Maejo Int. J. Sci. Technol. **2022**, *16*(02), 170-178

Maejo International Journal of Science and Technology

ISSN 1905-7873
Available online at www.mijst.mju.ac.th

Full Paper

A Study on single-dose oral toxicity of D-allose in ICR mice

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Received: 20 May 2022 / Accepted: 15 August 2022 / Published: 24 August 2022

Abstract: D-Allose is a rare natural sugar, which is attractive as a sweetener alternative to sugar because of its biological functions and low-calorie content. However, there is a lack of basic information regarding the use of D-allose as a food ingredient. In this study D-allose toxicity to ICR mice was investigated. The mice were treated with a single dose of D-allose (1,250, 2,500 or 5,000 mg/kg body weight). No organ injury was observed, nor any changes observed in the clinical sign, body weight and the blood serum chemistry. The approximate lethal dose was estimated to exceed 5,000 mg/kg in mice. These results suggest that D-allose is practically non-toxic to mice.

Keywords: D-allose, single-dose oral toxicity, ICR mice

INTRODUCTION

Over the past 40 years, overweight and obesity have rapidly increased. By 2030, more than 2.16 billion people will be overweight and 1.12 billion people will be obese [1, 2]. The World Health Organisation has identified excessive sugar intake as a cause of overweight and obesity and has issued a guideline for reducing sugar intake to less than 10% of total energy intake to prevent obesity in adults and children [3]. International government and regulatory agencies including the US Food and Drug Administration, European Food Safety Authority, Health Canada, and Food Standards Australia and New Zealand recommend consumption of sweeteners that are safe for the human body and low in calories, instead of sugar. Accordingly, the global market for artificial sweeteners such as saccharin, aspartame, acesulfame potassium, sucralose and neotame, and natural sweeteners such as stevia, erythritol, xylitol and allulose, has been rapidly growing [4-7].

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D-Allose, a C-3 epimer of D-glucose and an isomer of D-allulose, is a rare natural sugar [8]. It has several physiological functions including anti-inflammatory [9, 10] and anti-osteoporotic [11] effects and anti-cancer potential against ovarian [12], lung [13], hepatocellular [14, 15], prostate [16], cervical and skin [17] cancers. D-Allose exerts beneficial effects against stroke [9], ischaemia–reperfusion injury [10] and hypertension [18]. It has 80% sweetness of sucrose but is very low in calorie [19, 20]. Moreover, 91.2% and 67% of D-allose can be absorbed and excreted respectively in the urine in rats and humans [21, 22].

Chemical methods for the production of D-allose [23] suffer from the drawbacks of low productivity, complex reaction steps, bad selectivity, unwanted by-products and chemical pollution [19]. Accordingly, biological methods using enzymes including D-glucose isomerase, D-tagatose 3-epimerase, D-allulose 3-epimerase and L-rhamnose isomerase have been used for D-allose production [24]. Although various production methods have been developed, D-allose, unlike D-allulose and D-tagatose, has not yet been approved as Generally Recognised As Safe by the Food and Drug Administration for use as a food additive. In this study we examined the effects of oral administration of D-allose on mice to get basic information on the safety of using D-allose as a functional food ingredient and sweetener.

MATERIALS AND METHODS

Chemicals

D-Allose and D-allulose standards were purchased from Sigma Aldrich (USA). For treatment in mice, D-allose was produced from D-allulose by commercial glucose isomerase (Novozyme, Denmark) as previously reported [25]. Briefly, glucose isomerase in a 300-mL packed bed reactor was reacted with 500 g/L D-allulose at pH 8.0, 60°C and a dilution rate of 0.24/hr for 20 days. The produced D-allose was purified from the reactants using UBK-555 Ca calcium ion-exchange resin (Mitsubishi Chemical, Japan) and the purity of purified D-allose was analysed with a Bio-liquid-chromatography (LC) system (Dionex ICS-3000, USA).

Animals and Experimental Conditions

For animal study, 22 specific pathogen-free CrljOri:CD1(ICR) mice of either sex (30.6–34.0 g body weight of male, 25.3–28.2 g body weight of female, seven weeks old) were obtained from Orient Bio Co. (Seongnam, Republic of Korea). They were acclimatised for one week and 20 mice, males and females, were selected for treatment. They were kept in the following environmental conditions: temperature range of 23 ± 3°C, relative humidity range of 55 ± 15%, ventilation of 10–20 air changes/hr, 150–300 Lux of luminous intensity and a 12-hr light/dark cycle. All procedures and protocols for animal study were reviewed and approved (Serial no. 17-M446) by the Institutional Animal Care and Use Committee of Chemon Nonclinical Research Institute (Youngin, Republic of Korea), accredited by the AAALAC International, and they were performed in accordance with the GLP regulations for Nonclinical Laboratory Studies from the Ministry of Food and Drug Safety in the Republic of Korea as well as the guideline published by the Organisation for Economic Co-operation and Development.

