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Effect of Organic Acids Extracted from Brewing Lees of Rice Vinegar on the Blood-Glucose Levels of Diabetic Rats and Mice

Takashi TAJIRI* and Aritomo SUZUKI**

Synopsis

The effects of organic acids extracted from strained lees the vinegar *kuro-zu* and *oi-zu* on the blood-glucose level were studied in mice with inherited diabetes mice and rat with diabetes stroke-prone spontaneously hyprevtensive induced by alloxan. The organic acids were given orally. The organic acids from the *Oi-zu* strained lees lowered the blood-glucose level of the diabetic mice. The organic acids from the *Kuro-zu* lowered blood-glucose in both the mice and the rats. When the dose of organic acids was low, the effect on the blood-glucose levels of the rats was transitory. The effect of higher doses lasted longer. The results suggested that organic acids extracted from lees of rice vinegar promote health if added to food, as they reduced high blood-glucose levels in these diabetic animals.

INTRODUCTION

Starting about 200 years ago, naturally brewed rice vinegar (*yone-zu*)[1] has been produced in the Fukuyama district of Kagoshima Prefecture. Satsuma *yone-zu* is available in two main varieties, *kuro-zu* and *oi-zu* and is marketed as a health food. *Kuro-zu* is fermented for about 180 days and *Oi-zu* for about 5 years. The manufacturing methods for these rice vinegars are shown in Fig. 1.

In Japan diseases prevalent in aging such as hypertension and diabetes are on the increase as western-style eating habits spread. Vinegars may be helpful in preventing such diseases, and attention is being focussed on health foods containing organic acids, the main components of vinegar contributing to its effect. *Yone-zu* is rated particularly among the many kinds of vinegars available. Strained lees or dregs obtained as a by product in the manufacture of *yone-zu* have not been much used, although a small amount has been used in pickling. In powdered form, these strained lees and dregs could be used as a material for health food.

In this study strained leed and dregs were powdered by vacuum freeze-drying and the effects of the various organic acids extracted from the powder on diabetes in experimental animals were studied.

* Laboratory of Food Processing, Faculty of Agriculture, Kinki University, Kowakae, 3-4-1 Higashi-Osaka, Osaka, 577 (食品栄養学科食品加工学研究室)
** Department of Pharmacology, Kinki University School of Medicine, Ono-higashi, 377-2, Osaka-Sayama, Osaka, 589 (医学部薬理学研究室)

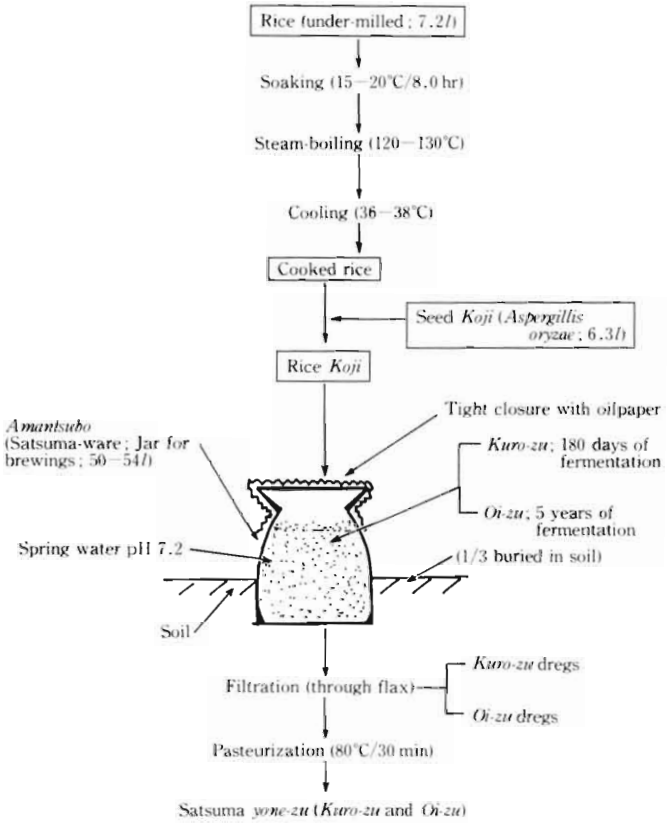

Fig. 1. Production of *Kuro-zu* and *Oi-zu* (Satsuma *yone-zu*).

