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Ninety-day oral toxicity study of rare sugar syrup in male Wistar rats

Tatsuhiro Matsuo[1,*], Reika Ishii[1], Tetsuo Iida[2], Takako Yamada[2], Satoshi Takamine[2], and Yoko Shirai[3]

[1]Faculty of Agriculture, Kagawa University, Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0795,
[2]Matsutani Chemical Industry Co., Ltd., Kitaitami, Itami-shi, Hyogo 664-8508,
[3]National Institute for Materials Science, Namiki, Tsukuba, Ibaraki 305-0044, Japan

ABSTRACT

High-fructose corn syrup (HFCS) is a cost-effective sweetener that has seen a marked increase in its use in developed countries. Some studies have shown that ingestion of HFCS can cause increase in body weight and body fat. Recently, we developed a new product, rare sugar syrup (RSS) made from HFCS, which can reduce the HFCS-induced obesity. As the solid element of RSS contained about 6% rare sugar D-psicose, a functional monosaccharide with no bioavailable energy, RSS might be useful as a sweetener to take the place of HFCS. Elucidation of the effects of sub-chronic feeding in rats with RSS is essential before it can be utilized as a physiologically functional food. In this study, male Wistar rats (3 weeks old) were fed diets containing 40% RSS or HFCS for 90 days. The RSS group ingested 1.31 g/kg body weight per day of D-psicose. Body weight gain and intra-abdominal adipose tissue weight were significantly lower in the RSS group than in the HFCS group. The weight of the liver and kidneys were significantly higher in the RSS group than in the HFCS group. However, no gross pathological findings were evident at dietary doses of 40% RSS or were correlated with hypertrophy of the liver and kidney. On clinical chemistry analysis, the aspartate aminotransferase and alanine amino-transferase values were significantly lower in the PS group. Therefore, the present study found no adverse effects of 40% RSS in the diet.

KEYWORDS: rare sugar syrup, high-fructose corn syrup, 90-day oral toxicity, pathological tests, rat

INTRODUCTION

The introduction of high-fructose corn syrup (HFCS) as a cost-effective sweetener in the developed countries has gradually led to a great increase in its use [1]. In America, from 1970 to 1990, consumption of HFCS increased by more than 1000% and currently accounts for 40% of all added caloric sweeteners [2]. The increase in HFCS use was accompanied by a decline in sucrose use during the same time period [3]. One common source of HFCS is caloric beverages, i.e., soft-drinks. It is also a primary ingredient in baked foods, many cereals, breads, canned fruits, jams, jellies, desserts, and fruit juices [4]. Vos *et al*. [5] demonstrated that over 10% of daily calories come from fructose, of which 75% in adults and 82% in children are attributed to added HFCS rather than naturally occurring fructose. Given its prevalence in the diet, it is crucial to understand the behavioral and physiological effects of dietary HFCS.

As obesity has escalated to epidemic proportions around the world, many causes, including dietary

*Corresponding author
matsuo@ag.kagawa-u.ac.jp

components, have been suggested [6]. Excessive caloric intake has been related to high-fat foods, increased portion sizes, and diets high in both simple sugars such as sucrose and in HFCS as a source of fructose [7-9]. A marked increase in the use of HFCS preceded the obesity epidemic and it may be an important contributor to this epidemic in advanced nations [1].

Recently, we developed a new product, rare sugar syrup (RSS) made from HFCS by the alkaline isomerization method, for the reduction of obesity caused by HFCS. The solid element of RSS contains about 6% rare sugar D-psicose, and small amounts of other sugars. One of them was presumed to be the rare sugar, D-allose. Previously, the useful effects of D-psicose as a food material have been suggested [10-15]. Furthermore, no adverse effects were seen at low doses of D-psicose in the diet [16-19]. As these rare sugars are still very expensive, they are difficult to use as food materials or sweeteners. RSS can be produced more easily and more cost-effectively than D-psicose. However, as RSS includes small amounts of other unidentified sugars, the safety of RSS as a functional low-energy sweetener must be determined more precisely.

In this study, to assess the safety of RSS, the 90-day toxicity of RSS was examined in rats. The objective of this study was to determine whether RSS can be safely used as a functional food compared to HFCS.

MATERIALS AND METHODS

All procedures involving animals were approved by the Animal Care Committee of Kagawa University (No.19, 2010).

