



# Propolis and its flavonoid compounds cause cytotoxicity on human urinary bladder transitional cell carcinoma in primary culture

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## Abstract

**Background and purpose:** The flavonoids present in propolis are considered to be a rich source of chemopreventive agents since they have various therapeutic biological activities. This study was carried out to find whether propolis and its polyphenolic/flavonoid compounds may induce cytotoxicity in primary culture of human urinary bladder transitional cell carcinoma (TCC) cells as compared to normal urinary bladder epithelial cells.

**Material and Methods:** Pieces of TCC or normal bladder epithelial tissue were collected by transurethral surgery from patients in different stages (grade G1, G2, G3) of TCC. Single cell suspension from pieces of either TCC or normal bladder epithelial tissue was prepared according to standard laboratory procedure. Incubation for cytotoxicity testing was carried out in RPMI-1640 medium with 20% FCS at 37°C and with 5% CO<sub>2</sub> with or without different concentration (50, 150, 300 µg/ml) of test components.

**Results:** The cytotoxicity of two preparations of propolis (water and ethanolic extract of propolis; WSDP or EEP) and its polyphenolic compounds (caffeic acid, naringin, chrysin, and quercetin) was determined using trypan blue exclusion assay. The findings suggest that EEP is the most effective in inhibition of urinary bladder TCC cell proliferation as compared to WSDP or single flavonoids derived from propolis. All test components showed no cytotoxic effect on normal epithelial cells.

**Conclusions:** The result of this study may have considerable impact on the potential use of EEP as an adjuvant to surgery to suppress or prevent tumor recurrence in urinary bladder since only a few anti-cancer drugs have been effective in tumor control. As immunomodulation by BCG has been used to improve the results of surgery it is likely that propolis preparation (EEP) as an immunomodulating compound may be a substitute for mycobacterial treatment since propolis preparation or its polyphenolic components have expressed no side effect after treatment. However, the exact cancer chemoprevention mechanisms of propolis have to be elucidated.

## INTRODUCTION

Transitional cell carcinoma is the most important cancer of the urinary tract and occurs in the renal pelvis, ureter, urinary bladder, and urethra. Bladder cancer is the fourth most common cancer among men and the eighth most common among women in the USA (1);

bladder cancer occurs about twice as often in males than females. The highest incidence of cancer of the urinary bladder is observed in developed countries, with the exception of Japan and Russia (e.g. North America 29.2/100,000 vs. Japan 8.8/100,000). However, the occurrence of urinary bladder cancer among Japanese emigrants in the USA, beyond the second generation, is twice that seen in those remaining in Japan (2). North Africa is well known as a high-risk area for squamous-cell carcinoma of the urinary bladder, which is certainly related to the presence of urinary schistosomiasis.

Neoplasms in the urinary-tract epithelium possess several biological characteristics, such as multistage and multifocal carcinogenesis (3). The majority of bladder tumors (90%) have been found to be transitional cell carcinoma (TCC) where variable morphology, natural history and prognosis demonstrate that it is not single disease but occurs in three distinct forms, each possessing characteristic features such as carcinoma *in situ*; low-grade papillary, non-invasive; and high grade, invasive malignancy (4–6). Moreover, a high recurrence rate (50–70%) of superficial urinary bladder tumors, even after curative transurethral resection (TUR) has often been reported (7). These successive, recurrent tumors may increase in their histological grade, and more than 15% of patients suffer a progression to muscle-invasive disease, with subsequent poor prognosis (8, 9).

A number of anti-cancer drugs have been used, mainly via local instillation into the urinary bladder, as an adjuvant to surgery to suppress or prevent tumor recurrence in urinary bladder but only a few anti-cancer drugs have been effective in tumor control. Thereafter, a new additional modality is required to achieve more satisfactory clinical control for this malignancy.

Large amounts of epidemiological data have supported the inverse relation between the consumption of fruits and vegetables and the incidence of cancer. Thus, a number of environmental causes have been identified for urinary bladder cancer, including such dietary factors as low vitamin A intake, infrequent consumption of carrots, milk and cruciferous vegetables, and high consumption of meat, animal fat and coffee. The flavonoids present in propolis are considered to be rich source of chemopreventive agents, since they have various therapeutic biological activities such as immunomodulatory, anti-bacterial, anti-viral, anti-fungal, anti-protozoal, anti-parasitic, anti-inflammatory, anti-ulcer, anti-tumor anti-oxidant anti-proliferative, anti-mutagenesis, anti-angiogenic and anti-allergic activities (10).

