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# The effects of 90-day feeding of D-psicose syrup in male Wistar rats

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## ABSTRACT

**D-Psicose is a rare sugar present in small quantities in natural products. In a previous study, we showed that D-psicose suppresses increase in plasma glucose and reduces body fat accumulation in rats. Based on acute and chronic toxicity testing in rats, D-psicose is classified as an ordinary and safe substance. Recently, we developed a high D-psicose syrup (PS) made from high fructose corn syrup (HFCS) by the alkaline isomerization method. However, the safety of PS as a food additive has not been demonstrated. In this study, we investigated the effects of 90-day feeding of PS in male Wistar rats. The rats were fed diets containing 3% D-psicose (control) or 4.3% PS for 90 days. The body weight gain and intra-abdominal adipose tissue weight did not differ between the control and PS group. The weights of the tissues did not differ between the two dietary groups. In clinical chemistry and hematological analyses, no differences were found between the control and PS groups. No gross pathological findings were evident at dietary doses of 4.3% PS. Therefore, the present study found no adverse effects of PS in rats fed a diet containing 4.3% PS for 90 days.**

**Keywords:** D-Psicose; D-Psicose Syrup; 90-Day Feeding; Pathological Tests; Rat

## 1. INTRODUCTION

D-Psicose (D-*ribo*-2-hexulose), a C-3 epimer of D-fructose, is a "rare sugar" present in small quantities in commercial mixtures of D-glucose and D-fructose obtained by hydrolysis of sucrose or isomerization of D-glucose [1]. D-Psicose is also present in processed cane and beet molasses [2], and is found in wheat [3], *Itea* plants [4], and in the antibiotic psicofranine [5]. In the 2000s, D-psicose began to be made using an enzymatic method on a large scale, making it possible to conduct biochemical and nutritional studies [6]. Examining the effects of D-psicose on glucose and lipid metabolism, we found that D-psicose is a sweet monosaccharide that provides no energy to growing rats and leads to less intra-abdominal fat accumulation than D-glucose and D-fructose in rats [7,8]. In addition, we suggested previously that supplemental D-psicose can lower plasma glucose levels [9]. Toyoda *et al.* [10] suggested that D-psicose can prevent postprandial hyperglycemia by improving the translocation of glucokinase from the nucleus to the cytoplasm in the liver of diabetic rats. D-psicose is expected to have a beneficial effect in the control of blood glucose levels in type 2 diabetes.

Based on acute toxicity testing in rats, D-psicose is classified as an ordinary substance, with an oral $LD_{50}$ value of 16 g/kg in male Wistar rats [11]. D-psicose, which is naturally present in foods such as fruit juice and fruit cereal, is derived from D-fructose by the cooking process [2,12]. Oshima *et al.* [13] reported that in high-sugar food products, heat processing had a marked effect on the production of D-psicose. In particular, confectionery products and seasoning sauces exhibited higher D-psicose content (0.005 - 1.3 mg/g) than other products [13]. As a result, most people ingest a limited amount of D-psicose on a daily basis. Furthermore, we examined the effects of sub-chronic (90 days) and long-term feeding (12 - 18 months) of D-psicose to rats prior to its utilization as a physiologically functional food [14,15]. We showed that 3% D-psicose in the diet had no adverse effects in rats.

Recently, we developed a new product, high D-psicose syrup (85% D-psicose) made from high fructose corn syrup (HFCS) by the alkaline isomerization method. D-Psicose syrup (PS) can be easily and cost-effectively produced compared to D-psicose. However, as PS includes small amounts of unknown ingredients (probably

several monosaccharides), the safety of PS as a low-energy sweetener or food additive is not clear.

In this study, to assess the safety of PS, the effects of 90-day feeding of PS was conducted in rats at a dietary dose of 4.3% PS (including 3% D-psicose). The objective of this study was to determine whether PS can be safety used as a functional food similar to D-psicose.

## 2. MATERIALS AND METHODS

All procedures involving animals were approved by the Animal Care Committee of Kagawa University.

