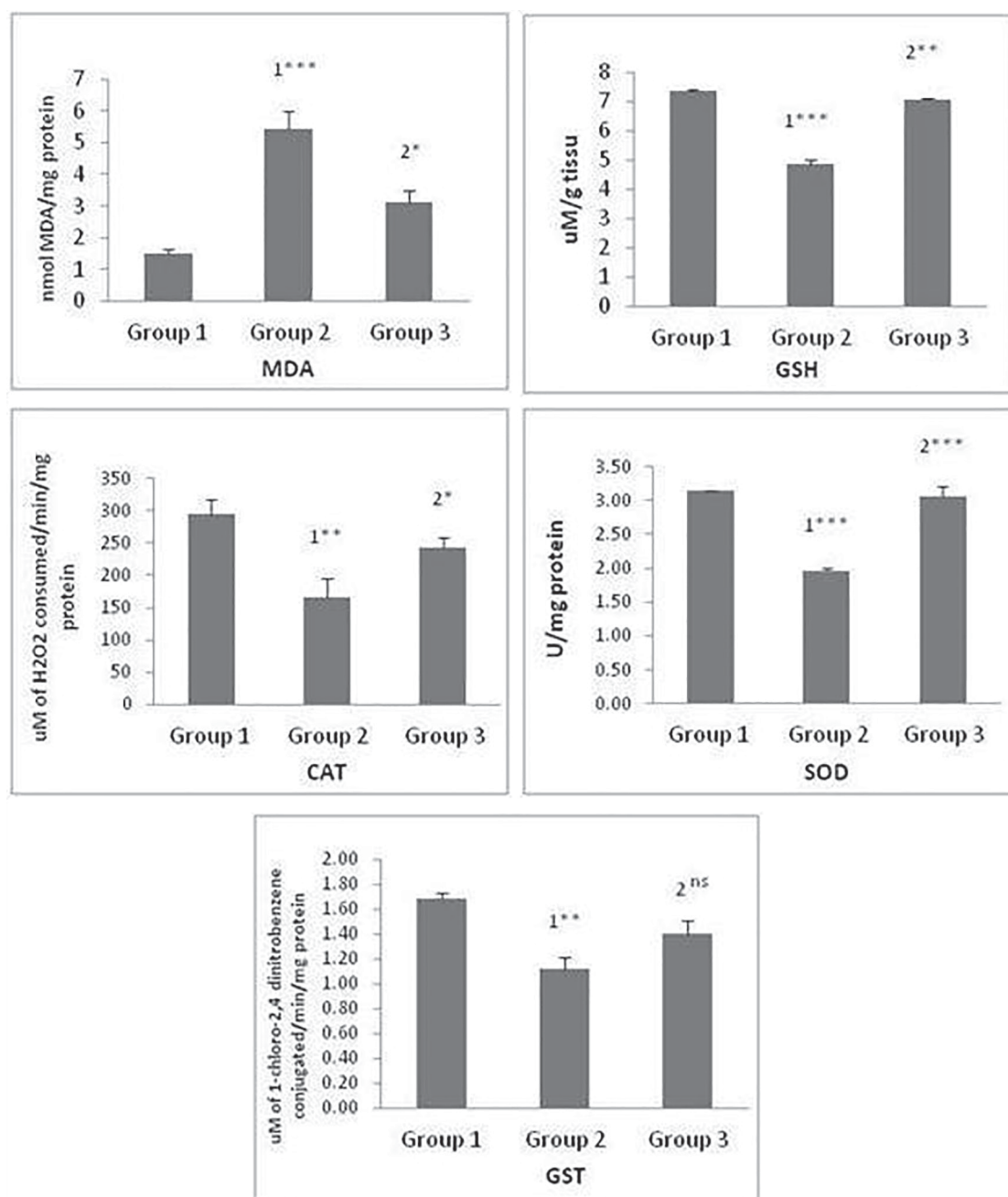


FIGURE 4 – Effect of the Algerian propolis extract on oxidative stress parameters. Each value is expressed as mean $\pm$ standard deviation ($n = 8/\text{group}$). p -value < 0.05 : significant (*), $p < 0.01$: very significant (**), $p < 0.001$: highly significant (***), NS (not significant). ¹group 2 vs. group 1. ²group 3 vs. group 2. Group 1: control group includes rats that received olive oil; group 2: rats treated with benzo(a)pyrene (B(a)P), Group 3: rats that received the Algerian propolis extract followed by B(a)P. MDA: malondialdehyde, GSH: reduced glutathione, CAT: catalase, SOD: superoxide dismutase, and GST: glutathione s-transferase

(protein kinase β) activation as well as specifically inhibits the phosphatidylinositol 3-kinase/Akt pathway (Ozturk *et al.*, 2012). Furthermore, treatment of several cancer cell types with CAPE inhibits nuclear factor-kappa B activity (Akyol *et al.*, 2013). In addition, caffeic acid and its derivatives can significantly inhibit UVA-mediated matrix metalloproteinase-3 upregulation by fibroblasts.

Further study on the antiproliferative activity of the EEP on the A549 cell line should be performed to elucidate the mechanism of anticancer activity.

Oxidative stress is responsible for the occurrence of a wide variety of human diseases, such as cancers (Sosa *et al.*, 2012). Several studies have suggested that propolis possesses cancer chemopreventive activity. We

