Naturally occurring inhibitors against the formation of AGEs, diabetes, and Maillard reaction products in health

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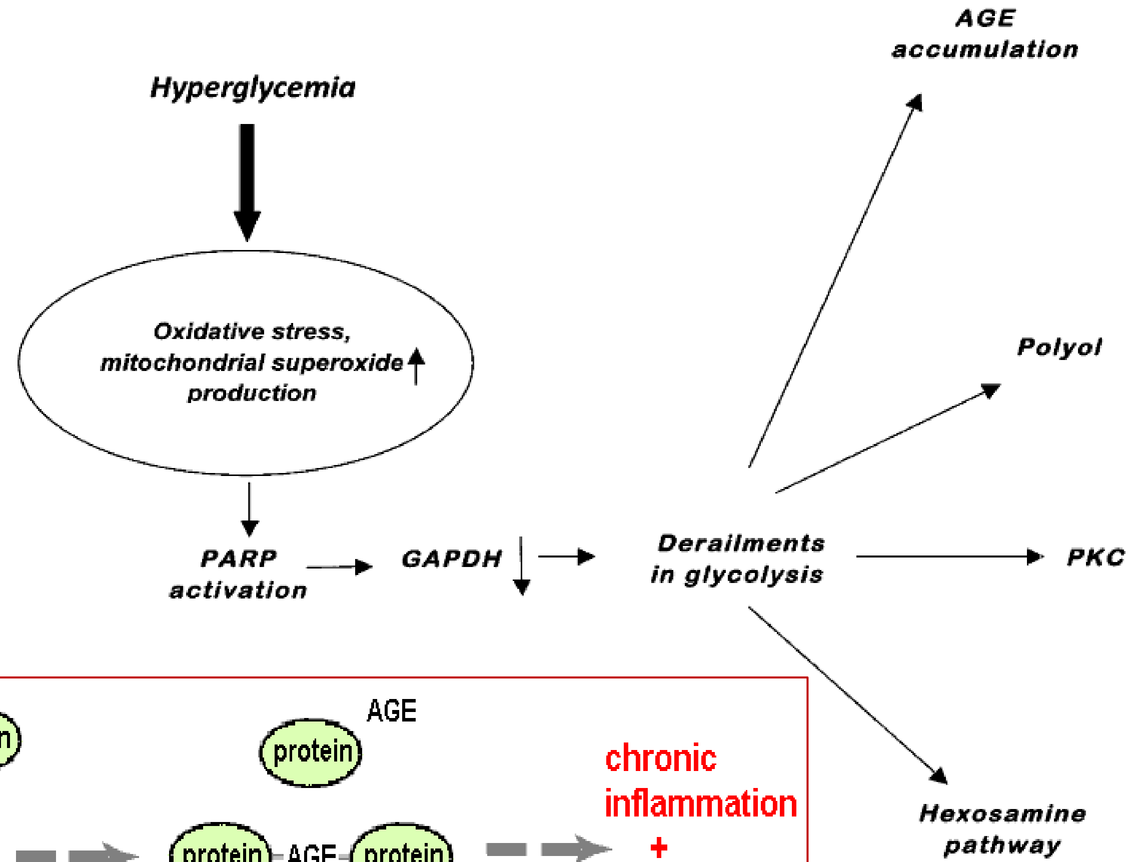
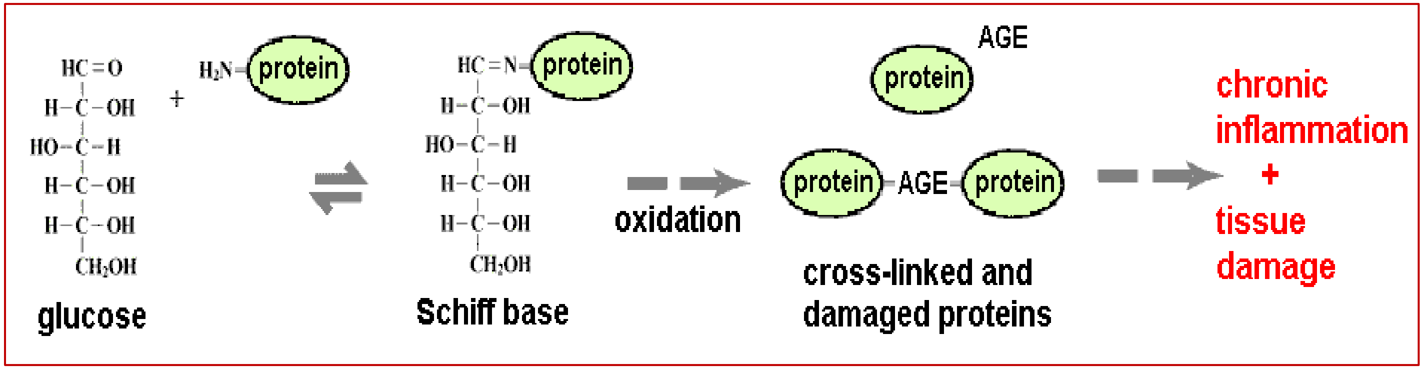
Hyperglycemia, Oxidative stress, AGEs

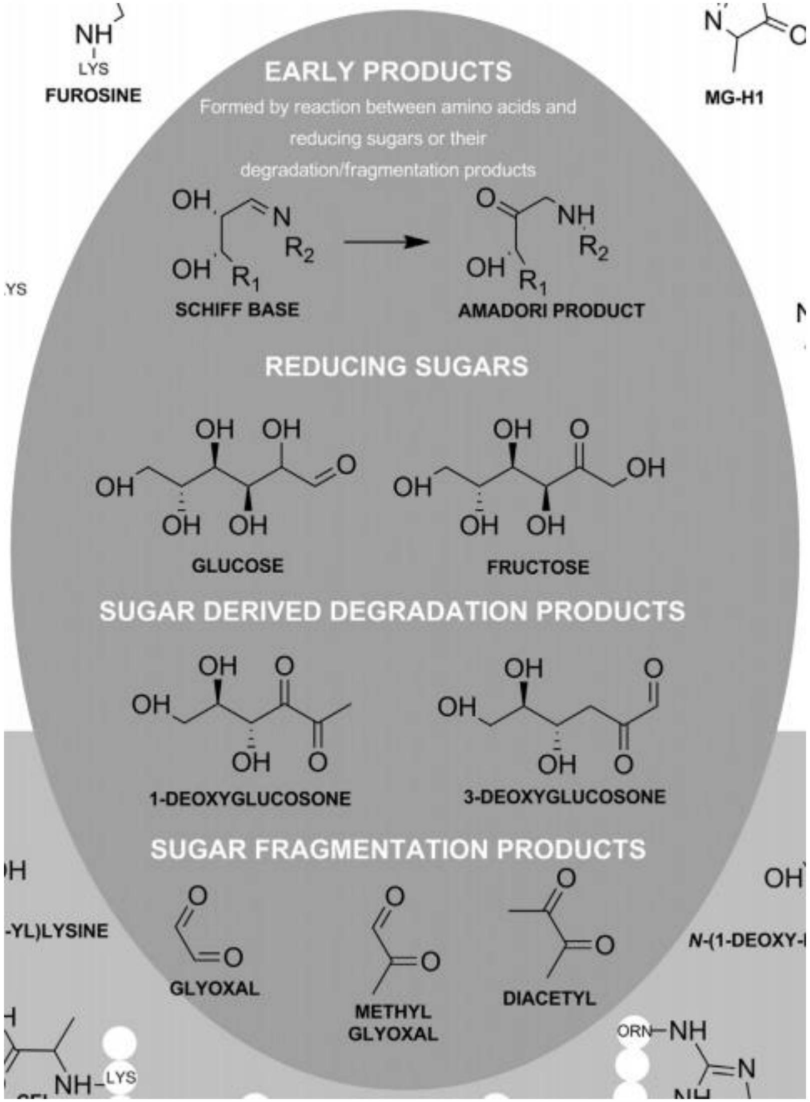




Advances in Experimental Medicine and Biology 2013, pp 272–287

Advanced glycation end-products (AGEs)

PROACTIVE KU

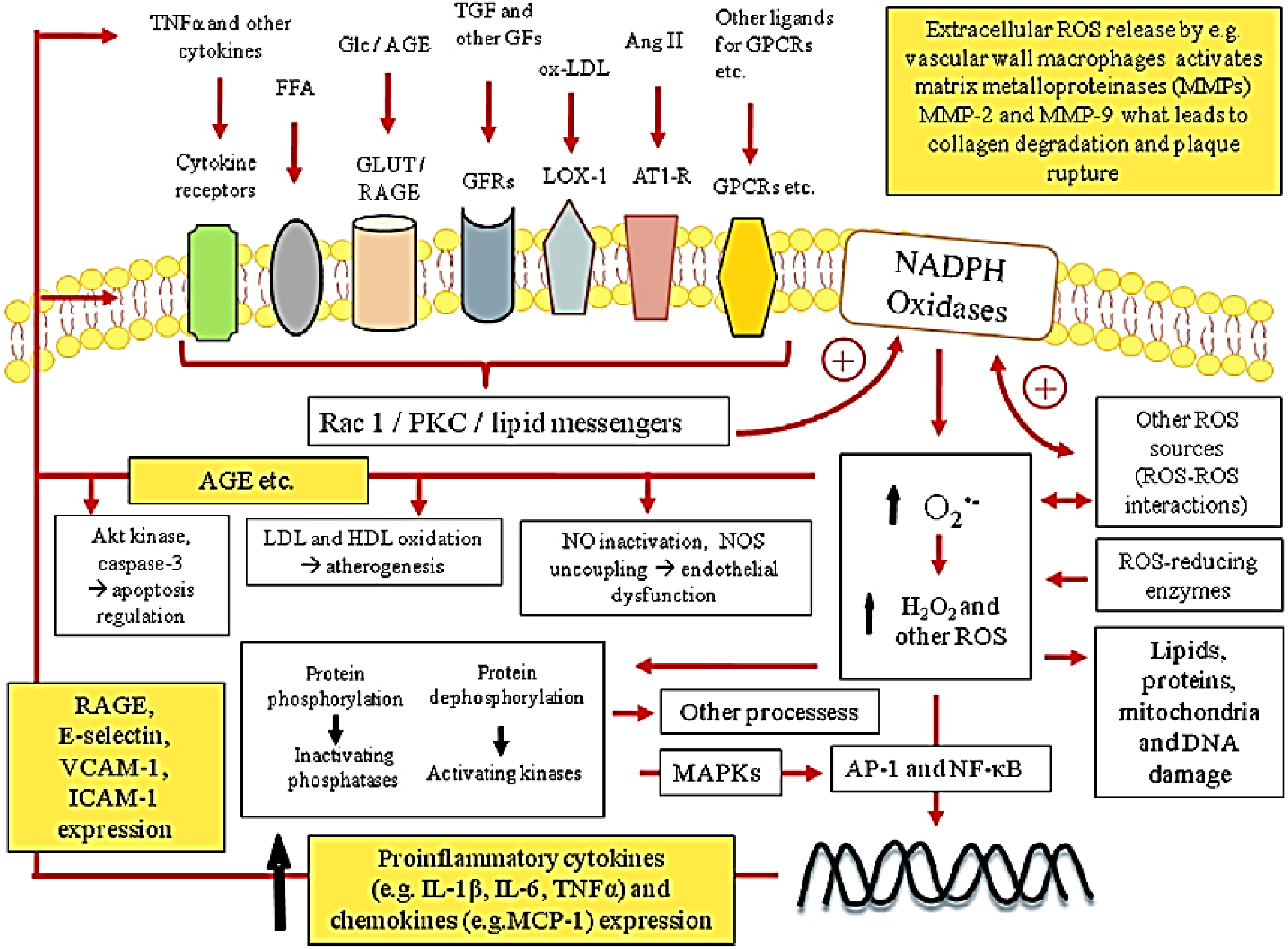


- AGEs can arise from intracellular auto-oxidation of glucose to glyoxal, decomposition of the Amadori product to 3-deoxyglu-cosone and nonenzymatic phos-phate elimination to form methylglyoxal.
- These reactive intracellular dicarbonyl react with amino groups of intracellular and extracellular proteins to form AGEs.

Food and Chemical Toxicology 60 (2013) 10–37

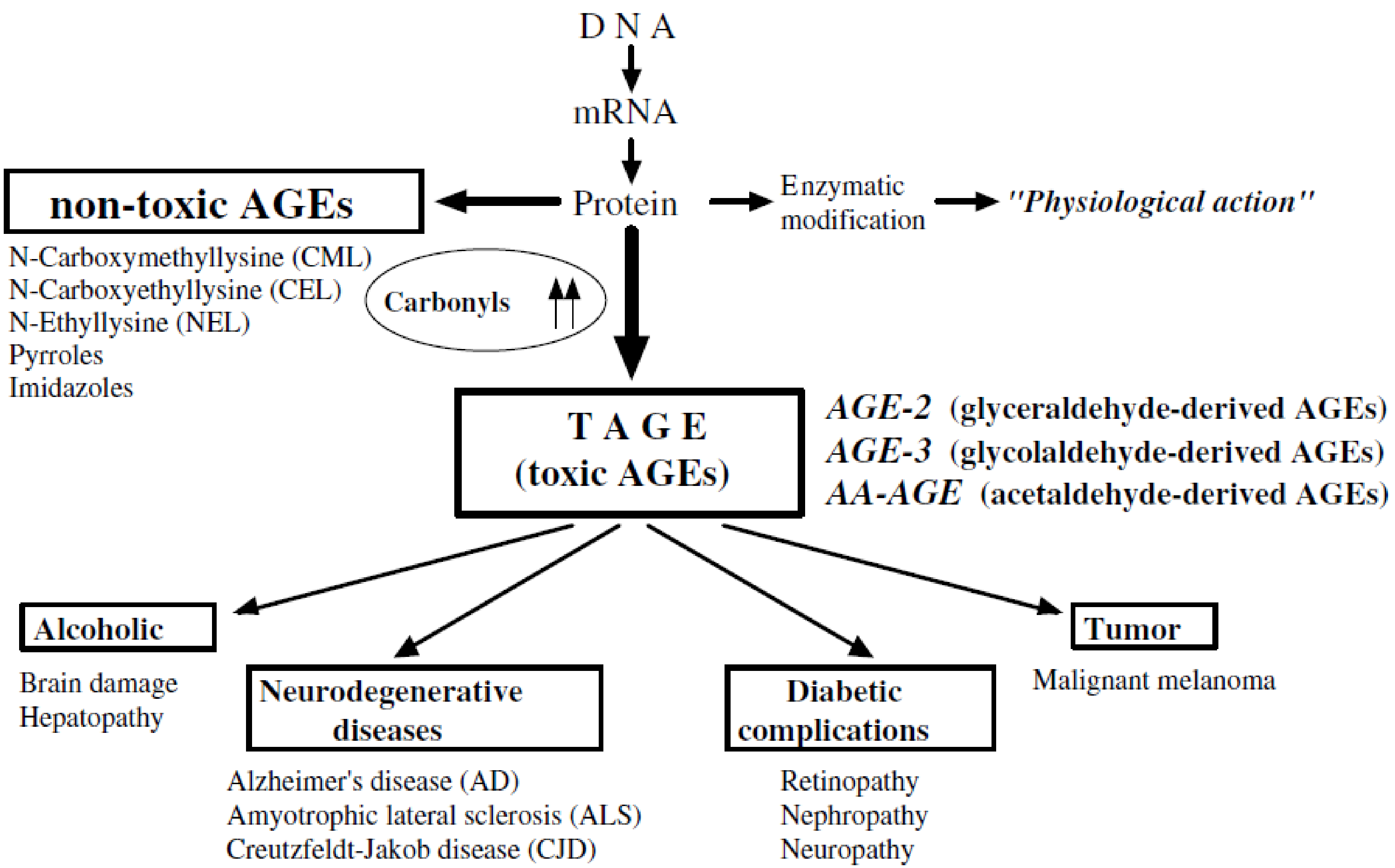
Production of AGE precursors can damage cells by three general mechanisms

- Intracellular proteins modified by AGEs have altered function
- Extracellular matrix components modified by AGE precursors interact abnormally with other matrix components and with matrix receptors that are expressed on the surface of cells
- Plasma proteins modified by AGE precursors bind to AGE receptors (such as RAGE and AGE-R1,2 and 3) on cells such as macrophages, vascular endothelial cells and vascular smooth muscle cells.
    - AGE receptors binding activates PKC, and induces the production of ROS.
    - It also activates NF-κB, and disturbs MAPK signaling



Vascular Pharmacology 56 (2012) 216–231

TAGE (toxic AGEs) hypothesis in various chronic diseases



Medical Hypotheses (2004) 63, 449–452

Endogenous/Dietary Sources

PROACTIVE KU

– Endogenous or dietary factors play a major role in the antioxidative defenses of the organism against the ROS generated during normal cellular aerobic respiration.

– As natural products have generally been proven to be relatively safe for human consumption, as compared to synthetic compounds, there has been an increasing interest in the use of natural plant compounds, such as anti-glycating agents .

