Lemon fruits lower the blood uric acid levels in humans and mice

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A B S T R A C T
Hyperuricemia is a chronic metabolic disorder leading to gouty arthritis, kidney stones, hypertension, renal failure and even cardiovascular diseases in humans. In the present study, the role of the lemon fruit juice and/or the water soluble extracts in lowering the blood uric acid level was evaluated in both human subjects and mice. Fresh lemon fruits, excluding the peel and seeds, were used to prepare the juice and/or water soluble extracts. Human subjects with hyperuricemia were given the freshly squeezed pure lemon fruit juice daily at 30 mL/day (equivalent to a lemon a day) for 6 weeks. Human serum samples were collected for biochemical assessments at weeks 0 (baseline), 3, and 6, respectively. At the end of clinical study, fasting blood samples were collected for blood tests. Mice were given oxonic acid potassium (OA) to induce hyperuricemia. Hyperuricemic Mice were orally given the lemon fruit water soluble extracts at 10 mg/kg body weight and/or allopurinol at 5 mg/body weight for 11 consecutive days. At the end of study, the mice were euthanized and the blood and liver tissues were collected for biochemical tests. The results showed that the lemon fruit juice and/or the water soluble extracts significantly lowered serum uric acid levels in both human subjects and mice. Neither renal nor liver dysfunction was observed. The mechanistic results indicated that lemon might exert the role in lowering serum uric acid independent of inhibition of xanthine oxidase. The results lay a foundation for the future development of dietary treatments of hyperuricemia in humans.

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1. Introduction

Hyperuricemia is a metabolic disease as presented by elevation of the blood uric acid level. Uric acid is the end product of purine metabolism in humans. A blood uric acid level, e.g., urate greater than 360 mmol/L (6 mg/dL) for women and 400 μmol/L (6.8 mg/dL) for men, is the diagnosis for hyperuricemia. The occurrence of hyperuricemia is related to a variety of factors, such as lifestyle, nutrition balance, medication, gender, age, and genetics (Musacchio et al., 2016). Imbalanced nutrition and increased consumption of alcohol leads to the increased prevalence of the disease in certain populations, particularly in that of young adults (Zhou and Liu, 2015). The prolonged on-set of hyperuricemia is associated with the development of hypertension, hyperlipidemia, hyperglycemia, gouty arthritis, kidney stones, renal failure, and cardiovascular diseases (Becker et al., 2005; Dehghan et al., 2008; Kang and Nakagawa, 2005; Krishnan et al., 2011; Mazzali et al., 2001; Tatsuno and Saito, 2001). According to the 2004 National Survey, conducted by the China National Research Center for Disease and Health, there are more than 120 million hyperuricemic cases reported in China. Therefore, there is an urgent need to develop a strategy to treat and prevent hyperuricemia.

Therapeutic drugs for hyperuricemia are now available, such as allopurinol and benz bromarone (Tung et al., 2015). These drugs are synthetic and often related to adverse side effects (Becker et al., 2005; Stamp and Jordan, 2011). For instance, allopurinol is associated with the increased, potential risk of rash, fever, renal, liver and kidney diseases (Haidari et al., 2009). Benz bromarone has the capability of causing fulminant hepatotoxicity (Yu et al., 2006). Therefore, natural fruits and vegetables, and their bioactive compounds as potential alternatives, have recently received considerable attention in the treatment of hyperuricemia.

Lemon fruits are rich in bioactive compounds such as vitamin C, citric acid, hesperidin, sodium, and potassium. Previous publications have reported that lemon and orange juice can be used to treat hyperuricemia urinary calculus (Aras et al., 2008), calcium oxalate calculus (Kulaksizoglu and Sofikerim, 2008), and kidney calculus (Touhami et al., 2007). However, it remains unclear whether lemon fruits lower the blood uric acid level in mammals. Therefore, the aim of the current study was to determine the role of lemon...
fruit juice and bioactive water soluble extracts in the treatment of hyperuricemia in human subjects and mice.

2. Materials and methods

2.1. Lemon, lemon fruit juice and the water soluble extracts

A large batch of fresh lemon fruits were purchased from the Ruili Experimental Station in the Industrial Crops Institute at Yunnan Academy of Agricultural Sciences, China. The fruits were transferred in a temperature controlled container and stored in the research laboratory at Huazhong Agricultural University in Wuhan, China. For the human lemon intervention study, the lemon fruit juice was freshly squeezed on a daily basis from lemon fruits, after removal of the peel and seeds. Individual subjects were given 30 mL pure lemon juice with no other additional ingredients (equivalent to the amount of a lemon per day). For the animal study, 6000 g lemon fruits were used and the peel and seeds were removed. The freshly squeezed juice (about 1400 mL) was filtered through three layers of cheesecloth to remove the cell debris and other large particles; then, the supernatant was freeze dried, which yielded 64.5 g of freeze dried powder of the lemon fruit juice. The powder was reconstituted in 645 mL distilled water. The clear supernatant solution was used as the lemon water soluble extract (LET) for the animal study.

