

## Effect of the magnetized water supplementation on blood glucose, lymphocyte DNA damage, antioxidant status, and lipid profiles in STZ-induced rats

Hye-Jin Lee and Myung-Hee Kang<sup>§</sup>

*Department of Food Science and Nutrition, Daedeok Valley Campus, Hannam University, 461-6 Jeonmin-dong, Yuseng-gu, Daejeon 305-811, Korea*

### Abstract

This study investigated the effects of magnetized water supplementation on blood glucose, DNA damage, antioxidant status, and lipid profiles in streptozotocin (STZ)-induced diabetic rats. There were three groups of 4-week-old male Sprague-Dawley rats used in the study: control group (normal control group without diabetes); diabetes group (STZ-induced diabetes control); and magnetized water group (magnetized water supplemented after the induction of diabetes using STZ). Before initiating the study, diabetes was confirmed by measuring fasting blood glucose (FBS > 200 dl), and the magnetized water group received magnetized water for 8 weeks instead of general water. After 8 weeks, rats were sacrificed to measure the fasting blood glucose, insulin concentration, glycated hemoglobin level, degree of DNA damage, antioxidant status, and lipid profiles. From the fourth week of magnetized water supplementation, blood glucose was decreased in the magnetized water group compared to the diabetes group, and such effect continued to the 8th week. The glycated hemoglobin content in the blood was increased in the diabetes group compared to the control group, but decreased significantly in the magnetized water group. However, decreased plasma insulin level due to induced diabetes was not increased by magnetized water supplementation. Increased blood and liver DNA damages in diabetes rats did significantly decrease after the administration of magnetized water. In addition, antioxidant enzyme activities and plasma lipid profiles were not different among the three groups. In conclusion, the supplementation of magnetized water not only decreased the blood glucose and glycated hemoglobin levels but also reduced blood and liver DNA damages in STZ-induced diabetic rats. From the above results, it is suggested that the long-term intake of the magnetized water over 8 weeks may be beneficial in both prevention and treatment of complications in diabetic patients.

**Key Words:** Magnetized water, blood glucose, DNA damage, lipid profile, STZ-induced rat

### Introduction

According to the recently announced data from the Statistics Korea [1], diabetes mellitus showed in 20.7 out of 100,000 individuals and ranked fourth in the 2010 Annual Report on the Cause of Death Statistics; and the mortality rate due to diabetes tended to increase 5.6% compared to the previous year. Diabetes mellitus has been considered as a disease developed by several mechanisms, but some hypotheses on diabetes and oxidative stress have been recently suggested. Hyperglycemia, one of the major clinical symptoms of diabetes, is the primary factor for several chronic complications of diabetes such as arteriosclerosis or cardiovascular diseases [2]. When chronically high blood glucose is maintained, the production of reactive oxygen species (ROS) is increased due to protein glycation and glucose autooxidation [3] and interferes with the anti-oxidative defense systems [4].

It has been reported by investigators that diabetic patients show higher oxidative stress and oxidative DNA damages and lower

antioxidant defense systems, compared to healthy individuals [5-7]. Also, it has been reported in animal experiments that higher DNA damages are observed in the kidney and liver of streptozotocin (STZ)-induced type 1 diabetic rats [8,9]. Therefore, it is considered important that not only blood glucose but also oxidative stress in the body needs to be reduced to treat the complications of diabetes. Various efforts have been made for the treatment of diabetic complications using antioxidants [10,11] such as quercetin, ascorbate, and beta-carotene, as well as antioxidant nutrients [12-14], the natural food extracts with antioxidant activity [15-18], in addition to the conventional treatment using hypoglycemic agents which have side effects. Although the development of health functional foods for the management of diabetic complications through the reduction of oxidative stress in the body has been actively promoted, it is expected that the health-promoting effects through functional water, which is the basis of our body, rather than health functional foods, may be more effective. However, the research on the physiological effects (particularly the anti-diabetic effect) of

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<sup>§</sup> **Corresponding Author:** Myung-Hee Kang, Tel. 82-42-629-8791, Fax. 82-42-629-8808, Email. mhkang@hnu.kr

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beverages which are not based on the extract of natural foods or functional drinking water is rather wanting.