### 2.1. Animals and Experimental Diets

Twenty male Wistar rats (3 weeks old) were obtained from Japan SLC (Shizuoka, Japan). They were fed CE-2, a commercial rodent diet (CLEA, Tokyo, Japan) and water *ad libitum* until they were 4 weeks old. They were caged individually at 22˚C ± 2˚C, with lights on from 08:00 to 20:00. The rats were randomly divided into two groups of 10 (control and PS groups). We adopted D-psicose as a control for PS because the safety of D-psicose was confirmed by our previous studies in animals and humans [11,14-16]. The compositions of experimental diets are shown in **Table 1**. The compositions of PS were as follows: D-psicose, 70%; water, 17%; unknown ingredients, 13%. The control and PS diets included 3% D-psicose in CE-2, a commercial rodent chow (CLEA). The amount of test carbohydrates was determined with reference to previous studies concerning D-psicose [13] or sucralose, the $LD_{50}$ of which is the same as that of D-psicose [17,18]. Each group of rats was given free access to food and water for 90 days. PS, which was made from HFCS by the alkaline isomerization method, and D-psicose, which was made enzymatically from D-fructose, were gifts from Rare Sugar Production Technical Research Laboratories, LLC (Kagawa, Japan). The HFCS used as a raw material for PS was supplied by Matsutani Chemical Industry, Co., Ltd. (Hyogo, Japan).

**Table 1.** Composition of experimental diets.

| Groups | Control | D-Psicose syrup |
|---|---|---|
| Ingredients | g/kg | |
| CE-2 | 95.7 | 95.7 |
| D-Psicose syrup* | 0.0 | 4.3 |
| D-Psicose | 3.0 | 0.0 |
| Sucrose | 0.5 | 0.0 |
| Distilled water | 0.7 | 0.0 |
| Total | 1000.0 | 1000.0 |

*17% of water is included. D-Psicose includs 85% of solid content.

### 2.2. Experimental Design

After 90 days of feeding, rats in each group were fasted for 4.5 h beginning at 06:00, and then anesthetized by intraperitoneal administration of sodium pentobarbital. Blood was collected from the abdominal aorta for clinical hematological analysis and to obtain serum for chemical analysis. The rats were exsanguinated. The brain, heart, lungs, liver, pancreas, kidneys, adrenal glands, spleen, testes, intra-abdominal adipose tissues (epididymal, perirenal, and mesenteric), and muscle tissues (soleus, gastrocnemius, and plantarius) were rapidly removed and weighed. Parts of the liver, kidneys, and small intestine (about 5 mm of the end of the jejunum) were preserved in 10% neutral buffered formalin for histopathological examinations. The stomach, small intestine, large intestine, and cecum were also rapidly removed and weighed. In addition, the small and large intestine length, surface area, and cecal content weight were measured.

### 2.3. Analysis

The following hematological and clinical chemistry parameters were evaluated white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), total protein (TP), ratio of albumin and globulin (A/G), albumin (ALBU), globulin (GLO), aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid (UA), urea nitrogen (BUN), creatinine (CREA), calcium (Ca), iron (Fe), total cholesterol (CHO), triglyceride (TG), glucose (GLU), and free fatty acids (FFA). The hematological and chemical analyses were performed by Shikokuchuken Co., Ltd. (Kagawa, Japan). The histopathological examinations were performed by Shikoku Cytopathology Center Co., Ltd. (Kagawa, Japan). Fixed tissue samples from the liver, kidney, and small intestine were embedded in paraffin and cut into sections 5 - 6 μm thick on a microtome. The tissue sections were stained with hematoxylin and eosin (HE) and examined by light microscopy. Next, the histopathological findings in each rat were subjectively quantified as follows: –, 0; ±, 1; +, 2; ++, 3; +++, 4.

### 2.4. Statistical Analysis

All values are expressed as the mean ±SD. Statistical analysis of the differences between the control and PS groups was performed using Student's *t*-test. Statistical significance was set at $p < 0.05$. All analyses were performed with a commercially available statistical software package (Excel Statistics 2008; SSRI, Co., Ltd.,

 

Tokyo, Japan).

## 3. RESULTS

### 3.1. Body and Tissue Weights, Food Intake, and Digestive Tract Size

The body and tissue weights, food intake, and digestive tract size in rats fed the two experimental diets for 90 days are presented in **Table 2**. The final body weight, weight gain, and food intake did not differ between the control and PS groups. The mean stomach and cecal content weights were significantly higher in the PS group than in the control group, but no differences were observed in any other tissues.

