Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : December 24, 2018
Accepted : February 14, 2019
Published online : March 31, 2019
doi:10.24659/gsr.6.1_31

Review article

Anti-glycation and improvement microbiota by *Geranium dielsianum* extract: Relation to health problems in athletes.

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Abstract

In Peru, *Geranium dielsianum* (GD) has been transmitted for its medical efficacies for diabetes and various inflammations for a long time, and it has been known as GD tea, in particular. We, in this paper, summarize the efficacies of GD extract (trade name, GDE: MISKAMISKA ™) that they had been investigating and explain the relationship between its anti-glycation action and the health problems of athletes. In an *in vitro* experiment, inhibitory effects on the formation of advanced glycation end products (AGEs) equivalent to the level of aminoguanidine or higher were confirmed in the anti-oxidant activity (ORAC = 5.1 × 10^3 μmolTE/g), α-glucosidase inhibitor, and glucose/type 1 collagen reaction system of the GDE. In an animal experiment, as the result of an assessment of their effects on intestinal bacterial flora, using Sprague Dawley (SD) rats and type-2 diabetes model (Otsuka Long-Evans Tokushima Fatty; OLETF) rats, an increase of lactobacilli and bifidobacteria in cecal contents and stool by the intake of GDE was observed. As a result of a human clinical experiment (open-label experiment without a control group), even though no improvement in skin autofluorescence (SAF) was found by the analysis using AGE Reader ™, there were findings suggesting the improvements of skin moisture content and the maintenance of transpidermal water loss in addition to the improvements of subjective symptoms relating to skin and constipation. Summarizing the efficacies of GDE against glycative stress reactions, as the first stage, the inhibition of postprandial hyperglycemia by the inhibitory activity of α-glucosidase and as the second stage, the inhibition of the formation of AGEs were shown; and in addition to the direct action of GDE, the involvements of its anti-oxidant effect and the enhanced production action of lactic acid and short-chain fatty acid in intestinal bacterial flora were suggested. Meanwhile, as a result of the literature research of the health situation of athletes, it was observed that athletes are actually suffering from skin symptoms and gastrointestinal symptoms such as diarrhea. It is possible that GDE contributes to the resolution of these problems in athletes, and future research is expected.

KEY WORDS: *Geranium dielsianum*, advanced glycation end products (AGEs), α-glucosidase inhibition, microbiota, anti-glycation

Introduction: *Geranium dielsanum*

Geranium dielsianum (GD) is a dicotyledonous perennial plant of the *Geraniaceae* family. It is known that this species grows only in highlands more than 3,500 m above sea level in the Central Andes mountain range in Peru. Its medical efficacies for diabetes, epipharyngitis and oral inflammation and its blood purification effect have been transmitted [1)], and it is popular as a brewed health tea in Peru. GD extract (GDE) has been commercialized as MISKAMISKA™ and its action to improve intestinal environment is reported [2)].

GDE has been sold as a commercial product, and its effects of improvements of intestinal environment and subjective symptoms relating to skin have been reported in a post-marketing survey. The results of the *in vitro* experiment, animal experiments relating to intestinal bacteria, and human clinical experiment concerning GDE that we conducted are introduced.

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Glycative Stress Research 2019; 6 (1): 031-038

Concept of Glycative Stress and its Countermeasure

Recently, lifestyle diseases relating to glycative stress, such as type-2 diabetes, metabolic syndrome, and dyslipidemia, have been remarkably increasing. Although this tendency is becoming common in Japan and Southeast Asian countries, it is also occurring worldwide.

Glycative stress is mainly a series of biological reactions where aldehyde derived from reducing sugar, fat, and alcohol becomes excessive; non-enzymatically then reacts with protein in the living body, and through a complicated processes, advanced glycation end products (AGEs) are produced [3, 4]. AGEs deposit in living tissue, and at the same time, combine with receptor for AGEs (RAGE) and generate inflammatory cytokines; and as a result, it causes problems in various organs and systems [3]. A portion of the AGEs is transferred into cytoplasm via scavenger receptors which then cause endoplasmic reticulum (ER) stress. Furthermore, if they reach the cell nucleus, it causes epigenome change [5]. A rapid increase in blood glucose after meals (140 mg/dL or more) is called a "glucose spike" [6], which simultaneously causes the formation of various kinds of aldehyde ("aldehyde spark") by exposed aldehyde of ring-opened glucose, and precipitate tissue disorders, such as vascular endothelial cell disorder [7]. In other words, postprandial hyperglycemia adversely affects the living body more than the amount which has recently been considered.