Recently, the interest in magnetized water has been increased along with the interest in the functionality of beverages. Magnetized water is hexagonal water obtained by passing water through specially manufactured permanent magnet that can activate and ionize water molecules to change its structure hexagonal, like water in our body. It has been known from drinking experiences and case reports that the magnetized water is effective in several chronic diseases including diabetes (caused by oxidative stress), but scientific experimental results are seldom reported. Among the efficacies of magnetized water, it was reported that magnetized water increased glutamate decarboxylase activity [19] and reduced dental plaque [20]. Some Chinese studies have shown the magnetized water to be effective in the treatment of urolithiasis [21] and litholysis [22]. In the recent preliminary study of our laboratory, the administration of magnetized water for at least 6 weeks suppressed the lymphocyte DNA damages in animals with DEN (diethyl nitrosamine)-induced cancer [23].

Thus, this study was performed to investigate the effects of the magnetized water administered for a period of time on blood glucose, lymphocyte DNA damage, antioxidant status, and lipid profiles in streptozotocin-induced diabetic rats.

## Materials and Methods

### *Animal breeding and experimental design*

For experimental animals, 24 male Sprague-Dawley rats, 4-week-old, were purchased from the Central Lab, Animal Inc. (Korea) and kept in the animal laboratory with automatic temperature and humidity control. Each animal was kept in a cage with free access to water and feed for 1-week of acclimation period before the experiment. Eight animals were assigned to the control group (C), and sixteen animals were assigned to the diabetic groups. To induce diabetes, 50 mg/kg streptozotocin (STZ) dissolved in 0.9% NaCl saline solution was injected

through the tail vein. After 3-4 days, rats with over 200 mg/dl of fasting blood glucose level were selected and assigned to two groups: the diabetes control group (6 rats, STZ-induced diabetes control, DC) and the magnetized water group (5 rats, magnetized water supplemented after the induction of diabetes using STZ, DMW), and kept for 8 weeks.

The magnetized water used in the experiment was produced by passing water through the magnetic field of 9,000-13,000 gauss at the Korea Clean System Co., and it was provided to the magnetized water group for drinking water. The magnetized water was changed every day, as the shelf life of the magnetized water was 1 day according to the instruction of the producer. The AIN-93 diet [24] was used for the basic experimental diet for animals (Table 1). All three groups were provided with the same diet.

### *Blood glucose and intra-peritoneal glucose tolerance test (IPGTT)*

For blood glucose measurement, blood samples were collected from the tail vein of the animal after 12-hour fasting at the 8th week of the experimental diet treatment and measured using the blood glucose monitoring system (Accutrend GC, Roche, Germany). For intra-peritoneal glucose tolerance test, the fasting blood glucose level was used as initial data, and 50% glucose solution (2 g glucose/1 kg BW) was administered intra-peritoneally using an incubation tube. Blood samples were collected from the tail vein at 30, 60, 90, 120, and 180 minutes, and the blood glucose concentration change of the venous blood was measured using the blood glucose monitoring system (Accutrend GC, Roche, Germany).

### *Blood and liver tissue sampling*

At the eighth week after the administration of experimental diet for control group, diabetes group, and magnetized water group, all animals in the three groups were fasted for 12 hours and then sacrificed to collect blood samples by heart puncture. From the whole blood samples, 70  $\mu$ l was saved for Comet analysis and 50  $\mu$ l for glycated hemoglobin analysis. The remaining blood was placed in a lithium-heparin treated polystyrene tube and centrifuged at 3,000 rpm for 15 minutes, and then stored at  $-80^{\circ}\text{C}$  in a freezer for plasma insulin analysis. Erythrocytes were mixed with iso-osmotic phosphate buffered saline (pH 7.4) and centrifuged at 3,000 rpm for 10 minutes, repeated 3 times, and then diluted 1:1 with buffer to obtain erythrocyte suspension. The plasma and erythrocytes were stored at  $-70^{\circ}\text{C}$  in a freezer until analysis. For liver tissue samples, the liver was dissected after sacrifice and rinsed in cold saline, and then blotted dry on a filter paper, frozen quickly in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$  until analysis.

**Table 1.** Composition of experimental diet

Ingredients	grams/kilogram diet
Casein	200.000
Cornstarch	397.486
Dextrose	132.000
Sucrose	100.000
Cellulose	50.000
Soybean Oil	70.000
t-Butylhydroquinone	0.014
Salt Mix	35.000
Vitamin Mix	10.000
L-Cystine	3.000
Choline Bitartrate	2.500

### *Glycated hemoglobin*

Glycated hemoglobin was measured using a Hemoglobin A1c kit (BioSystem, Ltd, Spain). A 50  $\mu$ l whole blood was mixed with 200  $\mu$ l potassium phthalate solution and left in the room temperature for 15 minutes for reaction. The hemolysate was passed through the column using phosphate buffer, and the absorbance was measured at 415 nm using the UV/VIS spectrophotometer (Shimadzu UV-1601, Japan).

