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Protective effect of Boysenberry Juice on Ferric Nitrilotriacetate-induced Renal Injury in Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Author TH designed the study, managed the analyses of the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MK and KK managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Boysenberry (*Rubus loganbaccus* × *baileyanus* Britt.) is one of the popular berries in Western countries. In the present study, the effect of the boysenberry juice on ferric nitrilotriacetate (Fe-NTA)-induced renal injury was investigated. ICR mice (7 weeks old, male) were *ad libitum* administered boysenberry juice for 1 week, and were intraperitoneally injected Fe-NTA (5 mg Fe/kg body weight) as an oxidant to renal. The consumption of boysenberry juice suppressed the Fe-NTA-increased thiobarbituric acid reactive substances (TBARS) in kidney and blood urea nitrogen (BUN). These results indicate that the consumption of boysenberry juice reduces the risk of oxidative stress.

Keywords: Boysenberry; ferric nitrilotriacetate; renal injury; antioxidative activity; anthocyanins; mice.

1. INTRODUCTION

Boysenberry (*Rubus loganbaccus* × *baileyanus* Britt.) originates in the United States and was imported into New Zealand [1]. Currently, this fruit is mainly cultivated in New Zealand and the United States. Boysenberry is rich in antioxidant functional ingredients such as anthocyanins, red

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and blue pigments, and ellagic acid [2]. Igarashi et al. indicated that anthocyanins derived from boysenberry suppressed galactosamine-induced liver damage in rats [3]. Sugimoto et al. demonstrated that boysenberry-derived anthocyanins showed the suppressive effect on the oxidative stress accompanied with diabetes in streptozotocin-induced diabetic rats [4]. Therefore, boysenberry intake is expected to have preventive effects on various diseases accompanied by the antioxidant activity.

Chronic kidney disease (CKD) is a common and serious illness that suffers 8-16% of the world's population [5]. Prevalence of CKD is associated with an increase in patients with diabetes, and diabetic nephropathy, which are CDK accompanied with diabetes, occurs in up to 40% of diabetics [6]. In 2019, 433 million people are suffering from diabetes, which is 1/11 of the world's adults. The International Diabetes Federation estimates that 578 and 700 million adults will have diabetes by 2030 and 2045, respectively [7]. In other words, it is thought that the number of patients with CDK will increase as the number of diabetic patients increases rapidly in the future.

To investigate the protective effects of boysenberry intake on CDK, the present study investigated the effects of boysenberry juice on ferric nitrilotriacetate (Fe-NTA)-induced renal injury in mice.

2. MATERIALS AND METHODS

2.1 Materials

Ferric nitrate enneahydrate and sodium bicarbonate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Nitriloacetic acid disodium salt was from Nacalai Tesque (Kyoto, Japan). As a standard for a HPLC analysis, cyanidin-3-glucoside was from Extrasynthese (Genay, France) and quercetin, quercetin-3-glucoside, caffeic acid and ellagic acid were from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals were of highest commercially available grade.

2.2 Preparation of Boysenberry Juice

The concentrated (9×) boysenberry juice was commercially produced in New Zealand, and kindly provided by Amuco (Kanagawa, Japan). Briefly explaining the productive process, fruits (brix, 7) were crushed and concentrated at 45-50°C for 7 h, and pressed and centrifuged to remove the sludge. The concentrate was pasteurized at 90°C for 30 min, filtrated through 1 μm of diatomite, and re-concentrated to adjust the brix to 62-64. This juice was stored at 5°C to preserve the quality. The concentrated boysenberry juice was diluted with 8-fold volume of distilled water for animal experiments in this study as boysenberry juice.

