Effects of "Chinese yam" on hepato-nephrotoxicity of acetaminophen in rats

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KEY WORDS Dioscorea alata L; paracetamol; hepatotoxicity; nephrotoxicity

ABSTRACT

AIM: To study the effect of yam in Taiwan, which is a commonly used Chinese medicine, on hepato-nephrotoxicity in rats. METHODS: Crude water extract of yam (Dioscorea alata L.), was used to treat rats with an acute toxicity induced by acetaminophen (APAP) challenge. RESULTS: The pharmacological and biochemical studies showed the extract of yam had the effect of kidney securesness and liver fortification (P < 0.01). The pathologic sections showed good improvements in renal tubular degranulation changes, necrosis and disintegration. The extract of yam also possessed a good protection against the inflammation of central vein in necrosis of liver tissue. CONCLUSION: The liver and kidneys are originated from the same source. Pathologically, deficiency of the life essence in the kidney may lead to the blood deficiency in the liver. The results showed that the yam could prevent the damages of the liver and kidneys, thus preserving their functions. This could be the reason why the yam was commonly used in traditional Chinese medicine, as seen in Liuxi Dihuang Wan be used in the case of deficiency of liver-yin and kidney-yin.

INTRODUCTION

Yams, the edible tubers of various species of the genus Dioscorea, are important items in the diets of many tropical countries and are also widely used in most parts of the world because of the carbohydrate they provide. Yam is composed mainly of starch with small amounts of proteins, lipids, and most vitamins and is very rich in minerals. In Brazil, yam has been considered as a source for food system because of its several desirable properties, such as the high stability to high temperature and low pH. In Natal, the yam is heated with water in the scoop out tuber and used to treat sores and wounds in humans and animals. In addition, extracts of yam have exhibited high antibacterial and antioxidant activities.

The yam is also a common source of food and herbal medicine in China, first described in "Shennong Bencao Jing" called "Chinese yam." The origin of Chinese yam has been clarified to be derived from Dioscorea (D) opposita Thunb. D fujii Prain et Burkill, D persimillis Prain et burkill, and D alata L. The Chinese yam produced in Xinxiaog prefecture of Henan Province in China is considered the best, known as "Shanyao." It is also produced in Hebei, Shanxi, Shandong, and some areas in southern and southwestern China. In Taiwan, it is usually used as a tonic nourishment.

The pharmacological effects of Dioscorea on blood sugar reduction, immune improvement, and antioxidation have been reported. The yam flour diet could also be a modulator of chemically induced toxicity. Yam also shows effects in reinforcing the spleen and stomach, promoting the production of body fluid to nourish the lung and kidney channels, improving the symptoms of poor appetite, lassitude or loose stool and diarrhea. It is also used to cure the cough and dyspnea due to lung deficiency, the spermatorrhea due to deficiency of the kidney, leukorrhagia, frequent urination, and diabetes in folk medication.

In spite of long use of "Dioscorea" species in medicine as a source of steroids, little work has been done on phytomedicine properties of this genus. Therefore, in the present study, water extract of yam
(Dioscorea alata L.) as well as the primary antidote of acetaminophen (APAP) poisoning\(^{9-12}\), the N-acetylcysteine (NAC), were used to treat rats with an acute toxicity induced by APAP challenge. Results of this study could help us to understand the action of yam on liver and kidney channels.

**MATERIALS AND METHODS**

**Animals** Male Wistar rats, 4 - 6 weeks old were obtained from the National Laboratory Animal Breeding and Research Center, National Science Council, Taiwan, and maintained at constant humidity (50 % ± 5 %) and temperature (22 ± 3 °C) under a 12-h light/dark cycle. They were allowed free access to standard laboratory diet and tap water at all times, unless otherwise specified.

**Preparation of water extract of Chinese yam**

The Chinese yam (rhizoma of D alata L.) used in this study was purchased from a local herb market at Ming-Jiann village in Taiwan, and later authenticated by Dr Sin-Yie LIFU, Taiwan Agricultural Research Institute. The yam was mashed and deocted with adequate boiling d-H\(_2\)O, two times for 1 h. The decoction was then filtered, mixed, concentrated, and lyophilized. Yield was approximately 9.6 % of dried starting material. A voucher specimen (No FS-nchu 900823) was lodged in the herbarium of the Department of Food Science, National Chung-Hsing University.

**APAP-induced hepato-nephrotoxicity in rats**

The method of acute toxicity induction used in this study has been described, with some modification, by Monto et al\(^{15}\). Rats were divided into five groups of eight animals each. All animals except controls received APAP (1200 mg/kg, ip). Group A received APAP only. Group B received APAP followed 1/2 h later by NAC (1000 mg/kg, sc). Group C and D received APAP 1/2 h later by crude extract of water yam (500 and 1000 mg/kg, ig). Group E received saline (10 mL/kg, ig) and was used as a normal control. After 24 h, all animals were killed and blood was drawn from carotid artery and serum was separated for different assays.