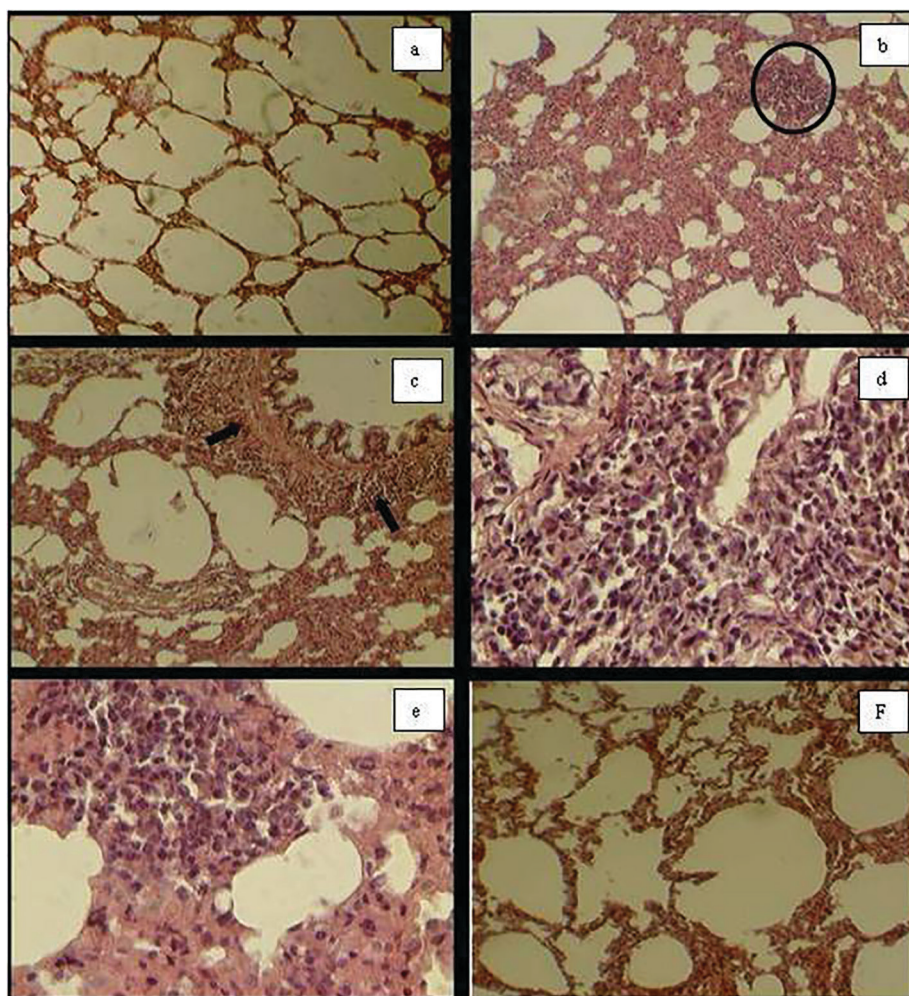


FIGURE 5 - The histopathological studies of lung sections viewed under a light microscope (hematoxylin and eosin [H&E] staining) in the control and experimental groups. (a) Control animals showing normal architecture with regular sized alveolar spaces (100× H&E) (b) and (c) Benzo(a)pyrene (B(a)P)-induced animals showing condensed architecture with destroyed alveoli, alveolar septal thickening, airway inflammation, and hyperchromatic nuclei in alveolar wall cells (100× H&E) (d) and (e) higher magnification shows infiltration of inflammatory cells (400× H&E) (F) Propolis pre-treated cancer-bearing animals showing slightly reduced alveolar damage compared to that of control animals (100× H&E). Arrows indicate peribronchial inflammatory infiltration and circles indicate intense alveolar septal infiltration.

were interested in this part in our study to evaluate the antioxidant capacity of the EEP.

In our study, the considerable weight loss observed in animals treated with B(a)P, probably occurred because of cancer cachexia, anorexia, or malabsorption due to the toxic effect of drug reactive metabolites. Moreover, the significant increase in tumorigenesis observed in the lung could be due to uncontrolled proliferation of cancerous cells. Our findings are in agreement with Kasala *et al.* (2016) who reported that decreases in body weight and increases in lung weight are a common symptom of B(a)P-induced lung carcinogenesis. The increase in body weight and the decrease in lung weight that we observed after administration of the EEP could be because of the

protective efficacy of this extract before drug treatment.