### *Plasma insulin*

The plasma insulin level of experimental animals was measured by using the insulin ELISA kit (Linco, Ltd, USA) according to the enzyme-linked immune sorbent assay. In a 96-plate well, 10  $\mu$ l assay buffer, 10  $\mu$ l matrix solution, and 10  $\mu$ l plasma were placed in order and then mixed with 80  $\mu$ l detection antibody and shaken at room temperature for 2 hours. It was rinsed 3 times with wash buffer, mixed with 100  $\mu$ l enzyme solution, again shaken at room temperature for 30 minutes, and rinsed with wash buffer. The 100  $\mu$ l substrate solution was added and shaken for 15 minutes; and the absorbance was measured at 590 nm using the ELISA reader (SUNRISE, Austria).

### *Plasma lipid analysis*

A 0.01 ml sample of plasma stored at  $-80^{\circ}\text{C}$  in a freezer was mixed with 1 ml enzyme solution of the kit reagent (CM Korea Co. Inc.) and reacted for 5 minutes at  $37^{\circ}\text{C}$  in a water bath. Plasma lipids such as total cholesterol and triglycerides were analyzed using the Photometric Auto Analyzer (ERBA CHEMPRO, India). For HDL-cholesterol, 0.2 ml plasma and 0.2 ml precipitation solution were mixed and left at room temperature for 5 minutes and then centrifuged for 10 minutes. Then a 0.1 ml supernatant was mixed with 3 ml enzyme solution and placed in a  $37^{\circ}\text{C}$  water bath for 5 minutes for reaction. It was analyzed using the Potometric Auto Analyzer. LDL-cholesterol was calculated using the Friedwald formula.

### *Erythrocyte antioxidant enzyme activity*

Erythrocyte catalase analysis was performed, as previously stated [25], using the UV/VIS spectrophotometer. Hemolyzed erythrocytes were mixed with 50 mM phosphate buffer (pH 7.0) and hydrogen peroxide, and the reduction of hydrogen peroxide was measured at 240 nm for 30 seconds, at  $20^{\circ}\text{C}$ .

For erythrocyte SOD (superoxide dismutase) activity, the erythrocyte suspension was hemolyzed with distilled water and mixed with ethanol and chloroform, and then centrifuged at 3,000 U/min for 2 minutes. The supernatant was divided into several concentrations and incubated at  $37^{\circ}\text{C}$  for 10 minutes and mixed with 20  $\mu$ l pyrogallol (1,2,3-Trihydroxybenzol), and the concentration was measured at 320 nm for 180 seconds using the UV/VIS spectrophotometer [25]. The SOD activity was defined

as the antioxidant ability that suppresses the auto-oxidation of pyrogallol by 50%.

For glutathione peroxidase (GSH-Px) measurement, hemolyzed erythrocytes were mixed with glutathione, glutathione reductase, and NADPH, and incubated at  $37^{\circ}\text{C}$  for 10 minutes, and then reacted with T-butylhydroperoxide. The reduced concentration of NADPH was measured at 340 nm for 90 seconds, using the UV/VIS spectrophotometer, to calculate the degree of anti-oxidation of GSH-Px [25].

### *Lymphocyte DNA damage measurement by comet assay*

Comet assay for the measurement of lymphocyte DNA damage, as described in the preliminary study [23], was performed by modifying and complementing the method of Singh *et al.* [26]. A sample of 70  $\mu$ l fresh whole blood was mixed in 900  $\mu$ l PBS, and only the lymphocytes were separated using 100  $\mu$ l Histo-paque 1077. Approximately 20  $\mu$ l of lymphocytes were collected to make slides using low melting agarose (LMA) gel and normal melting agarose (NMA). After the gel had hardened, a layer of 75  $\mu$ l LMA solution was applied to cover the slide, which was then soaked in prepared cold alkali lysis buffer (2.5 M NaCl, 100 mM  $\text{Na}_2\text{EDTA}$ , 10 mM Tris) for cell lysis and immersed for 1 hour at low temperature in a dark room to unfold the double helix structure of DNA. After the completion of lysis, slides were arranged in the electrophoresis tank, which was filled with  $4^{\circ}\text{C}$  cold electrophoresis buffer (300 mM NaOH, 10 mM  $\text{Na}_2\text{EDTA}$ , pH > 13). After unwinding, electrophoresis was performed at  $25\text{ V}/300 \pm 3\text{ mA}$  for 20 minutes. After the completion of electrophoresis, the slides were washed three times with neutralizing buffer (0.4 M Tris, pH 7.4) for 5 min at  $4^{\circ}\text{C}$  and dried. All of these steps following the lysis treatment were processed in the dark to prevent additional DNA damage. Ethidium bromide (20  $\mu\text{g}/\text{ml}$ ) was added to each slide which was then analyzed using a fluorescence microscope (LEICA DMLB, Germany). Images of 100 randomly selected cells (50 cells from each of two replicate slides) were analyzed from each rat. Measurements were made by image analysis (Kinetic Imaging, Komet 4.0, UK), determining the tail length (TL,  $\mu\text{m}$ ), percentage DNA in the tail (TD, %), and tail moment (TM, the product of multiplying percentage DNA in the tail with tail length). For all samples, the calculation of DNA damage was made by dividing the cases measured without  $\text{H}_2\text{O}_2$  treatment (spontaneous DNA damage) and those measured after  $\text{H}_2\text{O}_2$  treatment ( $\text{H}_2\text{O}_2$  DNA damage).

