MODULATOR IMPACTS OF PROPOLIS EXTRACT AGAINST DOXORUBICIN MEDIATED CARCINOGENESIS ON HEPATO-CELLULAR CARCINOMA AND *Drosophila* SOMATIC CELLS

NAGLAA M. EBEED[1] AND SAWSAN M. ABDELMEGEED[2]

1- Department of Genetics, Faculty of Agric., Ain Shams University, Cairo, Egypt

2- Department of Plant Protection, Faculty of Agric., Ain Shams University, Cairo, Egypt

Propolis is a sticky and rubbery resinous substance, known as bee glue collected by honeybees from buds and exudates of certain trees and various plants sources which mix it with their own salivary secretions and waxes (Zizic *et al.*, 2013). Honeybees used propolis in the beehives as a general sealer, draft excluder and as antibiotic and also as an embalming substance to seal any cracks and fissures in the hive and they 'line their front door' with it to prevent contamination (Ghisalberti, 1979; Crane, 1997; Burdock, 1998). More than 300 constituents in different propolis samples have been identified (Kamiya *et al*., 2012). Properties of propolis vary depending on its constituent parts, which might differ considerably with geographical seasonal variations and regional flora, consequently, altering its particular chemical composition (Moreira, *et al*., 2008; Da Silva *et al*., 2013).

The scientists have explored the differences in compound arrangements of propolis in regard to kind of honey bee, region, and plants that the resin is gathered (Daugsch *et al*., 2008; Sibel and Semiramis, 2005). Therefore, alongside along the increasing hobby of naturally derived compounds, the pharmacological houses concerning more studies on propolis extracts as natural product (Chirumbolo, 2012). Propolis is prosperous into a huge variety on bioactive constituents, including polyphenols (flavonoids, phenolic acids, and their esters), terpenoids, steroids, amino acids and various inorganic compounds, that is gainful to honey bees as well as have general pharmacological weight as a characteristic natural mixture (Umthong, *et al*., 2011; Watanabe *et al*., 2011; Barlak *et al*., 2011; Chirumbolo, 2012; Zizic *et al*., 2013). Propolis contains caffeic acid, ferulic acid, and phenethyl ester, which exhibited antioxidative effect (Kumazawa *et al*., 2010). Different extraction methods of propolis may affect its action through diverse mixes when distinctive solvents solubilization was utilized. The most regularly extracts used in biological assays are water, ethanol, and methanol utilizing different concentrations (Cunha, *et al*., 2004).

In vitro and *in vivo* studies investigated that various bee products hold a potential anticancer activity (Yusuf *et al.*, 2007). Propolis is looked for its

Egypt. J. Genet. Cytol., 46: 389-407, July, 2017
Web Site (www.esg.net.eg)

antiproliferative effect on cancer cells. Many investigations tended to address its anticancer activity (Vatansever *et al*., 2010; Eom *et al.*, 2010; Sawicka *et al.*, 2012). Propolis can be utilized as therapeutic medications for the human diseases such as tumors because it has many components that revealed antiangiogenic and antioxidant actions (Ahn *et al*., 2009). Sforcin, (2007) explore that propolis could affect antibody production; macrophage activation and lymphocyte proliferation. One of the most thing causing death is cancer disease overall the world, Extensive understanding has been picked up into the mechanisms of cancer by which some chemicals affect cellular growth and how this knowledge has been used to design new chemotherapeutic medications (Fry and Jacob, 2006) offering more selectivity towards malignancy cells than normal cells causing a little side effects.

Several researchers have addressed the antitumor potential of propolis *in vivo* and *in vitro*. Moreover, the human utilizes propolis in conventional drug dates to historical times for its pharmaceutical activities including anti-bacterial, anti-fungal, anti-protozoal, antiviral, anti-tumor, anti-oxidative, anti-mutagenic, anti-inflammatory activities, immune-stimulating, hepatoprotective and non-toxic natures. Additionally, it was utilized more as a functional ingredient in cosmetics, medicine, and food industries (Umthong *et al*., 2011; Borges *et al*., 2011 & 2013; Chirumbolo, 2012; Zizic *et al*., 2013).

Even though propolis flora is a broadly dispersed in Egypt, production of propolis is yet restricted by its differences in chemical composition. Consequently; determination of polyphenolic substance of propolis extract should be concern. It is critical to determine the possible adverse impacts, for example, cytotoxicity and the decreasing proliferation of a cell in target organ tissues.

The present investigation intended to extract and assess the chemical content, cytotoxic action, the growth inhibitory activity and anticancer capability of Egyptian propolis versus Chinese propolis. This was carried out using water extract (WE) and ethanolic extract (EE) on the human hepatocellular carcinoma (HEp-2) cell line and the loss of heterozygosity (LOH) assay of *Drosophila melanogaster* somatic cells against the direct genotoxicity of doxorubicin.

MATERIALS AND METHODS

Propolis sample collection

Two samples of propolis were studied, one of them; Egyptian samples that collected in plastic bags from colonies of honeybees located at Faculty of Agriculture, Qalyubi Governorate, Egypt. The other one, commercial Chinese propolis samples kindly provided by Bee Hive Botanicals the Korean Apiculture Society Company Apis Flora Commercial and Industrial.

Preparation of propolis phenolic extracts

Two extracts were applied, first, propolis water extract (PWE) and second, propolis ethanolic extract (PEE). PWE was prepared by the strategy of Orsolic *et al*. (2005). Under sterile conditions, one gram of brown powder of propolis was dissolved in 10 ml distilled water and mixed vigorously for 10 min. Finally, this suspension was centrifuged at 10,000/10 rpm /min at room temperature. The supernatant was gathered and stored at -20°C until utilization. PEE was prepared by dissolved one g of propolis powder in 10 ml 80% ethanol (1:10, w/v) at 20°C for two days in clean dark brown glass bottles. After the extraction period, the supernatant solution was filtered twice with Whatman no. 4 and no.1 filter papers. Then the residues were re-extracted under the same conditions. After the second extraction, the filtrates were combined. Ethyl alcohol extract was then evaporated to dryness under a vacuum (Falcão *et al*., 2010). Yielded sticky extract have been used to prepare different concentrations of propolis for applications. The doses were selected according to Czepiel *et al*. (2010) and Newairy *et al*. (2009).