Test carbohydrates

HFCS and RSS used as raw materials of experimental diets were supplied by Matsutani Chemical Industry, Co., Ltd. (Hyogo, Japan). HFCS and RSS included 30% water. The composition of HFCS was as follows; D-glucose, 50.2%; D-fructose, 44.6%; oligosaccharides, 5.2%. RSS is made from HFCS by the alkaline isomerization method. Briefly, HFCS is dissolved to 0.1 M NaOH solution, which is isomerized through a strong base ion exchange resin at 60°C. The solution obtained is neutralized, desalinated, and concentrated by standard methods, thus producing RSS (70% w/w). The composition of RSS is measured by high-performance liquid chromatography with refractive index detection. The analytic column was the MCI GEL CK 08EC (Mitsubishi Chemical Corporation, Tokyo, Japan). The composition of RSS was as follows: D-glucose, 43.7%; D-fructose, 31.4%; D-psicose, 6.1%; oligosaccharides, 4.4%; and other saccharides, 14.4%.

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 ± 2°C, with lights on from 08:00 to 20:00. The rats were randomly divided into two groups of 10 animals each (HFCS and RSS groups). We adopted HFCS as a control of RSS because HFCS is a very popular sweet additive and the safety of HFCS has already been established [20]. The experimental diets are shown in Table 1. The amount of test carbohydrate (weight ratio, 40%; D-psicose content, 1.7%) was determined with reference to previous studies concerning D-psicose [18] or sucralose with an LD_{50} level (16 g/kg weight) equivalent to that of D-psicose [21, 22]. Each group of rats were given free access to food and water for 90 days.

Experimental design

After 90 days of feeding, rats in each group were fasted for 4.5 h beginning at 06:00 h, then anesthetized by intraperitoneal administration of sodium pentobarbital (50 mg/kg). 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, adrenals, spleen, testes, intra-abdominal adipose tissues (epididymal, perirenal and mesenteric), and muscle tissues (soleus, gastrocnemius and plantarius) were quickly removed and weighed. Parts of the liver, kidney, and small intestine (about 5 mm of the end of the jejunum) were preserved in 10% neutral buffered formalin for histopathological examinations. The stomach,

Table 1. Composition of experimental diets.

Groups	HFCS	RSS[1]
Ingredients	g/kg	
Casein	200.0	200.0
DL-Methionine	3.0	3.0
Cornstarch	324.9	324.9
HFCS	464.3 (325.0)[2]	0.0
RS	0.0	464.3 (325.0)[2]
Powdered fat	50.0	50.0
Mineral mixture	35.0	35.0
Vitamin mixture	10.0	10.0
Cellulose	50.0	50.0
Choline chloride	2.0	2.0
Butylhydroxytoluene	0.1	0.1
Total	1139.3 (1000.0)[2]	1139.3 (1000.0)[2]

HFCS, high fructose corn syrup; RSS, rare sugar syrup.
[1]D-Psicose is included at 1.7% in the RS diet.
[2]The solid content is shown within parentheses.

small intestine, large intestine, and cecum were also quickly removed and weighed. In addition, the small and large intestine length, surface area, and cecal content weight were determined.

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), triglycerides (TG), glucose (GLU), and free fatty acid (FFA).

Hematological and chemical analyses were performed by Shikokuchuken Co., Ltd. (Kagawa, Japan). Evaluation of the histopathological examinations was performed by Shikoku Cytopathology Center Co., Ltd. (Kagawa, Japan). The tissue samples of the liver, kidney, and small intestine were fixed in 10% neutral formalin, embedded in paraffin, and cut into sections 5-6 μm thick with a microtome. The tissue sections were stained with hematoxylin and eosin (HE) and examined with a light microscope. Then, the histopathological levels in each rat were subjectively quantified as follows: –, 0; ±, 1; +, 2; ++, 3; +++, 4.

Statistical analysis

All values are expressed as the means ± SD. Statistical analysis of the differences between the HFCS and RSS 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 package (Excel Statistics 2008; SSRI, Co., Ltd., Tokyo, Japan).

RESULTS

Body and tissue weights, food intake, and digestive tract size

Results for the body and tissue weights, food intake, and digestive tract size in rats fed for

Table 2. Body weight, food intake and tissue weights.