D-Allose Treatment of Mice

Twenty each of male and female ICR mice were randomly distributed in groups of five mice. D-allose at 5,000 mg/kg body weight was set as the high dose as per the Organisation for

Economic Co-operation and Development guidelines for testing of chemicals (TG No. 425). The medium and low doses were set at 2,500 and 1,250 mg/kg body weight respectively. A total of four test groups including the control group, treated with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid only, were set. Administered volume of D-allose was 10 mL/kg body weight and calculated based on body weight measured after 3–4 hr of fasting on the day of administration (Table 1). The dorsal skin of mice was fixed and D-allose was directly administered into the stomach using a syringe with a feeding needle. Food was supplied approximately 1–2 hr after dosing.

Table 1. Experimental design and groups used in the study

Group	Gender	No. of mice	Mice ID	D-Allose dose volume (mL/kg)	D-Allose dose (mg/kg)
Control	M/F	5 / 5	1–5 / 21–25	10	0
A1	M / F	5 / 5	6–10 / 26–30	10	1250
A2	M / F	5 / 5	11–15 / 31–35	10	2500
A3	M / F	5 / 5	16–40 / 36–40	10	5000

Observation of Clinical Signs and Mortality and Measurement of Body Weight

Clinical signs and mortality were continuously observed in the first 1 hr after administration of D-allose and then every hour for the next 5 hr. The administration day was set as Day 1 and clinical signs and mortality were observed for 15 days. Body weights of mice were measured on Day 1, 2, 4, 8 and 15.

Necropsy

On Day 15, all surviving mice were anesthetised by inhalation of carbon dioxide. Blood samples were collected from the abdominal aorta to obtain serum for chemical analysis. The mice were exsanguinated and the organs were grossly examined.

Chemical Analysis of Blood Serum

Blood samples were incubated for 40 min. at 4°C and centrifuged at 3,000 × g for 10 min. to obtain blood serum. The following biochemical parameters were evaluated using a clinical chemical analytic equipment (Fuji Dri-Chem 3500, Fujifilm, Japan) and a control solution (QP-H, Fujifilm, Japan): alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactase dehydrogenase (LDH), triglycerides (TG), total cholesterol (TCHO), total protein (TP), total bilirubin (TBIL), direct bilirubin (DBIL), creatine phosphokinase (CPK), γ-glutamyl transferase (GGT), glucose (GLU), uric acid (UA) and calcium (Ca).

Analytical Methods

The purified D-allose was analysed using a Bio-LC system with an electrochemical detector and a CarboPac PA1 column. The column was eluted at a flow rate of 1 mL/min. at 30°C with water/200 mM NaOH in the following ratios (v/v): from 50:50 to 0:100 between 0–10 min., 0:100 to 50:50 between 10–15 min. and constant at 50:50 between 15–20 min.

SPSS 22.0 for medical science software was used for statistical analyses. Data of body weights were analysed by one-way analysis of variance (ANOVA); data of the treated group was compared with that of the vehicle control group. A value of $p < 0.05$ was considered as statistically

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significant. Because mortality was not observed, the median lethal dose (LD_{50}) values were not calculated.

RESULTS

Identification of Prepared D-Allose

The chemical structure of D-allose produced from D-allulose by glucose isomerase has already been verified by nuclear magnetic resonance spectroscopy in our previous study [25]. Therefore, in this study the LC chromatograms of reactant and product were compared with those of D-allose and D-allulose standards (Figure 1), which showed >97% purity for the prepared D-allose.

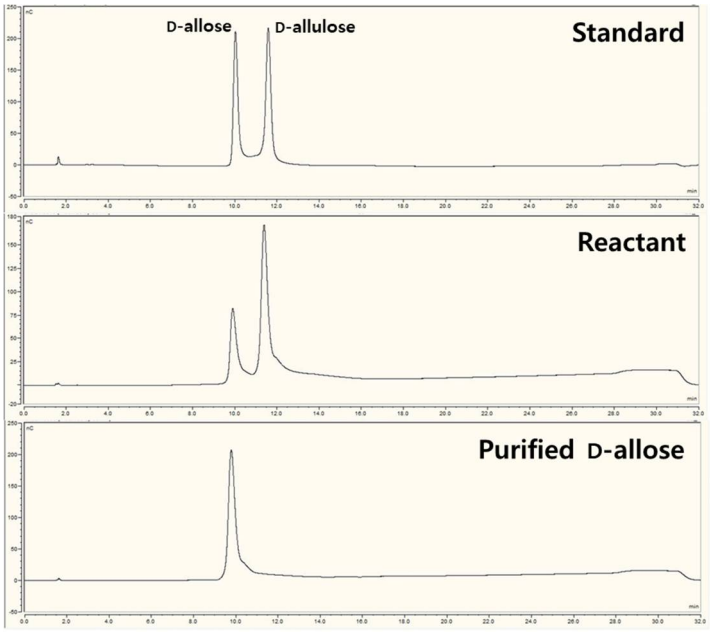

Figure 1. Bio-LC chromatograms of reactant and purified product compared with D-allose and D-allulose standards