### *Liver DNA damage measurement by comet assay*

A certain amount of liver tissue from each experimental animal was collected and mixed with 10 times volume of HBSS buffer (1 mg/g collagenase), placed in a shaking incubator (120 rpm,  $37^{\circ}\text{C}$ ) to separate the cells, and then mixed with low melting agarose gel to disperse on the slide. It was analyzed by the same procedure as the blood comet assay.

*Statistical analysis*

All data were analyzed using SPSS-PC+ statistics package (version 10.0). For each item, percentage and mean ± standard error (SE) were calculated. For significance verification by group, ANOVA was performed. For post-hoc analysis, the significance in the difference of the means among groups was verified through Duncan's Multiple Range Test. All statistical significances were evaluated at α = 0.05 level.

**Results**

*Body weight changes and food intake*

Body weight changes, food intake, and water intake of experimental animals were shown in Table 2. Body weight change was decreased and food intake and water intake were significantly increased in diabetes group and magnetized water group compared to control group, but no significant differences were observed between the diabetes group and the magnetized water group.

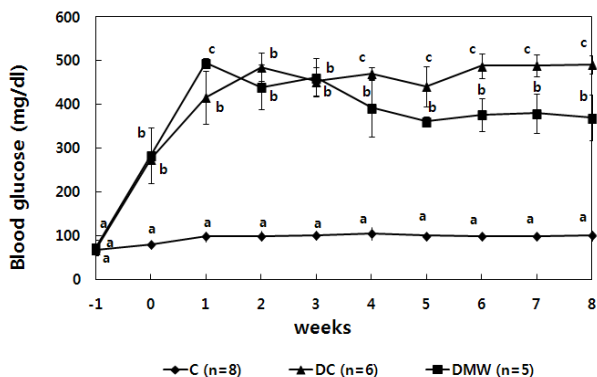
*Blood glucose and intra-peritoneal glucose*

The blood glucose changes in the three groups during 8 weeks of the experimental period were shown in Fig. 1. The blood

**Table 2.** Body weight gain, food intake, and water intake of rats

Groups	After STZ injection			Water intake (ml/day)
	Initial BW (g)	Final BW (g)	Food intake (g/day)	
C	140.70 ± 8.84 <sup>1)</sup>	473.97 ± 32.03 <sup>a</sup>	23.31 ± 1.11 <sup>a</sup>	65.49 ± 13.00 <sup>a</sup>
DC	139.70 ± 8.09	273.51 ± 66.01 <sup>b</sup>	26.13 ± 1.92 <sup>b</sup>	148.32 ± 16.68 <sup>b</sup>
DMW	149.33 ± 10.18	221.52 ± 23.00 <sup>b</sup>	25.28 ± 2.20 <sup>ab</sup>	156.44 ± 20.99 <sup>b</sup>

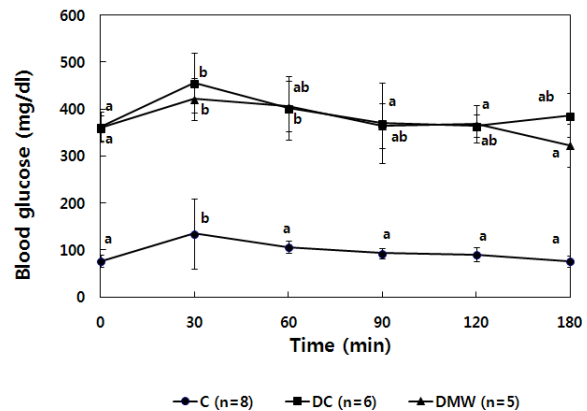
<sup>1)</sup> Mean ± SD, C, Control (n = 8); DC, Diabetes Control (n = 6); DMW, Diabetes + Magnetized Water (n = 5). Different letters are significantly different from control group (P < 0.05) by Duncan's multiple range test.



