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Sources of Polycyclic Aromatic Hydrocarbon Exposure in Non-Occupationally Exposed Koreans

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Urinary 1-hydroxypyrene (1-OHP), an exposure biomarker for polycyclic aromatic hydrocarbons (PAHs), was used to identify potential sources of PAH exposure for 660 Koreans who were not occupationally exposed to PAHs (65% male; 35% female; mean age, 36.5 ± 11.1 years). In this study, 74% of subjects had detectable levels of urinary 1-OHP, with a concentration range of 0.001–3.796 μg/L (median, 0.079 μg/L). A backward elimination was conducted: five variables were selected with a significance level for removal of $P \leq 0.1$. The results of this study showed that residence in areas with relatively poor environmental conditions (Seoul and Suwon) was strongly associated with high concentrations of urinary 1-OHP ($P = 0.007$), while consumption of fried chicken and length of time spent outdoors had marginal positive associations with urinary 1-OHP levels ($P = 0.06$ and $P = 0.09$, respectively). Compared with the above three factors, tobacco smoking and urinary cotinine levels were poorly associated with urinary 1-OHP ($P = 0.16$ and 0.23, respectively). Pear consumption had an inverse association with urinary 1-OHP levels ($P < 0.01$). Individual variations in urinary 1-OHP concentrations were evaluated by considering the subjects' age, sex, and genetic polymorphisms in enzymes involved in the metabolism of PAHs. Among the individual variations, *GSTT1*-present subjects showed higher 1-OHP levels than *GSTT1*-absent subjects in cities having 10-μm particulate matter (PM_{10}) levels and population density lower than those of Seoul and Suwon ($P < 0.05$). These epidemiological results suggest that the above factors that should be considered in preventing PAH exposure. Environ. Mol. Mutagen. 42:250–257, 2003. © 2003 Wiley-Liss, Inc.

Key words: polycyclic aromatic hydrocarbons; 1-hydroxypyrene; exposure route; biomarker; genetic polymorphism

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), which are environmental carcinogens formed by the incomplete combustion of organic material, have been a major focus of research [Yang et al., 1999]. Humans may be exposed to PAHs through the air, soil, food, their occupation, and their lifestyle [Jongeneelen, 1994]. To prevent exposure to PAHs effectively, it is necessary to identify the main sources of PAH exposure for the general population, particularly for those who are not occupationally exposed to PAHs. However, very few studies have clarified the main sources of PAH exposure. These types of studies are complicated by the varying bioavailability of PAHs from different exposure pathways (e.g., via inhalation, food consumption, skin contact), as well as individual differences in susceptibility to PAH exposure. Recently, genetic differences in the enzymes important for the metabolism of PAHs have been evaluated as susceptibility biomarkers for PAH biomonitoring [Yang et al., 1999; Nerurkar et al., 2000]. However, it is difficult to observe the effects of genetic differences on

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biological monitoring without considering all the major exposure routes of PAHs.

Urinary 1-hydroxypyrene (1-OHP) has been used routinely as a biomarker to estimate the total exposure level of PAHs [Jongeneelen, 1994]. Using this biomarker, we conducted an integrated study to clarify the main exposure routes of PAHs for Koreans and the factors that effect PAH exposure. The variables that were considered included indoor/outdoor air pollution and environmental tobacco smoking to assess inhalation exposure to PAHs, as well as drinking water, beverages, and food intake to assess gastrointestinal tract exposure to PAHs. Information on these variables was collected to reflect both the daily life of the subjects and the last 24 hr of exposure. Modulating factors that affect the strength of PAH exposure were also investigated. These included the presence of incinerators and traffic jams in the vicinity of housing areas or offices, cooking and heating fuels, and genetic polymorphisms in PAH-metabolic enzymes, e.g., cytochrome P-450 (CYP) 1A1, CYP1B1, and glutathione-*S*-transferase (GST) M1 and GSTT1. The study subjects comprised a nationwide population obtained from five provinces of Korea, i.e., Seoul, Gyeonggi, Chungnam, Chungbuk, and Kyongbuk (N = 660). Investigation of the simultaneous and multiple exposures to PAHs and the large sample size in this study should provide a reliable assessment of the sources of PAH exposure in the general Korean population that is not occupationally exposed to PAHs.

MATERIALS AND METHODS

Subjects, Questionnaire, and Sample Collection

The 660 Koreans (418 males; 242 females; age, 36.5 ± 11.1 years) who were included in this study lived in Seoul (northern Republic of Korea), Suwon in Gyeonggi province (northern Republic of Korea), Daejeon in Chungnam (central Republic of Korea), Chungju in Chungbuk (central Republic of Korea), and in Gyeongju/Pohang (southern Republic of Korea) (Fig. 1). All subjects provided informed consent. The subjects completed a questionnaire for PAH exposure that was composed of questions covering five major categories: air/soil pollution, time/activity, lifestyle, food consumption, and water (Appendix). None of the subjects was occupationally exposed to PAHs. Blood and spot urine samples were obtained from the subjects before breakfast (April to November 2000). Data on the levels of 10-μm particulate matter (PM_{10}) for the cities were obtained from monthly air pollution reports by the Ministry of Environment, Korea (available at: http://www.me.go.kr) for the period during which urine samples were collected.

Analyses of Urinary 1-OHP, Creatinine, and Cotinine

Urinary 1-OHP and creatinine and the modification index of urinary 1-OHP were determined using the methods of Hara et al. [1997] and Ogata and Taguchi [1987], with minor modification. The high-performance liquid chromatography (HPLC) system used to quantify 1-OHP included 515 HPLC pumps, an automated gradient controller, a 717-plus Autosampler, and a 474 Scanning Fluorescence Detector (excitation, 242 nm; emission, 388 nm)(all from Waters, Milford, MA). For creatinine analysis, a Waters™ 486 Tunable Absorbance Detector (225 nm) was used instead of the fluorescence detector. The HPLC parameters were as follows: column, TOSOH TSK-gel ODS-80TM (4.5 mm × 150 mm); mobile phase for 1-OHP, 60% acetonitrile in water at a flow rate of 1.0 ml/min; mobile phase for creatinine, a mixture of water/acetonitrile (85/15; pH, 3.3), containing 20 mM of KH_2PO_4 and 3 mM of 1-octanesulfonic acid, at a flow rate of 0.4 ml/min.

The urinary cotinine level, a biomarker of tobacco smoking, was analyzed using a previously reported method [Yang et al., 2001].

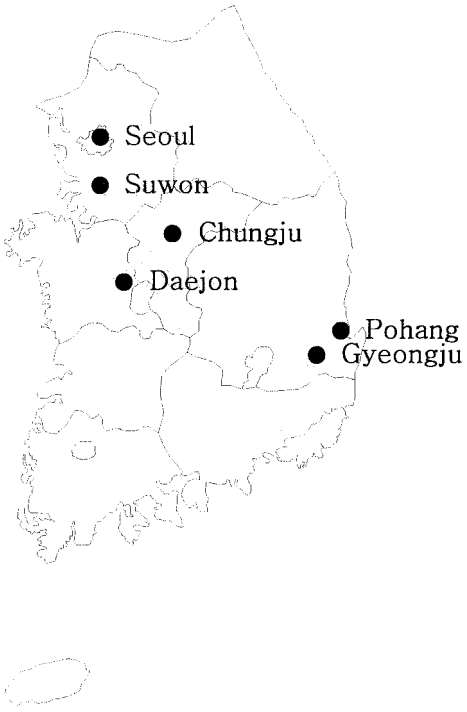

Fig. 1. Sampling areas in South Korea.

Determination of Genotypes

To minimize the effects of individual variation in urinary 1-OHP levels due to differences in metabolism efficiency, we determined genetic polymorphisms for *CYP1A1*, *CYP1B1*, *GSTM1*, and *GSTT1*, which are suspected to affect the urinary 1-OHP levels. Genomic DNA was isolated from the buffy coat fraction of each blood sample using a DNA isolation kit provided by Promega (Madison, WI). For determination of genotypes, the single base extension method and PCR-RFLP were employed as described previously [Chen et al., 1996; Katoh et al., 1998; Yang et al., 2003].

Chemicals

1-OHP was obtained from Aldrich (Milwaukee, WI). All other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan).

Statistical Analysis

Chi-square tests were performed to screen for associations between the 1-OHP groups (low and high) and the categorical covariates before estimating a logistic regression model. One-way analyses of variance were used for the continuous covariates. This study contains various covariates, which were expected to correlate with each other, for example, tobacco

TABLE I. Characteristics of Subjects Grouped by Place of Residence

City	No. of subjects	PM_{10} during sampling seasons[a] ($\mu g/m^3$)	Population density[b] (persons/km^2)	Sex (% males)	BMI (kg/m^2)	Smokers (% subjects)	Alcohol drinkers (% subjects)
Seoul	187	71	17,131.7	62.0	23.1 ± 3.1	46.4	77.7
Suwon	72	66	7,853.3	55.6	21.9 ± 3.0	20.8	77.8
Daejon/Chungju	239	54	2575.8	60.3	22.5 ± 2.7	33.6	73.8
Gyeongju/Pohang	162	42	220.1	72.8	22.9 ± 2.8	45.9	73.6
Total	660	—	—	63.3	22.8 ± 2.9	38.7	74.5

PM, particulate matter; BMI, body mass index.
[a]Particulate matter with a mass median aerodynamic diameter of <10 μm; based on monthly reports of Ministry of Environment, Korea.
[b]Based on data from the Korea National Statistical Office [2000].

smoking, and alcohol drinking. Furthermore, the correlations among the covariates can easily affect the regression estimates by the covariates that were in the regression model. Therefore, a backward elimination method with a $P = 0.1$ level of significance was used to select the variables in the model. The Hosmer and Lemeshow statistic was measured to support this model's adequacy for the data. The logistic regression model for the odds of the higher 1-OHP group was constructed using SAS 8.1 software (SAS Institute, Cary, NC).

RESULTS

Characteristics of Subjects

Table I shows characteristics of the study subjects in terms of their place of residence. The monthly air pollution reports of PM_{10} from the Ministry of Environment, Korea (available at: http://www.me.go.kr), which were used as a general measure of air quality, indicated that PM_{10} levels were higher in Seoul and Suwon than the other cities ($P = 0.02$). Thus, the PM_{10} data and the population density in Seoul and Suwon suggest that the environmental conditions (air, soil and water) in these cities are poor compared with the other cities. There were relatively lower percentages of males and smokers among the subjects in Suwon than those in the other cities. Body mass index (BMI) and the frequency of alcohol drinkers were similar among the subjects from all the cities; the mean frequency of alcohol drinking for all subjects was 1.8 ± 1.1 times/week. In addition, the average years of education for the subjects was ~4 years greater (14.7 years) than the average for all Koreans (10.1 years [Korean National Statistical Office, 2000]), and the subjects from Suwon had a somewhat higher level of education than the subjects from the other cities.

Table II shows the residential and traffic environment of the subjects. Most subjects lived in urban areas. Liquefied natural gas (LNG) boilers and kerosene heaters were the principal house-heating systems. As LNG was used as a cooking fuel by 96.5% of the subjects, cooking fuel was considered identical for all subjects. Gasoline, LPG (liquefied petroleum gas), and diesel, in that order, were the most commonly used fuels to power motor vehicles. More than one-half of the subjects experienced heavy traffic or traffic jams around their place of residence.

TABLE II. Residential and Traffic Environment of the Subjects

Variables	% of subjects
Housing	
Urban	83.1
Urban commercial area	4.8
Industrial area	1.4
Rural area	10.8
Heating fuel	
LNG	60.9
Kerosene	36.1
Coal	0.2
Electricity	0.8
Other	2.0
Cooking fuel	
LNG	96.5
Kerosene	1.3
Firewood	0.3
Electricity	1.3
Other	0.5
Automobile fuel[a]	
Gasoline	79.0
Diesel	8.8
LPG	9.6
Other	2.6
Traffic[b]	
Heavy with traffic jams	24.4
Heavy but no jams	53.7
Not heavy and no jams	17.4
Rare traffic	4.4

LNG, liquefied natural gas; LPG, liquefied petroleum gas.
[a]For automobile owners, n = 554; for other catagories, n = 660.
[b]During rush hour, between home and workplace.

Responses to the food questions of the questionnaire indicated that the subjects within the 24 hr before sampling consumed 159 food items.

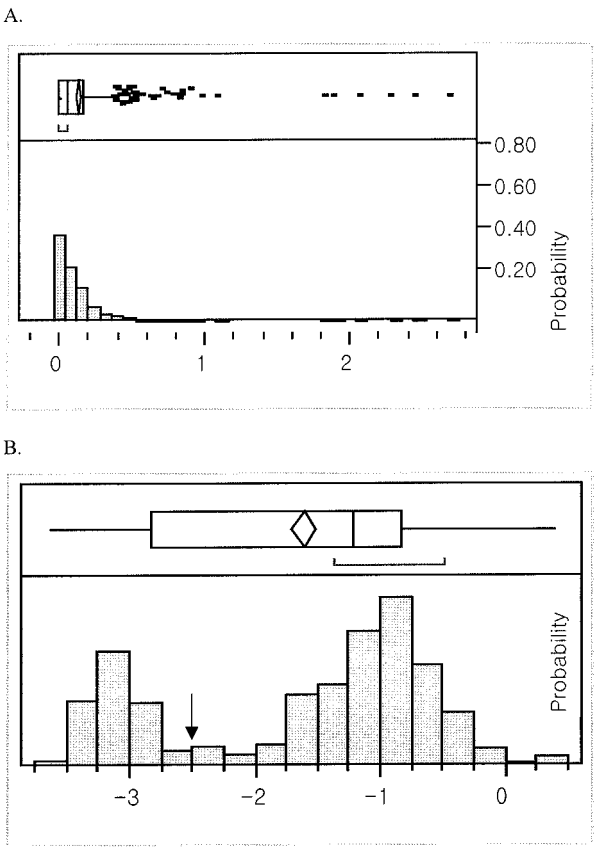

Fig. 2. Distribution of urinary 1-hydroxypyrene (1-OHP). Upper parts of A and B show an outlier box plot with the square in the box showing the interquartile range. **A:** Distribution of urinary 1-OHP (μmol/mol creatinine). **B:** Distribution of log of urinary 1-OHP (μmol/mol creatinine); ↓, demarcation (−2.7).

Urinary 1-OHP and Cotinine Level

Using 1-OHP-spiked urine (0.125, 0.25, 0.5, 1, and 2 ng of 1-OHP/ml of urine), a linear regression between the HPLC 1-OHP-peak area and the amounts of spiked 1-OHP was obtained ($P < 0.01$). The coefficient of the variation (CV) was 6.5–19.5% (N = 5). The detection limit for urinary 1-OHP was 0.001 μg/L. A total of 26% of subjects had undetectable levels of urinary 1-OHP; the urinary 1-OHP levels in the remaining 74% of subjects ranged from 0.001 to 3.796 μg/L (median, 0.079 μg/L). For the statistical analyses, subjects with undetectable levels of 1-OHP were assigned a value of one-half of the minimum detectable concentration of urinary 1-OHP, i.e., 0.0005 μg/L. After creatinine correction, the urinary 1-OHP values did not have a normal distribution (Fig. 2A: median of urinary 1-OHP, 0.012 μmol/mol creatinine). A geometric transformation of urinary 1-OHP values was conducted, which produced a bimodal distribution (Fig. 2B). Using a demarcation point of −2.7, i.e., 0.002 μmole/mole creatinine, we divided all subjects into two groups, a low and a high 1-OHP group. A logistic regression model was developed for the odds of being in the high 1-OHP group. This model was used to identify sources of PAH exposure.

In this study, 6% of subjects had undetectable levels of urinary cotinine; concentrations of urinary cotinine in the remaining 94% of subjects ranged from 0.016 to 35.953 mg/L (median, 0.358 mg/L). When subjects with undetectable levels of cotinine were assigned a value of half the minimum detectable urinary cotinine concentration, i.e., 0.008 mg/L, smokers showed significantly higher urinary cotinine levels than nonsmokers (geometric mean (GM) [geometric standard deviation (GSD)], 0.549 [0.006] mg/L vs. 0.034 [0.004]; $P < 0.01$). Therefore, urinary cotinine reflected environmental tobacco smoking in the subjects. However, there was no significant association between urinary 1-OHP and cotinine levels ($P = 0.23$).