There are several stages to reducing glycative stress. The first stage is the inhibitions of glucose spikes and aldehyde sparks. The second stage is the inhibition of AGE formation. The third stage is the promotion of AGE decomposition. The fourth stage is the control of AGEs/RAGE signals. The fifth, and final, stage is the method concerning exogenous AGEs. In the process of AGE formation of the second stage, oxidation reaction is involved in part of the process, as AGE formation is alleviated to some extent caused by functional ingredients having anti-oxidant action.

In this research, the results collected to date regarding what stage GDE exerts its action and alleviates glycative stress are shown.

In Vitro Action of *Geranium Dielsianum* Extract (GDE) α-Glucosidase Inhibitory Activity

Regarding α-glucosidase inhibitory activity by GD extract (GDE), the inhibitory activities of sucrase and maltase were measured. Fifty-percent inhibitory concentrations (IC_{50}) of each of sucrase and maltase show that the inhibitory activity of sucrase was 0.028 mg/mL and that of maltase was 0.016 mg/mL [8]. The blood glucose spike may be expected to be alleviated by reducing glucose absorption by α-glucosidase inhibition.

In an experiment using streptozotocin-induced diabetic rats, the blood glucose level-lowering effect by GD aqueous extract (500 mg/kg body weight) has been reported [9], a part of the action mechanism is considered to be α-glucosidase inhibition caused by GD aqueous extract.

AGE Formation Inhibitory Activity

AGE formation inhibitory activity in a glucose/type 1 collagen *in vitro* reaction system was assessed using GDE as a test product and aminoguanidine as a positive control. Their activities to inhibit the formation of fluorescent AGEs and N^{ε}-(carboxymethyl) lysine (CML) were measured as AGEs [8].

The inhibitory ratios of GDE and aminoguanidine as a positive control increased in a concentration-dependent manner, which shows their activities to inhibit the formation of fluorescent AGEs and CML. IC_{50} of GDE against the fluorescent AGE formation was 0.021 mg/mL, and that of aminoguanidine was 0.16 mg/mL (***Fig. 1-a***, original data). The comparison of IC_{50} shows that the intensity of the activity of GDE was approximately eight times of that of aminoguanidine. IC_{50} of GDE against the formation of CML was 0.0065 mg/mL, and that of aminoguanidine was 0.16 mg/mL (***Fig. 1-b***) [8]. From the comparison of IC_{50}, the intense of the activity of GDE was approximately 25 times that of aminoguanidine.

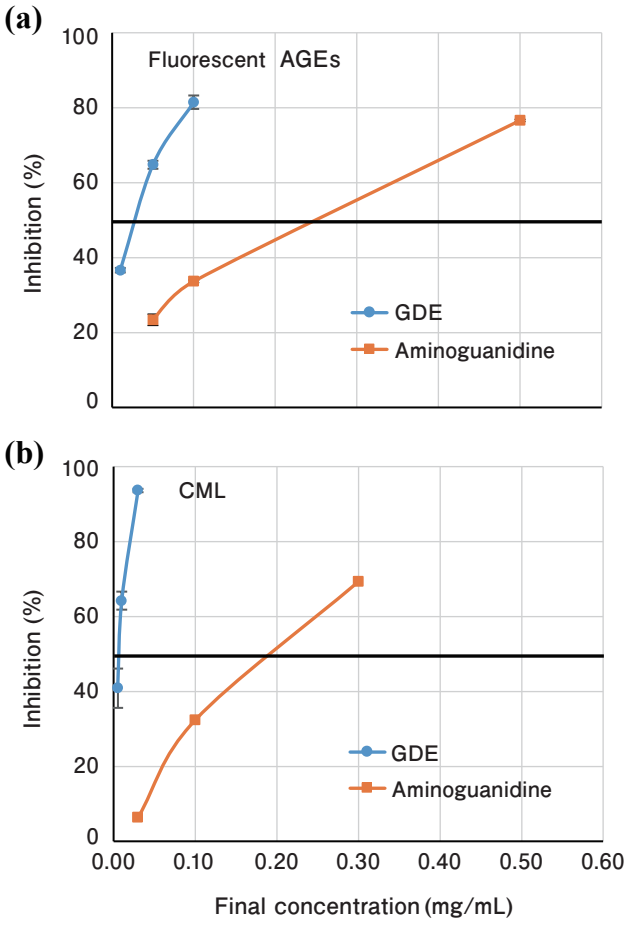

Fig. 1. **Inhibitory effect of GDE on AGE formation in the glucose/type 1 collagen reaction model.**

a: Fluorescent AGEs; results are expressed as mean ± SD, n = 3 (original data). **b**: CML; GDE, results are expressed as mean ± SD, n = 3 and aminoguanidine, results are expressed as mean values, n = 2, quoted from the Reference 8. AGE, advanced glycation end product; GDE, *Geranium dielsianum* extract; CML, N^{ε}-(carboxymethyl)lysine; SD, standard deviation.