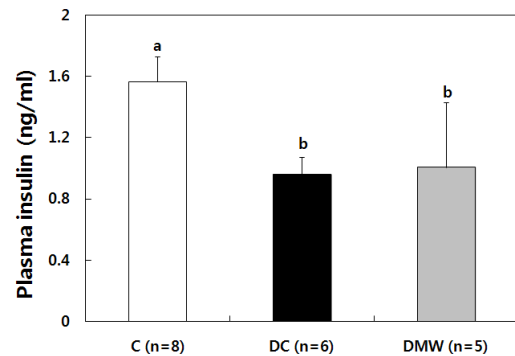
**Fig. 1.** Effect of magnetized water on blood glucose levels in STZ-induced diabetic rats. Mean ± SD, C, Control (n = 8); DC, Diabetes Control (n = 6); DMW, Diabetes + Magnetized Water (n = 5). Points with different letters between groups are significantly different at P < 0.05 after Duncan's multiple range test.

glucose level before inducing diabetes was not different among the control group, diabetes group, and magnetized water group. The blood glucose level during the first week (0 week) was significantly higher in streptozotocin-induced diabetes group and magnetized water group compared to the control group, but there was no difference between the diabetes group and magnetized water group. However, after 1 week and 4 weeks of the experiment, the blood glucose level in the magnetized water group was significantly decreased compared to the diabetes group, and such reduction effect was continued to the eighth week, the end of the experiment (Fig. 1).

The results of intra-peritoneal glucose tolerance test are shown in Fig. 2. In the control group, the blood glucose level was increased after 30 minutes of glucose administration and tended to decrease after 60 minutes, and then maintained the decreased level at 90, 120, and 180 minutes. In the diabetes group, the increased blood glucose level after 30 minutes of glucose administration tended to decrease after 90 minutes. In the magnetized water group, the increased blood glucose level after



**Fig. 2.** Effect of magnetized water on intra-peritoneal glucose tolerance in STZ-induced diabetic rats. Mean ± SD, C, Control (n = 8); DC, Diabetes Control (n = 6); DMW, Diabetes + Magnetized Water (n = 5). Points with different letters within each group are significantly different at P < 0.05 after Duncan's multiple range test.



**Fig. 3.** Effect of magnetized water on plasma insulin levels in STZ-induced diabetic rats. Mean ± SD, C, Control (n = 8); DC, Diabetes Control (n = 6); DMW, Diabetes + Magnetized Water (n = 5). Bars with different letters are significantly different at P < 0.05 after Duncan's multiple range test.

30 minutes of glucose administration tended to gradually decrease, and then was significantly decreased after 180 minutes.

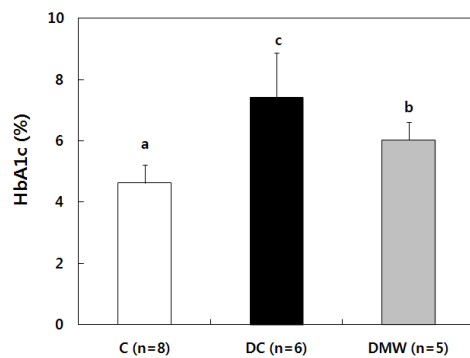
### Plasma insulin

In case of insulin-dependent type 1 diabetes, plasma insulin decreases due to the destruction of beta-cells of the pancreas. The results of insulin levels in the diabetes group and magnetized water group in the study were shown in Fig. 3. Compared to the control group ( $1.57 \pm 0.16$  ng/ml), the plasma insulin level in the STZ-induced diabetes group ( $0.96 \pm 0.11$  ng/ml), the type 1 diabetes model, was significantly low and tended to slightly

increase in the magnetized water group ( $1.01 \pm 0.42$  ng/ml), but not significantly different (Fig. 3).

### Glycated hemoglobin

The results of blood glycated hemoglobin content, which indicates the long-term blood glucose control showed that the content was about 1.6 times higher in the diabetes group (7.4%) compared to the control group (4.6%), but significantly low in the magnetized water group (6.0%) suggesting that the supplementation of magnetized water decreases the production of glycated hemoglobin in the diabetes group (Fig. 4).