2.2. Reagent and instrument

Oxyxazine acid potassium was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Allopurinol tablets were purchased from China WTO Tianjie Pharmacy (Jiangsu) Co., Ltd. (Nanjing, China). The xanthine oxidase assay kit and coomassie blue staining assay kit were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The automatic biochemical analyzer was manufactured by the Siemens Medical Diagnostic Products (Shanghai) co., Ltd. (Shanghai, China).

2.3. Clinical trial

Fourteen patients with hyperuricemia, between the age range of 20 and 55, were recruited for the study. Exclusion criteria include: hyperuricemia induced by blood disease, tumor radiotherapy/chemotherapy or related drugs; heart, cerebrovascular, liver, kidney and hematopoietic system and psychosis; medication for lowering uric acid; participation in other clinical trials; not recommended to consume lemon (those with gastric ulcer, gastric hyperchlorhydria, dental caries and diabetes); and/or other reasons not suitable for clinical trial studies. The subjects consumed 30 mL freshly prepared lemon fruit juice daily for 6 weeks (equivalent to a lemon per day), without any other dietary restrictions, except alcohol and hyperuricemia lowering drugs. During the experimental period, overnight fasting blood samples were collected at weeks 0 (basal line), 3, and 6 (see the trial work flow diagram in Fig. 1). Then the collected blood samples were subject to blood biochemical tests in the affiliated hospital at Huazhong Agricultural University. The work described herein has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. This study has been approved by the ethics committee of Huazhong Agricultural University (Ethical number HZAUHU-2016-005).

![Fig. 1. Clinical trial workflow.](image_url)
2.4. Animal experiments

A total of 32 male Kun-Ming strain mice (at 18±2 g body weight, BW) were purchased from the Laboratory Animal Research Center of Hubei Province (Wuhan, China). Animals were group housed with free access to water and a regular chow diet; throughout the study, the animals were kept in a controlled environment – 12-h light/dark cycle at a constant room temperature – in the animal facility at Huazhong Agricultural University. Mice were adapted to the environment for one week before experiments were performed. All experiments were conducted in accordance with the Animal Welfare Act and under institutionally approved IACUC animal protocols. At study termination, mice were sacrificed by CO2 followed by cervical dislocation, according to the approved protocols. Blood and liver tissues were collected for experiments.

The mice were randomly divided into four groups (eight mice per group): a blank control group, oxonic acid potassium (OA)-treated group (a hyperuricemia OA group), LET group (hyperuricemia mice treated with LET), and allopurinol group (a positive control group, hyperuricemia mice treated with allopurinol), respectively.

Daily, hyperuricemia was induced by gavage application of oxonic acid potassium (250 mg/kgBW) at 10:00 AM, while the mice from the other groups were given the same amount of distilled water. After 1 h, the mice from the LET group were treated via oral gavage with LET at 10 mg/kg BW (equivalent to 10 mL LET/kg BW) and the mice from the allopurinol group were given 5 mg/kg BW allopurinol. Accordingly, the mice from the OA and blank groups were treated with distilled water at 10 mL/kg BW. The mice were on the treatments for 11 consecutive days. At the end of study, mice were euthanized and the blood and liver tissues were collected for future analysis.

2.5. Biochemical analysis

The xanthine oxidase (XOD) activity in serum and liver tissues was determined spectrophotometrically using a XOD assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The contents of serum uric acid, creatinine (CRE), and blood urea nitrogen (BUN) in serum were measured by an automatic biochemical analyzer (Beckman Coulter 5811, USA).

2.6. Statistical analysis

The values were expressed as mean ± SD. N=8 mice per group. The statistical differences in measured variables were tested by one-way ANOVA using SPSS software, and/or Student’s t-test. Significance was set at p<0.05.

3. Results

3.1. Volunteer basic information analysis

Table 1 shows that the study participants were male subjects with hyperuricemia, within the age range of 22 and 50. Four subjects noted that they had taken drugs to maintain uric acid levels and six subjects had visual gout. Four out of 14 subjects with hyperuricemia exited the study after the primary screening, including volunteers #11, 12, 13 and 14. Volunteers 11 and 13 left the study because of frequently drinking (at least twice a week on average) and volunteers 12 and 14 left the study for personal issues. Volunteer #8 did not complete the study, because their blood uric acid level returned to a normal state after week 3 (from 538 μmol/L to 314 μmol/L). Further information about the volunteers is available in Table 1.