CML is formed from the reaction between lysine residue and the glyoxal derived from the oxidative cleavage of Amadori compounds and lipid peroxidation, as well as from glycol aldehyde derived from hypochlorous acid and serine [3]. Its formation is also accelerated by the existences of reactive oxygen species, such as hydroxyl radical (・OH) and superoxynitrite (ONOO・). CML is a typical AGE accumulation in skin and exists on the epidermal layer, which turnover is relatively rapid [10, 11]. It can be expected to reduce and alleviate skin disorders caused by glycative stress by inhibiting the formation of CML on skin.

Anti-oxidant Activity

In order to assess the anti-oxidant capacity, an oxygen radical absorbance capacity (ORAC) experiment [12] was conducted. The ORAC value of GDE was 5.1×10^3 μmolTE/g [8]. Although simple comparison is difficult because the extraction conditions and solid component amounts are different, the ORAC value of GDE showed its anti-oxidant activity to be stronger than those of *Lindera umbellate* extracts 1.5×10^3 μmolTE/g and *Perilla frutescens* extracts 1.1 × 103 μmolTE/g [13]. The measurement results were expressed by the activity unit (μmolTE/g) expressed by Trolox of 1 μmol (molecular weight: 250.29). TE means Trolox Equivalent.

Effects of *Geranium dielsianum* Extract (GDE) on Intestinal Bacteria

The result of a collaborative research between Tezukayama Gakuin University, Faculty of Human Sciences, Department of Food and Nutrition and TOWA CORPORATION Ltd. is introduced. The effects of GDE orally taken on intestinal bacterial flora, using Otsuka Long-Evans Tokushima Fatty (OLETF) rats of a type-2 diabetic model and Long-Evans Tokushima Otsuka (LETO) rats of their control model, were analyzed. The relative quantities of lactobacilli and bifidobacteria in cecal contents were measured by a quantitative polymerase chain reaction (PCR) method of genes (16SrDNA). As a result, lactobacilli increased about three times (***Fig. 2-a***) and bifidobacteria about 60 times (***Fig. 2-b***) in the GDE intake group (original data).

An experiment of the analysis of intestinal bacterial flora has been conducted, where Sprague Dawley (SD) rats were divided into the group of those grown with food mixed with GDE and control food group [2]. As a result, the amount of lactobacilli in cecal contents was significantly larger in the GDE-mixed food group than the control food group by the analysis using the quantitative PCR method (***Fig. 3-a***). The amounts of bifidobacteria both in stool (two weeks, four weeks) and cecal contents of the GDE mixed food group were significantly larger than those of the control food group (***Fig. 3-b***). The pH of the cecal contents of the GDE mixed food group was 7.28 ± 0.04, and that of the control food group was 8.0 ± 0.13 ($n = 6$, $p < 0.001$); the GDE mixed food group showed a significantly low value. It was considered that in the GDE food group, pH was kept at a low level owing to lactic acid and short-chain fatty acid (SCFA) produced by lactobacilli and bifidobacteria.

These results suggest that the GDE acts to improve intestinal environment. The increased productions of lactic acid and SCFA including acetic acid and butyric acid are part of the merits of improvement of intestinal environment. SCFA maintains intestinal pH from weak acid to neutral condition and can inhibit the growth of saprogenic bacteria. SCFA connects with specific receptors (G-protein coupled receptors such as APR41 and GPR43), and play various roles in the body. In sympathetic ganglion, the rises of heart rate and body temperature occur by the stimulation by SCFA [14, 15]. In white adipose tissue, fat decomposition is progressing and fat accumulation is inhibited [16]. This phenomenon is said to be a compensation action in energy metabolism, where intestinal bacteria increase the production of SCFA at the time of excessive consumption, and basal metabolism rises by it being sensed by receptors. In our *in vitro* experiment model where glucose and human albumin were reacted, the inhibitory effect of acetic acid and lactic acid on the AGE formation was confirmed (***Table 1***) [17]. The rise of basal metabolism and inhibitory action on fat storage (leading to the prevention of fatty liver and visceral fat) was associated with the restoration of healthy intestinal bacteria flora, as