Groups			HFCS	RSS
Initial weight		(g)	57 ± 8	56 ± 10
Final weight		(g)	344 ± 16	323 ± 23*
Weight gain		(g)	287 ± 14	267 ± 18*
Food intake		(g/day)	23.4 ± 1.1	21.7 ± 1.4*
Food efficiency		(mg/g)	136 ± 16	137 ± 19
Tissue weights				
Brain		(g)	1.82 ± 0.06	1.71 ± 0.28
Heart		(g)	0.81 ± 0.06	0.80 ± 0.04
Lungs		(g)	1.03 ± 0.09	0.99 ± 0.09
Liver		(g)	9.74 ± 1.05	11.3 ± 1.51*
Pancreas		(g)	0.43 ± 0.08	0.45 ± 0.02
Kidneys		(g)	2.01 ± 0.12	2.31 ± 0.26*
Adrenals		(g)	0.05 ± 0.01	0.05 ± 0.01
Spleen		(g)	0.66 ± 0.04	0.66 ± 0.04
Testicles		(g)	2.88 ± 0.14	2.87 ± 0.10
Intra-adipose tissues[1]		(g)	34.4 ± 2.70	22.5 ± 3.73*
Muscle tissues[2]		(g)	3.60 ± 0.25	3.61 ± 0.21
Intestinal tracts size				
Stomach	weight	(g)	1.84 ± 1.24	1.78 ± 0.29
Small intestine	weight	(g)	5.05 ± 0.43	5.22 ± 0.45
	length	(m)	1.04 ± 0.04	1.09 ± 0.03
Large intestine	weight	(g)	0.78 ± 0.16	0.66 ± 0.09
	length	($x10^{-2}$·m)	17.4 ± 2.22	15.6 ± 2.87
Cecum	content	(g)	2.50 ± 1.30	6.63 ± 2.00*
	weight	(g)	0.50 ± 0.12	0.82 ± 0.16*
	surface area	($x10^{3}$·mm^{2})	2.15 ± 0.63	3.52 ± 1.35*

Values are means ± SD for 10 rats. *Significant difference from the HFCS group ($p<0.05$, Student's t-test).
[1]Total weight of epididymal, perirenal and mesenteric adipose tissues.
[2]Total weight of soleus, gastrocnemius and plantaris muscles.

90 days are presented in Table 2. Final body weight, weight gain, intra-adipose tissue weight, and food intake were significantly lower in the RSS group than in the HFCS group, whereas food efficiency did not differ between the two groups. The liver, kidney, and cecal weights and cecal surface area, were significantly higher in the RSS group than in the HFCS group, but no differences were observed in any other tissue weights. Rats of the RSS group actually ingested 1.31 g/kg body weight per day of D-psicose (mean value for 90 days).

Serum chemical and blood hematological values

The serum chemical and blood hematology results for the rats are presented in Table 3. ALBU, AST, and ALT values were significantly lower in the RSS group than in the HFCS group, but no differences were observed in any other chemical values between the RSS and HFCS groups. WBC, RBC, and PLT were significantly higher, and MCH and MCHC were significantly lower in the RSS group than in the HFCS group. No differences were observed in Hb concentration and Ht value between the two dietary groups.

Table 3. Serum clinical chemistry and blood hematological values

Groups		HFCS	RSS
Serum			
TP	(g/100 ml)	6.58 ± 0.22	6.33 ± 0.26
A/G		5.33 ± 0.61	5.10 ± 1.03
ALBU	(g/100 ml)	5.53 ± 0.16	5.27 ± 0.29*
GLO	(g/100 ml)	1.05 ± 0.13	1.07 ± 0.19
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/l)	102.9 ± 13.3	80.7 ± 10.0*
ALT	(IU/l)	52.7 ± 16.2	38.8 ± 6.2*
UA	(mg/100 ml)	1.02 ± 0.10	0.92 ± 0.11
BUN	(mg/100 ml)	17.3 ± 4.0	17.5 ± 1.4
CREA	(mg/100 ml)	0.27 ± 0.03	0.21 ± 0.03
Ca	(mg/100 ml)	10.9 ± 0.3	11.1 ± 0.3
Fe	(μg/100 ml)	147 ± 26	139 ± 24
CHO	(mg/100 ml)	91.3 ± 16.4	87.4 ± 19.7
TG	(mg/100 ml)	95.9 ± 33.8	125.2 ± 68.4
FFA	(mEq/100 ml)	1.14 ± 0.14	0.96 ± 0.33
Blood			
WBC	($x10^2$/μl)	20.7 ± 2.58	29.6 ± 4.28*
RBC	($x10^4$/μl)	896 ± 24	924 ± 25*
Hb	(g/100 ml)	15.4 ± 0.3	15.2 ± 0.4
Ht	(%)	47.0 ± 1.0	45.3 ± 1.0
MCV	(fl)	52.4 ± 1.2	49.0 ± 0.9
MCH	(pg)	17.2 ± 0.4	16.5 ± 0.3*
MCHC	(%)	32.8 ± 0.4	33.6 ± 0.4*
PLT	($x10^4$/μl)	50.3 ± 4.8	55.0 ± 4.8*