Clinical Signs and Mortality

After a single oral dose of D-allose, clinical signs and mortality of the ICR mice were observed for 15 days. In both sexes no specific clinical signs were noticed in the control and treated mice, and no deaths were observed (data not shown). The approximate lethal dose of D-allose was considered to be greater than 5,000 mg/kg.

Changes in Body Weight

Changes in body weight of the mice following D-allose administration were measured for 15 days. During the experimental period, both male and female mice gained weight. However, no significant difference was observed in body weights between the control and treated groups ($p > 0.05$) (Table 2).

Necropsy and Histopathological Findings

Fifteen days after the administration of D-allose, necropsy of the mice was conducted. No significant lesions were observed as a result of D-allose administration (data not shown).

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Table 2. Changes in body weight of ICR mice after oral administration of D-allose

Group	No. of mice	Body weight (g)					Weight gain
		Day 1	Day 2	Day 4	Day 8	Day 15	(g)
Control	10	30.68 ± 2.87	31.52 ± 3.19	31.67 ± 2.83	32.90 ± 2.87	35.20 ± 2.97	4.52 ± 1.39
A1	10	31.05 ± 2.96	31.75 ± 3.16	31.52 ± 2.86	32.46 ± 2.67	34.77 ± 2.48	3.72 ± 1.67
A2	10	30.77 ± 2.99	31.24 ± 3.36	30.90 ± 3.19	31.25 ± 2.90	33.74 ± 3.36	2.97 ± 1.38
A3	10	30.89 ± 3.04	31.44 ± 3.25	31.49 ± 3.19	32.49 ± 3.82	34.69 ± 3.57	3.80 ± 1.36

Note: Values are means ± standard deviation (SD) for 10 mice.

Biochemical Parameters in Blood Serum

The results of biochemical analyses of the blood serum are shown in Table 3. The activities of serum (ALT, AST, LDH, CPK and GGT) and concentrations of TG, TP, TBIL, DBIL, UA and Ca are not significantly different among the four groups. The serum ALP activities are significantly higher in the A1 group and lower in the A2 and A3 groups than that of the control group. The TCHO concentration is significantly higher in the A3 groups than that in the other groups and the GLU concentration is significantly lower in the A1 group than that in the other groups.

Table 3. Biochemical parameters of serum samples of ICR mice at 15 days after oral administration of D-allose

	Control	A1	A2	A3
ALT (U/L)	48.2 ± 3.46	50.1 ± 2.39	50.7 ± 11.27	52 ± 3.02
AST (U/L)	58.5 ± 4.48	58.1 ± 2.88	58.3 ± 4.24	58.4 ± 3.13
ALP (U/L)	1167.4 ± 196.27^{a}	1528.5 ± 226.82^{b}	924.5 ± 124.99^{c}	951.3 ± 261.11^{c}
LDH (U/L)	72.7 ± 9.29	81.3 ± 13.78	74.3 ± 13.82	86 ± 13.08
TG (mg/dl)	95.3 ± 6.91	99.3 ± 19.86	91.3 ± 21.73	94.5 ± 14.58
TCHO (mg/dl)	72.2 ± 4.39^{a}	78.3 ± 6.57^{a}	76.3 ± 8.64^{a}	90.7 ± 7.93^{b}
TP (g/dl)	5.14 ± 0.10	5.01 ± 0.27	4.9 ± 0.24	4.95 ± 0.14
TBIL (mg/dl)	0.11 ± 0.03	0.14 ± 0.05	0.18 ± 0.15	0.15 ± 0.05
DBIL (mg/dl)	0.10 ± 0.00	0.12 ± 0.04	0.10 ± 0.00	0.10 ± 0.00
CPK (U/L)	93.7 ± 4.67	87.3 ± 7.99	90.7 ± 4.38	89.1 ± 3.48
GGT (U/L)	1 ± 0.00	1 ± 0.00	1.1 ± 0.30	1 ± 0.00
GLU (mg/dl)	158.3 ± 7.17^{a}	133.2 ± 25.43^{b}	163.2 ± 10.47^{a}	154.6 ± 14.05^{a}
UA (mg/dl)	0.50 ± 0.13	0.44 ± 0.09	0.53 ± 0.11	0.51 ± 0.07
Ca (mg/dl)	7.97 ± 0.18	7.44 ± 0.36	7.66 ± 0.18	7.7 ± 0.21

