Original Article

The 90-day oral toxicity of D-psicose in male Wistar rats

Tatsuhiro Matsuo,[1,*] Reika Ishii[1] and Yoko Shirai[2]

[1]Faculty of Agriculture, Kagawa University, Ikenobe, Miki-cho, Kita-gun, Kagawa 761-0795, Japan
[2]National Institute for Materials Science, Namiki, Tsukuba, Ibaraki 305-0044, Japan

(Received 11 May, 2011; Accepted 26 May, 2011; Published online 18 November, 2011)

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 toxicity testing in rats, D-psicose is classified as an ordinary substance (LD_{50} = 16 g/kg). Elucidating the effects of sub-chronic feeding of D-psicose in rats 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 3% D-psicose or sucrose for 90 days. The body weight gain and intra-abdominal adipose tissue weight did not differ between the sucrose and the D-psicose groups. The weights of the liver and kidneys were significantly higher in the D-psicose group than in the sucrose group. However, no gross pathological findings were evident at dietary doses of 3% D-psicose or were correlated with hypertrophy of the liver and kidney. In a clinical chemistry analysis, the erythrocyte and leukocyte courts were significantly higher in the D-psicose group, but that was not considered to be toxicologically significant. Therefore, the present study found no adverse effects of D-psicose in rats fed a diet containing 3% D-psicosefor 90 days.

***Key Words*:** **D-psicose, sucrose, 90-day oral toxicity, pathological tests, rat**

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 from 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] Examining the effects of D-psicose on glucose and lipid metabolism, we found that D-psicose is a sweet monosaccharaide that provides no energy to growing rats and leads to less intra-abdominal fat accumulation than D-glucose and D-fructose in rats.[6,7] In addition, we have suggested that supplemental D-psicose can lower plasma glucose levels.[8] Toyoda *et al.*[9] suggested that D-psicose can prevent postprandial hyperglycemia by improving the translocation of glucokinase from nucleus to 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; the oral LD_{50} value was 16 g/kg in male Wistar rats.[10] D-Psicose, which is naturally present in foods such as fruit juice and fruit cereal, is derived from D-fructose by the cooking process.[2,11] Oshima *et al.*[12] 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.[12] As a result, most humans ingest a limited amount of D-psicose on a daily basis. Recently, we examined the effects of long-term feeding (12–18 months) of D-psicose to rats prior its utilization as a physiologically functional food.[13] We have concluded that 3% D-psicose in the diet had no adverse effects in rats. However, in the previous 12-to-18-month toxicity study, because the effects of aging were strongly seen in rats, the toxicity of D-psicose may have been hidden. Therefore, an additional sub-chronic examination was necessary.

In this study, to assess the safety of D-psicose, 90-day oral toxicity studies were conducted with rats at dietary dose of 3% D-psicose. The objective was to determine whether D-psicose can be safety used as a functional food.

Materials and Methods

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

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 light from 08:00 to 20:00 h. The rats were randomly divided into two groups of 10 (sucrose and D-psicose groups). We adopted sucrose as a control for D-psicose because sucrose is a very popular sweet carbohydrate and is used in many studies as a control saccharide.[14–16] The experimental diets were 3% sucrose or D-psicose added to CE-2. The amount of test carbohydrates (3%) was determined with reference to previous studies concerning D-psicose[13] or sucralose, which has an LD_{50} that is the same as that of D-psicose (16 g/kg weight).[17,18] Each group of rats was given free access to food and water for 90 days. D-Psicose, which was made from high fructose corn syrup by the alkaline isomerization method, was donated by Rare Sugar Production Thecnical Research Laboratories, LLC (Kagawa, Japan). The high fructose corn syrup used as a raw material was supplied by Matsutani Chemical Industry, Co., Ltd. (Hyogo, Japan). Sucrose was purchased from Wako Pure Chem. Ind. (Osaka, Japan).

Experimental design. After 90 days of feeding, rats in each group were fasted for 4.5 h beginning at 06:00 h, and then were anesthetized via the intraperitoneal administration of sodium pentobarbital. Blood was collected from the abdominal aorta for clinical hematological analysis and to obtain serum for the chemical analysis. The rats were allowed to exsanguinate. The brain, heart, lungs, liver, pancreas, kidneys, adrenal glands, spleen, testicles, intra-abdominal adipose tissues (epididymal, perirenal and mesenteric) and muscle tissues (soleus, gastrocnemius and plantarius) were quickly removed and weighed. Parts of the liver, kidneys and small intestine (about 5 mm of the end of the jejunum)

*To whom correspondence should be addressed.
E-mail: matsuo@ag.kagawa-u.ac.jp

doi: 10.3164/jcbn.11-66

were preserved in 10% neutral buffered formalin for histopathological examinations. The stomach, 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 measured.

