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Detoxification effects of aloe polysaccharide and propolis on the urinary excretion of metabolites in smokers

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ABSTRACT

The aim of the present study was to investigate the detoxifying effects of aloe polysaccharide (APS), propolis, and the mixture of APS and propolis on the urinary excretion of major human tobacco carcinogens, BaP and an addictive stimulant alkaloid, nicotine. Smokers (≥20 cigarettes/day) were randomly classified into four subgroups (10 people/group) and were given 600 mg/day of APS, 600 mg/day of propolis, or 600 mg/day of the mixture of APS (420 mg/day) and propolis (180 mg/day) for four weeks. Urinary excretion of BaP and cotinine (a metabolite of nicotine) increased in a time-dependent manner increased after supplementation with APS (BaP, 2.23-fold; cotinine, 2.64-fold), propolis (BaP, 1.30-fold; cotinine, 2.08-fold), and the mixture (BaP, 2.33-fold; cotinine, 2.28-fold) compared with smoker control. Creatinine, glucose, and total bilirubin levels significantly decreased in a time-dependent manner after supplementation with APS (creatinine, 15.24%; glucose, 40.22%; total bilirubin, 48.82%), propolis (creatinine, 16.83%; glucose, 36.25%; total bilirubin, 52.59%), and the mixture (creatinine, 16.36%; glucose, 46.37%; total bilirubin, 39.20%) ($p < 0.05$). These results suggest that supplementation with APS, propolis, or the mixture could reduce the risk of cancer or other diseases associated with tobacco smoking by enhancing urinary excretion of BaP and nicotine.

1. Introduction

The epidemic of tobacco and tobacco-related diseases is one of the biggest threats to global public health. Tobacco is responsible for the deaths of more than 7 million people a year (WHO, 2017). More than 6 million of those deaths are the result of direct tobacco use, with the remainder resulting from exposure to second-hand smoke. Tobacco smoking has been clearly and unambiguously identified as a direct cause of cancers of the lung, prostate, oral cavity, esophagus, stomach, colon/rectum, cervix, pancreas, larynx, liver, bladder, and kidney as well as leukemia, especially acute myeloid leukemia (Islami et al., 2014; Lee et al., 2005, 2017; Morais et al., 2014; Sosnowski and Przewoźniak, 2014; USDHHS, 2004; Walter et al., 2014). Additionally, tobacco smoking is a direct cause of ischemic heart disease (the commonest cause of death in Western countries), respiratory disease, aortic aneurysm, chronic obstructive lung disease, stroke, inflammatory diseases, pneumonia, and hepatocirrhosis (Boyle, 1997; Pacheco et al., 2013). Tobacco smoke contains about 7000 characterized compounds, of which at least 70 are human carcinogens including benzo[a]pyrene (BaP), tobacco-specific nitrosamines (TSNs) such as N-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), acetaldehyde, cadmium, and several other organic and inorganic compounds (CalEPA, 2005; Hecht, 1999; IARC, 2004; Mallock et al., 2018; Piasek et al., 2016; Smith et al., 2003; USDHHS, 2010, 2014; WHO, 2017). Among 70 human carcinogens classified by IARC, asbestos was also detected in cigarette filter and mainstream smoke, which might be associated with respiratory diseases including lung cancer (Casalone et al., 2018; Longo et al., 1995; Yanamala et al., 2018).

Aloe vera is a perennial plant belonging to the family Liliaceae (Grindlay and Reynolds, 1986). For many years, products of *Aloe vera* (same as *Aloe barbadensis* Miller), whether these are the fresh gel, juice, or formulated products, have been also used for health, medical, and cosmetic purposes (Kwack et al., 2014; Lee et al., 1980; Morton, 1961). The aloe plant produces anthraquinone glycosides (10%–30%) such as aloins (A and B); mucilage (30%), a resinous material (16%–63%); polysaccharides; sugars (about 25%); imucopolysaccharides such as acemannan and betamannan; fatty acids (cholesterol, campesterol, and β-sitosterol), glycoproteins (aloctins A and B); enzymes (inducing cyclooxygenase and bradykininase); and other compounds such as lupeol,

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salicylic acid, urea nitrogen, cinnamic acid, phenol, sulfur, magnesium lactate, prostanoids, and fiber (Capasso et al., 1997). Of these, aloe polysaccharides were shown to have chemopreventive and immunomodulating effects (Kim and Lee, 1997; Kim et al., 1999; Yoo and Lee, 2005).

Aloe vera has been used as a folk medicine since ancient times due to its curative and healing qualities, extensively in the areas of wound healing and thermal injury healing (Coats and Ahola, 1979; Davis et al., 1989; Morton, 1961). There have been numerous reports that support the biological and pharmacological activities of *Aloe vera* such as antibacterial, antifungal, and antiviral properties along with antioxidant effects (Kim et al., 1999; Lee et al., 2000; Mantle et al., 2001). Reports include evidence of anti-tumor, anti-diabetic, anti-tyrosinase, and analgesic properties for bones, joints, and crippling diseases in addition to *Aloe vera*'s efficacy in treating gastric ulcers, arthritis, skin cancer, burns, eczema, and psoriasis (Beppu et al., 1993; Heggers et al., 1993; Radha and Laxmipriya, 2014; Rodriguez-Bigas et al., 1988; Visuthikosol et al., 1995; Yagi et al., 1987).

Propolis is a resinous material collected by bees from plant buds and exudates that the bees use to build and repair honeycombs (Ghisalberti, 1979; Gülçin et al., 2010). Propolis has been used as a remedy in folk medicine since ancient times for its pharmaceutical properties, as a constituent in bio-cosmetics and health foods, and for numerous other purposes (Ghisalberti, 1979; Wollenweber and Buchmann, 1997). Propolis is composed of resin and vegetable balsam (50%), wax (30%), essential and aromatic oils (10%), pollen (5%), and various other substances (5%) including organic debris (Cisarino et al., 1987). Propolis contains more than 160 constituents such as polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones), sesquiterpene quinones, coumarins, steroids, amino acids, and inorganic compounds (Bonvehi et al., 1994; Ghisalberti, 1979). Flavonoids identified from propolis included galangine, kaempferol, quercetin, pinocembrin, pinostobin, and pinobanksin (Volpi, 2004). Some of the phenolics are cinnamyl alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic acid, and caffeic and ferulic acids. Propolis has been reported to have a broad spectrum of biological activities such as anti-neoplastic, anti-inflammatory, antibiotic, free radical-scavenging, anti-viral, anti-fungal, anesthetic, cytostatic, spasmolytic, and anti-ulcerous activities (Koo et al., 2000; Kujumgiev et al., 1999; Manolova et al., 1985; Marcucci, 1995; Moreno et al., 2000; Nna et al., 2018; Park et al., 1998; Pascual et al., 1994; Yamauchi et al., 1992).