**Assessments of liver and kidney functions**

The drawn blood was centrifuged at 2000 x g, using the centrifuge (KUBOTA 8800, Japan) at 4 °C for 10 min to separate the sera. To assess the liver function, activities of serum glutamate-oxalate-transaminase (sGOT), glutamate-pyruvate-transaminase (sGPT) and y-glutamyl transferase (sGGT) were measured with clinical test kits spectrophotometrically on a “Home Screen” chemistry analyzer system (ARTAX\(^{49}\)). For renal function assessment, levels of blood urea nitrogen (BUN), creatinine and uric acid were also measured using the ARTAX\(^{49}\).

**Histopathological observation** After blood draining, kidney and liver sections were taken from each lobe of the organ. The tissue was fixed in 10 % neutral-buffered formalin, dehydrated with different ethanol solutions from 50 % - 100 % and embedded in paraffin, then cut into 4 - 5 μm thick sections, stained with haematoxylin-eosin and were observed under a photomicroscope.

**Statistical analysis** Data were express as \( \bar{x} \pm s \) (\( n = 6 \)) and statistically assessed by one-way analysis of variance (ANOVA). The difference between yam-treated and control group was evaluated by Student’s \( t \)-test using the Sigma plot software program. \( P < 0.05 \) was regarded as statistically different.

**RESULTS**

**Effects of yam on APAP-induced hepatotoxicity** Administration of APAP resulted in a marked increase in liver transaminase activities, which were significantly different from those of the vehicle control group (Fig 1; bar B vs bar A). The sGOT, sGPT, and sGGT activities of drugs-treated groups are summarized in Fig 1. Treatments by the extracts of yam (500 mg/kg, 1000 mg/kg) and NAC (1000 mg/kg) significantly reduced the enzyme activities promotion caused by APAP-intoxication (\( P < 0.01 \)), except sGGT activity in NAC group.

APAP insult to rats caused significant derangement in cytoarchitecture of the liver. The slice showed a focal centrilobular necrosis (bridging confluent necrosis, in zonal 3), heterophill infiltration and sinusoid extension in Fig 2B. Treatment of animals with yam reversed, to a large extent, the hepatic lesions produced by APAP, as were obvious from the absence of cellular necrosis, lymphocytes infiltration around central vein, but with some extent of sinusoid extension (Fig 2C and 2D). In the reference group, ie, treatment with NAC (1000 mg/kg), the hepatic architecture pattern, with mild eosinophylc change scattered throughout the parenchyma in zonal 3, was as the same as in the yam treating groups (Fig 2E).

**Effects of yam on APAP-induced nephrotoxicity** Tab 1 showed the serum markers of kidney in
creatinine, and uric acid, which were significantly different from those of the control group. Treatment of rats with yam exhibited a significant reduction of the biochemical parameters viz. BUN, creatinine, and uric acid, induced by APAP-intoxication \( (P < 0.01) \).

The kidney of APAP-intoxicated rats showed acute damages in glomerulus and proximal tubule severely. There were glomacular bleeding and partial endothelial rupture in Bowman’s capsule. Proximal tubules showed loss of cellular boundary, aggregation of chromatin in nucleus, loss of brush border, necrosis, and disintegration. Epithelium of convoluted tubule was swelling, budding off of vesicles. Cytoplasm contained many minute granules. Some epithelia were broken, debris and granules leaked into tubular lumen (clogging of lumen) in Fig 3B. The histological pattern of the kidney of the rats treated with yam showed a normal tubular pattern with a mild degree of swelling, necrosis, and degranulation (Fig 3C and 3D).

**DISCUSSION**

APAP is a widely used analgesic-antipyretic agent, which has few adverse effects when taken in the usual therapeutic dose. If taken in large overdoses, it becomes a potent hepato-toxin, producing fulminant hepatic and renal tubular necrosis, which can be lethal both in humans and animals\(^{14}\). Most instances of APAP-related hepatic injury have resulted from large, single overdoses taken in an attempt at suicide. A few cases have involved accidental resulted from large single or multiple doses taken with therapeutic intent\(^{15}\). The characteristic zone 3 necrosis of APAP appears to be produced by an electrophilic metabolite of the drug \( N\)-acetyl-p-benzoquinonimine, NAPQI\) that binds covalently to tissue macromolecules and probably also oxidizes lipoprotein and the critical sulphhydryl groups (protein thiols) and alters the homeostasis of calcium. The zone