– "Yellow Myroblan", "Asiatic plantain" and "Seaweed fulvescenes" are introduced in this presentation

I. Natural Inhibitors against Formation of Advanced Glycation Endproducts (AGEs)

Terminalia chebular (Yellow Myrobalan)

PROACTIVE KU

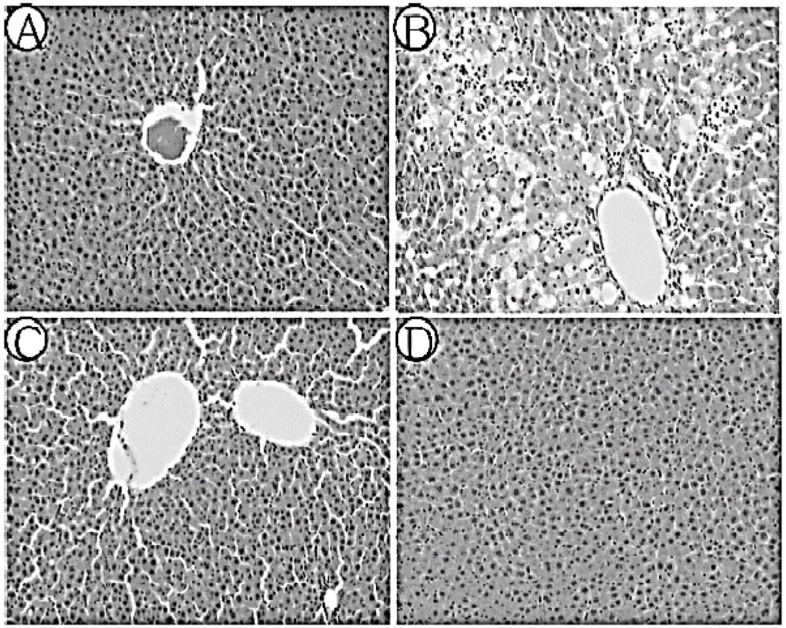
- Dried ripe fruit of *T. chebula*, which is a native plant in India and Southeast Asia
- Known as yellow myroblans in English and harad in Hindi
- A popular folk medicine for homeostatic, antitussive, laxative, diuretic, and cardiotonic treatments
- Exhibits *in vitro* antioxidant and free radical scavenging activities
- Antimicrobial, antiviral, anticancer, anti-anaphylaxis, and anti-diabetic activities have been reported

September 2005 *Biol. Pharm. Bull.* 28(9) 1639—1644 (2005)

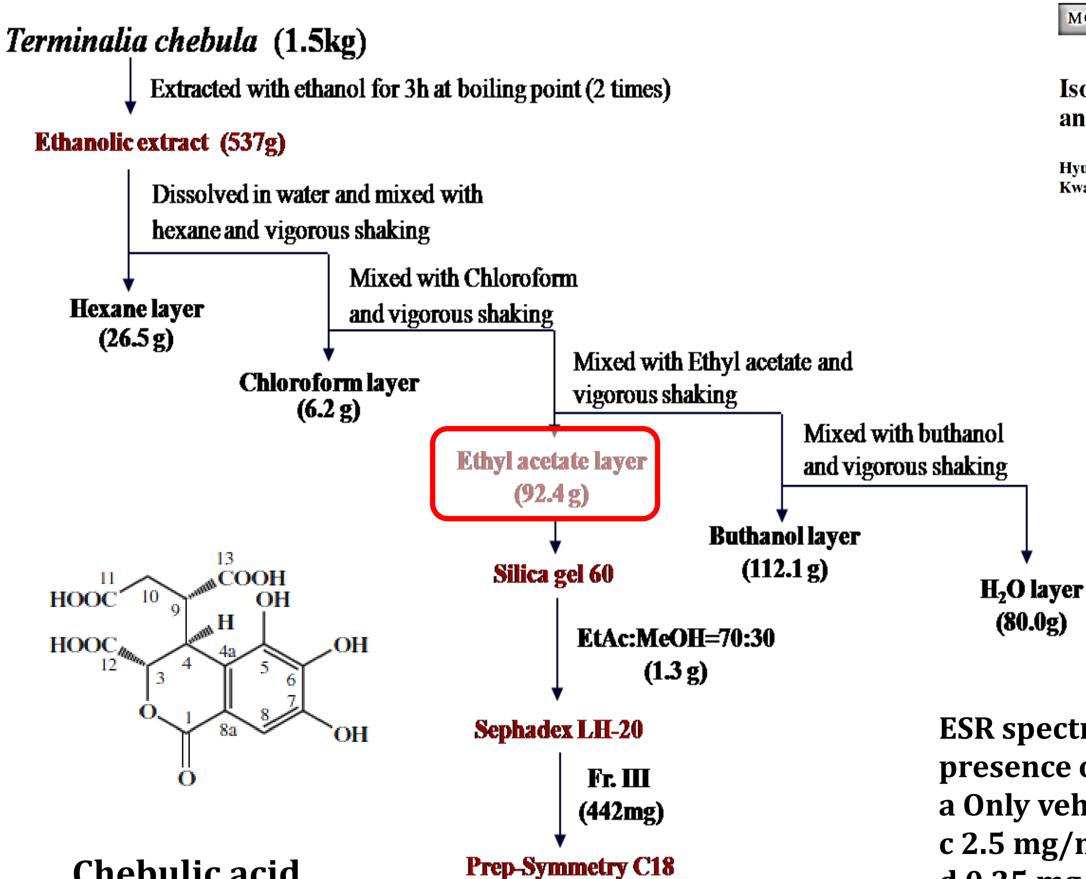
Antioxidant Effects of Aqueous Extract of *Terminalia chebula* *in Vivo* and *in Vitro*

Hyun-Sun LEE,[a] Nam Hee WON,[b] Kyoung Heon KIM,[a] Hojoung LEE[c], Woojin JUN,[d] and Kwang-Won LEE*,[a]

Treatment	AST (IU/l)	ALT (IU/l)	MDA (mmol/g liver)	Glutathione (nmol/mg protein)	
				Reduced	Oxidized (GSSG)
Untreated control	114.5±8.1	12.0±7.3	63.5±6.2	70.5±3.7	23.1±3.9
t-BHP (0.1 mmol/kg)	381.1±24.3[b)]	49.3±4.5[b)]	133±20[b)]	59.2±0.2[d)]	69.7±2.6[d)]
TCE[a)] (500 mg/kg)	148.9±25.6[c)]	35.6±5.0[c)]	79.4±8.8[c)]	69.8±10	18.3±3.9[e)]
(1000 mg/kg)	112.1±7.7[c)]	14.7±4.4[c)]	63.9±9.5[c)]	65.7±16	17.3±3.4[e)]



Chebulic acid from *Terminalia chebular*



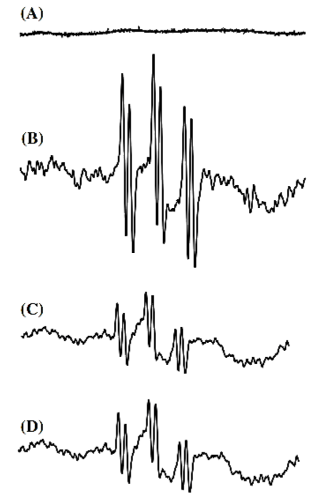
Chebulic acid

Arch Toxicol (2007) 81:211–218
DOI 10.1007/s00204-006-0139-4

MOLECULAR TOXICOLOGY

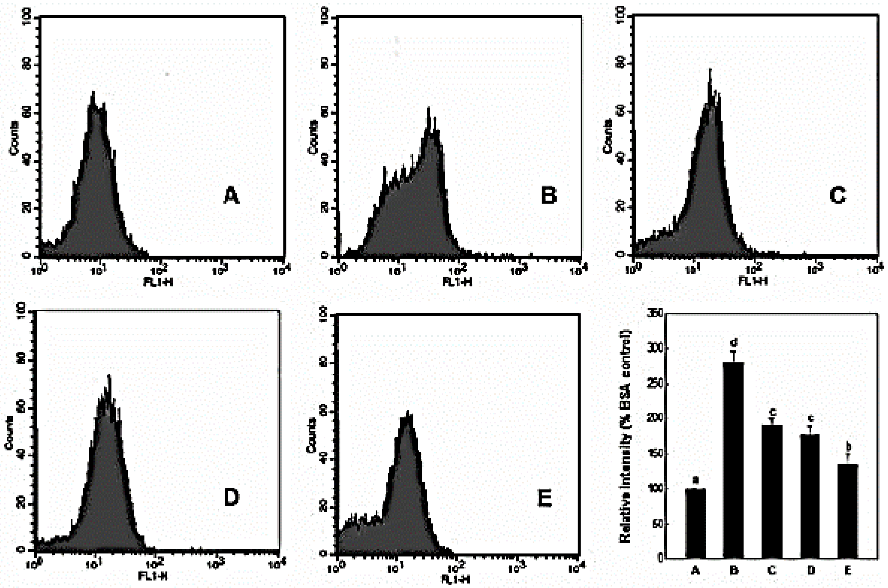
Isolation of chebulic acid from *Terminalia chebula* Retz. and its antioxidant effect in isolated rat hepatocytes

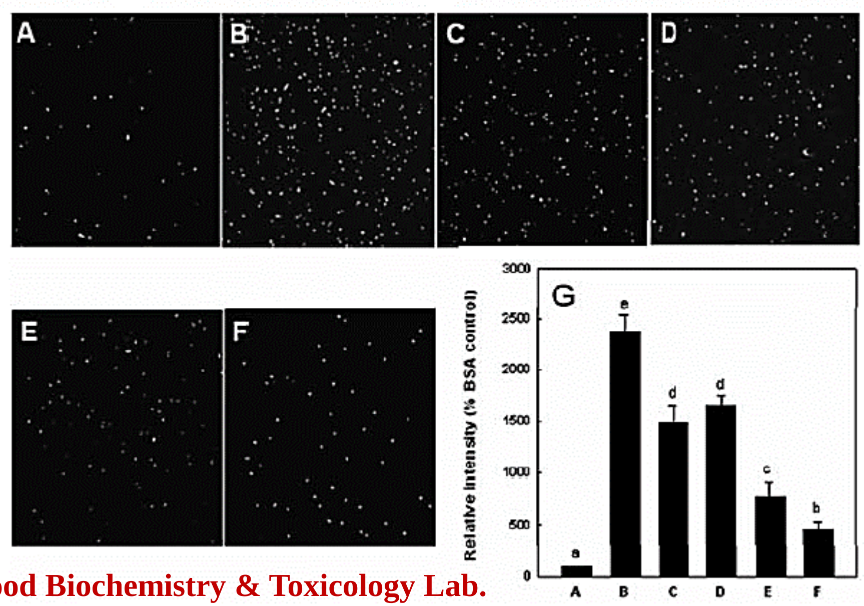
Hyun-Sun Lee · Sung-Hoon Jung · Bong-Sik Yun · Kwang-Won Lee



ESR spectrum observed after incubation for 90 s in the presence of 50 M *N-tert*-butyl—phenylnitron (BPN).
a Only vehicle, b 0.25 M *t*-BHP,
c 2.5 mg/ml chebulic acid and 0.25 M *t*-BHP,
d 0.25 mg/ml chebulic acid and 0.25 M *t*-BHP

	DPPH$^{\bullet}$ SC_{50}[a] (μg DM[b] ml^{-1})	FRAP[c] (mmol $FeSO_4 \cdot 7H_2O$ g^{-1} DM
Chebulic acid	48.5 ± 1.3	360.6 ± 1.4
EGCG	19.5 ± 1.7	417.5 ± 10.4





1060 Original Papers

Inhibitory Effects of *Terminalia chebula* Extract on Glycation and Endothelial Cell Adhesion

Lee H-S et al. Inhibitory Effects of … Planta Med 2011; 77: 1060–1067

Authors Hyun-Sun Lee[1], Hong Yon Cho[2], Kuen-Woo Park[3], In-Hwan Kim[4], Jong-Tak Kim[5], Mi-Hyun Nam[6], Kwang-Won Lee[6]

(A) HUVEC exposed to BSA
(B) 100 μg/mL glycer-AGE
(C) 100 μg/mL glycer-AGE + 5mM AG
(D) 100 μg/mL glycer-AGE + T. chebula 50 μg/mL
(E) 100 μg/mL glycer-AGE + T. chebula 100 μg/mL for 24h

(A) HUVEC exposed to BSA
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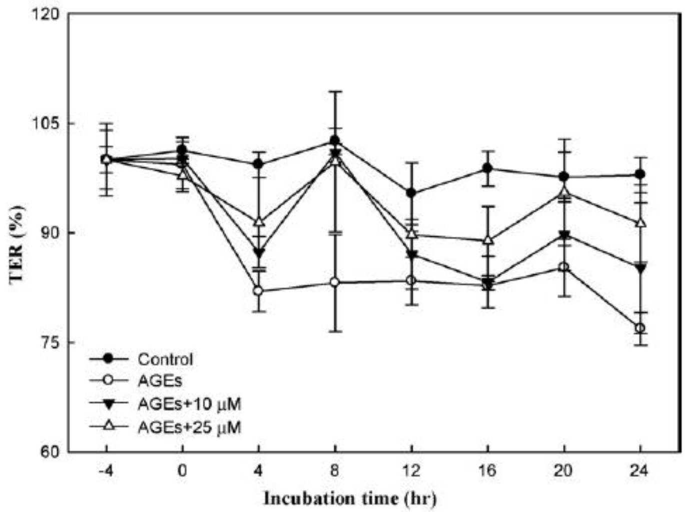
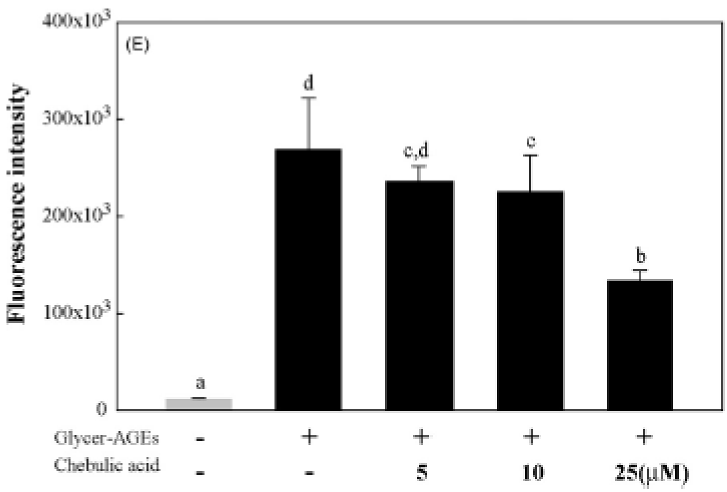
(D) 100 μg/mL glycer-AGE + T. chebula 50 μg/mL
(E) 100 μg/mL glycer-AGE + T. chebula 100 μg/mL
(F) 100 μg/mL glycer-AGE + 25 μM chebulic acid for 24h

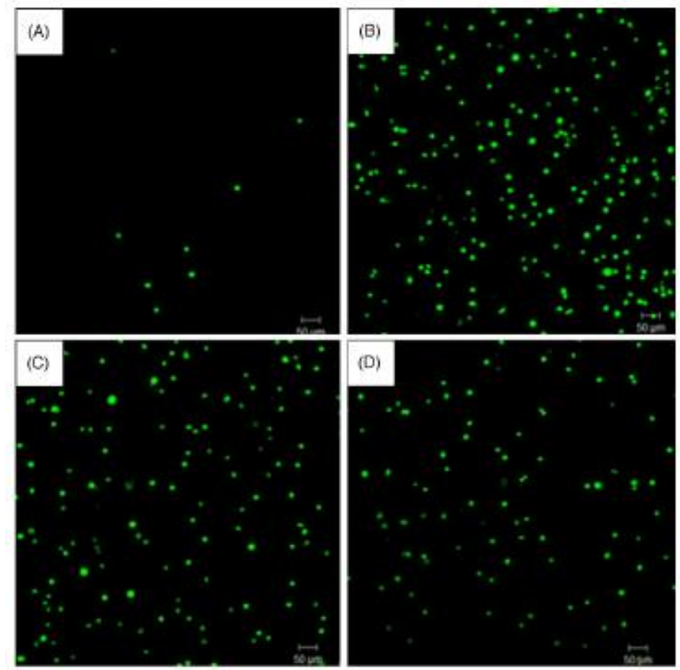




Journal of Ethnopharmacology 131 (2010) 567–574
Contents lists available at ScienceDirect
Journal of Ethnopharmacology
journal homepage: www.elsevier.com/locate/jethpharm
ELSEVIER

Preventive effects of chebulic acid isolated from *Terminalia chebula* on advanced glycation endproduct-induced endothelial cell dysfunction

Hyun-Sun Lee[a], Yoon-Chang Koo[d], Hyung Joo Suh[b], Kyung-Yong Kim[c], Kwang-Won Lee[d,*]

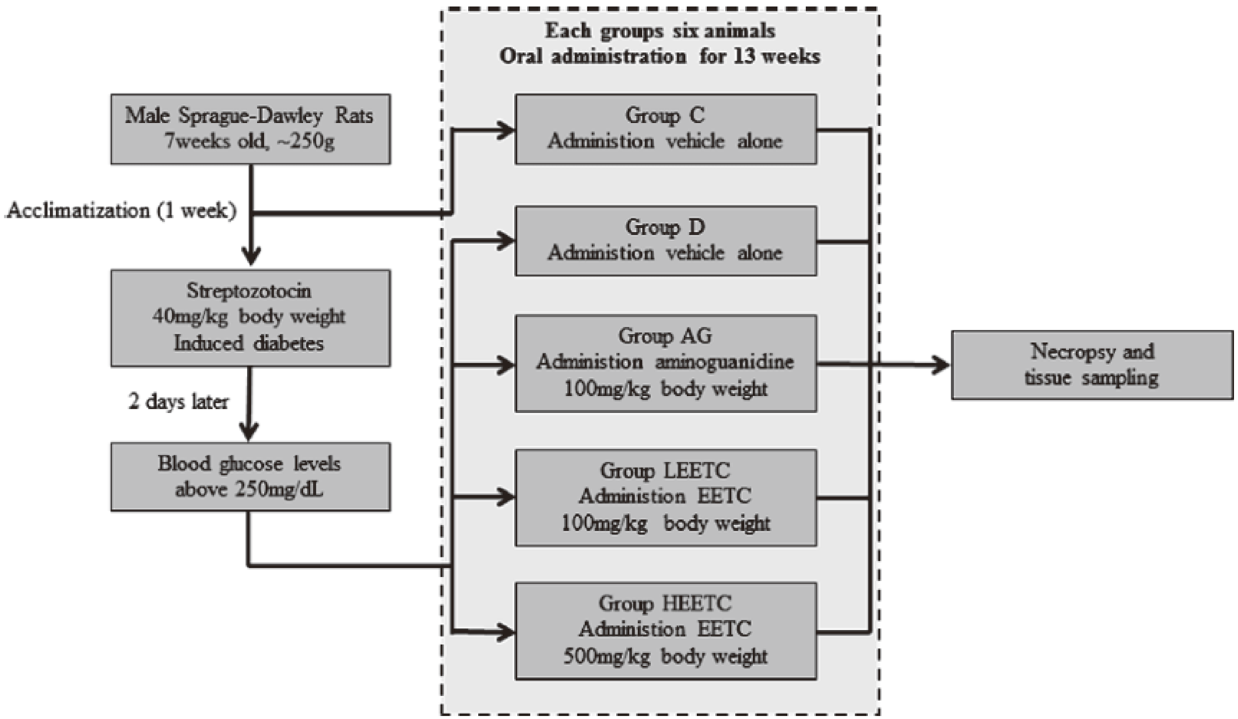


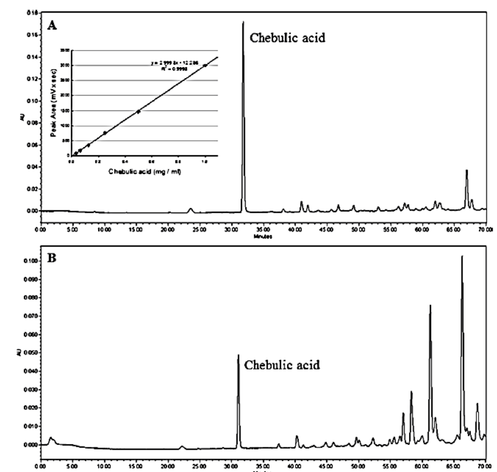
(A) HUVEC exposed to BSA
(B) 100 μg/mL glycer-AGE
(C) 100 μg/mL glycer-AGE + 10 μM chebulic acid
(D) 100 μg/mL glycer-AGE + 25 μM chebulic acid for 24h to the adhesion assay

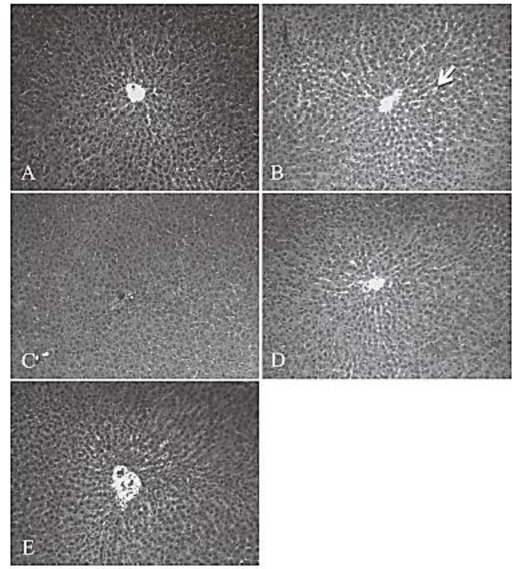
1702 Regular Article *Biol. Pharm. Bull.* 34(11) 1702—1709 (2011)