Exposure Routes of PAHs

A backward elimination was conducted on possible exposure routes for PAHs, including air, soil, water, lifestyle, and food intake; five variables were selected with a *P*-value of ≤ 0.1, i.e., city of residence, time spent outdoors, consumption of fried chicken, the consumption of pears, and tobacco smoking. The interaction terms of the five selected variables were not significant. Table III shows the results of the logistical regression using the five variables. Among the variables, residence in Seoul and Suwon, cities predicted to have poor environments, showed a significant positive association with the high 1-OHP group ($P = 0.007$). Fried chicken consumption ($P = 0.06$) and time spent outdoors ($P = 0.09$) were marginally associated with 1-OHP level, while tobacco smoking was not significantly associated ($P = 0.16$).

In contrast, pear consumption significantly reduced the odds for the high 1-OHP group. Therefore, pear consumption appears to prevent the bioproduction of 1-OHP from PAHs.

Effect of Individual Difference Factors on Urinary 1-OHP

Considering the above exposure routes, the effects of the individual differences on urinary 1-OHP levels were investigated. The individual differences considered were age, sex, BMI, and genetic polymorphisms in *CYP1A1*, *CYP1B1*, *GSTM1*, and *GSTT1* (Table IV). After adjusting for the main PAH exposure source, i.e., city of residence, only the *GSTT1* genetic polymorphism was borderline significantly associated with urinary 1-OHP levels: *GSTT1*-present subjects had slightly higher 1-OHP levels than the *GSTT1*-absent subjects ($P = 0.06$). In particular, for residents of cities other than Seoul/Suwon, this trend was significant (GM [GSD] of urinary 1-OHP in the *GSTT1*-present subjects and the *GSTT1*-absent subjects, 0.022 μg/L [0.001] vs. 0.012 μg/L [0.001], respectively: $P < 0.05$).

TABLE III. Factors Associated With Urinary 1-OHP Levels

Parameter	Distribution (n)	1-OHP levels (μg/L) GM (GSD)	1-OHP levels (μmol/mol creatinine) GM (GSD)	Adjusted[a] *P*-value	Adjusted[a] OR	Adjusted[a] 95% CI
City of residence	Seoul/Suwon (259)	0.026 (0.001)	0.034 (0.001)	0.007	1.664**	(1.164–2.415)
	Other cities (401)	0.017 (0.001)	0.025 (0.001)			
Time outdoors	1.29 hr ± 2.58	0.020 (0.001)	0.029 (0.001)	0.093	1.068*	(0.989–1.153)
Fried-chicken consumption[b]	Consumers (30)	0.089 (0.001)	0.101 (0.001)	0.062	2.583*	(0.954–6.994)
	Nonconsumers (630)	0.019 (0.001)	0.027 (0.001)			
Pear consumption[b]	Consumers (36)	0.005 (0.001)	0.006 (0.001)	0.000	0.247**	(0.117–0.518)
	Nonconsumers (624)	0.021 (0.001)	0.030 (0.001)			
Smoking	Smokers (255)	0.025 (0.001)	0.031 (0.001)	0.164	1.303	(0.897–1.896)
	Nonsmokers (405)	0.018 (0.001)	0.028 (0.001)			

1-OHP, 1-hydroxypyrene; GM, geometric mean; GSD, geometric standard deviation; OR, odds ratio; CI, confidence interval.
[a]Adjusted for other parameters: analysis of maximum likelihood estimates: OR for high 1-OHP group (≥0.002 μmol/mol creatinine.
[b]Consumption of food within 24 hr before urine sampling
*$P < 0.1$.
**Statistically significant, $P < 0.05$.

TABLE IV. Distribution of Genotypes

Polymorphism	Allele frequency		% in all subjects		
CYP1BI					
R48G (C → G)	*C*	*G*	*C/C*	*C/G*	*G/G*
	0.82	0.18	70	25	5
A119S (G → T)	*G*	*T*	*G/G*	*G/T*	*T/T*
	0.83	0.17	69	29	2
L432V (C → G)	*C*	*G*	*C/C*	*C/G*	*G/G*
	0.90	0.10	81	18	1
N453S (A → G)	*A*	*G*	*A/A*	*A/G*	*G/G*
	1.00	0.00	99	1	0
CYP1A1					
I462V (A → G)	*A*	*G*	*A/A*	*A/G*	*G/G*
	0.77	0.23	60	35	5
GSTM1					
present or absent			Present		Absent
			57		43
GSTT1					
present or absent			Present		Absent
			51		49

DISCUSSION

Urinary 1-OHP Levels in the Subjects

As few published studies indicate when the urine specimens were collected and how they handled subjects with undetectable levels of 1-OHP, it is difficult to compare the degree of PAH exposure in our Korean subjects with those found in other studies. Focusing on measurements made on people who were not occupationally exposed to PAHs, the average urinary 1-OHP levels found in other studies were 0.22 μg/L in Japanese (N = 527) [Kawamoto et al., 1997]; ~0.3 nmol/12 hr in Japanese/Caucasians/Hawaiians (N = 188] [Nerurkar et al., 2000]; 0.04 μmol/mol creatinine in Korean college students (N = 128) [Nan et al., 2001]; and 0.2 μg/24 hr in Germans (N = 69) [Scherer et al., 2000]. Excluding the subjects with undetectable levels of 1-OHP, we found a median concentration of 0.079 μg/L of urinary 1-OHP for our subjects (N = 499). Because people who are occupationally exposed to PAHs have very high urinary 1-OHP levels, e.g., 9 mg/g creatinine [Wu et al., 1998] compared with non-occupationally exposed people, the level of urinary 1-OHP in our Korean subjects was consistent with people who are not occupationally exposed to PAHs.

Exposure Routes of PAHs

In this study, subjects living in a city with a relatively poor environment had significantly higher odds for being in the high 1-OHP group (Table III). These results provide evidence that the total environment of a residential area, including the air, soil, and water, is a source of PAHs. In addition, the time spent outdoors was marginally associated with urinary 1-OHP concentration (Table III). Thus, air pollution may be the main PAH exposure source for the Korean population. However, the urinary 1-OHP level was not associated with a single questionnaire item, e.g., the degree of urbanization such as residence in rural, urban or industrial areas; the fuel used for house/office heating, cooking, or transportation; the extent of traffic jams during the rush hour; or the distance between the residence and roads. Even though there was a simple correlation between the urinary 1-OHP levels and tobacco smoking (smoker or nonsmoker), the effects of the smoking status on urinary 1-OHP level were weaker than those of the city of resi-

dence, and no significant association could be found between the urinary 1-OHP and cotinine level, which is an exposure biomarker for environmental tobacco smoking. Furthermore, the urinary cotinine levels were associated with the urinary 1-OHP levels only in smokers, and not in nonsmokers or in the low 1-OHP group. Therefore, routes other than environmental tobacco smoking are the major sources of PAH exposure sources among nonsmokers and low-PAH-exposure people.

Besides the city of residence, the consumption of fried chicken was marginally associated with the high 1-OHP group (Table III). Our questionnaire included several chicken dishes besides fried chicken, e.g., Sam-gye-tang (Ginseng chicken soup), and Dak-Jjim (smothered chicken), but these were not positively associated with urinary 1-OHP levels. Therefore, it is not chicken itself but the method of cooking the chicken that is important for producing PAHs. Charbroiling and grilling of chicken produce PAHs [McDonald et al., 2003], and cooking with oil at high temperatures has been reported to be one of the largest contributors to the daily PAH intake [de Vos et al., 1990]. Therefore, the present results suggest that cooking with hot oil contributes to PAH exposure.

Factors That Reduce PAH Exposure

Solid fruits such as pears and apples have shown protective effects for chronic obstructive pulmonary disease (COPD). The protection is believed to be due to the anti-inflammatory and antioxidative properties of high levels of flavonoids in the fruits [Tabak et al., 2001]. Furthermore, some flavonoids reduce 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-mediated processes, such as *CYP1A* induction, by functioning as antagonists of the aryl hydrocarbon receptor (AHR) [Reiners et al., 1999]. In this study, pear intake significantly reduced the odds of being in the high 1-OHP group (Table III). Since air pollutants including PAHs, are risk factors for both COPD and lung cancer [Walter et al., 2000; Cohen, 2000], our observation that pear intake reduced urinary 1-OHP levels suggests the chemopreventive potential of pear consumption via reduction of bioactive intermediates of PAHs. No association was found between the urinary 1-OHP levels and the consumption of several foods that contain high levels of benzo[*a*]pyrene [Kazerouni et al., 2002], such as steak, grilled fish, and beef/pork barbecue. The Korean pear (*Purus fructus*) is known as a traditional medicine that promotes digestion, and reduces fever and respiratory tract diseases (MediMedia Korea, available at: http://www.kimsonline.co.kr/tradimed.htrad). Koreans usually consume pears as dessert after meals of greasy foods such as pork or beef barbecue. Therefore, Korean dietary practices may attenuate the effects of greasy foods on urinary 1-OHP levels. In addition, accumulation of PAHs was significantly lower on pear leaves than other similar-sized leaves, such as linden leaves [Jouraeva et al., 2002], and characteristics of the pear plant, such as the presence of epicuticular waxes, may also contribute to low PAH exposure via pear consumption.

Susceptibility Biomarkers for Exposure Assessment of PAHs

In this study, several individual variation factors that might affect urinary 1-OHP levels were examined. Considering the main PAH-exposure source, city of residence, the *GSTT1* polymorphism showed a trend indicating that *GSTT1*-present individuals in the low 1-OHP cities have higher urinary 1-OHP levels than *GSTT1*-deficient individuals. The low-1-OHP-cities, Daejeon/Chungu and Gyeonju/Pohang, have better environments than Seoul or Suwon based on PM_{10} levels and population density (Table I). GSTT1 shows different metabolic effects resulting in both bioactivation and detoxification, depending on the xenobiotic [Their et al., 1996]. Therefore, GSTT1 may promote mutation or carcinogenesis by some chemicals. Although the role of GSTT1 on carcinogenic PAH metabolism is unclear, our epidemiological study suggests that genetic differences have their strongest effects under conditions of low environmental pollution. Gene-gene interactions and gene-environment interaction have been the subject of extensive research. The effects of pure genetic factors on the exposure responses, such as cancer, may be weak compared with environmental effects. Therefore, genetic variations, susceptibility biomarkers for an exposure assessment, may be especially important under low PAH-exposure conditions.

In conclusion, our epidemiological results suggest several factors that should be considered for the prevention of PAH exposure. Residential areas with poor environments were associated with high urinary 1-OHP levels, with the consumption of fried chicken and the time spent outdoors being possible contributing factors. Compared with these factors, tobacco smoking had a weak association with urinary 1-OHP levels, while pear consumption reduced the odds for a high level of urinary 1-OHP. Finally, among the individual difference factors, *GSTT1* genetic polymorphism showed an association with the urinary 1-OHP level in cities with low levels of pollution.

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APPENDIX. QUESTIONNAIRE USED TO ESTIMATE EXPOSURE TO PAHS

Exposure routes	No. of questions	Main content of questionnaire
Air/Soil	40	Environment of residence and work place: Urban or rural area; fuel for automobile (e.g., diesel, gasoline, LNG) and for heating/cooling and cooking (coal, petroleum, electric, solar energy, other); ventilation method; traffic on the road between office and house; distance between house and the nearest road; size of the road; specific facilities near residence and work place (incinerator, waste-reclaimed land, factory, gas station, bus or taxi stops, other); smokers among family, friends or colleagues (side steam); house repair or building in a recent month; reasons for moving home or job change, etc.
Time-activity pattern	31	Time spent indoors/outdoors; kinds of indoor environments (houses, offices, factories, schools, restaurants/cafés/karaoke bars/night clubs/beer halls, stores, banks, hospitals, church, theater, greenhouse) and outdoor environments (yards, parks, markets, other); kinds of transportation and time spent for transportation (bicycle, subway, automobile, bus, trucks, other); time spent waiting for transportation, walking, other
Lifestyle	22	Job characteristics; income; tobacco smoking; alcohol consumption; exercise; diet; intake of oriental medicine, multivitamins and dietary supplements; leisure time activities; education; age, sex, body weight, height
Food consumption	214	Food intake in daily life and within 24 hr before urine sampling: Foods usually consumed among 13 categories of food (161 items) in the general Korean population, i.e., 10 kinds of boiled rice (Bap), 11 kinds of noodle (Myon), 3 kinds of bread, 18 kinds of soup (Guk or Tang), 12 kinds of stews or casseroles (Jji-gae and Jeon-gol), 38 kinds of broiled and fried dishes (Gui and Jeon), 14 kinds of smothered and soy-glazed dishes (Jjim and Jorim), 9 kinds of pickle (Kimchis), 5 kinds of salted fish (Jeot-gal), 6 kinds of vegetable dishes (Na-mul), 13 kinds of vegetable salads, 7 kinds of sauces (including various fermented sauces such as Doen-jang), 9 kinds of fruit, 3 kinds of fast food (e.g., hamburgers); places where food consumed; washing hands before eating; preferred food items; type of cooking (e.g., raw, boiled, fried) and degree of doneness of fried meat/fish (rare, medium, well, and very well done); frequency of consuming vegetables
Water/fluid	25	Sources of drinking water (tap water, filtered water, mineral water, commercial water, underground water, other); volumes consumed daily of drinking water, milk, liquid yogurts, coffee, tea, soft drinks, other
Total:	332	

LNG, liquefied natural gas.

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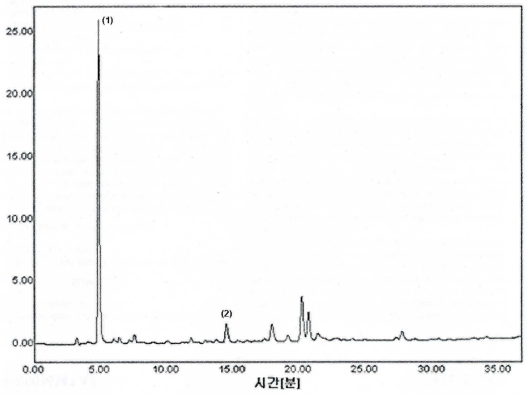
전체 청구항 수 : 총 2 항

(54) 배 유래 활성성분을 포함하는 숙취예방 및 해소용 조성물

(57) 요 약

본 발명은 한국산 배(pears)를 이용한 숙취예방 및 해소용 조성물에 관한 것으로, 특히 한국산 배에서 유래된 활성성분을 포함하는 숙취예방 및 해소용 조성물이다. 본 발명에서는 한국산 배에서 숙취해독작용을 나타내는 활성성분이 배 껍질의 수용성 부분에 많이 존재하는 알부틴임을 확인한다.

대 표 도 - 도2



(1) arbutin, (2) (+)-catechin.

특허청구의 범위

청구항 1

배 껍질의 수용성(peel water soluble) 분획을 유효성분으로 하는 숙취예방 및 해소용 조성물.

청구항 2

배 유래 알부틴(arbutin)을 유효성분으로 하는 숙취예방 및 해소용 조성물.

명 세 서

발명의 상세한 설명

기 술 분 야

[0001] 본 발명은 숙취해소용 조성물에 관한 것으로, 특히 한국산 배(pears)를 이용한 숙취예방 및 해소용 조성물에 관한 것이다.

배 경 기 술

[0002] 음주로 인해 야기되는 개인의 건강 및 사회 전반에 걸친 폐해는 보건 의료뿐 아니라 국가차원에서 다루어져야 할 주요 과제로 대두되었다. 일반적으로 음주는 상부 소화-호흡기관 (upper aerodigestive tract, 구강, 인두, 하 인두, 후두, 식도)암, 직장암, 유방암 등의 발생빈도를 증가시키고 간암화 (hepatocarcinogenesis)의 주요한 병인학적 요인이다 (Poschl et al, 2004). 뿐만 아니라, 음주로 인한 사회경제적 손실규모가 대부분의 국가에서 GDP 대비 약 0.5-2.7% 에 이르는 것으로 보고되고 있고 우리나라의 경우, 2000년 한해 음주로 인한 생산성 감소 및 손실액이 6조 2,845 억 원으로 추정되고 있다 (정우진 등, 2006).

[0003] 최근에는 유전적으로 결정된 알코올 관련 효소의 유전자 다형으로 인해 나타나는 에탄올 및 아세트알데하이드 대사의 변화가 만성알코올 섭취 후 나타나는 장기 손상의 취약성뿐 아니라 급성, 만성 알코올 섭취에 따른 반응, 알코올 남용 등의 개인 및 인종간의 차이를 결정한다고 보고하고 있다. 특히, 알코올 섭취에 따른 홍조반응, 간 질환과 미토콘드리아의 ALDH(aldehyde dehydrogenase) 2의 유전자 다형이 높은 상관관계를 가지고 있다. ADH(Alcohol dehydrogenase)가 알코올 제거에 중요한 역할을 하지만 알코올 중독 등 관련 질환에 대한 역할에 있어서는 아직 논란의 여지가 있다.