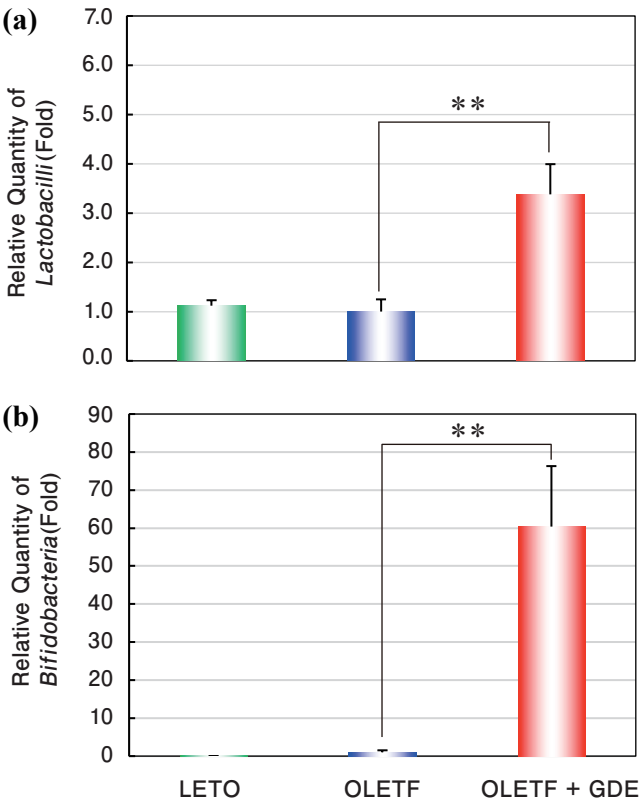

Fig. 2. **Effect of GDE on bacteria count in cecum: evaluation in the type-2 diabetic model rat (OLETF).**

a: Lactobacilli, **b**: Bifidobacteria. Bar indicates SEM, **p < 0.01 vs OLETF group, n = 6 each. Bacteria count was measured by the quantitative PCR method using 16SrDNA. GDE, *Geranium dielsianum* extracts; PCR, polymerase chain reaction; OLETF, Otsuka Long-Evans Tokushima Fatty; LETO, Long-Evans Tokushima Otsuka = control of OLETF; SEM, standard error mean. Original data.

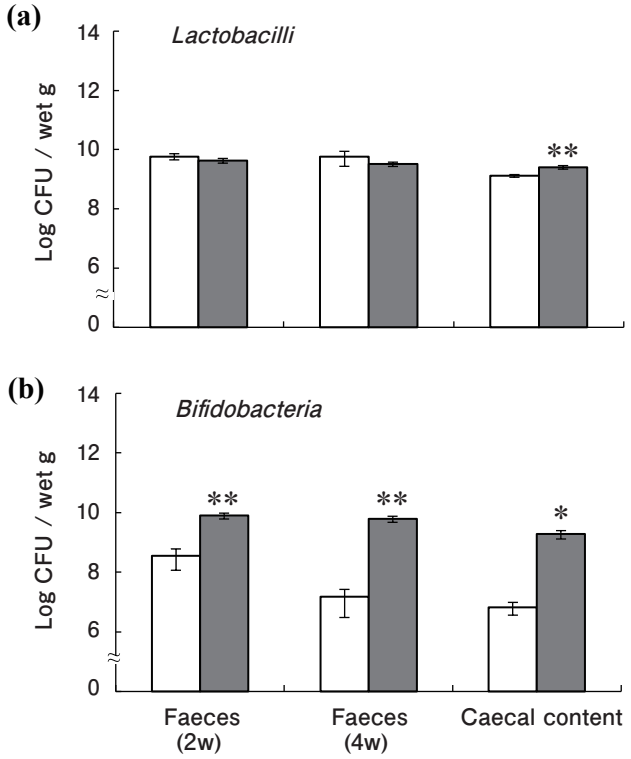

Fig. 3. **Effect of GDE on bacteria count in cecum and stool: evaluation in the SD rat.**

a: Lactobacilli, **b**: Bifidobacteria. White column: control bait group, gray column: GDE-containing bait group. Results are expressed as mean ± SEM, *p < 0.05, **p < 0.01 vs. control bait group, n = 6 each. Bacteria count was measured by the quantitative PCR method using 16SrDNA. GDE, *Geranium dielsianum* extracts; PCR, polymerase chain reaction; SD, Sprague Dawley; SEM, standard error mean; CFU, Colony Forming Unit. Quoted from the Reference 2.