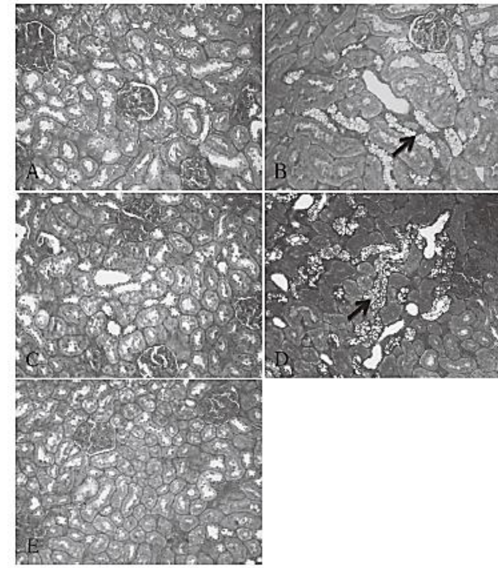
Oral Administration of Ethyl Acetate-Soluble Portion of *Terminalia chebula* Conferring Protection from Streptozotocin-Induced Diabetic Mellitus and Its Complications

Ji-hoon KIM, Chung-Oui HONG, Yun-chang KOO, Su-Jung KIM,† and Kwang-Won LEE*



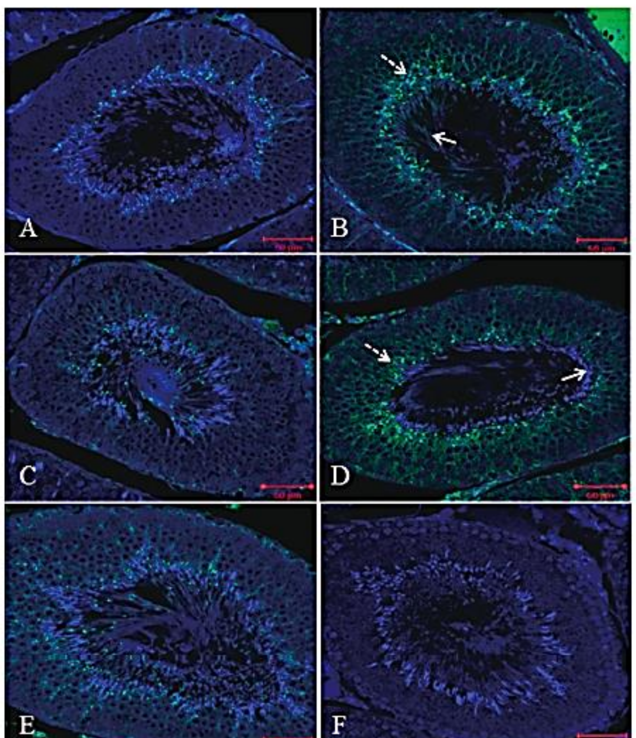




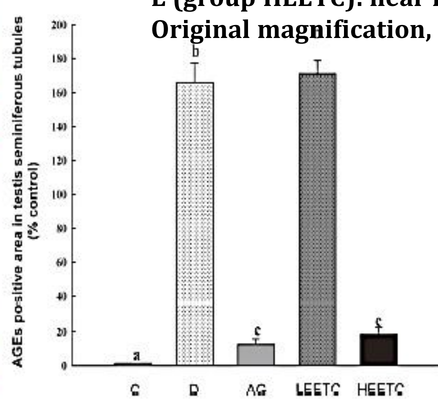


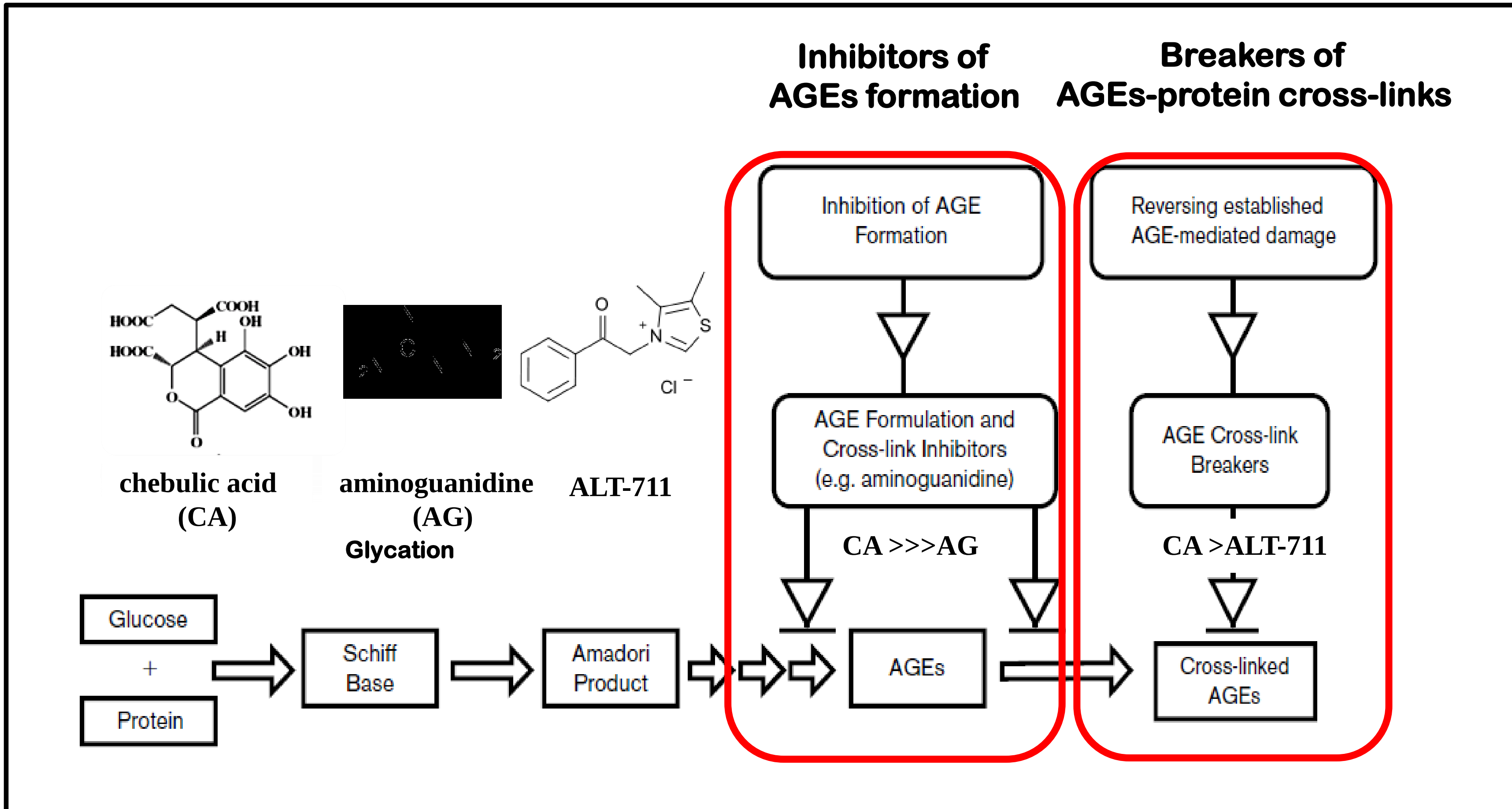
A (group C): normal;
B (group D): glycogen-granule accumulation (solid arrow) in tubular epithelium;
C (group AG): near normal appearance of kidney tubules;
D (group LEETC): glycogen-granule accumulation (solid arrow) in the tubular epithelium;
E (group HEETC): near normal appearance of kidney tubules.
Original magnification, x200.

(A)



(B)



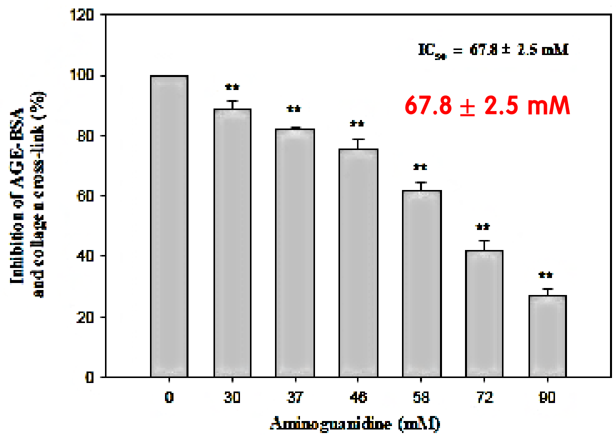

Inhibitors of
AGEs formation
Breakers of
AGEs-protein cross-links
chebulic acid
(CA)
aminoguanidine
(AG)
ALT-711
Glycation
Inhibition of AGE
Formation
Reversing established
AGE-mediated damage
AGE Formulation and
Cross-link Inhibitors
(e.g. aminoguanidine)
AGE Cross-link
Breakers
CA >>>AG
CA >ALT-711
Glucose
+
Protein
Schiff
Base
Amadori
Product
AGEs
Cross-linked
AGEs

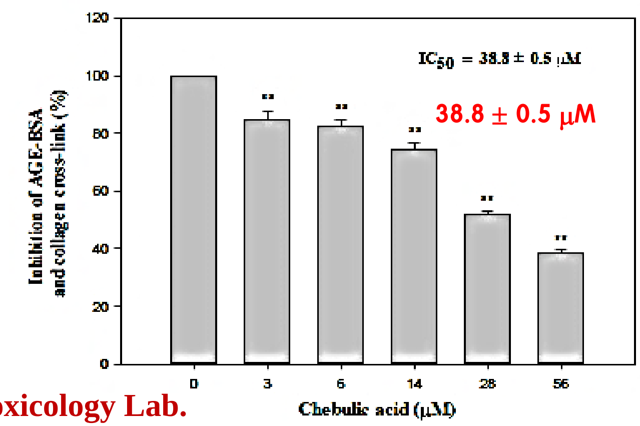
Inhibitory effect of chebulic acid (CA) on Glycol-BSA and collagen cross-link *in vitro*

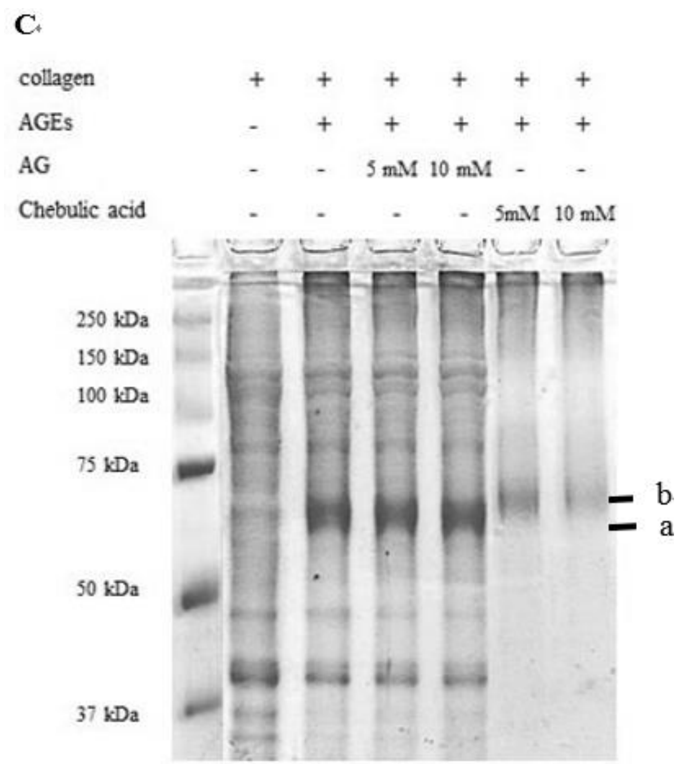
PROACTIVE KU

	DPPH• SC_{50}[a] (μg/DM[b] ml)	FRAP[c] (mmol $FeSO_4 \cdot 7H_2O$/g DM)
Chebulic acid	39.2±4.9	4.70±0.06
Ascorbic acid	19.0±1.2	11.4±0.1

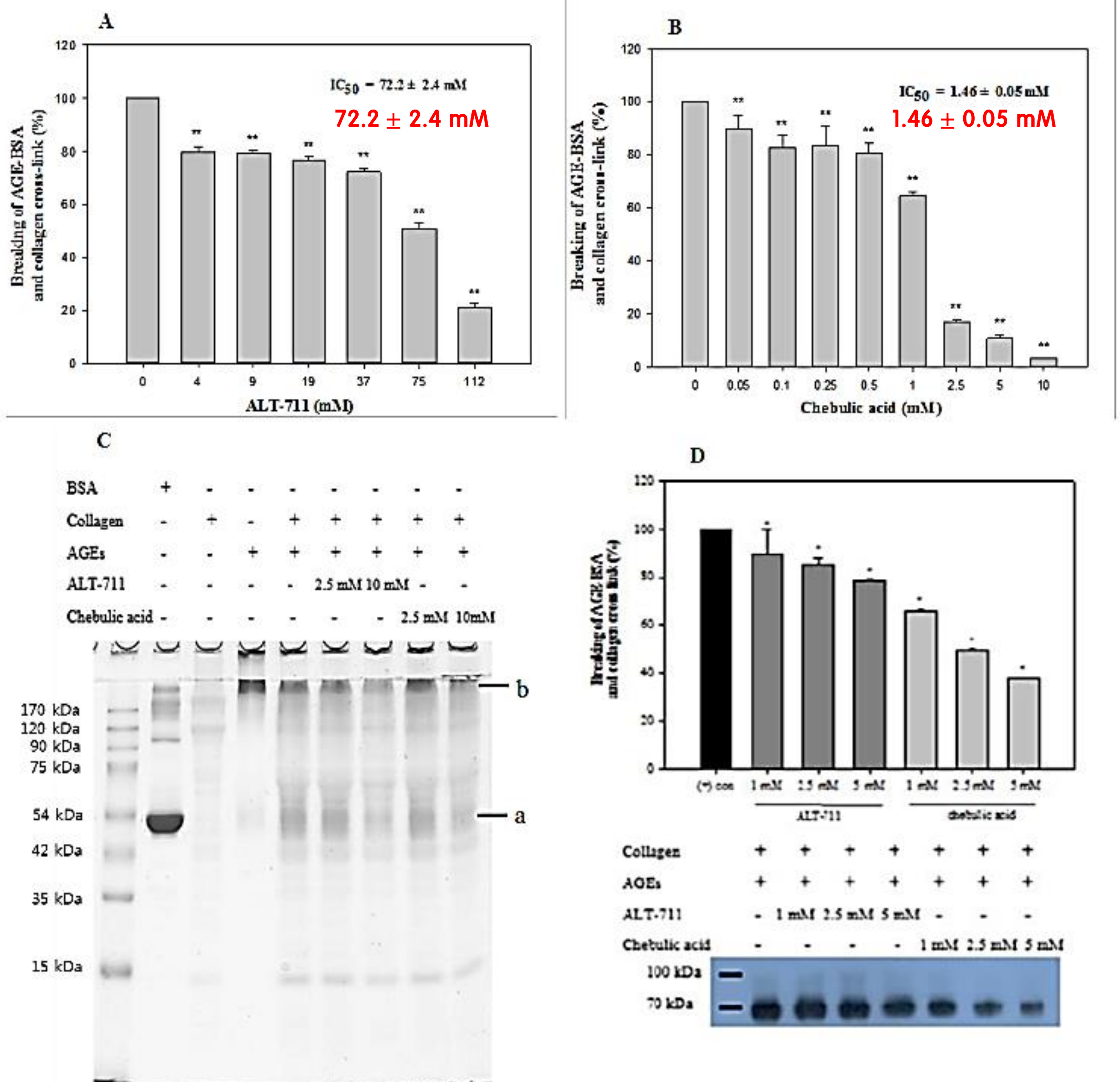
Each value is mean ± SD of three replicate experiments (*n* = 3)





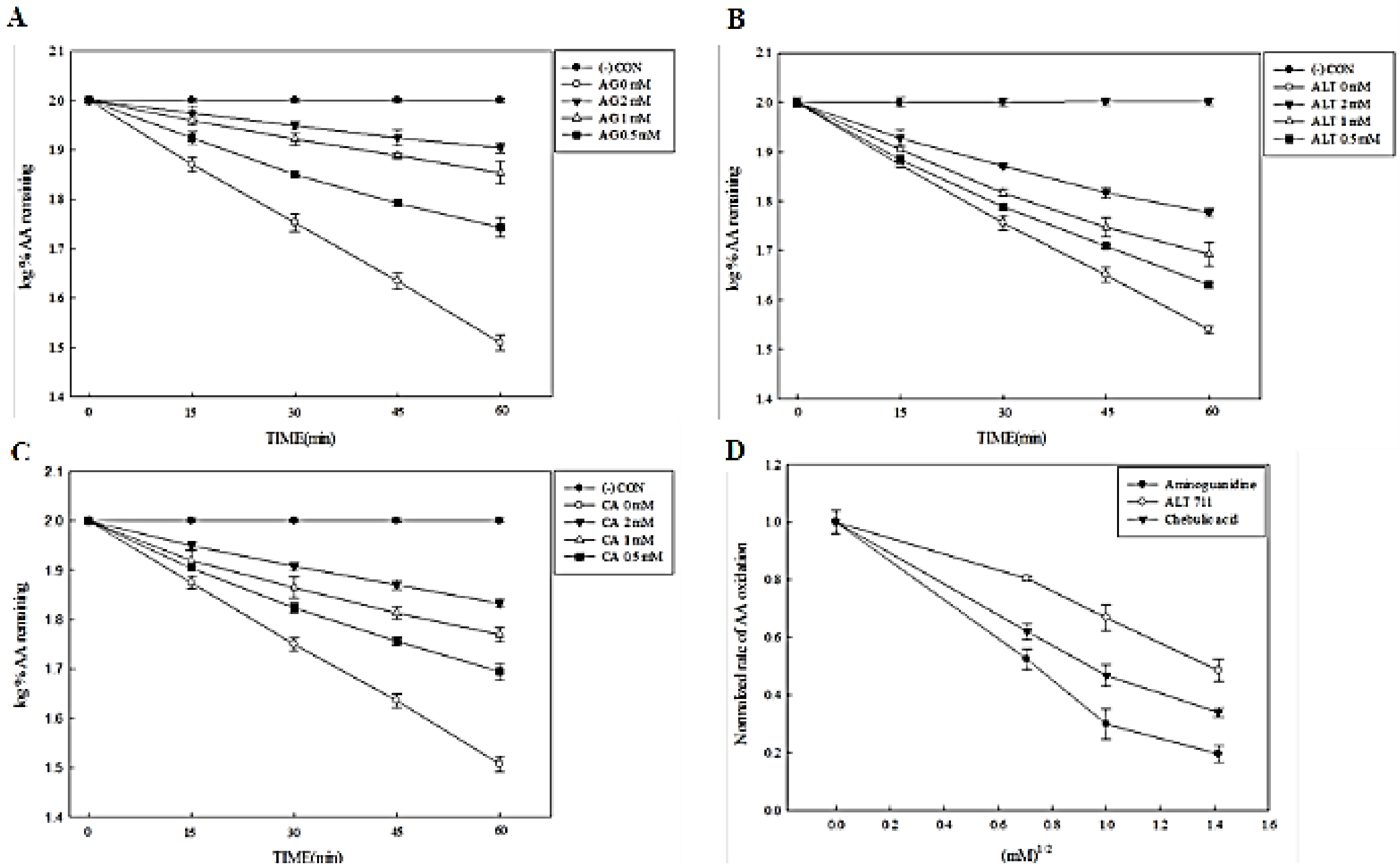


Breaking effect of CA on Glycol-BSA induced collagen cross-link *in vitro*



Kinetics of oxidation of ascorbic acid (AA) with and without different levels of AG (A), ALT 711