Significant progress has been exemplified by the translation of laboratory discovery correlating well with the influence of chemopreventive natural products on experimental tumors, heralding further advances in the near future. In a recent review (10) we reported that propolis and propolis-related polyphenolic compounds used as cancer chemopreventive agents may play an important role in prevention and therapy of experimental tumors *in vivo* and *in vitro*. Chemoprevention is one of the most promising new approaches in cancer research, i.e. the ad-

ministration of an agent to inhibit, delay or reverse the process of carcinogenesis or inhibit proliferation of tumor cells (11). The chemopreventive activity of propolis and related polyphenolic/flavonoid compounds in animal tumor models and in *in vitro* cultures are likely to be the result of their ability to inhibit DNA synthesis in tumor cells, their capability to induce apoptosis of tumor cells, and their property to activate macrophage to produce factors capable of regulating the function of B-, T- and NK-cells, and direct toxicity to tumor cells (10).

In all, intravesical instillations with a chemotherapeutic drug can clearly reduce the risk of recurrence for patients with non-muscle-involved bladder carcinomas in the short term (8). However, in the long term it has only a modest effect on the risk of recurrence after TUR without reducing the risk of progression. In case of highly recurrent non-muscle-involved carcinoma or multiple recurrences, the BCG therapy is advocated. Since propolis preparations (WSDP or EEP) and propolis-related polyphenolic/flavonoid components exerted strong antitumor activity in *in vitro* cultures (10), we proposed that their potential as an intravesical chemopreventive/chemotherapeutic agent may prevent the recurrence rate of transitional cell carcinoma directly by their direct cytotoxic action as well as indirectly by their antitumor activity described elsewhere (10). In addition to this, a combined intravesical treatment and systemic use of propolis and related flavonoids may in general have great impact on transitional cell carcinoma since no toxicity or induced side effects of propolis and related polyphenols regarding dose and the time of duration of treatment were observed in experimental animals (10, 11).

This study was carried out to find whether propolis or its polyphenolic/flavonoid compounds interfere with TCC growth ability by induction of cytotoxicity in primary culture of human urinary bladder carcinoma cells of different grades as compared to normal urinary bladder epithelial cells.

## MATERIALS AND METHODS

### Patients

There were 30 patients (10 grade G1, 10 grade G2, and 10 grade G3 tumors). They were 65 to 75 years of age. Patients were consecutive, not treated with any intravesical therapy and an informed consent for these studies from each subject was obtained. In addition, the performed studies were approved by local Human Investigation Committee.

### Water-soluble derivative of propolis (WSDP)

Water-soluble derivative of propolis (WSDP) was prepared by the method described in our previous paper (11). Briefly, Croatian propolis from beehives kept at the outskirts of Zagreb was extracted with 96% ethanol which was filtered and evaporated to dryness in vacuum evapo-

rator. The resultant resinous product was added to a stirred solution of 8% L-lysine (Sigma Chemie, Deisenhofer, Germany) and freeze-dried to yield the WSDP, a yellow-brown powder.

The chemical profile of propolis from the northern hemisphere, often named as »poplar-type« propolis can be characterized by three analytical parameters: total flavonol and flavone content, total flavanone and dihydroflavonol content, and total polyphenolics content. According to (12), spectrophotometric procedures for quantification of the three main groups of bioactive substances in propolis could be used for quality assessment of different propolis samples, and results of those analyses correlate with biological activity, especially in the »poplar-type« of propolis. The spectrophotometric assay based on the formation of aluminum chloride complex was applied for quantification of total flavones/flavonols and expressed as a quercetin equivalent. For the quantification of propolis in flavanones and dihydroflavonols, we used 2,4-dinitrophenylhydrazine method (13). Total polyphenolic content was measured by the Folin-Ciocalteu procedure (14). WSDP contains: flavones and flavonols 2.13%, flavanones and dihydroflavonols 9.06%, total flavonoids 11.19%, total polyphenols 70.48%.

The WSDP was stored under sterile conditions at  $-20^{\circ}\text{C}$  to minimize bacterial contamination. Before use the WSDP was dissolved in distilled water.

### Ethanolic extract of propolis (EEP)

Raw Croatian propolis was collected by scraping it off hive frames. The collected propolis samples were kept desiccated in the dark until analysis at room temperature. Ethanolic propolis extract (EEP) was prepared by the method described elsewhere (15). Briefly, propolis (10 g) was crushed into small pieces in a mortar and mixed vigorously with 34.85 ml 80% (V/V) ethanol during 48 h at  $37 \pm 1^{\circ}\text{C}$ . After extraction, the ethanolic extract of propolis was filtered through Whatman N<sub>0</sub>.4 paper and then the extract was lyophilized. Recent spectrophotometric analysis has shown that EEP contains: flavones and flavonols 1.6%, flavanones and dihydroflavonols 38.60%, total flavonoids 40.20, total polyphenols 84.40%.

EEP was dissolved in ethanol and further dilutions were made in water. The final concentration of ethanol was less than or equal to 0.1%. Ethanol (0.1%) was used in the control group.