Analysis of propolis extracts by HPLC

Phenolic contents and flavonoid substance were analyzed in propolis extracts using High-Performance Liquid chromatography (HPLC). HPLC was achieved on an Agilent 1260 Infinity HLPC Series (Agilent, USA) equipped with Quaternary pump, Zorbax Eclipse plusC18 column 150 mm X 4.6 mm i.d., 5 µm particle (Agilent, technologies, USA), operated at 25°C. The separation was achieved using a ternary linear elution gradient with a) HLPC grade water 0.2% H_3PO_4 (v/v), b) methanol and c) acetonitrile. The injected volume was 20 µL. Detection: VWD detector set at 284 nm (Ivanauskas *et al*., 2008).

Cytotoxicity test using HEp-2 cell lines

EPWE, EPEE, CPWE and CPEE propolis extracts and doxorubicin (DOX) (positive control) cytotoxicity were tested on the human carcinoma (HEp-2) cell line. Cell viability was determined as described by Mosmann (1983).

HEp-2 cells were seeded at the density of 4 x 10^4/ml into 96-well microtiter plates and allowed to adhere for 24 h at 37°C in humidified atmosphere with 5% carbon dioxide (CO_2) in complete culture medium (Dulbecco's Modified Eagles Medium (DMEM, SIGMA, USA) supplemented with 5% of fetal calf serum, and 100 U/mL of penicillin and 100 µg/mL streptomycin and adjusted to pH 7.2 with 0.2% sodium bicarbonate. 24 h later, after the cell adherence, EPWE, EPEE, CPWE and CPEE propolis extracts and DOX (50 µg/mL) were applied to cells. The final concentrations of extracts applied to cells were 100 and 200 µg/ml (triplicate wells per condition). The cultures were incubated for 48 h. control considered as untreated cells, and in all cases, DMSO was below 0.1%.

Neutral red uptake (NRU) assay

Assay was applied carried out by methods of Siddiqui *et al*. (2008). The solutions had been removed from all plates after treatment period and using phosphate buffered saline twice to wash cells. Cells had been then incubated for 3h in medium supplemented with neutral red stain (50 μg/ml), then washing cells with a solution containing 0.5% formaldehyde and 1% calcium chloride. Afterwards, cells were subjected to incubate further in a solution of acetic acid (1%) and the ethanol (50%) for 20 min/37°C to extract the dye. The plates were then read at 540 nm using UV- visible spectrophotometer multiplate reader (Synergy HT, Bio-Tek, USA). The values have been in comparison with control sets, run under identical conditions without the test compound.

Detection of loss of heterozygosity (LOH) causing tumor in D. melanogaster

This assay was conducted to identify and characterize the potential tumorigenesis or anti-tumorigenesis of propolis extracts and was scored for loss of LOH on *D. melanogaster* (Eeken *et al.*, 2002; Nepomuceno, 2015).

Drosophila strains and cross

Wts/TM3, Sb^1. strain of drosophila with accession number (Bloomington/7052). It has wart (wts) tumor suppressor lethal allele, balanced by TM3 on chromosome 3. This strain was given by Bloomington Drosophila Stock Center of the University of Indiana, USA and Oregon R wild type *D. melanogaster* stocks according to Eeken *et al.* (2002) and Fly (2008).

Experimental procedures

To obtain the wts/+ heterozygous larvae, virgin females ♀♀ wts/TM3, Sb1 were mated with (OR) ♂♂ wild type males +/+ to obtain the larvae free from balancer chromosome. Eggs of the cross were collected during an 8h period. After 72±4 h, third-instar larvae were washed, gathered utilizing 20% glycerol solution, and transferred to treatment vials containing *Drosophila* medium with 500 mg/ml of EPWE, EPEE, CPWE and CPEE propolis extracts for 24 h. afterwards, they were transferred to a standard *Drosophila* medium.

The anti-carcinogenic effect was established by means of pretreatment (Propolis then Dox) and post-treatments (Dox then Propolis) to wts/+ larvae of *D. melanogaster*. DOX was used for positive control and water for negative control. Only flies with genetic structure wts/+ were examined.

In the pre-treatment experiments (Propolis then Dox), larvae were fed for 24 hours on a medium supported with propolis extract, then were transferred to DOX treated medium. In post-treatment experiments (Dox then Propolis), larvae were firstly treated with DOX, then reared on the medium contained propolis extracts until pupation. All Drosophila stocks and crosses were maintained at 25°C (Eeken *et al*., 2002; Oršolić *et al*., 2012).

Scoring of wts tumors

Males and females of wts/+ genotype were microscopically examined for tumors and were bearing no dominant markers (TM3, Sb1) of the balancer. The flies were examined utilizing a Leica stereoscopic at 25X magnification. Only tumors that were large enough have been recorded. The tumor frequencies were calculated as number of tumors/number of wts/+ flies (Oršolić *et al*., 2012; Nepomuceno, 2015).

Statistical analysis

The statistical significance differences of tumor frequencies in the experiment and control was calculated by Mann, Whitney and Wilcoxon Nonparametric U Test, using P = 0.05 level of significance (Nepomuceno, 2015).

RESULTS AND DISCUSSION

Chemical characterization of propolis extracts by HPLC

The present investigation was designed to extract and evaluate the difference in chemical content of Egyptian and Chinese propolis extracted with water (WE) and ethanol (EE).

Table (1) shows the total polyphenol contents and flavonoid of propolis substance of EPWE, EPEE, CPWE and CPEE extracts using HPLC. Its fingerprints revealed main phenolic and polyphenolic acids contents Caffeic, Vanillin, Ferulic, Ellagic, Benzoic acid and Cinnamic acids, except Catechol and Caffeine.