PROACTIVE KU

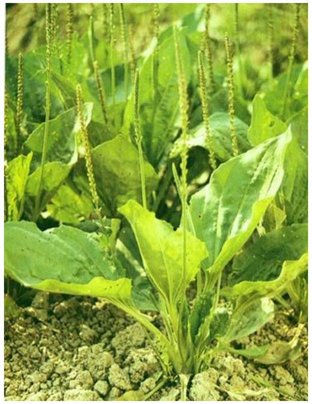


ALT-711(IC$_{50}$ of 1.92±0.20 mM) <CA (IC$_{50}$ of 0.96±0.07 mM) <AG (0.47±0.05 mM)

Plantago asiatica (Asiatic plantain)

PROACTIVE KU

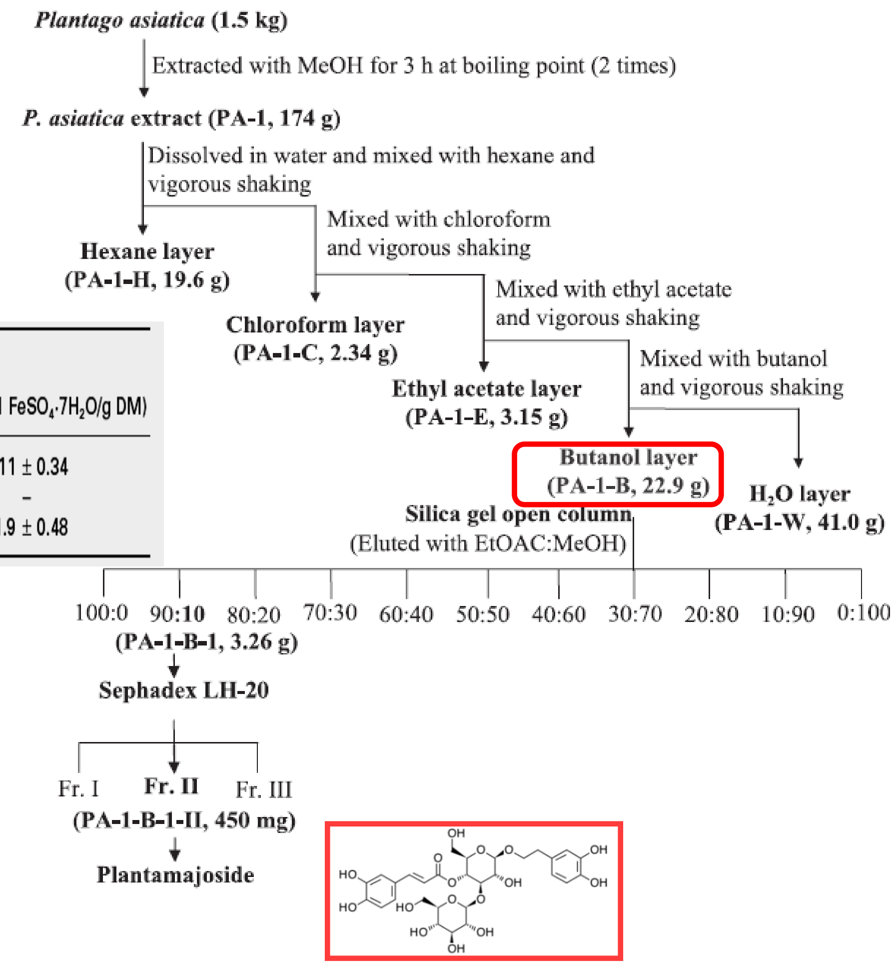
- A native plant in East Asia
- Traditionally been used as a popular folk medicine for nephritis, cough, eye disease, gonorrhea and heart disease
- Mostly known for antipyretic, antitussive, diuretic and wound healing properties



PROACTIVE KU

● Plantamajoside from *Plantago asiatica* (Phyto. Res. 22:323–329 (2008))

	Glycation inhibition (%)[b]			
	10 mM	25 mM	DPPH˙EC_{50} (μM)[c]	FRAP (mmol $FeSO_4 \cdot 7H_2O$/g DM)
Plantamajoside	95.2 ± 0.25	96.1 ± 0.20	36.7 ± 1.1	4.11 ± 0.34
Aminoguanidine	89.8 ± 0.65	95.0 ± 0.90	–	–
Ascorbate	–		80.6 ± 3.2	11.9 ± 0.48



Plantamajoside

J. Fd Hyg. Safety
Vol. 26, No. 3, pp. 260~265 (2011)

Protective Effect of *Plantago asiatica* L. Leaf Ethanolic Extract Against Ferric Nitrilotriacetate-Induced Prostate Oxidative Damage in Rats

Seung-Taek Hong, Chung-Oui Hong, Mi-Hyun Nam, YuanYuan Ma, Yun-Jin Hong, Da-Hee Son, Su-Hyun Chun and Kwang-Won Lee*

J. Fd Hyg. Safety
Vol. 26, No. 2, pp. 107~113 (2011)

Protective Effect of *Plantago asiatica* L. Extract Against Ferric Nitrilotriacetate (Fe-NTA) Induced Renal Oxidative Stress in Wistar Rats

Chung-Oui Hong, Seung-Teak Hong, Yun-Chang Koo, Sung-Yong Yang, Ji-Young Lee, Yanhouy Lee, Young-Min Ha, and Kwang-Won Lee*

Table 1. Effects of pre-treatment with *Plantago asiatica* extract (PAE) on Fe-NTA-mediated accretion of renal serum blood urea nitrogen (BUN) and serum creatinine content in rat

Treatment groups	BUN (mg/dL)	Creatinine (mg/dL)
Saline (control)	$18.54 \pm 1.29^{b**}$	$0.52 \pm 0.04^{b**}$
Fe-NTA (13.5 mg Fe/kg body weight)	116.82 ± 5.20^{a}	2.70 ± 0.20^{a}
PAE (1 g/kg body weight) + Fe-NTA (13.5 mg Fe/kg body weight)	$95.76 \pm 7.89^{c**}$	$2.16 \pm 0.34^{c*}$
PAE (2 g/kg body weight) + Fe-NTA (13.5 mg Fe/kg body weight)	89.34 ± 26.93^{ac}	$2.20 \pm 0.51^{ac*}$
PAE (4 g/kg body weight) + Fe-NTA (13.5 mg Fe/kg body weight)	$39.54 \pm 21.93^{d**}$	$1.16 \pm 0.55^{d**}$

Values are expressed as mean ± SD (n = 5), $*P < 0.05$, $**P < 0.01$ compared with rat treated with Fe-NTA alone.

Table 1. Effects of pretreatment with *Plantago asiatica* leaf extract (PLE) on Fe-NTA-mediated depletion of prostate glutathione content and decrease in the activities of glutathione metabolizing enzymes, glutathione-S-transferase and glutathione reductase in rat

Treatment groups	Reduced glutathione (mmol GSH/g tissue)	Glutathione-S-transferase (Units/g tissue)	Glutathione reductase (Units/g tissue)
Saline (control)	193.84 ± 37.78^{b**}	96.11 ± 6.23^{b***}	31.32 ± 3.85^{b***}
Fe-NTA (0.24 mmol Fe/kg body weight)	101.87 ± 24.31	53.29 ± 11.45	15.74 ± 2.92
PLE (1 g/kg body weight) + Fe-NTA (0.24 mmol Fe/kg body weight)	119.01 ± 1.23	65.74 ± 9.79	19.24 ± 0.53
PLE (2 g/kg body weight) + Fe-NTA (0.24 mmol Fe/kg body weight)	150.80 ± 34.11	76.54 ± 4.44^{b**}	19.17 ± 3.31
PLE (4 g/kg body weight) + Fe-NTA (0.24 mmol Fe/kg body weight)	182.99 ± 10.89^{b**}	91.66 ± 5.53^{b***}	26.88 ± 4.40^{b**}

Value are expressed as mean ± SD (n = 3), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with rat treated with Fe-NTA alone.

Table 2. Effects of pre-treatment with *Plantago asiatica* extract (PAE) on Fe-NTA-mediated depletion of renal glutathione content and decrease in the activities of glutathione metabolizing enzymes, glutathione-S-transferase and glutathione reductase in rat

Treatment groups	Reduced glutathione (mmol GSH/g tissue)	Glutathione-S-transferase (Units/g tissue)	Glutathione reductase (Units/g tissue)
Saline (control)	142.82 ± 16.51^{b**}	124.69 ± 13.07^{b**}	107.31 ± 8.70^{b**}
Fe-NTA (13.5 mg Fe/kg body weight)	59.45 ± 12.32^{a}	49.88 ± 4.55^{a}	56.70 ± 5.40^{a}
PAE (1 g/kg body weight) + Fe-NTA (13.5 mg Fe/kg body weight)	77.86 ± 12.62^{ac}	66.59 ± 5.01^{ad*}	67.37 ± 8.66^{c}
PAE (2 g/kg body weight) + Fe-NTA (13.5 mg Fe/kg body weight)	123.11 ± 12.72^{b**}	83.25 ± 8.38^{cd**}	80.34 ± 6.06^{cd**}
PAE (4 g/kg body weight) + Fe-NTA (13.5 mg Fe/kg body weight)	147.97 ± 26.27^{b**}	124.68 ± 13.67^{b**}	98.67 ± 10.11^{bd**}

Values are expressed as mean ± SD (n=3), $*P < 0.05$, $**P < 0.01$ compared with rat treated with Fe-NTA alone.

NUTRITIONRESEARCH33(2013)704–710

A beverage of Asiatic plantain extracts alleviated postprandial oxidative stress in overweight hyperlipidemic subjects challenged with a high-fat meal: a preliminary study

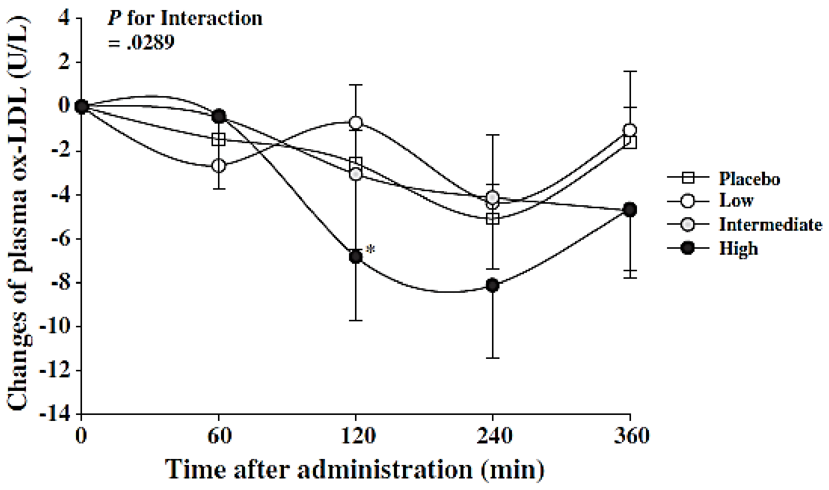
Yeni Lim[a], Kwang Won Lee[b], Ji Yeon Kim[c,*], Oran Kwon[a,*]

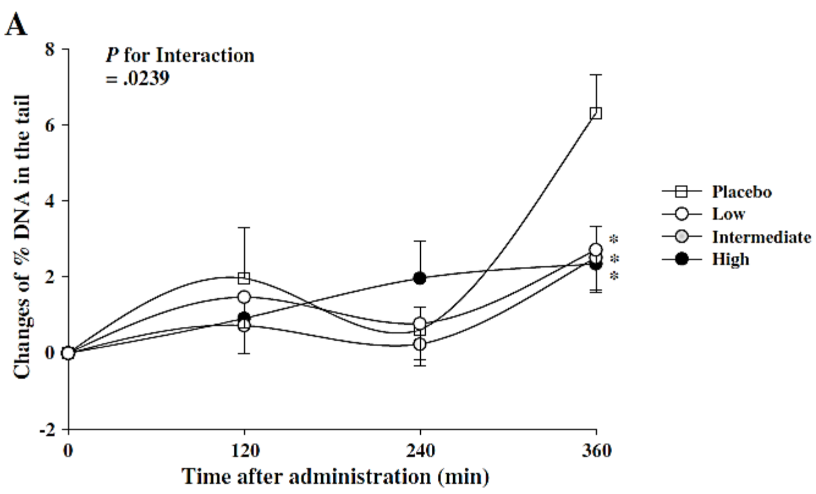
A randomized, double-blind, placebo-controlled parallel design

Table – Baseline characteristics of subjects prior to the study

Variable	Placebo (n = 10)	Asiatic plantain			P
		Low (n = 10)	Intermediate (n = 10)	High (n = 10)	
Sex (male/female)	7/3	3/7	6/4	5/5	–
Age (y)	42.40 ± 3.41	45.30 ± 2.94	38.50 ± 1.93	43.60 ± 3.77	.4633
Height (cm)	164.96 ± 2.51	166.72 ± 2.28	163.36 ± 3.25	167.23 ± 2.65	.7336
Weight (kg)	76.36 ± 2.20	76.75 ± 3.06	75.65 ± 2.78	81.12 ± 3.34	.2954
BMI (kg/m^2)	26.97 ± 0.62	27.53 ± 0.59	28.40 ± 0.88	28.91 ± 0.56	.1914
SBP (mm Hg)	123.50 ± 4.56	122.80 ± 2.44	128.60 ± 3.61	128.60 ± 3.44	.5162
DBP (mm Hg)	72.5 ± 2.73	75.40 ± 2.17	77.90 ± 3.04	78.00 ± 2.80	.4396
FBG (mg/dL)	83.50 ± 4.25	95.80 ± 7.13	89.90 ± 4.36	91.90 ± 4.53	.4158
TC (mg/dL)	202.20 ± 7.52	217.40 ±15.22	228.10 ± 5.45	236.80 ± 7.52	.0863
TG (mg/dL)	135.00 ±18.64	163.50 ±10.03	138.80 ±18.31	170.00 ±21.07	.4046
HDL-C (mg/dL)	51.00 ± 2.05	48.60 ± 4.73	54.10 ± 5.43	46.40 ± 1.88	.5393
LDL-C (mg/dL)	128.00 ± 6.78	139.60 ±12.92	147.90 ± 4.92	161.00 ± 6.96	.0598

- High fat meal (976 kcal, 51% from fat)
- Low 5 g, Intermediate 10 g, High 20 g doses of PAE or a placebo per 80 mL
- Blood samples were obtained at fasting and 60, 120, 240, and 360 minutes (total of 5 samples) after intervention.



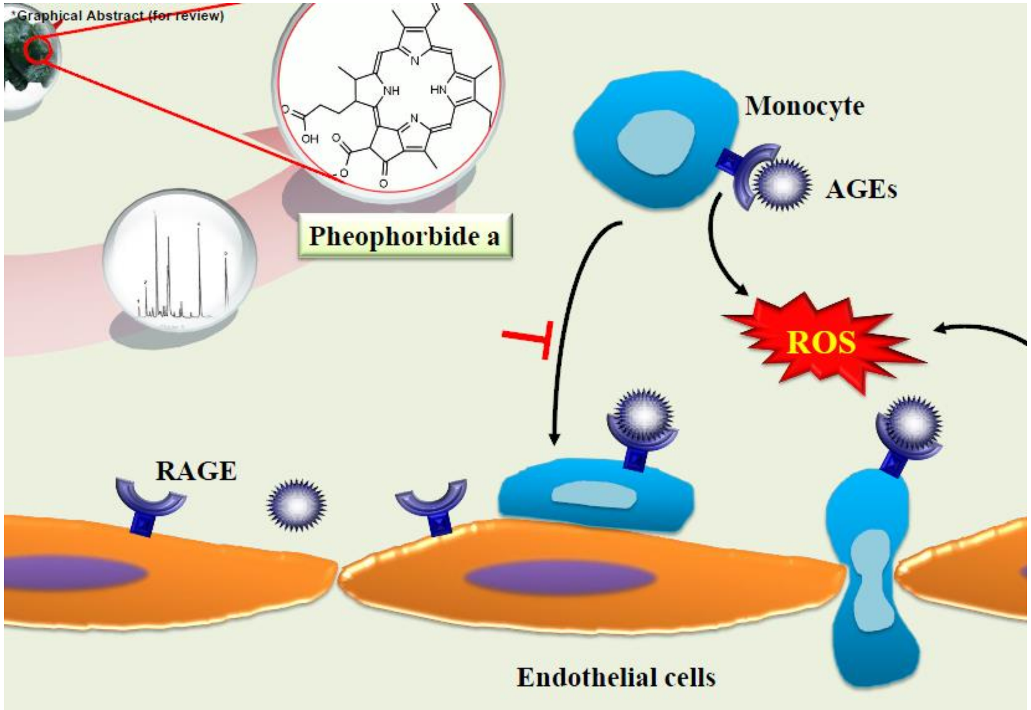


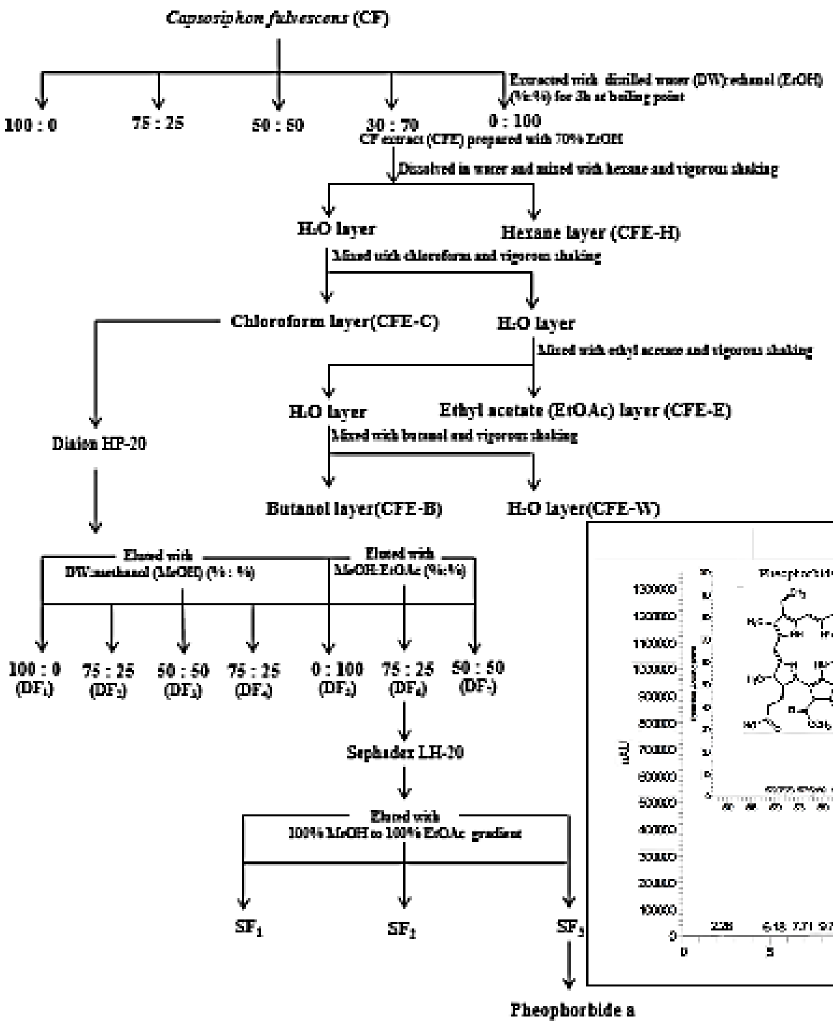
Capsosiphon fulvescens (Seaweed fulvescenes, Maesaengi)

