Food and Chemical Toxicology 131 (2019) 110548

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

A study of D-allulose-associated reproductive toxicity in rats

Hyejung Kim[a], Chongjin Park[a], Soonok Sa[a], Iris Case[b], Chunmei Li[c,*], Yonglin Gao[d], Hongbo Wang[c], Jingwei Tian[c]

[a] Samyang Corp., 295 Pangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, 13488, South Korea
[b] NutraSource, Inc., Maryland, 21029, USA
[c] School of Pharmacy, Key Laboratory of Molecular Pharmacology and Drug Evaluation (Yantai University), Ministry of Education, Collaborative Innovation Center of Advanced Drug Delivery System and Biotech Drugs in Universities of Shandong, Yantai University, Yantai, 264005, PR China
[d] School of Life Science, Yantai University, Yantai, 264005, PR China

ARTICLE INFO

Keywords:
D-allulose
One-generation reproductive toxicity
Rat

ABSTRACT

In this study, we assessed whether D-allulose was associated with reproductive toxicity in rats, assessing reproduction and offspring growth following gavage of parents with 0, 500, 1000, and 2000 mg/kg of this compound. Specifically, female rats were continuously dose from 2 weeks prior to mating until day 21 of lactation, while males were dose for the 10 weeks before mating. We did not observe any direct toxicity or mortality upon D-allulose administration, with no changes in body weight or eating behavior between study and control groups. We also did not observe any significant alterations in precoital time, copulation index, fertility index (male), or pregnancy index (male) between groups. Relative to controls, there was also no effect of D-allulose treatment on pregnancy rates, implantation, pregnancy length, gender ratios, viability indexes, lactation indexes, prenatal death rates, or number of live young at time of birth. Organ weights and indexes were also comparable between groups at time of sacrifice, and treatment was not linked to any obvious manifestations upon necropsy or histopathological examination. In the F1 offspring, the body weights of pups born to parents administered D-allulose (2000, 1000, and 500 mg/kg) were slightly higher on days 1–9 postnatally relative to controls ($p < 0.05$), however after day 9 these effects were no longer evident. Together, these results indicate a no-observed-adverse-effect level (NOAEL) of D-allulose of 2000 mg/kg, the highest dose tested, in parental animals and their offspring.

1. Introduction

D-allulose, is a rare sugar that is not commonly found in nature and which is a C3 epimer of D-fructose. The United States department of agriculture (USDA) has deemed D-allulose to be generally recognized as safe (GRAS) (GRN 693, FDA, 2017), with several reports indicating an impact of D-allulose on the metabolism of lipids (Choi et al., 2018; Nagata et al., 2018). D-allulose can increase liver glucose uptake and suppress the activity of lipid-generating enzymes within the liver (Nagata et al., 2015), and it can further both decrease food intake and increase energy usage and activity of soleus muscle lipoprotein lipase in rats on a high-sucrose diet (Ochiai et al., 2014). Work in mice indicates that D-allulose further reduces the absorption of dietary fat absorption *via* the small intestine, and increases fat tissue oxidation upon high-fat diet feeding (Han et al., 2016). Many researchers using animal models have found D-allulose to be linked with decreases in overall fat mass, body weight, and/or energy intake (Matsuo et al., 2001). Preliminary work in a clinical setting has similarly observed reductions in body fat mass in those patients that are obese or overweight upon D-allulose administration (Han et al., 2018). Even in healthy humans, D-allulose can increase rates of postprandial fat oxidation, highlighting its potential as a possible novel sweetening agent useful for achieving and maintaining a healthy weight based on enhancements to overall rates of energy metabolism (Hossain et al., 2015).

Toxicological evaluation plays a very important role in the research and sue of substance (Li et al., 2017; Gao et al., 2014). Previous studies have indicated that long-term administration of D-allulose caused no harmful effects in dogs up to 0.2 g/kg (Nishii et al., 2017). In acute toxicity, some dogs (1/6) experienced vomiting, and others (5/6) showed transient diarrhea after single treatment 4 g/kg of D-allulose (Nishiiet al., 2016). The present study aimed to assess the reproductive toxicity linked with D-allulose based on a one-generation assessment of reproductive outcomes in rats, in compliance with OECD Test Guideline 415 (OECD, 1983). In total, we administered D-allulose by gavage to 24

* Corresponding author.
E-mail address: gylbill@163.com (C. Li).

https://doi.org/10.1016/j.fct.2019.05.056
Received 28 January 2019; Received in revised form 28 May 2019; Accepted 29 May 2019
Available online 30 May 2019
0278-6915/

male and 48 female rats per group (0, 500, 1000, and 2000 mg/kg/day. These dosages, if extrapolated on a body weight basis to humans, correspond to 5, 10, 20 g/person/day, respectively), with our results indicating a no-observed-adverse-effect level (NOAEL) for D-allulose of 2000 mg/kg for both parental animals and their offspring.