### Polyphenolic compounds

For experiments using polyphenolic compounds we used Caffeic acid (CA) – 3,4-dihydroxycinnamic acid (Aldrich-chemie, Milwaukee, WI, USA), Quercetin dihydrate (QU) (Fluka, BioChemica, Switzerland), Chrysin and Naringenin (Sigma, Germany).

All polyphenolic compounds were dissolved in ethanol and further dilutions were made in water. The final concentration of ethanol was less than or equal to 0.1%.

Ethanol (0.1%) was also used in the control group. No difference between water as control and 0.1% of ethanol in water was observed in preliminary experiments.

### Human tumour and normal epithelial tissue samples

Pieces of urinary bladder TCC from the cavity of urinary bladder or pieces of normal epithelial tissue from urinary bladder wall were obtained with informed consent from patients (General Hospital, Urology Department, Varaždin, Croatia) and collected after surgical resection. Single cell suspension was prepared by digestion with trypsin and DNase (11). Each suspension was passed through a stainless steel mesh (200 wires/inch), centrifuged three times at 24g for 5 minutes in saline and then resuspended in medium RPMI-1640 (Institute of Immunology, Zagreb) supplemented with 5% of fetal calf serum (FCS). Viability was determined using trypan blue exclusion assay. Percent of viability of bladder cancer cells was 69.23–94.8, 66.4–95.15 and 76.23–95.46 for G1, G2, and G3, respectively.

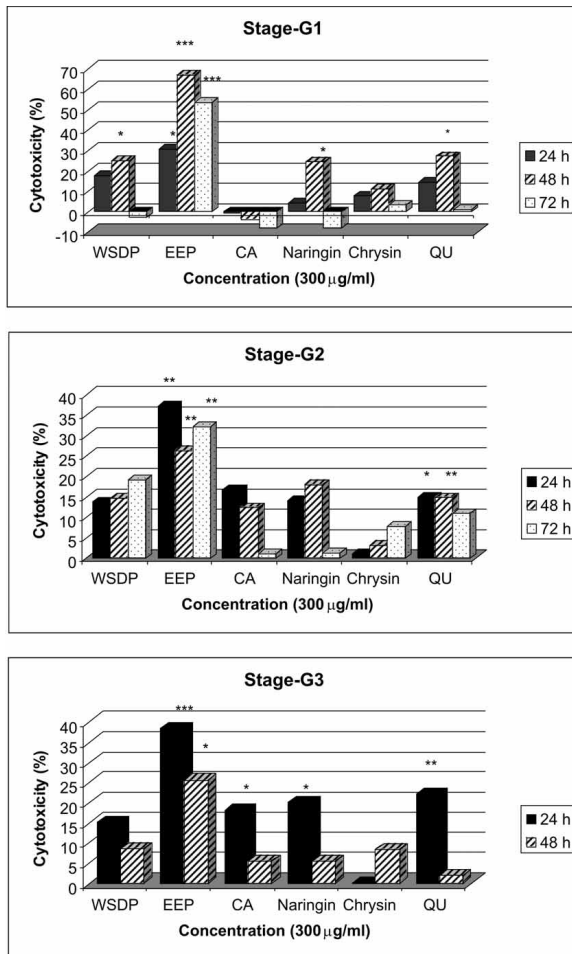
*Urinary bladder cancer*-Pieces of urinary bladder TCC from urinary bladder wall were collected by transurethral surgery from patients in different stages (grade G1, G2, G3) of bladder TCC. The tissues were embedded in paraffin and stained with hematoxylin and eosin for investigating the grade of carcinomas and the depth of invasion in more detail. The criteria for histological grade and clinical stage were as follows. Grade 1 (G1) indicates well-differentiated tumors in cellular atypism and histological features as transitional epithelium. Grade 3 (G3) indicates poorly differentiated tumors lacking in the features of transitional epithelium. Grade 2 (G2) indicates moderately differentiated tumors and medium features between G1 and G3 (16). Samples of bladder cancer tissue contained 95% transitional cell carcinoma cells as determined by histochemical staining by Mallory method and immunohistochemical staining by monoclonal antibody leukocyte common antigen (LCA), CD 68 and S-100 proteins to exclude non tumor tissue components (17).

*Normal epithelial tissue*-Pieces of normal epithelial tissue from urinary bladder wall were taken from patients by transurethral surgery.

*Tumor- and normal epithelial-cell suspension*-Single cell suspension from pieces of either TCC cells or normal epithelial tissue from the urinary bladder was prepared according to standard laboratory procedure (11).