Note: Values are means ± SD for 10 mice. Different superscripts represent significant differences ($p < 0.05$). ALT=alanine aminotransferase, AST=aspartate aminotransferase, ALP=alkaline phosphatase, LDH=lactase dehydrogenase, TG=triglycerides, TCHO=total cholesterol, TP=total protein, TBIL=total bilirubin, DBIL=direct bilirubin, CPK=creatine phosphokinase, GGT=γ-glutamyl transferase, GLU=glucose, UA=uric acid, Ca=calcium

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DISCUSSION

D-Allose is a rare natural sugar with various biological functions and benefits to human health. Nevertheless, the study on the use of D-allose as a food ingredient has rarely been conducted. The effects of D-allose administration on clinical signs, organs, changes in body weight and biochemical changes in the blood serum of mice were assessed in this study.

Mice administered D-allose up to 5,000 mg/kg body weight showed no clinical signs or mortality at Day 15 (data not shown). An LD_{50} value represents the dose that kills 50% of the members of a population and this value is used to compare relative acute hazards of a substance. According to Loomis and Hayes [26], substances with LD_{50} exceeding 5,000 mg/kg body weight are practically non-toxic and those with LD_{50} exceeding 15,000 mg/kg body weight are relatively harmless. Herein, the LD_{50} value of D-allose is estimated to exceed 5,000 mg/kg body weight. Matsuo et al. [27] reported that the LD_{50} value of D-allose was 20.5 g/kg body weight for rats, which was higher than that of other rare sugars, such as D-allulose (16 gm/kg body weight) and D-tagatose (20 gm/kg body weight) [28, 29]. Among the natural sweeteners, the LD_{50} value of xylitol was 25.7 gm/kg body weight for rats and that of stevia was 5.2 and 6.1 gm/kg body weight for male and female hamsters respectively [30, 31]. Erythritol showed LD_{50} values of 13.1 and 13.5 gm/kg body weight for male and female rats respectively [32, 33]. LD_{50} values of sucralose were 16 and 10 gm/kg body weight for mice and rats respectively [34].

The mice were also observed for any increase in body weight for 15 days after administration of D-allose at 1,250, 2,500 and 5,000 mg/kg body weight. However, their weight gain was not related with the administration of D-allose (Table 2). Administration of 3% D-allose solution for six months was reported to have a significant effect on reducing the body weight [21]. Hossain et al. [35] reported that high doses of D-allose induced a slight decrease in body weight. However, no change in body weight was observed upon administration of D-allose at 2,000 mg/kg body weight to rats for two weeks [18]. These results suggest that long term (more than six months) administration of D-allose at high doses may be effective in reducing body weight.

The necropsy of the mice showed no abnormal tissues in the present study (data not shown). No significant relationship between D-allose administration and development of organ or tissue lesions was found. D-Allose was reported to inhibit acute renal injury in systemic inflammation [36] and to protect the liver [35], brain [37], and retina from ischemia reperfusion injury [38]. Long-term administration of D-allose was found to lead to significant reduction in weight of the lung and skeletal muscle in rats, and these symptoms might be related to the body weight loss [27].

The blood serum analysis provides important information about the health of internal organs such as the liver and kidneys. In our results most of the indicators show no significant difference compared to control and only some of them show a difference among groups (Table 3). Of the activities of indicators such as ALT, AST, ALP, LDH, CPK and GGT, significant difference is observed in ALP activity only. However, there is no correlation between D-allose concentration and ALP activity. Among the other indicators, viz. TG, TCHO, TP, TBIL, DBIL, GLU, UA and Ca, TCHO and GLU concentrations are significantly different among the groups, although they do not correlate with D-allose dose. Iga et al. [28] reported that significant differences in TCHO and GLU concentrations among the groups were not observed when D-allose was administered for six months. Therefore, these differences among the groups in our study may be considered as a temporary change following single administration of D-allose.

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CONCLUSIONS

No evidence of D-allose toxicity to mice was found for single administration of D-allose. Further study on multiple administration of D-allose is required.

ACKNOWLEDGEMENTS

This study was supported by the KU Research Professor Program of Konkuk University. It was also supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (Project no. 2018R1D1A1B07050820). Support from the R&D Programme for Forest Science Technology (Project no. 2020197A00-2222-BA01) provided by the Korea Forest Service (Korea Forestry Promotion Institute) was also gratefully acknowledged.

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