2. Materials and methods

2.1. Chemicals

D-allulose derived from a non-genetically modified *Microbacterium foilorum* was provided by Samyang Corp. (Republic of Korea).

2.2. Animals and treatment

2.2.1. F0 animals

Prior to mating, males were dosed with D-allulose for a total of 10 weeks, while females were dosed from 2 weeks before mating until day 21 of lactation. Mating was conducted with a ratio of 1 male to 2 females for a 14-day mating period, with successful mating detected either based on vaginal plug detection or identification of sperm upon a vaginal smear, with both protocols being conducted daily. The day a successful mating event was identified was denotes as gestation day (GD) 0 of the pregnancy period, and males and females were then separated and individually housed.

If after the 14-day period a female had not undergone mating, female was re-mated with a male of proven fertility in the same exposure group. A continued lack of successful mating led to separation of these females who were then sacrificed 21 days later and subjected to necropsy. Rats were able to give birth to and rear their own F1 offspring until lactation day (LD) 21. Normal maternal clinical signs (i.e., changes in eyes, mucous membranes, loss of fur or scabbing) were observed in all groups. In addition, we calculated copulation, fertility, and pregnancy indices, as well as the precoital interval, and duration of pregnancy. Male rats were euthanized by carbon dioxide inhalation after the mating period (Guo et al., 2018), while females who gave birth were euthanized following weaning.

Females who did not deliver were euthanized on day 3 after the final expected parturition date. Upon euthanasia, all rats underwent a complete gross necropsy, with special attention paid to reproductive tissues. We also preserved organs for microscopy including the ovaries, uterus, cervix, vagina, testes, epididymides, seminal vesicles, prostate, coagulating gland, and pituitary gland (Li et al., 2016). Briefly, the organs/tissues were fixed in formalin (10%) and embedded in paraffin, and were sectioned following hematoxylin eosin (H&E) staining. Then, the histological sections were examined by the study pathologist. If test substance related pathological changes were note in some organs/tissues, the middle dosage group was checked until no abnormity was found (Li et al., 2014, 2018a).

2.2.2. F1 animals

The day upon which parturition was completed was deemed postnatal day (PND) 0. On this day, pups were examined and number of liver and stillborn pups were assessed. Live pups further underwent counting, sexing, weighing, and external examinations. Pups were then monitored daily, with numbers of live and dead pups recorded on PNDs 4, 7, 14, and 21. Extra pups present on PND 4 were eliminated at random to reduce litter sizes to 4 males and 4 females when possible, with the goal of avoiding any influences of litter size on study outcomes. Deceased pups were assessed for any evident structural anomalies or pathological findings. On PNDs 0, 3, 6, 9, 12, 15, 18, and 21, body weights of F1 animals were measured and physical findings were assessed for 1 random male and female in each litter.

We additionally assessed behavior in 1 random male and female pup from each litter, measuring the righting reflex on PND 2 by calculating the time it takes the pup to right itself following placement on a flat surface with the dorsal side down (Palanza et al., 2001). If subjects failed to right after 2 min, the test was terminated. On PND 4, cliff avoidance was assessed by positioning animals on the edge of a platform with their forelimbs and snout positioned such that the eyes were directly over the platform edge (Palanza et al., 2001). On PND 10, negative geotaxis was assessed *via* placing an animal head down on a board positioned at a 45-degree angle, and measuring the time needed for the animal to turn 180° up to a maximum of 2 min (Cada et al., 2000). On PND 21, a rotarod test was performed by assessing how long it took animals to fall off of a 6-cm-diameter rod spinning at 15 rpm (Meyer et al., 1979).

2.3. Statistical analysis

All data are given as mean ± standard deviation. Non-parametric data were tested *via* Kruskal-Wallis analysis of variance (ANOVA) followed by a Mann-Whitney *U* test where appropriate, while a one-way ANOVA was used to test parametric data. When significant differences were detected, a multiple comparisons test was performed based on the Dunnett method. Incidence data, including clinical signs and histopathologic findings, were compared by *via* Fisher's exact probability test. Significance is indicated as: $p < 0.05$ (*).