Egyptian propolis has total of phenolic and polyphenolic acids contents ranged between 18.83 and 39.29 mg/ml in EPWE and EPEE, respectively. Chinese propolis has total phenolic and polyphenolic acids contents ranged between 34.87 and 180.89 mg/ml for CPWE and CPEE, respectively.

EPWE, EPEE, CPWE and CPEE extracts analyzed by HPLC showed that there were sensible and various concentrations of phenolic compounds in both. However, concentrations of some phenolic compounds (Caffeic acid) within the CPWE propolis was higher than that in Egyptian one (EPWE). Of the contrary, the concentration of Vanillin acid in Egyptian propolis (EPWE) was higher than that in Chinese one. These variations in phenolic compounds percentage may the reason of the differences in antitumor potentiality between Egyptian and Chinese propolis extracts.

Contrasts were seen in absolute phenolic and polyphenolic acids and flavonoid substance among PWE and PEE samples. Also, the PEE implicates phenolic and polyphenolic compounds were more than PWE in both kinds of propolis. The results also indicated that Chinese propolis ethanol extract (CPEE) had significantly the highest amounts of total polyphenols and flavonoids (rutine) 180.89 and 188.90 μg/mL, respectively.

The obtained results agreed with previous phytochemical studies, which measured the phenolic content of propolis (Bankova, *et al*., 2000; Hegazi and Abd El Hady, 2002; Salonen *et al*., 2012; Hegazi, *et al.*, 2014). There are many factors affecting the percentage of phenolic compounds; solvents types, heat temperature during extract, blending and the origin root of propolis (Salonen *et al*., 2012; Ramanauskien *et al*., 2013). Dobrowolski *et al.* (1991) and Kędzia (2009) determined more than 38 flavonoids in propolis. Table (1) shows PEE contains on average 6 to 9 flavonoid compounds. Belonging to flavonoids, terpenes and phenolic acids, which have been distinguished in Chinese propolis ethanol extracts (CPEE), confer strong free radical scavenging capacities through taking out ROS instantly in relation to the higher polyphenols substance in propolis extract and enhance endogenous cancer prevention agent and antioxidant.

In vitro cultures experiments of propolis extracts including polyphenols and flavonoid contents exerted antitumor activity (Oršolić and Basic, 2007). In addition, several studies (Silici and Semiramis, 2005; Sawicka *et al*., 2012) confirmed that different compounds might be found in propolis content, depending on the varieties of the plants and geographical areas from which the resin is collected, and the strain of bees involved. On the other hand, chemical composition of propolis including caffeic acid, caffeic phenyl ester, artepillin C, quercetin are known to be promoters that stimulate cell proliferation or apoptosis (Díaz-Carballo *et al*., 2008).

Cell survival and cytotoxicity of propolis extracts on HEp-2 tumor cell line

The cytotoxicity and cell viability of EPWE, EPEE, CPWE and CPEE extracts were evaluated *in vitro* against HEp-2 cell lines in contrast with doxorubicin, the positive control, by using NRU Assay according to Repetto *et al.* (2008).

Cell viability percentage of carcinoma cell line after treatment with extracts at two doses (100 and 500 µg/ml) is shown in Fig. (1). The obtained results in Table (2) showed a concentration, dependent activity on both doses. All studied propolis extracts induced partially suppression of cell growth of HEp-2 tumor cell line except CPWE which gave 100% cell viability.

On the other hand, most extreme cytotoxic impacts were seen after treatment with CPEE; 65% and 77% at concentrations of 100 and 500 µg/ml, respectively (Table 2). The great majority of propolis which was strongly cytotoxic against carcinoma cell line was at 500 µg/ml of CPEE. PEE exhibited strong anti-proliferative effects than PWE against HEp-2 cancer cell and showed a higher cytotoxicity when contrasted with Dox, a fact that supports their anti-cancer activity. These data indicated that the induced cytotoxicity to tumor cells by CPEE was higher than CPWE and it relies upon the chemical composition of each extract.

The study of anti-proliferative efficiency of propolis extract versus HEp-2 cells showed that the PEE from Egypt and China could suppress the proliferative of HEp-2 cells in dose-dependent manner. It was observed that CPEE samples have major condensation of total phenolics and polyphenolic acids (Table 1). The present research findings suggested that PEE is the most effective and promising inhibition of human liver carcinoma cells. These effects are ascribed to the chemical constituents of propolis, which highly relies on the geographical location of the flora.

The identification of phenolic and polyphenolic acids and flavonoids content present in propolis is extremely valuable and promising with respect to standardization and practical applications in therapy. The present investigation is a new perspective for future research in cancer disease through *in vivo* assessment of propolis.

These results were confirmed by Matsuno (1995) who isolated PMS-1, active component from Brazilian propolis which suppresses the tumor of hepatoma cells and arrested the tumor cells at S phase. Also, PRF-1 is a natural component which isolated from propolis water extract and gave cytotoxic to HeLa human cells hepatocellular carcinoma and HLC-2 human cells lung carcinoma (Matsuno *et al*., 1997).

Oršolić *et al.* (2005) found that propolis ethanol extract inhibited DNA synthesis in tumor cell cultures, induction of apoptosis of tumor cells and antitumor action of PEE were highly dependent on dose (Propolis and its active compounds, mainly CAPE were shown to suppress cell cycle proliferation and induce cell death in various cancer types. The antiproliferative effects were mainly rely on the dose and the region which affect the chemical component of propolis (Watabe *et al*., 2004; Jin *et al.*, 2008; Chen *et al.*, 2008).

Vatansever *et al.* (2010) demonstrated that anticancer action of propolis ethanol extract, which is rich in phenolic acids and flavonoids content from Turkey, showed apoptosis induction strongly dependent upon the concentration and dilutions of PEE on breast cancer cell line MCF-7. Barlak *et al.* (2011) demonstrated antiproliferative action of raw propolis on prostate cancer. The antitumor activity of propolis was because of phenolic compounds and its ability to induce cytotoxicity at low concentration against cancer cell lines besides safeguarding normal cells even at elevated doses (Mouse *et al*., 2012).

