Food and Chemical Toxicology 62 (2013) 573–578

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Subchronic toxicity study of ulvan from *Ulva pertusa* (Chlorophyta) in Wistar rats

Huimin Qi [a,b], Xiaolei Liu [c], Kai Wang [a], Dongmei Liu [a], Liye Huang [d], Shunmei Liu [a], Quanbin Zhang [b,*]

[a] Weifang Medical University, No. 7166 Baotong Road, Weifang 261053, PR China
[b] Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China
[c] Weifang University, No. 5147 Dongfeng Road, Weifang 261061, PR China
[d] Shandong Institute of Scientific and Technical Information, Jinan 250101, PR China

ARTICLE INFO

Article history:
Received 17 June 2013
Accepted 17 September 2013
Available online 29 September 2013

Keywords:
Ulvan
Ulva pertusa
Subchronic toxicity
Rats

ABSTRACT

Ulvan extracted from *Ulva pertusa* (Chlorophyta) is a group of sulfated heteropolysaccharide, for simplicity, the sulfated polysaccharide is referred to as ulvan in this paper. To our knowledge, there is no detailed report investigating the toxicity of ulvan. In this study, the subchronic (6 months) toxicity of varying levels of ulvan extracted from *U. pertusa* was investigated in Wistar rats after oral administration. ALT, ALB, ALP, WBC, PLT, and liver relative organ weigh of female rats showed significantly difference at 3000 mg/kg body weight per day, compared with control group. On the other hand, TG, T-CHO concentrations of female rats (6 months) were significantly decreased at 600, 1200 and 3000 mg/kg body weight per day. This result proved that ulvan had antihyperlipidemic activity. Beside, ulvan showed anticoagulant activity in this study. Overall, our findings indicated that ulvan had affected specific hematology, serum biochemistry parameters and liver, and had great differences between males and females rats.

1. Introduction

Ulvan is a kind of sulfated polysaccharide extracted from green algae, and the main constituents of ulvan are sulfated rhamnose residues linked to uronic acids, resulting in a repeated disaccharide unit β-D-glucuronosyl-(1,4)-α-L-rhamnose 3-sulfate, called aldobiouronic acid (Lahaye and Robic, 2007). In recent years, ulvan has already proven to be a remarkable polysaccharide with different attractive properties that make it suitable for a wide range of applications (Cluzet et al., 2004; Kaeffer et al., 1999; Mao et al., 2009; Lahaye and Robic, 2007; Tabarsa et al., 2012).

The green alga, *Ulva pertusa*, is an important food source in many parts of the world. *U. pertusa* is nutritious with low calorie, abundant vitamins, trace elements and dietary fibers (Lahaye and Jegon, 1993). In China, *U. pertusa* is distributed in the intertidal zone of the Yellow Sea and the Bohai Sea. Furthermore, it has been used as a drug in Traditional Chinese Medicine for hyperlipidemia, sunstroke and urinary diseases. In our laboratory, we isolated ulvan from *U. pertusa* collected in Qingdao, China, and the structures were [β-D-GlcpA-(1 → 4)-α-L-Rhap3s] and [α-L-Idop A-(1 → 4)-α-L-Rhap 3s] (Qi et al., 2005a; Yu et al., 2003a), furthermore, the study demonstrated that ulvan of *U. pertusa* had a protective effect against hyperlipidemia of rats (Qi et al., 2012; Yu et al., 2003b). The results suggest that ulvan could have potential clinical importance as antihyperlipidemic agents.

Although diverse studies on the biological activities of ulvan have been performed by now, no detailed toxicological assessment of ulvan has been reported. In the present study, we tested the toxicity of a 6-month oral trial of ulvan extracted from *U. pertusa* in Wistar rats. In our previous study (Qi et al., 2012), ulvan showed antihyperlipidemic activity at the dose of 125–500 mg/kg body weight per day in Wistar rats. Considering the moderate solubility of ulvan in water, the maximum dose that can be administered by single oral gavage is about 4000 mg/kg body weight. In acute toxicity study (data not shown), the fixed dose of 4000 mg/kg body weight was selected and in the subchronic toxicity study, the low and high dose of ulvan were fixed at 600 and 3000 mg/kg bw per day, respectively. In order to precisely reflect the toxicity of ulvan, we also select a middle dose of 1200 mg/kg bw per day.

