



Anti-aging Effect and Gene Expression Profiling of Aged Rats Treated with *G. bimaculatus* Extract

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(Received April 24, 2015; Revised May 13, 2015; Accepted June 14, 2015)

Extract from *Gryllus bimaculatus* crickets inhibits oxidation at the DNA level, with reduced production of 8-hydroxy-2'-deoxyguanosine (8-OHdG). Microarray analyses were performed with a rat 28K cDNA clone set array to identify the gene expression profiles of aged (10 months old) Wistar Kyoto rats treated for one month with 100 mg/kg *G. bimaculatus* ethanol extract to assess the effects. The extract produced a meaningful anti-edema effect, evident by the inhibition of creatinine phosphokinase activity. The weights of abdominal and ovarian adipose tissues were reduced and the proportion of unsaturated fatty acids in adipose tissues was increased in an extract dose-dependent manner. Compared with untreated control rats, rats treated with the extract displayed the upregulation of 1053 genes including Fas (tumor necrosis factor receptor superfamily, member 6), Amigo3 (adhesion molecule with an immunoglobulin-like domain), Reticulon 4, 3-hydroxy-3-methylglutaryl-coenzyme (Hmgcr; a reductase), related anti-fatigue (enzyme metabolism), and Rtn antioxidant, and the downregulation of 73 genes including Ugt2b (UDP glycosyl-transferase 2 family), Early growth response 1, and Glycoprotein m6a. Data suggest that *G. bimaculatus* extract may have value in lessening the effects of aging, resulting in a differential gene expression pattern indicative of a marked stress response and lower expression of metabolic and biosynthetic genes.

Key words: Anti-aging effect, *G. bimaculatus*, Wistar Kyoto rats, Microarray

INTRODUCTION

Water extracts of cricket (*Gryllus bimaculatu*, Gb) have been used in oriental medicine to treat fever and hypertension. Crickets are also reared in Korea as an amphibian food (1). The main components of Gb are protein; fat including essential fatty acids like oleic acid, linoleic acid and γ -linoleic acid; ash; and moisture (2). *G. bimaculatus* extract significantly decreases blood ethanol concentrations by enhancing liver mitochondrial alcohol metabolizing enzymes (3). Grasshoppers that are roasted can also be a food source, but these are not typically consumed in Korea. The observed adverse effect level (NOAEL) of Gb is reportedly higher than 5 g/kg body weight/day, as shown in a 13-week oral

dose toxicity study in Sprague-Dawley rats (4).

Aging is a process of progressive decline in the physiological capacity of an organism, manifested by accumulated alteration and destabilization at the whole system level (5). Generally, oxidative stress is a cause of aging in lipid peroxidation, protein carbonyl content increase, and DNA damage. Aging is also associated with a differential gene expression pattern indicative of a marked or lower stress response of metabolic and biosynthetic genes (6). A recent report regarding protein and healthy aging showed that an evenly distributed protein diet provides a framework that allows older adults to benefit from the synergistic anabolic effect of protein and physical activity (7). Gb contains rich crude protein (52%), which can be one of these protein sources. Aging is not prevented with an antioxidant agent or fruits instead of a sufficient daily meal. Nevertheless, we compared Gb extract to blueberry as a positive control of anti-aging activity, which decreases D-galactose-induced oxidative stress and brain damage in rats (8), and its supplementation improved memory in middle-aged mice fed a high-fat diet (9).

In the present study, we demonstrate the potential value of Gb ethanol extract in lessening the deleterious aspects of

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aging in serum and the gene expression level of 10-month aged WKY rats, with treatment for one month.

MATERIALS AND METHODS

Materials. *G. bimaculatus* were reared on a cricket farm located in Jungsun, Kangwon-Do, South Korea. Crickets were freeze-dried in the Department of Agricultural Biology, National Academy of Agricultural Science, Korea.

Preparation of *G. bimaculatus* extract. To prepare the ethanol extract, 1 kg dried *G. bimaculatus* was homogenized, soaked, and extracted three times with 70% ethanol by ultrasonification for 30 min. The samples were filtered through Whatman filter paper and concentrated by evaporation and freeze-drying. The dried powder was dissolved in saline prior to use. Blueberry (frozen, imported) was purchased in the commercial Mart, extracted with 70% ethanol by ultrasonification and freeze-dried in an identical manner.