Table 1.
Anti-glycation activity of organic acid and inorganic acid.

	Sample	IC_{50} (mg/mL)
Organic acid	Acetic acid	0.018
	Lactic acid	0.022
	Gluconic acid	0.021
	Oxalic acid	0.004
	Malonic acid	0.008
	Succinic acid	0.007
	Malic acid	0.004
	Tartaric acid	0.005
	Citric acid	0.003
Inorganic acid	Hydrochloric acid	0.316
	Perchloric acid	0.013
	Phosphoric acid	0.026
	Sulfuric acid	0.025

Inhibitory actions on fluorescent AGE formation in the glucose/human serum albumin reaction model were evaluated. Results are expressed as IC_{50}, n = 3. AGE, advanced glycation end product; IC_{50}, 50% inhibitory concentration. Quoted from the Reference 17.

well as with any action to inhibit the AGE formation as a direct action work toward the alleviation of glycation stress. It is greatly significant to maintain intestinal bacteria flora in healthy conditions more than has been considered. GDE supportively acts on the maintenance of the homeostasis of intestinal bacterial flora.

Result of an Experiment Using *Geranium dielsianum* Extract (GDE)

We are implementing an open-label experiment without a control group in order to verify the effect and safety of GDE on glycation stress, skin function and intestinal environment of women by using a GDE-based test product [18]. Thirty-three women were recruited from the those aged from 30 to 50 years, and who tended to be constipated, suffered from rough skin, and liked to eat sweets and carbohydrates; and 12 subjects (41.5 ± 5.0 years) who were ranked at the highest in AGE autofluorescence (skin autofluorescence: SAF) [19] by AGE Reader ™ (DiagnOptics, Groningen, the Netherlands) were selected and were administered with GDE (1.200 mg/day) for 12 weeks. They received biochemical examination of blood before the intake (0 week) and 8 and 12 weeks after the start of administration, a survey on lifestyle habits by the Anti-Aging QOL Common Questionnaire (AAQOL), a questionnaire regarding skin and also regarding bowel movements was conducted. SAF was measured for glycation stress index, water content in stratum corneum for the items for the assessment of skin by using a corneometer (Courage & Khazaka, Cologne, Germany) and transepidermal water loss by using Tewameter (Courage & Khazaka). Blood oxidative stress (bOS) and blood anti-oxidant power (bAP) [20] of the serum samples were measured for the index of oxidative stress by using Spotchem IM (Arkray, Kyoto, Japan).

As a result, according to the questionnaire concerning skin, the subjective symptoms after the intake of GDE for 12 weeks, “Dryness of skin”, “Cosmetic collapse”, “Dullness of skin” and “Fine grain and smooth skin” significantly improved ($p < 0.05$). “Moist-feeling skin” significantly improved from eight weeks to 12 weeks after the intake ($p < 0.05$). According to the survey with AAQOL, the score of “Constipation” significantly improved and the number of bowel movements significantly increased from the first week since the intake and continued until the twelfth week ($p < 0.001$).

In the blood biochemical examination, the comparison of before and after the intake of GDE showed that the level of HDL cholesterol significantly rose (previous value: 66.3 ± 14.7 mg/dL, after value: 71.5 ± 12.7 mg/mL, +7.8%, $p = 0.034$). Regarding oxidative stress index, even though there was no differences in bOS, bAP significantly rose (+14.5%, $p = 0.013$).

In the skin moisture test, the moisture content in stratum coreum at the region of right upper arm was significantly improved twelve weeks after the intake (+14.0%, $p = 0.040$). Transepidarmal water loss at the region of the right upper arm significantly decreased eight weeks after the intake (−18.9%, $p = 0.020$) and 12 weeks after intake (−25.3%, $p = 0.002$). No significant improvement was recognized in dermal elasticity.

In the analysis as a whole, a significant increase of SAF was recognized (previous value 2.14 ± 0.30, after value: 2.26 ± 0.22, +5.6%, p = 0.028). It is pointed out that the fluorescence wavelength detected by AGE Reader TM has a commonality with the autofluorescent wavelength of flavonoids included in a part of the herbs. As an example, in the test where the mixed extracts of *Anthemis nobilis*, *Houttuynia coradata*, *Crataegus oxyacantha* and *Vitis vinifera* were applied to the skin, although there was no significant difference, the upward tendency of SAF was recognized in spite of an improving trend of skin elasticity (***Fig.4***)[21]. Generally speaking, fluorescent AGEs are assessed by an excitation wavelength of 370 nm and a detection wavelength of 440 nm; however, the fluorescence spectrum of the mixed herb extract liquid used in this test has fluorescence in the neighborhood of 440 nm (***Fig. 5***). This is due to the flavonoids included in these herbs having a high possibility of an autofluorescence similar to AGEs. It will be a future task to investigate the functional ingredients included in GDEs and the wavelength characteristics of the fluorescence.