2. Materials and methods

2.1. Ulvan

The test substance, ulvan, was prepared from *U. pertusa* cultured in Qingdao, China, as described previously (Yu et al., 2003a) with minor modification. Its chemical composition was analyzed as follows: total sugar content (49.1%), sulfate

Abbreviations: U, ulvan; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein; Alb, albumin; T. Bil, total bilirubin; ALP, asparatate aminotransferase; BUN, total blood urea nitrogrn; Crea, creatinine; CK, creatine kinase; PT, Prothrombin Time; APPT, Part prothrombin time; Na, sodium; K, potassium; Cl, chloride; TG, total glyceride; T-CHO, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.

* Corresponding author. Tel./fax: +86 532 82898703.
E-mail addresses: qbzhang@ms.qdio.ac.cn, wfqihuimin@126.com (Q. Zhang).

0278-6915/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2013.09.022

content (19.5%), uronic acids (19.2%). Its average molecular weight was determined to be 151.7 kDa by high performance steric exclusion chromatography analysis. Ulvan was dissolved in distilled in 0.5% CMC water for animal treatment.

2.2. Experimental animals in subchronic toxicity and housing conditions

Male and female Wistar rats were obtained from the Chinese of Medicinal Academy of Science Animal Institute (Tianjin) at 6–7 weeks of age. Rats were allowed to adapt to the concentrations of the animal house for 1 week before the experiments. The animals were maintained on a 12 h dark/light cycle at about 24 ± 3 °C and allowed free access to standard laboratory diet (purchased from Tianjin Animal Center) and tap water ad libitum during the experiments.

2.3. Subchronic toxicity study design

Hundred and sixty Wistar rats were randomly divided into control (Group 1, $n = 40$, 20 males and 20 females) and three doses level groups. Animals of the low dose level group (Group 2, $n = 40$, 20 males and 20 females) were treated with ulvan at the dose of 600 mg/kg body weight per day, those from middle dose level group (Group 3, $n = 40$, 20 males and 20 females) and high dose level group (Group 4, $n = 40$, 20 males and 20 females) were treated with 1200 mg/kg and 3000 mg/kg bw per day ulvan, respectively. All the samples were administered by oral gavage in a volume of 1 mL/100 g body weight once daily for 6 days per week over a period of 6 months. The control was treated with the same volume of 0.5% CMC during the experiments. The ulvan solutions were prepared by dissolving ulvan in 0.5% CMC. The final concentration of ulvan in the solutions was 40 mg/mL for Group 2, 80 mg/mL for Group 3, and 200 mg/mL for Group 4, respectively. Twenty-four hours after the last ulvan administration after 6 months part rats (28, 14/14, male/female for each groups) were necropsied after blood samples were collected from the abdominal aorta under ether anesthesia for evaluation of clinical hematology, biochemistry and organ pathology. The remaining rats (12, 6/6, male/female for each groups) were placed on the control diet for an additional 1-month recovery period to determine reversibility or delayed occurrence of any treatment related change. Following the 1-month recovery period, animals from the recovery groups were sacrificed for collection of blood and organs for histopathologic analysis.

2.4. Clinical examinations

Clinical signs were observed once daily, body weights and food in-taking were measured every one week administration before 10 weeks and every two weeks administration after 10 weeks during the experiments.

Hematological parameters, measured with an automated hematology analyzer (KX-21, Japanese), consist of red blood cell count (RBC), hemoglobin (Hb), platelet count (PLT), white blood cell count (WBC), and white blood cell differential count. prothrombin time (PT), part prothrombin time (APTT) were measured with coagulation factor analyzer (LG-PABER).

Clinical biochemical parameters, measured with an automated biochemical analyzer (Olympus Au640) were: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), albumin (Alb), total bilirubin (T. Bil), asparatate aminotransferase (ALP), total blood urea nitrogrn (BUN), creatinine (Crea), Sodium (Na), Potassium (K), Chloride (Cl), glucose, total glyceride (TG), Total cholesterol (T-CHO), high density lipoprotein (HDL), and low density lipoprotein (LDL).