2.3 Polyphenol Analysis

Anthocyanins were extracted from the concentrated boysenberry juice with 9-fold volume of methanol containing 0.1% HCl, and centrifuged at 1200 *g* for 10 min followed by the filtration with 0.2 μm (Millipore, Bedford, MA). The extract was analyzed using a HPLC system (Hitachi, Tokyo, Japan) equipped with a UV-VIS detector (Hitachi L-7420). The analytical column employed was a C18 ODS column 5 μm, 250 mm × 4.6 mm i.d. (Nacalai Tesque) maintained at 35°C. The binay mobile phase consisted of (A) methanol containing 0.1% HCl, 50 mM NaH_2PO_4, pH 2.1 (1:9, v/v) and (B) methanol containing 0.1% HCl, 50 mM NaH_2PO_4, pH 2.1 (7:3, v/v). The gradient method started at 1 ml/min from 0 to 30% B in 5 min, and 30% B for 5 min, followed by 30 to 100% B in 5 min, and then 100% B for 15 min. In all cases, the columns were re-equilibrated between injections with the equivalent of 15 ml of the initial mobile phase. The sample injection volume was 10 μl, and detection wavelength was 530 nm. Polyphenols in the boysenberry juice, except anthocyanins, were analyzed by a HPLC as described previously [8].

2.4 Preparation of Fe-NTA Solution

A solution of Fe-NTA was prepared immediately before use by the method of Awai et al. [9] with slight modification. Briefly, ferric nitrate enneahydrate and nitriloacetic acid disodium salt were each dissolved in distilled water to form 300 mM and 600 mM solutions, respectively. They were mixed at a volume ration of 1:2 (molar ration, 1:4). The pH was adjusted with sodium carbonate to 7.4.

2.5 Animals

This study was approved by the Institutional Animal Care and Use Committee and carried out according to the Guidelines of Animal Experimentation of Kobe University. Male ICR mice (7 weeks old) were purchased from Japan

SLC (Shizuoka, Japan) and acclimated to the facility for 1 week followed by all animal experiments. Mice were *ad libitum* given boysenberry juice or drinking water for 1 week and then intraperitoneally injected Fe-NTA (5 mg Fe/kg body weight) or saline. One hour after the injection, mice were killed, and the kidney and blood were removed. During the 1 h, these mice were *ad libitum* given the boysenberry juice or drinking water continuously.

2.6 Determination of Thiobarbituric Acid Reactive Substances (TBARS) in Kidney

TBARS in kidney was measured by the method of Kikugawa et al. [10]. Kidney was homogenized in 9-fold volume of ice-cold 1.15% potassium chloride using a Potter-type Teflon glass homogenizer. An aliquot (0.3 ml) of the homogenate was added 50 µl of 0.8% butylated hydroxytoluene in glacial acetic acid, 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetic acid adjusted pH with 1 M NaOH to 3.5, and 1.5 ml of 0.8% thiobarbituric acid. The mixture was incubated at 4°C for 60 min and then at 100°C for 60 min. After addition of 1 ml of distilled water and 5 ml of *n*-butanol: pyridine (15:1, v/v), the mixture was centrifuged at 800 × *g* for 10 min at room temperature. MDA was determined on the basis of spectrophotometric absorbance measurement of the pink colored product of TBARS at 532 nm. TBARS were expressed as malondialdehyde (MDA) equivalents.

2.7 Determination of Blood Urea Nitrogen (BUN)

Plasma was prepared from blood, and then BUN was determined by the diacetylmonoxime method [11]. An aliquot (20 µl) of plasma was incubated with 3 ml of reaction buffer containing 0.01 M diacetylmonoxime, 0.55 mM thiosemicarbazide and 0.42 M phosphoric acid in boiling water for 20 min. Urea was determined by an absorbance of red chromophores formed with urea and diacetylmonoxime at 540 nm.

2.8 Statistical Analysis

Data were expressed as the mean ± standard deviation (SD). Statistical analyses were performed using the Turkey-Kramer test. The results were considered to be significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