3.2. The effect of LET on the serum uric acid level in subjects with hyperuricemia

The study of lemon fruit juice in the reduction of blood uric acid level was completely open to volunteers. There were no restrictions
to the individual’s diet and drink, except for alcohol consumption and anti-hyperuricemia drugs. The results in Fig. 2 showed that the blood uric acid levels in volunteers # 1, 2, and 3 were elevated at week 3 but declined at week 6. According to the information provided in Table 1, those three subjects relied on anti-hyperuricemia drugs to control their uric acid levels prior to this clinical trial. They stopped taking the drugs when they started drinking lemon juice in the study. The elevation of blood uric acid levels at week 3 may have been caused by the removal of the drug. The blood uric acid levels in volunteer # 6 at week 3 and week 6 did not decline because the volunteer drank alcohol during those time periods. The blood uric acid level in volunteer # 10 declined at week 6, after rising at week 3.

Further, the serum uric acid levels in volunteers # 4, 8 and 9 showed an almost straight, downward trend; that of volunteer #4 returned to a normal value after drinking the lemon fruit juice for three weeks (Fig. 3). According to food intake questionnaire, these three volunteers drank lemon fruit juice strictly following the principle of one lemon a day and never consumed alcohol during the study.

Moreover, the serum uric acid level of volunteer # 5 dropped at week 3, then rebounded at week 6. This was caused by the subject’s heavy drinking before the third serologic examination. The uric acid level in volunteer #7 increased because he took other drugs for hair loss during the trial study.

To sum up, a total of 14 patients with hyperuricemia were included in this study, and ten volunteers completed the test. In particular, uric acid levels in 3 subjects showed a great downward trend. That indicated that the consumption of the lemon fruit juice had a certain effect of lowering uric acid in hyperuricemic subjects.

3.3. The effect of LET on blood metabolic parameters in patients with hyperuricemia

The content of fasting blood glucose (Glc), CRE and BUN were detected. As shown in Table 2, volunteer # 8 at week 3 had a Glc of 2.4 mmol/L, due to overtime fasting. In the other volunteers, the Glc levels were within healthy range. CRE and BUN are biomarkers of renal function. The CRE and BUN contents were at the normal range, at each time point, suggesting that the consumption of lemon fruit juice for 6 weeks at a dose of a lemon a day, had no side effects on the kidney function of the subjects.

For blood lipid profiling, total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), triglyceride (TG), apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1) were detected. As shown in Table 3, the lemon fruit juice did not cause changes to the blood lipid profiles in all of the subjects. Biomarkers for liver function were also assessed, including: total bilirubin (T-BIL), indirect bilirubin (I-BIL), direct bilirubin (D-BIL), total protein (TP), albumin (ALB), globulin (GLB), the albumin/globulin ratio (ALB/GLB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), AST/ALT, alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) (Table 4). The results showed that drinking the lemon fruit juice lowered the levels of T-BIL, D-BIL and I-BIL in volunteers # 4 and 5, which failed to fall
in the normal range after intervention. I-BIL, D-BIL, and T-BIL levels were under the normal range in other subjects before and after the intervention. GLB concentrations are high, but the ratios of ALB and GLB were all in the normal range for all subjects. The AST/ALT ratios were also in the normal range. Some of the volunteer’s ALP and GGT concentrations changed, but were still within a controllable range. Collectively, the results revealed that the lemon fruit juice had no side effects on the liver function in human subjects.

3.4. The effect of LET on serum uric acid levels in hyperuricemic mice

The results in Fig. 4 show that the serum uric acid levels were significantly higher in the hyperuricemic group than that of the blank control group after 11 days’ gavage of allopurinol; these results indicated the success of establishing the hyperuricemic mouse model. The serum uric acid levels in the LET group and the allopurinol control group were significantly lower than those of the hyperuricemia model group, revealing that LET significantly lowered the serum uric acid level in hyperuricemic mice.

3.5. The effect of LET on XOD activity in mice

In the serum, the XOD activity had no significant change among the hyperuricemia model group, the blank control group, and the LET group; however, it was significantly lower in the allopurinol control group (Fig. 5). There was no change in the liver XOD activity in all 4 of the mice groups.

3.6. The effect of LET on kidney function

Finally, the effect of LET on kidney function was assessed, as shown in Fig. 6. The results demonstrated that serum BUN and CRE levels of the hyperuricemia model group did not significantly increase, as compared to those of the blank control group after LET.
treatment for 11 days. The serum BUN levels had no significant differences between the allopurinol control group and the hyperuricemia model group. The CRE level was significantly lower in the allopurinol control group than that of the hyperuricemia model group, but there was no significant difference when compared to the blank control group. In addition, serum BUN levels of LET group were significantly higher than that of the hyperuricemia model group after treatment. The serum CRE levels of LET mice were lower than that of the hyperuricemia model group and there were no significant differences compared with control group. These results indicate that the effect of LET on kidney function in mice would warrant further studies.