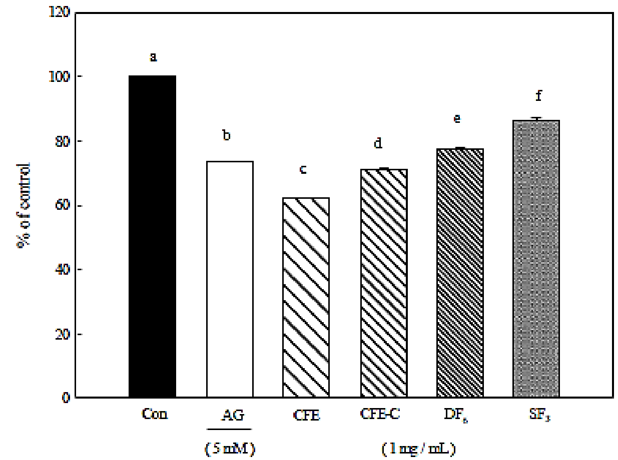
- A native seaweed in South Korea
- Hypocholesterolemic effect
- Heavy metal binding activity and high contents of basic ions (K^{+}, Ca^{2+}, Fe^{2+})

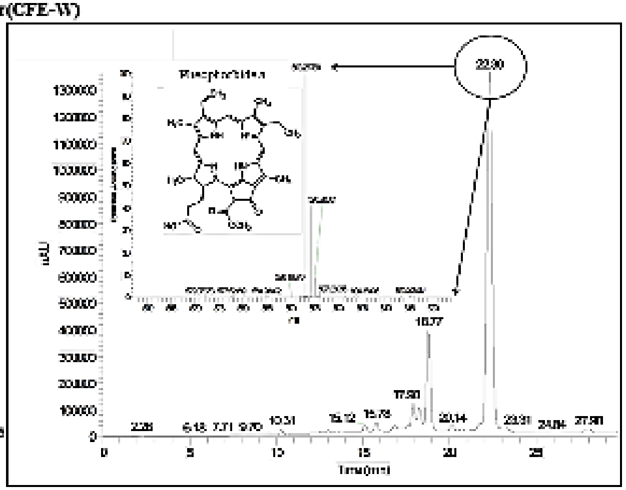
Bioorganic & Medicinal Chemistry Letters (under revision)

Pheophorbide a from *Capsosiphon fulvescens* down-regulates vascular endothelium dysfunction and advanced glycation endproducts mediated proinflammatory cytokines

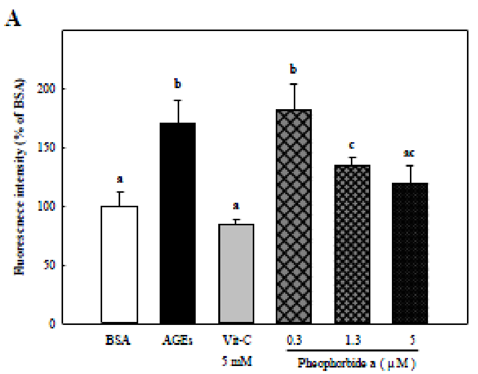



Capsosiphon fulvescens (CF)
Extracted with distilled water (DW):ethanol (EtOH) (%:%) for 3h at boiling point
100 : 0
75 : 25
50 : 50
30 : 70
0 : 100
CF extract (CFE) prepared with 70% EtOH
Dissolved in water and mixed with hexane and vigorous shaking
H2O layer
Hexane layer (CFE-H)
Mixed with chloroform and vigorous shaking
Chloroform layer(CFE-C)
H2O layer
Mixed with ethyl acetate and vigorous shaking
H2O layer
Ethyl acetate (EtOAc) layer (CFE-E)
Mixed with butanol and vigorous shaking
Butanol layer(CFE-B)
H2O layer(CFE-W)
Diaion HP-20
Eluted with DW:methanol (MeOH) (%:%)
Eluted with MeOH:EtOAc (%:%)
100 : 0 (DF1)
75 : 25 (DF2)
50 : 50 (DF3)
75 : 25 (DF4)
0 : 100 (DF5)
75 : 25 (DF6)
50 : 50 (DF7)
Sephadex LH-20
Eluted with 100% MeOH to 100% EtOAc gradient
SF1
SF2
SF3
Pheophorbide a

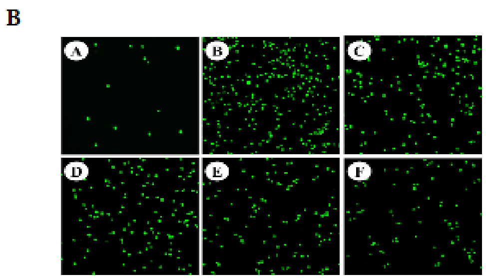

% of control
120
100
80
60
40
20
0
a
b
c
d
e
f
Con
AG
CFE
CFE-C
DF6
SF3
(5 mM)
(1 mg / mL)

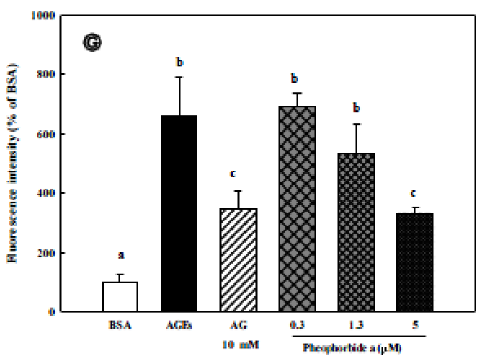

Pheophorbide a
22.30
18.77
mAU
Time(min)
0
5
10
15
20
25

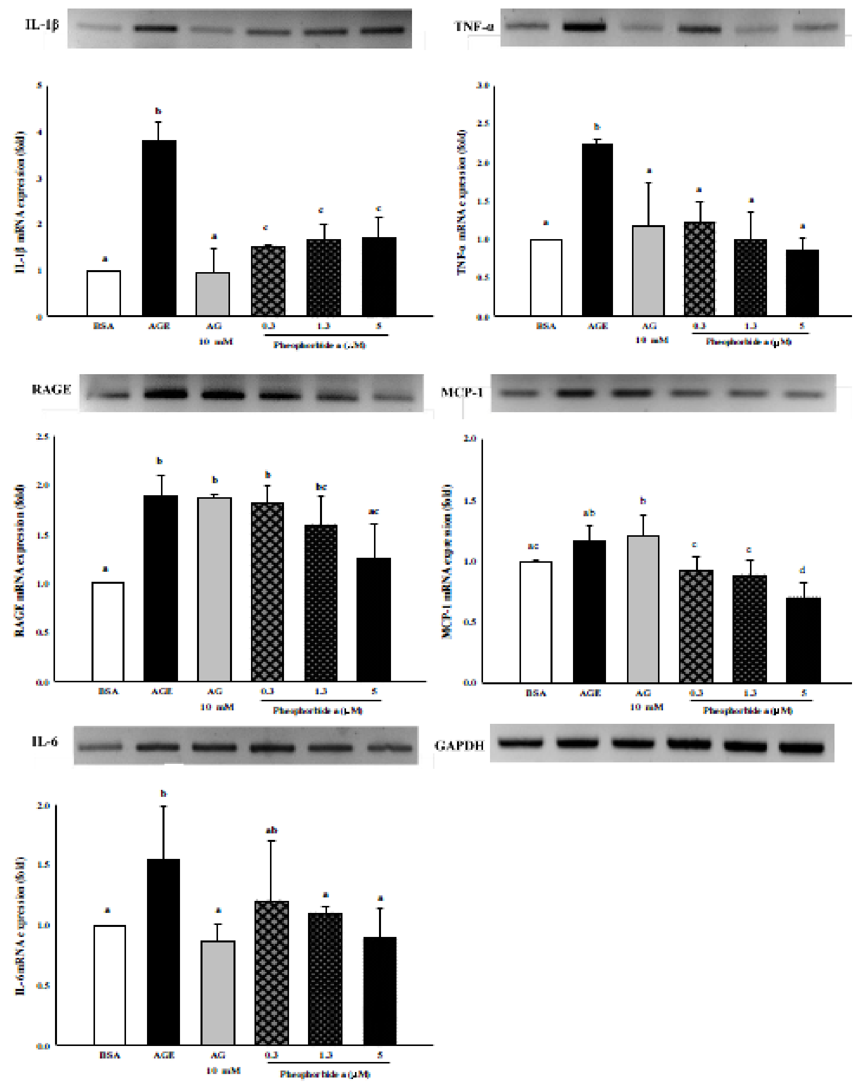
A


Fluorescence intensity (% of BSA)
BSA
AGEs
Vit-C
5 mM
0.3
1.3
5
Pheophorbide a (μM)

B


A
B
C
D
E
F


G
Fluorescence intensity (% of BSA)
BSA
AGEs
AG
10 mM
0.3
1.3
5
Pheophorbide a (μM)

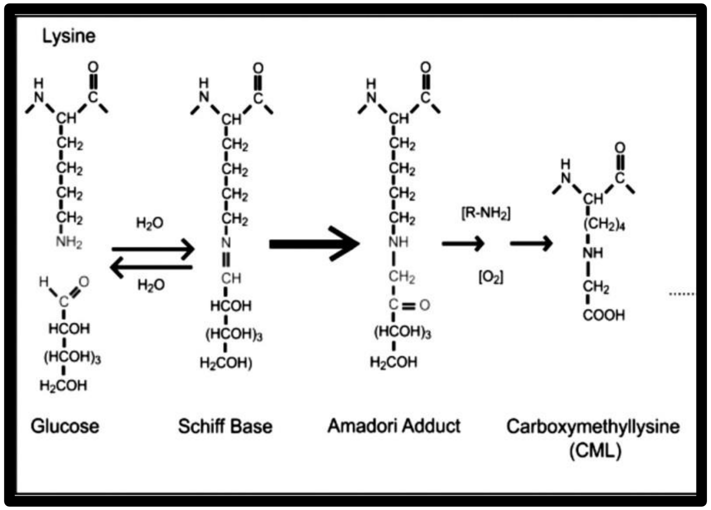

IL-1β
TNF-α
RAGE
MCP-1
IL-6
GAPDH
BSA
AGE
AG
10 mM
0.3
1.3
5

II. Determination of N^{ε}-(carboxymethyl)lysine in Representative Korean Food

N^{ε}-(carboxymethyl)lysine (*CML)*

PROACTIVE KU

- **N^{ε}-carboxymethyl-lysine (CML) is a frequently used marker of AGEs formed in food (or endogenously in living organisms)**
- **A diet rich in AGE contributes to the body's AGE pool and may contribute to these health effects**
- **Although it is generally assumed CML (as an AGE) binds to RAGE in the human body, this is not absolutely certain and should be evidenced.**



- **Assuming the body's CML interacts with the receptor RAGE, dietary CML impairs human health.**

- **CML is one of the most widely studied AGE, and is frequently used as a marker for AGE formation in food.**
- **It is stable and exists in the bound form.**
- **It is known that dietary CML contributes to the body's CML pool.**
- **Therefore, it is worthwhile to obtain scientific insight into aspects related to dietary CML with the view of reducing as much as possible the presence of this compound in food products.**

Determination of N^{ε}-(carboxymethyl)lysine in representative Korean food by a modified HPLC

PROACTIVE KU

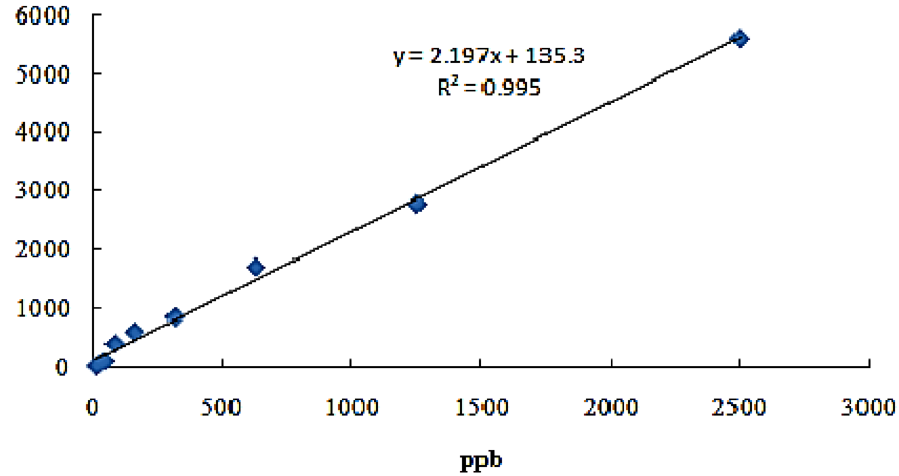
– **Five main ingredient of food that mostly consumed was selected according to 2008 National Health Statistics by Korean** (Korea Centers Disease Control & Prevention, 2008)
  1. Rice of grains
  2. Soy bean of legume
  3. Pork and beef of meat
  4. Mackerel of fish
  5. Egg of chicken

– **Two cooking recipes**
  1. Boiling or steam
  2. Grilling



<Table 4> Optimal conditions of HPLC analysis

Apparatus	Varian(model 210)
Column	Waters Spherisorb® 5um ODS2(4.6×250mm, Analytical column)
Mobile phase	A: D. W.
	B: MeOH
Gradient condition	0min: A=100%, B=0%
	15min: A=50%, B=50%
	16min: A=0%, B=100%
	20min: A=0%, B=100%
Flow rate	0.5ml/min
Injection volume	20µl
Dectector	Fluorescence(excitation: 340nm, emisstion: 455nm)
o-phtalaldehyde(OPA)	8mM



<Figure 4> Area-dependent CML standard calibration

<Table 6> The value of LOD and LOQ-according to area base

Noise (LU)	Average (LU)	Noise area(χ)[a] (LU)	LOQ area (Noise area×10, LU)	Ratio[b]	LOQ concentration (ppb)	LOD concentration (ppb, LOQ/3)
0.0015						
0.0015						
0.0016	0.0015	0.0971	0.9707	23.5162	0.4153	0.1384
0.0014						
0.0017						

[a] Noise area(χ)=(Noise height×Known area)/ Known height. This equation derived by next relation; Noise height: Noise area(X)=Known height: Known area

[b] Ratio was calculated using known result of 9.7656ppb CML(area: 22.8283LU).

CML content in representative foods

PROACTIVE KU

Foods	CML (mg/100 g protein)
Godeungeo-jorim (braised mackerel in soy sauce)	585.34 ± 55.92
Samgyeopsal (Korean-style bacon)	558.14 ± 74.75
Godeungeo-gui (broiled mackerel)	281.72 ± 11.06
Bulgogi (roasted beef)	248.92 ± 62.30
Suyuk (boiled pork)	247.71 ± 67.00
Dubu-jorim (braised pan-fried tofu in soy sauce)	99.20 ± 6.60
Gyeran-jjim (steamed egg)	89.42 ± 34.41
Gyeran-mari (rolled omelette)	63.52 ± 9.33
Nokdujeon (mung-bean pancake)	63.17 ± 11.31
Sirutteok (steamed rice cake)	19.07 ± 6.01
Bab (cooked white rice)	10.31 ± 1.45

III. Health Effects of Dietary MRPs

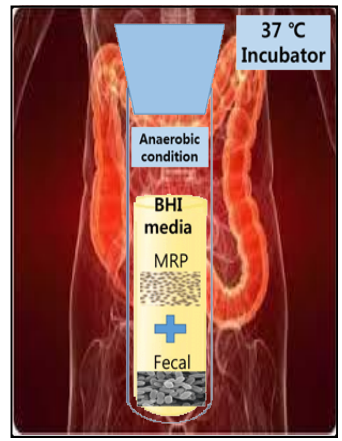
0.8 M Sugar
(glucose, fructose, galactose)

0.8 M Lysine

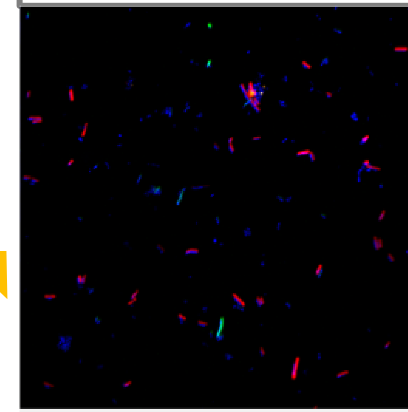
Maillard reaction products

Shaking water bath
110℃, 60 rpm
for 30 min

Batch fermentation



Fluorescence *in situ* hybridization



Metabolite (short chain fatty acid) by HPLC

Impact of MRPs on human intestinal bacteria

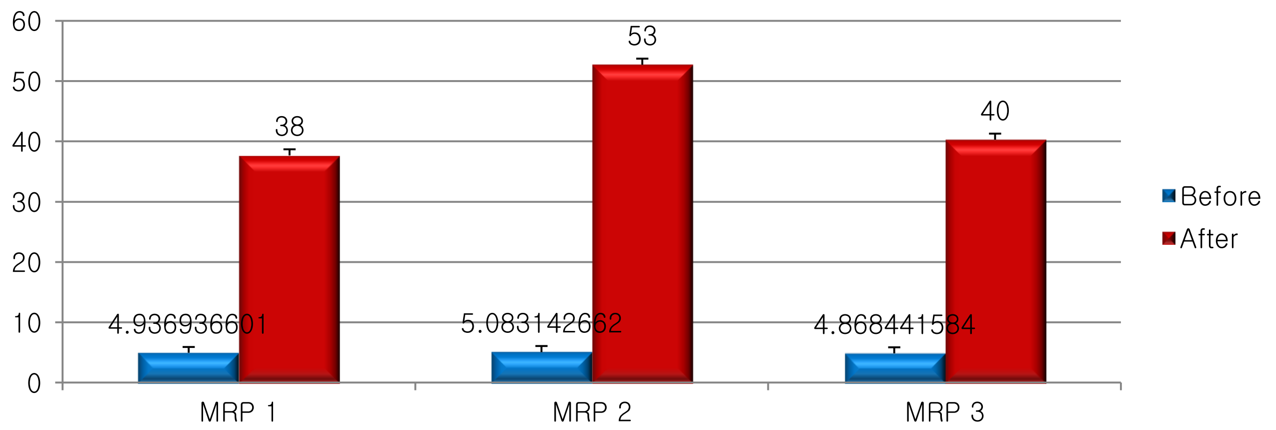
110℃, 0.5 h

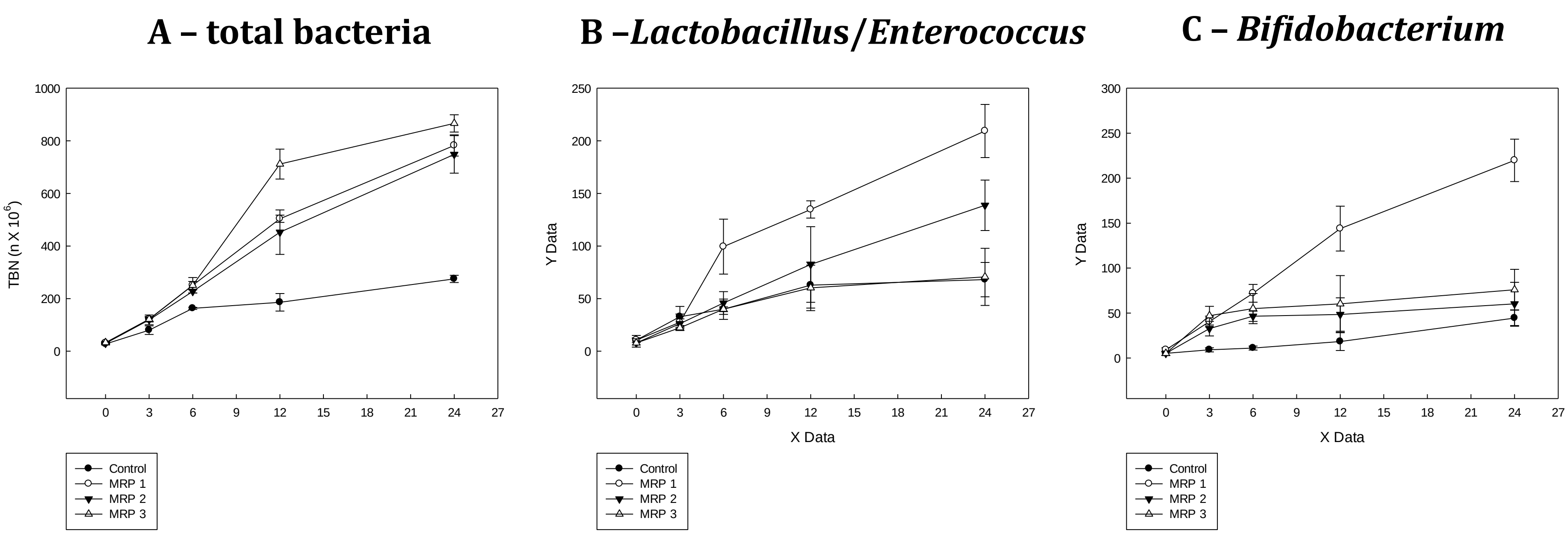
0.8 M Lysine + 0.8 M Glucose → MRP1

0.8 M Lysine + 0.8 M Fructose → MRP2

0.8 M Lysine + 0.8 M Fructose → MRP3

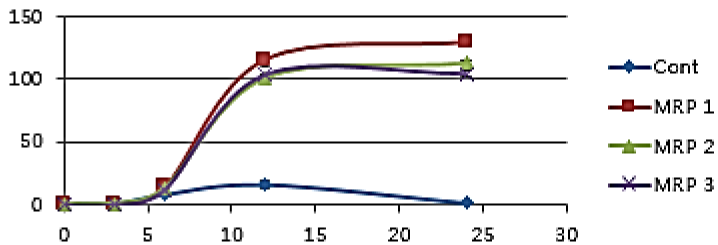
CML contents of MRPs (μg/1g)