Analysis of tumor marker enzymes, such as GGT and LDH, serves as a proxy of the cancer response to therapy and are indicators of lung damage. GGT activity is a specific marker for diagnosis and also has extrapolative value in malignancies, such as lung cancer, whereas LDH is a fairly sensitive marker for solid neoplasms. Cells of lung cancer-bearing mice express increased levels of GGT and LDH in serum (Anandakumar *et al.*, 2009). In the present study, significant increases in these two tissue marker enzymes were observed in the B(a)P-treated animals. The EEP pre-treatment brought down the levels of these marker enzymes close to normal, suggesting its beneficial effect.

The antioxidant activities of the EEP were studied *in vitro* using the DPPH method. Compared to quercetin, a strong and well-known antioxidant used as a positive control, the EEP showed a high scavenging effect. Our results are in agreement with Piccinelli *et al.* (2013) who reported that different Algerian propolis samples possess strong antioxidant activity with an IC_{50} range of 32.3–82.5 $\mu\text{g/mL}$. A positive correlation was detected between the flavonoid content of the propolis and its antioxidant activity. This antioxidant activity is due to the presence of certain elements in the flavonoid structure, such the presence of a double bond between C-2 and C-3 in the B ring and the presence of highly reactive hydroxyl groups (3' and 4'-dihydroxy) in the ring, which is capable of donating an electron and hydrogen to ROS; thus, interrupting the free radical propagation reaction leading to lipid peroxidation (LPO) (Kurek-Górecka *et al.*, 2014).