### Primary cell culture and reagents

Briefly, cells were seeded in triplicate in 24-well plates (Falcon, USA) at a concentration of  $1 \times 10^6$  cells/ml for TCC cells or normal epithelial cells. Incubation for cytotoxicity testing were carried out in RPMI-1640 medium with 20% FCS at  $37^{\circ}\text{C}$  with 5%  $\text{CO}_2$  with or without different concentration (50, 150, 300  $\mu\text{g/ml}$ ) of test components. The concentrations were selected based on result

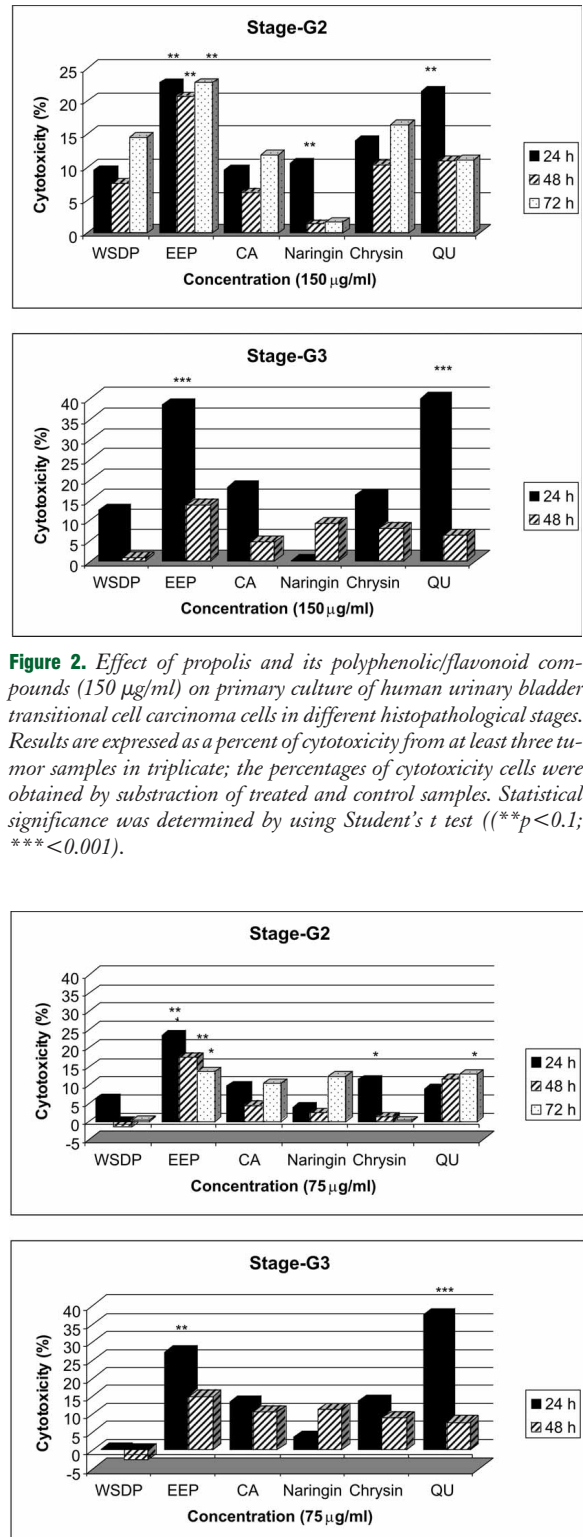


**Figure 1.** Effect of propolis and its polyphenolic/flavonoid compounds (300 µg/ml) on primary culture of human urinary bladder transitional cell carcinoma cells in different histopathological stages. Results are expressed as a percent of cytotoxicity from at least three tumor samples in triplicate; the percentages of cytotoxicity cells were obtained by subtraction of treated and control samples. Statistical significance was determined by using Student's *t* test (\**p*<0.05; \*\*<0.1; \*\*\*<0.001).

of pilot experiments, as described in our previous publications (10, 11, 18-20) as well as the experience related to the work with propolis and flavonoids. After exposure to test components for 24, 48, or 72 h, the cytotoxicity of two propolis preparations (WSDP or EEP) and their polyphenolic compounds (caffeic acid, naringin, chrysin, and quercetin) was determined using trypan blue exclusion assay. Briefly, after cultivation, 100 µl of cell suspension from the well was used and mixed with equal volume of trypan blue. Following this procedure, the cells were counted manually in a hemocytometer by three independent observers.

