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Full Length Research Paper

The water decoction of *Aristolochia debilis* Sieb.et Zucc induces renal toxic effects in rats

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The present study was designed to investigate toxic effects of different concentration of the *Aristolochia debilis* Sieb.et Zucc. on renal functions in rats. Wistar rats were randomly divided into low dosage group (treated with *A. debilis* Sieb.et Zucc at a dose of 0.81 g.kg^{-1}.d^{-1} for three months), moderate dosage group (at a dose of 4.05 g.kg^{-1}.d^{-1}), high dosage group (at a dose of 8.1 g.kg^{-1}.d^{-1}) and control group. The renal function and urine nacety-β-D-amino-glucosidase (NAG), blood urea nitrogen (BUN) and serum creatinine (Scr) was measured in 1, 2 and 3 months. The histopathological changes were examined by light and electron microscopy. The urine NAG level was elevated in High dosage group and Moderate dosage group, but there were no significant differences of BUN and serum creatinine among four groups. The renal histopathological examination showed that slight tubular-interstitial injury was detected in moderate dosage group and obvious renal injury was observed in High dosage group. Nevertheless, there were no renal abnormalities in Low dosage group. In summary, low dosage of *A. debilis* Sieb.et Zucc was rather safety and did not cause the renal toxicity. Nevertheless, moderate and high dosage of *A. debilis* Sieb.et Zucc may impair renal function and induce renal tubular-interstitial injury.

Key words: *Aristolochia debilis* Sieb.et Zucc., aristolochic acid, renal injury, NAG, BUN.

INTRODUCTION

During the past decades, clinical evidence presented that some herbs containing aristolochic acid often caused renal injury, which has drawn wide attention of the medical field (Zhu et al., 2005; Stiborova et al., 2008). The renal toxicity of herbs which contain aristolochic acid was usually called as “Aristolochic Acid Nephropathy” (Debelle et al., 2008). However, it is undeniable that the concept of “Aristolochic Acid Nephropathy” has brought great negative influence on the application of herbs worldwide (Debelle et al., 2008). For example, the Food and Drug Administration (FDA) had published two announcements continuously to point out that products which contain aristolochic acid were potential carcinogen and even had renal toxicity. Thus, it is very necessary to clarify the toxic dosage and safety dosage of *Aristolochia debilis* Sieb.et Zucc on kidney. The present study was therefore to study toxic effects of different doses of aristolochia debilis Sieb.et Zucc on kidney. Wistar rats were treated orally with different doses of water decoction of *Aristolochia debilis* Sieb.et Zucc for three months. Successive observation lasted for three months on the relationship among nephrotoxicity of aristolochia debilis Sieb.et Zucc, drug dosage and administration time. A safety-dosage evaluation of aristolochia debilis Sieb.et Zucc in kidney was made. The urine NAG, BUN and serum creatinine was determined by urine and blood auto-analysator. The light and electron microscopy were employed to observe the histopathological changes in rat kidney.

MATERIALS AND METHODS

Experimental animal

The 40 male Wistar rats with a weight between 140 and 160 g were

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Table 1. Effects of *Aristolochia debilis* Sieb.et Zucc. on urine specific gravity.

Group	No.1 month	No.2 month	No.3 month
Low dosage group	1.021 ± 0.007	1.021 ± 0.008	1.019 ± 0.007
Moderate dosage group	1.019 ± 0.007	1.021 ± 0.007	1.020 ± 0.007
High dosage group	1.019 ± 0.008	1.020 ± 0.008	1.019 ± 0.007
Control group	1.020 ± 0.007	1.021 ± 0.006	1.020 ± 0.006

provided by Experimental Animal Center of Harbin Medica University. The 40 rats were randomly divided into four groups: control group, low dosage group, moderate dosage group and high dosage group.

Low dosage group: 0.81 $g.kg^{-1}.d^{-1}$ water boiled aristolochia debilis Sieb.et Zucc was given to rats for three months. Moderate dosage group: 4.05 $g.kg^{-1}.d^{-1}$ water boiled *A. debilis* Sieb.et Zucc was given to rats for three months. High dosage group: 8.1 $g.kg^{-1}.d^{-1}$ water boiled aristolochia debilis Sieb.et Zucc was given to rats for three months. Control group: Water was a dose given to rats for three months.