Chemoprevention of cancer is the use of natural or synthetic agents to block, reverse, or prevent the development of invasive cancers (Greenwald and Kelloff, 1996; Koo et al., 2013; Shureiqi et al., 2000; Wattenberg, 1985). There are at least three mechanisms of chemoprevention: anti-mutagenesis, anti-proliferation/anti-progression, and antioxidant/anti-inflammatory. Anti-mutagenesis activities encompass inhibiting carcinogen uptake, excretion, formation/activation, and deactivation/detoxification, blocking carcinogen–DNA bindings, and enhancing the fidelity of DNA repair (Yoo and Lee, 2005).

Anti-proliferation/anti-progression activities comprise modifying signal transduction, modulating hormonal and growth factor activity, inhibiting aberrant oncogene activity, telomerase activity, basement membrane degradation, angiogenesis, and polyamine metabolism; inducing terminal differentiation and apoptosis; restoring immune responses such as tumor suppressor function; enhancing intercellular communication; correcting DNA methylation imbalances, and activating anti-metastasis genes. Antioxidant/anti-inflammatory activities include scavenging reactive electrophiles and oxygen radicals and inhibiting arachidonic acid metabolism (Kelloff et al., 1996; Shureiqi et al., 2000). All these above mentioned approaches are based on inhibiting carcinogenesis progress after exposure to carcinogens. As a chemopreventive approach, the enhanced excretion of carcinogen is more effective before the reaction of electrophilic carcinogen to biomolecules in the body (Lee and Park, 2003).

With the present study, we aimed to investigate the detoxifying effects of aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis on the urinary excretion of one of major human tobacco carcinogens, BaP and cotinine (a metabolic product of nicotine, a precursor of TSN), in smokers as a possible mode of chemoprevention (Brajenović et al., 2015; Yuan et al., 2014).

2. Materials and methods

2.1. Chemical

(−) Cotinine, 2-phenylimidazole, triethylamine, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium hydroxide, and BaP were obtained from Sigma (St. Louis, MO, USA). BaP-7,8-dihydrodiol was also purchased from the National Cancer Institute, Chemical Carcinogen Reference Standard Repository (Bethesda, MD, USA). Solvents of analytical or high-performance liquid chromatograph (HPLC) grade were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ, USA). Aloe polysaccharide (APS) prepared from *Aloe barbadensis* Miller was kindly provided by ALOECORP (Harlingen, TX, USA). APS consists of 85.1% polysaccharides (73% being mannose) and 0.5% protein as previously described (Kim et al., 1999). Propolis was kindly provided by Morikawa Kenkodo Co., Ltd. (Tokyo, Japan). A propolis water-soluble powder was obtained from 80% ethanol extract of propolis by freeze-drying and its composition is as described previously (Takeda et al., 2018). Briefly, propolis used in this study consists artepillin C 13 mg/kg, p-coumaric acid 3.5 mg/kg, and drupanin 6.5 mg/kg (Takeda et al., 2018).

2.2. Study design and subjects

We recruited 40 healthy smokers and 10 healthy nonsmokers, all male students between the ages of 20 and 28 years, from Sungkyunkwan University, Suwon, South Korea. All subjects gave informed consent and completed a questionnaire on the state of their health, diets, smoking habits, alcohol consumption, and medical history. All procedures performed in studies involving human participants were in accordance with the ethical standards of The Institutional Review Committee of Sungkyunkwan University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Smokers were defined as individuals who had smoked more than 20 cigarettes per day for the last year, and nonsmokers were individuals who had never smoked cigarettes (Table 2). Smokers were randomly classified into four subgroups (10 people/group) to receive placebo, aloe polysaccaride, propolis, or the mixture of aloe polysaccaride and propolis. The subjects did not take any drugs before the start of the study. During the study period, they were given 600 mg/day of aloe polysaccaride, 600 mg/day of propolis, or 600 mg/day of the mixture of aloe polysaccaride (420 mg/day) and propolis (180 mg/day) for four weeks based on the daily recommended dosage (Table 1).

2.3. Blood and urine collection

Blood and urine samples were collected from all subjects before and

Table 1
Experimental groups and treatment protocol.

Experimental groups (n)	Treatments
1. Nonsmokers (10)	Placebo
2. Smokers (10)	Placebo
3. Smokers + Aloe (10)	600 mg/day of aloe polysaccaride
4. Smokers + Propolis (10)	600 mg/day of propolis
5. Smokers + (Aloe + Propolis) (10)	600 mg/day of the mixture of aloe polysaccharide (420 mg/day) & propolis (180 mg/day)

Table 2
Basic information, cotinine, BaP, creatinine, total bilirubin, and glucose levels in smokers and nonsmokers.

Characteristics	Smokers	Nonsmokers
Number of subjects	40	10
Age (years)		
Mean	24.15 ± 2.43	23.70 ± 2.16
Range	20–28	20–26
Body weight (kg)		
Mean	66.83 ± 7.17	66.60 ± 8.95
Range	53–84	55–83
Height (cm)		
Mean	174.48 ± 4.32	173.90 ± 6.62
Range	165–185	164–185
BMI (kg/m^2)		
Mean	21.97 ± 2.44	21.95 ± 1.93
Range	7.51–28.39	18.93–25.47
Alcohol consumption (bottles/week)		
Mean	1.65 ± 1.3	1.67 ± 0.87
Range	0.15–5.5	1–3
Tobacco smoking (cigarettes/day)		
Mean	22.75 ± 5.06	-
Range	20–40	-
Smoking period (years)		
Mean	4.88 ± 2.11	-
Range	1–10	-
BaP (ng/mL)		
Mean	0.34 ± 0.02	0.29 ± 0.04[a]
Range	0.28–0.40	0.25–0.39
Cotinine (μg/mL)		
Mean	8.59 ± 2.81	0.10 ± 0.03[a]
Range	3.52–18.32	0.03–0.20
Creatinine (mg/dL)		
Mean	1.01 ± 0.09	0.95 ± 0.05
Range	0.9–1.2	0.9–1.0
Glucose (mg/dL)		
Mean	95.36 ± 10.34	85.00 ± 2.88
Range	61–124	78–93
Total bilirubin (mg/dL)		
Mean	1.19 ± 0.19	1.01 ± 0.17
Range	0.8–1.7	0.8–1.6

[a] Significantly different from smokers ($p < 0.01$).

after supplementation with aloe polysaccaride, propolis, or the mixture of both. Blood samples (20 mL) were taken from all subjects by venipuncture in a heparinized tube. At the same time, a spot urine sample was collected in the morning. The plasma was separated from erythrocytes by centrifugation at 3000 × *g* for 15 min at −4 °C. Plasma and urine samples were stored at −80 °C until analysis.