**Table 2.** Body weight, food intake, tissue weights and digestive tract size.

| Groups | | Control | D-Psicose syrup |
|---|---|---|---|
| Initial weight | (g) | 60 ± 6 | 59 ± 5 |
| Final weight | (g) | 334 ± 19 | 348 ± 25 |
| Weight gain | (g) | 274 ± 16 | 289 ± 22 |
| Food intake | (g/day) | 19.3 ± 1.1 | 19.5 ± 0.8 |
| *Tissue weights* | | | |
| Brain | (g) | 1.39 ± 0.33 | 1.71 ± 0.25 |
| Heart | (g) | 0.78 ± 0.05 | 0.82 ± 0.06 |
| Lungs | (g) | 0.93 ± 0.07 | 1.00 ± 0.10 |
| Liver | (g) | 10.4 ± 0.90 | 10.4 ± 1.34 |
| Pancreas | (g) | 0.51 ± 0.07 | 0.55 ± 0.08 |
| Kidneys | (g) | 2.27 ± 0.18 | 2.36 ± 0.20 |
| Adrenals | (g) | 0.05 ± 0.01 | 0.05 ± 0.01 |
| Spleen | (g) | 0.72 ± 0.08 | 0.73 ± 0.06 |
| Testicles | (g) | 3.10 ± 0.14 | 3.11 ± 0.11 |
| Intra-adipose tissues[1] | (g) | 21.5 ± 2.79 | 20.8 ± 4.02 |
| Muscle tissues[2] | (g) | 3.77 ± 0.18 | 3.91 ± 0.23 |
| *Digestive tracts* | | | |
| Stomach weight | (g) | 1.98 ± 0.49 | 2.62 ± 0.49 |
| Small intestine weight | (g) | 5.28 ± 0.48 | 5.66 ± 0.52 |
| Small intestine length | (m) | 1.06 ± 0.04 | 1.10 ± 0.05 |
| Large intestine weight | (g) | 1.09 ± 0.11 | 1.09 ± 0.18 |
| Large intestine length | ($\times10^{-2}$·m) | 16.9 ± 2.16 | 18.3 ± 2.36 |
| Cecal weight | (g) | 0.74 ± 0.15 | 0.77 ± 0.05 |
| Cecal surface area | ($\times10^{3}$·mm$^2$) | 3.76 ± 0.54 | 4.06 ± 0.40 |

Values are means ±SD for 10 rats. [1]Total weight of epididymal, perirenal and mesenteric adipose tissues. [2]Total weight of soleus, gastrocnemius and plantaris muscles.

**Table 3.** Blood hematological and serum chemical values.

| Group | | Control | D-Psicose syrup |
|---|---|---|---|
| *Blood* | | | |
| WBC | ($\times10^2$/μl) | 30.2 ± 3.0 | 29.6 ± 4.6 |
| RBC | ($\times10^4$/μl) | 892 ± 24 | 892 ± 23 |
| Hb | (g/100 ml) | 14.4 ± 0.4 | 14.7 ± 0.5 |
| Ht | (%) | 46.3 ± 1.4 | 46.4 ± 1.4 |
| MCV | (fl) | 52.0 ± 1.0 | 52.0 ± 1.0 |
| MCH | (pg) | 16.2 ± 0.2 | 16.4 ± 0.3 |
| MCHC | (%) | 31.2 ± 0.6 | 31.6 ± 0.5 |
| PLT | ($\times10^4$/μl) | 56.6 ± 8.5 | 56.1 ± 2.0 |
| *Serum* | | | |
| TP | (g/100 ml) | 6.23 ± 0.18 | 6.15 ± 0.20 |
| A/G | | 3.17 ± 0.49 | 3.04 ± 0.34 |
| ALBU | (g/100 ml) | 4.72 ± 0.25 | 4.62 ± 0.21 |
| GLO | (g/100 ml) | 1.51 ± 0.15 | 1.53 ± 0.13 |
| TBIL | (mg/100 ml) | 0.20 ± 0.00 | 0.20 ± 0.00 |
| DBIL | (mg/100 ml) | 0.10 ± 0.00 | 0.10 ± 0.00 |
| IBIL | (mg/100 ml) | 0.10 ± 0.00 | 0.10 ± 0.00 |
| AST | (IU/I) | 230 ± 32 | 172 ± 92 |
| ALT | (IU/I) | 76.1 ± 19.4 | 87.9 ± 48.2 |
| UA | (mg/100 ml) | 1.22 ± 0.26 | 1.14 ± 0.36 |
| BUN | (mg/100 ml) | 20.8 ± 1.7 | 22.2 ± 2.1 |
| CREA | (mg/100 ml) | 0.27 ± 0.03 | 0.30 ± 0.05 |
| Ca | (mg/100 ml) | 10.2 ± 0.2 | 10.4 ± 0.3 |
| Fe | (μg/100 ml) | 110 ± 25 | 111 ± 13 |
| CHO | (mg/100 ml) | 70.1 ± 10.0 | 68.8 ± 12.5 |
| TG | (mg/100 ml) | 79.6 ± 36.1 | 69.0 ± 32.4 |
| GLU | (mg/100 ml) | 156 ± 14 | 167 ± 14 |
| FFA | (mEq/100 ml) | 0.87 ± 0.24 | 0.70 ± 0.11 |

Values are means ±SD for 10 rats.