Values are means ± SD for 10 rats.
*Significant difference from the HFCS group ($p<0.05$, Student's t-test).

Histopathological examination

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 RSS were observed. Histopathological observation showed no difference in the total damage in the liver, kidney, and small intestine between the RSS and the HFCS groups.

DISCUSSION

In the present 90-day feeding study of RSS at a dose of 40% in male Wistar rats, no mortality occurred, and systemic toxicity was not evident. Although the present study demonstrated that the effects of 40% RSS in the diet after its long-term administration to rats were increase in liver and kidney weights, chemical and histopathological examinations revealed no values suggestive of overt RSS treatment-related toxicity.

Previously, we found that the LD_{50} value of D-psicose administered orally in rats was 16g/kg [10]. In the present study, rats actually ingested 1.31 g/kg body weight per day of D-psicose. Body weight gain and intra-abdominal adipose tissue weight were significantly lower in rats fed the RSS diet than in rats fed the HFCS diet for 90 days. As food efficiency did not differ between the two dietary groups, the percentage of intra-abdominal adipose tissue weight was significantly lower in

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

Groups		HFCS	RSS
Ogans	Findings		
Liver	Bile duct proliferation	0.0 ± 0.0	0.0 ± 0.0
	Necrosis	1.5 ± 0.7	1.4 ± 0.7
	Microgranuloma	1.9 ± 1.2	1.6 ± 1.2
	Lipid deposition	3.2 ± 0.8	1.9 ± 0.9*
	Fatty change	0.9 ± 0.7	1.8 ± 1.2
	Total score of damage	7.5 ± 2.4	6.7 ± 3.2
Kidney	Basophilic change in the tubule	1.0 ± 0.9	1.2 ± 0.8
	Hyaline cast in the tubule	0.6 ± 0.8	0.4 ± 0.9
	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	0.0 ± 0.0
	Total score of damage	1.8 ± 1.4	1.7 ± 1.1
Small intestine	Villous damage	0.1 ± 0.3	1.2 ± 0.7
	Crypt damage	0.3 ± 0.7	0.0 ± 0.0
	Cellular infiltration	0.3 ± 0.5	0.3 ± 0.5
	Goblet cell depletion	0.5 ± 0.7	0.0 ± 0.0*
	Total score of damage	1.2 ± 1.9	0.6 ± 1.0

Values are means ± SD for 10 rats.
*Significant difference from the HFCS group ($p<0.05$, Student's t-test).
[1]Quantifiation of damage levels in each rat: -, 0; ±, 1; +, 2; ++, 3; +++, 4.

rats fed the RSS diet than in rats fed the HFCS diet (7.0 ± 1.1% versus 10.0 ± 0.8%, $p<0.05$). We reported previously that D-psicose supplements suppress hepatic lipogenic enzyme activity and reduce intra-abdominal fat accumulation more effectively than D-glucose or D-fructose supplements in rats [10, 12]. In addition, we found that D-psicose is a sweet monosaccharide that provides no energy to growing rats [11]. The present findings also support our previous results, indicating that this may be due to the synergistic effect of D-allose with D-psicose.

The RSS decreased rat food intake during the 90-day experimental period. This phenomenon was confirmed even by our previous work using D-psicose in the diet [13, 17]. D-Psicose or RSS might have a satiety effect, but D-psicose did not affect serum leptin, adiponectin, or TNF-α concentrations [23]. The elucidation of appetite control mechanism of D-psicose, including hormones involved in the central regulation of feeding behavior, in the gastrointestinal tract, are not known.