In three months, the rat bodyweight were recorded every week, the dosage of *A debilis* Sieb.et Zucc was adjusted according to rats' bodyweight at that week. The experimental protocols for this animals study were approved by our Institutional Animal Care and Use Committee and the animals were cared in accordance with the Guidelines for Animal Experiments of the Heilongjiang University of Traditional Chinese Medicine. The temperature of laboratory was 20 ± 2℃.

Water decoction of *Aristolochia debilis* Sieb.et Zucc

A. debilis Sieb.et Zucc was provided by the department of traditional Chinese medicine at the first affiliated hospital of Heilongjiang University of Traditional Chinese Medicine and passed through general characteristic identification and microscopically identification.

The 500g Aristolochia debilis Sieb.et Zucc was soaking with water for 1 h, then to heat up to boiling, to drench drug juice. After 30 min, 2000 ml water was added into herb residue, to heat up to boiling, to drench drug juice to filter and concentrate the two drug juice to make 500ml herb liquor after 20 min (1 g crude drug/ml), to conserve at 4℃, to reheat it to room temperature by electric furnace before use.

Urine assay

The 2 ml urine samples were collected from the rats in 1, 2 and 3 months. The urine specific gravity and urine glucose were measured by using urine analysator. Nacety-β-D-amino-glucosidase (NAG) was detected by Nanjing bioengineering institute kit according to the instruction of kit.

Blood assay

In 1, 2 months, the 3 ml blood was taken from mice's epicanthic intravenous of each group. In 3 months, the 3 ml blood samples were collected in tubes after rat's head removed. The blood serum was obtained under the centrifuge condition (4℃, 3000 r/min, 10 min) and Scr and BUN were determined by blood biochemistry auto-analyzer. Animals were fasting, but water was given on the day before blood collected.

Renal histopathology

After All Wistar rats were killed by removing head, one unilateral renal from rat was taken and fixed in 4% formaldehyde for 8 h. The HE staining was made in order to observe the effects of aristolochia debilis Sieb.et Zucc on renal histopathology by light microscope. Another renal was snapped and immediately placed it in 3% glutaraldehyde solution and then was stored in 4℃ in refrigerator. The electron microscope was employed to observe the effects of aristolochia debilis Sieb.et Zucc on renal microstructure.

Statistics analysis

The experimental data was presented as $\bar{x} \pm s$ examination and analyzed with SPSS 12.0 statistics software. All statistical comparisons were made by the means of one-way analysis of variance (ANOVA). The difference showing a level of $p < 0.05$ was considered to be statistically significant.

RESULTS

Effects of *Aristolochia debilis* Sieb.et Zucc on urine specific gravity

There were no significant differences of urine specific gravity among three *A. debilis* Sieb.et Zucc groups and control group at the end of 1, 2 and 3 months ($P > 0.05$) (Table 1). Additionally, urine glucose was not also detected among these four groups.

Effects of *Aristolochia debilis* Sieb.et Zucc on urine NAG

In 1 and 2 months, urine NAG level only in High dosage group was significantly higher than control group ($P < 0.05$). In 3 months, urine NAG in Moderate dosage group was also higher than control group ($P < 0.05$). There was no significant difference of urine NAG between Low dosage group and control group in 1, 2 and 3 months ($P > 0.05$) (Table 2).

Effects of *Aristolochia debilis* Sieb.et Zucc on urine BUN

There were no significant differences of urine BUN among three *A. debilis* Sieb.et Zucc groups and control

Table 2. Effects of *A. debilis* Sieb.et Zucc. on urine NAG (u/g.Cr).

Group	No.1 month	No.2 month	No.3 month
Low dosage group	30.31 ± 6.21	32.93 ± 3.66	32.67 ± 2.21
Moderate dosage group	30.81 ± 5.02	33.82 ± 2.92	35.91 ± 4.22
High dosage group	30.76 ± 8.36	37.78 ± 3.03	41.08 ± 3.57
Control group	31.73 ± 8.36	32.23 ± 4.46	33.29 ± 4.58

Notes: $P < 0.01$, compared with the control group.

Table 3. Effects of *Aristolochia debilis* Sieb.et Zucc. on BUN (mmol/l).

Group	No.1 month	No.2 month	No.3 month
Low dosage group	6.65 ± 0.82	6.97 ± 0.72	6.87 ± 0.66
Moderate dosage group	6.24 ± 0.72	6.67 ± 0.57	7.01 ± 0.96
High dosage group	6.20 ± 0.67	6.98 ± 0.47	6.99 ± 0.42
Control group.	6.15 ± 0.65	6.66 ± 0.73	6.79 ± 0.99

Table 4. Effects of *Aristolochia debilis* Sieb.et Zucc. on serum creatinine (μmol/l).