Analysis. The following hematological and clinical chemistry parameters were evaluated WBC, RBC, Hb, Ht, MCV, MCH, MCHC, PLT, TP, A/G, ALUB, GLO, AST, UA, BUN, CREA, Ca, Fe, CHO, TG, GLU, and 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). The fixed tissue samples from the liver, kidney and small intestine were embedded in paraffin and cut with a microtome set at a thickness of 5–6 μm. The tissue sections were stained with hematoxylin and eosin (HE) and examined with a light microscope. Next, the histopathological findings in each rat were subjectively quantified as follows: –, 0; ±, 1; +, 2; ++, 3; +++, 4.

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

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 the two substances for 90 days are presented in Table 1. The final body weight, weight gain and food intake did not differ between the sucrose group and the D-psicose group. Rats actually ingested 1.67 g/kg body weight per day D-psicose or 1.65 g/kg body weight per day of sucrose (mean values for 90 days). The mean liver and kidney weights were significantly higher in the D-psicose group than in the sucrose group, but no differences were observed in any other tissues.

Serum chemical and blood hematological values. The serum chemical and blood hematology results for the rats are presented in Table 2. The TP and ALBU values were significantly higher, and the UA concentration was significantly lower in the D-psicose group than in the sucrose group, but no differences were observed in any other serum chemistry values between the sucrose and D-psicose groups. The WBC, RBC, MCHC and PLT were significantly higher, and the MCV and MCH were significantly lower, in the D-psicose group than in the sucrose group. No differences were observed in the Hb concentration and Ht value between the two dietary groups.

Histopathological examination. The histopathological observations of the liver, kidney and small intestine are presented in Table 3. Age-related naturally-occurring lesions were observed in the tissues, but no abnormalities due to the ingestion of D-psicose were observed. The histopathological examination showed no differences in the total damage in the liver, kidneys and small intestine between the sucrose and the D-psicose groups.

Discussion

In the present 90-day feeding study of D-psicose at the dose of 3.0% in male Wistar rats, no mortality occurred, and systemic toxicity was not evident. Although the present study demonstrated that long-term administration of 3% D-psicose to rats led to increases in the weights of the liver and kidneys, chemical and histopathological examinations revealed no values suggestive of any overt D-psicose treatment-related toxicity.

Previous testing found that the LD_{50} value of D-psicose administered orally to rats was 16 g/kg.[10] In the present study, rats actually ingested 1.67 g/kg body weight per day of D-psicose or 1.65 g/kg body weight per day of sucrose. The body weight gain and intra-abdominal adipose tissue weight in rats did not differ between the two dietary groups.

We previously reported that D-psicose supplements suppress

Table 1. Body weight, food intake and tissue size

Groups			Sucrose	D-Psicose
Initial weight		(g)	59 ± 4	59 ± 4
Final weight		(g)	343 ± 24	346 ± 29
Weight gain		(g)	284 ± 20	287 ± 27
Food intake		(g/day)	18.9 ± 0.6	19.3 ± 1.1
Tissue weights				
Brain		(g)	1.73 ± 0.21	1.77 ± 0.16
Heart		(g)	0.80 ± 0.05	0.79 ± 0.04
Lungs		(g)	0.99 ± 0.04	0.99 ± 0.07
Liver		(g)	8.98 ± 0.92	10.1 ± 1.26*
Pancreas		(g)	0.43 ± 0.07	0.43 ± 0.09
Kidneys		(g)	2.01 ± 0.13	2.30 ± 0.18*
Adrenals		(g)	0.05 ± 0.01	0.05 ± 0.01
Spleen		(g)	0.70 ± 0.08	0.69 ± 0.08
Testicles		(g)	2.98 ± 0.09	3.01 ± 0.16
Intra-adipose tissues[1]		(g)	21.0 ± 3.89	19.9 ± 2.53
Muscle tissues[2]		(g)	3.95 ± 0.25	3.88 ± 0.25
Intestinal tracts size				
Small intestine	weight	(g)	5.13 ± 0.60	5.21 ± 0.49
	length	(m)	1.02 ± 0.06	1.04 ± 0.04
Large intestine	weight	(g)	1.07 ± 0.14	1.19 ± 0.16
	length	($\times 10^{-2} \cdot$ m)	18.4 ± 1.97	19.6 ± 1.90
Cecum	content	(g)	3.80 ± 1.07	4.13 ± 0.57
	weight	(g)	0.75 ± 0.12	0.78 ± 0.10
	surface area	($\times 10^3 \cdot mm^2$)	3.60 ± 0.31	3.54 ± 0.15

Values are means ± SD for 10 rats. *Significant difference from the Sucrose 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.