[0004] 숙취는 많은 양의 알코올을 섭취하였을 때 나타나는 불쾌한 신체적, 정신적 증상으로 일반적으로 알코올 섭취 후 8-16시간 후에 일어나고 그 결과로 두통, 구토, 설사, 식욕부진, 피로 등이 나타난다.

[0005] 한국산 배 (*Pyrus Pyrifolia* cv. shingo)는 예로부터 숙취해소에 사용되어왔다. 배의 세포벽은 다당류인 20-30%의 셀룰로즈, 25%의 헤미셀룰로즈, 35%의 팩틴과, 5-10%의 당 단백질, 그리고 미량의 페놀계 물질로 구성되어 있다 (농림부 보고서, 2002). 본 발명자는 배의 건강 기능성 연구에서 한국산 배가 발암물질인 PAHs(polycyclic aromatic hydrocarbons)의 배출효과, 나아가 PAHs관련 발암에 억제 효과가 기대된다고 발표한 바 있다 (양미희 등, 2005). 나아가 본 발명자는 한국공개특허 10-2009-0083752호에서 한국산 배의 알코올에 대한 숙취 해독효과를 과학적으로 규명하고 한국산 배의 숙취 해독효과가 에탄올의 체내 흡수 저해 또는 에탄올의 체내 대사 촉진에 있음을 확인하였다 (양미희, 2008). 이 외에도 한국산 배는 ACE(angiotensin converting enzyme)의 활성 억제효과 (Zhang et al, 2003), 알러지 반응 억제 효과 (Lee et al, 2004), 항 당뇨효과 (김정상 등, 2002), xanthine oxidase활성의 억제효과, 암세포 성장 저해효과, superoxide dismutase 유사활성 (안봉전 등, 2004)을 가진다고 보고 되었다.

[0006] 종래에 숙취해소를 위해 여러 소재들이 이용되어 왔다. 한국공개특허 10-1996-0000088호에서는 갈화 추출물을 숙취해소를 위한 주성분으로 이용하고 있으며, 한국공개특허 10-1997-0073403호에서는 헛개나무 추출물을, 한국공개특허 10-1998-0000173호에서는 노근 추출물을 이용하고 있다. 한국등록특허 제0378830호에서는 호깨나무와 함께 국산 배를 주성분으로 하는 숙취해소음료를 만들고 있다. 본 발명자의 한국공개특허 10-2009-0083752호에서는 한국산 배의 숙취 해독효과에 대한 메커니즘 확인을 통해 한국산 배를 유효성분으로 하는 숙취해소용 조성물을 제공하고 있다. 본 발명자의 한국공개특허 10-2009-0083752호를 제외하고는 숙취해소와 관련된 종래기술에서 배는 대부분 다른 주성분과 함께 보조적으로 사용되고 있으며, 주로 수분 보충 등을 위한 목적으로 사용되고

있다. 한국등록특허 제0378830호의 경우는 주성분에 의해 발생하는 부작용을 완화시키기 위해 배를 사용하였다.

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발명의 내용

해결 하고자하는 과제

[0017] 본 발명은 한국산 배에서 숙취 해독 효과를 나타내는 활성성분을 확인하고 이를 이용하여 보다 효과적인 숙취예방 및 해소용 조성물을 제공하는 것을 목적으로 한다.

과제 해결수단

[0018] 본 발명에서는 시험을 통해 한국산 배의 숙취 해독 효과가 배 껍질 부분의 수용성 성분에 기인함을 확인하고, 이러한 시험결과를 토대로 배 껍질의 수용성(peel water soluble) 분획을 유효성분으로 하는 숙취예방 및 해소용 조성물을 제공한다.

[0019] 또한, 본 발명에서는 한국산 배의 숙취 해독 효과가 배 껍질 부분의 수용성 성분 중에서도 알부틴(arbutin)에 기인함을 확인하고, 이러한 시험결과를 토대로 배 유래 알부틴을 유효성분으로 하는 숙취예방 및 해소용 조성물을 제공한다.

[0020] 본 발명자는 본 발명에 앞서 선출원한 한국공개특허 10-2009-0083752호에서 한국산 배의 숙취 해독 효과를 과학적으로 확인하고 그 기작이 ADH(alcohol dehydrogenase) 촉진작용을 통한 체내 알코올 농도 감소에 있음을 밝힌 바 있다. 본 발명에서는 이러한 ADH(alcohol dehydrogenase) 촉진작용을 통해 체내 알코올 해독효과를 가져오는 배의 활성성분을 확인한다. 먼저 본 발명에서는 배를 부위별로 수용성 정도에 따라 분리한 각 분획별로 ADH 활성에 미치는 영향을 조사하였으며, 그 결과 배 껍질의 수용성 분획에서 배즙에 가장 가까운 ADH 상승을 농도 의존적으로 발견하였다. 이에 따라 배의 숙취해소 본체로 의심되는 폴리페놀류 중 알부틴(arbutin), 클로로겐산(chlorogenic acid), (+)-카테킨(catechin)에 대한 ADH 활성을 조사하였으며, 그 결과 알부틴에서 농도 의존적으로 ADH 활성을 촉진함을 확인하였다. 본 발명을 통해 한국산 배의 숙취 해독 기작은 ADH 활성에 대한 관여가 주작용이며, 이러한 작용을 하는 활성성분은 배 껍질의 수용성 부분에 많이 존재하는 알부틴임을 확인할 수 있

다.

효 과

[0021] 본 발명에서는 ADH 촉진작용을 통해 체내 알코올 농도를 감소시키는 효과를 나타내는 배의 활성성분이 구명되며, 이러한 활성성분을 이용하여 보다 효과적인 숙취예방 및 해소용 조성물이 제공된다. 본 발명은 천연물 활성성분의 새로운 효능 확인을 통해 유용한 용도를 제공하는 효과가 있다

발명의 실시를 위한 구체적인 내용

[0022] 이하 구체적인 실시예를 통해 본 발명을 보다 상세히 설명한다. 그러나 이들 실시예는 오로지 본 발명을 보다 구체적으로 설명하기 위한 것으로, 본 발명의 범위가 이들 실시예에 의해 한정되는 것은 아니다.

[0023] **1. 재료 및 방법**

[0024] (1) 재료

[0025] 우리나라 중부지방에서 재배된 한국산 신고배(*Pyrus pyrifolia* cv. Shingo)를 실험에 사용하였다. 먼저 배를 증류수로 씻고 물기를 제거한 후 과육, 과피, 과심의 3부위로 분리하였다. 또한, 같은 배를 사용하여 한국공개특허 10-2009-0083752호에서 알코올 해독 시험에 사용한 것과 동일한 배즙을 만들었다. 즉, 압착기 타입의 쥬서기(NJE-2005SY, NUC, 대구, 한국)를 사용하여 배즙을 만든 다음, 진공 하에서 여과시키고(Whatman filter paper No.2, Maidstone, England), 110 ㎖ 폴리에틸렌 백에 진공포장한 후 -20℃ 정도로 냉동보관하면서 실험에 사용하였다.

[0026] (2) 배의 추출, 분획 및 정제

[0027] 껍질을 벗긴 후 분리된 배의 과육, 과피, 과심 각각을 즉시 메탄올에 넣고 80℃에서 4시간 동안 3회 추출하였다. 메탄올 추출액을 증류수에 용해시킨 후 여과지로 여과하여 수용성과 수불용성 부분으로 나누었다.

[0028] 수용성 부분은 증류수에 녹여, 아세톤을 이용하여 불순물을 제거 및 활성화시킨 후 50% 아세톤과 물로 치환한 Diaion HP-20 수지에 하룻밤 흡착시킨 후, 메탄올/물 (메탄올/물의 비가 0:1-> 1:0임)을 용매로 한 실리카겔 컬럼 크로마토그래피 (25×500 ㎜, 25×400 ㎜)로 처리하여 정제하였다.

[0029] 수불용성 부분은 에틸아세테이트로 추출하였으며, 얻어진 추출물은 다시 헥산/에틸아세테이트(헥산/에틸아세테이트의 비가 4:1-> 1:50임)를 용매로 한 실리카겔 컬럼 크로마토그래피(230-400 메쉬, 30×400 ㎜)로 처리하여 8개의 분획을 얻었다. 전체적인 배의 분리 및 분획 개요를 도 1에 도시하였다.

[0030] (2) 각 분획별 효능 실험

[0031] 상기 재료에서 만든 배즙 50 ㎕에 해당하는 용량의 각 분획에 대해 ADH 활성에 미치는 영향을 조사, 비교하였다.

[0032] (3) 배의 폴리페놀류에서 ADH 활성 증진효과 분석 및 동정

[0033] 배의 숙취해소 본체로 의심되는 페놀성 화합물(알부틴(arbutin), 클로로겐산(chlorogenic acid), (+)-카테킨 등)을 배 껍질의 수용성 분획(peel water soluble), 과육의 수용성 분획(pulp water soluble)별로 HPLC로 분석, 정량하였다.

[0034] **2. 결과**

[0035] (1) 분획별 효능실험

[0036] 배즙의 ADH(alcohol dehydrogenase) 촉진작용을 통한 체내 알코올 농도(alcohol level) 감소효과를 나타내는 활성성분을 구명하기 위하여, 상기 배즙으로 환산 시 50 ㎕에 상응하는 각 분획의 상응량, 즉 생배즙 50 ㎕에 함유되는 각 분획의 상응량으로 *in vitro* 실험을 실시하였으며, 그 결과는 표 1과 같다. 즉, 배 껍질의 수용성 분획과 과육의 수용성 분획을 합치면 본래 배즙에 상응하는 ADH 상승 효과를 얻을 수 있었다.

표 1

배의 분획별 ADH 활성의 변화

Fractions	ADH activity (uM of generated NADH/min)		
	Volumes of corresponded pear juice (ul)		
	25	50	100
Pear juice [a]	40 ±8.26	59.1 ±7.35	85.76±9.27
Peel water soluble [b]	24.2 ±3.27	40.3 ±4.76	74.7 ±6.25
Peel EA [c]	4.21 ±3.20	5.88 ±2.65	-
Pulp Water soluble [d]	11.4 ±2.22	18.1 ±5.30	21.4 ±1.25
Pulp EA [e]	3.66 ±2.52	3.66 ±1.20	4.80 ±2.43

EA; ethylacetate extraction
[a] Pulp and peels of pears were reached to approximately 90 and 10 % in pear juice, respectively.
[b] Real doses: Half, 1 and 2 mg of peel water soluble are corresponded to 25, 50, and 100 ul of pear juice.
[c] Real doses: 0.0016, 0.0033 and 0.033 mg of peel EA are corresponded to 25, 50, and 100 ul of pear juice.
[d] Real doses: 2.5, 5 and 10 mg of pulp water soluble are corresponded to 25, 50, and 100 ul of pear juice.
[e] Real doses: 2.5, 5 and 10 ug of pulp water soluble are corresponded to 25, 50, and 100 ul of pear juice.

[0037]

[0038] **(2) 배의 폴리페놀류에서 ADH 활성 증진효과 분석 및 동정**

[0039] 상기와 같은 결과를 토대로 배의 수용성 분획의 유효성분을 분석하였다. 먼저 문헌을 통한 조사 결과, 배의 수용성 분획에는 폴리페놀류(polyphenols)가 들어있고, 주로 이들은 클로로겐산(chlorogenic acid), 알부틴(arbutin), (+)-카테킨(catechin) 등으로 보고 되어 있다. 이에 따라 배의 숙취해소 본체로 의심되는 페놀성 화합물을 배 껍질 수용성 분획, 과육 수용성 분획별로 HPLC로 분석, 정량하였으며, 배 껍질의 수용성 분획을 분석, 정량한 결과는 도 2와 같다. 또한, 이들 폴리페놀 성분 각각에 대한 ADH 활성을 조사하였으며, 그 결과는 표 2, 3과 같다. 조사 결과, 폴리페놀류 표준품, 배에서 추출한 알부틴, 클로로겐산, (+)-카테킨에 대한 ADH 활성 조사에서, 알부틴에서 농도의존적으로 ADH 활성이 촉진되는 것을 발견하였다. 이에 따라, 배의 숙취해소 기작은 ADH 활성에의 관여가 주작용이며, 이러한 작용을 하는 활성성분은 배 껍질에 많이 존재하는 (과육에도 다소 존재하지만) 폴리페놀류로서, 그 중에서도 특히 알부틴인 것을 확인할 수 있다.

표 2

배의 폴리페놀류에 대한 표준품의 ADH 활성의 변화

Compounds	ADH activity (uM of generated NADH/min)		
	Concentration of each compound		
	25uM	50uM	100uM
Arbutin	26.5	34.48	38.17
(+)-Catechin	22.16	25.14	26.47
Chlorogenic acid	26.5	30.78	23.39

[0040]

표 3

배에서 추출한 폴리페놀류의 ADH 활성의 변화

Compounds	ADH activity (uM of generated NADH/min)		
	Volumes of corresponded pear juice (ul)		
	12.5	25	50
Pear juice	36 ± 5.25	42.2±5.35	59.1 ±6.31
Arbutin[a]	34.39±9.46	41.65±3.98	50.66±5.91
Chlorogenic acid[b]	29.46±5.59	30.56±8.17	38.58±10.10
(+)- catechin[c]	32.39±6.23	37.86±5.59	31.05±14.94

[a] Real doses: 1.25 2.5 and 5 ug of arbutin are corresponded to 12.5, 25 and 50 ul of pear juice.
[b] Real doses: 0.25, 0.5 and 1 ug of chlorogenic acid are corresponded to12.5, 25 and 50 ul of pear juice.
[c] Real doses: 0.09, 0.18 and 0.361 ug of (+)-catechin are corresponded to12.5, 25 and 50 ul of pear juice.

[0041]

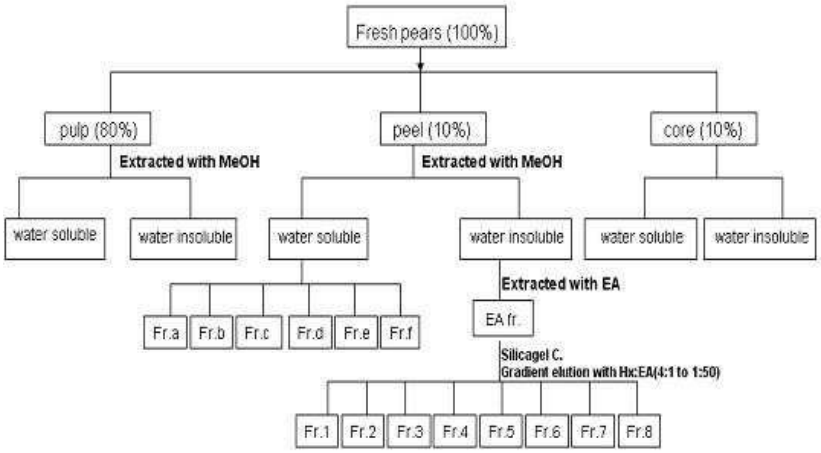
도면의 간단한 설명

[0042] 도 1은 부위와 물성에 따른 배의 분리 및 분획을 나타낸 것이다.

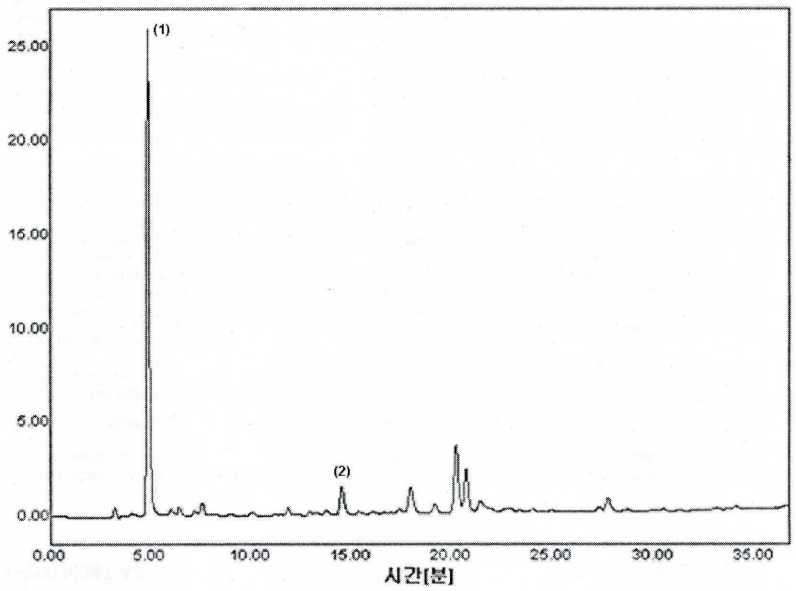
[0043] 도 2는 배 껍질 수용성 분획의 HPLC 프로파일이다.

도면

도면1



도면2



(1) arbutin, (2) (+)-catechin.