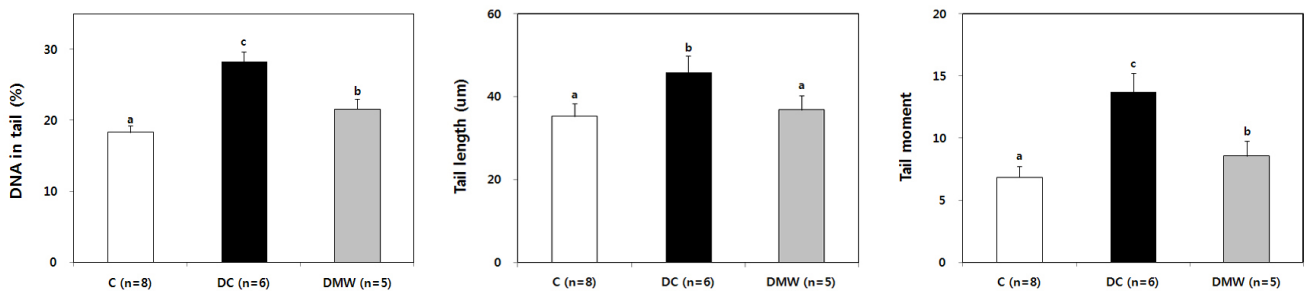


**Fig. 4.** Effect of magnetized water on blood HbA1c levels in STZ-induced diabetic rats. Mean  $\pm$  SD, C, Control (n=8); DC, Diabetes Control (n=6); DMW, Diabetes + Magnetized Water (n=5). Bars with different letters are significantly different at  $P < 0.05$  after Duncan's multiple range test.

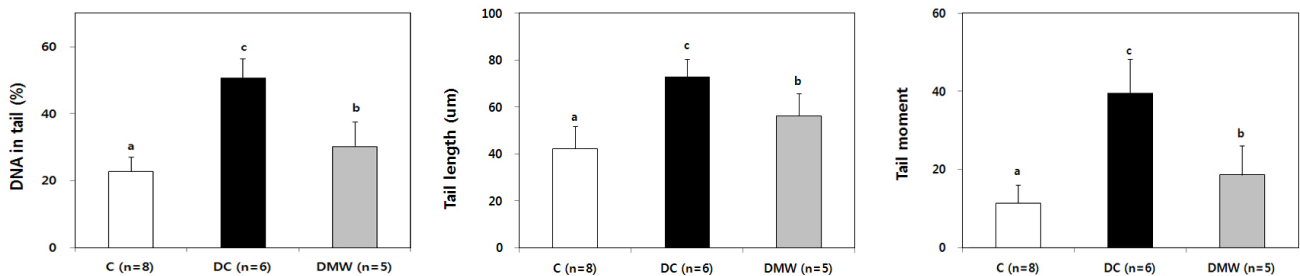
### DNA damage

The results of the comet assay for the degree of lymphocyte DNA damages in the three groups are shown in Fig. 5. Three indicators of DNA damage, such as DNA in tail, tail length, and tail moment, showed that DNA damage was significantly higher in the diabetes group and significantly lower in the magnetized water group than the control group, 23.3% in DNA in tail, 19.7% in tail length, and 37.2% in tail moment. This suggests that 8-week supplementation of magnetized water effectively reduced the increase of DNA damages due to induced diabetes (Fig. 5).

The degree of DNA damages in the liver of animals was similar to that observed in the blood DNA damages. The increased DNA damages due to induced diabetes significantly decreased in the



**Fig. 5.** Protective effect of magnetized water on blood DNA damages in STZ-induced diabetic rats. Mean  $\pm$  SD, C, Control (n=8); DC, Diabetes Control (n=6); DMW, Diabetes + Magnetized Water (n=5). Bars with different letters are significantly different at  $P < 0.05$  after Duncan's multiple range test.



**Fig. 6.** Protective effect of magnetized water on liver DNA damages in STZ-induced diabetic rats. Mean  $\pm$  SD, C, Control (n=8); DC, Diabetes Control (n=6); DMW, Diabetes + Magnetized Water (n=5). Bars with different letters are significantly different at  $P < 0.05$  after Duncan's multiple range test.

**Table 3.** Effect of magnetized water on erythrocyte antioxidant enzyme activities in STZ-induced diabetic rats

Variable	Catalase (K/gHb)	GSH-Px (U/gHb)	SOD (U/gHb)
C	49.0 ± 1.9 <sup>1)</sup>	383.3 ± 48.2	2456.8 ± 499.2
DM	45.3 ± 3.9	282.8 ± 25.5	2212.8 ± 381.6
DMW	48.8 ± 3.4	296.7 ± 31.8	2538.0 ± 188.3

<sup>1)</sup> Mean ± SD.

C, Control (n = 8); DC, Diabetes Control (n = 6); DMW, Diabetes + Magnetized Water (n = 5).