Ibrahim *et al.* (2015) studied the cytotoxic impact of Turkish propolis on different tumor cell lines including, liver, colon, breast, cervix, and prostate. The results demonstrated that Turkish propolis ethanol extracts was a perfect source of a substance that inhibits oxidation and a natural antitumor agent having the ability to suppress cancer cell proliferation.

The anticarcinogenic impact of propolis extracts on Drosophila

This study planned to estimate the potential genotoxic effect of EPWE, EPEE, CPWE and CPEE extracts using test for the detection of clones of epithelial tumors (warts) in *D. melanogaster* as a model organism.

The assay was applied on F_1 flies of three independent experiments, including the water as negative control and DOX 0.125 mg/mL as positive control and 5mg/ml of propolis water or ethanol extracts. Treatment with propolis extracts was firstly examined to detect tumor clones in *D. melanogaster* cells to evaluate its carcinogenic activity. The numbers of F_1 flies scored, number of examined tumors and Frequency (no. of tumors/fly) were given in Table (3). An investigation of data showed that propolis extract did not have any cytotoxic influence on the offspring when contrasted with the negative control.

Data presented in Table (3) revealed that EPWE, EPEE, CPWE and CPEE extracts did not show any statistically significant changes in the frequency of tumors when compared to the negative control ($P>0.05$). Therefore, no carcinogenic effect of EPWE, EPEE, CPWE and CPEE extracts, in these experimental conditions, were found in tumor test in *D. melanogaster*.

Furthermore, EPWE, EPEE, CPWE and CPEE exhibited reductions in the average of tumors than spontaneous tumors. With regard to the impact of extracts on the frequency of warts tumor, Egyptian propolis treatments, as well as Chinese propolis, showed significant decrease in tumor frequency than negative control (0.00677), where, the rate of tumors was 0.00242, 0.00214, 0.0024 and 0.00197 for EPWE, EPEE, CPWE and CPEE, respectively.

From the statistically analysis in data summarized in Table (3), it can be pointed out that, tested propolis extracts did not stimulate significant increase in the tumor frequency. In addition, reduction in the frequency of spontaneous mutations was observed for these extracts. However, doxorubicin (DOX), used as a positive control, showed high value in warts tumor frequency (0.91377 per fly) with a high significant response ($p<0.001$) for tumor induction when contrast with the negative control (Fig. 1). These tumors arose in every part of the flies analyzed and the size of the tumors varied in examined flies.

DOX showed that it is clearly carcinogenic compound (Fig. 2). A high value of genotoxic activity with a highly significant response ($p<0.001$) was detected for tumor induction as compared with the negative control. Treatments with propolis extracts were not toxic in the chronic feeding. At the tested concentrations, no effect was exhibited on the frequencies of somatic clones with respect to their particular negative controls. The data obtained showed that all tested proptosis samples

did not induce carcinogenicity at the selected doses.

Modulator impacts of propolis extractors

The study extends to identify the anticarcinogenic effect of propolis extractors in combination with doxorubicin (DOX) as tumor agent. The anticarcinogenic effect was established by means of pre-and post-treatment of propolis extractors against DOX-treated wts/+ larvae of *D. melanogaster*. The frequencies of induced tumor in pre-and post-treatments of propolis extractors associated with doxorubicin DOX (0.125 mg/mL) experiments exhibited highly significant decreases in *wts* tumor has appeared in Table (4) and Fig. (2).

The data showed statistically significant reductions (α =0.05) in tumor frequency when as compared with positive control. Whereas, in post-treatment experiment, larvae exposed to EPWE and EPEE (5 mg/ml) after DOX treatment showed highly significant reduction of induced tumors (55 and 58%) with tumor average of 0.4515 and 0.4191 tumor/fly. At the same direction, the frequency of induced tumor after DOX treatment of pretreated larvae with EPWE or EPEE (5 mg/ml) was also significantly reduced (0.3478 and 0.3034 per fly), showing 65 and 70% reductions in induced tumors, respectively (Table 4 and Fig. 3). Meanwhile, post-treatments experiment of Chinese extract can reduce frequencies of induced tumors to 0.45 and 0.41 tumor/fly, respectively, with reduction rates of 55 and 59% respectively, as appeared in Table (4) and Fig. (3).

In pre-treatment experiments of Chinese extracts, larvae were exposed to 5 mg/ml propolis extract before DOX treatment. The average of tumor induction was significantly decreased to 0.33 and 0.30 tumor/fly, respectively, (Table 4) which gave reduction rates of 67% and 70 % for CPWE and CPEE treatments, respectively, (Fig. 2). In general, treatment with water or ethanol extractors of propolis either of Egyptian or Chinese did not induce tumor induction at a significant level as contrasted to control. CPEE showed an anticarcinogenic activity slightly, higher than Egyptian extracts either with EPEE or EPWE treatments although there is no significant difference between them. Egyptian and Chinese, propolis showed significant qualitative similarities. All propolis extractors showed antimutagenic and anticarcinogenic effects but the inhibition varied as stated by the propolis origin and kind of extract. Pre-treatments of propolis extract followed by DOX treatment inhibit carcinogenicity of DOX by showing a significant decrease in tumor frequencies (Table 4). It was obvious that EPEE or CPEE showed the highest anticarcinogenic activity against DOX when treatment of propolis extractors was firstly applied (Table 3).

Pre-treatments of propolis extractors followed by DOX treatment inhibit carcinogenicity of DOX by showing a significant decrease in tumor frequencies (Table 4). It was obvious that EPEE or

CPEE showed the highest anticarcinogenic activity against DOX when treatments of propolis extractors were firstly applied (Table 3). The pre-treatments with chronic propolis extractors was effective significantly in reducing the frequencies of tumor induced by DOX than post-treatment and the ethanol extract was more functional than water extract.