MATERIALS and METHODS

Yone-zu can be brewed both naturally and artificially; here naturally brewed *yone-zu* was used. Both the kinds and amounts of organic acids differ depending on the method of brewing. The *kuro-zu* and *oi-zu* used here were manufactured as shown in Fig. 1. Powder of the brewing less of *Kuro-zu* and *Oi-zu* were prepared by use of a vacuum freeze-drying apparatus (Model ER-90; Shimadzu Corp.). The strained lees were frozen at -40 ± 2°C at the reduced pressure of 5×10^{-1} mm Hg for 4 hr and then dried at 20°C at the same pressure for 5 hr. The strained lees of *kuro-zu* and *oi-zu* used here were the kind gift of the Sakamoto Brewing Co., Ltd. (Kagoshima). The organic acids[2] extracted with alcohol are listed in Table 1.

As one kind of experimental animal, we used 19 Kasukabe (KK) mice with inherited diseases, divides into four groups. One group (n=4) received a solution of the organic acids from the lees of *kuro-zu*, one group (n=5) received the organic acids from the lees of *oi-zu*, and the first control group (n=5) received the same volume of water instead of the solution of organic acids. All three of these groups were provided with a set amount of a special feed without organic acids. The second control group (n=5) was treated in the same way as the first, except that ordinary feed was made available ad libitum.

As the other kind of experimental animal, we used 8 stroke-prone spontaneously hypertensive rats (SP-SHR) with a blood-glucose level of 80-100 mg/100 ml. Two groups with 4 rats each

Table 1. Organic acids (mg/100 g of vinegar) extracted from brewing lees of the rice vinegars (*kuro-zu* and *oi-zu*).

Organic acids	Vinegar	
	Kuro-zu lees	*Oi-zu* lees
Pyroglutamic acid	0.46	0.38
Lactic acid	6.24	5.66
Acetic acid	28.17	24.43
Citric acid	0.22	0.19
Succinic acid	0.18	0.21
Malic acid	0.01	0.23
Tartaric acid	0.01	0.31
Adipic acid	—	0.34
Total	35.29	31.75

Extraction of alcohol-insoluble solids (AIS).
Kuro-zu and *Oi-zu* : Satsuma *yone-zu* from Kagoshima, Japan.

were prepared, one of rats with blood-glucose levels of about 200 mg/100 m*l* and the other with blood-glucose levels of 500-600 mg/100 m*l*, by the intravenous administration of alloxan.

The organic acids extracted from lees of *oi-zu* (1.0 mg/1.0 of water, pH 3-4) was diluted 1 : 50 with water. The dilute organic acids solution (pH 4.0) was forcibly administered to the KK mice at the dose of 0.2 mg/kg of body weight (2 ug of organic acids/0.1 m*l* solution) by use of stomach probe if the animals did not drink the solution volantarily. Administration was done three times in a 1-week period. The same volume of water was administered to (or drunk by) the first control group. The mice in the second control group were not given water by stomach probe. Body weights and blood-glucose levels were measured repeatedly up to 4 weeks after the first administration. The blood-glucose level was assayed colorimetrically by use of Dextrostix test paper (Miles-Sankyo Co., Ltd).

Organic acids extracted from the strained lees of *kuro-zu* (0.16 mg of organic acids/g of powder) were mixed with the feed provided to five of the KK mice and to all 10 of the diabetic rats for four weeks. For both species, one was used as a control, one received 0.16 mg/g body weight, one received 1.6 mg/g, one received 4.0 mg/g and one received 16 mg/g. Body weight and the blood-glucose levels were measured once a week.