3. Results

3.1. Clinical observations

We observed no evidence of toxicity or mortality linked with D-allulose at any time in this study, with no significant differences in body weights in rats of any sex or at any time relative to the mating period (Figs. 1–4). Male body weights did trend slight lower in the 2000 and 1000 mg/kg groups, but these differences were not significant ($p > 0.05$). There were also no differences in food consumption between treatment groups (data not shown).

3.2. Fertility data results

Copulation index, fertility index (male), and pregnancy index (male) did not differ significantly relative to control at any D-allulose dose (Table 1). Precoital time was slightly shorter in the D-allulose groups (2000, 1000, and 500 mg/kg), but these differences were not statistically significant ($p > 0.05$).

3.3. Reproductive and litter outcomes

As shown in Table 2, we did not detect any abnormalities in

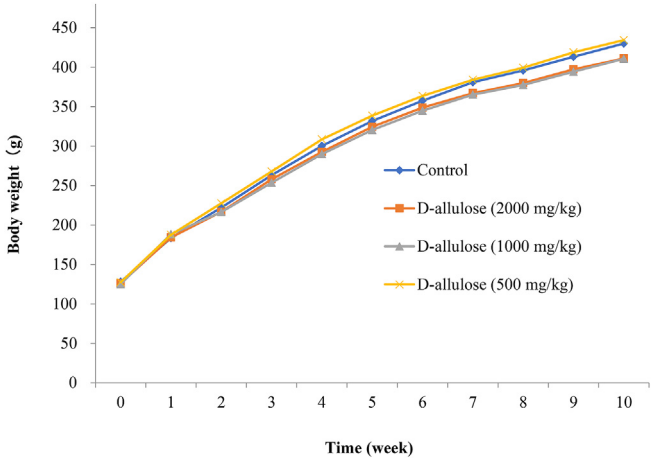

Fig. 1. Mean body weights for male rats.

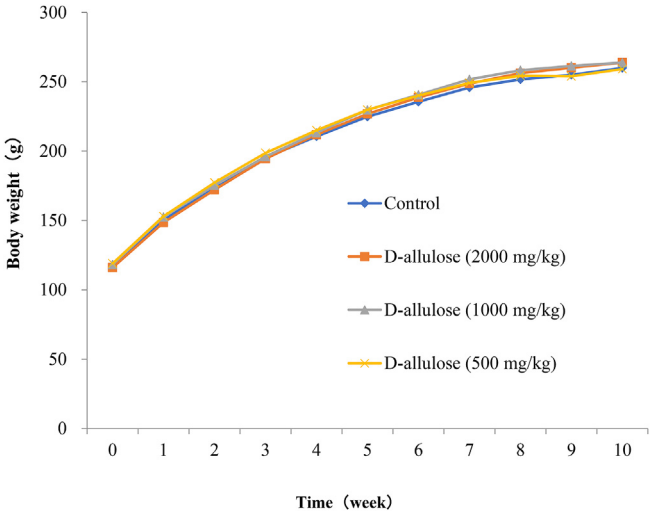

Fig. 2. Mean body weights for female rats (Pre-mating). Animals were treated with D-allulose during 9–10 week.

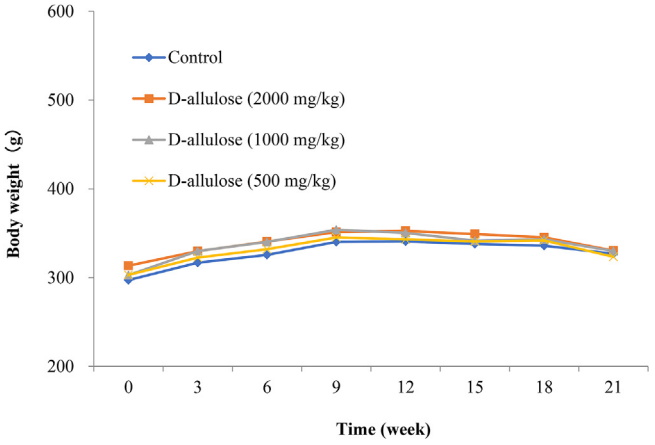

Fig. 3. Mean body weights for female rats (Lactation).