### 3.2. Serum Chemical and Blood Hematological Values

No differences were observed in any chemical or hematological values between the control and PS groups (**Table 3**). These values remained within the normal ranges, indicating that there was no overt PS toxicity.

### 3.3. Histopathological Examination

The histopathological observations of the liver, kidney,

 

and small intestine are presented in **Table 4**. Age-related naturally-occurring lesions were observed in the tissues, but no abnormalities due to the ingestion of PS were observed. The histopathological examination showed no differences in the total damage in the liver, kidneys, and small intestine between the control and PS groups.

## 4. DISCUSSION

In the present 90-day feeding study of PS at a dose of 4.3% in male Wistar rats, no mortality occurred, and systemic toxicity was not evident. We have examined the effects of acute [11], sub-chronic (90 days) [15], and long-term feeding (12 - 18 months) [14] of D-psicose to rats prior to its utilization as a physiologically functional food. We concluded that a small amount of D-psicose in the diet had no adverse effects in rats. In the present study, we focused on the safety of impurities (0.5% unknown ingredients) of the PS rather than safety of the PS itself. Therefore, the control diet included 0.5% sucrose for comparison with 0.5% impurities in the PS diet.

Generally, small amounts of impurities are included in natural products and processed foods [19]. For example, royal jelly has been widely used as a dietary supplement and in cosmetics in many countries [20]. With regard to chemical composition, royal jelly was reported to consist mainly of proteins, sugars, lipids, vitamins, and many unknown ingredients [21,22]. The main active ingredients of royal jelly are not clear, but royal jelly is recognized as a safe food or supplement [23]. On the other hand, HFCS also contains about 6% unknown ingredients [24]. However, HFCS has already been generally used in many developed countries [25]. PS includes un-

**Table 4.** Histopathological observations of liver, kidney and small intestine[1].

| Groups | | Control | D-Psicose syrup |
|---|---|---|---|
| *Ogans* | *Findings* | | |
| Liver | Bile duct proliferation | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Necrosis | 0.2 ± 0.4 | 1.9 ± 1.0 |
| | Microgranuloma | 0.4 ± 0.5 | 1.2 ± 1.0 |
| | Lipid deposition | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Fatty change | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Total score of damage | 0.6 ± 0.7 | 3.1 ± 1.4 |
| Kidney | Basophilic change in the tubule | 1.0 ± 1.3 | 1.2 ± 1.2 |
| | Hyaline cast in the tubule | 0.8 ± 0.8 | 1.0 ± 0.8 |
| | Brown pigment deposition in the tubule | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Atrophy of the glomerulus | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Hyalinization in the glomerulus | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Thickening of Bowman's capsule basement membrane | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | Lymphocyte infiltration in the interstitium | 0.2 ± 0.4 | 0.4 ± 0.5 |
| | Total score of damage | 1.5 ± 1.3 | 2.0 ± 1.5 |
| Small intestine | Villous damage | 0.4 ± 0.7 | 1.2 ± 1.0 |
| | Crypt damage | 0.1 ± 0.3 | 0.4 ± 0.5 |
| | Cellular infiltration | 0.9 ± 0.6 | 0.8 ± 0.9 |
| | Goblet cell depletion | 0.1 ± 0.3 | 0.5 ± 0.9 |
| | Total score of damage | 1.5 ± 1.7 | 2.9 ± 2.2 |

Values are means ±SD for 10 rats. [1]Quantify the findings level of damage in each rats: –, 0; ±, 1; +, 2; ++, 3; +++, 4.

 

known impurities, but it is likely to be a safe food product. The PS was made from HFCS by the alkaline isomerization method. The alkaline isomerization method is used widely, resulted in production of many sugar products, such as cyclodextrin, maltitol, and erythritol [26].

D-Psicose can be produced by the enzymatic method on a large scale, making it possible to conduct biochemical and nutritional studies [6]. We found that D-psicose is a sweet monosaccharide that provides no energy and leads to less body fat accumulation than D-glucose and D-fructose in rats [7,8]. In addition, we have suggested that supplemental D-psicose can lower plasma glucose levels [9]. D-Psicose is expected to have a beneficial effect in the control of blood glucose levels in type 2 diabetes. However, D-psicose is more expensive than other substitute sugars. However, PS can be produced more easily and cost-effectively than D-psicose. PS may be effective for the prevention obese or type 2 diabetes as functional foods.

In conclusion, the present study evaluated the effects of 90-day 4.3% PS administration to rats, and there were no gross pathological findings. The hematological and chemical values were not suggestive of any overt PS toxicity. Overall, no adverse effects were seen at this low dose level of PS in the diet.

## 5. ACKNOWLEDGEMENTS

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