At necropsy, the killed rats had the following histopathological examined macroscopically and the heart, liver, spleen, lungs, kidneys, adrenals, thymus, brain, testicle (in male), epididymis (in male), uterus (in female), and ovaries (in female) were weighted and their organ weight per 100 g body weight (relative weight) was calculated based on the fasted animal's body weight. All organs/tissues, including heart, aorta, lungs, windpipe, brain, pituitary gland, liver, kidneys, spleen, thymus gland, lymph gland, stomach, oesophagus, intestines, jaw gland, thyroids, parathyroid glands, adrenal gland, pancreas, testicle (in male), epididymis (in male), prostate gland (in male), uterus (in female), ovary (in female), bladder, marrow and sciatic, were fixed and preserved in 10% phosphate buffered formalin. Fixed tissues were routinely processed for embedding in paraffin, sectioned and stained with hematoxylin and eosin. Collected tissues were grossly and microscopically examined during histopathological examination.

2.5. Statistical analysis

Data were presented as means ± SD. The data were analyzed by one-way analysis of variance (ANOVA) and the Student's *t*-test was used to determine the level of significance of differences in population means. A significant difference was accepted with $P < 0.05$.

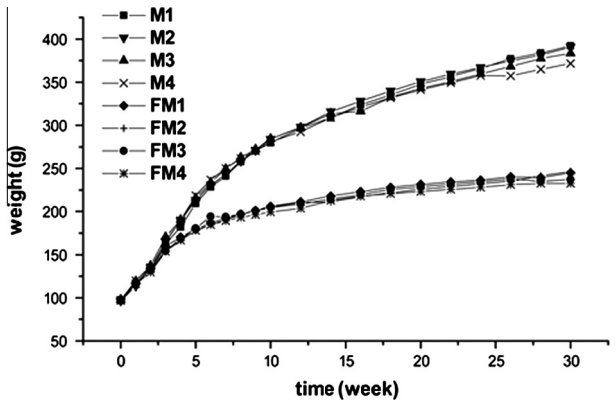

Fig. 1. Growth curves for Wistar rats fed the diet containing ulvan for 6 months and 1 month recovery. M1: male rats in group 1 (control); M2: male rats in group 2 (600 mg/kg); M3: male rats in group 3 (1200 mg/kg); M4: male rats in group 3 (3000 mg/kg); FM1: female rats in group 1 (control); FM2: female rats in group 2 (600 mg/kg); FM3: female rats in group 3 (1200 mg/kg); FM4: female rats in group 4 (3000 mg/kg).

Table 1
Food in-taking data in male and female Wistar rats fed the diet containing ulvan for 30 weeks (g/rat).

Weeks	Group 1 (0)	Group 2 (600 mg/kg)	Group 3 (1200 mg/kg)	Group 4 (3000 mg/kg)
Male				
0	19.5	18.1	18.4	19.8
1	21.1	19.3	19.7	21.0
2	25.5	23.4	24.2	23.6
3	28.9	25.1	23.1	25.9
4	27.4	24.4	24.2	25.8
5	26.5	27.6	24.7	25.4
6	23.0	22.2	23.4	21.3
7	21.5	21.5	21.3	21.8
8	20.0	21.5	22.8	20.4
9	20.5	22.3	23.1	20.8
10	21.5	22.4	22.7	21.3
12	21.0	22.8	21.5	22.2
14	20.0	22.6	22.6	22.4
16	21.7	21.0	20.1	21.3
18	21.4	19.6	20.4	20.7
20	19.1	22.7	21.7	20.7
22	18.1	20.9	20.1	20.3
24	18.4	20.1	19.4	18.3
26	18.1	20.5	19.1	20.0
28	20.3	20.1	20.1	20.7
30	21.8	19.1	19.6	20.1
Female				
0	18.2	17.2	16.9	16.6
1	19.5	18.4	17.5	17.3
2	19.4	20.3	19.5	18.4
3	19.3	19.1	18.9	18.5
4	18.4	18.6	20.1	19.8
5	17.2	17.9	18.8	19.1
6	16.8	17.1	18.2	18.3
7	15.4	16.0	14.9	15.5
8	14.9	16.5	16.7	17.7
9	15.1	16.3	15.9	17.4
10	14.3	15.9	15.7	16.8
12	14.6	15.1	15.9	15.5
14	14.6	14.9	15.0	15.2
16	13.5	14.1	15.0	14.2
18	15.0	14.1	15.2	15.0
20	14.3	14.0	14.7	14.5
22	14.0	14.2	14.2	14.8
24	15.0	14.1	14.0	14.8
26	14.7	14.1	14.1	14.3
28	14.3	14.0	14.1	14.0
30	13.1	12.3	13.0	12.3