2.4. Analysis of BaP

We performed chromatographic analyses using a Hitachi HPLC (Tokyo, Japan) equipped with a Hitachi Model L-7200 autosampler and a Hitachi Model L-7100 pump. The output from the detector was connected to a Hitachi Model D-7000 Interface Module, and the data were recorded on an HP deskjet printer. In a 15-mL conical tube containing 150 μL of BaP-7,8-diol (200 ng/mL) as an internal standard, we added 4 mL of chloroform, and 4 mL of urine; the tubes were agitated for 1 h in a rotary shaker. After centrifugation (3500 × *g*; 10 min), the organic phase was transferred to a fresh test tube and evaporated under N_2 gas in a 40 °C. The extracts were dissolved in 300 μL of methanol and passed through a 0.45 μm filter (Millipore, Milford, MA, USA), and then aliquots (40 μL) were injected into a chromatographic system. The effluents were monitored with a Jasco Model 920-FP Fluorescence Detector (Japan Spectroscopic, Tokyo, Japan) at an emission wavelength of λ_{em} = 450 nm (excitation wavelength: λ_{ex} = 265 nm). The elution was performed using a Supelcosil LC-18, 5 μm ODS column with an eluent of 20–80% B solution, employed as a linear gradient at a flow rate of 1.2 mL/min for 20 min (eluent A - 50:50 (water:methanol)/ eluent B – methanol).

2.5. Analysis of cotinine

We analyzed cotinine with Hitachi's HPLC system using a Supelcosil LC 18 reversed-phase column (150 × 4.6 mm, 5 μm particle, Supelco, Bellefonte, PA, USA) operating at room temperature. Cotinine and the internal standard were eluted isocratically with a mobile phase consisting of phosphate buffer (adjusted to pH 7.4): acetonitrile:methanol mixture (70:10:20, by vol.) at a flow rate of 1.0 mL/min. The mobile phase was filtered through a 0.45-mm membrane filter (Millipore) and degassed before use. In a 15-mL conical tube containing 2 mL of 5 M sodium hydroxide, 100 μL of phenylimidazole (20 μg/mL) as an internal standard, 4 mL of chloroform and 4 mL of urine were added. The tubes were agitated for 1 h in a rotary shaker. After centrifugation (3500 × *g*; 10 min), the organic phase was transferred to a fresh test tube and evaporated under N_2 gas. The extracts were dissolved in 200 μL of mobile phase (70:10:20, by vol.) and then passed through a 0.45-μm filter (Millipore). Aliquots (20 μL) were injected into a chromatographic system with a detector UV L-7400 operating at 260 nm.

2.6. Blood biochemistry

Serum biochemical parameters (creatinine, glucose, and total bilirubin) were measured using an autoanalyzer.

2.7. Statistical analysis

Quantitative differences between group values were statistically analyzed using ANOVA (analysis of variance) with a multiple comparison post-test by the Bonferroni method. *P* values of < 0.05 were considered statistically significant. All values are expressed as mean ± SD.

3. Results

3.1. Baseline characteristics

Ten subjects were healthy nonsmokers, and 40 were healthy smokers; smokers and nonsmokers ranged in age from 20 to 28 years and 20–26 years (24.15 ± 2.43 years and 23.70 ± 2.16 years), respectively. The 40 smokers smoked an average of 20–40 cigarettes/day (22.75 ± 5.06 cigarettes/day) and had smoked for 1–10 years (4.88 ± 2.11 years). The four treatment groups (aloe polysaccaride, propolis, the mixture of aloe polysaccaride and propolis, or placebo) were not significantly different with respect to alcohol consumption, age, or body mass index (BMI), as expected by design (Table 2). The treatment groups were not also significantly different with respect to cigarette consumption/day, baseline urinary excretion levels of cotinine and BaP, or background of creatinine, glucose, and total bilirubin level in the sera.

3.2. Urinary excretion of BaP

Effects of aloe polysaccaride, propolis and the mixture of aloe polysaccaride and propolis (600 mg/day of aloe polysaccaride, 600 mg/day of propolis, 600 mg/day of the mixture of aloe polysaccaride [420 mg/day] and propolis [180 mg/day]) were investigated on the urinary excretion of BaP in smokers (10 people/group, including placebo) and nonsmokers for the study period of 4 weeks. Fig. 1 shows standard profiles of BaP and BaP-7,8–diol by HPLC, and the profiles of the smokers' urine. Urinary excretion of BaP was 0.34 ± 0.02 ng/mL in the urine of the smokers and 0.29 ± 0.04 ng/mL in nonsmokers. After supplementation with aloe polysaccaride for 4 weeks, the smokers' urinary BaP excretion increased significantly in a time-dependent manner (1 week; 0.34 ± 0.01, 2 week; 0.41 ± 0.01 ($p < 0.01$), 3 week; 0.42 ± 0.02 ($p < 0.01$), and 4 week; 0.75 ± 0.02 ng/mL ($p < 0.01$)) compared with the zero week (0.34 ± 0.02 ng/mL)