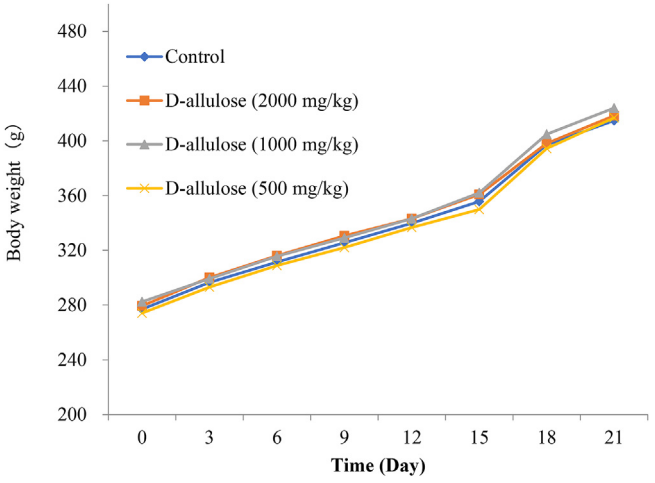

Fig. 4. Mean body weights for female rats (Gestation).

newborn pups. We also observed no significant differences in numbers of pregnant females, corpora lutea, and implantations. No significant differences were found in the pregnancy period (day), prenatal death, gender ratio, viability index, lactation index, prenatal death rates, and number of live young at birth.

3.4. Necropsy and histopathology findings

Tables 3 and 4 show the terminal organ weights and indexes for study animals, with no evidence of significant differences between groups. While there was a significant difference was found in testes coefficient (relative organ weight) in the 500 mg/kg D-allulose group ($p < 0.05$). However, this change was only found in low dose animals, and there was no clear dose-response relationship, and the determined value was within the normal range observed in our facility. This finding was therefore not believed to be a toxicological effect. In gross necropsy examinations, we have checked all organs/tissues above mentioned in all test substance treatment group, and no abnormities were found. On histopathological investigations, no substance-related changes in all samples in high dose group (2000 mg/kg D-allulose) when compared with control group. Taken together, D-allulose did not induced any obvious reproductive organs toxicity under our test conditions.

3.5. F1 findings

Pup body weights in the D-allulose groups (2000, 1000, and 500 mg/kg) were slightly increased on PNDs 1–9 ($p < 0.05$) relative to control (Fig. 5). However, these changes were no longer evident after PND 9. Physical development and behavioral functions were not altered in response to D-allulose treatment (data not shown).

4. Discussion

D-Allulose is a unique form of bioactive monosaccharide with desirable effects on human healthy. Recent studies have ascribed many functions to D-allulose, with evidence it can combat hyperglycemia and obesity (Hossain et al., 2015; Iwasaki et al., 2018; Shintani et al., 2017). Herein, we assessed any potential adverse effects of D-allulose on reproduction in male and female rats, monitoring key reproductive parameters including mating behavior, conception, parturition, lactation, and weaning in SD rats.

Adverse outcomes of food or chemical-related toxicity often manifest as differences in body weight or food intake (Gao et al., 2013, 2017b). Here, we did observe a trend towards lower body weight in D-allulose treated groups (2000 and 1000 mg/kg), and this result maybe the enlargement of test substance activity. The outcome was consistent with previous findings regarding the anti-obesity effects of this compound. Indeed, some studies have found that subchronic ingestion of D-allulose in an obese mouse model system can effectively combat obesity and obesity-related disorders (Itoh et al., 2015). While we only observed differences in weight in the male rats, this is likely due to the shorter treatment time used for female rats.

Our findings did not detect significant effects of D-allulose treatment of pre-mating, mating, gestation, or lactation parameters. Although a slight decrease in precoital time in D-allulose treated rats (2000, 1000, and 500 mg/kg), this was believed to be incidental as this value was within historical control values in the laboratory. There were no significant differences in fertility data, or in reproductive and litter findings. Increases in relative testes weights were observed in the D-allulose 500 mg/kg group, but as this effect was not dose-dependent and no apparent changes in histology were observed, this was not believed to be due to toxicity.

Among F1 offspring, the number of corpora lutea and implantations, percent of prenatal death and live young, sex ratio, viability index, lactation index and pup abnormalities were unaffected by D-allulose treatment. No malformed pups were evident in any group. There was a slight increase in pup body weight on PNDs 1–9 in the D-allulose groups (2000, 1000, and 500 mg/kg) relative to controls. However, these changes were small, with no obvious dose dependence. While decreased body weights are often a sign of toxicity, increased body weights are not generally of toxicological significance (Gao et al., 2017a; Li et al., 2018b). We also conducted behavioral assessments of F1 offspring,

Table 1
Fertility data of parent animals treated with D-allulose during premating periods.