Animals. Male, 10-month old Wistar Kyoto (WKY) rats weighing 469.9 ± 9.4 g, obtained from Japan SLC (Shizuoka, Japan), were divided into three groups of six rats. Control rats were purchased from Jung Ang Lab Animals (Seoul, Korea). All procedures were in accordance with the NIH Guidelines for Care and Use of Laboratory Animals. The rats were acclimated for one week under normal husbandry conditions ($23 \pm 2^\circ\text{C}$, $55 \pm 10\%$ humidity and 12-hr light/dark cycle) and fed a standard diet (Rat and Mouse 18% 5L79; PMI Nutrition International, Brentwood, MO, USA) and water *ad libitum*. The rats were distributed into groups based on similar weights. The control group (I) was untreated. Group II was treated with 100 mg/kg *G. bimaculatus* ethanol extract in phosphate buffered saline. Group III was treated with 100 mg/kg blueberry ethanol extract. Each group was maintained for one month.

Organ weights. Absolute and relative (organ-to-body weight ratios) weights of the adrenal glands, kidneys, heart, liver, lung, spleen, stomach, pancreas, thymus, and ovaries were determined.

Measurement of blood pressure. Measurement of the direct model *in vivo* and the tail-cuff method (indirect) was performed on a weekly basis according to a previous report (10). Using a blood monitoring system (Japan Muromachi Co., MK-100), we monitored WKY rats that had received a single daily dose of Gb extract (100 mg/kg) orally (diluted in saline) over one month. Measurements were performed one week before, and four weeks after treatments.

Blood sampling and plasma assay. Following one month of treatment, blood (~3 mL) was collected from the posterior vena cava following light CO₂ inhalation, and

used for serum chemistry measurements of total protein, total bilirubin, glucose, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), γ -glutamyl transferase (GGT), alkaline phosphatase (ALP), creatinine phosphokinase (CK), lactic dehydrogenase (LDH), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, blood urea nitrogen (BUN), creatinine, triglycerides, uric acid, sodium, potassium, and chloride. All were evaluated using a model 7060 automatic clinical analyzer (Hitachi, Tokyo, Japan).

Oxidative DNA (8-OHdG) damage quantitation. Among numerous types of oxidative DNA damage, the formation of 8-hydroxydeoxyguanosine (8-OHdG) was determined with an enzyme-linked immunoassay according to the manufacturer's protocol (OxiselectTM oxidative DNA damage ELISA kit, Cell Biolabs, Inc., San Diego, CA, USA).

DNA microarray. Following histopathological analysis, microarray hybridization was performed on liver samples as described previously (11). Total RNA was isolated from the liver using a Qiagen RNeasy Midi Kit (Qiagen, Valencia, CA, USA). A regular microarray was carried out according to the manufacturer's instructions for the FairPlayTM microarray labeling kit (Stratagene, La Jolla, CA, USA). Briefly, 20 μg total RNA was reverse-transcribed to single stranded cDNA. The cDNA was purified with ethanol precipitation and resuspended in 5 μL 2 \times coupling buffer, and coupled with 5 μL Cy3 or Cy5 dye for 1 hr in the dark. The labeled control liver cDNA and the treated liver cDNA were combined and purified. The labeled cDNA was mixed with 1.5 μL 10 $\mu\text{g}/\mu\text{L}$ salmon DNA, 1.5 μL 8 $\mu\text{g}/\mu\text{L}$ poly d(A), 1.5 μL 4 $\mu\text{g}/\mu\text{L}$ yeast tRNA, 4.5 μL 20 \times SSC, and 0.75 μL 10% sodium dodecyl sulfate (SDS), heated at 99 $^\circ\text{C}$ for 2 min, and incubated at 45 $^\circ\text{C}$ for 15 min. The labeled DNA was loaded onto a microarray chip. A hybridization chamber was assembled with the microarray chip and submerged in a water bath overnight at 60 $^\circ\text{C}$. The microarray chip was washed in wash buffer I (2 \times SSC, 0.1% SDS) for 15 min, then in wash buffer II (1 \times SSC) for 5 min and wash buffer III (0.2 \times SSC) for 15 min. The slide was dried by centrifugation at 500 \times g for 15 min, and scanned using a BMS Array Scanner and an applied precision Array WoRx eBiochip Reader (BioRad, Hercules, CA, USA) using the Cy3 and Cy5 channels (12).