Table 2. Serum chemical and blood hematological test results

Groups		Sucrose	D-Psicose
Serum			
TP	(g/100 ml)	6.01 ± 0.12	6.22 ± 0.22*
A/G		3.39 ± 0.39	3.37 ± 0.27
ALBU	(g/100 ml)	4.63 ± 0.13	4.79 ± 0.15*
GLO	(g/100 ml)	1.38 ± 0.13	1.43 ± 0.12
AST	(IU/l)	161 ± 29	167 ± 47
UA	(mg/100 ml)	1.06 ± 0.16	0.81 ± 0.07*
BUN	(mg/100 ml)	18.3 ± 3.6	20.5 ± 1.9
CREA	(mg/100 ml)	0.32 ± 0.02	0.30 ± 0.07
Ca	(mg/100 ml)	10.0 ± 0.1	10.3 ± 0.2
Fe	(μg/100 ml)	119 ± 19	107 ± 16
CHO	(mg/100 ml)	63.0 ± 11.8	65.1 ± 13.1
TG	(mg/100 ml)	42.2 ± 21.9	68.1 ± 28.0
GLU	(mg/100 ml)	142 ± 17	173 ± 18
FFA	(mEq/100 ml)	0.67 ± 0.15	0.69 ± 0.10
Blood			
WBC	($\times 10^2$/μl)	30.9 ± 5.4	44.4 ± 3.7*
RBC	($\times 10^4$/μl)	896 ± 27	936 ± 28*
Hb	(g/100 ml)	15.3 ± 0.3	15.3 ± 0.3
Ht	(%)	45.6 ± 0.8	45.0 ± 0.9
MCV	(fl)	51.0 ± 1.4	48.2 ± 0.8*
MCH	(pg)	17.1 ± 0.4	16.3 ± 0.2*
MCHC	(%)	33.5 ± 0.4	33.9 ± 0.4*
PLT	($\times 10^4$/μl)	64.7 ± 6.3	71.8 ± 6.3*

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

hepatic lipogenic enzyme activity and reduce intra-abdominal fat accumulation more effectively than D-glucose or D-fructose supplements in rats.[7,19] In addition, we found that D-psicose is a sweet monosaccharide that provides no energy to growing rats.[6] The present findings did not support our previous results. However, another our study demonstrated that rats fed a 3% D-psicose diet for 12 months did not differ in most of the parameters from rats fed 3% sucrose, which indicates that D-psicose did not inhibit rat growth.[13] Therefore, low dietary levels (3% of diet or less) of D-psicose over an extended period primarily affected fat accumulation, with either no effects or only minimal effects on other organs.

In a previous short-term toxicity test in rats, we showed that the feeding of diets with extremely high levels of D-psicose appeared to be harmful to the intestinal tract.[10,20] Moreover, we previously reported that the cecal weight, cecal surface area and cecal content weight increased with increases of D-psicose in the diet (above 10%).[10,20] 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 by intestinal microflora, producing short-chain fatty acids as soluble dietary fiber.[21,22] In this study, no adverse effects on the intestinal tract were seen when the rats were fed 3% D-psicose in the diet. Therefore, 3% D-psicose in the diet does not appear to be harmful to the intestinal tract.

Dietary D-psicose increased the weights of the animal's liver (12% lager than the sucrose group) and kidneys (14% lager). This finding agrees with our previous studies.[7,8,10,13] The serum level of AST is used as an index of hepatic damage. These values in this study did not differ between the D-psicose and sucrose groups. In addition, histopathological observations of the liver and kidneys revealed no abnormalities due to the ingestion of D-psicose. Liver enlargement occurs in animals and humans under a variety of conditions, with different consequences for health.[23] For example, it can be the result of a physiological adaptation to an enhanced workload or metabolic demand, metabolic abnormalities, toxicity, an inflammatory process, or due to proliferative disease.[24–26] Bar *et al.*[27] found that D-tagatose, another rare sugar, increased liver glycogen deposition and relative liver weights in non-fasting rats at dietary levels of 5–20%. 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 that D-psicose treatment induced an increase in glycogen deposition.[8] In addition, it was reported that D-psicose could increase the glucokinase activity by enhancing the translocation of glucokinase from the nucleus to the cytoplasm in the liver of diabetic rats.[9] However, it is unknown whether these mechanisms of liver enlargement induced by D-psicose and D-tagatose are the same.