Our previous short-term toxicity test showed that the feeding of diets extremely high in D-psicose appeared to be harmful to the intestinal tract in rats [16, 17]. Moreover, we previously reported that cecal weight, cecal surface area, and cecal content weight increased with increase of D-psicose in the diet (above 10%) [16, 17]. D-Psicose is partly absorbed in the digestive tract and is excreted into the urine and feces. However, it is also fermented in the cecum in rats by intestinal microflora, producing short-chain fatty acids as a soluble dietary fiber [17, 24]. In this study, cecal content, weight and surface area were significantly higher in the RSS group than in the HFCS group. As the D-psicose concentration of RSS was only 1.7%, other sugars in RSS may not be completely absorbed in the upper digestive tract, and fermentation may take place in the cecum of rats. The absorption rates of RSS in the upper digestive tract and its prebiotic effects require further research.

Dietary RSS increased the weight of the animal's liver (16% larger than the HFCS group) and kidneys (16% larger). These findings agreed with our previous studies [10, 12, 13, 18]. The serum levels of AST and ALT are used as indexes of hepatic damages. These values in this study were significantly lower in the RSS group than in the HFCS group, which reflects that RSS does not create toxicity to the rat liver. In addition, histopathological observations of the liver and kidneys revealed no abnormalities due to the ingestion of RSS. Liver enlargement occurs in animals and humans, under a variety of conditions with different consequences for health [25]. For example, it can be the result of a physiological adaptation to an enhanced workload or metabolic demand, a metabolic abnormality, a toxic effect, an inflammatory process, or a proliferative disease [26-28]. This phenomenon in the liver has been well studied for D-tagatose, a rare sugar, which has been approved as a novel food, and has been widely adopted in the European food industry. In the safety reports of D-tagatose, liver enlargement in rats is reversible on cessation of D-tagatose administration, and in humans D-tagatose treatment did not significantly alter liver volume compared to the non-treated control group, and thus species differences must be considered in this variation [29-30]. Bar *et al*. [30] also found that dietary D-tagatose (5-20% w/w) increased liver glycogen content and relative liver weight in non-fasting rats. D-Tagatose is an incompletely absorbed ketohexose that has potential as an energy-reduced alternative sweetener. They concluded that the liver enlargement seen in response to the consumption of D-tagatose was a physiological response to the treatment-induced increase in glycogen deposition. We previously found a D-psicose-treatment-induced increase in glycogen deposition [13]. In addition, it was reported that D-psicose could increase glucokinase activity by enhancing the translocation of glucokinase from nucleus to cytoplasm in the liver of diabetic rats [31]. Although it is likely that these mechanisms of liver enlargement induced by RSS are similar to D-tagatose, other mechanisms may not be excluded. Glycogen deposition and liver enlargement (increased relative liver weights) were also seen in rats fed diets with high level of various saccharides [32-34]. Thus, other sugars in RSS might also have been related to liver enlargement. It was hypothesized that the formation of a phosphorylated metabolites plays a crucial role in this process, either by activating glucokinase or by changing intracellular concentrations of

phosphate compounds [31]. We postulated that D-psicose, of which about 50% is absorbed [24], is more active than D-fructose in promoting glycogen deposition. The slower degradation of psicose-1-phosphate may account for this phenomenon. With regard to kidney enlargement, the same effect was observed with rats fed erythritol, which is also a rare sugar. In a four-week safety study on erythritol, Til *et al*. [35] reported that kidney enlargement takes place by natural adaptation, which was caused by higher intake of water in rats. According to Til *et al*., it is also necessary to quantify the water intake levels when rats are fed RSS.

In the hematological analysis in this study, dietary RSS significantly increased the WBC, RBC, and PLT values compared to HFCS, and significantly decreased the MCH and MCHC values. These findings suggest the existence of no overt RSS toxicity, because the values remained within the normal range.

In conclusion, the present study found the effects of 90-day 40% RSS administration in rats to be increase in the liver and kidney weights, with no gross pathological findings correlated with this hypertrophy. Hematological and chemical values were not suggestive of overt RSS toxicity. Overall, no adverse effects were seen at this low dose of RSS in the diet. Thus, it seems that RSS can be used as a food material and sweetener in place of HFCS.

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