**Statistics**

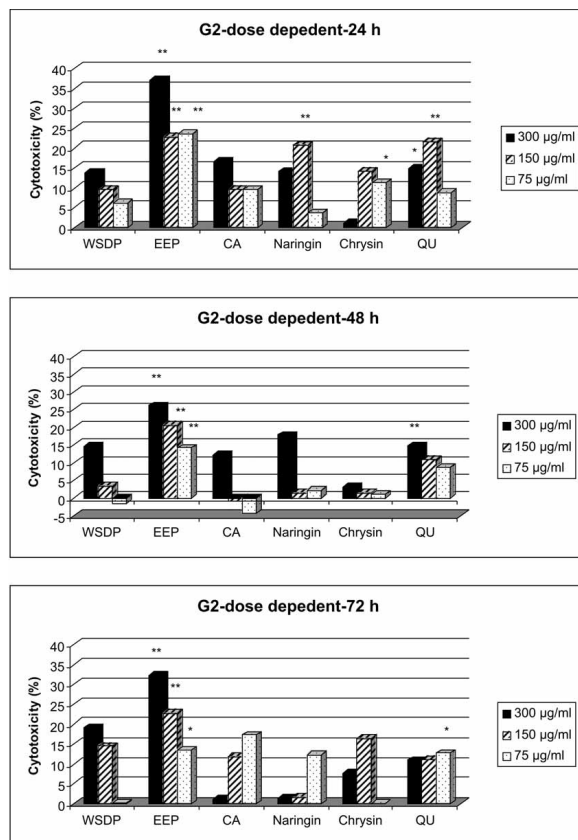
Differences between sample values were evaluated by Student's *t* test.



**Figure 2.** Effect of propolis and its polyphenolic/flavonoid compounds (150 µg/ml) on primary culture of human urinary bladder transitional cell carcinoma cells in different histopathological stages. Results are expressed as a percent of cytotoxicity from at least three tumor samples in triplicate; the percentages of cytotoxicity cells were obtained by subtraction of treated and control samples. Statistical significance was determined by using Student's *t* test (\*\**p*<0.1; \*\*\*<0.001).

**Figure 3.** Effect of propolis and its polyphenolic/flavonoid compounds (75 µg/ml) on primary culture of human urinary bladder transitional cell carcinoma cells in different histopathological stages. Results are expressed as a percent of cytotoxicity from at least three tumor samples in triplicate; the percentages of cytotoxicity cells were obtained by subtraction of treated and control samples. Statistical significance was determined by using Student's *t* test (\**p*<0.05; \*\*<0.1; \*\*\*<0.001).



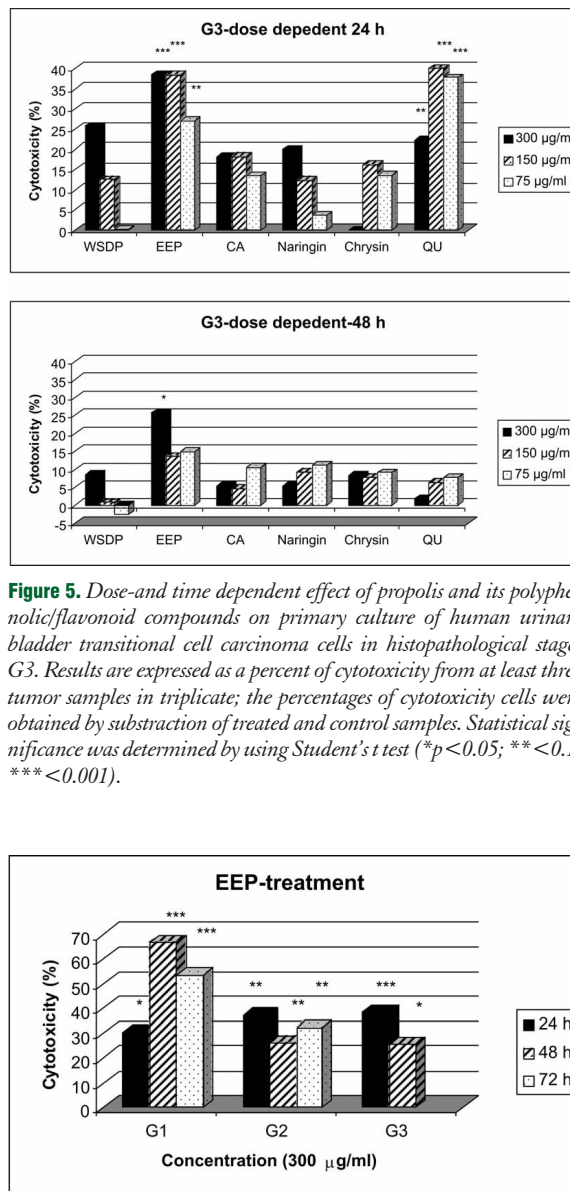


**Figure 4.** Dose- and time dependent effect of propolis and its polyphenolic/flavonoid compounds on primary culture of human urinary bladder transitional cell carcinoma cells in histopathological stage G2. Results are expressed as a percent of cytotoxicity from at least three tumor samples in triplicate; the percentages of cytotoxicity cells were obtained by subtraction of treated and control samples. Statistical significance was determined by using Student's *t* test (\* $p < 0.05$ ; \*\* $< 0.1$ ).