Items	Control	2000 mg/kg WB	1000 mg/kg WB	500 mg/kg WB
Male				
No. of males mated	12	12	12	12
Copulation index[a] (%)	12/12 (100)	12/12 (100)	12/12 (100)	12/12 (100)
Fertility index[b] (%)	23/25 (92)	22/25 (88)	23/25 (92)	22/25 (88)
Female				
No. of females mated	25	25	25	25
Copulation index[a] (%)	25/25 (100)	25/25 (100)	25/25 (100)	25/25 (100)
Pregnancy index[c] (%)	23/25 (92)	22/25 (88)	23/25 (92)	22/25 (88)
Precoital time (day)	4.0 ± 3.7	2.3 ± 1.9	3.3 ± 3.3	2.5 ± 1.5

[a] Number of animals with successful copulation/number of mated animals.
[b] Number of impregnating animals/number of animals with successful copulation.
[c] Number of pregnant animals/number of animals with successful copulation.

Table 2
Reproductive and littering findings of dams treated with D-allulose during premating, gestation, and lactation periods.

Items	Control	2000 mg/kg WB	1000 mg/kg WB	500 mg/kg WB
No. of pregnant females	23	22	23	22
No. of corpora lutea	17.3 ± 1.8	16.0 ± 2.7	18.0 ± 2.2	17.6 ± 3.0
No. of implantations	14.3 ± 1.9	12.7 ± 3.8	14.0 ± 2.0	12.5 ± 2.7
Pregnancy period (day)	21.7 ± 0.6	21.8 ± 0.5	21.7 ± 0.6	21.6 ± 0.6
Percent of prenatal death	1.4 ± 4.1	4.1 ± 7.1	2.7 ± 4.8	1.0 ± 2.6
Female/Male	0.9 ± 0.4	1.1 ± 1.0	1.1 ± 0.7	0.9 ± 0.6
Viability index (%)[a]	99.7 ± 1.2	99.7 ± 1.6	100	100
Lactation index (%)[b]	100	100	100	100
No. of neonates with external anomalies	0	0	0	0
Percent of live young at birth	98.6 ± 4.1	95.9 ± 7.1	97.3 ± 4.8	99.0 ± 2.6

[a] Viability index = (no. of live pups at day 4/no. of live pups at birth) × 100.
[b] Lactation index = (no. of live pups at day 21/no. of live pups after litter-size control) × 100.

Table 3
Organ weights of male rats treated with D-allulose during premating periods.

Parameter	Control	2000 mg/kg WB	1000 mg/kg WB	500 mg/kg WB
Mean weight (g)				
Liver	11.34 ± 1.68	10.78 ± 1.56	11.10 ± 1.40	10.96 ± 1.23
Kidneys	2.90 ± 0.48	2.79 ± 0.35	2.83 ± 0.35	2.97 ± 0.37
Spleen	0.77 ± 0.20	0.73 ± 0.10	0.78 ± 0.13	0.76 ± 0.11
Heart	1.36 ± 0.17	1.44 ± 0.21	1.49 ± 0.18	1.50 ± 0.12
Lung	1.72 ± 0.33	1.70 ± 0.28	1.66 ± 0.19	1.74 ± 0.27
Testes	3.80 ± 0.46	3.26 ± 0.72	3.65 ± 0.46	3.59 ± 0.39
Epididymides	1.43 ± 0.25	1.39 ± 0.27	1.46 ± 0.18	1.39 ± 0.26
Prostate	1.32 ± 0.40	1.16 ± 0.39	1.07 ± 0.37	1.44 ± 0.81
Seminal vesicle	1.42 ± 0.25	1.47 ± 0.26	1.57 ± 0.36	1.51 ± 0.29
Pituitary	0.0113 ± 0.0017	0.0109 ± 0.0022	0.0112 ± 0.0022	0.0113 ± 0.0023
Mean organ-to-terminal body weight ratios				
Liver	2.50 ± 0.20	2.46 ± 0.22	2.50 ± 0.15	2.36 ± 0.19
Kidneys	0.64 ± 0.06	0.64 ± 0.05	0.64 ± 0.05	0.64 ± 0.05
Spleen	0.17 ± 0.05	0.17 ± 0.02	0.18 ± 0.02	0.16 ± 0.02
Heart	0.30 ± 0.03	0.33 ± 0.03	0.34 ± 0.04	0.33 ± 0.03
Lung	0.38 ± 0.07	0.39 ± 0.05	0.38 ± 0.04	0.37 ± 0.04
Testes	0.84 ± 0.06	0.75 ± 0.15	0.82 ± 0.09	0.77 ± 0.06*
Epididymides	0.32 ± 0.04	0.32 ± 0.07	0.33 ± 0.02	0.30 ± 0.05
Prostate	0.30 ± 0.10	0.27 ± 0.09	0.24 ± 0.09	0.32 ± 0.20
Seminal vesicle	0.32 ± 0.05	0.34 ± 0.06	0.36 ± 0.08	0.33 ± 0.06
Pituitary	0.0025 ± 0.0004	0.0025 ± 0.0005	0.0025 ± 0.0005	0.0024 ± 0.0004