In our study, the B(a)P treatment produced large quantities of free radicals, which, in turn, interacted with membrane lipids and consequently induced LPO in the lung tissues of rats. Progressive cellular architectural changes due to oxidative stress and LPO generated during the cytochrome p450 (CYP1A1)-dependent metabolism of B(a)P has been implicated in the pathogenesis of lung carcinogenesis. The products of LPO include MDA, which is involved in the formation of tumors by interacting with DNA to form MDA–DNA adducts, that induce genetic alterations and inhibit protective enzymes, leading to carcinogenesis during B(a)P-induced oxidative stress (Kim, Lee, 1997). Many studies have demonstrated the protective effects of polyphenols in propolis, such as chrysin and CAPE, against oxidative damage in the lungs of rats, which suppress LPO and inhibit lipoxygenase activity (Yildiz *et al.*, 2008; Kasala *et al.*, 2016). This result agrees with our results in which MDA concentration was inhibited in the group treated with B(a)P, which was added after the EEP treatment, and confirms the antioxidant and free-radical scavenging activity of our EEP in reducing the deleterious effects of B(a)P.

Another important detoxification pathway consists of the conjugation of B(a)P metabolites with GSH by GSTs, including GST-a, GST-p, and GST-m. El-Khawaga *et al.* (2003) reported that administration of propolis is accompanied by increased oxidant status as the concentration of GST increases, but which decreases significantly in cancer-bearing mice (El-Khawaga, Salem, Elshal, 2003). These results agree with those obtained in our study where we found a significant increase in the GST level after administering the EEP.

Some studies have reported that a depletion of non-enzymatic antioxidants, such as GSH, is probably due to

consumption and utilization by lung cells in conjugation reactions with BPDE, the ultimate carcinogenic metabolites of B(a)P, as well as the products of LPO and H_2O_2 (Rahman, Macnee, 1999), which oxidize GSH sulfhydryl groups to the disulfide compound GSSG (oxidized form), leading to their depletion. The current study shows that the EEP increased and normalized the depleted level of GSH caused by B(a)P. Studies have also reported that administration of some of the flavonoids found in propolis can increase GSH levels (Al-Jasabi, Abdullah, 2013; Kasala *et al.*, 2016) which serves as free radical scavenger by neutralizing the hydroxyl radicals via donation of a hydrogen atom (Deponte, 2013).

Conversion of ROS to less toxic intermediates by enzymatic antioxidants, such SOD and CAT, represents another important detoxification pathway against oxidative damage induced by B(a)P. The main function of SOD is to protect the cell from oxidative damage caused by superoxide anions and LPO, whereas that of CAT is to catalyze the breakdown of H_2O_2 generated during oxidative stress in tumor cells. Decreased SOD and CAT activities are observed in lung cancer (Kasala *et al.*, 2016) and could be related to increased oxidative damage to DNA and proteins or to the accumulation of superoxide anions and H_2O_2 which consumes these enzymes. We observed that treatment with the EEP significantly reversed all changes induced by B(a)P by increasing SOD and CAT.

In line with the present results, it has been reported that some components of propolis, such as CAPE, have a regulatory effect on activities of antioxidant enzymes, such as CAT, SOD, and GSH. Moreover, administering CAPE to rats modifies the enzyme activity of CYP P450 isoforms such as CYP1A1 (Akyol *et al.*, 2016), which are involved in activating of B(a)P.

Our findings show that the chemopreventive efficacy of the EEP against B(a)P-induced progression of lung tumorigenesis may be due, in part, to inhibiting LPO and inducing antioxidant activity to quench ROS-mediated oxidative stress, which in turn maintains the cellular oxidant/antioxidant balance and protects against oxidative damage exerted by B(a)P. A histopathological study of lung tissue from each group was performed to substantiate the enhanced effect of the EEP.

CONCLUSION

The EEP inhibited proliferation of A549 cancer cells and revealed anti-adhesive activity by the integrin family of adhesion receptors. Furthermore, histological and biochemical studies of lung tissues showed that the EEP markedly reduced oxidative stress caused by B(a)P

and prevented damage by increasing enzymatic and non-enzymatic antioxidants, as well as decreasing LPO. These results suggest that Algerian propolis can act against lung cancer, and may lead to the potential use of these natural compounds for treating and preventing lung cancer.

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