Analysis of fatty acid composition of rat adipose tissue. For epididymidal and abdominal fat analysis, the concentration of 29 free fatty acids and fatty acid composition were analyzed in adipose tissues by gas chromatography-mass spectroscopy (GC-MS). Test tissue fatty acid of each removed adipose or epididymidal tissue (0.1 g) was prepared by chloroform: methanol (2:1) extraction over-

night and the filtrated solution was removed under nitrogen gas. Subsequently, the lipids were saponified by alkaline hydrolysis of the phospholipid at 100°C with 0.5 N methanolic sodium hydroxide, and methylated at 100°C with 14% BF₃ for 15 min. The top layer was transferred in petroleum ether and analyzed by GC/MS (Agilent 6890GC, Agilent 5973N mass detector, EI mode) with a HP-5 capillary column (Agilent Technologies, Palo Alto, CA, USA). The inlet temperature was 250°C and the MS transfer line was kept constant at 230°C. The oven temperature was held at 180°C for 20 min, then programmed at a 10°C/min increase to 230°C and held 10 min. Quantification was achieved using a mixed 37 fatty acid standard: Sigma L9405, 10 µg/mL (Sigma-Aldrich Inc.), and linoleic acid (C18:2n6) as an internal standard.

Statistical analyses. The mean and standard error of all parameters studied were determined for each group using an ANOVA test. A Student's *t*-test was carried out to determine significant differences between the control and treated groups. A *p* value < 0.05 was considered significant.

RESULTS

Clinical signs and food consumption. No deaths or adverse clinical signs were apparent due to the ingestion of the *G. bimaculatus* or blueberry extract. The level of food consumption was similar in all treated groups during the course of the study (data not shown).

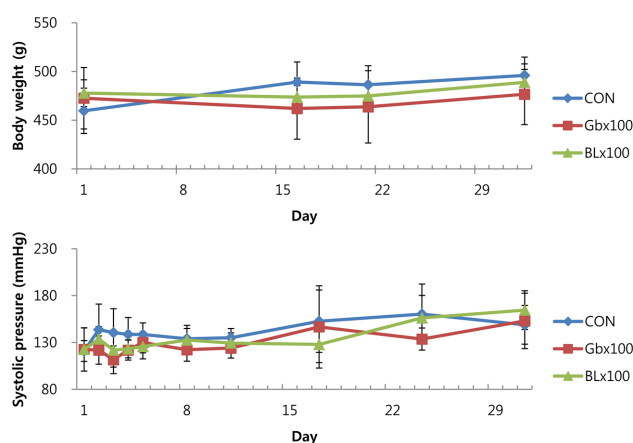


Fig. 1. Effect of Gb extract on body weight and systolic blood pressure in 10-month old WKY rats treated orally with Gb ethanol extract for 1 month.

Body weight, blood pressure, and heart rate changes.

There were no toxicologically significant differences in the mean body weight between the treatment groups (Fig. 1). During the 1-month administration period, the body weights of the male WKY rats in the treatment and control groups were comparable. The mean weekly body weight versus time data are presented in Fig. 1. No statistically significant differences in blood pressure (systolic blood pressure and heart rate) were observed between the 100 mg/kg *G. bimaculatus* extract-treated group and the control group.

Table 1. Hematological findings of male WKY (10-month old) rats treated orally with Gb extract for 1 month

Item	Unit	CON ^a	Gb ex. 100	BL ex.100
RBC	10 ³ /mm ³	9.14 ± 0.39	9.17 ± 0.24	9.07 ± 0.27
WBC	10 ⁶ /mm ³	6.54 ± 2.26	7.72 ± 2.76	8.90 ± 2.08
Hgb	g/dL	16.93 ± 0.40	16.72 ± 0.33	16.50 ± 0.59
Hct	%	56.35 ± 2.56	56.10 ± 1.26	55.52 ± 2.45
MCV	fL	62.0 ± 0.5	61.2 ± 0.5	61.3 ± 1.5
MCH	pg	18.5 ± 0.5	18.3 ± 0.2	18.2 ± 0.1
MCHC	g/dL	30.0 ± 1.1	29.8 ± 0.2	29.8 ± 0.6
PLT	10 ³ /mm ³	807.0 ± 73.3	775.6 ± 103.1	802.6 ± 16.8
PTT	sec	37.2 ± 6.8	44.2 ± 7.7	32.2 ± 8.6
Thrombin time	sec	37.2 ± 12.4	38.1 ± 5.34	44.0 ± 20.0
Factor I	mg/dL	333 ± 16.0	262 ± 57.4	275.3 ± 74.3
PT	sec	1.43 ± 0.04	1.44 ± 0.09	1.52 ± 0.05
Neutrophil	%	30.23 ± 6.88	28.08 ± 6.30	20.2 ± 2.67
Lymphocyte	%	67.1 ± 7.3	69.2 ± 6.5	76.5 ± 2.8
Monocyte	%	1.15 ± 0.31	1.38 ± 0.33	1.96 ± 0.27
Eosinophil	%	1.03 ± 0.39	0.88 ± 0.15	1.00 ± 0.188
Basophil	%	0.53 ± 0.17	0.46 ± 0.09	0.36 ± 0.09