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(54) 발명의 명칭 **ＰＡＨｓ관련 암예방 식품 및 그러한 식품을 확인하는 방법**

요약

본 발명은 각종 환경적 요인으로 인한 발암물질 PAHs에 노출된 경우 암을 예방할 수 있는 식품 및 그러한 식품을 찾는 방법적 수단을 제공한다. 본 발명에서는 식품 섭취 후 뇨 중의 1-OHP농도를 일정 시간 간격으로 측정하므로써, 배가 PAHs의 체외 배출을 촉진한다는 것을 제시하였다. 또한, 동물실험에 의해 배가 폐선암 발생을 억제하는 효과가 있음을 확인하였다. 나아가, 면역기능 조절작용의 가능성을 확인하였다.

대표도

도2

색인어

배, PAHs, 항암식품, 폐선암

명세서

도면의 간단한 설명

[0001] 도1: 약물 동력학 실험 스케줄

도2: 배섭취에 따른 뇨 1-OHP의 동력학

도3: 동물처치법

도4: 열처리배즙이 *in vitro*에서 마우스 비장세포 증식에 미치는 영향

도5: 열처리배즙이 *in vitro*에서 T세포 마이토젠(Con A)으로 유도되는 마우스 비장세포 증식에 미치는 영향

도6: 열처리배즙이 *in vitro*에서 B세포 마이토젠(LPS)으로 유도되는 마우스 비장세포 증식에 미치는 영향

도7: B세포 마이토젠과 동시투여시 열처리배즙의 비장세포 증식효과

도8: NO생성에 미치는 영향

도9: TNF-α생성에 미치는 영향

도10: 단세포전기영동법

도11a: CHO.1A2BR2662 세포주, 24시간 처리, 소핵실험결과

도11b: CHO.1A2BRN2E422 세포주, 24시간 처리, 소핵실험결과

도12a: CHO.1A2BR2662 세포주, 2시간 처리, DNA손상방지 실험결과

도12b: CHO.1A2BRN2E422 세포주, 24시간 처리, DNA손상방지 실험결과

발명의 상세한 설명

발명의 목적

발명이 속하는 기술분야 및 그 분야의 종래기술

[0002] 유기물질의 불완전 연소로 형성된 다환족 방향성 탄화수소(PAHs)는 공기, 토양, 음식을 통하여 직업상 및 일상생활에서 인간에게 노출되는 환경적인 발암물질이다. 일반적으로 소변 중의 1-하이드록시파이렌(1-OHP)은 PAHs 노출 정도의 측정에 있어서 생물학적 지표로 사용되어 왔다[Jongeneelen, 1994].

[0003] 본 발명자는 PAHs의 노출 원인으로는 공기, 토양 및 물을 포함하는 환경 그 중에서도 대기 오염이 가장 큰 영향을 미치는 것임을 밝혔다. 또한, 튀김닭을 섭취한 경우에도 소변 중의 1-OHP의 레벨이 급상승하는 것으로 관찰되어 고온의 오일을 이용하는 조리법으로 만들어진 음식이 PAHs 노출의 중요한 원인이 되는 것으로 예상된다.

[0004] 상술한 바와 같이 각종 공해 및 음식물로 인한 PAHs에의 노출은 환경적 요인으로서 현대 사회에 있어서 지속적이며 누적된 효과로서 암을 유발하는 원인이 된다. 따라서, 이에 대항하기 위해서 항암제 등 약물의 투여가 아니라 일상 식품으로 암을 예방할 수 있는 방법을 모색하고자 하는 노력이 계속되고 있다.

발명이 이루고자 하는 기술적 과제

[0005] 따라서, 본 발명은 각종 환경적 요인으로 인해 PAHs에 노출된 경우 그로인한 암을 예방할 수 있는 식품을 제공하는 것을 목적으로 한다.

[0006] 또한, 본 발명은 그러한 식품을 찾는 방법적 측면에서의 수단을 제공하는 것을 목적으로 한다.

발명의 구성 및 작용

[0007] 상술한 목적을 달성하기 위하여 본 발명은 PAHs의 체외 배출을 촉진하는 것을 특징으로 하는 PAHs관련 암 예방 식품을 제공한다.

[0008] 상기 항암식품은 섭취 후 12시간 이내에 PAHs의 체외 배출을 증가시키는 것을 특징으로 한다.

[0009] 그러한 기능을 가지는 항암식품의 일 실시예로서 본 발명은 배를 제공한다.

[0010] 상기 배는 열처리된 즙의 형태임을 특징으로 한다.

[0011] 본 발명은 일 실시예로서 상기 열처리는 140℃ 내지 150℃에서 2시간 내지 5시간 가압추출하는 것임을 특징으로 하는 PAHs관련 암예방 식품을 제공한다.

[0012] 또한, PAHs의 체외 배출 능력을 측정하여 PAHs관련 암 예방 식품을 확인하는 방법을 제공한다.

[0013] 상기 PAHs의 체외 배출 능력은 식품 섭취 후 뇨 중의 1-OHP농도를 시간 간격으로 측정하는 것을 특징으로 한다.

[0014] PAHs의 노출 정도 지표로서는 그 대사물 중의 하나인 1-OHP를 사용하였다. 즉, PAHs의 노출로 인해 높아진 1-OHP의 농도가 어떠한 식품을 섭취하므로써 낮아지면, 체내 유전자 변형 등을 초래하여 발암화를 유발하는 시간을 감소시키므로, 그 식품은 PAHs 노출에 대해 항암 효능이 있는 것으로 예상할 수 있다. 특히, 본 발명에서는 식품 섭취 후 일정 시간 간격으로 뇨 중의 1-OHP농도를 측정하므로써, 약물 동력학적 결과에 근거하여 PAHs 유래 발암 물질 배출에 신속한 효과가 있음을 예측할 수 있게 된다.

[0015] 나아가, 본 발명은 비장세포 증식능력과 대식세포에서의 종양괴사인자(TNF) 생성능력을 측정하므로써, 항종양 및 면역기능에 미치는 영향을 평가하였다.

[0016] 특히, 본 발명에서는 항암식품으로서 배를 선택하여 항암 효능 및 면역계에 미치는 영향을 규명하는 실험을 수행하였다.

[0017] 실험예1 (PAHs 체외 배출 촉진 효과)

[0018] 배 섭취가 PAHs 노출에 미치는 영향을 평가하기 위하여 배 섭취 전·후 각각 소변 중의 1-OHP의 레벨을 측정하여 비교하였다.

[0019] 배 섭취 후의 영향을 평가하기 위해 24시간 후에 소변을 채취하였다.

[0020] 소변 중의 1-OHP 농도 측정을 위해 채취된 소변 1ml에 2M NaAc 100㎕, β-글루커로니다제(Beta-glucuronidase) 25유닛 및 아릴설페이타아제(Arylsulfatase) 25유닛을 투여한 후 37℃에서 3시간 인큐베이션 시켰다. 그런 다음, 아세토니트닐 400㎕를 넣고 원심분리하였다. 원심분리 상등액의 HPLC를 측정하였다.

[0021] 배 섭취 유·무에 따른 소변 중의 1-OHP 농도를 표1에 나타내었다. 실험대상자는 660명(남자:418명, 여자:242명, 연령:36.5±11.1)으로 하였다.

【표 1】

[0022]

구분	소변 중의 1-OHP 농도 평균(㎍/L)
배 섭취 전	0.021
배 섭취 후	0.005

[0023] 상기 표로부터 배 섭취에 의해 소변 중의 1-OHP의 농도가 현저히 감소된다는 사실을 확인할 수 있었다.

[0024] 배의 섭취가 소변 중의 1-OHP의 레벨을 감소시킨다는 사실은 배 섭취가 PAHs 생활성 중간체인 1-OHP 생성을 저지하는 등 배의 항암 식품으로서의 화학예방적인 가능성을 시사하는 것이다.

[0025] 그러나, 상기 실험만으로는 배의 어떤 작용으로 소변 중의 1-OHP 농도가 낮아지게 되는지 정확히 알 수 없다.

[0026] 따라서, 하기의 약물 동력학적 실험을 수행하였다.

[0027] PAHs의 노출 원인으로 튀김닭을 설정하였다. 20세 전후 남녀 40명을 대상으로 튀김닭만 섭취한 경우 및 튀김닭 섭취 후 배를 섭취한 경우 소변 중의 1-OHP 농도 변화 측정을 위해 도1의 타임 스케줄에 따라 튀김닭과 배의 섭취 및 소변 채취를 실시하였다. 배는 1개(약 775g)/1일을 섭취하게 하였으며 결과를 도2에 나타내었다.

[0028] 도2에 의하면 튀김닭만 섭취한 경우는 섭취 후 12시간 후부터 36시간이 지나도록 1-OHP의 농도가 꾸준히 증가하였다. 반면, 배를 함께 섭취한 경우는 12시간 이내에 1-OHP 농도가 높았고, 그 후 급격히 감소하는 것을 확인할 수 있었다. 1-OHP 체외 배출에 있어서 반감기가 12시간 정도임을 감안할 때 배는 12시간 이내에 1-OHP의 체외 배설을 거의 완료시키는 것이다. 따라서, 배는 PAHs 또는 그 대사물질을 체외로 신속하게 배출하므로써 이들이 체내에 머무르며 암을 유발하는 것을 막는 것이다.

[0029] 즉, 약물 동력학적인 방법에 의해 배의 PAHs유래 암유발 억제 작용을 확인하였다.

[0030] 한편, 실험용 마우스의 복강에 발암성 물질로 파이렌(pyrene)을 투여하고, 열처리 배즙 및 비열처리 배즙을 도3의 스케줄로 처치하였다. 뇨중의 1-OHP함량을 도4에 나타내었다.

[0031] 열처리배즙은 6시간까지 1-OHP의 농도가 급상승함에 비하여, 비열처리 배즙은 벤조파이렌만 투여한 경우와 동일한 농도 변화를 보였다. 이것으로 열처리함에 의해 배의 PAHs 체외 배출 촉진 효과가 특히 향상된다는 것을 확인할 수 있다.

[0032] 실험예2 (종양 발생 억제 효과)

[0033] 배의 폐종양 발생 억제 효능을 평가하였다.

[0034] 폐암모델로 A/J마우스를 생후 10~12일 사이에 벤조(a)파이렌(B[a]P)를 복강으로 투여하였다. 이유가 시작되는 시기인 21령 이후부터 추출된 배즙식이가 이루어졌고, 투여 후 약 10~12주에 부검하여 폐암(폐과형성소 및 폐샘종)에 대한 발생빈도 및 마리당 발생 개수를 병리조직학적으로 판독하였다.

[0035] 비열처리 배즙으로는 배를 6 내지 8 등분 한 후 녹즙기로 조분쇄, 블랜더로 미분쇄한 다음 착즙기로 착즙하고, 원심분리(2000g에서 10분)하여 얻은 것을 사용하였다. 열처리 배즙은 6 내지 8 등분한 배를 가압추출기에 넣어 140℃에서 3시간 가압추출 추출하므로써 얻었다.

[0036] A/J마우스에서 벤조파이렌으로 유발한 마리당 폐종양(폐선종) 발생갯수를 표2에 나타내었다.

【표 2】

[0037]

		<0.5㎜	≥0.5㎜	≥1㎜	합계
수컷	B[a]P 단독처리군	24.43±13.02	13.00±6.24	1.14±1.07	37.14±15.26
	B[a]P→열처리배즙	24.75±11.36	6.88±5.17	0.38±0.52	31.63±14.33
	B[a]P→비열처리배즙	7.14±8.41	4.33±2.57	0.14±0.38	11.57±10.49
암컷	B[a]P 단독처리군	11.6±6.54	12.00±7.21	0.60±1.34	23.40±11.10
	B[a]P→열처리배즙	15.17±6.84	6.00±3.35	0.33±0.52	21.17±8.28
	B[a]P→비열처리배즙	17.17±9.64	6.17±2.48	0.33±0.52	23.33±10.98

[0038] 종양의 크기가 0.5㎜이상인 것을 비교할 때, 수컷에서는 B[a]P 단독 투여군에서 약 13개 발생하였으나, 열처리 배즙 처리군에서 약 6.8개, 비열처리 배즙 처리군에서 약 4.4개로 감소하였다. 한편, 암컷에서는 각각, 12개, 6개 및 6.1개로 감소하였다.

[0039] 이것은 배가 폐암 발생을 억제하는 효능이 있다는 것을 시사한다.

[0040] 실험예3 (항산화 효능)

[0041] 배의 항산화 효능을 측정하기 위하여 성분 중의 폴리페놀 함량 및 DPPH(1,1-diphenyl-2-picrylhydrazyl radical) 에세이를 통한 전자공급능력(electron donating ability, EDA)의 IC_{50}을 측정하였다. 표3과 같은 온도 및 시간으로 열처리 한 배즙으로 실험하였다.

[0042] 비열처리 배즙 및 열처리 배즙은 실험예2에서와 동일한 방법으로 준비하였다.

[0043] 배의 성분 중 항산화성 성분으로 알려진 폴리페놀의 함량 및 EDA의 IC_{50}을 측정하였다.

【표 3】

[0044]

가공조건(온도, 시간)	폴리페놀 함량(mg/100g)	IC_{50}(g/L)
비열처리 배즙	801.31	17.0675
110℃, 1시간	1036.48	1.9768
110℃, 2시간	1092.70	1.6473
110℃, 3시간	1230.49	1.7867
110℃, 4시간	1298.72	1.6556
110℃, 5시간	1497.88	1.5085
120℃, 1시간	1338.45	1.4384
120℃, 2시간	1371.30	1.3522
120℃, 3시간	1376.03	1.4629
120℃, 4시간	1392.36	1.1191
120℃, 5시간	1413.04	1.0554
130℃, 1시간	1078.50	1.2210
130℃, 2시간	1638.68	0.7314
130℃, 3시간	1988.61	0.6406
130℃, 4시간	2218.23	0.5289
130℃, 5시간	2222.84	0.6390
140℃, 1시간	1824.99	0.7093
140℃, 2시간	2567.08	0.4846
140℃, 3시간	3201.48	0.4025
140℃, 4시간	3108.52	0.4719
140℃, 5시간	2960.07	0.4616
150℃, 1시간	3777.45	0.2098
150℃, 2시간	3746.34	0.3480
150℃, 3시간	3711.74	0.3243
150℃, 4시간	3061.08	0.3004
150℃, 5시간	2556.23	0.3126
BHT(합성항산화제)		0.1360

[0045] 상기 표로부터, 배의 폴리페놀의 함량 및 황산화 활성은 열처리에 의해 향상된다는 것을 알 수 있다. 구체적으로, 배를 140℃ 내지 150℃에서 2시간 내지 5시간 가압추출한 것이 2500(mg/100g) 이상의 폴리페놀 함량 및 0.5(g/L) 이하의 EDA IC_{50}값을 나타내었다.

[0046] 실험예4 (면역계에 미치는 영향)

[0047] 실험동물에서 비장세포증식능력을 측정하였다.

[0048] 실험동물로는 암컷 ICR 마우스 6~8주령을 사용하였다. 열처리 배즙을 감압농축한 후 동결건조하여 냉장보관하였으며, *in vitro* 실험을 시작하기 직전에 배지액으로 농도별로 희석하여 사용하였다.

[0049] 마우스에서 추출한 비장세포를 열처리 배즙이 농도별로 첨가된 배지에서 3일간 배양하여 마이토젠(mitogen) 첨가여부에 따른 비장세포 증식능력을 MTT 에세이로 측정하였다.

[0050] 결과를 열처리 배즙 단독 배지, T세포 마이토젠(Con A)(2㎍/ml) 및 열처리 배즙 배지 및 B 세포 마이토젠(LPS)(50㎍/ml) 및 열처리 배즙 배지에서의 배양 결과 비장세포 증식을 도 5, 6 및 7에 각각 나타내었다. * 및 ** 표시는 대조군과 현저한 차이를 보이는 경우를 표시한다.

[0051] 다음으로, 실험동물의 복강 대식세포의 산화질소 및 종양괴사인자(TNF-α) 생성에 미치는 영향을 실험하였다.

[0052] 실험동물로 ICR계 암컷 마우스를 사용하였다. 정상 마우스당 빙냉의 DMEM 배지 8ml을 복강 내로 주사한 후, 채취한 세포액을 합하여 원심분리하였다. 적혈구를 용혈시키고, 원심분리한 후 $4x10^6$세포수/ml로 조정한 세포액을 24웰 플랫 바텀드 플레이트에 세포가 웰 바닥에 부착되도록 하기 위해 2시간 방치(37℃, 5% CO_2)한 후, 열처리 배즙의 최종 농도가 0, 0.16, 0.31, 0.63, 1.25, 2.50mg/ml이 되도록 가하고, 배즙 단독 또는 LPS(Lipopolysaccharide) 1㎍/ml과 함께 가하여 배양하고 48시간(TNF-α 측정) 또는 96시간(NO 측정) 후에 취한 세포배양액을 원심분리 (2000rpm, 5분, 4℃)하여 얻은 상등액을 산화질소(NO)측정에 사용하였다.