**Tab 1.** Renal protective effects of water extract of Yam and NSC on APAP-induced toxicity in rats. \( n = 6 \). \( \bar{x} \pm s \). \( P < 0.01 \) vs normal group. \( P < 0.05 \), \( P < 0.01 \) vs APAP group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose/mg·kg(^{-1})</th>
<th>BUN/mg·L(^{-1})</th>
<th>Creatinine/mg·L(^{-1})</th>
<th>Uric acid/mg·L(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
<td>132 ± 17</td>
<td>4.18 ± 0.03</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>APAP</td>
<td>1200 (ip)</td>
<td>184 ± 8(^f)</td>
<td>5.432 ± 0.025(^s)</td>
<td>15 ± 4(^s)</td>
</tr>
<tr>
<td>APAP + yam</td>
<td>500 (ig)</td>
<td>147 ± 7(^f)</td>
<td>4.757 ± 0.010(^s)</td>
<td>9.67 ± 0.05(^s)</td>
</tr>
<tr>
<td>APAP + yam</td>
<td>1000 (ig)</td>
<td>140 ± 8(^f)</td>
<td>4.548 ± 0.022(^s)</td>
<td>9.50 ± 0.11(^s)</td>
</tr>
<tr>
<td>APAP + NAC</td>
<td>1000 (ac)</td>
<td>217 ± 47</td>
<td>5.4 ± 0.4</td>
<td>14.00 ± 0.21</td>
</tr>
</tbody>
</table>
Fig 2. The photomicrographs of liver section taken from rats. A: Control (saline injection only). B: APAP (1200 mg/kg) injection only. C: APAP + pam (500 mg/kg). D: APAP + pam (1000 mg/kg). E: APAP + NAC (1000 mg/kg). Note: CV for central vein, P for portal area. HE stain, ×700.

3 location of the necrosis is a consequence of the location in that zone of the enzyme system (cytochrome P-450) responsible for converting APAP to its active metabolite-NAPQI. The amount of the metabolite formed normally is small, since the therapeutic dose of APAP taken is not large and its metabolic fate is largely in the direction of conjugation with glucuronate and sulfate. The small amounts of active metabolite formed are readily detoxified by reaction with glutathione (GSH) to form mercapturic acid. Hepatic necrosis occurs only when the amount of active NAPQI produced exceeds the binding capacity of GSH. This occurs when the dose of drug taken is large. The adverse effects of a large dose or even smaller dose are enhanced by factors that increase the fraction of drug converted to an active NAPQI or decrease the availability of GSH. The likelihood, accordingly, that a dose of APAP will lead to hepatic injury depends on the quantity ingested, the activity of the cytochrome P-450 system, and the adequacy of GSH stores[16].

In kidney, cytochrome P-450 is localized almost exclusively in the proximal tubule with negligible (if any) activity in the glomerulus, distal tubules, or collecting ducts. Thus, nephrotoxicity requiring P-450 mediated bioactivation will most certainly be localized in the proximal tubule[16]. Because of their active absorptive and secretory activities, the proximal tubules often have higher concentration of toxicants[19]. Blood urea nitrogen is derived from normal metabolism of
protein and excreted in the urine. Elevated BUN usually indicates glomerular damages. Its level can also be affected by hepatotoxicity resulted from toxicants. Creatinine is a metabolite of creatine and excreted in the urine via glomerular filtration and the tubule. Elevation of creatinine in the blood thus can be an indication of impaired kidney function.

Non-narcotic analgesics are effective inhibitors of prostaglandin synthetase; a lack of endogenous prostaglandins, which act as vasodilators, could lead to ischemia of the tissue and eventually to necrosis[20]. The kidney of APAP-intoxicated rats showed acute damages in proximal tubule severely in Fig 3B. The morphological changes of this study are consistent with the principles mentioned above. Through the observation of pathological specimens and the examinations of BUN, creatinine and uric acid, the extract of yam shows a better improvement in repairing the liver cells, damaged by administering the APAP to the animals, as compared to the NAC.

All these results show that the yam is beneficial to the cells of the liver as well as the kidneys. This could be the reason why the yam is used in traditional Chinese medicine, as seen in Liuwei Dihuang Wan to treat the deficiency of liver-yin and kidney-yin.

This study lends some supports to traditional knowledge and can serve as a basis for use in traditional medicine practices in the future.
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REFERENCES


山药保护对乙酰氨基酚诱导的大鼠肝肾损伤

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目的：研究山药的常用中药一山药对肝肾毒性的作用。方法：对乙酰氨基酚诱导大鼠急性损伤方式进行山药水提取物之疗效评价。结果：山药显示突出的保肝护肾效果( P < 0.01)。病理组织学上发现山药对肾小管萎缩、坏死、骨髓凋亡有显著的保护作用，并且对肝中央静脉炎症及实质组织坏死都有显著的保护作用。结论：山药能同时对肝及肾细胞有益，而达保肝护肾效果。这是山药常常被使用如“六味地黄丸”之中的用于肝肾耐盐的机制。

关键词：山药；对乙酰氨基酚；肝毒性；肾毒性

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