Abbreviations: WBC, white blood cell; RBC, red blood cell; Hgb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet. PTT, partial thromboplastin time; PT, prothrombin time.

^aCON: PBS (as a vehicle) treated with murine normal diet.

Each value represents the mean ± S.E. * Statistically significant from the control (*p* < 0.05 vs. control).

Hematology and blood chemistry. Some dose-dependent changes were observed between the treated and control groups with respect to the hematological parameters at the end of the experiment. An increase in activated partial thromboplastin time (PTT) was observed in the WKY rats in the treated groups (PTT: control, 37.2 ± 6.8 secs; *G. bimaculatus* extract 100 mg/kg, 44.2 ± 7.7 secs; blueberry extract 100 mg/kg, 32.2 ± 8.6 secs) but the differences were not significant. Minor changes were found in hematological parameters (eosinophils, neutrophils, lymphocytes, monocytes, and basophils) for some extract-treated (one side) male rat groups. However, the effects of the *G. bimaculatus* extract were not considered adverse because all changes in hematological data were within the normal physiological range (Table 1).

Serum biochemistry. In the sera of the *G. bimaculatus* extract- and blueberry extract-treated WKY rat groups, creatinine phosphokinase (CK) levels were significantly lower than those in the control group after 1 month of treatment [control, 444.5 ± 297.9 IU/L; *G. bimaculatus* extract, 335.2 ± 179.1 IU/L (25% reduction); blueberry extract $309.0 \pm$

204.5 IU/L (30.5% reduction)]. Serum glucose levels were also lower versus the control (control, 218.6 ± 68.0 mg/dL; *G. bimaculatus* extract, 244.6 ± 50.3 mg/dL; blueberry extract, 273.6 ± 4.3 mg/dL), however the differences were not significant.

DNA microarray. Microarray analysis using a mouse 28K cDNA clone set array was performed to identify the gene-expression profiles in the livers of *G. bimaculatus* extract-treated WKY rats, in order to clarify potential markers for aging. Compared with the control group, treated rats showed 1053 upregulated genes, including Fas (tumor necrosis factor receptor superfamily, member 6), Amigo3 (adhesion molecule with immunoglobulin-like domain), and Reticulon 4 (Rtn4) (Table 4). Rats treated with 100 mg/kg *G. bimaculatus* extract displayed the down-regulation of 73 genes including UDP glycosyltransferase 2 (Ugt2b), Early growth response 1 (Egr1), and Glycoprotein m6a (Gpm6a) (Table 4). 3-Hydroxy-3-methylglutaryl-coenzyme (Hmgcr; a reductase), related anti-fatigue (enzyme metabolism), and Rtn antioxidant were also upregulated.

Table 2. Serological findings of male WKY (10-month old) rats treated orally with Gb extract for 1 month