Glycogen deposition and liver enlargement (increased relative

Table 3. Histopathological observations of liver, kidney and small intestine[1]

Groups		Sucrose	D-Psicose
Organs	Findings		
Liver	Bile duct proliferation	0.1 ± 0.3	0.0 ± 0.0
	Necrosis	0.4 ± 0.5	0.3 ± 0.5
	Microgranuloma	0.7 ± 0.5	1.0 ± 0.8
	Lipid deposition	0.3 ± 0.5	0.0 ± 0.0
	Fatty change	0.1 ± 0.3	0.0 ± 0.0
	Total score of damage	1.6 ± 1.2	1.3 ± 0.8
Kidney	Basophilic change in the tubule	1.1 ± 0.9	1.1 ± 1.0
	Hyaline cast in the tubule	0.0 ± 0.0	0.4 ± 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	0.4 ± 0.5	0.5 ± 0.7
	Total score of damage	1.5 ± 1.3	2.0 ± 1.5
Small intestine	Villous damage	1.1 ± 1.0	1.1 ± 1.0
	Crypt damage	0.1 ± 0.3	0.0 ± 0.0
	Cellular infiltration	0.8 ± 0.6	1.2 ± 1.0
	Goblet cell depletion	0.2 ± 0.6	0.0 ± 0.0
	Total score of damage	2.2 ± 2.3	2.3 ± 1.9

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

doi: 10.3164/jcbn.11-66

liver weights) were also seen in rats fed diets with high levels of various other accharides.[28–30] It was hypothesized that the formation of phosphorylated metabolites plays a crucial role in this process, either by activating glucokinase or by changing the intracellular concentrations of phosphate compounds.[9] It is possible that D-psicose, of which about 50% is absorbed,[21] is more active than D-fructose in promoting glycogen deposition. The slower degradation of psicose-1-phosphate may account for this phenomenon.

In the hematological analysis performed as part of this study, dietary D-psicose significantly increased the WBC, RBC and PLT values compared to sucrose, and significantly decreased the MCV and MCH values. However, these values remained within the normal rage, indicating that there was no overt D-psicose toxicity.

In conclusion, the present study evaluated the effects of 90-day 3% D-psicose administration to rats, and found that there were to be increases in the liver and kidney weights, with no gross pathological findings associated with this hypertrophy. The hematological and chemical values were not suggestive of any overt D-psicose toxicity. Overall, no adverse effects were seen at this low dose level of D-psicose in the diet.

Acknowledgments

We would like to thank Dr. Koji Kondo, Rare Sugar Production Thecnical Research Laboratories, LLC (Kagawa, Japan) for donating D-psicose. This work was supported by the Regional Innovation Creation R&D Programs, Ministry of Economy, Trade and Industry, Japan.

Abbreviations

A/G	ratio of albumin and globulin
ALBU	albumin
AST	aspartate aminotransferase
BUN	urea nitrogen
Ca	calcium
CHO	total cholesterol
CREA	creatinine
Fe	iron
FFA	free fatty acid
GLO	globulin
GLU	glucose
Hb	hemoglobin
HCT	hematocrit
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
PLT	platelet count
RBC	erythrocyte count
TG	triglycerides
TP	total protein
UA	uric acid
WBC	leukocyte