The propolis extractors could inhibit the mutagenesis of DOX. The present findings indicated that the analyzed propolis extractors might be deduced to contain some constituents fit for repressing the mutagenicity of direct or indirect-acting mutagens. There is a relationship between propolis chemical constituents and antitumor activity. It has many polyphenols, flavonoid aglycones, phenolic, and ketones compound. Many studies supported that polyphenolic compounds can exhibit anti-tumor effects in murine tumor models (Scheller *et al*., 1989; Hayashi *et al.*, 2000; Femia *et al*., 2001; Bissery *et al.*, 1988).

The differences saw in the propolis composition in the three kinds of EEP, because of the different in vegetal source available in the collecting area (Egypt and China) (Table 1), indicated that the chemical structure of propolis is reliant on its geographical features; subsequently, its biological action is firmly related to the vegetation local to the site of summation (Park *et al*., 2002; Christov *et al*., 2005).

Ethanolic extractors of propolis showed a greater amount of tannins and glycosides presence worked as antibacterial and antioxidative characteristic, of propolis (Banskota *et al*., 2001) and the, retrieval of chemical components was the best in case of the extraction with ethanol (Kalia *et al*., 2013). Hegazi and Abd El Hady (2001 & 2002) and Popova *et al*. (2005) discovered that the antimicrobial action diverges as indicated by the differences in the chemical constituents and propolis source.

SUMMARY

The antitumor action of propolis is of clinical interest because of the need for new anticancer treatment agents. The present investigation intended to extract and assess the chemical content, cytotoxic action, the growth inhibitory activity and anticancer capability of Egyptian propolis versus Chinese propolis. This was carried out using water extract (WE) and ethanolic extract (EE) on the human hepatocellular carcinoma (HEp-2) cell line and the loss of heterozygosity (LOH) assay of Drosophila melanogaster somatic cells against the direct genotoxicity of doxorubicin.

EPWE, EPEE, CPWE and CPEE extracts analyzed by HPLC showed that there were sensible and various concentrations of phenolic compounds in both.

Total phenolics were determined to be 18.83, 34.87, 39.29 and 180.89 μg-1 by using EPWE, CPWE, EPEE and CPEE extracts, respectively. Chinese propolis ethanol extract (CPEE) have major concentrations of total phenolics and phenolic acids and contained high concentrations of

rutin (188.90 μg/mL). The study of the antiproliferative capacity of propolis extractors against HEp-2 cancer cell lines showed that all the studied propolis extracts induce suppression of cell growth except CPWE extract; it gave 100% cell viability. The great majority of the propolis are strongly cytotoxic against HEp-2 cell line with 500 µg/ml CPEE. Also, PEE is the most effective in inhibition of HEp-2 cell proliferation compared to PWE. In *Drosophila* assay, treatment with propolis extract and DOX carcinogenic agent led to a reduction in the frequency of recombination compared to the treatment with DOX alone either in the post- and pre-treatments. In general, PEE exhibited powerful anti-proliferative effects than PWE. The ethanol extract provided the highest protection against Doxorubicin (DOR) induced genotoxicity, a fact that supports their anti-cancer activity. The results demonstrate that PEE is a good source of a natural antitumor operator able to inhibit cancer cell proliferation.

REFERENCES

Ahn, M. R., K. Kazuhiro, K. Shigenori, N. Tsutomu, K. Kazuhiko, U. Yoshihiro, H. Hitoshi, N. Hideko and O. Toshiro (2009). Correlation between antiangiogenic activity and antioxidant activity of various components from propolis. Molecular Nutrition & Food Research, 53: 643-651.

Bankova, V. S., S. L. De Castro and M. C. Marcucci (2000). Propolis: recent advances in chemistry and plant origin. Apidologie, 31: 3-15.

Banskota, A. H., Y. Tezuka and S. H. Kadota (2001). Recent progress in pharmacological research of propolis. Phytotherapy Research, 15: 561-571.

Barlak, Y., O. Deger, M. Colak, S. C. Karatayli, A. M. Bozdayi and F. Yucesan (2011). Effect of Turkish propolis extracts on proteome of prostate cancer cell line. Proteome Sci., 9: 74.

Bissery, M. C., F. A. Valeriote, G. G. Chabot, J. D. Crisman, C. Yost and T. H Corbett (1988). Flavone acetic acid (NSC-34 7512)-induced DNA damage in Glasgow osteogenic sarcoma *in vivo*. Cancer Research, 48: 1279-1285.

Borges, J., M. Tagliamento, A. Silva, P. Sobral and R. Carvalho (2013). Development and characterization of orally-disintegrating films for propolis delivery. Food Science and Technology, Ciênc. Tecnol. Aliment., 33: 28-33.

Borges, K. S., M. S. Brassesco, C. A. Scrideli, A. E. Soares and L. G. Tone (2011). Anti-proliferative effects of Tubi-bee propolis in glioblastoma cell lines 2. Genet. Mol. Biol., 34: 310-314.

Burdock, G. A. (1998). Review of the biological properties and toxicity

of bee propolis, Food Chem. Toxicol., 36: 347-363.

Chen, M. J., W. H. Chang, C. C. Lin, C. Y. Liu, T. E. Wang, C. H. Chu, S. C. Shih and Y. J. Chen (2008). Caffeic acid phenethyl ester induces apoptosis of human pancreatic cancer cells involving caspase and mitochondrial dysfunction. Pancreatology, 8: 566-76.

Chirumbolo, S. (2012). Flavonoids in propolis acting on mast cellmediated wound healing. Inflammopharmacology, 20: 99-101.

Christov, R., B. Trusheva, M. Popova, V. Bankova and M. Bertrand (2005). Chemical composition of propolis from Canada, its antiradical activity and plant origin. Natural Product Research, 19: 673-678

Crane, E. (1997). The past and present importance of bee products to man. Bee Products: Properties, Applications and Apitherapy. Plenum, New York.