## RESULTS

### Water and ethanolic extract of propolis and its polyphenolic compounds inhibit the growth of primary culture of human urinary bladder TCC cells

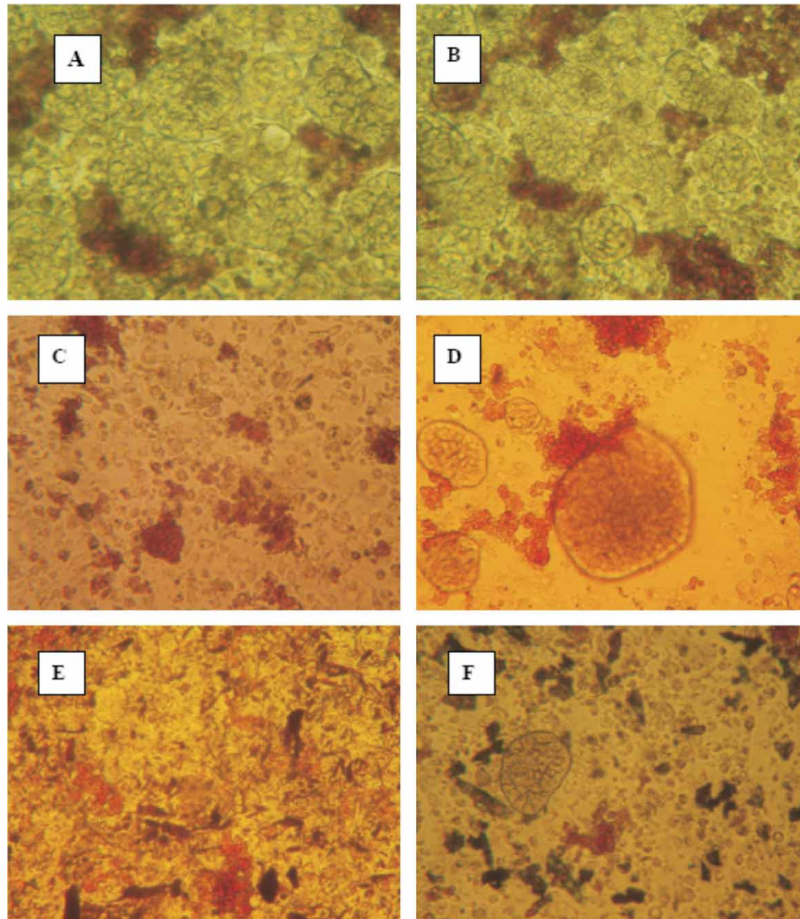
Our first aim was to investigate whether treatment with water or ethanolic extract of propolis or its polyphenolic compounds induce cytotoxic effect on primary culture of human urinary bladder TCC cells. As shown in Figures 1, 2 and 3, propolis and its polyphenolic compounds induce cytotoxicity of primary culture of human urinary bladder cancer cells in all histopathological grades of cancer. Comparing the cytotoxic efficacy of test components in different stages of urinary bladder TCC cells, the greatest cytotoxicity was in G1 stage (Figures 1, 2, 3). Majority of test components exhibited a time- and dose-dependent cytotoxicity (Figures 4, 5). Thus, WSDP treatment at concentrations of 75, 150, and 300 mg/ml resulted in 6-14% greater cytotoxicity than that in control tumor cells in G2 histopathological cancer grade after 24 h; EEP



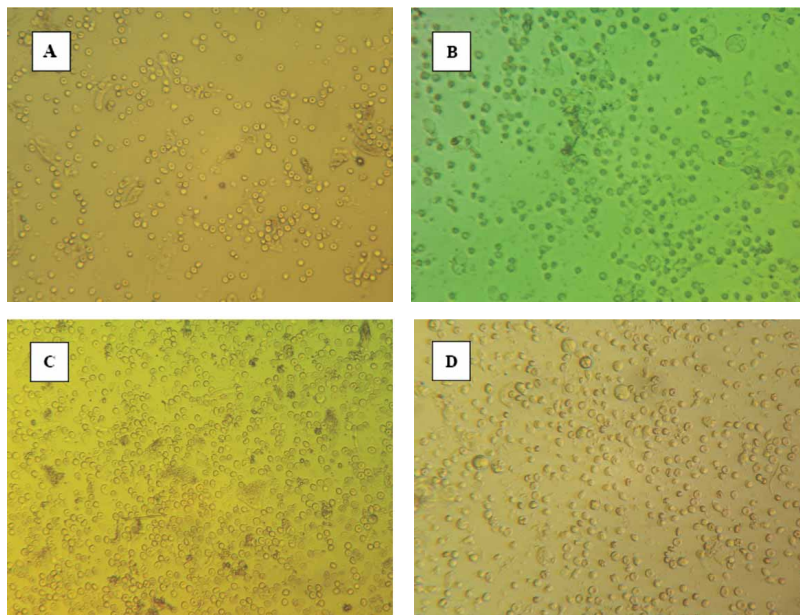
**Figure 5.** Dose- and time dependent effect of propolis and its polyphenolic/flavonoid compounds on primary culture of human urinary bladder transitional cell carcinoma cells in histopathological stages G3. Results are expressed as a percent of cytotoxicity from at least three tumor samples in triplicate; the percentages of cytotoxicity cells were obtained by subtraction of treated and control samples. Statistical significance was determined by using Student's *t* test (\* $p < 0.05$ ; \*\* $< 0.1$ ; \*\*\* $< 0.001$ ).