**Table 4.** Effect of magnetized water on plasma lipid profiles in STZ-induced diabetic rats

Variable	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
C	83.1 ± 14.0 <sup>1)</sup>	66.9 ± 19.7 <sup>a</sup>	41.3 ± 12.6	28.4 ± 19.2
DM	74.9 ± 28.2	93.0 ± 31.8 <sup>ab</sup>	43.1 ± 20.2	13.1 ± 11.7
DMW	69.3 ± 37.6	58.7 ± 14.3 <sup>a</sup>	40.4 ± 28.1	17.2 ± 12.2

<sup>1)</sup> Mean ± SD.

C, Control (n = 8); DC, Diabetes Control (n = 6); DMW, Diabetes + Magnetized Water (n = 5).

Different letters are significantly different from control group ( $P < 0.05$ ) by Duncan's multiple range test.

three indicators of DNA damages by magnetized water supplementation: 40%, 23%, and 53% in DNA in tail, tail length, and tail moment, respectively (Fig. 6).

#### *Erythrocyte antioxidant enzyme activity*

The activity of erythrocyte antioxidant enzymes such as catalase, SOD, and GSH-Px was measured in the diabetic rats to observe the antioxidant effect of the magnetized water. There were no significant differences in the activity of erythrocyte antioxidant enzymes among the groups (Table 3).

#### *Blood lipid profiles*

For blood lipid profiles, triglycerides tended to increase in the diabetes group and decrease in the magnetized water group compared to the control group, but the difference was not statistically significant. There were no differences in total cholesterol, HDL-cholesterol, and LDL-cholesterol among the three groups (Table 4).

## **Discussion**

Both increased reactive oxygen species (ROS) and decreased antioxidant defense systems increase the oxidative stress of diabetic patients. If a substance has ROS scavenging ability, it can have potential effectiveness in diabetic animals with high oxidative stress level. As severe DNA damages along with decreased blood glucose in diabetes have been reported in several studies [5-7], this study was conducted to investigate the effect of magnetized water supplementation on the improvement of diabetes through the reduction of blood glucose level and of DNA damages in STZ-induced type 1 diabetic rats.

Magnetized water is hexagonal water obtained by passing through specially manufactured permanent magnet that can activate water molecules to change its structure hexagonal, like the water in our body [27]. The magnetized hexagonal water has been known to be effective in the prevention and treatment of diseases such as diabetes and cancer by promoting metabolism in the body and improving bio activation. However, the scientific basis or experimental study on the physiological activity of magnetized water intake in the prevention and treatment of diabetes or cancer in humans is very limited worldwide. Some studies on the physiological activity of magnetized water have been reported during 1980s and 1990s, mainly in China. It has been reported that the intake of magnetized water treated ascariasis [28] or dissolved calculus [29], and increased glutamate decarboxylase activity [19]. Ma *et al.* [19] presented the possibility that magnetized water can prevent aging and fatigue by increasing the cell membrane permeability. In addition, some clinical cases claim that it is effective in asthma, arthritis, diabetes, and obesity, but most of the results are not based on solid science.

In this study, the intake of magnetized water in STZ-induced type 1 diabetic white rats distinctively showed the blood glucose and glycated hemoglobin lowering effects, but blood insulin concentration was not increased. When STZ is administered to experimental animals to induce type 1 diabetes, pancreatic  $\beta$ -cells become destructed and insulin secretion is decreased, which then causes abnormal metabolism and increased blood glucose level. However, after 4 weeks of magnetized water supplementation, the blood glucose level in STZ-administered animals was significantly decreased compared to the diabetes group. The glycated hemoglobin level, which reflects the long-term average blood glucose level, also decreased after 8 weeks supplementation of magnetized water. Thus it is thought that the steady intake of magnetized water is effective in lowering blood glucose in STZ-administered animals, but the reason for the blood glucose level decrease is not clear. The plasma insulin concentration in the magnetized water group increased most significantly. Therefore, more detailed and in-depth study is needed to investigate the mechanism of the blood-glucose lowering effect of magnetized water, using various biomarkers.

In the study, blood and liver DNA damages were significantly higher in the STZ-induced diabetes group than the control group. When magnetized water was administered to this diabetes group, blood lymphocyte DNA and liver DNA damages were significantly improved. This result is similar to that observed in the recent study of our laboratory, in which the administration of magnetized water reduced lymphocyte DNA damage in experimental animals with cancer induced by DEN (diethyl nitrosamine), a known potent carcinogen. The reduction effect was greater as the period of magnetized water intake was longer [23]. Oxidative stress is generated with diabetes mellitus and is involved in the progress of pancreatic beta-cell dysfunction. The results that the intake of magnetized water reduces lymphocyte

DNA damage through the reduction of oxidative stress in diabetic rats may be used in the prevention and treatment of diabetic complications. However, the mechanism of the effect of magnetized water on DNA damages in the body and further the physiological influence on the microstructure of water are not clear.