RESULTS and DISCUSSION

1. Effects of organic acids from strained lees of *oi-zu* on blood-glucose levels

The blood-glucose levels of the group given organic acids from *oi-zu* had decreased significantly by 24 hr after the first administration and was still low after 4 weeks. The mean blood-glucose level of the group given organic acids was significantly lower than that of first control group at first. At four weeks, the blood-glucose level in the group given organic acids tended to be lower than in the first control group, but not significantly ; at this time, the blood-glucose level of this control group was low, for an unknown reason. (Fig. 2).

2. Effects of organic acids from strained lees of *kuro-zu* blood-glucose levels

KK mice develop bending of the tail when hyperglycemia (150-200 mg/100 m*l*) with accompanying hyperinsulinois occurs because of obesity. This symptom in same ways resembles symptom of diabetes in aging[2,3].

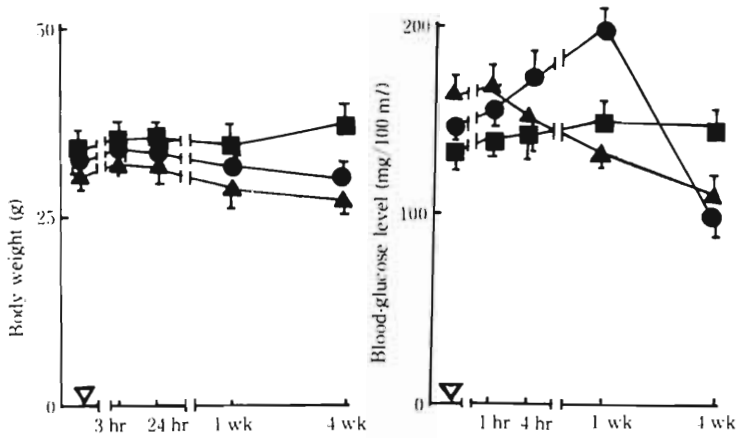

Fig. 2. Effects of organic acids from brewing lees of *oi-zu* on body weight and blood-glucose levels in KK mice.
▲ : Group given 0.2 mg of organic acids/kg
● : First control group (given water)
■ : Second control group (ordinary feed)
▽ : Start of oral administration

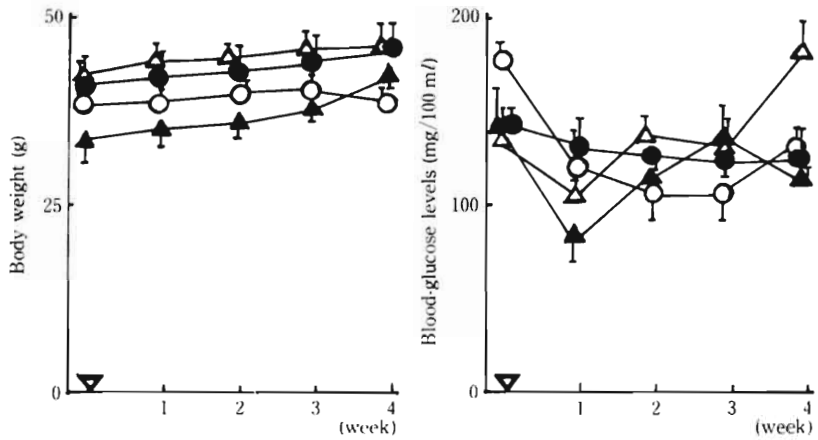

Fig. 3. Effect of organic acids from brewing lees of *kuro-zu* on body weight and blood-glucose levels in KK mice.
▲ : Mouse given 0.16 mg/kg
△ : Mouse given 1.6 mg/kg
○ : Mouse given 16 mg/kg
● : Control
▽ : Start of oral administation