Item	g/kg	CON ^a	GBex 100	BL ex 100
Toal protein	g/dL	7.76 ± 0.50	7.66 ± 0.31	7.18 ± 0.31
CRP(HS)		0.24 ± 0.11	0.24 ± 0.06	0.26 ± 0.06
ALP	IU/L	117.8 ± 10.8	144 ± 6.88	12.97 ± 5.08
AST	IU/L	137.0 ± 37.8	133.2 ± 32.4	138.8 ± 20.6
ALT	IU/L	49.6 ± 10.4	49.6 ± 3.8	54.0 ± 13.8
γ GT	g/dL	3	3	3
CK	IU/L	444.5 ± 297.9	335.2 ± 179.1	309.0 ± 204.5
LDH	IU/L	2452 ± 2029	1709 ± 1108	1372 ± 855
Na	nmol/L	136 ± 6.3	134 ± 4.4	134 ± 6.6
K	nmol/L	22.4 ± 8.4	25.4 ± 5.10	25.4 ± 8.05
Cl	nmol/L	95.2 ± 1.92	96.0 ± 2.0	96.0 ± 2.2
BUN	mg/dL	21.4 ± 2.19	17.5 ± 3.00	17.6 ± 1.01
Uric acid	mg/dL	5.74 ± 1.21	5.16 ± 0.46	4.82 ± 1.54
T.Chol	mg/dL	205.6 ± 26.2	203.4 ± 7.47	200.6 ± 17.01
H.Chol	mg/dL	177.4 ± 17.0	176.4 ± 2.8	175.0 ± 12.6
TG	mg/dL	73.8 ± 20.2	79.2 ± 7.0	81.2 ± 16.4
Glucose	mg/dL	218.6 ± 68.0	244.6 ± 50.3	273.6 ± 104.3
Creatine	mg/dL	0.64 ± 0.4	0.61 ± 0.02	0.68 ± 0.08
Calcium	nmol/L	11.86 ± 0.89	12.3 ± 0.18	11.96 ± 0.53
IP	mg/dL	166.7 ± 4.2	15.34 ± 3.31	16.88 ± 4.13
Phoholipid	mg/dL	216 ± 22.73	227 ± 8.01	222.8 ± 14.7
Insulin	mU/mL	0.2	0.2	0.2
FFA	mEq/L	749 ± 129.9	807.4 ± 189.0	642.6 ± 155.5
Albumin	g/dL	4.82 ± 0.24	4.92 ± 0.08	4.56 ± 0.21
T. Bil	mg/dL	0.16 ± 0.09	0.14 ± 0.06	0.14 ± 0.06

Abbreviations: ALP: alkaline phosphatase; AST(GOT), glutamate oxaloacetate transaminase; ALT(GPT), glutamate pyruvate transaminase; GGT, γ -glutamyl transferase; CK: creatinine phosphokinase; LDH, lactate dehydrogenase; Na, Sodium; K, potassium; Cl, chloride; BUN, blood urea nitrogen; T. Chol: total cholesterol; H. Chol: high cholesterol; L.Chol: low cholesterol; TG, triglyceride; Ca, calcium; IP, inorganic phosphorus.

^aCON : PBS (vehicle) treated with murine normal diet.

Each value represents mean \pm S.D. Statistically significant from control ($p < 0.05$).

Table 3. Upregulated genes differentially expressed in the liver tissue of WKY 10-month aged rats treated with *G. bimaculatus* ethanol extract for 1 month

NO.	PairMean ratio* (test/control)	Description	Gene symbol
1	1.479	Sema domain, immunoglobulin domain (Ig), short basic domain, (semaphorin) 3C	Sema3c
2	1.361	Similar to hypothetical protein MGC52110	RGD1565095
3	1.349	Calsequestrin 1 (fast-twitch, skeletal muscle)	Casq1
4	1.335	3-Hydroxy-3-methylglutaryl-Coenzyme A reductase	Hmgcr
5	1.315	ADP-ribosylation factor guanine nucleotide-exchange factor 1	Arfgef1
6	1.311	HAUS augmin-like complex, subunit 1	Haus1
7	1.310	Reticulon 4	Rtn4
8	1.310	Ligase III, DNA, ATP-dependent	Lig3
9	1.308	WD repeat domain 89	Wdr89
10	1.301	Hypothetical protein LOC690785	LOC690785
11	1.301	Kelch-like 5 (<i>Drosophila</i>)	Klhl5
12	1.300	Myeloid or mixed-lineage leukemia (trithorax homolog, <i>Drosophila</i>); translocated to, 3	Mllt3
13	1.295	Endothelin receptor type A	Ednra
14	1.291	URB2 ribosome biogenesis 2 homolog (<i>S. cerevisiae</i>)	Urb2
15	1.288	Casein kinase 1, epsilon	Csnk1e
16	1.287	Mastermind like 2 (<i>Drosophila</i>)	Maml2
17	1.287	Solute carrier family 2, (facilitated glucose transporter) member 8	Slc2a8
18	1.286	Fas (TNF receptor superfamily, member 6)	Fas
19	1.283	Claudin 1	Cldn1
20	1.275	Toll-like receptor 4	Tlr4
21	1.270	Adaptor-related protein complex 1, sigma 3 subunit	Ap1s3
22	1.270	Chemokine (C-C motif) ligand 24	Ccl24
23	1.270	Adhesion molecule with Ig like domain 3	Amigo3
24	1.269	Eph receptor A2	Epha2
25	1.268	Vacuolar protein sorting 52 homolog (<i>S. cerevisiae</i>)	Vps52
26	1.268	Similar to hypothetical protein MGC47256	RGD1308694
27	1.268	Secernin 1	Scrn1
28	1.267	tRNA methyltransferase 61 homolog A (<i>S. cerevisiae</i>)	Trmt61a
29	1.256	Growth differentiation factor 10	Gdf10
30	1.254	Spermatogenesis associated 5	Spata5

*Corrected for background intensity using local background correction.