As a subclass analysis, an analysis was conducted by classifying subjects into an upper group (2.19 or more) and lower group (less than 2.19) based upon the SAF

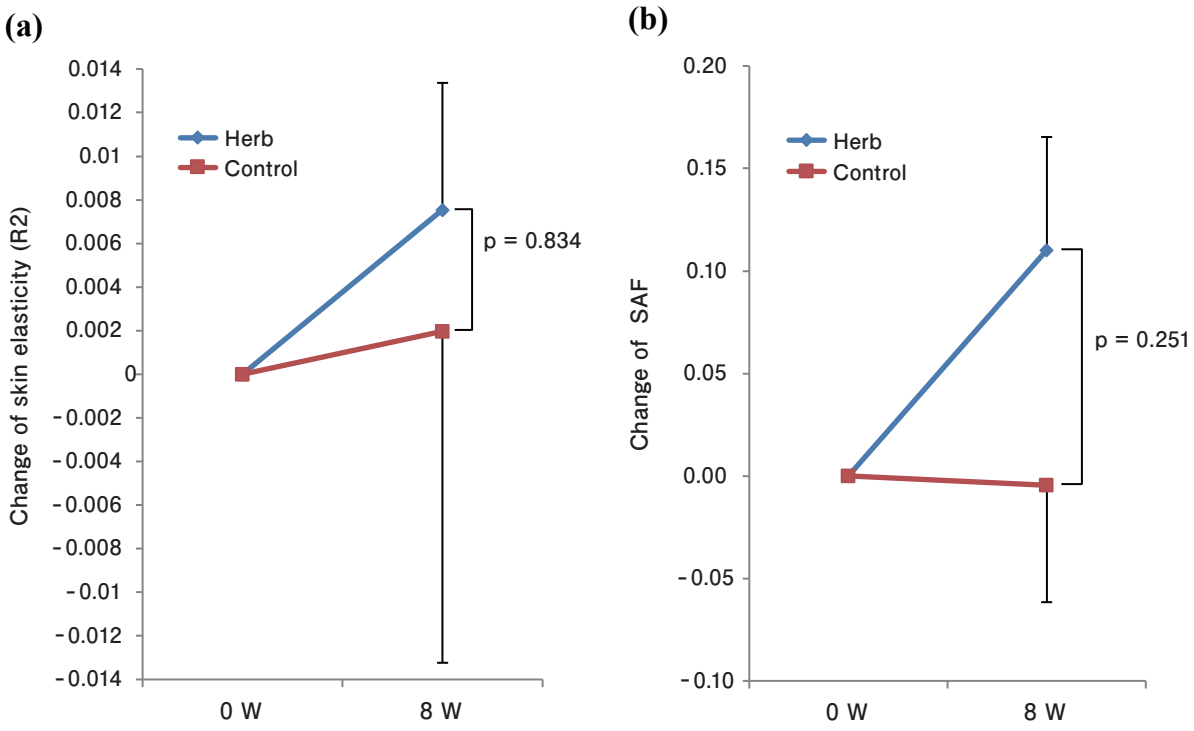

Fig. 4. **Change of skin function after the 8-week topical application of mixed herb.**

a: Skin elasticity (R2), **b**: SAF. Bar indicates SEM, n = 5. The mixed herbs consist of *Anthemis nobilis*, *Houttuynia coradata*, *Crataegus oxyacantha* and *Vitis vinifera*. R2 was measured by Cutometer (Courage & Khazaka, Cologne, Germany). The skin area without topical application was used as control. SAF, skin autofluorescence measured by AGE Reader; SEM, standard error mean. Quoted from the Reference 21.