Values were means ± SD.
*Indicates a significant difference at the $p < 0.05$ level, when compared with the control group.

detecting and no treatment-related effects on behavioral development in these rats.

As we detected no adverse developmental or reproductive outcomes, the NOAEL for D-allulose is at least 2000 mg/kg, which was the maximum dose in the present study. Administration of 2000 mg/kg does thus not affect the reproductive abilities of parental rats, or the development of F1 offspring of these rats.

Conflicts of interest

None declared.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

Table 4
Organ weights of female rats treated with D-allulose.

Parameter	Control	2000 mg/kg WB	1000 mg/kg WB	500 mg/kg WB
Mean weight (g)				
Heart	1.08 ± 0.10	1.05 ± 0.12	1.07 ± 0.10	1.05 ± 0.12
Liver	7.51 ± 0.82	7.56 ± 0.86	7.62 ± 0.89	7.16 ± 0.65
Spleen	0.51 ± 0.05	0.54 ± 0.06	0.53 ± 0.07	0.48 ± 0.08
Lung	1.33 ± 0.14	1.31 ± 0.14	1.32 ± 0.18	1.28 ± 0.21
Kidneys	1.83 ± 0.20	1.87 ± 0.21	1.87 ± 0.21	1.80 ± 0.17
Uterus	0.50 ± 0.12	0.52 ± 0.17	0.47 ± 0.09	0.48 ± 0.13
Ovary	0.16 ± 0.03	0.16 ± 0.05	0.16 ± 0.02	0.16 ± 0.02
Pituitary gland	0.0109 ± 0.0038	0.0105 ± 0.0031	0.0101 ± 0.0038	0.0090 ± 0.0036
Mean organ-to-terminal body weight ratios				
Heart	0.33 ± 0.03	0.32 ± 0.02	0.32 ± 0.03	0.33 ± 0.05
Liver	2.30 ± 0.18	2.29 ± 0.19	2.31 ± 0.24	2.22 ± 0.15
Spleen	0.16 ± 0.02	0.16 ± 0.02	0.16 ± 0.02	0.15 ± 0.02
Lung	0.41 ± 0.05	0.40 ± 0.05	0.40 ± 0.05	0.40 ± 0.07
Kidneys	0.56 ± 0.05	0.57 ± 0.05	0.57 ± 0.05	0.56 ± 0.05
Uterus	0.15 ± 0.04	0.16 ± 0.05	0.14 ± 0.03	0.15 ± 0.04
Ovary	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Pituitary gland	0.0033 ± 0.0011	0.0032 ± 0.0008	0.0030 ± 0.0010	0.0028 ± 0.0011

Values were means ± SD.

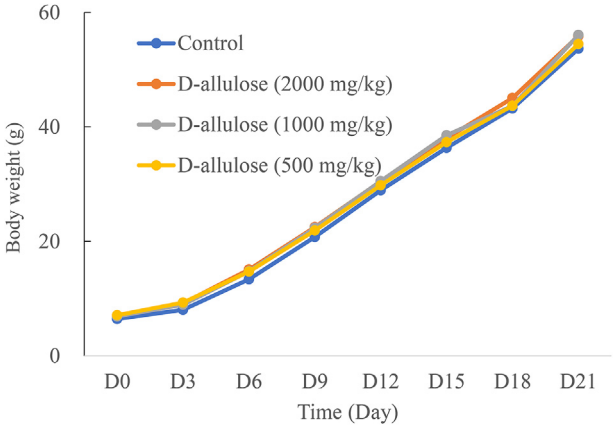

Fig. 5. Mean body weights for neonatal rats (Lactation).

influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.056.