DNA oxidative damage (8-OHdG) quantitation. The formation of 8-hydroxydeoxyguanosine (8-OHdG) in serum was decreased in a treatment dose-dependent manner showing a decline in DNA oxidative damage (Fig. 2).

Fatty acid composition in rat adipose tissue. The fatty acid profile as indicated by GC-MS, showed a slight decrease in the arachidonic acid (C20: 4n6, AFA) concentration in epididymal and abdominal fat of the male WKY rats in the Gb extract-fed group over a 1-month period compared with the control group (Table 5 and 6). There were also increases in the unsaturated fatty acid (FA) ratio especially single (mono) FA and decreases in saturated fatty acids in the Gb extract-fed groups.

DISCUSSION

Aging can be defined as a process of progressive decline in the physiological capacity of an organism, manifested by

accumulated alteration and destabilization at the whole system level (5). In seeking to lessen the deleterious effects of aging, safer and more effective drugs developed from substances obtained from natural products including sericulture products and crude insect-based preparations, have been studied (13,14). Slowing aging using a range of approaches has included ingestion of anti-oxidants for repairing oxidative damage in DNA, protein, and lipid, as well as stem cell-mediated tissue regeneration, and gene therapy (15-17).

DNA microarray analysis is a powerful tool for examining the mechanisms of complex conditions including aging. The gene expression profile of the gastrocnemius muscle from 5- and 30-month old male C57BL/6 mice revealed that aging is associated with alterations at the mRNA level (6).

Gb extract contains many components such as amino acids, nucleic acids, sugar, lipid and some active substances (unpublished data). Thus, it can access multiple receptors and can prevent the causes of aging due to the fact that it

Table 4. Downregulated genes differentially expressed in the liver tissue of WKY 10-month aged rats treated with *G. bimaculatus* ethanol extract for 1 month

NO.	PairMean ratio* (test/control)	Description	Gene Symbol
1	0.562	UDP glycosyltransferase 2 family, polypeptide B	Ugt2b
2	0.588	RT1 class Ia, locus A2 /// RT1 class I, locus A3 /// RT1 class Ib, locus EC2	RT1-A2 /// RT1-A3 /// RT1-EC2
3	0.636	Ubiquitin D	Ubd
4	0.668	Glycoprotein m6a	Gpm6a
5	0.690	Early growth response 1	Egr1
6	0.703	sprouty homolog 2 (Drosophila)	Spry2
7	0.703	Serine dehydratase	Sds
8	0.711	Insulin-like growth factor binding protein 1	Igfbp1
9	0.725	Elastin	Eln
10	0.728	Dual specificity phosphatase 1	Dusp1
11	0.731	Similar to interferon-inducible GTPase	RGD1309362
12	0.734	Myelocytomatosis oncogene	Myc
13	0.734	EGF-like, fibronectin type III and laminin G domains	Egflam
14	0.735	MAX gene associated	Mga
15	0.742	Insulin receptor substrate 2	Irs2
16	0.745	HtrA serine peptidase 3	Htra3
17	0.746	Similar to interferon-inducible GTPase	MGC108823
18	0.750	RT1 class Ib, locus EC2	RT1-EC2
19	0.751	Nuclear receptor subfamily 1, group D, member 2	Nr1d2
20	0.752	Hemoglobin, zeta	Hbz
21	0.753	Activating transcription factor 3	Atf3
22	0.754	Hypothetical protein LOC680687	LOC680687
23	0.762	Phosphorylase kinase, alpha 1	Phka1
24	0.763	AF4/FMR2 family, member 4	Aff4
25	0.767	TSPY-like 4	Tspyl4
26	0.769	UBX domain protein 4	Ubxn4
27	0.769	Similar to putative protein (5S487)	RGD1310819
28	0.770	Tissue factor pathway inhibitor 2	Tfpi2
29	0.772	Interferon (alpha, beta and omega) receptor 1	Ifnar1
30	0.772	KRIT1, ankyrin repeat containing	Krit1

PairMean ratio* means pair Mean ratio (test/control).