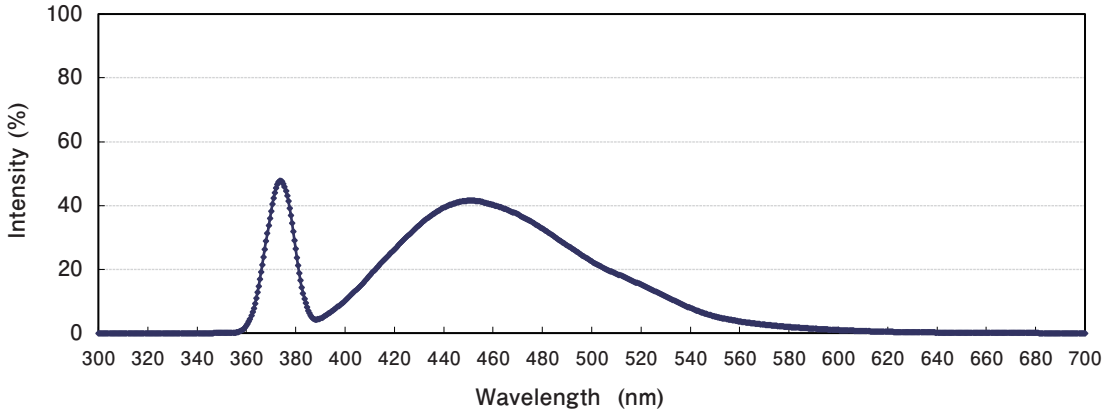

Fig. 5. **The fluorescence spectrum of mixed herb.**

The mixed herbs (0.2% solution) consist of *Anthemis nobilis*, *Houttuynia coradata*, *Crataegus oxyacantha* and *Vitis vinifera*. Excitation wavelength: 370 nm. Quoted from the Reference 21.

median value of 2.19 before the intake of the test product. No differences were observed in SAF and fasting blood glucose in the upper group; however, bA1c significantly decrease twelve weeks after the intake (previous value: 5.45 ± 0.22%, after value: 5.33 ± 0.23%, −2.2%, p = 0.034). As an additional analysis, the relationship between SAF and actual age was analyzed as an additional analysis. SAF was estimated by calculation from actual age. After twelve weeks, the reduction in SAF was recognized in three out of four subjects whose SAF was higher than 25% compared with the actual age at the condition where they had not taken GDEs yet. From these findings, it is suggested that there is no room for improvement in those whose SAF value was not high, and room for improvement in glycation stress is left for those whose SAF was high.

Regarding the safety evaluation of this research, no serious adverse events were recognized during or after the experiment, and the safety of GDE was confirmed.

In conclusion, the improvement of bowel movements and the effects on skin moisture maintenance were shown in the open-label experiment without a control group. Even though the anti-glycation action of GDE was not clarified in this experiment, in the *in vitro* experiment, its strong anti-glycation action and inhibitory action on the absorption of carbohydrate were recognized. Furthermore, from the result that SAF value was lowered in additional analysis centered on the subjects whose SAF value adjusted by age was high, GDE's anti-glycation can be expected by implementing a double blind experiment by narrowing down the conditions of the subjects in the future.

Relationship with Health Problem of Athletes

Generally, moderate exercise is said to be good for health. According to the health investigation of masters athletes 88 cases (56.8 ± 10.8 years), the scores of subjective symptoms of AAQOL were kept lower than those of the men of the same age [22]. It can be said that this is the result of appropriate self-discipline over a long period. However, in the cases of athletes, they often ruin their health due to excessive exercise.

In the research discussing the effects of walking, targeting 31 healthy subjects (59.1 ± 5.0 years) by classifying them into a testing group and a control group, the findings of exacerbation of subjective symptom were observed [23]. In the test group, the subjects worked at an exercise intensity of 40%~60% using a wristwatch-type walking meter equipped with pulse monitor (Pullsse: Seiko, Tokyo, Japan), and in the control group, they walked without using a walking meter. In both groups, the working amount was 45 minutes a day, five times a week for eight weeks. Furthermore, although significant improvements in several items were recognized by the assessment of subjective symptom using AAQOL, exacerbating items were also found. In the test group, only one item of "Skin problems" was recognized as an exacerbating item and in the control group, significant exacerbating scores were observed, such as "Liable to catch colds," "Tinnitus," and "Arthralgia." In the control group, they tend to walk at a somewhat fast pace, which may possibly lead to "Arthralgia." However, even the walking, which is considered to be appropriate for the test group, may cause "Skin problems."

In many cases of athletes, the amount of exercise associated with training substantially exceeds the exercise amount of the walking mentioned above. The health problems of athletes are greatly different in relation to damaged region, intensity of exercise, kind of sport, gender, and age.