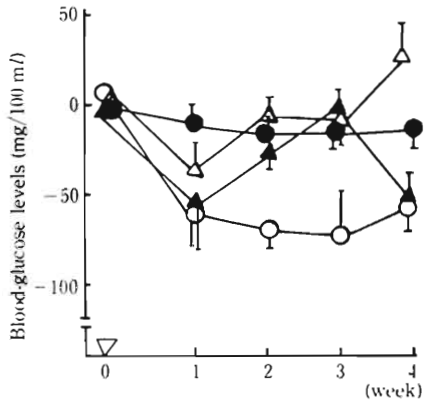

Fig. 4. Extent of decrease in blood-glucose of KK mice caused by organic acids from brewing lees of *kuro-zu*. Symbols are the same as in the legend of Fig. 3.

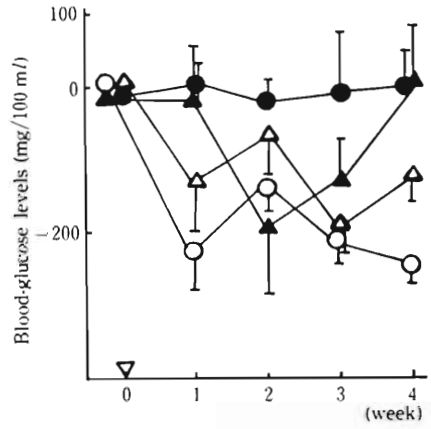

Fig. 6. Extent of decrease in blood-glucose in diabetic rats with very high starting levels of blood-glucose caused by organic acids from brewing lees of *kuro-zu*. Symbols are the same as in the legend of Fig. 5.

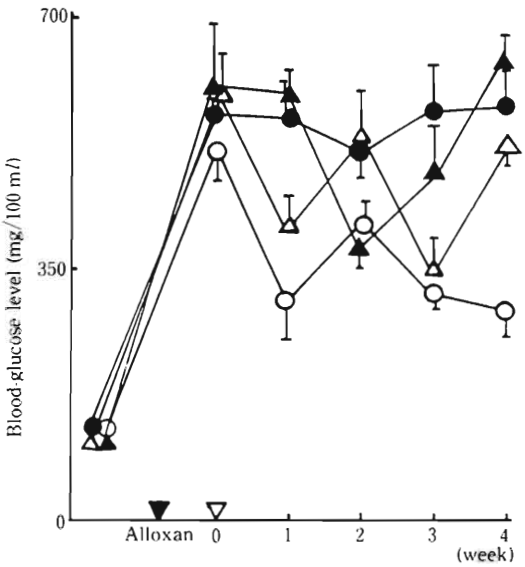

Fig. 5. Effect of organic acids from brewing lees of *kuro-zu* on blood-glucose levels in diabetic rats with very high starting levels of blood-glucose.
▲ : Rat given 0.16 mg/kg
△ : Rat given 1.6 mg/kg
○ : Rat given 16 mg/kg
● : Control
▼ : Intravenous administration of alloxan
▽ : Start of oral administration of organic acids

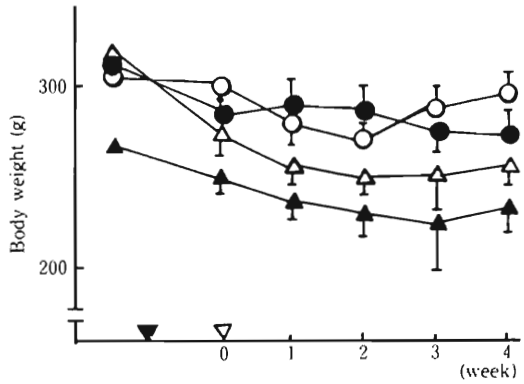

Fig. 7. Effect of organic acids from brewing lees of *kuro-zu* on body weight of diabetic rats with very high starting levels of blood-glucose. Symbols are the same as in the legend of Fig. 5.