Anthocyanins is one of the major polyphenols in boysenberry. The boysenberry juice used in the study contained totally 130 mg of anthocyanins in 100 ml of the juice, as an equivalent of cyanidin-3-glucosids (Table 1). A previous analysis indicated that 100 ml of commercial boysenberry juice contained totally 105 mg anthocyanins as cyanidin-3-glucoside equivalent [12]. The result indicates that the boysenberry juice used in the present study was almost same concentration as commercial juice. Torre and Barritt [13] identified and quantified cyanidin-3-sophoroside, cyanidin-3-glucoside, cyanidin-3-rutinoside, and cyanidin-3-glucosylrutinosid as cyanidin-3-glucosids. The boysenberry juice contained also other antioxidants, such as caffeic acid, ellagic acid, quercetin and vitamin C (Table 1). These constituents are consistent with previous reports [12,14]. Barnett et al. [15] showed that boysenberry extract functioned as an *in vivo* antioxidant in rats fed basal diets containing fish or soybean oils, because it raised the antioxidant status of plasma but decreased some biomarkers of oxidative damage. The antioxidants-containing boysenberry might lead the suppressive effect on the oxidative stress accompanied with diabetes in streptozotocin-induced diabetic rats [4].

To investigate protective effects of boysenberry juice on renal injury with oxidative stress, ICR mice were *ad libitum* administered the boysenberry juice or deionized water for 7 days and intraperitoneally injected Fe-NTA as an oxidant or 0.85% NaCl as a control. There is no difference between the amounts of ingested juice and water; 9.4 ± 3.3 and 8.5 ± 0.5 ml/day/mouse, respectively (data not shown). The administration of boysenberry juice showed the transient decrease in body weight on Day 1, while there is also no difference of body weight between the groups during the experiment (Fig. 1). In addition, there was also no significant difference in kidney and liver weights among four groups ($n = 6$) after killing (data not shown). Taken together, it was considered that boysenberry juice was not toxic.

In control mice given water, the injection of Fe-NTA significantly increased 1.5-fold TBARS in kidney, *i.e.*, 0.821 ± 0.198 nmols MDA/mg protein in 0.85% NaCl-injected mice ($n = 6$) and 1.218 ± 0.333 nmols MDA/mg protein in Fe-NTA-injected mice ($n = 6$), as shown in Fig. 2A. The administration of boysenberry juice reduced the increase in TBARS to a level untreated with Fe-

NTA. This result suggests that intake of boysenberry juice may exert a protective effect on renal injury with oxidative stress induced by Fe-NTA. BUN is known as a marker for renal dysfunctions. Injection of Fe-NTA significantly increased 1.8-fold BUN in control mice, *i.e.*, 13.2 ± 1.2 mg/dl in 0.85% NaCl-injected mice (*n* = 6) and 27.7 ± 8.6 mg/dl in Fe-NTA-injected mice (*n* = 6), as shown in Fig. 2B. The administration of boysenberry juice significantly suppressed 71% of the increase in BUN by Fe-NTA. The BUN was 17.3 ± 3.0 mg/dl. Thus, *ad libitum* administration of boysenberry juice showed the suppressive effect of renal damage by oxidative stress without the toxicity.

The antioxidant effects of boysenberry have been reported in studies on anti-diabetes [4] and other studies [15-17]. The present study indicated the preventive effect of daily intake of boysenberry juice on renal damage induced by oxidative stress. Taken together, intake of boysenberry juice is considered to be effective in preventing diabetic nephropathy. On the other hand, many studies indicated the antioxidant activity of anthocyanins [18,19]. According to the daily juice intake (9.4 ml) and anthocyanin content in the juice (Table 1), mice ingested approximately 12.2 mg of anthocyanin/day. Assuming that average body weight of mice was 35 g, it corresponds to daily ingestion of 0.43 mg anthocyanin per kg body weight. If the suppressing effect on renal damage by oxidative stress in this experiment is due to anthocyanins, this daily intake of 0.43 mg/kg may be the estimated amount for preventing diabetic nephropathy. However, as shown in Table 1, boysenberry juice contains also other antioxidants. so further studies are needed to clarify the details related to the preventive effect of boysenberry juice on nephropathy.