**Figure 6.** Comparative study of EEP on primary culture of human urinary bladder transitional cell carcinoma cells in different histopathological stages (G1, G2, G3) at concentration of 300 µg/ml. Results are expressed as a percent of cytotoxicity from at least three tumor samples in triplicate; the percentages of cytotoxicity cells were obtained by subtraction of treated and control samples. Statistical significance was determined by using Student's *t* test (\* $p < 0.05$ ; \*\* $< 0.1$ ; \*\*\* $< 0.001$ ).

23.5–37.16%, CA 9.61–16.76%, naringin 3.76–22.66%, chrysin 1.13–14.07% and QU 8.77–21.45%. In stage G3, test components at doses of 75, 150, and 300 mg/ml increased cytotoxicity to primary culture of human urinary bladder TCC cells in relation of control tumor cells after 24 h incubation of primary culture in the following manner: WSDP 0.25-25.71%, EEP 27.13-38.46%, CA 13.5–18.18%, naringin 3.7-20%, chrysin 0-16.16%, and QU 22.22-40%, respectively. The most effective agent in these



**Figure 7.** Primary culture of human urinary bladder transitional cell carcinoma cells: nontreated-control cells (A, B); urinary bladder carcinoma cells treated with EEP (C), WSDP (D) quercetin (E) chrysin (F) after 72 hours. Primary culture of human urinary bladder transitional cell carcinoma cells were photographed at x 400 magnification with a phase-contrast microscope.



**Figure 8.** Primary culture of human normal epithelial cells from urinary bladder wall: nontreated-control cells after 24 hours (A), 72 hours (B); normal epithelial cells treated with WSDP (C) or treated with EEP (D) after 72 hours. Primary culture of human normal epithelial cells were photographed at x 400 magnification with a phase-contrast microscope.

studies was EEP (Figures 6, 7). Test components exhibited no cytotoxic effect on normal urinary bladder epithelial cells (Figure 8).

## DISCUSSION

This study is the first, according to the available literature, to demonstrate that treatment of primary culture of human urinary bladder TCC cells with either preparation of water and/or ethanolic extract of propolis or with their polyphenolic/flavonoid compounds caused direct toxicity *in vitro* as compared to untreated control cells. These results demonstrated that test components induced time- and dose-dependent cytotoxicity on urinary bladder TCC cells. The test components exhibited toxicity on primary culture of human urinary bladder TCC cells, and no toxicity to normal urinary bladder epithelial cells. EEP was the most effective agent in these studies. The percent (%) of cytotoxicity of EEP was reversible to the histopathological cancer grade. Moreover, it is likely that synergistic action of different flavonoids present in EEP were more effective than any single compound. These results are in line with our earlier studies using animal cancer model (11, 18-20). In addition, the cooperative action of different isoflavones on cell growth, DNA synthesis, and apoptosis was also shown (21). Ike-moto *et al.* (22) demonstrated the antitumor effect of *Scutellariae radix* and its flavonoid components such as baicalein, baicalin, and wogonin on human bladder cancer cell lines (KU-1 and EJ-19 and murine bladder cancer cell line (MBT-2). Authors showed that flavonoids inhibited cell proliferation in dose-dependent manner, but baicalin exhibited the greatest antiproliferative activity. The same authors in an *in vivo* experiment investigated the antitumor effect of *Scutellariae radix* on C3H/HeN mice with MBT-2 cells and demonstrated a significant inhibition of tumor growth. Moreover, Yang *et al.* (23) demonstrated the chemopreventive effect of two flavonoids (diosmin and hesperidin) on N-butyl-N-(4-hydroxybutyl) nitrosamine (OH-BBN)-induced urinary bladder carcinogenesis in male ICR mice. These authors showed that hesperidin was more chemopreventive than diosmin when mice were fed during the initiation stage. This may be explained by differences in ring responsibility for anti-oxidant activity in flavonoids. Cell proliferation is thought to play an important role (24) in multi-stage carcinogenesis, including bladder tumorigenes. The result of the present study indicate that two preparations of propolis (WSDP and EEP) and their polyphenolic/flavonoid components could effectively inhibit three histopathological grades (G1, G2, G3) of human bladder cancer. The greatest percent of cytotoxicity was in grade 1 (G1) while the effect of test components in grade 2 (G2) or 3 (G3) was essentially similar; the somehow unusual findings for grade G1 as opposed to grade G2 and G3 could be explained by heterogeneity of tumor cells which in earlier phase of tumor growth is less expressed than in the later phase (25). It is possible that propolis and its flavonoids might induce cell differentiation as suggested by Csokay *et al.* (26). Several biochemical targets have