[0053] 복강 대식세포 배양 후 얻은 상등액 중의 산화질소(NO) 생성량을 그리에스(Griess) 시약을 사용하는 그린(Green) 등의 방법을 이용하여 NO의 안정된 산화물인 nitrite(NO_2^-)량으로 측정하였다. 또한, TNF-α 생성량을 PharMingen에서 제공한 sandwich ELISA법에 준하여 측정하였다.

[0054] 산화질소 및 TNF생성에 미치는 영향을 도8 및 9에 나타내었다. * 및 ** 표시는 대조군과 현저한 차이를 보이는 경우를 표시한다.

[0055] 도8로부터 열처리 배시료액을 단독으로 복강 대식세포에 가하여 72시간 배양했을 경우, 상등액 중의 NO 생성량은 저농도인 0.16mg/ml에서는 대조군에 비하여 약 45% 유의성 있게($P<0.05$) 증가하였으나 0.31mg/ml 농도 이상에서는 농도 의존적으로 감소하여 고농도에서는 약 50% 감소하는 양상을 보였다. 또한, 복강 대식세포에 LPS를 첨가하고 열처리 배시료액을 가하여 배양했을 경우에도 저농도인 0.16mg/ml와 0.31mg/ml에서 대조군에 비해 각각 약 67%와 34% 증가하였으나 고농도에서는 약 40% 감소하였으며 모두 유의성을 보였다.

[0056] 도9로부터 열처리 배시료액을 단독으로 복강 대식세포에 가하여 48시간 배양했을 경우 상등액 중의 TNF-α 생성량은 0.16mg/ml, 0.31mg/ml, 0.63mg/ml에서는 대조군에 비하여 약 20%정도 유의성있게 증가하였고 고농도에서는 약 10%정도 증가하였으나 유의성이 보이지 않았다. 또한 복강 대식세포에 LPS를 첨가하고 열처리 배시료액을 가하여 배양했을 경우에는 대조군과 큰 차이가 없었다.

[0057] 실험예5 (세포수준에서의 발암억제효과 및 기전)

[0058] 사람의 대사 기능 모델을 세포수준에서 만들기 위하여 사람 사이토크롬 p450 1A2와 사이토크롬 환원효소를 동시에 발현하는 CHO.1A2BR2662 세포주와 N-아세틸트랜스퍼라제를 cotransfection 시킨 CHO.1A2BRN2E422 세포주를 확립하여 이에 PAHs 발암·돌연변이 물질인 2-아미노안트라센을 배즙과 같이 처리하였다. 소핵시험에서는 24시간 처리후 고정하였고, 단세포전기영동법에서는 CHO.1A2BR2662 세포주는 처리 2시간 후에, CHO.1A2BRN2E422 세포주는 처리 24시간 후에 세포를 수거하여 배즙의 항돌연변이성을 측정하였다.

[0059] 도10은 단세포전기영동법에서의 각 파라메터 정의를 보여준다.

[0060] A+B: DNA 이동

[0061] 테일거리: 헤드 중심점 및 테일 중심점간의 거리

[0062] 올리브테일모멘트: 테일거리 X 테일 중의 %DNA

[0063] 각 세포주에서의 소핵형성억제 결과는 도11a 및 b에 DNA 손상방지효과는 도12a 및 b에 나타내었다.

[0064] 소핵시험에서는 열처리배즙 0.5% 이상에서 소핵형성이 억제되었으며, 비열처리배즙은 2% 이상에서 소핵형성이 억제되었다. 이 결과는 CHO.1A2BR2662(도11a) 및 CHO.1A2BRN2E422(도11b) 세포주 모두에서 동일한 결과를 나타내었다.

[0065] 단세포전기영동법에서는 열처리배즙과 비열처리배즙의 차이를 관찰할 수 없었다. 하지만 CHO.1A2BR2662 세포주를 이용한 경우(도12a) 열처리배즙 0.25% 이상에서 DNA 손상을 방지하였으나, 시료를 24시간 처리를 하는 CHO.1A2BRN2E422 세포주(도12b)에서는 2% 이상의 배즙을 투여한 경우 DNA 손상 방지 효과가 나타났다. 이상의 결과로 배즙이 2-아미노안트라센에 의한 소핵형성 및 DNA 손상을 억제하였으며, 열처리 배즙이 비열처리배즙에 비해 소핵형성을 더 민감하게 억제하였다.

발명의 효과

[0066] 배는 발암 물질인 PAHs의 체외 배출을 촉진하므로써 항암작용을 한다. 이것은 PAHs에 노출 후 배를 섭취한 경우 6시간 내에 소변 중의 1-OHP 농도가 급상승하고 그 후 감소하였으나, 배를 섭취하지 않은 경우에는 노출 후 12시간이 지나서부터 1-OHP 농도가 차츰 증가하는 것을 보여 PAHs의 체외 배출을 촉진하는 배의 효능을 확인할 수 있었다.

[0067] 또한, 배는 동물의 폐종양 발생 개수 및 크기를 억제하여 폐선암 발생 억제효과도 가지는 것으로 나타났다. 그리고, 소핵형성 억제 및 DNA 손상방지 등 항암 전단계인 항돌연변이 작용을 하는 것도 확인할 수 있었다.

[0068] 본원발명에 의하면 약물동력학적인 방법으로 PHAs 노출관련 암 예방 식품을 확인할 수 있었다.

청구의 범위

청구항 1

PAHs의 체외 배출을 촉진하는 것을 특징으로 하는 PAHs관련 암예방 식품.

청구항 2

제 1 항에서, 섭취 후 12시간 이내에서 PAHs의 체외 배출을 증가시키는 것을 특징으로 하는 PAHs관련 암예

방 식품.

청구항 3

제 2 항에서, 상기 항암식품은 배임을 특징으로 하는 PAHs관련 암예방 식품.

청구항 4

제 3 항에서, 상기 배는 열처리된 즙의 형태임을 특징으로 하는 PAHs관련 암예방 식품.

청구항 5

제 4 항에서, 상기 열처리는 140℃ 내지 150℃에서 2시간 내지 5시간 가압추출하는 것임을 특징으로 하는 PAHs관련 암예방 식품.

청구항 6

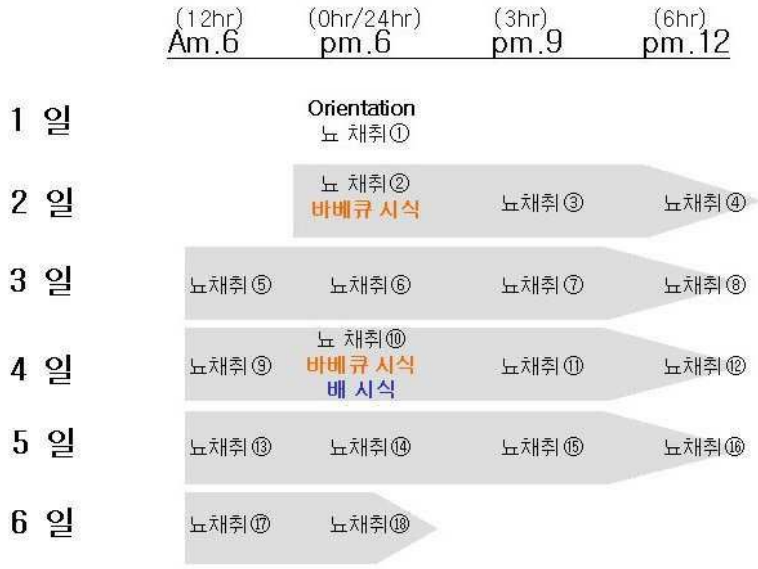
PAHs의 체외 배출 능력을 측정하여 PAHs관련 암 예방 식품을 확인하는 방법.

청구항 7

제 6 항에서, PAHs의 체외 배출 능력은 식품 섭취 후 뇨 중의 1-OHP농도를 시간 간격으로 측정하는 것을 특징으로 하는 PAHs관련 암 예방 식품을 확인하는 방법.

도면

도면1



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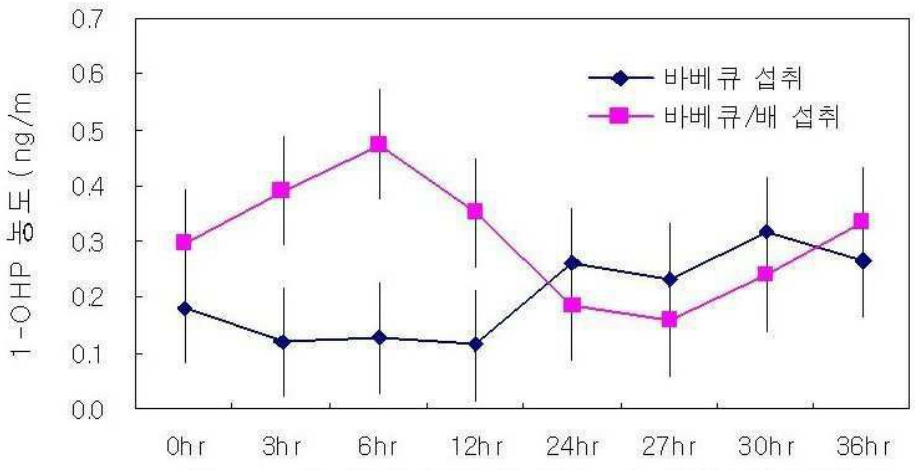
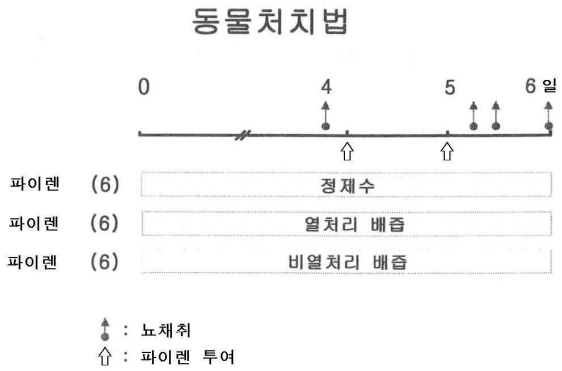
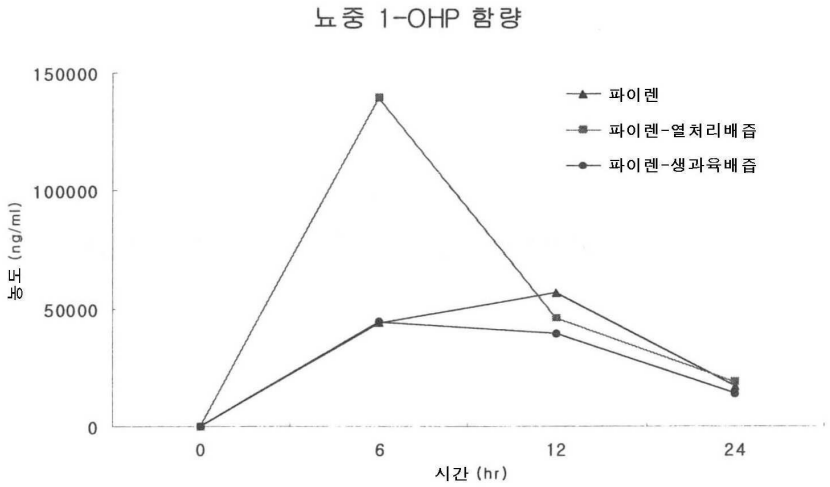


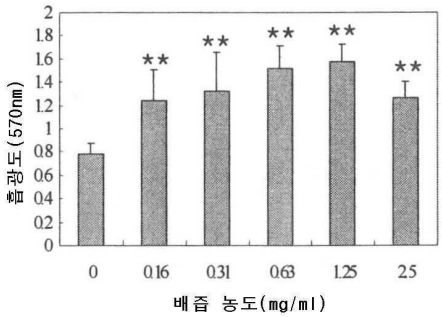
도면3


동물처치법
0
4
5
6 일
파이렌 (6) 정제수
파이렌 (6) 열처리 배즙
파이렌 (6) 비열처리 배즙
: 뇨채취
: 파이렌 투여

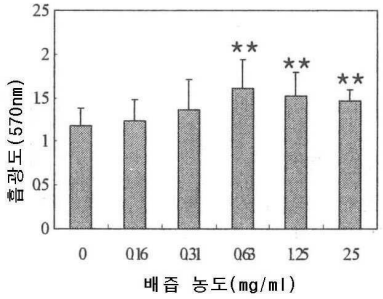
도면4


뇨중 1-OHP 함량
150000
100000
50000
0
농도 (ng/ml)
0
6
12
24
시간 (hr)
파이렌
파이렌-열처리배즙
파이렌-생과육배즙

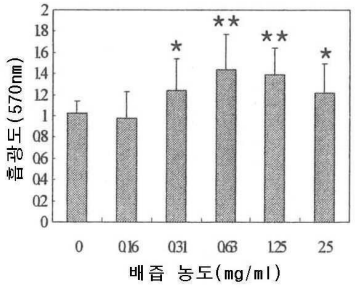
도면5


흡광도(570nm)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
0
0.16
0.31
0.63
1.25
2.5
배즙 농도(mg/ml)

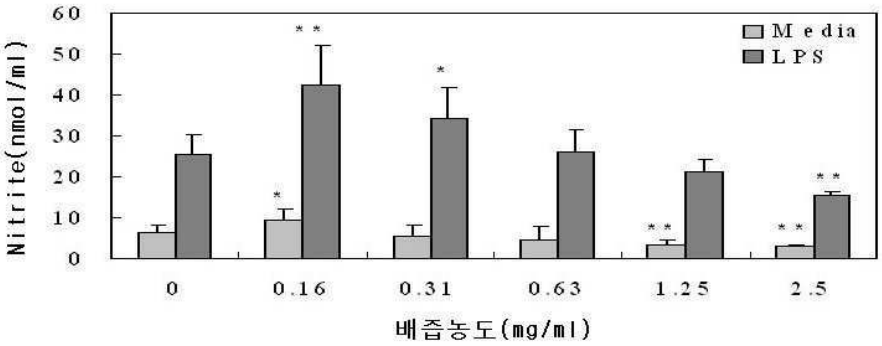
도면6



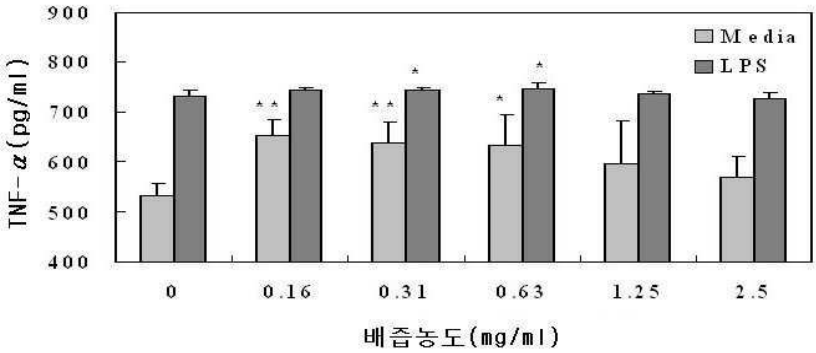
도면7



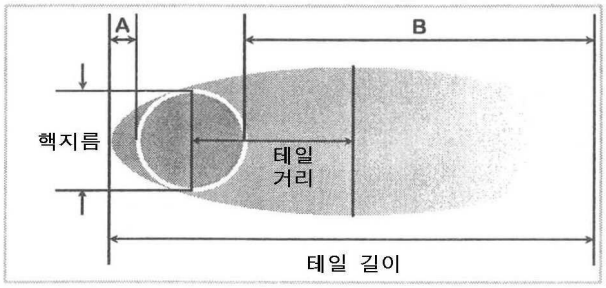
도면8



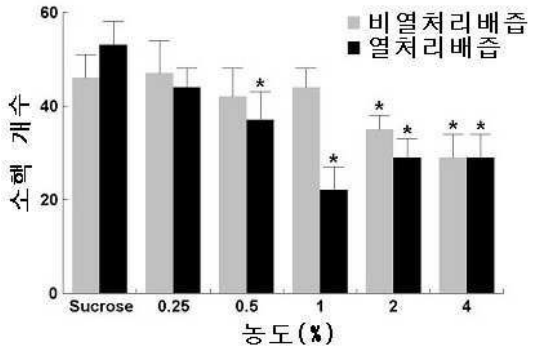
도면9



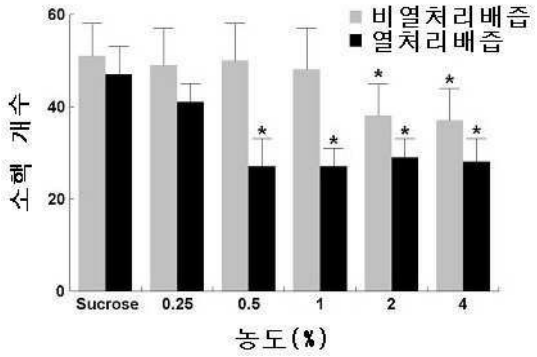
도면10



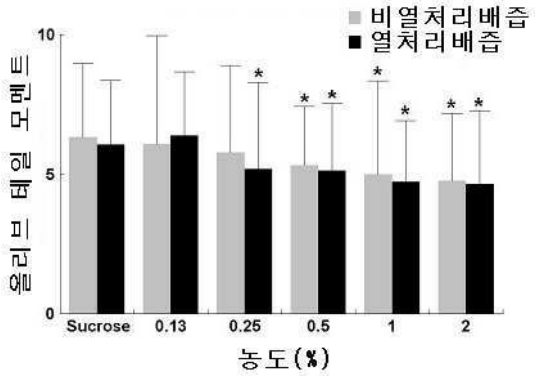
도면11a



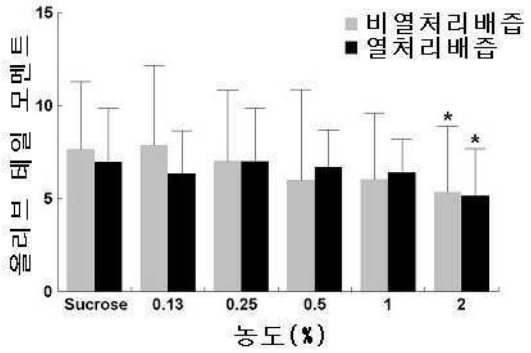
도면11b



도면12a



도면12b



BMC Complementary Medicine and Therapies

REVIEW Open Access

A review of pears (*Pyrus* spp.), ancient functional food for modern times

Sung-Yong Hong[1], Ephraim Lansky[2], Sam-Sog Kang[3] and Mihi Yang[1*]

Abstract

Background: Pears have been world-widely used as a sweet and nutritious food and a folk medicine for more than two millennia.