In the American Medical Society for Sports Medicine, the three cardinal symptoms of common health problems in female athletes: 1) lack of available energy, 2) hypothalamic amenorrhea and 3) osteoporosis [24]. For Japanese female gymnasts, as well, the problems of irregular menstruation, inappropriate intake of fat and protein, chronic muscle fatigue, and lowering of immune function are reported [25].

Cases of skin lesions of athletes often occur. Traumatic skin lesions often occur with soccer players and wrestlers, and fungal infections are often observed in swimmers and soccer players [26]. There are many cases where climbing is associated with problems related to muscles and the skeletal system. As the result of diagnosing the skin of the limbs of 60 climbers (51 males and 9 females), exfoliation of the skin of fingers (93.3%), formation of calluses on the fingers (90.0%) and athlete nodule (83.3%) were recognized [27]. Excessive exercise and weight restriction starting from before puberty causes the reduction of ovarian function, such as amenorrhea [28], and the reduction of the secretion of estrogen adversely affected the skin condition. Facial localization-type atopic dermatitis is often directly caused by sports during puberty [29].

It has been reported that long distance runners tend to have gastrointestinal symptoms including diarrhea and lower gastrointestinal bleeding. As a result of the research of literature using PubMed, 1,184 cases of runner's diarrhea were extracted, and in the cases where genders were identified, the number of runners having gastrointestinal symptoms was 1,084 (817 male cases and 274 female cases). The average age was 33.3 (16~67 years), the average running distance was 26 miles/week and the average rate of developing diarrhea was 40% [30]. There is a high possibility that it is caused by the disturbance of intestinal bacterial flora (dysbiosis).

Recently, the relationship between the brain and intestine has attracted attention. It has been known that dysbiosis brings about mental and physical stress symptoms, such as anxiety and depression, and the replenishment of probiotics, such as lactobacilli and bifidobacteria, can alleviate these symptoms. There is a report targeting male long distance runners who are university students, in which it was investigated whether the intestinal bacterial flora significantly changed when they subjectively felt bad symptoms [31]. A significant relationship between emotional stability and performance was recognized; however, no conclusion was obtained because there are individual differences in intestinal bacterial flora.

Athletes are often exposed to mental stress, and psychological elements greatly affect performance, leading them to need mental support. It is important to maintain high sleep quality in order to also maintain mental stability. Although appropriate exercise raises the quality of sleep, excessive exercise causes sleep disorders [32]. The use of appropriate bedding will favorably affect the performance of an athlete.

Conclusion

It was shown that GDE has anti-glycation action, anti-oxidative action and an intestinal environment improving action. Finally, the following is the relationship between GDE and anti-glycative stress, summarizing at which stage of the countermeasure against glycation stress these actions work. At the first stage, regarding the inhibition of blood glucose spikes, because GDE has an α-glucosidase inhibitory activity, it is assumed that the absorption of glucose is delayed and as a result postprandial hyperglycemia is alleviated. At the second stage, the inhibition on the AGE formation was shown in *in vitro* experiments. Anti-oxidant activity also contributes to the alleviation of this reaction. The promotion of AGE decomposition at the third stage, and the control of AGEs/RAGE signal at the fourth stage are problems to be solved in the future. The action caused by the intake of GDE to increase lactobacilli and bifidobacteria in the intestinal bacterial flora greatly contributes to the alleviation of glycation stress. SCFA has an inhibitory action on the AGE formation. SCFA raises basal metabolism (rises of body temperature and heart rate) via receptor and adjusts insulin action so that fat and carbohydrates do not become excessive. It is assumed that owing to the above mechanism, GDE supports the alleviation of glycation stress.

It is also assumed that athletes have great problems handling mental and physical stresses, skin disorders, digestive symptoms, and dysbiosis associated with it. The improvement of the balance of intestinal bacteria flora and improvement of skin disorders out of the actions of GDE that have been clarified so far contribute to the solution of some of the problems that athletes face.

Acknowledgements

This study was supported by the Japanese Council for Science, Technology and Innovation, SIP (Project ID 14533567), "Technologies for creating next-generation agriculture, forestry and fisheries" (funding agency: Bio-oriented Technology Research Advancement Institution, NARO), and by ifia Japan 2019 ("Sports and Nutrition Project" organized by Food Chemicals Newspaper Inc., Tokyo, Japan). A part of this study was published in the journal "Food Style 21" and presented at The 7th Symposium of Peruvian Herb Functional Research, November 20, 2018, Tokyo.

Conflict of interest

A part of this study was supported by TOWA CORPORATON Ltd.

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