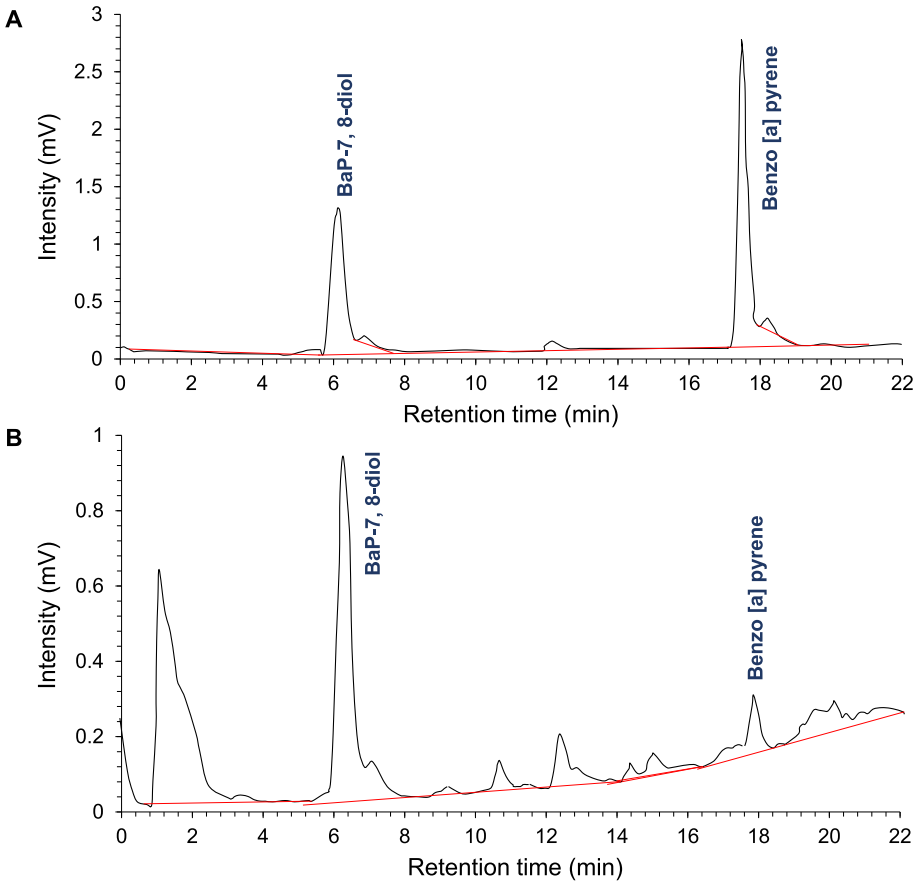

Fig. 1. The HPLC chromatographic profiles of BaP in the urine of smokers. (A) Standard profile of BaP and BaP-7,8–diol, (B) Profile of the smokers' urine.

(Fig. 3). In the case of smokers supplemented with propolis, the urinary BaP excretion increased marginally but significantly (1 week; 0.32 ± 0.03, 2 week; 0.40 ± 0.02 ($p < 0.01$), 3 week; 0.41 ± 0.01 ($p < 0.01$), and 4 week; 0.43 ± 0.02 ng/mL ($p < 0.01$)) compared with the zero week (0.34 ± 0.02 ng/mL). However, in smokers supplemented with the mixture, the urinary BaP excretion increased more significantly (1 week; 0.31 ± 0.02, 2 week; 0.41 ± 0.01 ($p < 0.01$), 3 week; 0.45 ± 0.02 ($p < 0.01$), and 4 week; 0.77 ± 0.03 ng/mL ($p < 0.01$)) than zero week (0.33 ± 0.01 ng/mL). After 4 weeks supplementation, the urinary excretion of BaP increased in a time-dependent manner as the total intake dose increased after supplementation with aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis, by 2.23-fold, 1.30-fold, and 2.33-fold, respectively. The urinary excretion of BaP in control remained constant during the study period (Fig. 3).

3.3. Urinary excretion of cotinine

The subjects' urinary excretions of cotinine were measured in the urine. Fig. 2 shows (A) standard HPLC profiles of cotinine and 2-phenylimidazole, and (B) the profiles of the smokers' urine. The baseline concentrations of cotinine in the smokers and nonsmokers were 8.59 ± 2.81 μg/mL and 0.10 ± 0.03 μg/mL ($p < 0.05$, smokers versus nonsmokers), respectively (Table 2). After 4 weeks supplementation with aloe polysaccaride, the smokers' urinary cotinine excretion in smokers increased markedly during the four weeks (1 week; 7.57 ± 1.98, 2 week; 9.25 ± 2.78, 3 week; 14.82 ± 2.87 ($p < 0.01$), and 4 week; 18.82 ± 2.98 μg/mL ($p < 0.01$)) compared with the zero week (7.13 ± 2.36 μg/mL) (Fig. 4). The urinary cotinine excretions in smokers supplemented with propolis also increased gradually during the 4-week study period (1 week; 8.65 ± 2.44, 2 week; 10.04 ± 3.89, 3 week; 11.11 ± 3.99, and 4 week; 14.98 ± 2.71 μg/mL ($p < 0.01$)) compared with the zero week (7.17 ± 1.10 μg/mL). However, in smokers supplemented with the mixture of aloe polysaccaride and propolis, the urinary cotinine excretion increased more markedly during the 4 weeks (1 week; 10.16 ± 3.18, 2 week; 10.46 ± 2.05, 3 week; 21.14 ± 3.04 ($p < 0.01$), and 4 week; 22.58 ± 3.11 μg/mL ($p < 0.01$)) compared with the zero week (9.86 ± 2.12 μg/mL). Similar to BaP, the urinary excretion of cotinine increased in smokers supplemented with aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis by 2.64-fold, 2.08-fold, and 2.28-fold, respectively, and that in the control remained constant during the study period. Overall, smokers supplemented with aloe polysaccaride, propolis, or the mixture of aloe polysaccaride and propolis showed time-dependent increases of cotinine excretion (Fig. 4).

3.4. Blood biochemistry

3.4.1. Analysis of creatinine level

Creatinine levels were measured in the peripheral blood of smokers and nonsmokers. Background levels of creatinine in smokers and nonsmokers were 1.01 ± 0.09 mg/dL and 0.95 ± 0.05 mg/dL (Table 2). During the study period, creatinine levels remained unchanged in control, but the mean levels of creatinine decreased gradually in smokers supplemented with aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis, by 15.24%, 16.83%, and 16.36%, respectively (Fig. 5); these smokers all showed a time-dependent decrease of creatinine, and their creatinine levels were close to those of nonsmokers after 3–4 weeks of supplementation.

