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Original

Hepatotoxicity Induced by Excessive Intake of Rhubarb

Kang Jin[1)], You Tian[1)], Jin Xin[2)], Masahisa Inoue[3)], Kojun Setsu[3)], Kan Rui[4)] and Ryo Tamamura[4)]

[1)]Department of Emergency Medicine, The First Hospital, China Medical University, Shenyang, 110001, China
[2)]China Medical University, 87K, English Class Three
[3)]Laboratory for Structure and Function Research, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8515 Japan
[4)]Department of Oral Pathology and Medicine, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 2-5-1 Shikata-cho, Okayama 700-8525, Japan
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Abstract: We studied the toxicity dose and toxicity mechanism of rhubarb, a Chinese traditional medicine, by administering different doses to rats and examining the effects on the liver. Kunming rats were given intragastric administration of large, medium, small and minimum doses of rhubarb twice daily for five consecutive days. Then the general status of the rats, the hepatoenzymological data, histopathology of liver, and TNFα mRNA expression were examined. The following results were obtained. 1. The general status of rats deteriorated with the increase in rhubarb dosage. The physical status of rats in the control group and minimum dose group were normal, but the status in other groups gradually deteriorated along with the increase in rhubarb dose. 2. Regarding the changes of hepatoenzymology profile, alanine aminotransferase and gamma-glutamyltransferase were elevated with increase in rhubarb dose; while the difference between normal control group and minimum dose group was unremarkable, the differences among other groups were obvious. 3. Examination of H&E-stained slides showed fatty degeneration of the liver starting from the minimum dosage group. This phenomenon is aggravated with increase in rhubarb dosage. 4. TNFα expression was elevated significantly with increase in rhubarb dosage, beginning from the minimum dosage group. The present results showed that even a small dose of rhubarb given to rats by the intragastric route for five consecutive days was toxic to the liver, and the toxic effect was enhanced with increase in rhubarb dosage. The toxic effect of excessive intake of rhubarb to the liver is mainly manifested as fatty degeneration. TNFα, an injury factor leading to fatty degeneration of cells, was probably involved in the hepatotoxicity by excessive intake of rhubarb.

Key words: Rhubarb, Alanine aminotransferase, Gamma-glutamyltransferase, TNFα, Fatty degeneration

Introduction

In recent years, along with a global trend of return to natural cure, an increasing number of people are trying to use natural medications to treat many kinds of diseases. However, there is a general misconception that Chinese herbal medicine, which consists mainly of natural drugs, is safe and reliable. This belief has led to indiscriminate use of large amounts of Chinese herbal medicine by some people, which sometimes results in adverse side effects, which may even become life-threatening.

Rhubarb has been the most popular medication used in the clinic since the old days, but currently cognition of the adverse effects of rhubarb is lacking[1-3)]. In the present study, we evaluated the toxic effect of different dosages of rhubarb by intragastrically administering large, medium, small and minimum doses of rhubarb to Kunming rats, and examining the general status of the rats, hepatoenzymological data, histopathology of liver, and TNFα mRNA expression. This study aimed to provide a safety profile for rhubarb based on more scientific data in order to promote safe use of this kind of medication.

Corresponding author: Kang Jin , Department of Emergency Medicine, The First Hospital, China Medical University, Shenyang Heping District Nanjing North Town, Jinkang 110001, China. Tel:+86-24-8631-2830, E-mail: jinkang@hotmail.com

Materials and methods

1. Animals

The experiments were conducted on sixty male Kunming rats weighing 20 to 24 g (supplied by the animal center of Chinese Medical University)

2. Preparation of the animal model

A rhubarb solution was prepared from 1000 g of dry rhubarb. Animals were randomized into four experimental groups and a control group. The experimental groups were given rhubarb at dosages of 4 (minimum dose), 8 (small dose), 16 (medium dose) and 32 g/kg (large dose). Rhubarb solution was given by the intragastric route to rats twice daily for five consecutive days. The control group was administered water in the same manner as

in the experimental groups. During this period, the general state of the rats was observed. At the completion of the last intragastric administration, food was withdrawn but water was supplied. Sixteen hours later, blood was collected from the eye and serum was collected for measurements of alanine aminotransferase (ALT) and gamma-glutamyltransferase (γ-GT).

3. Hematoxylin and eosin staining of liver sections

Rats were killed by cervical dislocation, and the liver was removed. The same liver lobe from each rat was fixed in the 4% polyformaldehyde and processed for HE staining.

4. Semi-quantitative examination for TNF α expression

Total RNA of was extracted from rat liver by TRIZOL reagent. TNFα mRNA expression of the rat liver was examined using a semi-quantitative RT-PCR method. The PCR products were electrophoresed on a gel and the densities of the bands were scanned. TNFα mRNA expression was expressed as a ratio of GAPDH mRNA expression.

5. Statistic analysis

Quantitative data are expressed as mean ± SD. The significance of differences among groups was evaluated by single factor analysis. Correlation between parameters was tested by Pearson correlation analysis. Statistics analyses were performed using the software SPSS12 (SPSS, Inc., Chicago, IL). P values less than 0.05 were considered statistical significant.