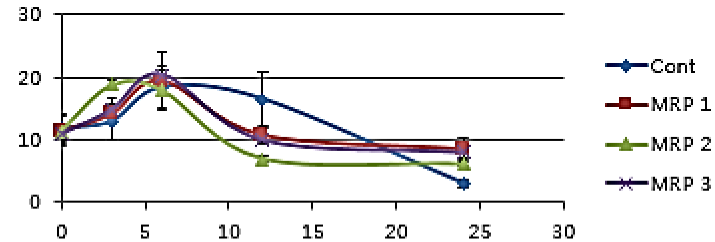


Measurement of changes in the levels of total and specific bacterial groups in a batch fermentation supplemented with MRPs.
The bacterial numbers were assessed by FISH using specific 16S rDNA probes.

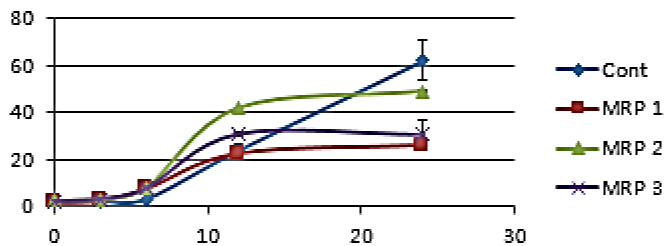
Contents of lactic acid



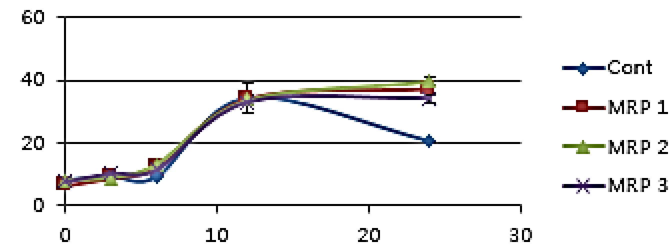
Contents of formic acid



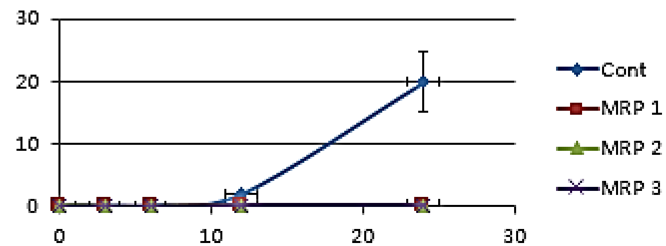
Contents of acetic acid



Contents of propionic acid



Contents of butyric acid



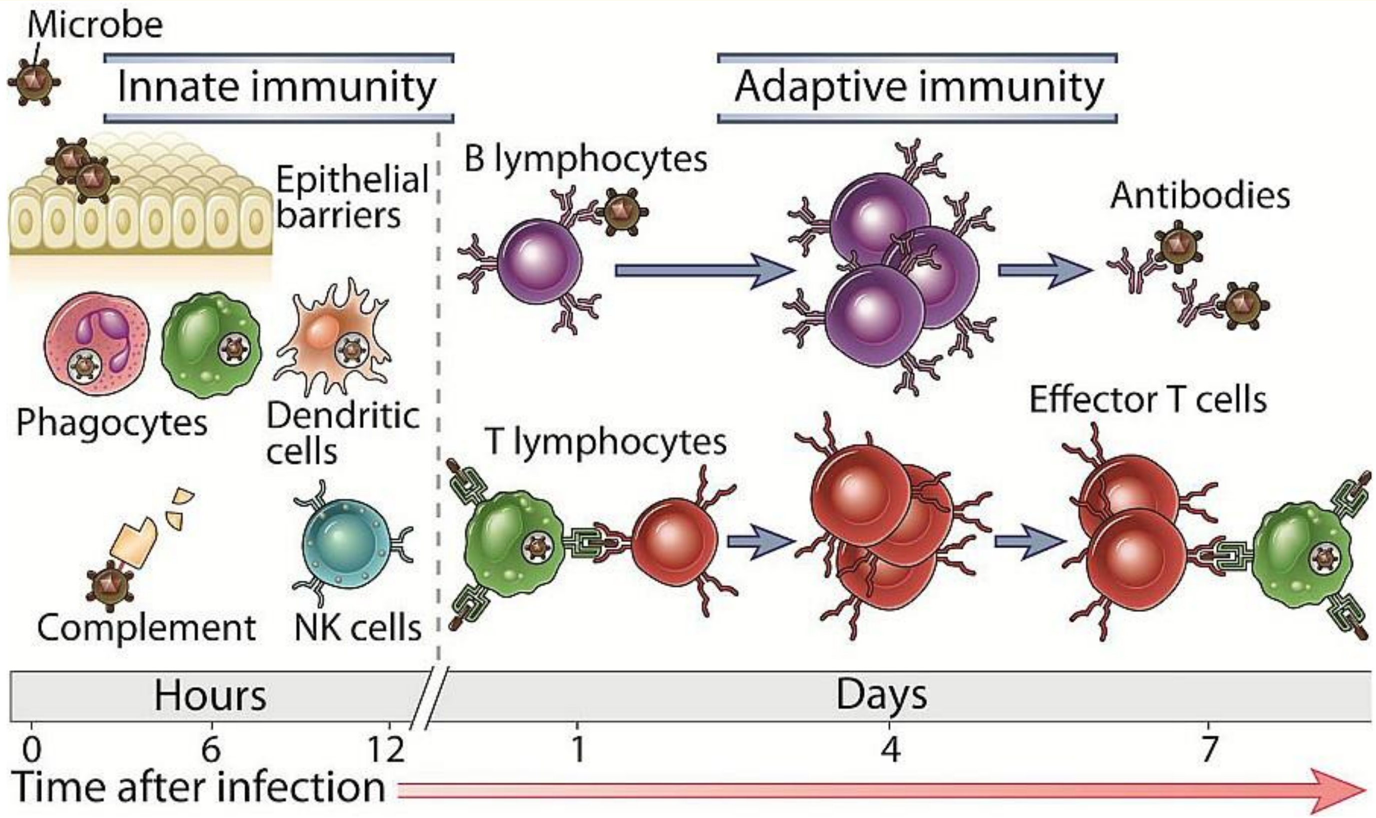
The impacts of the examined MRPs on the production of short chain fatty acids in batch fermentation.

Health effects of dietary MRPs: Immune-modulating Effect of MRPs

PROACTIVE KU

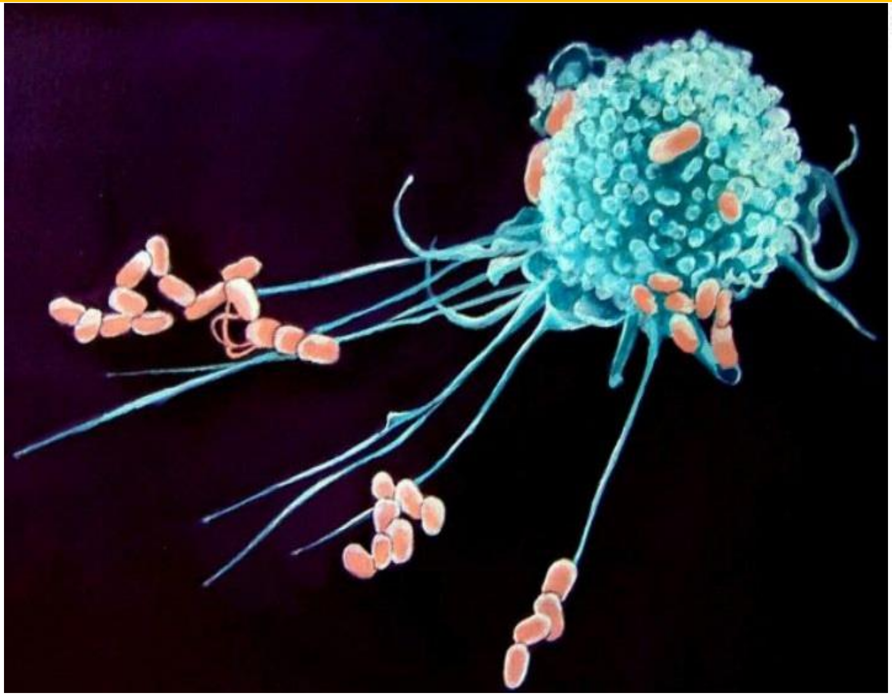
Immune System

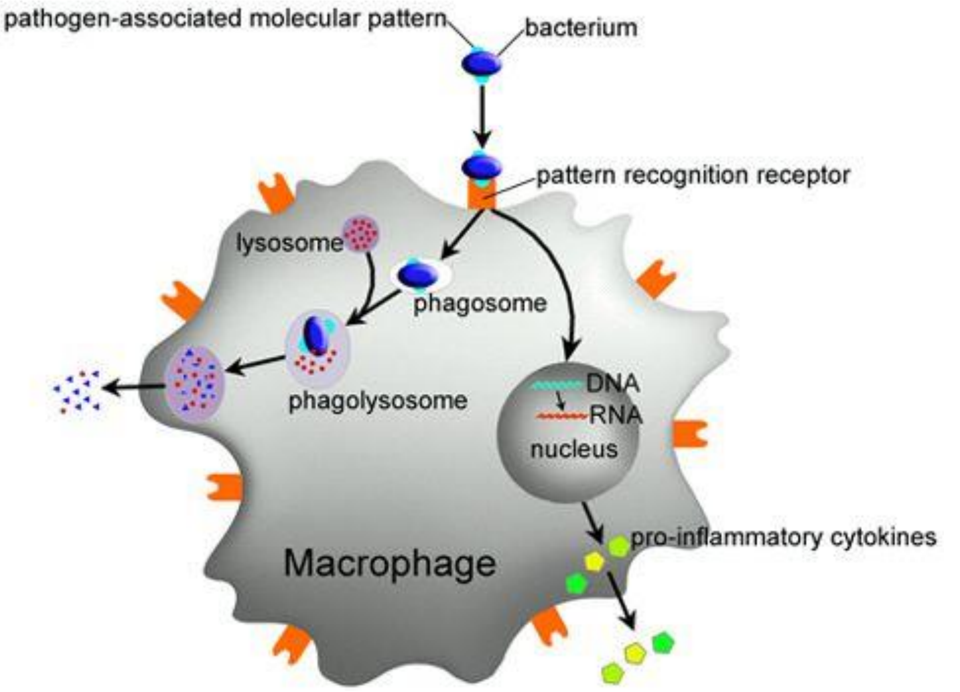
- Immune response is induced by recognizing pathogen or other complex on host cells. *(Ahmad-Nejad, Häcker et al. 2002; An, Yu et al. 2002)*
- Innate immunity's function : 1st defense against pathogen & acquired immunity modulatory *(Oriss, Ostroukhova et al. 2005)*



Macrophage

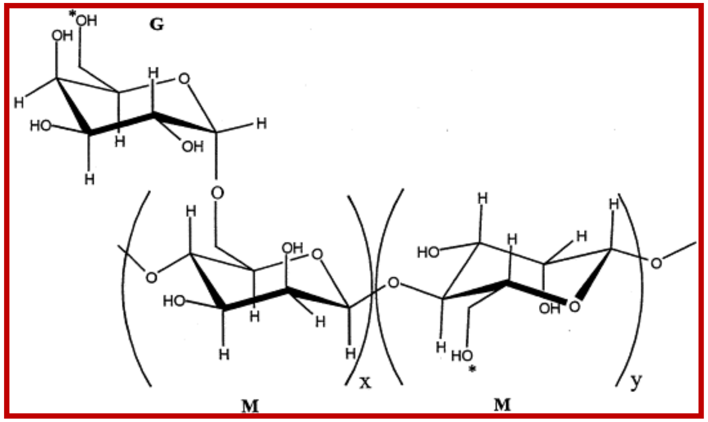
- Phagocytes : the major cell group of innate immune response & 1st response immunocytes
- Macrophage : invasion of bacteria or other foreign objects → chemical reaction of receptors → alerted to the presence of foreign matter
- Activated macrophages → nitric oxide (NO) & cytokines *(Uthaisangsook, Day et al. 2002)*





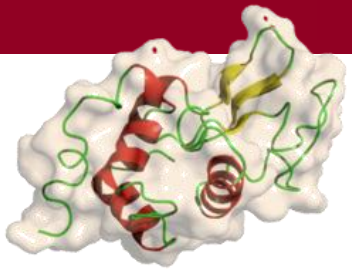
Galactomannan

- Polysaccharides consisting of a mannose backbone with galactose side chain



- In food industry, being used in foods as stabilizers

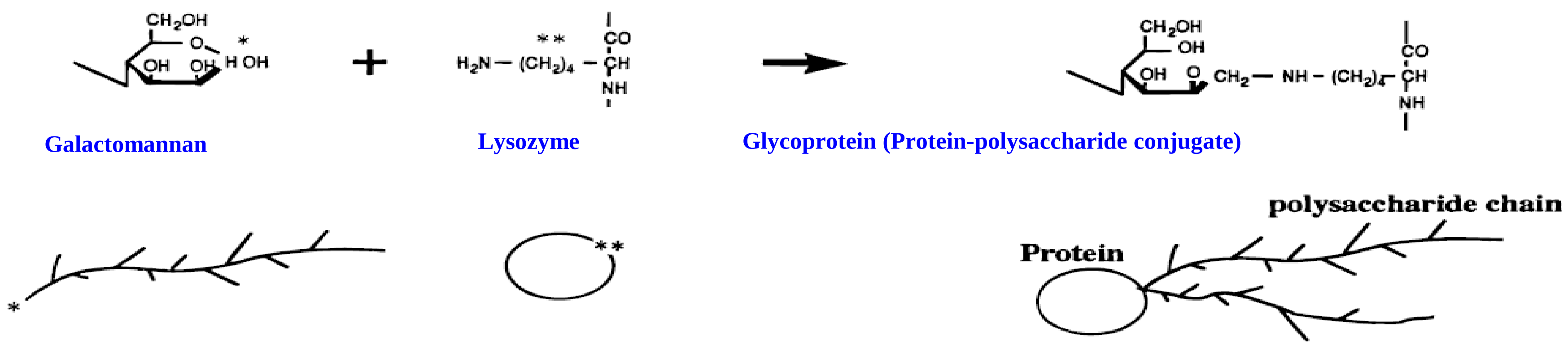
- It was reported that it has immune-modulating effect (Badia et al., 2012)



Lysozyme

- N-acetylmuramide glycanhydrolases, are glycoside hydrolase enzymes
- Antimicrobial activity to gram positive bacteria attacking peptidoglycan
- In mammalian cells, lysozyme plays an important defense role in the earlier stage of bacterial infection
- Widely distributed in food, especially fish (Takahashi et al., 1986)

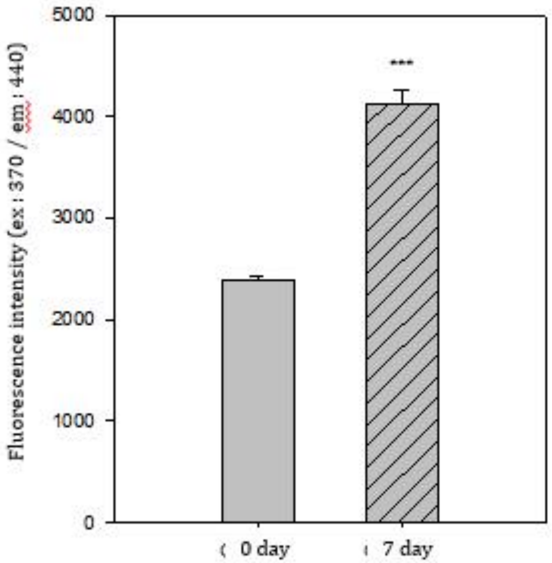
(Nakamura et al., 1998)