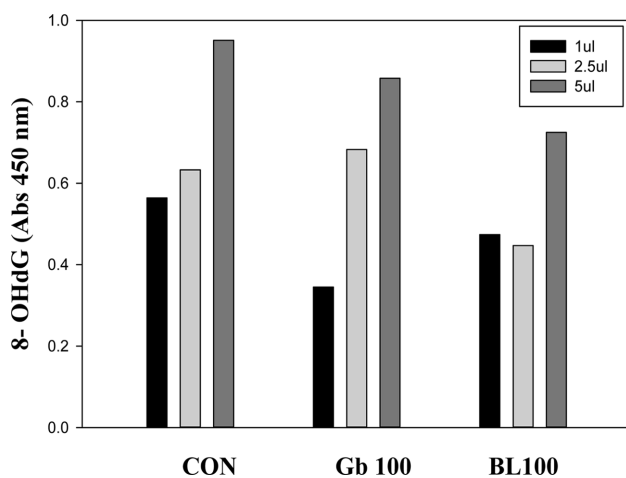


Fig. 2. 8-OHdG level in 10-month old WKY rats treated orally with Gb ethanol extract for 1 month. Gb100 or BL100 corresponds to the 100 mg/kg Gb ethanol or 100 mg/kg BL ethanol extract-treated group.

contains amino acids as a protein source for healthy aging (7). For instance, Gb extract also contains trehalose (insect sugar), chitosan derivatives, essential fatty acids, nucleic acids and gypin (anti-hypertensive agent). Therefore, Gb extract showed a critical anti-aging effect at the DNA oxidative damage decrease level according to the reduction of 8-hydroxy-2'-deoxyguanosine. In addition, creatinine phosphokinase activity exhibited a 25% reduction with an anti-edema effect on 10-month old male Wistar Kyoto rats in the present aging study. The glucose level of the treated groups was slightly increased, 11.9% with the Gb extract and 25% with the blueberry extract (25%), however this increase was not statistically significant compared with the control group. In the present study, with respect to the serum glucose level, Gb extract has a lesser effect than blueberry extract.

Furthermore, data generated from the DNA microarray supported the anti-aging effect of the extracted *G. bimaculatus*, with meaningful gene expression profiles in WKY rats

Table 5. Fatty acid composition of abdominal fat of WKY 10-month aged rats treated with *G. bimaculatus* ethanol extract for 1 month

Fatty acid	Control	Gb extract	Blueberry extract
Myristoleic acid (C14:1)	0.10 ± 0.01	0.22 ± 0.05*	0.10 ± 0.03
Myristic acid (C14:0)	1.32 ± 0.23	1.48 ± 0.17	1.15 ± 0.17
Pentadecanoic acid (C15:0)	0.41 ± 0.06	0.35 ± 0.06	0.36 ± 0.05
Palmitoleic acid (C16:1)	4.76 ± 0.56	6.66 ± 3.41	5.84 ± 1.33
Palmitic acid (C16:0)	19.59 ± 0.37	19.67 ± 1.08	20.42 ± 1.62
Heptadecanoic acid (C17:0)	0.34 ± 0.03	0.30 ± 0.06	0.35 ± 0.01
Linoleic acid (C18:2)	42.90 ± 3.87	42.76 ± 1.92	44.81 ± 0.69
Oleic acid (C18:1)	24.07 ± 3.00	22.62 ± 3.19	21.13 ± 3.25
Stearic acid (C18:0)	4.07 ± 0.16	3.47 ± 1.13	3.32 ± 0.61
Arachidonic acid (C20:4)	0.83 ± 0.08	0.78 ± 0.07	0.86 ± 0.02
Eicosapentaenoic acid (C20:5)	0.13 ± 0.01	0.12 ± 0.03	0.14 ± 0.02
Eicosatrienoic acid (C20:3)	0.21 ± 0.01	0.22 ± 0.05	0.22 ± 0.01
Eicosadienoic acid (C20:2)	0.25 ± 0.02	0.25 ± 0.02	0.25 ± 0.01
Eicosenoic acid (C20:1)	0.40 ± 0.02	0.42 ± 0.05	0.37 ± 0.02
Eicosanoic acid (C20:0)	0.09 ± 0.01	0.10 ± 0.03	0.08 ± 0.01
Docosahexaenoic acid (C22:6)	0.53 ± 0.03	0.57 ± 0.22	0.60 ± 0.06
Saturated fatty acid	25.82 ± 0.25	25.38 ± 2.42	25.68 ± 1.34
Unsaturated fatty acid (UFA)	74.18 ± 0.25	74.62 ± 2.42	74.32 ± 1.34
Single UFA	29.33 ± 3.56	29.92 ± 0.56	27.43 ± 2.01
Poly UFA	44.85 ± 3.76	44.70 ± 2.11	46.89 ± 0.71

Each value represents mean ± S.D.