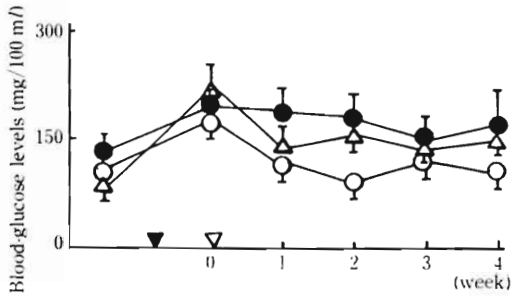

Fig. 8. Effects of organic acids from brewing lees of *kuro-zu* and of tolbutamide on blood-glucose levels in with lower starting levels of blood-glucose.
○ : Rat given 4.0 mg/kg organic acids
● : Control
△ . Rat given 200 mg/kg tolbutamide
▼ . Intravenous administration of alloxan
▽ · Start of oral administration of organic acids or tolbutamide

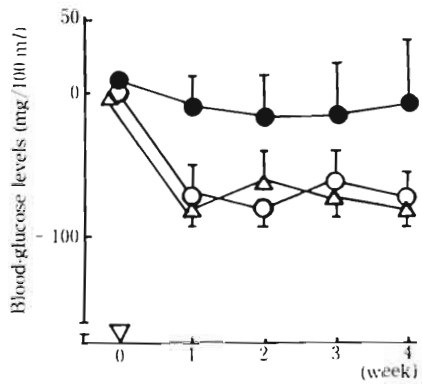

Fig. 9. Extent of decrease in blood-glucose in diabetic rats with lower starting levels of blood-glucose caused by organic acids from brewing lees of *kuro-zu* or by tolbutamide. Symbols are the same as in the legend of Fig. 5.

The blood-glucose levels of the mice lowered by 40-60 mg/100 ml within one week in the animals given 0.16, 1.6, or 16 mg/kg, and the difference with the control mouse was significant at each of these doses (Fig. 3). However, blood-glucose gradually rose in the mice given 0.16 or 1.6 mg/kg, returning to almost the base-line level. The blood-glucose level of the mouse given 16 mg/kg remained low. The organic acids from strained lees of *kuro-zu* did not affect body weight (Fig. 4).

Alloxan induces experimental diabetes in rats by destroying beta cells of the pancreas selectively[4,5]. In the diabetic rats we used that were given high blood-glucose levels (500-600 mg/100 ml), the administration 0.16 or 1.6 mg/kg had a transitory lowering effect on blood-glucose. The administration of 16 mg/kg lowered the blood-glucose level one week after the start of administration and this effect continued to the end of testing at four weeks (Figs. 5 and 6).

The body weights of rats given the organic acids tended to decrease during the administration period (Fig. 7).

In the diabetic rats that were given less elevated blood-glucose (about 200 mg/100 ml), the blood-glucose level was lower after one week of the start of administration of 4.0 mg/kg, and the effect was continued to the end of testing. The effect was similar to that of the administration of 200 mg/kg tolbutamide (given orally once), which has been used to treat diabetes (Figs. 8 and 9).

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米酢搾汁粕中有機酸の糖尿病動物の血糖降下作用

田尻尚士・鈴木有朋

摘　要

不老酢(おいず)搾汁粕及び黒酢搾汁粕中の有機酸の血糖降下作用の有無を遺伝性糖尿病マウス及びAlloxan糖尿病ラットを用いて検討した．その結果を報告する．不老酢搾汁粕有機酸は糖尿病マウスには血糖降下作用を示した．黒酢搾汁粕有機酸は糖尿病マウス及びAlloxan糖尿病ラットで用量依存性の血糖降下作用を示した．Alloxan糖尿病ラットでは血糖降下効果は少量では一過性であるが，大量を用いると効果の持続性が認められた．

以上の結果より，不老酢搾汁粕，黒酢搾汁粕中より抽出した有機酸は血糖降下作用を持つことが認められた．しかし，その有効成分及び機序については今後の検討が必要である．