Results

1. General status

The general status of the rats deteriorated with increase in rhubarb dosage. Rats in the normal control group and minimum dose group appeared healthy. Rats administered rhubarb at small, medium and large doses showed decreased activity, less food intake, loose stool or even diarrhea, piloerection, dull coat, yellow and soiled fur, dark red eyeball, and retarded weight gain or even weight loss.

2. Hepatoenzymology

The serum ALT and γ-GT levels were elevated with increase in rhubarb dose. Compared with the normal control group, the differences were unremarkable in the minimum dose group, but the differences were significant in the other groups (Table 1). Analysis of the relation of ALT and γ-GT in different dose groups with the SPSS12 software showed a correlation coefficient of 0.92 ($p<0.05$).

Table 1. Serrum ALT and Serum ALT and g-GT levels (mean ± SD)

Group	N	ALT(IU/L)	g-GT (IU/L)
Control	11	26.9 ± 3.0	42.0 ± 2.5
Minimum dose	11	31.2 ± 3.5	46.5 ± 3.2*
Small dose	10	55.4 ± 4.2	62.7 ± 4.0#
Medium dose	10	90.2 ± 5.4	91.3 ± 4.5#
Large dose	11	170.5 ± 7.1	138.2 ±5.5#

*: P>0.05 compared with control group;
#: p<0.01, compared with control group.

Table 2. TNFα mRNA expression expressed as a ratio of the GAPDH gene as an internal control.

Group	TNF±˜ : GAPDH
Control	0.34 ± 0.05
Minimum dose	0.36 ± 0.12*
Small dose	0.66 ± 0.11#
Medium dose	0.89 ± 0.15#
Large dose	1.14 ±s0.17#

*: P>0.05 compared with control group, #: P<0.05 compared with control group.

3. HE staining of liver tissue

The hepatocytes in normal control group and minimum dose group showed no obvious changes. However, fatty degeneration appeared in the rat liver sections of the small dose group, and the phenomenon aggravated with increase in rhubarb dose (Figs. 1, 2, 3, 4 and 5).

4. TNF α gene expression

The Trizol reagent one-step method was used to extract total RNA from rat liver, which was reverse transcribed to cDNA. The amplified product of the TNFα was 397bp, while that of GAPDH was 190 bp. The RT-PCR was performed using GAPDH as an internal control (Fig. 6).

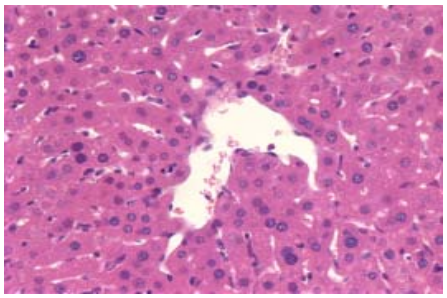

Fig. 1. HE staining of rats liver section in normal control group (×400)

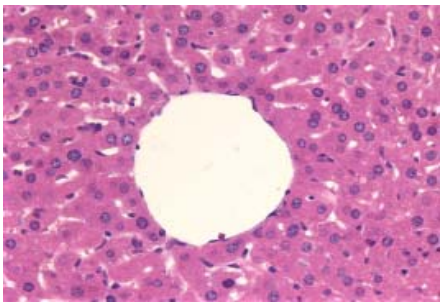

Fig. 2.HE staining of rats liver section in minimum dose group (×400)

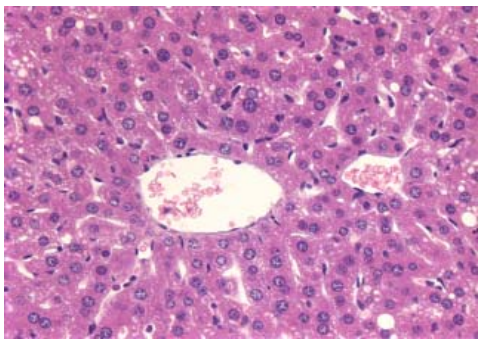

Fig. 3. HE staining of rats liver tissue section in small dose group (×400)

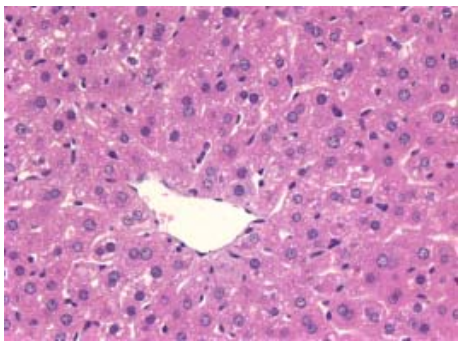

Fig. 4. HE staining of rats liver section in medium dose group (×400)