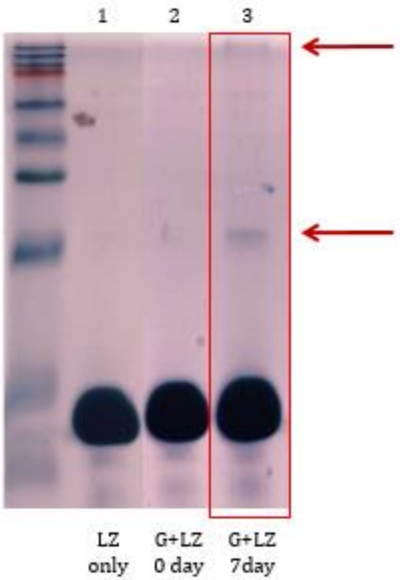
1 : 5

Lysozyme + **Galactomannan** → **Freeze-dry** → **Incubation** → **1d** / **7d**

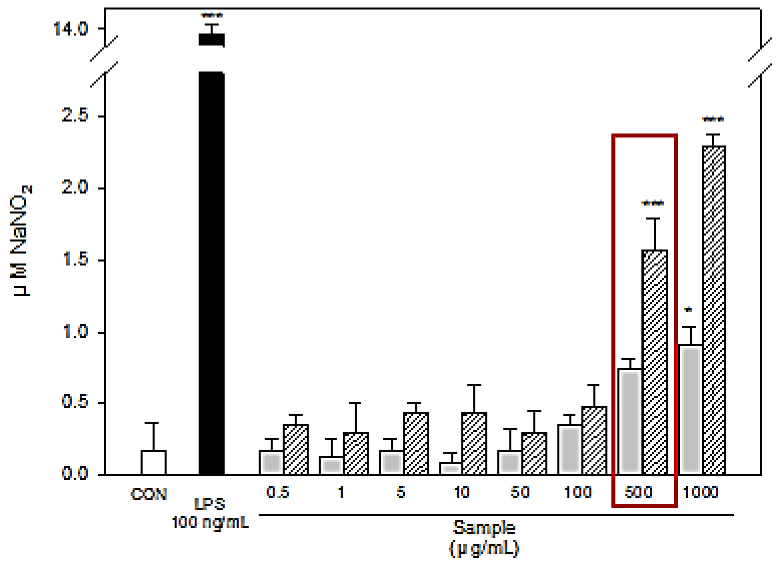
60℃ and 79% relative humidity using saturated KBr solution

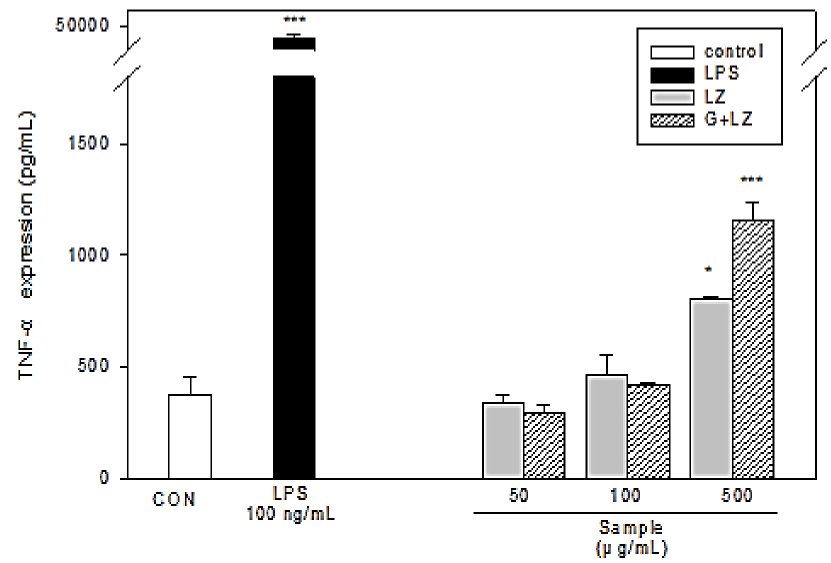


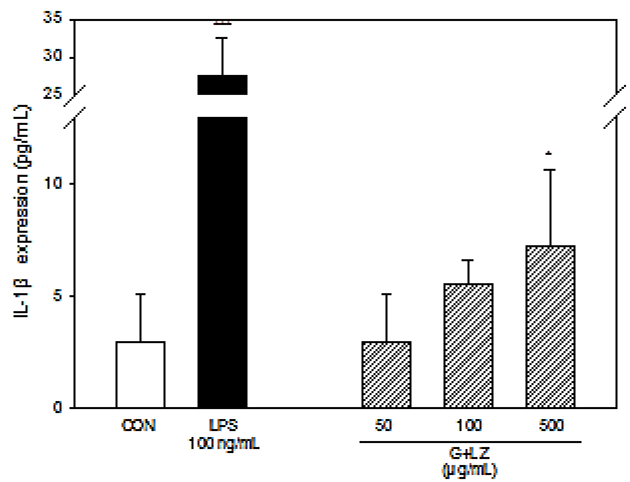
Fluorescence of LGC (Ex:370/Em:440)

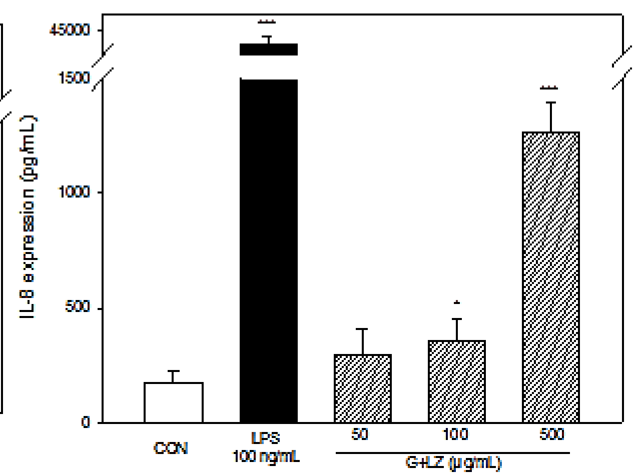


LGC with incubation at 60℃ (1) LZ only (2) LGC 0 d (3) LGC 7 d


μ M NaNO2
14.0
2.5
2.0
1.5
1.0
0.5
0.0
***
*
CON
LPS
100 ng/mL
0.5
1
5
10
50
100
500
1000
Sample
(μ g/mL)


TNF-α expression (pg/mL)
50000
1500
1000
500
0
***
*
control
LPS
LZ
G+LZ
CON
LPS
100 ng/mL
50
100
500
Sample
(μ g/mL)


IL-1β expression (pg/mL)
35
30
25
10
5
0
CON
LPS
100 ng/mL
50
100
500
G+LZ
(μg/mL)


IL-8 expression (pg/mL)
45000
1500
1000
500
0
***
CON
LPS
100 ng/mL
50
100
500
G+LZ (μg/mL)

Whey Protein Concentrate (WPC)

- A by-product generated from cheese and casein manufacture *(McIntosh, Royle et al. 1998)*
- 20% of milk protein → 22% α-lactalbumin, 50% β-lactoglobulin & other proteins

- WPC : 30~80% protein → ultrafiltration or reverse osmosis *(Wong, Camirand et al. 1996)*
  - Improvement of the oxidative damage by alcohol indicates *(Tseng, Chen et al. 2008)*
  - Anti-cancer effects *(Bounous, Batist et al. 1991; Hakkak, Korourian et al. 1999)*
  - Immunomodulatory effect in the mouse dietary *(Wong and Watson 1995)*

To develop new source product having an useful functions from the by-product generated in manufacturing cheese and casein via Maillard reaction

Glycation of WPC

Characterization of WPC

WPC

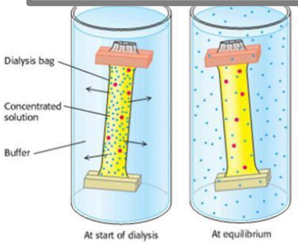
10 g / 1L
0.1 M sodium phosphate buffer (pH 7.4)

Glycation

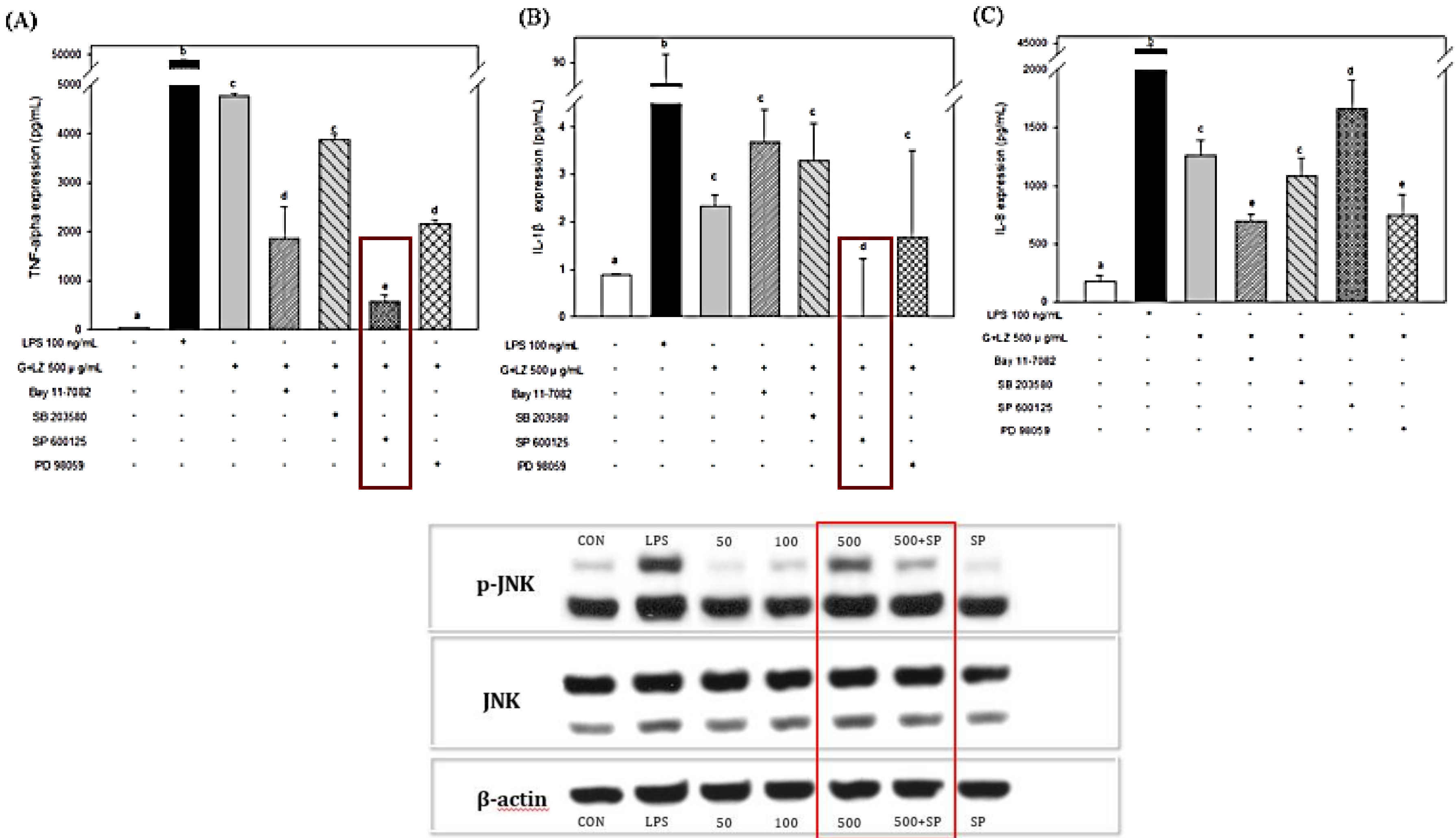
Shaking water bath
55℃, 60 rpm
for 24 h

Glycation Degree

Dialysis (24 h)



Freeze dry



(A)
TNF-alpha expression (pg/mL)
LPS 100 ng/mL
G+LZ 500 μ g/mL
Bay 11-7082
SB 203580
SP 600125
PD 98059
(B)
IL-1β expression (pg/mL)
(C)
IL-8 expression (pg/mL)
p-JNK
JNK
β-actin
CON
LPS
50
100
500
500+SP
SP

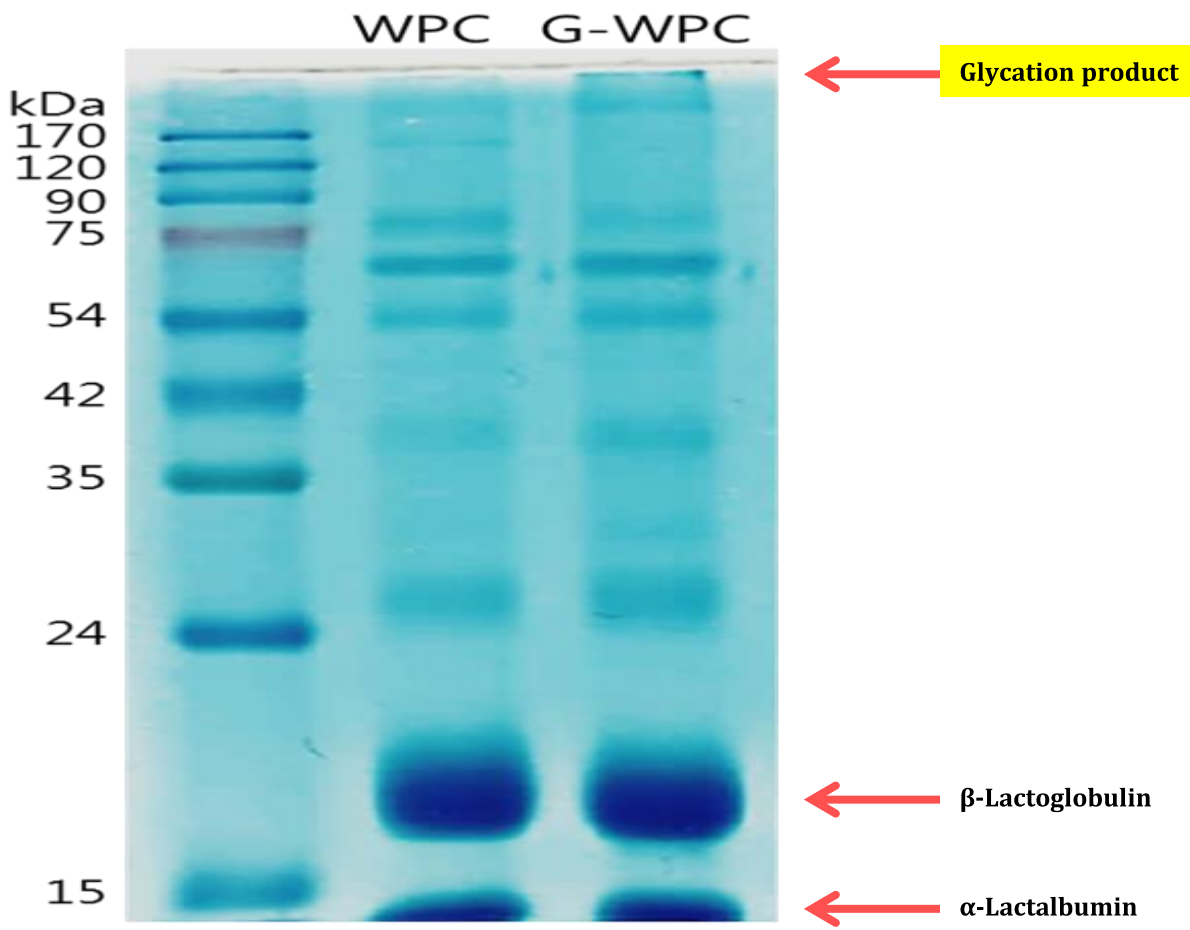
Composition of WPC

Composition	WPC (%, w/w)
Moisture	5.17±0.04
Crude protein	73.62±0.16
Crude fat	3.41±0.25
Lactose	7.66±0.14
Crude ash	2.64±0.00

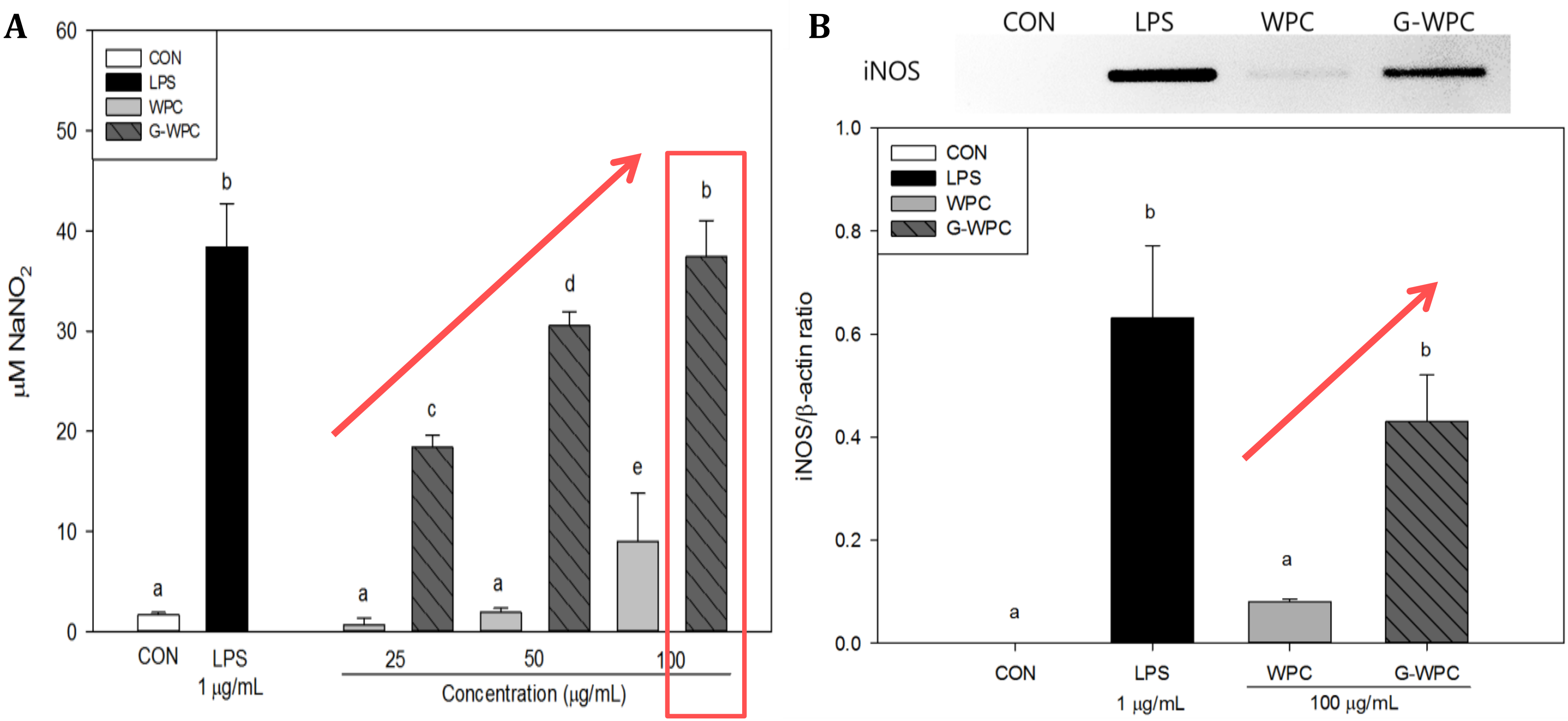
10

1

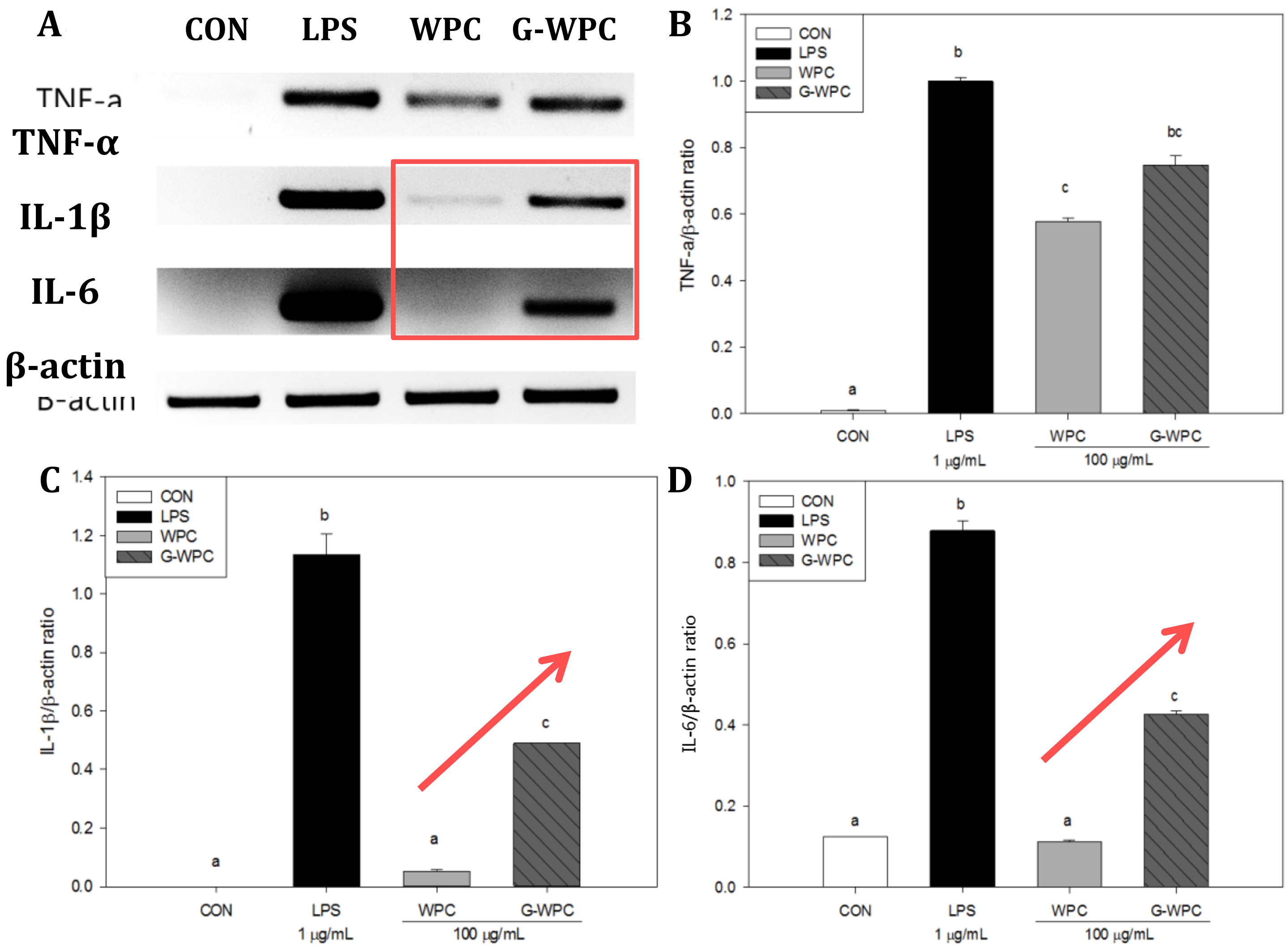
All tests were performed using Korea Food Code (2013) offered Ministry of Food and Drug Safety in Korea.