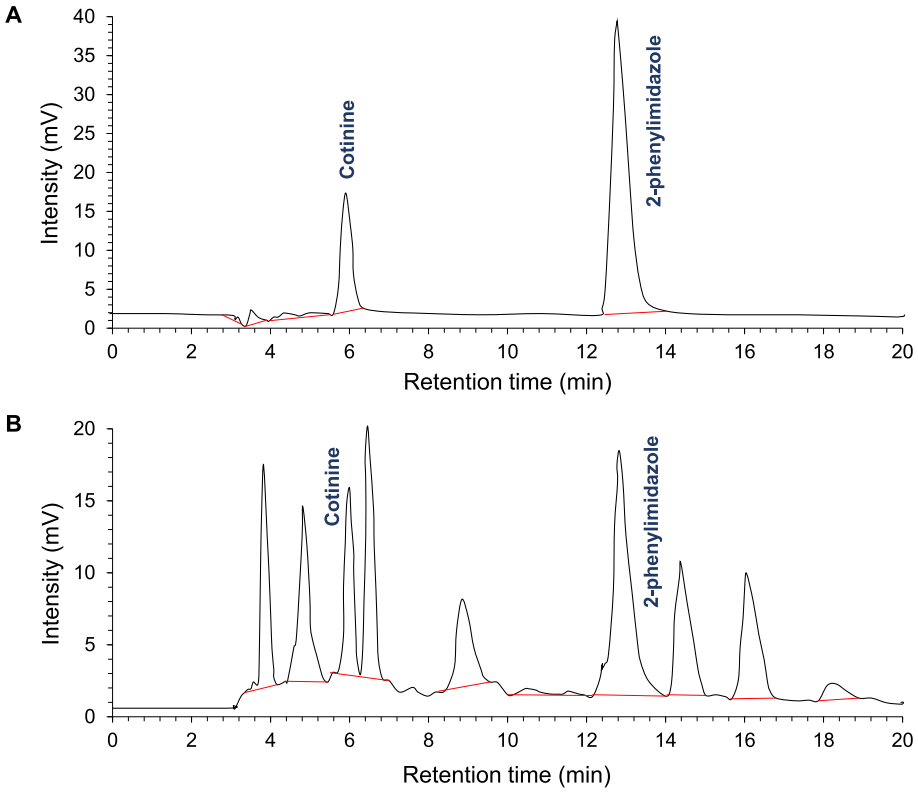

Fig. 2. The HPLC chromatographic profiles of cotinine in the urine of smokers. (A) Standard profile of cotinine and 2-phenylimidazole; (B) Profile of the smokers' urine.

3.4.2. Analysis of glucose and total bilirubin level

Glucose levels were measured in the peripheral blood of smokers and nonsmokers. The baseline level in smokers was 95.36 ± 10.34 mg/dL and 85.00 ± 2.88 mg/dL in nonsmokers ($p < 0.05$, smokers versus nonsmokers) (Table 2). During the study period, glucose levels remained unchanged in control, but the mean levels decreased significantly in smokers supplemented with aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis, by 40.22%, 36.25%, and 46.37%, respectively (Fig. 6A); a time-dependent decrease in glucose was clearly observed in these smokers, and their glucose levels were close to those of nonsmokers after 2 weeks.

Total bilirubin levels were measured in the peripheral blood of smokers; the background levels in smokers and nonsmokers were

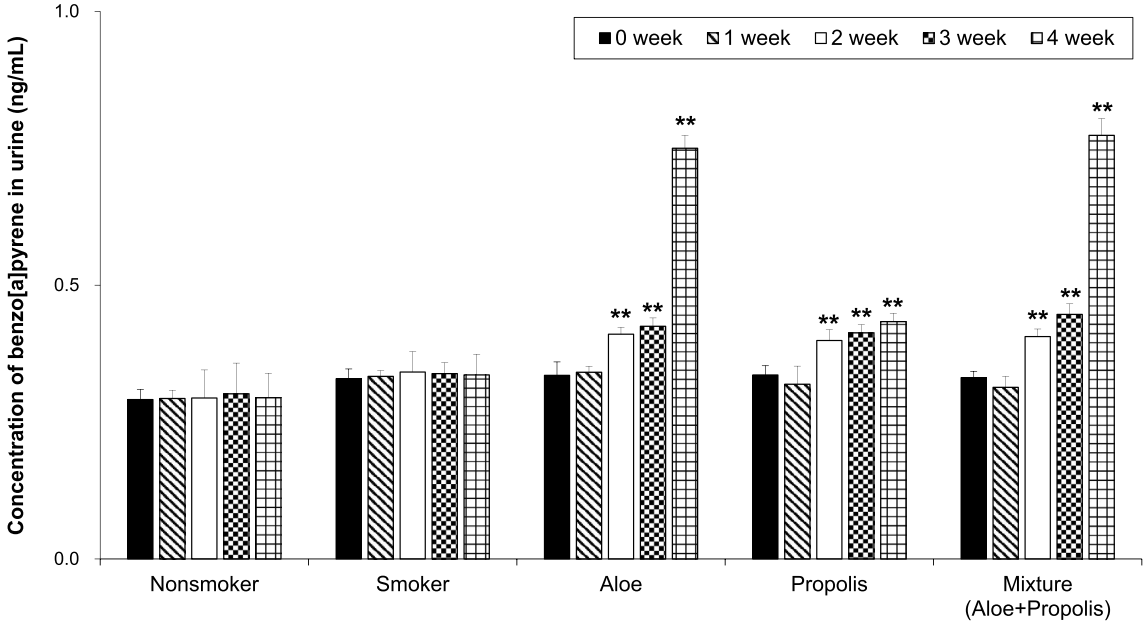

Fig. 3. Effects of aloe polysaccaride, propolis, and mixture of aloe polysaccaride and propolis supplementation on urinary excretion of BaP in smokers. Aloe polysaccaride; 600 mg/day of, propolis; 600 mg/day, the mixture of aloe polysaccaride (420 mg/day) and propolis (180 mg/day). Values are the mean ± SD of triplicate experiments. Significant differences are represented by asterisks (**$p < 0.01$ compared with the smoker group).