been proposed to explain the cytotoxic effect of propolis and its polyphenolic compounds, such as their antioxidant properties to inhibit prooxidant enzymes (cyclooxygenase, COX; lipoxygenase, LOX; xanthine oxidase), inhibition of signal molecules, modulation activity of oncogenes encouraging the process apoptosis/necrosis, a change of redox state of tumor cells, angiogenesis inhibition and enzymes metalloproteinase, telomerase and topoisomerase, ornithin decarboxylase, synthesis polyamine, as well as numerous kinases (tyrosine protein kinase, cAMP-dependent protein kinase, phosphoinositide 3-kinase, mitogen-activating protein kinase, cyclin-dependent kinase) involved in cell proliferation (10, 26-28). It also showed that the inhibition of prooxidative enzymes with flavonoids leads to inhibition of angiogenesis process, and that it reduces the growth of human bladder cancer *in vitro* by apoptotic mechanism (29-34). Propolis, flavonoid galangin and other flavonoids from propolis are potent COX-2 inhibitors among several natural products tested (35-37). Therefore, it is possible that galangin as a COX-2 inhibitor, as well as other flavonoids present in propolis, may inhibit the proliferation of urinary bladder cancer cells.

A large body of evidence suggests that inhibiting COX-2, the inducible form of COX, will be an important strategy for prevention of cancer. COX-2 overexpression has been observed in colorectal, non-small-cell lung, gastric, breast, cervical, prostate and bladder cancer (38-41), which is associated with an increase in angiogenesis, tumor invasiveness, and immunosuppression in various tumors along with decrease in apoptosis (42-46). COX-2 inhibitors can also be expected to lead to efficacy of their use as adjuncts to various anti-cancer agents (47). The molecular mechanism of cancer chemoprevention by flavonoids may involve the inhibition of the prooxidant processes that cause tumor promotion. Flavonoids and caffeic acid analogues are particularly effective at inhibiting the prooxidant enzymes xanthine oxidase (48, 49), COX or LOX (50). However, additional studies are needed on the chemoprevention of urinary bladder cancer via this pathway.

Knowledge on various molecular chemopreventive or therapeutic mechanisms that may be involved in the mechanism of cancer chemoprevention by polyphenolic/flavonoid components was summarized in papers (10, 34, 51).

Moreover, inhibition of urinary bladder cancer by green tea which contains polyphenolic catechin and other components such as vitamin C, vitamin A, chlorophyll, caffeine and quercetin (the components present in propolis) was demonstrated *in vitro* and *in vivo* (16, 52, 53).

The incidence of bladder cancer has increased, and responses to therapy have been limited. Several studies have shown the effectiveness of immediate post-resection chemotherapy with agents such as doxorubicin and mitomycin C (54, 55). Here we reported that water and/or ethanolic extract of propolis or their polyphenolic/flavonoid compounds may induce cytotoxicity in primary



culture of human urinary bladder cancer cells and have selective effect as compared to normal urinary bladder epithelial cells. Although bacillus Calmette-Guerin is one of the most effective agents for preventing recurrence, it cannot be used immediately after resection secondary to the systemic absorption. Propolis and its polyphenolic/flavonoid components are not harmful if absorbed and they reportedly do not affect normal tissue, making it an ideal potential agent. Our previous data *in vivo* and numerous literature data about chemopreventive mechanism and local effect of these components on tumor cells (56) suggest that propolis and its compounds could be potential candidates in therapy of urinary bladder cancer. Widely available, safe and inexpensive, propolis and its compounds may prove to be of considerable benefit in the prevention and treatment of urinary bladder cancer.

The result of this study may provide great impact on the potential activity of EEP as an adjuvant to surgery, to suppress or prevent tumor recurrence in urinary bladder since only a few anti-cancer drugs have been effective in tumor control. Since immunomodulation by BCG has been used to improve the results of surgery it is likely that propolis preparation (EEP) as immunomodulating compound (57, 58) may be a substitute for mycobacterial treatment as propolis preparation have expressed no side effect after treatment. However, the exact cancer chemoprevention mechanisms of propolis effectiveness have to be elucidated. The data presented in this study also support and warrant propolis efficacy studies in preclinical urinary bladder models.

## CONCLUSIONS

In conclusion, these results suggest that propolis and its polyphenolic/flavonoid components may become attractive and promising treatment for urinary bladder cancer but further animal and human *in vivo* studies are warranted to evaluate the safety and clinical utility of these test components in patients with urinary bladder cancer.

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