Cunha, I. B. S., A. C. H. F. Sawaya, F. M. Caetano, M. T. Shimizu, M. C. Marcucci, F. T. Drezza, G. S. Povia and P. O. Carvalho (2004). Factors that influence the yield and composition of Brazilian propolis extracts. Journal of the Brazilian Chemical Society, 15: 964-970.

Czepiel, J., G. Biesiada, M. Gajda, W. Szczepan´ski, K. Szypuła, Z. Dabrowski and T. Mach (2010). The effect of TCDD dioxin on the rat liver in biochemical and histological assessment. Folia Biologica (Krakow), 58: 85-90.

Da Silva, F. C. O., G. C. S. Celi, G. Gambato, M. D. Oliveira de Souza, M. Salvador, S. Moura, F. F. Padilha, F. K. Seixas, T. Collares, S. Borsuk, O. A. Dellagostin, J. A. P. Henriques and M. Roesch-Ely (2013). Chemical characterization, antioxidant and cytotoxic activities of Brazilian red propolis. Food Chem. Toxicol., 52: 137-142.

Daugsch, A., C. S. Moraes, P. Fort and Y. K. Park (2008). Brazilian red propolis - chemical composition and botanical origin. Evid Based Complement Alternat. Med., 5: 435-441.

Díaz-Carballo, D., S. Malak, W. Bardenheuer, M. Freistuehler and H. P. Reusch (2008). The contribution of plukenetione A to the antitumoral activity of Cuban propolis. Bioorg. Med. Chem., 16: 9635-9643.

Dobrowolski, J. W., S. B. Vohora, K. Sharma, S. A. Shah, S. A. Naqvi and P. C. Dandiya (1991). Antibacterial, antifungal, antiamoebic, antiinflammatory, antipyretic studies on propolis bee products. J. Ethnopharmacol., 35: 77-82.

Eeken, J. C., I. Klink, B. L. van Veen and A. Pastink (2002). Induction of epithelial tumors in *Drosophila melanogaster* heterozygous for the tumor suppressor gene wts. Environ. Mol. Mutagen, 40: 277-282.

Eom, H. S., E. J. Lee, B. S. Yoon and B. S. Yoo (2010). Propolis inhibits the proliferation of human leukaemia HL-60 cells by inducing apoptosis through the mitochondrial pathway. Nat. Prod. Res., 24: 375-386.

Falcão, S. I., M. Vilas-Boas, L. M. Estevinho, C. Barros, M. R. M. Domingues and S. M. Cardoso (2010). Phenolic characterization of Northeast Portuguese propolis: usual and unusual compounds. Analytical and Bioanalytical Chemistry, 396: 887-897.

Femia, A. P., G. Caderni, C. Buzzigoli, E. Cocca, M. Salvadori and P. Dolara (2001). Effect of simple phenolic compounds on azoxymethane-induced aberrant crypt foci in rat colon. Nutrition and Cancer, 41: 107-110.

Fly, B. (2008). A database for the *Drosophila* research community. Drysdale R. and The FlyBase Consortium, Methods Mol. Biol., 420: 45-59.

Fry, F. H. and C. Jacob (2006). Sensor/effector drug design with potential relevance to cancer. Curr. Pharm., 12: 4479-4499.

Ghisalberti, E. L. (1979). Propolis. A review. Bee World, 60: 59-84.

Hayashi, A., A. C. Gillen and J. R. Lott (2000). Effects of daily oral administration of quercetin chalcone and modified citrus pectin on implanted colon tumor growth in Balb-c mice. Alternative Medicine Review, 5: 546-552.

Hegazi, A. G. and F. K. Abd El Hady (2001). Egyptian propolis. 1. Antimicrobial activity and chemical composition of Upper Egypt propolis. Z. Naturforsch., 56: 82-88

Hegazi, A. G. and F. K. Abd El Hady (2002). Egyptian propolis: 3. Antioxidant, antimicrobial activity and chemical composition of propolis from reclaimed land. Z. Naturforsch., 57: 395-402.

Hegazi, A. G., A. M. Abdou and F. Abd Allah (2014). Egyptian Propolis 11: Its antimicrobial activity with comparison with different localities. Int. J. Curr. Microbiol. App. Sci., 3: 530-538.

Ibrahim, T., S. Demir, S. Misir, K. Kilinc, A. Mentese, Y. Aliyazicioglu and O. Deger (2015). Cytotoxic effect of Turkish propolis on liver, colon, breast, cervix and prostate cancer cell lines. Tropical Journal of Pharmaceutical Research, 14: 777-782.

Ivanauskas, L., V. Jakstas, J. Radusiene, A. Lukosius and A. Baranauskas (2008). Evaluation of phenolic acids and phenylpropanoids in the crude drugs. Medicina, 44: 48-55.

Jin, U. H., K. H. Song, M. Motomura, I. Suzuki, Y. H. Gu, Y. J. Kang, T. C. Moon and C. H. Kim (2008). Caffeic acid phenethyl ester induces mitochondria-mediated apoptosis in human myeloid leukemia U937 cells. Mol. Cell Biochem., 310: 43-48.

Kalia, P., Kumar, R. K. and K. Harjai (2013). Phytochemical screening and antibacterial activity of different extracts of Propolis. International Journal of Pharmaceutical and Biological Research, 3: 219-222.

Kamiya, T., H. Nishihara, H. Hara and T. Adachi (2012). Ethanol extract of Brazilian red propolis induces apoptosis in human breast cancer MCF- 7 cells through endoplasmic reticulum stress. J. Agric. Food Chem., 60: 11065-11070.

Kędzia, B. (2009). Chemical composition of polish propolis. Part I.: The initial period of investigations. Post. Fitoter., 1: 39-44.

Kumazawa, S., M. R. Ahn, T. Fujimoto and M. Kato (2010). Radical-scavenging activity and phenolic constituents of propolis from different regions of Argentina. Natural Product Research, 24: 804-812.