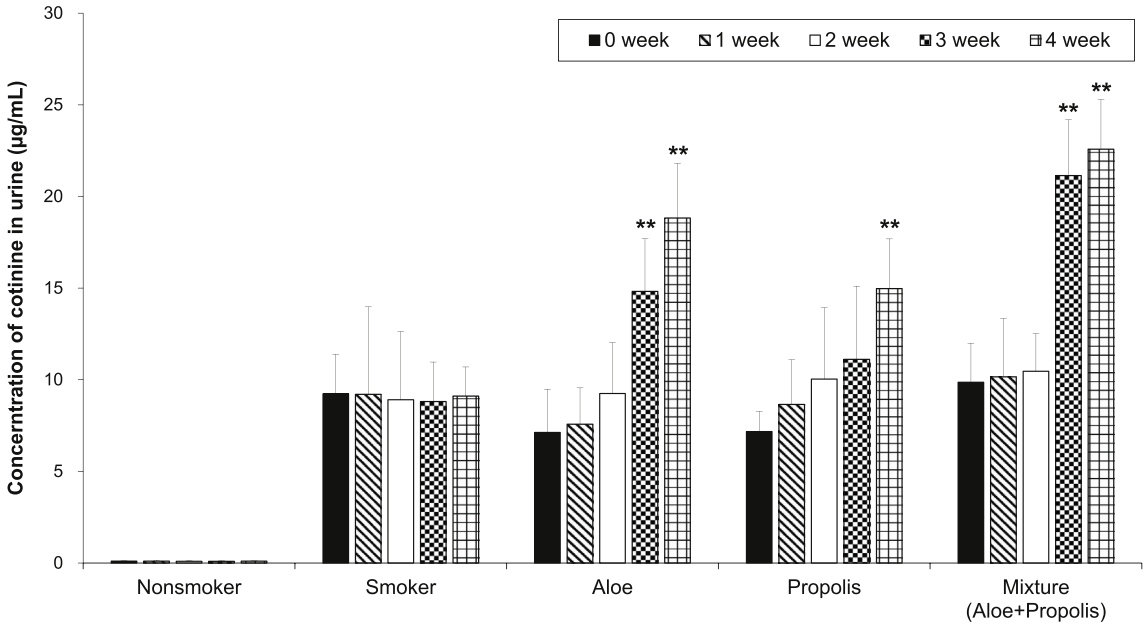

Fig. 4. Effects of aloe polysaccaride, propolis, and mixture of aloe polysaccaride and propolis supplementation on urinary excretion of cotinine in smokers. Aloe polysaccaride; 600 mg/day of, propolis; 600 mg/day, the mixture of aloe polysaccaride (420 mg/day) and propolis (180 mg/day). Values are the mean ± SD of triplicate experiments. Significant differences are represented by asterisks (**$p < 0.01$ compared with the smoker group).

1.19 ± 0.19 mg/dL and 1.01 ± 0.17 mg/dL ($p < 0.05$, smokers versus nonsmokers) (Table 2). Total bilirubin levels in control remained unchanged during the study period, but the mean levels decreased significantly in smokers supplemented with aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis, by 48.82%, 52.59%, and 39.20%, respectively (Fig. 6B). A time-dependent decrease in total bilirubin was observed in the smokers supplemented with aloe polysaccaride, propolis and the mixture of aloe polysaccaride and propolis, and their bilirubin levels were close to those of nonsmokers after 1 week.

4. Discussion

There has been increased interest during recent years in the protective effects of natural plant products on smoking-induced diseases. *Aloe vera* gel contains two fractions of glycoproteins (lectins) and polysaccharides have been claimed for such activity (Javed and Rahman, 2014; Reynolds and Dweck, 1999). The commercial polysaccharide fraction Acemannan™, an acetylated mannan from A. vera, regressed the growth of a murine sarcoma implanted in mice, probably through an immune attack (Peng et al., 1991). Our previous study indicated that aloe polysaccharide exerted anti-carcinogenic effects on

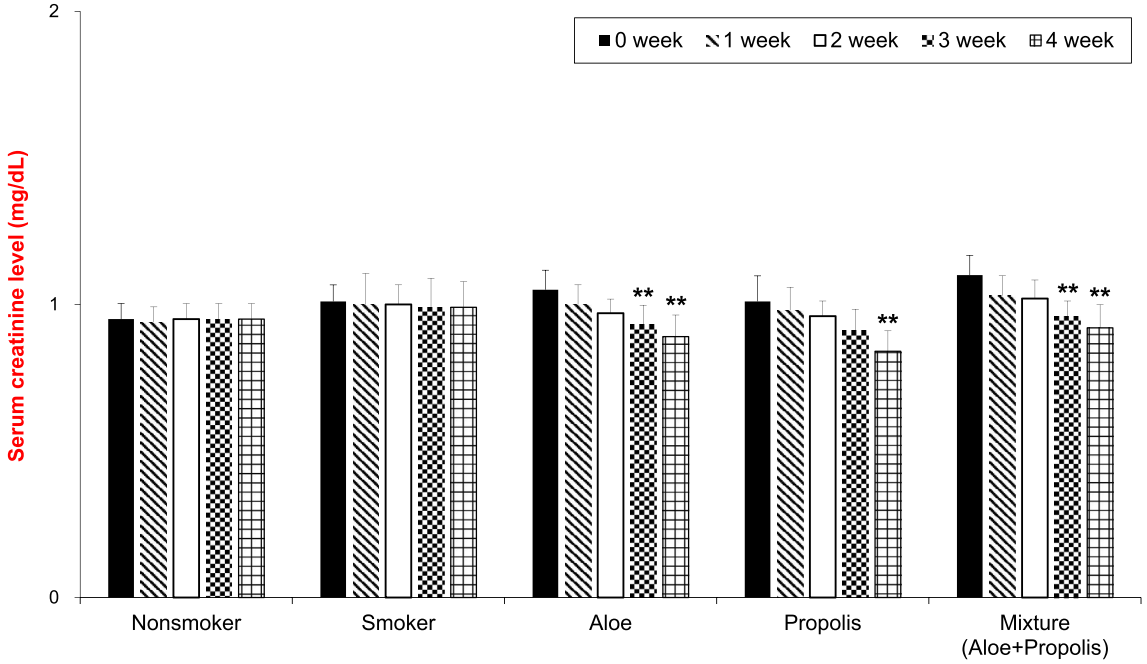

Fig. 5. Effects of aloe polysaccaride, propolis, and mixture of aloe polysaccaride and propolis supplementation on creatinine levels in smokers. Aloe polysaccaride; 600 mg/day of, propolis; 600 mg/day, the mixture of aloe polysaccaride (420 mg/day) and propolis (180 mg/day). Values are the mean ± SD of triplicate experiments. Significant differences are represented by asterisks (**$p < 0.01$ compared with the smoker group).