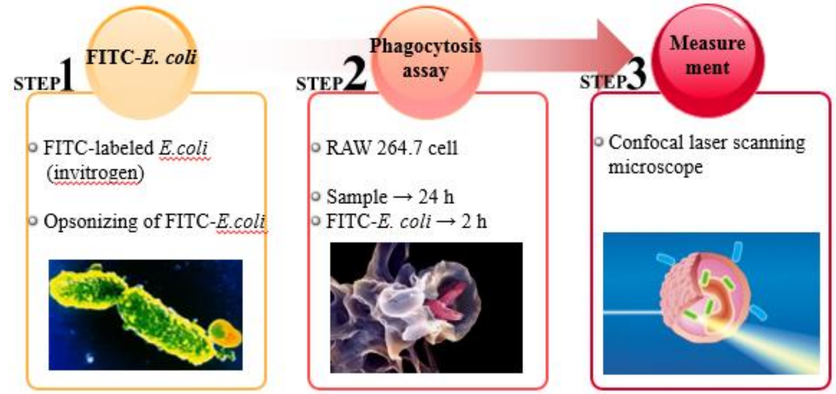
SDS-PAGE analysis of G-WPC

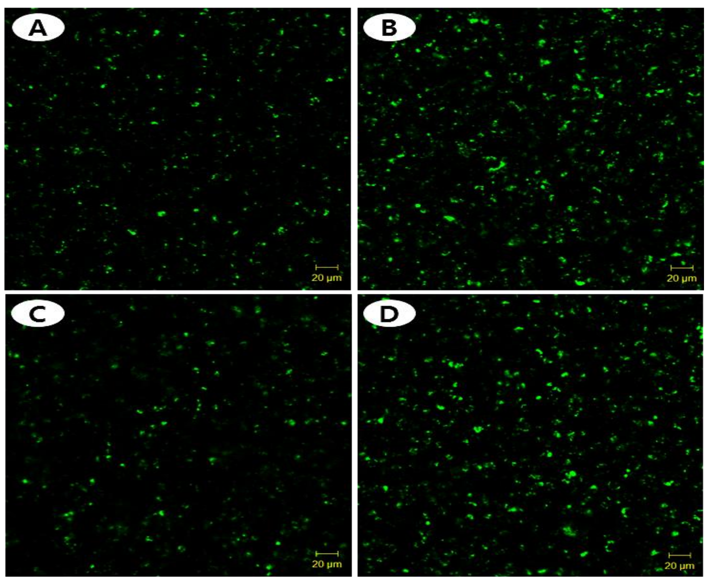


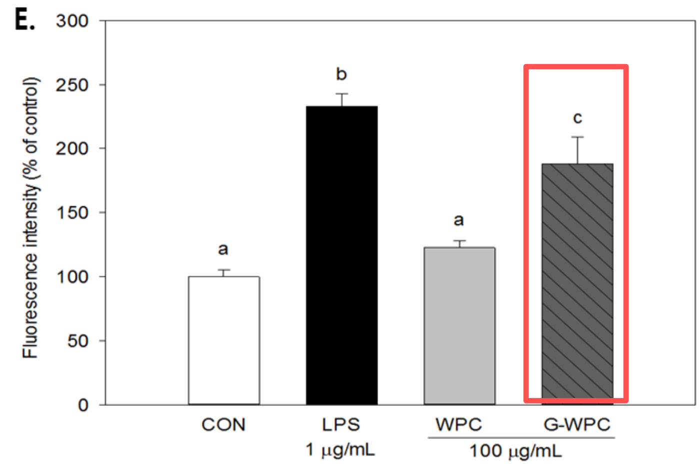
NO production and iNOS mRNA expression of G-WPC on RAW 264.7 cells



Expression of TNF-α, IL-1β and IL-6 mRNA in RAW 264.7 cells







Effect of phagocytosis on RAW 264.7 cells.

A conforcal laser scanning microscope image (A~D) and image analysis using image J (E).
(A): Control. (B): LPS (1 μg/mL). (C): WPC. (D): G-WPC.

Fermentation of G-WPC

Glycated WPC

+

Glucose, Proteose peptone, Sodium acetate, Ammonium citrate, Dipotassium phosphate, Magnesium sulfate, Manganese sulfate, Tween 80

+

Lactobacillus fermentum LC01

10^7 CFU/ml

37℃, 48 h

→ F-WPC

Control of F-WPC

Glucose, Proteose peptone, Sodium acetate, Ammonium citrate, Dipotassium phosphate, Magnesium sulfate, Manganese sulfate, Tween 80

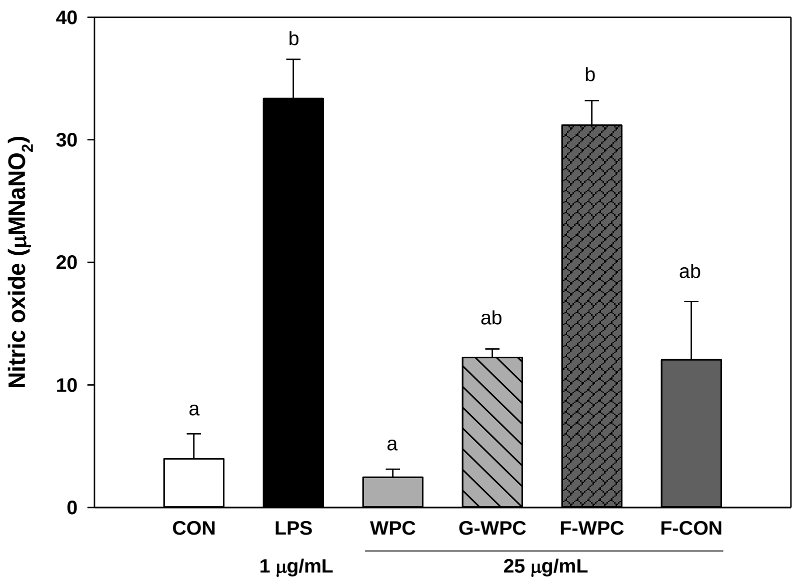
+

Lactobacillus fermentum LC01

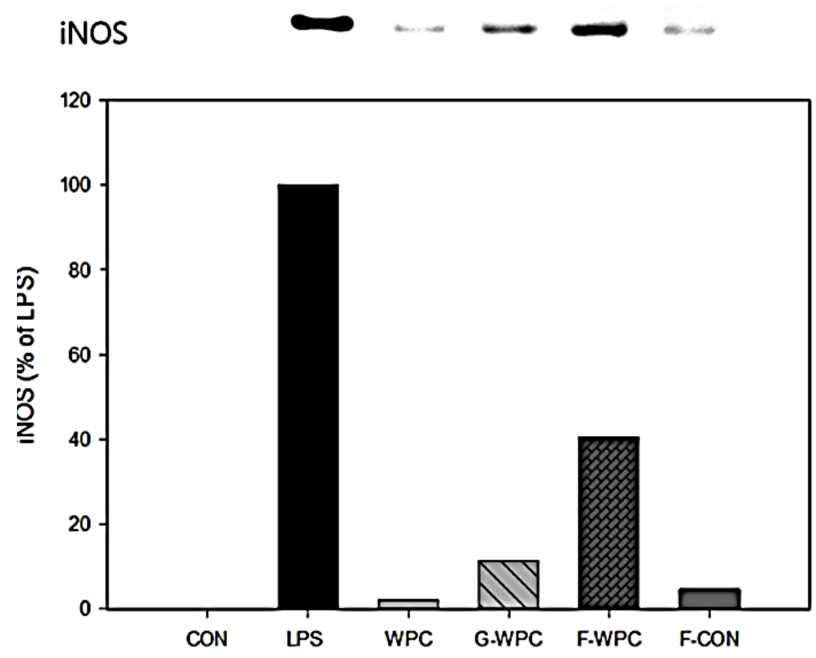
10^7 CFU/ml

37℃, 48 h

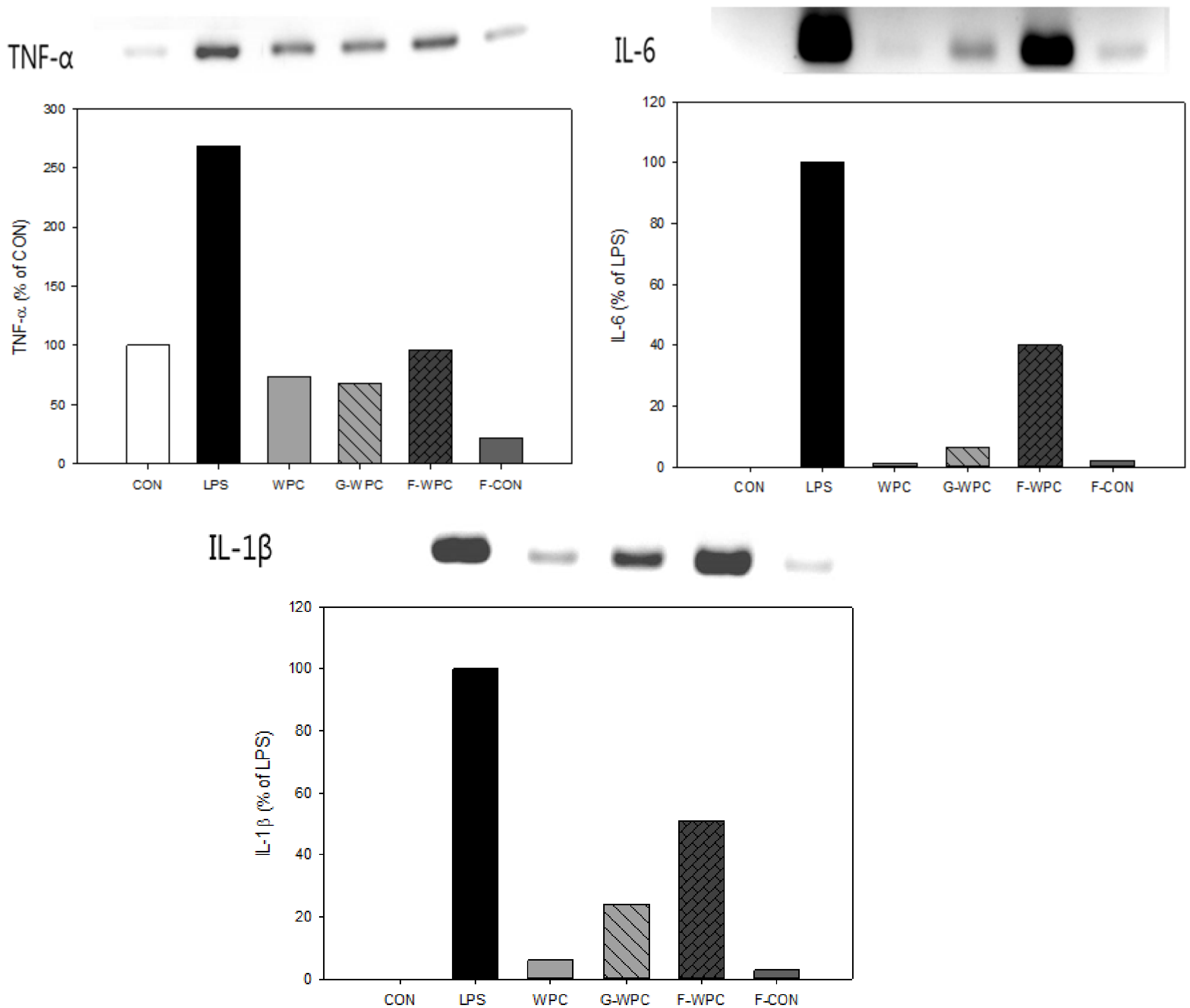
→ F-WPC CON



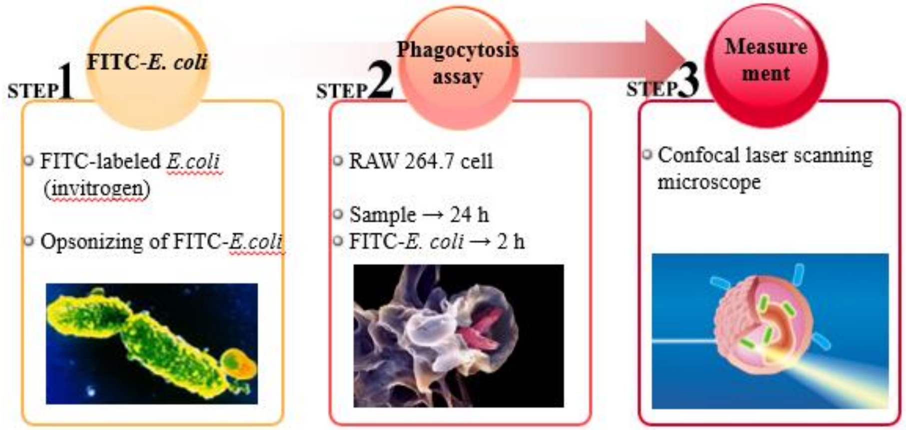
Effect of G-WPC and F-WPC on NO production

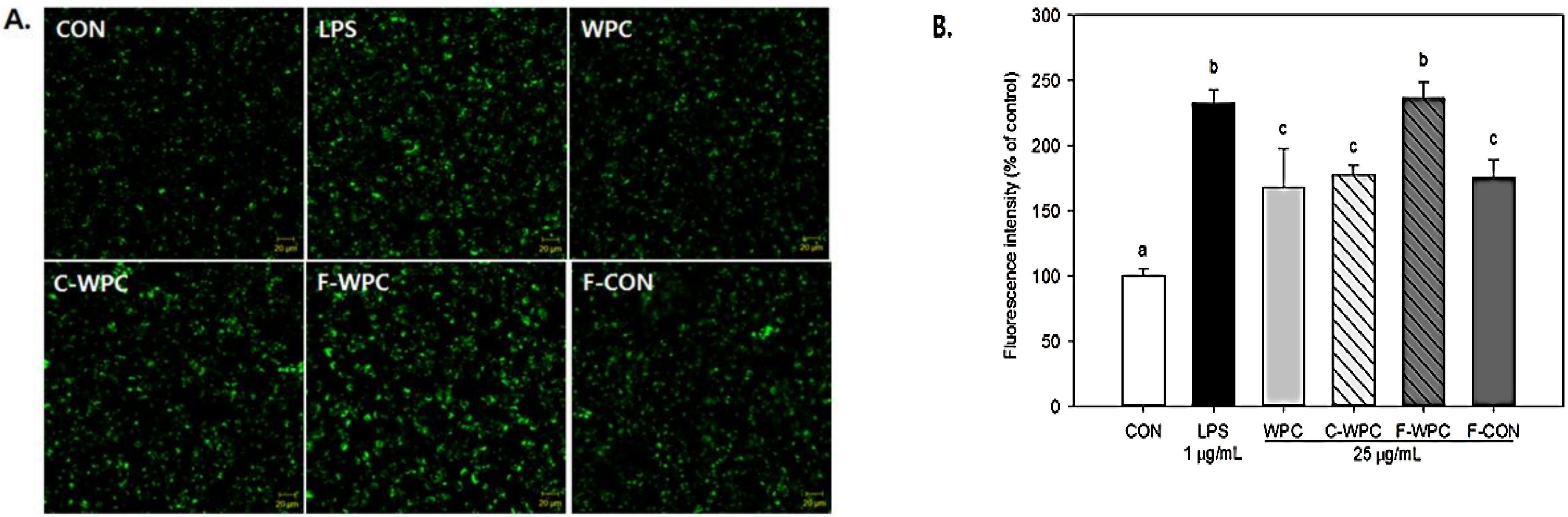


Effects of iNOS expression in mRNA levels by G-WPC and F-WPC on RAW 264.7 cells



Effects of TNF-α, IL-6, IL-1β and β-actin expression in mRNA levels by G-WPC and F-WPC on RAW 264.7 cells





Phagocytosis effect of G-WPC and F-WPC on RAW 264.7 cells

Conclusions

PROACTIVE KU

- **The aqueous extracts of yellow myrobalan (*Terminalia chebula* Retz.), asiatic plantain (*Plantago asiatica* L.) and seaweed fulvescenes (*Capsosiphon fulvescens*), and their compounds, CA, PM, and PhA showed effects for alleviating diabetic complications.**

- **Grilling cooking method showed higher CML contents than boiling or steaming one, and food items including pork, beef and mackerel have higher CML levels than ones including rice, tofu and eggs.**

Conclusions

- Model MRPs applied to experimental batch-type simulator models increased the growth of gut commensal bacteria, particularly lactobacilli and bifidobacteria, and produced more level of lactate and less one of butyric acid.

- MRPs showed immune-modulating effects contributing to host immune defense.

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