References

1 Cree GM, Perlin AS. O-isopropylidene derivatives of D-allulose (D-psicose) and D-erythro-hexopyranos-2,3-diulose. *Can J Biochem* 1968; **46**: 765–770.
2 Binkley WW. The fate of cane juice simple sugars during molasses formation. IV. Probable conversion of D-fructose to D-psicose. *Int Sugar J* 1963; **65**: 105–106.
3 Miller BS, Swain T. Chromatographic analyses of the free amino acids, organic acids and sugars in wheat plant extracts. *J Sci Food Agric* 1965; **11**: 344–348.
4 Hough L, Stacey BE. Variation in the allitol content of Itea plants during photosynthesis. *Phytochemistry* 1966; **5**: 171–175.
5 Eble TE, Hoeksema H, Boyack GA, Savage GM. Psicofuranine I. Discovery, isolation, and properties. *Antibio Chemother* 1959; **9**: 419–420.
6 Matsuo T, Suzuki H, Hashiguchi M, Izumori K. D-psicose is a rare sugar that provides no energy to growing rats. *J Nutr Sci Vitaminol (Tokyo)* 2002; **48**: 77–80.
7 Matsuo T, Baba Y, Hashiguchi M, Takeshita K, Izumori K, Suzuki H. Less body fat accumulation with D-psicose diet versus D-fructose diet. *J Clin Biochem Nutr* 2001; **30**: 55–65.
8 Matsuo T, Izumori K. Effects of dietary D-psicose on diurnal variation in plasma glucose and insulin concentrations of rats. *Biosci Biotechnol Biochem* 2006; **70**: 2081–2085.
9 Toyoda Y, Mori S, Umemura N, *et al*. Suppression of blood glucose levels by D-psicose in glucose tolerance test in diabetic rats. *Jpn Pharmacol Ther* 2010; **38**: 261–269.
10 Matsuo T, Tanaka T, Hashiguchi M, Izumori K, Suzuki H. Effects of oral acute administration and subchronic feeding of several levels of D-psicose in rats. *J Nutr Sci Vitaminol (Tokyo)* 2002; **48**: 512–516.
11 Oshima H, Kimura I, Izumori K. Synthesis and structure analysis of novel disaccharides containing D-psicose produced by endo-1,4-β-D-xylanase from Aspergillus sojae. *J Biosci Bioeng* 2006; **101**: 280–283.
12 Oshima H, Kimura I, Izumori K. Psicose contents in various food products and its origin. *Food Sci Technol Res* 2006; **12**: 137–143.
13 Yagi K, Matsuo T. The study on long-term toxicity of D-psicose in rats. *J Clin Biochem Nutr* 2009; **45**: 271–277.
14 Suzuki M, Tamura T. Sweeteners: low-energetic and low-insulinogenic. In: Bray GA, ed. *Diet and Obesity*, Basel: Karger, 1988; 163–173.
15 Boesch C, Ith M, Jung B, *et al*. Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers. *Regul Toxicol Pharmacol* 2001; **33**: 257–267.
16 Suzuki M, Nishiyama K, Ohmori S, Yoshioka M, Shimomura Y. Effects of dietary palatinose and reduced-palatinose on body energy composition. *J Clin Biochem Nutr* 1992; **13**: 117–125.
17 Goldsmith LA. Acute and subchronic toxicity of sucralose. *Food Chem Toxicol* 2000; **38**: S53–S69.
18 Mann SW, Yuschak MM, Amyes SJ, Aughton P, Finn JP. A combined chronic toxicity/carcinogenicity study of sucralose in Sprague-Dawley rats. *Food Chem Toxicol* 2000; **38**: S71–S89.
19 Matsuo T, Baba Y, Hashiguchi M, Takeshita K, Izumori K, Suzuki H. Dietary D-psicose, a C-3 epimer of D-fructose, suppresses the activity of hepatic lipogenic enzymes in rats. *Asia Pac J Clin Nutr* 2001; **10**: 233–237.
20 Matsuo T, Tanaka T, Hashiguchi M, Izumori K, Suzuki H. Metabolic effects of D-psicose in rats: studies on faecal and urinary excretion and caecal fermentation. *Asia Pac J Clin Nutr* 2003; **12**: 225–231.
21 Whistler RL, Singh PP, Lake WC. D-Psicose metabolism in the rat. *Carbohydr Res* 1974; **34**: 200–202.
22 Matsuo T, Izumori K. Effects of supplemental D-psicose on glucose tolerance and serum adipocytokine levels in rats fed a high-fat diet or a low-fat diet. *J Oleo Sci* 2004; **53**: 453–460.
23 Walker WA, Mathis RK. Hepatomegaly: an approach to differential diagnosis. *Pediatr Clin North Am* 1975; **22**: 929–942.
24 Goldberg L. Liver enlargement produced by drugs: its significance. *Proc Eur Soc Study Drug Toxicity* 1966; **7**: 171–184.
25 Fukuhara M, Takabatake E. Comparative study of liver enlargement induced by various chemicals. *J Pharm Dyn* 1978; **1**: 153–159.
26 Grasso P. Liver growth and tumorigenesis in rats. *Arch Toxicol Suppl* 1979; **2**: 171–180.
27 Bär A, Lina BA, de Groot DM, de Bie B, Appel MJ. Effect of D-tagatose on liver weight and glycogen content of rats. *Regul Toxicol Pharmacol* 1999; **29**: S11–S28.
28 Goldblatt PJ, Witschi H, Freidman MA, Sullivan RJ, Shull KH. Some structural and functional consequences of hepatic adenosine triphosphate deficiency induced by intraperitoneal D-fructose administration. *Lab Invest* 1970; **23**: 378–385.
29 Yu DT, Burch HB, Phillips MJ. Pathogenesis of fructose hepatotoxicity. *Lab Invest* 1974; **30**: 85–92.
30 Yu DT, Phillips MJ. Hepatic ultrastructural changes in acute fructose overload. *J Ultrastruct Res* 1971; **36**: 222–236.