4. Discussion

The study was an open clinical trial including ten patients with hyperuricemia. Intriguingly enough, a significant downward trend in serum uric acid levels was identified in three of the volunteers. Those results, suggested that the lemon fruit juice had a certain effect on lowering uric acid. In addition, the blood GLc, blood lipid, liver and renal functions were detected in the patients with hyperuricemia. The results clearly demonstrated that the lemon fruit juice did not produce side effects on the liver and kidney function in human subjects. Also, it had no significant effect on blood lipid profiles and the glucose level.

Patterns of age-dependent uric acid increase are markedly different in men and women (Musacchio et al., 2016). For this study, we recruited only male patients with hyperuricemia. On the other hand, this study also had some limitations, such as a short intervention time period and a small sample size. However, this study provides promising evidence about the health benefit of lemon fruit juice in reduction of serum uric acid in hyperuricemic patients.

Hyperuricemia occurs as a result of overproduction of uric acid caused by purine metabolic disorder. Hyperuricemia is associated with glycolipid metabolism disorder and dietary factors (Zhu et al., 2016). Foods containing high purine increase the body purine content and the consequent risk of high blood uric acid level. Previous studies have shown that the high fruit and soybean dietary intervention could be an effective alternative to reduce blood uric acid in asymptomatic hyperuricemia patients (Zhang et al., 2016). Patients with high uric acid are recommended to not take drugs that inhibit the excretion of uric acid, such as aspirin and diuretics (Caspi et al., 2000; Lin et al., 2000). However, high blood uric acid can lead to the occurrence of gout (Schlesinger et al., 2014), therefore, Febuxostat and allopurinol are common drugs used to inhibit the production of uric acid. Febuxostat is a potent inhibitor of both the oxidized and reduced forms of XOD (Takano et al., 2005). The side effects of febuxostat and allopurinol are similar (Becker et al., 2005). Patients are more tolerant to febuxostat than allopurinol (Mayer et al., 2005).

XOD is distributed in most mammalian tissues, including blood, with its highest concentrations in the liver (Battelli et al., 1999). XOD is a form of molybdoenzyme protein and it plays an important role in the purine metabolism of the human body (Zhang et al., 2015). First, XOD catalyzes the oxidation of hypoxanthine to produce xanthine; then, it catalyzes the oxidation of xanthine to produce uric acid in the purine catabolic pathway (Harrison, 2002; Hille, 2005). The inhibitors of XOD are commonly used in the control of hyperuricemia, as they can prevent the synthesis of uric acid and reduce the symptoms of the disease (Emmerson, 1996). Allopurinol, an inhibitor of XOD, is often used to lower uric acid (Kuo et al., 2012). In this study, the XOD activity in the serum of the allopurinol control group was significantly lower than in the hyperuricemic model group. The data might suggest that the mechanism of the LET lowering blood uric acid might differ from allopurinol, independent of XOD inhibition.

It has been reported that hyperuricemia is associated with different types of kidney disease. Since the kidney is the primary site of excretion of uric acid, plasma concentration of uric acid is mainly determined by renal handling of uric acid (Fathallah-Shaykh and Cramer, 2014). Serum BUN and CRE levels are considered as biochemical markers for early kidney damage (Zhang et al., 2015). The content of BUN and CRE was also detected in this study.

Uric acid is the end product of purine metabolism in the human body (Petru et al., 2016). Gradually, guanine nucleotide and adenine nucleotide are transformed into xanthine; then, they are turned into uric acid under the action of xanthine oxidase. Uric acid can be further decomposed into soluble allantoin under the action of uric acid oxidase, which exist in most mammals except for human beings (Wilson and Berns, 2012). Potassium oxonate is an urate oxidase inhibitor; it can raise the serum uric acid content by inhibiting the decomposition of uric acid by uricase in mice (Tung and Chang, 2010). Thus, this modeling method is the most commonly used, because it is easy to practice and repeat with laboratory animals.

The lemon fruit juice not only enhances the glomerular filtration rate and the ability of anti-lipid peroxidation, but also reduces the saturation of urine calcium oxalate and calcium phosphate, thereby reducing the occurrence of urinary stones (Aras et al., 2008; Touhami et al., 2007). Based on the mouse experimental model and the clinical trial, this current study presents that lemon fruit juice has a certain effect on lowering blood uric acid, which lays a foundation for the future development of dietary treatment for hyperuricemia in humans.

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References