Matsuno, T. (1995). A new clerodane diterpenoid isolated from propolis. Zeitschrift fur Naturforschung, 50: 93-97.

Matsuno, T., C. Chen and P. Basnet (1997). A tumoricidal and antioxidant compound isolated from an aqueous extract of propolis. Medical Science Research, 25: 583-584.

Moreira, L., L.G. Dias, J. A. Pereira and L. Estevinho (2008). Antioxidant properties, total phenols and pollen analysis of propolis samples from Portugal. Food Chem. Toxicol., 46: 3482-3485.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55-63.

Mouse, H. A., M. Tilaoui, A. Jaafari, L. A. Mbarek, R. Aboufatima and A. Chait (2012). Evaluation of the *in vitro* and *in vivo* anticancer properties of Moroccan propolis extracts. Rev. Bras. Farmacogn., 22: 558-567.

Nepomuceno, J. C. (2015). Using the *Drosophila melanogaster* to assessment carcinogenic agents through the test for detection of epithelial tumor clones (Warts). Adv. Tech. Biol. Med., 3: 149-156.

Newairy, A. A., A. F. Salama, H. M. Hussien and M. I. Yousef (2009). Aluminium induced alterations in biochemical parameters and lipid peroxidation of male rats: protective role of propolis. Food Chem. Toxicol., 47: 1093-1098

Oršolić, N. and I. Basic (2007). Cancer chemoprevention by propolis and its polyphenolic compounds in experimental animals. Recent Progress in Medicinal Plants, 17: 55-113.

Oršolić, N., A. H. Knezevic, L. Sver, S. Terzic and Basic, I. (2004). Immunomodulatory and antimetastatic action of propolis and related polyphenolic compounds. J. Ethnopharmacol. 94: 307-315.

Oršolić, N., S. Terzić, Ž. Mihaljević, L. Šver and I. Bašić (2005). Effects of local administration of propolis and its polyphenolic compounds on tumor formation and growth. Biological and Pharmaceutical Bulletin., 28: 1928-1933.

Orsolin, P. C., R. G. Silva-Oliveira and J. C. Nepomuceno (2012). Assessment of the mutagenic, recombinagenic and carcinogenic potential of orlistat in somatic cells of *Drosophila melanogaster*. Food Chem. Toxicol., 50: 2598-2604.

Park, Y. K., S. M. Alencar and C. L. Aguiar (2002). Botanical origin and chemical composition of Brazilian propolis. J. Agric. Food Chem., 50: 2502-2506.

Popova, M., S. Silici, O. Kaftanoglu and V. Bankova (2005). Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. Phytomedicine, 12: 221-228.

Ramanauskien, K., A. M. Inknien, V. Petrikait and V. Briedis (2013). Total phenolic content and antimicrobial activity of different Lithuanian propolis solutions. Hindawi Publishing Corporation, Evidence-Based Complementary and Alternative Medicine, 2-5.

Repetto, G., A. Peso and J. L. Zurita (2008). Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nature Protocols, 3: 1152-1135.

Salonen, A., S. Saarnio and R. Julkunen-Tiitto (2012). Phenolic compounds of propolis from the boreal coniferous zone. Journal of Agicultural Science, 56: 13-22.

Sawicka, D., H. Car, M. H. Borawska and J. Niklinski, (2012). The anticancer activity of proplis. Folia Histochemica et Cytobiologica, 50: 25-37.

Scheller, S., W. Krol, J. Swiacik, S. Owczarek, J. Gabrys and J. Shani (1989) Antitumoral property of ethanolic extract of propolis in mice-bearing Ehrlich carcinoma, as

compared to bleomycin. Zeitschrift fur Naturforschung, 44c: 1063-1065.

Sforcin, J. M. (2007). Propolis and the immune system: A Review. J. Ethnopharmacol., 113: 1-14.

Sibel, S. and K. Semiramis (2005). Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. Journal of Ethnopharmacology, 99: 69-73

Siddiqui, M. A., G. Singh, M. P. Kashyap, V. K. Khanna, S. Yadav, D. Chandra and A. B. Pant (2008). Influence of cytotoxic doses of 4-hydroxynonenal on selected neurotransmitter receptors in PC-12 cells. Toxicol: *In Vitro*, 22: 1681-1688.

Silici, S. and K. Semiramis (2005). Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. J. Ethnopharmacol., 99: 69-73.

Umthong, S., P. Phuwapraisirisan, S. Puthong and Chanchao, C. (2011). *In vitro* antiproliferative activity of partially purified Trigona laeviceps propolis from Thailand on human cancer cell lines. BMC Complement Altern. Med., 11: 37.

Vatansever, H. S., K. Sorkun, S. I. D. Gurhan, F. O. Kurt, E. Turkoz, O. Gencay and B. Salih (2010). Propolis from Turkey induces apoptosis through activating caspases in human breast carcinoma cell lines. Acta Histochem., 112: 546-556.

Watabe, M., K. Hishikawa, A. Takayanagi, N. Shimizu and T. Nakaki (2004). Caffeic acid phenethyl ester induces apoptosis by inhibition of NFkappaB and activation of Fas in human breast cancer MCF-7 cells. J. Biol. Chem., 279: 6017-6026.

Watanabe, M. A. E., M. K. Amarante, B. J. Conti and J. M. Sforcin (2011). Cytotoxic constituents of propolis inducing anticancer effects: A review. J. Pharm. Pharmacol., 63: 1378-1386.

Yusuf, N., C. Irby, S. K. Katiyar and C. A. Elmets (2007). Photoprotective effects of green tea polyphenols. Photodermatol Photoimmunol Photomed, 23: 48-56.

Zizic, J. B., N. L. Vukovic, M. B. Jadranin, B. D. Andelkovic, V. V. Tesevic, M. M. Kacaniova, S. B. Sukdolak and S. D. Markovic (2013). Chemical composition, cytotoxic and antioxidative activities of ethanolic extracts of propolis on HCT-116 cell line. J. Sci. Food Agric., 93: 3001-3009.