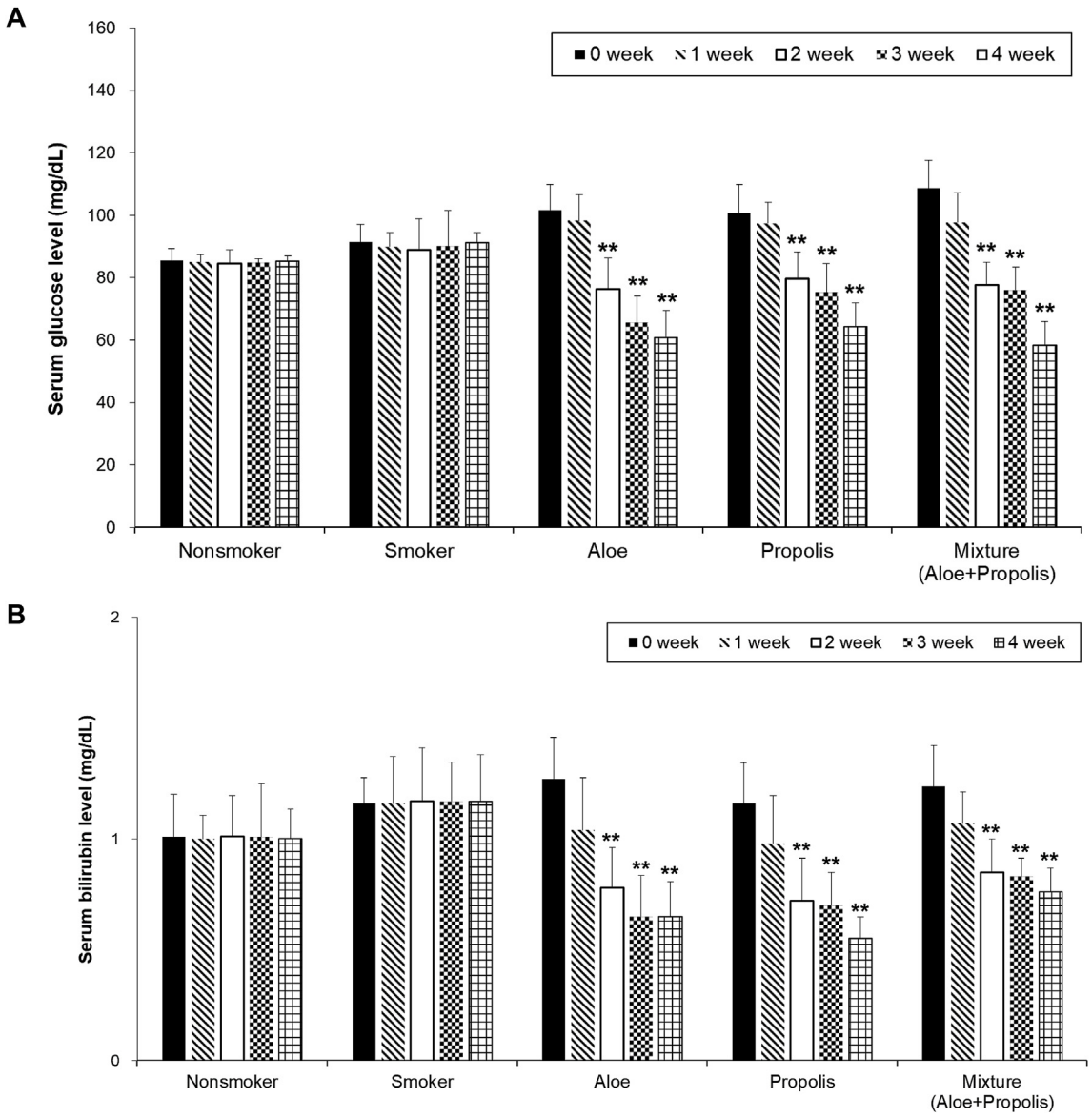

Fig. 6. Effects of aloe polysaccaride, propolis, and mixture of aloe polysaccaride and propolis supplementation on glucose levels (A) and (B) total bilirubin levels in smokers. Aloe polysaccaride; 600 mg/day of, propolis; 600 mg/day, the mixture of aloe polysaccaride (420 mg/day) and propolis (180 mg/day). Values are the mean ± SD of triplicate experiments. Significant differences are represented by asterisks (**$p < 0.01$ compared with the smoker group).

inhibiting BaP–DNA adduct formation by interfering with BaP absorption in vitro (Kim and Lee, 1997). This polysaccharide also inhibited the uptake of BaP and subsequent binding to cellular DNA, and it was shown to have other chemopreventive effects such as antioxidant, antimutagenic, anti-proliferating, and free radical scavenging properties (Chaudhary et al., 2007; Kim et al., 1999; Yoo and Lee, 2005).

Several components isolated from propolis have been reported to have anti-tumor activity against malignant tumor cells in vitro and in vivo (Banskota et al., 1998; Huang et al., 1996; Kimoto et al., 1998; Matsuno et al., 1997). In one recent study, PM-3 (3-[2-dimethyl-8-(3-methyl-2-butenyl)benzopyran]-6-propenoic acid) isolated from Brazilian propolis markedly inhibited the growth of MCF-7 human breast cancer cells by inhibiting cell cycle progression and inducing apoptosis (Luo et al., 2001). Galangin of propolis also showed anti-mutagenic and anti-clastogenic activities against polycyclic aromatic hydrocarbons such as BaP, aflatoxin B_1 and 7,12-dimethyl benzo[a]anthracene (Heo et al., 2001). Brazilian propolis and Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) as an active component of propolis scavenged hydroxyl radicals and inhibited lipid peroxidation, which protected mice from renal damage and carcinogenesis induced by ferric nitrilotriacetate (Kimoto et al., 2000). Caffeic acid phenethyl ester (CAPE) as one of the main components of propolis inhibited some metabolic enzymes including acetylcholinesterase, butyrylcholinesterase, glutathione s-transferase, lactoperoxidase and carbonic anhydrase isoenzymes I, II, IX and XII. Also it scavenged reactive oxgen species and demonstrated putative antioxidant activities (Göçer and Gülçin, 2011; Gülçin et al., 2016).

In the present study, we examined the chemopreventive effects of aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis in smokers based on the excretion of BaP and cotinine. We had previously conducted a bioactivity study of aloe polysaccaride and have designed this study to reveal synergistic effects when combined with other natural products, in addition to the positive effects of aloe polysaccaride. Many studies have been reported that oral administration of aloe combined with bee products including propolis can improve host composition and can reduce tumor progression and oxidative stress. It has been suggested that several compounds of aloe and bee products can exert synergistic effects and exhibit positive bioactive properties

(Kabbash et al., 2018; Tomasin et al., 2015). Based on these studies, we have chosen the doses and frequency of aloe polysaccaride (600 mg/day) according to the recommended dose and frequency for commercial products of aloe, and administered propolis equivalent for comparison. And, the dose of the mixture for aloe polysaccaride and propolis was determined to be 420:180 mg/day considered ADImax (daily maximum intake) as previously reported (Kwack et al., 2014).