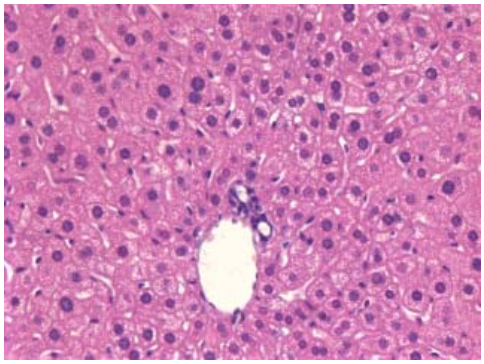

Fig. 5. HE staining of rats liver section in large dose group (×400)

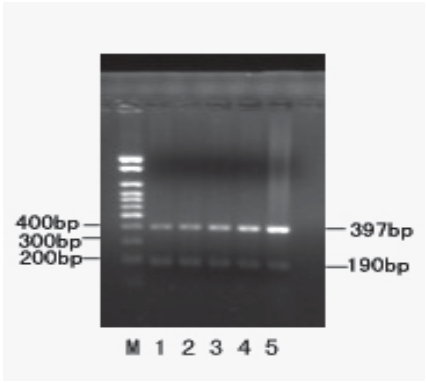

Fig 6. Semi-quantitative PT-PCR result showing electrophoresis of TNFα gene. Lanes 1-5 indicate the groups, normal, minimum, small, medium and large dose groups, respectively.

An ultraviolet-ray automatic imaging system was used to measure the densities of the bands. TNFα mRNA was expressed as a ratio to GAPDH.

TNFα gene expression was elevated significantly compared with the control beginning from the small dose group, and the magnitude increased with increase in rhubarb dose (Table 2).

Discussion

Rhubarb is a Chinese traditional medicine, which is widely used in the world[1]. It is used in internal medicine, surgery, gynecology, pediatrics clinics as single, complex or combined prescription. According to the Chinese Pharmacopoeia, the daily dosage of rhubarb for adult is 12-15 g[2]. The main function of rhubarb is effusion, but other functions such as antibacterial, antiviral and anti-neoplasmic actions are also known. Less than 1 g of rhubarb has stomachic, astringent and anti-diarrheal effects. Qiu et al[3] conducted an experiment to evaluate the acute toxicity of common bitter and cold medicine. They administered rhubarb solution into the rat stomach and observed emaciation. They reported the highest tolerant dose of rhubarb in rats as 120 g/kg/d. However, there are few recent reports on the toxic and adverse effects of rhubarb.

In order to evaluate the toxic dose of rhubarb and provide experimental evidence for safe use of rhubarb, this research used different doses of rhubarb administered by an intragastric route to rats for 5 consecutive days. The experimental results showed that rats given rhubarb at a dose of 4 g/kg/d remained normal, while those given a dose of 8 g/kg/d started to exhibit toxic findings such as slightly reduced activity, slight lack of luster of the coat, slightly loose stool, and slightly slow weight gain. These phenomena deteriorated with the increase in rhubarb dose. At a dose of 32 g/kg/d, the rats showed definite toxic adverse effects such as markedly reduced activity and food intake, soiled fur, piloerection, obvious diarrhea, and markedly retarded weight gain or even weight loss. From the hepatoenzymology study, slight increases of ALT and γ-GT were observed in rats administered 4 g/kg/d (small dose), but there were no significant differences compared with the controls. From doses of 8 g/kg/d and upward, ALT and γ-GT were significantly elevated compared to the control group. From the results of HE-stained liver sections, hepatocellular swelling and fatty degeneration were observed starting from the 8 g/kg/d group, and fatty degeneration aggravated markedly with increase in rhubarb dose. Analysis of correlation showed a positive correlation between ALT and γ-GT. These results indicate that rhubarb begins to produce toxic effect on the liver from 8 g/kg/d, and the toxic effects aggravate with increase in rhubarb dose. We speculate that the hepatotoxicity is caused by an excessively strong effusion effect of rhubarb.

The phenol and acid in rhubarb can increase gastrointestinal peristalsis leading to effusion. Excessive intake of rhubarb causes gastrointestinal function disturbance, and may cause breakdown of the gastrointestinal mucus barrier and promote transit of the

bowel bacteria into blood. Endotoxin has been shown to activate liver Kupffer cells[4,5] to secrete TNFα, IL-1 and other factors to induce biological membrane injury. TNFα is the main cellular factor to induce liver injury. The present experimental results indicate that intragastric administration of 8 g/kg/d of rhubarb upregulates expression of TNFα mRNA in rat liver, and upregulation is enhanced with increase in rhubarb dose. This finding correlates with hepatoenzymology and fatty degeneration injury. This study suggests that TNFα is an important cellular factor to induce fatty degeneration. Excessive rhubarb induces TNFα production through endotoxin. TNF is a strong inhibitor of lipoprotein lipase so that a high concentration of TNFα can reduce peripheral tissue fat decomposition, promote hepatocyte triglyceride synthesis and aggregation, causing hepatocellular fatty degeneration. Recent research has provided evidence that TNFα has an important effect in the development of fatty liver[6,7]. The present experiment suggests that TNFα participates in the proces of excessive rhubarb-induced fatty liver development. Therefore blocking the TNFα signaling pathway may block rhubarb-induced toxic effect to the liver.

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