Table (1): Composition and quantities of phenolic and polyphenolic acids and flavonoids present in water and ethanol extracts of Egyptian and Chinse propolis by using HPLC.

	Chemical marker	Content in μg/mL			
		EPWE	EPEE	CPWE	CPEE
Phenolic and polyphenolic acids content	Catechol	ND	ND	ND	ND
	Caffeine	ND	ND	ND	ND
	Caffeic	2.99	ND	3.90	5.71
	Vanillin	4.37	ND	1.70	13.38
	Ferulic	11.47	1.80	3.76	12.40
	Ellagic	ND	10.50	14.11	129.30
	Benzoic	ND	23.80	11.40	20.10
	Cinnamic	ND	3.19	ND	ND
	Total	18.83	39.29	34.87	180.89
Flavonoides	Rutine	49.48	NA	20.8	188.90

Note: EPWE= Egyptian propolis water extract EPEE = Egyptian propolis ethanol extract
CPWE= Chinese propolis water extract CPEE= Chinese propolis ethanol extract
ND = Not detected

Table (2): Cell viability from NRU cytotoxicity assay after 48h exposure of HEp-2 cells to propolis extracts.

Extraction	Viability%	
	100 µg/ml	500 µg/ml
EPWE	94	82
CPWE	102	97
EPEE	50	24
CPEE	35	23

Table (3): Number of F_1 flies scored, number of tumors examined, tumor frequencies (%) induced by Propolis water and ethanol extracts on developing flies, heterozygous for a recessive lethal allele of wts gene.

Treatments	Egyptian			Chinese		
	No. of F_1 flies scored	Number of tumors examined	Frequency (No. of tumors/fly)	No. of F_1 flies scored	Number of tumors examined	Frequency (No. of tumors/fly)
Control	3248	22	0.00677	3248	22	0.00677
DOX	1530	1200	0.91377	1530	1200	0.91377
WE	1650	4	0.00242	1250	3	0.00240
EE	2809	6	0.00214	1523	3	0.00197

Table (4): Effect of Pre-and post- treatments of propolis extracts against DOX treated *D. melanogaster* larvae.

	Treatments	Egyptian				Chinese			
		No. of scored F_1 flies	No. of tumors examined	Tumor Frequency	Inhibition or reduc-tion	No. of F_1 flies scored	No. of tumors examined	Tumor Frequency	Inhibition or reduction
Post	DOX / WE	1772	800	0.4515	55 %	1520	680	0.4474	55%
	DOX / EE	1954	819	0.4191	58 %	1240	513	0.4137	59%
Pre	WE / DOX	1150	400	0.3478	65 %	1412	468	0.3314	67%
	EE / DOX	1282	889	0.3034	70 %	1430	431	0.3014	70%

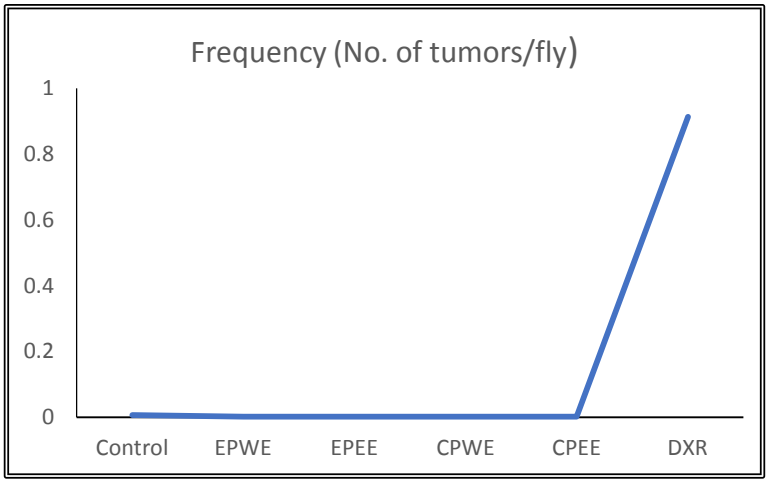

Fig. (1): Tumor frequencies induced by treatments of Propolis water and ethanol extracts on developing flies, heterozygous for a recessive lethal allele of wts gene compared with DOX.

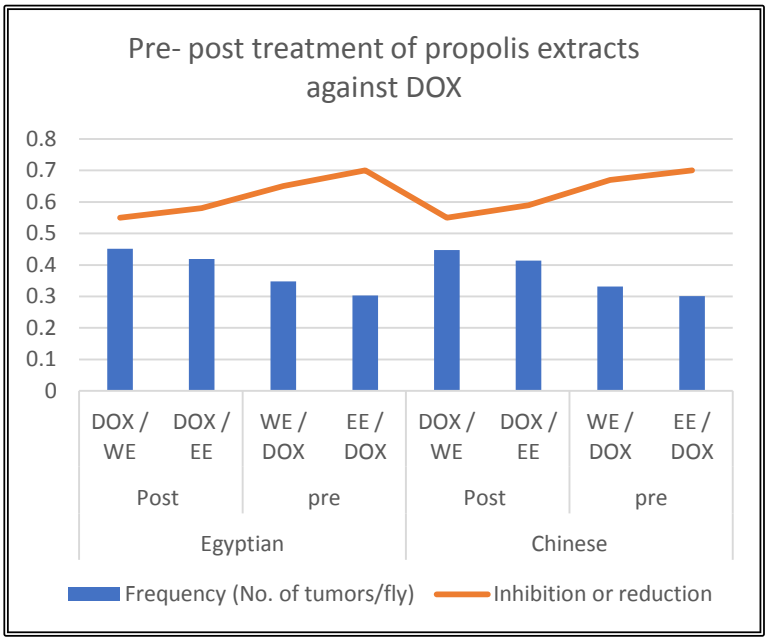

Fig. (2): Effect of pre-and post-treatments of propolis extracts against DOX treated D. *melanogaster* larvae.