Methods: We conducted a review from ancient literatures to current reports to extract evidence-based functions of pears.

Results: We found that pears have many active compounds, e.g., flavonoids, triterpenoids, and phenolic acids including arbutin, chlorogenic acid, malaxinic acid, etc. Most of researchers agree that the beneficial compounds are concentrated in the peels. From various in vitro, in vivo, and human studies, the medicinal functions of pears can be summarized as anti-diabetic,-obese, –hyperlipidemic, –inflammatory, –mutagenic, and -carcinogenic effects, detoxification of xenobiotics, respiratory and cardio-protective effects, and skin whitening effects. Therefore, pears seem to be even effective for prevention from Covid-19 or $PM_{2.5}$ among high susceptible people with multiple underlying diseases.

Conclusion: For the current or post Covid-19 era, pears have potential for functional food or medicine for both of communicable and non-communicable disease.

Keywords: Pears, Medicinal function, Detoxification, fiber, Arbutin, Flavonoids

Background

Pears (*Pyrus spp.*) have been used as folk medicine and healthy food for more than two millennia [1]. The genus name *Pyrus,* of the family Rosaceae, may be applied for both pears and apples, though *Malus* is the genus name most commonly used for apples, while *Pyrus* (*P.*) is more frequently used for pears. Pears comprise two major types, the 'European' or 'Western' pears, exemplified by *P. communis*, and the 'Asian' pears, typically, *P. pyrifolia* [2], although at least 22 species of pear with over 5000 subspecies or accessions have been recognized [3]. Terms such as 'Korean pear', 'Japanese pear' and 'Chinese pear' are often used interchangeably, but more accurately reflect the geographic milieux from which differing accessions evolved, especially of *P. pyrifolia*, but also from *P. bretschneideri*, *P. sinkiangensis*, and *P. ussuriensis*. Based on evolutionary, morphological and geographical characteristics, pears can be classified as two major accessions, i.e., European and Asian. Asian pears including *P. pyrifolia*, which are round-shaped, possess crisp flesh, high sugar content, especially fructose, low acid content, minimal aroma, and mild flavor, relative to Western or European pears, especially *P. communis*, featuring gourd-shape with soft and smooth flesh, few stone cells, and a stronger aroma and flavor [4]. Among Asian pears, traditional Korean pears, *P. faurie* and *P. serotina,* are smaller than modern cultivars and were developed for local tastes, flavor, and various needs [5–7]. Asian pear cultivars have been reported to contain higher amounts of phenolics, arbutin, and chlorogenic acid than Western pears [8, 9]. In particular, Korean pears hold higher contents of sugar, potassium, and

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Table 1 Summary of scientific names, synonyms, and common names of pear species shown in this review

Scientific name	Synonym	Common name
Pyrus anatolica	*Pyrus anatolica* Browicz	Turkey pear
Pyrus bretschneideri	–	Chinese white pear
Pyrus calleryana var. *fauriei*	*Pyrus fauriei* C.K.Schneider	Korean Sun pear
Pyrus communis	*Pyrus communis* subsp. *communis*	European pear
Pyrus malus var. *ussuriensis*	*M. domestica* (Suckow) Borkh	Ussurian pear, Manchurian pear, Harbin pear
Pyrus pashia	*Malus pashia* (Buch.-Ham. ex D. DON) Wenzig	Himalayan pear
P. pyrifolia	*Pyrus serotina*	Asian pear : Korean pear, Japanese pear a part of Chinese pears
Pyrus sinkiangensis	–	Xinjiang pear

water, relative to Western pears [10]. Scientific names, synonyms, and common names of various pear species are shown in Table 1.

In East Asia including Korea, Japan and China, pears have been used for diverse medicinal applications, e.g., respiratory symptoms relievers, fever managements, inflammation treatment, alcohol hangover, etc. [11, 12]. In particular, pears have been used for digestion of meat, such as a tenderizer in cooking of beef and desserts after consumption of Korean BBQ, Bulgogi. In addition, the traditional function of pears on alcohol hangover was confirmed by recent scientific evidence. That is, in vitro and in vivo studies showed that Korean pears (*P. pyrifolia* cv. Shingo) stimulate main alcohol metabolizing enzymes and eliminates body burden of alcohol and aldehyde [10]. Clinical trials also revealed that the pears alleviate hangover symptoms [13]. Moreover, many researchers have recently found new medicinal functions of pears by diverse studies including chemical analyses, nutritional factors, and in vitro, in vivo, and human studies [4]. If we further know the traditional functions and local usages of pears, it may help to find out new medicinal functions of pears. Therefore, we conducted a systematic review to provide current information of functional studies of pears, in conjunction with ancient and local applications.

Literature searches were performed from ancient literature to current reports in PubMed at the National Center for Biotechnology Information (NCBI), in Google Scholar, and in Research Information Sharing Service (RISS) at the Korea Education and Research Information Service (KERIS). We extracted evidence-based data of pears for usage, active compounds, and medical function. To obtain reproducibility and avoid publication bias, we collected the information that was confirmed by different researchers as possible as we can.

Traditional uses of pears

In Korea, pears have been cultivated as a folk medicine and as a sweet fruit since the Samhan period (ca. 300 BCE - 300 CE) [5]. The Taylor–Schechter Genizah collection of a Jewish community mentioned pears in medicinal prescriptions more than one millennium ago [14]. The *Shen Nong Ben Cao Jing* (神農本草經), the first traditional Chinese pharmacopoeia published ca. CE 220, described the uses of pears for relieving fever, quenching thirst, and suppressing cough [1]. The *Ben Cao Gang Mu* (本草綱目) [15], a Chinese pharmaceutical encyclopedia, elaborated on the characteristics of and myriad uses of pears in China, namely that pears are sweet and a little sour, cold, and harmless, however, too much consumption of pears makes people thin and weak and brings out diarrhea. For main treatment, the text goes on: they treat fever, suppress cough, and quench thirst. A slice of a pear is used for relieving pain and preventing decomposition in burn wounds. They are useful for dysarthria caused by irregular fever, stroke, and hypothermia, mitigate fever caused by ague, and provide benefits for urination and defecation. They mollify chest tightness, dyspnea, and mental symptoms caused by hyperpyrexia. They further moisturize lungs, cool down the heart, remove sputum, and detoxify abscesses and alcohol poisoning. Pear flowers were said to cleanse dirt on a face, and decoction of pear tree bark provides benefits for seasonal diseases caused by cold weather. The leaves were enlisted for treating scrotal hernia, while pounded leaves extracts were used against mushroom poisoning [15].

In addition, Jun Heo, a royal physician, described the usage of pears for treating irregular recurrent fever, relieving chest tightness, and quenching thirst, particularly, after drinking alcohol in his Korean traditional medical

book, *DongUiBoGam* (東醫寶鑑) [11]. He also depicted the contraindications of pears, e.g., swords cuts or pregnancy, and described that too many pears make the stomach or intestines sick, however, cleaning with water boiled with pear barks provides benefits for scabies and tinea scores (Fig. 1).

Leading compounds of pears

The major components of pears are water (approx. 80%), sugar and fructose (approx. 15%), and fiber (approx. 2%): Korean pears showed some higher composition of water, sugar, potassium than Western pears (Bartlett: *P. communis*), while Bartlett showed some higher levels of fiber and calcium [10].

Various active compounds in pears have been identified, such as polyphenols (phenolic acids, flavonoids), triterpenes, and glucosides [16, 17]. The highest concentration of these phenolic compounds occurs in the leaves, followed by the seeds, peels, and pulps. The phytonutrients in general are richer in peels than the pulp [18, 19]. Pears have thick peels containing pectin, and stone cells with highly thickened, lignified wall of abundant lignin and cellulose [20, 21]. The development of stone cells may be closely related to the synthesis, transfer, and deposition of lignin. As one might expect, the chemical composition in different parts of pears varies. For the monomeric compounds, arbutin, oleanolic acid, ursolic acid, chlorogenic acid, epicatechin, and rutin were reported to be the dominant in various pear cultivars both in peel and in flesh [9]. Figure 2 summerizes the leading functional pear compounds.

Arbutin

Arbutin, hydroquinone-β-D-glucopyranoside (Fig. 2), is a well-known antibiotic [22], and skin whitening compound [23, 24]. It is degraded into hydroquinone, a skin bleaching agent, and is used in cosmetics as a fragrance, reducing agent, and melanin polymerization inhibitor. Hydroquinone mediates immune function in vitro and in vivo, however, these effects have not yet been established in humans [24, 25]. A Chinese group reported the peel of imported Korean pears (Chinese name, Youran) contained approx. 1.5–20 fold higher amounts of arbutin (6982.0 μg/g dry weight) than other 9 different pear varieties cultivated (323.3–4395.8 μg/g dry weight) in China and South Africa [9]. In Oriental pears, the greatest concentration of arbutin was found in the peel (1.20 mg/g fresh weight), which was 3–5 times greater than that found in the core and 10–45 times greater than the level in the pulp [26, 27]. Therefore, pear skins, especially those of Korean pears, are one of the richest food sources of natural arbutin [28]. As such, arbutin can serve as a potential pear-specific intake biomarker [29].

Chlorogenic acid

Following arbutin, chlorogenic acid, 5-*O*-caffeoylquinic acid, is the second most abundant phenolic compound (Fig. 2) in pear flesh and peel [30]. In particular, chlorogenic acid reached 106.7–247.5 mg/100 g of fresh weight in immature Korean pears [23]. Chlorogenic acid has been studied and reported to have biological functions such as anti-inflammatory and antioxidant activity [31, 32]. Mechanistic studies on its medicinal function revealed that chlorogenic acid reduces TNF-α, downregulates IL-8 production in Caco-2 cells and RAW264.7 cells, protects

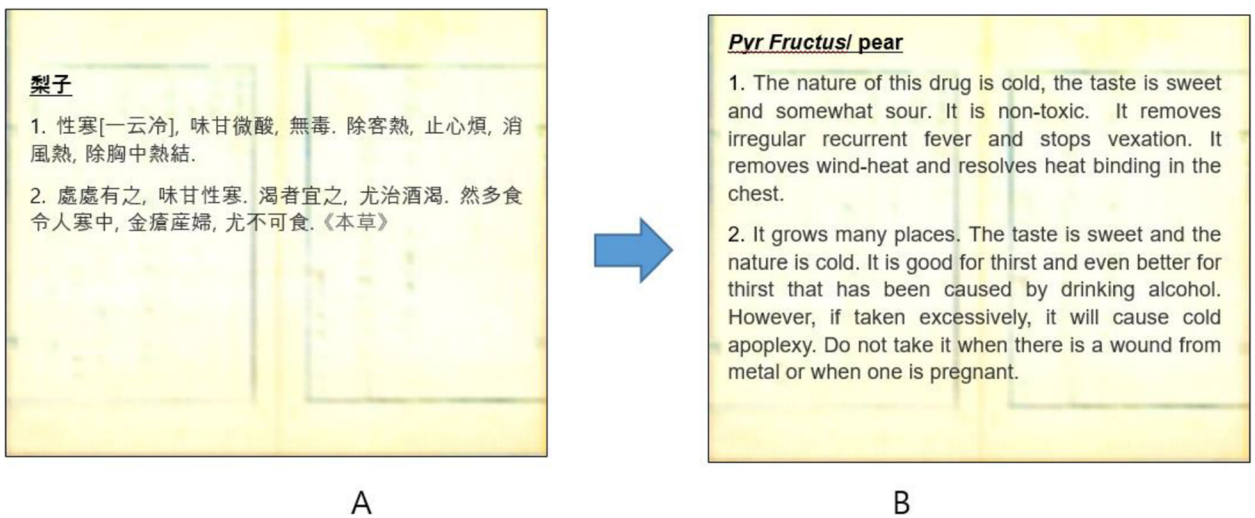

Fig. 1 'DongUiBoGam' (1613): principles and practice of Eastern medicine, one of memory of the world (UNESCO, 2009), described various usages of Korean pears: **A**, original version; **B**, English translation by Dr. Sang-Woo Ahn of Global DoungUiBoGam Center with minor modification by Mihi Yang

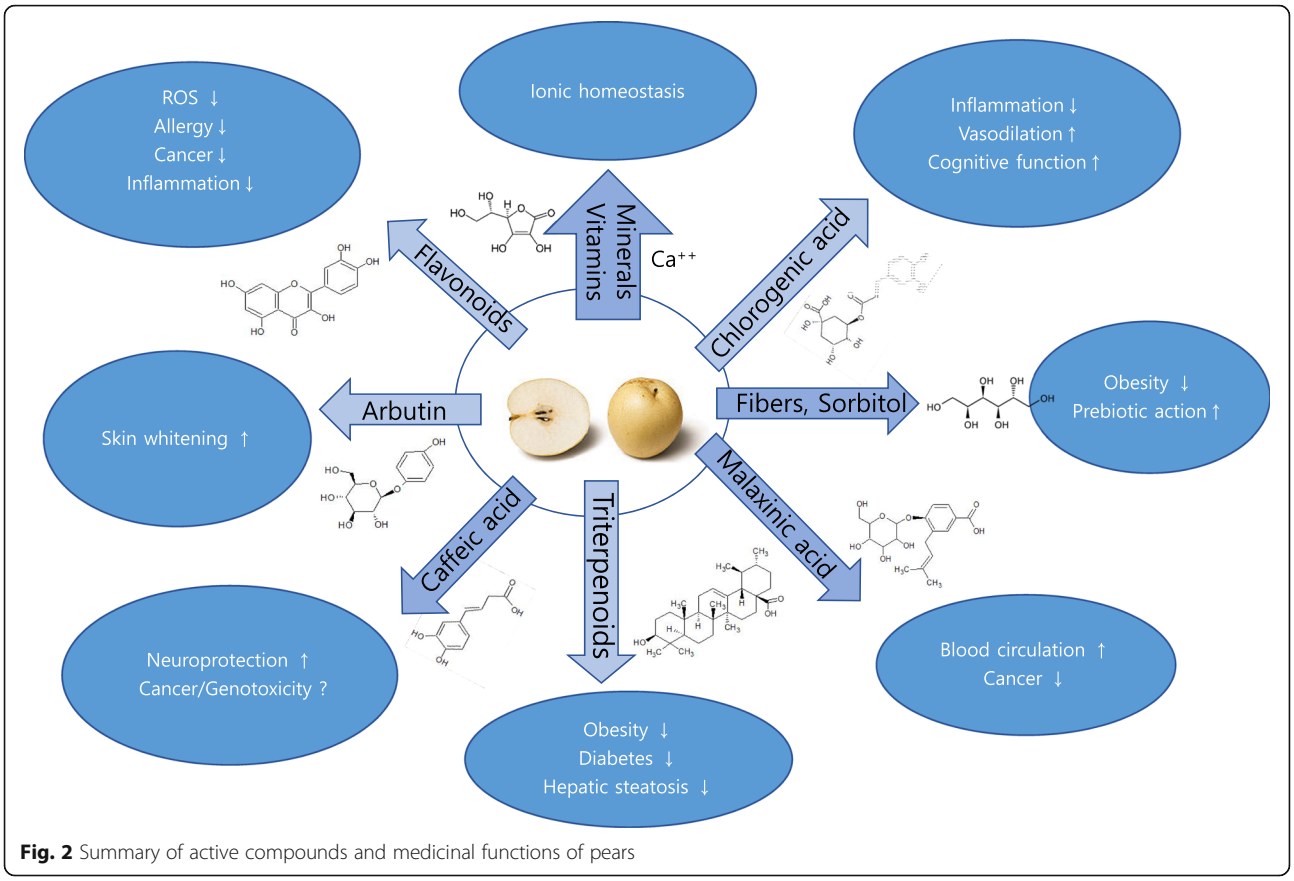

Fig. 2 Summary of active compounds and medicinal functions of pears

neurons, and improves wound healing in vivo, suggesting inhibition of inflammation [33, 34]. In addition, chlorogenic acid showed to induce a direct endothelium-dependent vasodilation by increasing NOS, COX, and endothelium-derived hyperpolarizing factor signalling pathways [35]. Recently, a Japanese study showed 6-month intake of chlorogenic acid (330 mg/100 ml of water) significant improved cognitive function from the One Back Test of the Cogstate, the Shifting Attention Test, and Finger Tapping Test as well as in the composite memory, verbal memory, complex attention, cognitive flexibility, executive function, and motor speed domains of the CNS Vital Signs test battery [36]. In addition, chlorogenic acid protected against DNA damage induced by ionizing radiation, suggesting significant radioprotective effects of these compounds [37].