Asterisk (*) indicates significant difference compared with the control ($p < 0.05$).

Table 6. Fatty acid composition of epididymal fat of WKY 10-month aged rats treated with *G. bimaculatus* ethanol extract for 1 month

Fatty acid	Control	GE extract	Blueberry extract
Myristoleic acid (C14:1)	0.15 ± 0.09	0.08 ± 0.02	0.11 ± 0.04
Myristic acid (C14:0)	1.49 ± 0.15	1.19 ± 0.28	1.51 ± 0.47
Pentadecanoic acid (C15:0)	0.42 ± 0.08	0.34 ± 0.04	0.43 ± 0.08
Palmitoleic acid (C16:1)	5.73 ± 2.11	4.03 ± 1.12	4.34 ± 0.34
Palmitic acid (C16:0)	21.15 ± 3.92	19.00 ± 5.21	22.47 ± 2.42
Heptadecanoic acid (C17:0)	0.31 ± 0.07	0.33 ± 0.08	0.41 ± 0.11
Linoleic acid (C18:2)	43.46 ± 5.90	44.23 ± 1.36	40.06 ± 5.58
Oleic acid (C18:1)	21.04 ± 3.84	24.07 ± 4.94	23.20 ± 3.04
Stearic acid (C18:0)	3.85 ± 1.32	4.55 ± 0.86	4.83 ± 1.30
Arachidonic acid (C20:4)	0.80 ± 0.26	0.73 ± 0.05	0.85 ± 0.18
Eicosapentaenoic acid (C20:5)	0.12 ± 0.04	0.11 ± 0.00	0.13 ± 0.03
Eicosatrienoic acid (C20:3)	0.21 ± 0.07	0.19 ± 0.03	0.22 ± 0.05
Eicosadienoic acid (C20:2)	0.28 ± 0.08	0.25 ± 0.06	0.30 ± 0.05
Eicosenoic acid (C20:1)	0.45 ± 0.03	0.46 ± 0.10	0.58 ± 0.11
Eicosanoic acid (C20:0)	0.12 ± 0.03	0.12 ± 0.03	0.15 ± 0.02
Docosahexaenoic acid (C22:6)	0.43 ± 0.26	0.35 ± 0.03	0.40 ± 0.11
Saturated fatty acid	27.33 ± 5.51	25.67 ± 4.93	29.89 ± 3.42
Unsaturated fatty acid (UFA)	72.67 ± 5.51	74.33 ± 4.93	70.11 ± 3.42
Single UFA	27.33 ± 2.31	28.33 ± 4.16	28.12 ± 2.79
Poly UFA	45.67 ± 6.66	45.67 ± 1.53	41.99 ± 6.02

being observed after a 1-month treatment. Fas (pro-apoptotic and matrix-degradation gene expression following induction of osteoarthritis in mature and aged rabbits (18), Amigo3 (19), and Rtn4 (whose expression protects SH-SY5Y cells against multiple death insults (20) were upregu-

lated (Table 4). Ugt2b, Egr1, and Gpm6a were downregulated (Table 5). Ugt2b and Egr1 play an important role in the regulation of cell proliferation, differentiation, and angiogenesis (21,22). Gpm6q, the sole member of the proteolipid protein family of tetraspan proteins to be expressed

exclusively by neurons in the central nervous system (23,24), was down-regulated 0.67 fold.

In conclusion, *G. bimaculatus* extract has potential efficacy in the treatment of aging in WKY rats by significantly reducing CK in serum. Consequently, *G. bimaculatus* extract may be a protective nutraceutical for aging-related disorders including circulatory disorders. The gene expression profile of WKY rats treated with *G. bimaculatus* extract could be a valuable prognostic marker to identify potential therapeutic targets for aging.

ACKNOWLEDGEMENTS

This work was supported by the Rural Development Administration National Research project, PJ009827.

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