References

Cada, A.M., Gray, E.P., Ferguson, S.A., 2000. Minimal behavioral effects from developmental cerebellar stunting in young rats induced by postnatal treatment with alpha-difluoromethylornithine. Neurotoxicol. Teratol. 22, 415–420.

Choi, B.R., Kwon, E.Y., Kim, H.J., Choi, M.S., 2018. Role of synbiotics containing d-Allulose in the alteration of body fat and hepatic lipids in diet-induced obese mice. Nutrients 10 (11), E1797. https://doi.org/10.3390/nu10111797. pii.

FDA, 2017. GRN 693. D-Psicose, submitted by Samyang Corp. Available at. https://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices&id=693&sort=GRN_No&order=DESC&startrow=1&type=basic&search=693.

Gao, Y., Li, C., Kang, L., Hang, B., Yan, M., Li, S., Jin, H., Lee, A.W., Cho, S.S., 2014. A subchronic toxicity study, preceded by an in utero exposure phase, with refined arachidonic acid-rich oil (RAO) derived from Mortierella alpina XM027 in rats. Regul. Toxicol. Pharmacol. 70, 696–703.

Gao, Y., Li, C., Wang, Y., Liu, Y., Li, G., Fan, X., Li, Y., Tian, J., Lee, A.W., 2017b. Nonclinical safety of astilbin: a 4-week oral toxicity study in rats with genotoxicity, chromosomal aberration, and mammalian micronucleus tests. Food Chem. Toxicol. 107 (Pt A), 1–9.

Gao, Y., Shen, J., Yin, J., Li, C., Fu, C., Cho, S., 2013. A subchronic dietary toxicity study of rice hull fiber in rats. Food Chem. Toxicol. 51, 137–142.

Gao, Y., Wang, Y., Wang, K., Zhu, J., Li, G., Tian, J., Li, C., Wang, Z., Li, J., Lee, A.W., Guo, C., 2017a. Acute and a 28-day repeated-dose toxicity study of total flavonoids from Clinopodium chinense (Benth.) O. Ktze in mice and rats. Regul. Toxicol. Pharmacol. 91, 117–123.

Guo, W., Gao, Y., Jiang, W., Li, C., Lin, F., Zhu, H., Wang, H., Ye, L., Qi, J.G., Cen, X., Tian, J., 2018. Toxicity effects of a novel potent triple reuptake inhibitor, LPM570065, on the fertility and early embryonic development in Sprague-Dawley rats. Regul. Toxicol. Pharmacol. 100, 45–51.

Han, Y., Han, H.J., Kim, A., Choi, J., Cho, S., Park, Y.B., Jung, U.J., Choi, M., 2016. D-allulose supplementation normalized the body weight and fat-pad mass in diet-induced obese mice via the regulation of lipid metabolism under isocaloric fed condition. Mol. Nutr. Food Res. 60, 1695–1706.

Han, Y., Kwon, E.Y., Yu, M.K., Lee, S.J., Kim, H.J., Kim, S.B., Kim, Y.H., Choi, M.S., 2018. A preliminary study for evaluating the dose-dependent effect of d-Allulose for fat mass reduction in adult humans: a randomized, double-blind, placebo-controlled trial. Nutrients 10 (2), E160 pii.

Hossain, A., Yamaguchi, F., Matsuo, T., Tsukamoto, I., Toyoda, Y., Ogawa, M., Nagata, Y., Tokuda, M., 2015. Rare sugar D-allulose: potential role and therapeutic monitoring in maintaining obesity and type 2 diabetes mellitus. Pharmacol. Ther. 155, 49–59.

Itoh, K., Mizuno, S., Hama, S., Oshima, W., Kawamata, M., Hossain, A., Ishihara, Y., Tokuda, M., 2015. Beneficial effects of supplementation of the rare sugar "D-allulose" against hepatic steatosis and severe obesity in lep (ob)/lep (ob) mice. J. Food Sci. 80 (7), H1619–H1626.

Iwasaki, Y., Sendo, M., Dezaki, K., Hira, T., Sato, T., Nakata, M., Goswami, C., Aoki, R., Arai, T., Kumari, P., Hayakawa, M., Masuda, C., Okada, T., Hara, H., Drucker, D.J., Yamada, Y., Tokuda, M., Yada, T., 2018. GLP-1 release and vagal afferent activation mediate the beneficial metabolic and chronotherapeutic effects of D-allulose. Nat. Commun. 9 (1), 113.