Caffeic acid

Caffeic acid, one of phenolic acids, is a minor compound in the flesh and peel (56.2 vs. 73.5 mg/kg) of Turkish [22, 24]. Although not structurally related, caffeic acid has been reported to elicit neuroprotective properties with caffeine [38]. Some studies also showed that caffeic acid enhanced collagen production [39, 40]. For colon cancer cells, such as the HCT 15, caffeic acid induced apoptosis, ROS generation and reduction in the mitochondrial membrane potential and showed chemopreventive potential [41].

Flavonoids

Immature Korean pears contain flavonoids at 182.5–368.9 mg/100 g of fresh weight [23]. B-ring dihydroxylated flavonol derivatives, such as quercetin and isorhamnetin, and monomeric and polymeric flavan 3-ols, such as epicatechin and proanthocyanidins, are dominant among the flavonoids found in ten pears including Radana cultivar [42, 43]. These chemicals have been thought to contribute to color, fruit quality, and plant resistance. In the case of European and Tunisian pear cultivars including the pulp and peel, the predominant flavonoid is (−)-epicatechin (Fig. 2) as terminal and extension units [16].

Following (−)-epicatechin, the anthocyanins, water-soluble pigments composed of an anthocyanidin aglycone, were mainly found in the red skinned pear cultivars [16, 44]. Other flavonoids found in Korean pears (*P. pyrifolia* Chuhwangbae) include quercetin 3-O-glucoside, its aglycone quercetin [18, 45], and dulcisflavan, a catechol [46]. Quercetin 3-O-glucoside is one of the dominant flavonols among leaves and fruits of pears [16].

Flavonoids have been emphasized due to their observed biological effects in vitro, e.g., free-radical scavenging, modulation of enzymatic activity, and inhibition of cellular proliferation, as well as their potential use as antibiotic, anti-allergic, anti-diarrheal, anti-ulcer, and anti-inflammatory, and anticancer agents [47]. However, epidemiologic studies exploring the role of flavonoids in human health have been inconclusive [48].

Malaxinic acid

Malaxinic acid, 4-(O-β-d-glucopyranosyl)-3-(3′-methyl-2′-butenyl)benzoic *acid,* is a major glucoside in Korean pears [49]. The amounts of malaxinic acid in immature Korean pears reached 0.76—5.86 mg/100 g of fresh weight, although the amounts of malaxinic acid were significantly less, as the pears matured [23]. Several reports have described some medicinal functions of malaxinic acid in Korean pears, e.g., anti-oxidative defense in blood circulation and inhibition of growth of cancer cells, such as BAEC, HT1080, HeLa, and B16/BL6 [23, 50–52]. The isoprenyl side chain in malaxinic acid may contribute to inhibition of a 21–26 kDa protein involved in cancer cell proliferation [53]. Therefore, malaxinic acid can be a candidate for one of major active compounds of pears, however, the evidence should be further collected.

Triterpenoids

Among triterpenoids, particularly ursolic (Fig. 2), oleanolic, and betulinic acids have been identified in European pear cultivars (*P. communis*), more than 17 fold higher in the peels than flesh (3460.5 ± 1255.9 vs. 201.4 ± 77.1 μg/g of dry weight) [30].

Because of their steroid-like chemical structures, endocrine-related functions can be expected from triterpenoids. For example, ursolic acid showed stimulation of lipolysis in primary-cultured rat adipocytes [54], inhibition of aromatase, which converts androgens into estrogens, and increased energy expenditure, leading to reduced obesity, improved glucose tolerance, and decreased hepatic steatosis [55]. Its isomer, oleanolic acid, has been speculated to have anti-oxidative, anti-tumor, anti-inflammatory, anti-diabetic, and anti-microbial effects [56].

Other compounds

Plenty of fibers in pears can be prebiotics, which selectively stimulate growth and/or activities of microbial species in the gut microbiota and confer health benefits to the host [57]. For example, pear mixture with carrot, sea buckthorn, plum, or beetroot containing inulin showed growth stimulation in Lactobacillus/Enterococcus [58]. Along with fructose and exceptionally rich fibers, i.e., insoluble cellulose and hemicellulose, and soluble pectin, sorbitol in pears is likely to be responsible for the known laxative properties of pears [1, 30]. Sorbitol, a sugar alcohol, accounts for up to 90% of the total carbohydrate in pears, especially Korean pears. In addition, ascorbic, citric, and malic acids, and minerals, such as magnesium, potassium, calcium, and iron, in pears are likely to support blood pH and ionic homeostasis [16].

Functional uses of pears

Anti-diabetic effects

Recent studies have shown that pears possess anti-hyperglycemic effects [59, 60]. Combined apple or other fruits, such as acai, cherry and pear also inhibited diabetic parameters [61, 62]. For example, consumption of apples and pears reduced the risk of Type 2 diabetes mellitus (T2DM) by 18% (95% confidence interval: 0.75–0.88) [62]. In the case of animals, diabetic mice treated with peel extracts of Yaguang pear (*P. ussuriensis* Maxim cv. Yaguang) exhibited significantly lower fasting glucose levels than the diabetic control group, which are possibly related to inhibition of alpha-glucosidase [63]. Similar in vivo results were obtained from the treatment with extracts of immature Asian pears, such as Hosui and Kosui pear cultivars (*P. pyrifolia*) [64]. Furthermore, the diabetic rats, treated with ethyl acetate and ethanol extracts of *P. communis,* exhibited significant reductions in blood glucose compared to diabetic controls and the anti-hyperglycemic effects of pears putatively was due to increased insulin secretion from the pancreatic β-cells [59].

As hyperglycemia is mediated by alpha-amylase and alpha-glucosidase, which promote digestion, absorption, and metabolism of dietary carbohydrates, these enzymes have been pharmacological targets for treating hyperglycemia, because ordinary pharmacological regulators were associated with adverse abdominal effects and poor patient compliance [60, 65]. Pears may serve as a suitable alternative regulating postprandial hyperglycemia by inhibiting alpha-amylase and alpha-glucosidase without any significant side effects. Comparison of 22 different fruit juices in vitro showed that juice extracted from pearple (Chinese white pear, *P. bretchneideri* Rehd.) had the highest inhibition activity against alpha-glucosidase [66]. In vitro examination of extracts of European pear cultivars, Red D'Anjou, Green D'Anjou, Barlett, Bosco, and Comice, also revealed significant inhibition of alpha-amylase and alpha-glucosidase with more potent suppression of alpha-amylase by pulp extracts and more potent inhibition of alpha-glucosidase by peel extracts [65]. Furthermore, an in vitro study with Barlett and Stakrimson pear cultivars showed that the phenolic compounds in Stakimson pear extract might be bioactive and responsible for anti-hyperglycemic property, as it is positively correlated with alpha-glucosidase inhibition [60].

These alpha-amylase and alpha-glucosidase inhibitory activities of pears were observed in many in vitro and in vivo studies and they could provide the foundation for further research on pear extracts aimed to elucidate whether it can play a role in management of T2DM.

The beneficial effects of pear consumption in reducing T2DM risk have been well established in previous observational studies, and the subsequent in vivo and in vitro data have shown a strong positive correlation between pear consumption and improvement in T2DM parameters. Further investigations, particularly studies in humans, might put pears as an ideal phytoceutical option in the management of T2DM patients.

Anti-obesity activity

Dietary modification and exercise have been recommended for overweight people as beneficial lifestyle interventions. As fruits are low-energy dense and rich in dietary fiber, they can provide stomach satiety with less caloric intake. Specifically, pears have a low energy density of 0.64 kcal/g with plentiful dietary fiber and showed beneficial effects on weight management in a number of different studies [67–70]. For example, the rats on high-fat diets containing pear insoluble dietary fiber (IDF) did not share the same pattern of weight gain as the rats fed diets without pear IDF co-administration and had weights as low as the normal chow-fed group [67]. Therefore, Chang et al. speculated the IDFs extracted from pears exhibited anti-obesity effects, such as acceleration of fat metabolism and reduction of levels of low density lipoprotein –cholestrol (LDL-C) and total cholesterol (TC), by promoting the growth of Bacteriodetes in rats' gut microbiota. In addition, the pear extract (PE) and *Garcinia cambogia* extract (GE) treated groups showed 4.1 and 14.7% reduction in maturation of pre-adipocytes into adipocytes, respectively, while combined PE and GE synergized to exhibit a 26.9% inhibition, highlighting their potential to prevent weight gain [70].

Some clinical studies have also shown anti-obesity effects of pears. After 12 weeks of daily consumption of fresh pears, green Bartlett or D′ Anjou, leptin concentrations and waist circumference were lower in the pear group than control [71]. Bosc pears decreased the amount of post-exercise exposure to metabolites, leading to improved exercise performance [69]: Pears reduced cortisol levels in the participants by 22% immediately after exercise, promoting faster recovery from strenuous exercises. Many studies support the use of pears for obesity prevention by decreasing caloric intake and promoting exercise.

Anti-hyperlipidemic effects

As abnormal plasma lipid concentrations are major contributors to CVDs, development of safe anti-hyperlipidemic materials has been desired. Therefore, natural products, such as green tea, onions and garlics, have been studied. Anti-hyperlipidemic effects of pears are particularly observed in hyperglycemic status, since hyperlipidemia is common in diabetic patients. To control lipid levels is even more important in diabetic patients, since they have higher risks of CVDs. Velmurugan and Bhargava found that pear feeding significantly reduced the levels of TC, triglyceride (TG), and LDL-C in hyperglycemic rats, while it increased the level of HDL-C in a dose dependent manner [59]. In addition, the pulp extracts of *P. Communis* L. var. Blanquilla showed the decreased levels of TC by 14.6%, TG by 6.8%, and LDL-C by 17.4% in rats fed cholesterol-containing diets [72]. However, pear peels had more potent lipid-lowering properties than pulp (19.4% for TC, 14.6% for TG, and 33.3% for LDL-C), compared to the control. Therefore, the lipid lowering effects of pears seem to be related to the components such as catechin, which are more condensed in peels than in pulp.

Anti-mutagenic and -carcinogenic effects

Pears showed some anti-mutagenic and anti-cancer activities by several mechanisms. Firstly, pears can inhibit carcinogenesis of polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene, which have two main carcinogenic mechanisms, formation of DNA-adducts and production of ROS [73, 74]. We observed that Korean pears reduce benzo(a)pyrene-induced lung cancer in A/J mice, particularly in males [75]. In a biological monitoring study, we also found chemopreventive effects of Korean pears on exposure to PAHs in approx.. 700 Koreans [76]: Urinary concentrations of 1-hydroxypyrene (1-OHP), a major metabolite of PAHs, were analyzed as a biomarker of exposure to PAHs and were significantly decreased in the pear consumers. These results were also confirmed by pharmacokinetic methods in a clinical trial with the subjects who were exposed to PAHs through eating fried chicken with and without pears (*P. pyrifolia* Shingo) [75, 77]: Rapid excretion of urinary 1-OHP was observed in the pear consumers, compared to the non-pear eaters. Therefore, we speculate that Korean pears mediate absorption, distribution, metabolism, and excretion (ADME) of PAHs, particularly excretion of PAHs. The acceleration of PAH excretion may reduce the retention of carcinogens in the pear consumers. In addition, urinary levels of malondialdehyde, a biomarker of lipid peroxidation and oxidative stress, were also decreased in the Korean pear consumers [77, 78]. Considering bioproduction of ROS as another major carcinogenic mechanism of PAHs, we suggest that Korean pears protect PAH-induced oxidative stress. With regard to the botanical characteristics of pear trees, the accumulation of PAHs was also

decreased on pear leaves than on other similar-sized plant leaves, such as linden leaves, and the presence of epicuticular waxes on pears may contribute to the low storing of PAHs [76]. Considering PAHs are a main components of $PM_{2.5}$ of air pollution, we suggest pears are effective for prevention from $PM_{2.5}$.

Secondly, pears can be anti-carcinogenic by virtue of their nitrite scavenging activity [79, 80]. As nitrites are widely used during food processing and storage, they may easily react with amines to produce strong carcinogens, such as N-nitrosamines, in the processed food. The nitrite scavenging activity of Asian pears, such as Baekwoon and Niitaka, was 80.7–86.7% [79, 80]. These pears showed comparably high nitrite scavenging activity than other plants, such as onions (50%) or kiwis (75.3–81.8%) [12].

Lastly, there are many functional phytochemicals in pears, such as phenolic compounds including chlorogenic acid and malaxinic acid, which have shown quite diverse anti-carcinogenicity, such as anti-proliferative activities against breast and liver cancer cells [81, 82]. Particularly, Korean pears showed anti-carcinogenic potential due to functions related to mediation of ADME for PAHs, reduction of ROS, nitrite scavenging activity, and antioxidant properties of phenolic compounds.

Anti-inflammatory effects

Excessive inflammatory responses are a leading cause of non-communicable diseases [83]. However, dietary ingestion of pears, apple, red wine, and strawberries showed inverse associations with inflammation scores (IS) in food-based analyses: Higher dietary anthocyanin and flavonol intakes showed strong association with anti-inflammatory effects in a population of US adults [84]. Anthocyanin intakes reduced IS, such as acute inflammation, cytokines, and oxidative stress, by 73%. Higher intakes of flavan-3-ols, such as catechins, epicatechins, etc., and their polymers were associated with significant reduction in biomarkers of oxidative stress, which included myeloperoxidase, LPL-A2, and isoprostanes, an index of creatinine.

The anti-inflammatory effects of different pear species were compared to those of dexamethasone in carrageenan-induced mice hind paw edema and xylene-induced mice ear edema models [9]. The methanol extracts of pears including *P. ussuriensis* Maxim species (Yaguang) and *P. communis* varieties (Hongpi, Qingpi and Guifei) reduced the mice paw and ear edema to a certain degree and exhibited dose-dependent anti-inflammatory effects. In the case of *P. bretschneideri* Rehd, its ethyl acetate fraction showed the strong inhibition of carrageenan-induced rat paw edema formation and displayed the potent anti-inflammatory activity against xylene-induced ear edema and acetic acid-induced extravasation of Evan's blue dye at the dose of 200 mg/kg and 400 mg/kg [85]. Triterpenoids and flavonoids, such as 2β,19α-dihydroxy ursolic acid, α-amyrin and quercitrin, were suggested to have these anti-inflammatory effects.

Azuma et al. evaluated the suppressive and anti-inflammatory effects of cellulose nanofibers from Japanese pears (*P. pyrifolia*, Nijuseiki) on inflammatory bowel disease (IBD): The pear nanofiber demonstrated anti-inflammatory effects via suppression of fibroses or by butyrate-mediated inhibition of NF-κB in an IBD murine model [86].