Advances in chemical analytical techniques and increased knowledge of genotoxic environmental agents brought the number of carcinogens identified in tobacco smoke to 69 by the year 2000 (Hoffmann et al., 2001; Smith et al., 2003; USDHHS, 2010). BaP was identified as the first chemical carcinogen in cigarette smoke in 1954 (Cooper et al., 1954), and since then it has been associated with various cancers such as gastric carcinomas, squamous cell carcinomas, pulmonary adenomas, leukemia, tumors of the spleen and pancreas, and mammary carcinomas (ACGIH, 2001; Clayton and Clayton, 1994; Hathaway et al., 1996; Lee et al., 1998, 2005) in several experimental animal species. In our study, BaP excretion level was measured in the urine of smokers and nonsmokers. The mean BaP in the smokers' urine was 1.17-fold higher than that in the nonsmokers. After daily supplementation with aloe polysaccaride, propolis, or the mixture of aloe polysaccaride and propolis for 4 weeks, urinary BaP excretion increased in all supplemented groups, with greater urinary excretory effects in the groups supplemented with aloe polysaccaride alone and the mixture of aloe polysaccaride and propolis rather than with propolis alone (Fig. 3). Based on these results, we suggest that aloe polysaccaride might inhibit the cellular uptake of BaP and subsequently enhance elimination by urinary excretion. BaP was measured in this study because APS did not affect the P-450 enzyme system to influence BaP metabolism (Kim and Lee, 1997).

Although nicotine is not known to be carcinogenic, it is the precursor to a group of carcinogens called the tobacco-specific nitrosamines (Hoffmann and Hecht, 1985). Nicotine-derived N-nitrosamines including NNK, NNN, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were recognized as potent chemical carcinogens, and they induced cancer in many organs (in the oral cavity, lung, upper aerodigestive tract, and pancreas) of experimental animals at very low doses (Bartsh and Montesano, 1984; Hecht and Hoffmann, 1988; Mirvish, 1995; Wynder and Hoffmann, 1994). Cotinine is a major metabolite of nicotine, an addictive constituent of cigarette smoke. A significant and positive association was observed between total cotinine in urine or serum and lung cancer risk in smokers (Yuan et al., 2011); urinary cotinine is currently considered the marker of choice for tobacco smoke exposure assessment (Benowitz, 1996; Chiu et al., 2017). The half-life of nicotine in the body is approximately 2–3 h, while that of cotinine is 19 h. Because of the longer half-life measured in urine and the unique source of cotinine is nicotine metabolization in the absence of ambient contamination during sample acquisition, cotinine is a commonly used biomarker of tobacco smoke exposure (Benowitz et al., 1983; Willers et al., 1995). Therefore, we measured urinary cotinine excretion as a marker for urinary excretion of nicotine in smokers. After daily supplementation with aloe polysaccaride, propolis, or and the mixture of aloe polysaccaride and propolis for 4 weeks, the urinary cotinine excretion had increased in all supplemented groups. Aloe polysaccaride was more effective than propolis, and the mixture of aloe polysaccaride and propolis was the most effective at enhancing urinary excretion of cotinine (Fig. 4). These results suggest that supplementation with aloe polysaccaride, propolis, or the mixture of the two could reduce cancer risk or other diseases caused by carcinogens associated with smoking by enhancing urinary excretion of BaP and cotinine.

Serum creatinine level is an index of renal function and renal excretion ability. All groups participating in the experiment were observed to be in the normal range of serum creatinine (0.6–1.5 mg/dL) (Guyton and Hall, 2006). However, the mean level of creatinine in serum from smokers was 1.07-fold higher than that in the nonsmokers ($p < 0.05$). After 4 weeks supplementation with aloe polysaccaride, propolis, or the mixture of aloe polysaccaride and propolis, creatinine levels in the sera decreased in a time-dependent manner. The decreased serum creatinine levels were the same in both the aloe polysaccaride alone and propolis alone treatment groups, but the levels decreased the most effective in the aloe polysaccaride and propolis treatment group (Fig. 5). These results suggest that marked decrease in serum creatinine levels may be related to increase urinary excretion by supplementation of aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis.

In the past 30 years, there have been reports on the anti-diabetic activity of aloe extracts (Agarwal, 1985; Ajabnoor, 1990; Beppu et al., 1993; Bunyapraphatsara et al., 1995, 1996; Ghannam et al., 1986; Hikino et al., 1986; Yongchaiyudha et al., 1996). Some flavonoids such as (−)epicatechin, quercetin, naringenin, and chrysin, a group of naturally occurring pigments have been also claimed to possess anti-diabetic activities (Hii and Howell, 1985). In general, smoking was reported to produce insulin resistance associated with the risk of type 2 diabetes in smokers (Chiolero et al., 2008; Eliasson et al., 1994; Facchini et al., 1992). In the present study, the smokers' mean serum glucose level was 1.12-fold higher than that of the nonsmokers ($p < 0.05$). The aloe polysaccaride, propolis, and aloe polysaccaride and propolis combined supplements were all effective in decreasing serum glucose levels, with the highest decrease observed in the mixture of aloe polysaccaride and propolis treatment group (Fig. 6A).

A. arborescens inhibited development of preneoplastic focal liver lesions in rats challenged with carcinogen, diethylnitrosamine, that acted on the liver (Tsuda et al., 1993; Yagi et al., 1977). Aloe-emodin appeared to have some protective effect not only against hepatocyte death but also on the inflammatory response subsequent to lipid peroxidation in the liver (Arosio et al., 2000). Alcoholic extract of tropical Brazilian propolis showed hepatoprotective activity on D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes that was mainly due to phenolic compounds including flavonoids (Banskota et al., 2000, 2001). In the present study, the mean level of total bilirubin in serum from smokers was also approximately 1.2-fold higher than that from the nonsmokers. Time-dependent decreases in total serum bilirubin level was observed in all supplemented groups after daily supplementation with aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis for 4 weeks.

In summary, these data demonstrated that the urinary excretion of BaP and cotinine was significantly enhanced by aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis supplementation. In particular, aloe polysaccaride treatment alone was more effective on the urinary BaP and cotinine excretion than was propolis treatment at the same dose (600 mg/day), but the mixture of the two exerted a similar effect to that of the aloe polysaccaride alone, even though it was given at a lower dose. During the study period, blood biochemical parameters (creatinine, glucose, and total bilirubin) also decreased in smokers by treatment with aloe polysaccaride, propolis, and the mixture of aloe polysaccaride and propolis. Further studies are needed to demonstrate the use of this supplement as a clinical and other disease-related agent and as an effective detoxifying agents, but we suggested that aloe polysaccaride, propolis and the mixture of aloe polysaccaride and propolis could be potential candidates for the chemopreventive agent by detoxifying approach for eliminating BaP and cotinine in the urine.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Conflicts of interest

The authors declare that there are no conflicts of interest.

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