Li, C., Gao, Y., Wang, Y., Li, G., Fan, X., Li, Y., Guo, C., Tao, J., 2017. Genotoxicity testing of sodium formononetin-3'-sulphonate (Sul-F) by assessing bacterial reverse mutation, chromosomal aberrations and micronucleus tests. Regul. Toxicol. Pharmacol. 86, 374–378.

Li, C., Jiang, W., Gao, Y., Lin, F., Zhu, H., Wang, H., Ye, L., Qi, J.G., Tian, J., 2018b. Acute, subchronic oral toxicity, and genotoxicity evaluations of LPM570065, a new potent triple reuptake inhibitor. Regul. Toxicol. Pharmacol. 98, 129–139.

Li, C., Li, G., Gao, Y., Sun, C., Wang, X., 2016. A 90-day subchronic toxicity study with sodium formononetin-3'-sulphonate (Sul-F) delivered to dogs via intravenous administration. Regul. Toxicol. Pharmacol. 77, 87–92.

Li, C., Wang, Z., Li, G., Wang, Z., Yang, J., Li, Y., Wang, H., Jin, H., Qiao, J., Wang, H., Tian, J., Lee, A.W., Gao, Y., 2018a. Acute and repeated dose 26-week oral toxicity study of 20(S)-ginsenoside Rg3 in Kunming mice and Sprague-Dawley rats. J. Ginseng Res. https://doi.org/10.1016/j.jgr.2018.10.001.

Li, C., He, J., Gao, Y., Xing, Y., Hou, J., Tian, J., 2014. Preventive effect of total flavones of Choerospondias axillaries on ischemia/reperfusion-induced myocardial infarction-related MAPK signaling pathway. Cardiovasc. Toxicol. 14, 145–152.

Matsuo, T., Baba, Y., Hashiguchi, M., Takeshita, K., Izumori, K., Suzuki, H., 2001. Dietary d-psicose, a C-3 epimer of d-fructose, suppresses the activity of hepatic lipogenic enzymes in rats. Asia Pac. J. Clin. Nutr. 10, 233–237.

Meyer, D.A., Tilson, H.A., Byrd, W.C., Riley, M.T., 1979. A method of the routine assessment of fore- and hindlimb grip strength of rats and mice. Neurobehav. Toxicol. 1, 233–236.

Nagata, Y., Kanasaki, A., Tamaru, S., Tanaka, K., 2015. D-Psicose,an epimer of d-Fructose, favorably alters lipid metabolism in Sprague-Dawley Rats. J. Agric. Food Chem. 63, 3168–3176.

Nagata, Y., Mizuta, N., Kanasaki, A., Tanaka, K., 2018. Rare sugars, d-allulose, d-tagatose and d-sorbose, differently modulate lipid metabolism in rats. J. Sci. Food Agric. 98 (5), 2020–2026.

Nishii, N., Nomizo, T., Takashima, S., Matsubara, T., Tokuda, M., Kitagawa, H., 2016. Single oral dose safety of D-allulose in dogs. J. Vet. Med. Sci. 78 (6), 1079–1083.

Nishii, N., Takashima, S., Kobatake, Y., Tokuda, M., Kitagawa, H., 2017. The long-term safety of D-allulose administration in healthy dogs. J. Vet. Med. Sci. 79 (11), 1780–1784.
Ochiai, M., Onishi, K., Yamada, T., Iida, T., Matsuo, T., 2014. D-Psicose increases energy expenditure and decreases body fat accumulation in rats fed a high-sucrose diet. Int. J. Food Sci. Nutr. 65, 245–250.
OECD (Organisation for Economic Cooperation and Development), 1983. Guidelines for Testing of Chemicals, No. 415 "One-Generation Reproduction Toxicity Study", Adopted. pp. 1–8.
Palanza, P., Parmigiani, S., vom Saal, F.S., 2001. Effects of prenatal exposure to low doses of diethylstilbestrol, o,p'DDT, and methoxychlor on postnatal growth and neurobehavioral development in male and female mice. Horm. Behav. 40, 252–265.
Shintani, T., Sakoguchi, H., Yoshihara, A., Izumori, K., Sato, M., 2017. d-Allulose, a stereoisomer of d-fructose, extends Caenorhabditis elegans lifespan through a dietary restriction mechanism: a new candidate dietary restriction mimetic. Biochem. Biophys. Res. Commun. 493 (4), 1528–1533.