Pear vinegar (PV) also alleviated ulcerative colitis with the disease activity index, which was significantly reduced in rats fed with 9% PV (4.0 ± 0.8), compared to control (7.4 ± 1.4) [87]: Histopathological scoring of severity of tissue damage was also less in the PV treated rats than in controls. PV also suppressed the myeloperoxidase-mediated activation of inflammatory cells and decreased the serum concentration of IL-6. In addition, the leaf extract of *P. ussuriensis* Maxim (Sandolbae) significantly reduced the level of NO in RAW 264.7 cells, as well as IL-6 and IL-1β in TNF-α-induced HaCaT cells [88]. The extract significantly ameliorated the dermatitis severity, scratching tendency, and transepidermal water loss, compared to the negative control among 2,4-dinitrochloro-benzene-treated NC/Nga mice. It normalized skin barriers with decreased production of IgE in mice serum. In addition, some studies showed that arbutin of different pear parts exhibited anti-inflammatory effects on pro-inflammatory cytokines, such as IL-1β, TNF-α, and MCP-1 [9, 89]. These proinflammatory cytokines attribute the cytokine storm of COVID-19. In the absence of an immediate and appropriate therapeutic intervention, COVID-19 patients develop acute respiratory distress syndrome as a result of acute lung damage followed by multi-organ failure and resulting in death [90]. Hence, pears have a strong potential to treat the cytokine storm.

In short, pears showed quite diverse anti-inflammatory effects through suppression of immune responses and this function seems to be related to the combination of diverse chemicals rather than one chemical in pears.

Respiratory protective effects

A recent systematic review on fruit and vegetable consumption showed that increased intakes of apples and pears was strongly associated with lower incidence of asthma symptoms, less diagnosed asthma, and less bronchial hyper-reactivity, leading these authors to establish an inverse correlation between pear/apple consumption and asthma [91]. Another prospective cohort study concluded that consumption of one or more servings of apples or pears led to significantly lowered risks of chronic

obstructive pulmonary disease (COPD) in ex-smokers, with a hazard ratio of 0.70 [92].

In in vitro studies, pears have also shown bronchodilatory effects. Ethanol extracts of '*Pyrus pashia* Buch.-Ham. ex D. Don' exerted a relaxant (0.01–5.0 mg/mL) effect on K^+ (80 mM) induced contractions in isolated rabbit trachea smooth muscle cells and caused shifting of the Ca^{2+} curves (1.0–3.0 mg/mL) toward right in a manner similar to that of verapamil (3 μM), possibly suggesting presence of Ca^{2+} channel blocking activity [2].

In animal studies, Lee et al. reported that the sensitivity of tracheal smooth muscle of mice to electrical field stimulation and acetylcholine was significantly decreased after treatment with pectins of Asian pear (*P. pyrifolia*): Trachea of mice also showed significantly fewer inflammatory signs, such as thickened bronchial mucosa, loss and/or abnormalities of cilia, lymphocyte proliferation, and sticky mucus plugs along the bronchi. Furthermore, there was 70% reduction in the serum allergen-specific IgE [93]. In addition, another study found that treatment with a combination of extracts from *P. bretschneideri* (pear fruit) and *Fritillaria ussuriensis* (bulb) inhibited tissue edema and reduced vascular permeability, compared to monotherapy with either extract alone among rats [94].

The mechanism by which pears play a beneficial role in the treatment of allergic inflammatory and respiratory diseases such as asthma may be related to their unique combination of polyphenols and flavonoids [2]. Research on flavonoids, including the rutin and quercetin present in pears, indicates some potential activities for the treatment of allergic diseases through the down-regulation of mast cell activation [95].

In addition, Yang et al. (2006) performed a clinical trial with COPD patients using heated Korean pear (*P. pyrifolia* cv. Shingo) juice; however, there was no significant improvement of respiratory health outcomes, including the George's respiratory questionnaire score or forced expiatory volume for 1 s among COPD patients after 1 month consumption of pear juice [77, 78]. Not heated but unheated pear juice reduced benzo(a)pyrene- induced lung cancer in A/J mice [75]. Therefore, heating for preservation can destroy bioactive compounds such as enzymes in pears.

Cardio-protective effects

Cardiovascular diseases are the leading global cause of death with 17.9 million mortality events per year [96]. Pear components have shown cardio-protective effects. Concerning active compounds in pear, chlorogenic acid showed to improve ex vivo vessel function and protect endothelial cells against HOCl-induced oxidative damage, via increased production of nitric oxide and induction of Hmox-1 [97].

Among pear species including Red D'Anjou, Green D'Anjou, Bartlett, Bosc and Comice, aqueous pulp extracts of Bartlett showed moderate (18–28%) ACE-I inhibition through in vitro enzyme models, however, no correlation was observed between ACE inhibition and total phenolics or antioxidant capacity [60, 65]. Cardioprotective functions of pears via ACE inhibition were confirmed in vivo systems. However, the mechanisms and active compounds are still obscure.

Alcohol detoxification and hepato-protection

Pears have been used as a traditional medicine to alleviate hangover symptoms [11]. However, the scientific mechanisms of alcohol detoxification by pears were obscure. Therefore, initial investigations were focused on the effects of pears on ADME of alcohol. The metabolic pathway of alcohol is relatively well-known. Alcohol is mainly metabolized in liver by alcohol dehydrogenase (ADH) into acetaldehyde, a toxic metabolite that increases hepatic lipid peroxidation and oxidative stress [10, 13, 98, 99]. Acetaldehyde is further metabolized by aldehyde dehydrogenase (ALDH) into acetate, which is finally eliminated by the kidney. Several Studies showed that Korean pears (*P. pyrifolia* cv. Shingo) fortified alcohol metabolism by stimulating ADH and ALDH activities in in vitro studies, consequently lowering the levels of blood alcohol or acetaldehyde in in vivo and human studies [10, 13]. In detail, pharmacokinetic analyses showed that Korean pears decreased levels of blood alcohol in *Aldh2* KO mice more significantly than those in normal mice [10], suggesting that the clinical alcohol detoxification effects of Korean pears might be greater in ALDH2 deficient persons than in normal people. Finally, total and average scores of hangover severity were significantly reduced in human subjects who consumed Korean pear juice before alcohol consumption [13].

Korean pears also enhanced the growth of hepatocytes via the increase of ATP synthesis in the cells [100]: Cell proliferation and DNA synthesis in hepatocytes were increased with treatment of [^{3}H]-thymidine and pear extracts. Moreover, the pears increased expression of CDK-2 and CDK-4, which are essential for the G1/S transition, but decreased expression of their inhibitors, p21Cip1/CDKNIA and p27Kip1.

For liver, the water extract of pear pomace from *P. pyrifolia* showed suppression of hepatic lipid peroxidation and protection against liver damage in rats fed a high fat/cholesterol diet [101]. In addition, the pear (*P. pyrifolia*) peel extracts significantly prevented the increase of levels of serum alanine aminotransferase and aspartate aminotransferase in acute liver injury among mice [102], mainly

showing antioxidant, anti-inflammation, and/or anti-apoptotic properties [103]. When taken together, Korean pears may contribute to prevention of liver from alcohol and non-alcoholic damages.

Skin whitening effects

Skin whitening is a desirable characteristic in the field of cosmetology, alongside slowing down the aging process and removing wrinkles. As we mentioned above, arbutin was discovered as a skin whitening substance. Pears are a naturally abundant source of arbutin [24, 104]: Skin-lightening action is related to its inhibition of the enzyme tyrosinase, which is critical for generating dark pigments, specifically melanin. Extracts of four cultivars of Korean pears (*P. pyrifolia*), namely Hanareum, Manpungbae, Shingo, and Chuwhangbae, inhibited tyrosinase activity by 50% in melanocytes of mice treated with melanocyte stimulating hormone, α-MSH. It was reported that a high concentration of arbutin was distributed in pear peels [12].

Based on the high contents of arbutin in Korean pears, five unripe Korean pears, i.e., *P. pyrifolia* cultivars, were tested for whitening activities [105]: For whitening

Table 2 Summary of medical functions of Korean pears and other pears

Function	Mechanism[a]	Material	Method	Reference
Antidiabetic	Blood glucose levels ↓	Yaguang (peel extracts); *P. communis*	in vivo	[59, 63]
Anti-obesity	Acceleration of fat metabolism and decreased LDL and TG	Insoluble dietary fiber from pear pomace (China)	in vivo	[67]
	Low calorie intake due to high fiber	*P. communis*	Human	[68]
Alcohol detoxification and hepato-protection	ADH and ALDH ↑	Korean pear (*P. pyrifolia* cv. Shingo)	in vivo	[10]
	Blood alcohol and acetaldehyde ↓	Korean pear (*P. pyrifolia* cv. Shingo)	Human	[13]
	Blockage of lipid accumulation in hepatocytes	Korean pear (*P. pyrifolia* cv. Shingo)	in vitro	[109]
Anti-inflammatory	Fibrosis and inhibition of NFκB ↓	Japanese pear (Nijuseiki)	in vivo	[86]
	MPO-mediated inflammatory cells ↑ serum level of IL-6 ↓	Japanese pear Vinegar	in vivo	[87]
Anti-asthmatic	Suppression of allergic asthma reaction due to reduced serum allergen-specific IgE levels and inflammatory signs	Korean pears (*P. pyrifolia* N. cv. Chuhwangbae)	in vivo	[93]
	Mast cell activation, histamine release ↓	Flavonoids in pears	in vitro	[95]
Bronchodilatory	Ca^{2+} channel blockade in trachea muscle cells	*P. pashia* Buch.-Ham	in vivo	[110]
Anti-hyperlipidemic	Plasma LDL, TG, and Cholesterol ↓; HDL ↑	*P. communis*	in vivo	[59, 72]
Skin Whitening Effects	Formation of cellular melanin ↓	Unripe Korean pears (*P. pyrifolia* cv. Manpungbae)	in vitro	[105]
	Expression of melanogenesis-related enzymes ↓	Korean pear extracts (*P. pyrifolia* cv. Chuhwangbae)	in vitro	[51]
Anti-mutagenic or carcinogenic	Rapid excretion of PAHs	Korean pear (Shingo)	Human in vitro	[75]

[a]*ADH* alcohol dehydrogenase; *ALDH* aldehyde dehydrogenase; *MPO* myeloperoxidase; *LDL* low density lipoprotein; *TG* triglyceride; *HDL* high density lipoprotein; *PAHs* polycyclic aromatic hydrocarbons

activity related to tyrosinase and cellular melanin formation, Manpungbae among the pears showed the strongest tyrosinase inhibition (4.9%) and achieved 74% reduction of the cellular melanin, compared to the non-treated cells. In addition, Yim et al. found that arbutin levels in pear cultivars decreased as the fruit matured. In B16F10 mouse melanoma cells, most of the cultivar extracts inhibited melanin synthesis by about 50% at a 100 μg/mL concentration until 90 days after full bloom [106]. In the case of *P. Anatolica*, which is endemic to Turkey, the leaves and branches showed higher levels of arbutin than fruits (4.74, 4.46, and 0.11%, respectively) [107]. In addition, arbutin-conjugated gold nanoparticles displayed enhanced whitening capabilities, compared to arbutin itself [108].

Protocatechuic acid (PCA) is another phenolic compound with anti-melanogenic and skin-lightening properties in pear peels. PCA significantly suppressed melanogenesis through the inhibition of tyrosinase as well as the co-inhibition of expression of other melanogenesis-related enzymes in mouse melanoma cells treated with Korean pear extracts (*P. pyrifolia* cv. Chuhwangbae) [51].

Given the high amounts of skin whitening agents such as arbutin and PCA, pears, particularly Korean pears, can be a safe and natural source for the therapeutics of hyperpigmentation. In the near future, new pharmaceutical formulation containing pears could be developed.

A summary of medicinal functions of Korean pears and other pears is shown in Table 2.

Limitations and methodological suggestions

For comprehensiveness, we have simultaneously tried to review adverse effects of pears. Pears are usually a safely consumed fruit for most people, but there may be some publications biased against pears emphasized as functional foods. With even thorough search, not many reports described pear-related drawbacks. A limited number of reports have addressed carbohydrate malabsorption, such as irritable bowel syndrome and nonspecific diarrhea in infancy and childhood, due to pear or apple juice [111]. Reminding the reader of the contraindication of pears on 'Ben Cao Gang Mu' [15] and the high incidence of self-treatment with herbal products among vulnerable populations [112], pears might be avoided for wasting syndrome patients, children, or pregnant women, due to malabsorption. For specific components of pears, the safety of hydroquinone, a metabolite of arbutin, has been concerned after the recognition that benzene caused aplastic anaemia and leukaemia in humans [113]. However, results on hydroquinone from topical, oral, or industrial exposure showed that it is quite safe, compared to the toxicity of benzene. Nevertheless, long term safety was not established, yet.

For other medicinal functions of pears, excretion of urinary stones [114] and wound healing for poor wound healing patients, such as diabetes [115], have been carefully described in some reports as new applications of pears. In addition, a wide plethora of traditional, integrative, complementary and alternative medicines have been touted as the solution for COVID-19, despite the paucity of evidence surrounding the safety and effectiveness of such therapies [116]. Based on the potential of pears as a modulator of pro-inflammatory cytokines via various anti-oxidative flavonoids [117], evidence-based studies of pears are needed for a new application, e.g., prevention and treatment of new communicable diseases.

Conclusions

For medicinal functions of pears, we learn from the old and make it a new (Fig. 3). Traditional functions of pears have been gradually confirmed with scientific evidence.

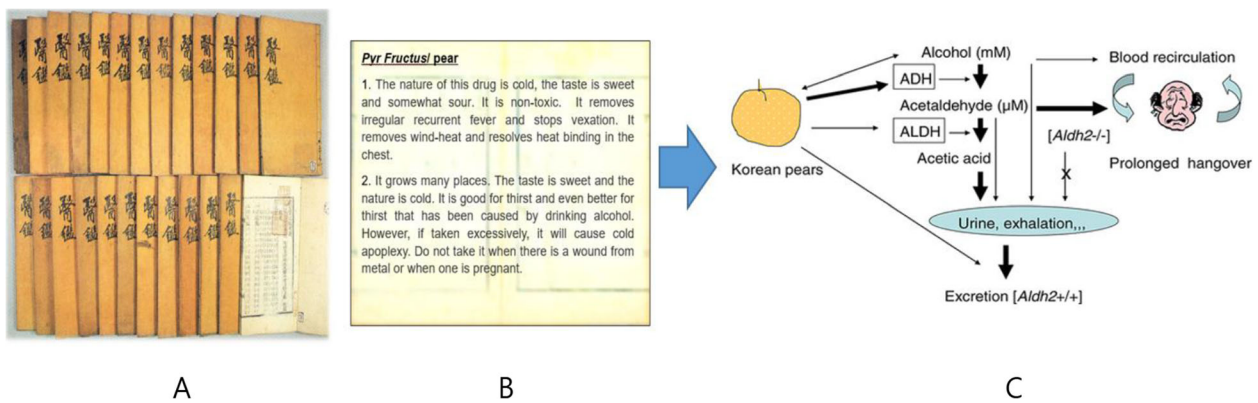

Fig. 3 New medicinal function of pears from the old: **A**, DongUiBoGam; **B**, medicinal uses and toxicity warning of pears in DongUiBoGam; **C**, potential mechanisms of Korean pears on alcohol detoxification [10]

Pears, an old and new fruit, show beneficial effects on various degenerative diseases and have a strong potential as functional food or medicine for high susceptible people with underlying diseases to prevent from new communicable diseases in the current or post COVID-19 era.

Abbreviations

ADH: Alcohol dehydrogenase; ADME: Absorption, distribution, metabolism, and excretion; ALDH: Aldehyde dehydrogenase; COPD: Chronic obstructive pulmonary disease; GE: *Garcinia cambogia* extract; HDL-C: High density lipoprotein- cholesterol; IBD: Inflammatory bowel disease; IDF: Insoluble dietary fiber; IS: Inflammation scores; LDL-C: Low density lipoprotein-cholesterol; 1-OHP: 1-hydroxypyrene; *P. : Pyrus*; PAHs: Polycyclic aromatic hydrocarbons; PCA: Protocatechuic acid; PE: Pear extract; PV: Pear vinegar; T2DM: Type 2 diabetes mellitus; TC: Total cholesterol; TG: Triglyceride

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Authors' contributions

S-Y H: 1st drafting of the most of manuscript; EL, and S-SK: designing the study; MY: corresponded, writing, searching data and designing study. All authors read and approved the final manuscript.

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Declarations

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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