Table 1. Main antioxidants in the boysenberry juice

Antioxidant	Content (mg/100ml)
Anthocyanins*[1]	130
Caffeic acid	0.17
Ellagic acid	19.0
Quercetin	0.50
Quercetin glycosides*[2]	0.53
Vitamin C	27.0

*[1] *As a quercetin-3-glucoside*
*[2] *As a cyanidin-3-glucoside*

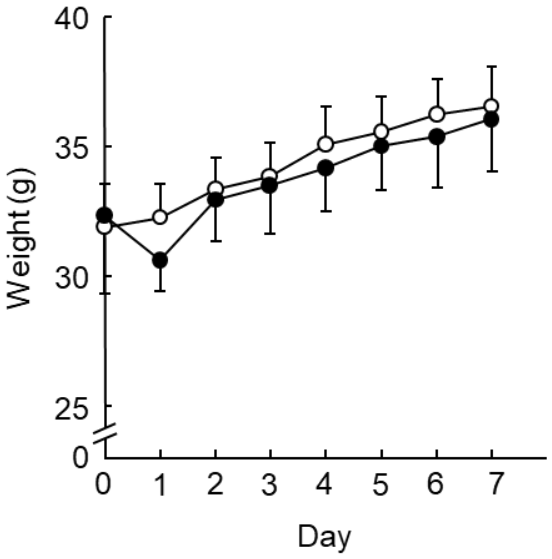

Fig. 1. Changes in body weights during animal experiments
Mice were ad libitum given boysenberry juice or drinking water for 1 week. Mice were daily weighed during the administration. Data are the means ± SD (n = 12)

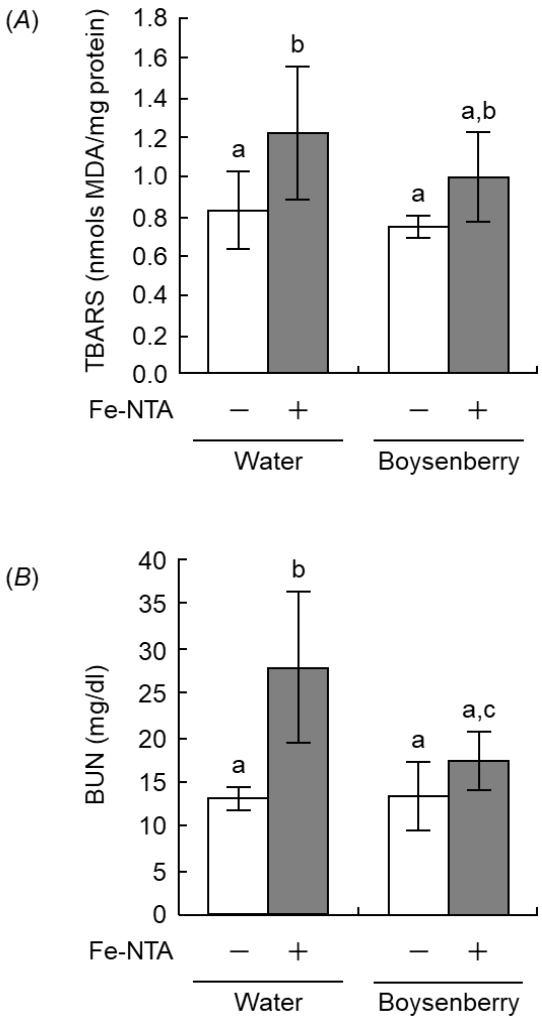

Fig. 2. Effects of boysenberry juice intake on oxidative stress and renal injury

Each group (n =12) described in Fig. 1 was further divided into 2 groups (n = 6), and then were intraperitoneally injected Fe-NTA (5 mg Fe/kg body weight) or saline. One hour after the injection, mice were killed, and the kidney and blood were removed. TBARS (A) and BUN (B) were analyzed as described in MATERIALS AND METHODS. Data are the means ± SD (n = 6). Different letters indicate significant differences ($p < 0.05$) according to the Turkey-Kramer test

4. CONCLUSIONS

Intake of boysenberry (*Rubus loganbaccus* × *baileyanus* Britt.) juice rich in anthocyanins obviously indicated protective effects on renal injury induced by Fe-NTA in mice. This result suggests that daily intake boysenberry juice may be a preventative effect on diabetic nephropathy.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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