Magnetized water has higher pH and electric conductivity compared to general drinking water [30]. Some study results have presented that the magnetization of water increased the permeability through cell membranes [31,32], and that the magnetic field directly affected intracellular fluid and intracellular substances to activate enzymes inside the cells and to accelerate biochemical reactions in the body [33]. Thus it is possible that drinking magnetized water activates antioxidant enzymes in the body and reduces DNA damages, but the detailed mechanism of how the magnetized water can reduce DNA damages is not yet known.

Ma *et al.* [19] observed physiological effects that magnetized water increased glutamate decarboxylase activity by about 30%. It was interpreted as the interaction among the magnetized water molecules becoming reduced, while the interaction between enzymes and magnetized water was promoted in the weak magnetic field established by magnetized water, finally affecting the structure of enzymes. Electrolytes in the water have higher degree of ionization, and magnetic treatment on such water changes chemical and physical characteristics of water by converting the kinetic energy of ions into electrical energy, and thus electrolyte ions become activated to the state that can bind others easily [23]. Such activated electrolytes are quickly absorbed in the body and promote biological activation of tissues and cells, and activate enzyme actions in the metabolism [34]. The study on the effect of drinking electrolyzed reduced water, which had a higher pH and effective in ROS scavenging capacity, showed that the intake of electrolyzed reduced water protected  $\beta$ -cell damages and improved blood glucose status in db/db diabetic rats [35].

Magnetized water, after being absorbed in the body, is transported to the heart through the portal vein and circulated in the body, and a part of kinetic energy of the blood is converted to electrical energy to generate new electricity in the blood by which unionized electrolytes can be ionized [34]. Such ions, when acting on the autonomic nerves, can improve blood circulation and thus improve the treatment effect on several diseases related to blood circulation.

It is considered that the effect of magnetized water on the improvement of DNA damages in STZ-induced diabetic rats in this study is due to increased ionization of water by magnetized water and the ensuing increase in the activity of antioxidant enzymes. However, the activity of erythrocyte antioxidant enzymes such as catalase, GSH-Px, and SOD was not changed after the intake of magnetized water in this study, failing to confirm the above result. Many enzymes interact with one another in the metabolism of the body. As the activities of various enzymes other than catalase, GSH-Px, and SOD, were not

measured, it could not be confirmed whether such effect of magnetized water was due to the action of a certain enzyme or other mechanisms.

When diabetes is developed, lipid metabolism is abnormally affected and excessive lipid peroxides and blood lipids are increased. In the effect of magnetized water on lipid profiles, the blood triglycerides level was significantly decreased in magnetized water group compared to diabetes group. This result may be an additional evidence that the intake of magnetized water can be used in prevention and treatment of diabetic complications, in addition to the blood glucose lowering effect in diabetes mellitus.

Masaru Emoto performed studies on the treatment effect of magnetized water in double-blind conditions, on the basis of the theory that changing plain water into magnetized water affects the ice crystals of water and makes it hexagonal water [36,37]. In 1998, Johnson *et al.* [20] studied the cleansing effect of a magnetized water on cavities, plaque, and gum health and observed that plaque was significantly reduced by 64 % in the group using magnetized water as washing water, compared to control group, and confirmed the similar results by Watt *et al.* [29]. However, studies in humans are very limited. In Russia, Merkulova and Mikhel'son [38] reported that magnetized water had biological and bactericidal actions [39]. In China, magnetized water has been reported to be effective in urinary calculi [21] and prevention of diarrhea [40].

Although previous studies with magnetized water have shown promising results on certain beneficial effects on human and animal health, these studies are very limited and poorly designed experimentally. The well-designed double-blind studies are seldom found on the subject. Also, the studies reported are mostly non-controlled observational studies that were performed between 20 and 30 years ago, and few scientists were involved in the area. Thus, rigorous and serious scientific analysis is needed, utilizing well-designed clinical studies in experimental animals and humans in the future to confirm the health-beneficial effects of magnetized water [41].

In conclusion, the 8-week intake of magnetized water in this study not only decreased the blood glucose and glycated hemoglobin levels but also reduced blood and liver DNA damages in STZ-induced diabetic rats. From these results, it is suggested that the long-term intake of magnetized water (over 8 weeks) may be beneficial in both prevention and treatment of complications in diabetic patients. More extensive studies are needed to find out the mechanism of action of the magnetized water.

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