Group	No.1 month	No.2 month	No.3 month
Low dosage group	29.64 ± 3.69	28.47 ± 5.45	31.96 ± 3.42
Moderate dosage group	30.95 ± 4.12	32.83 ± 2.10	31.17 ± 2.65
High dosage group	28.13 ± 2.30	30.94 ± 4.04	31.24 ± 2.70
Control group	30.62 ± 2.80	31.23 ± 2.79	30.74 ± 2.36

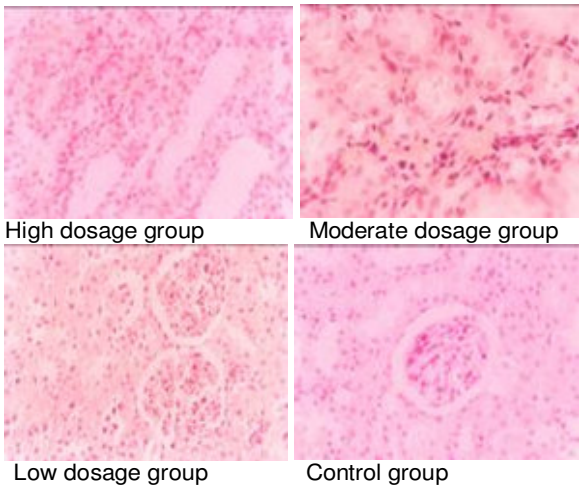

Figure 1. Effects of Aristolochia debilis Sieb.et Zucc. on histomorphology (Light microscope).

control group in 1, 2 and 3 months ($p > 0.05$) (Table 3).

Effects of *Aristolochia debilis* Sieb.et Zucc on serum creatinine

Although serum creatinine was increased with the time prolonged in every group, there were no significant differences among three *A. debilis* Sieb.et Zucc groups and control group ($p > 0.05$) (Table 4).

Effects of *Aristolochia debilis* Sieb.et Zucc on renal histopathology

In the light microscope, the lumen of renal tubule in the boundary between cortex and medulla was significantly dilated in High dosage group (Figure 1). The protein casts which have been stained red also appeared. Component of the renal interstitium was locally increased. The same symptoms in Moderate dosage group, but those were slighter. Histopathology abnormality was not found in Low dosage group and Control group. Glomerular did not have abnormal changes in all groups.

In the electron microscope, floc could be seen at the lumen of renal tubule and the lumen of renal tubule was dilated in High dosage group (Figure 2). Renal tubular epithelial cells did not have clear boundary, arranged in disorder and locally ruptured. Nuclei were pyknotic. Microvillus of renal tubule was sparsely distributed and unclear, part of microvillus vanished. Mitochondria were swollen significantly. Mitochondrial cristae and mitochondrial membrane dissolved. Hyperplasia and fibrosis could

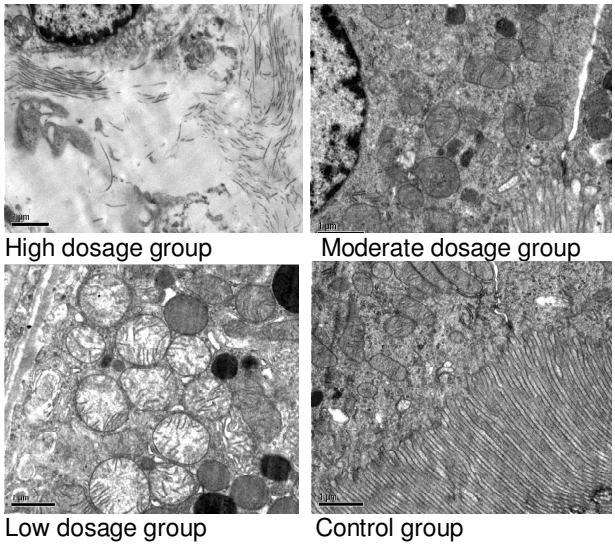

Figure 2. Effects of *A. debilis* Sieb.et Zucc. on histomorphology (Electron microscope).

be observed in renal interstitium. Glomerulus did not have abnormal changes. Moderate dosage group: microvilli of renal tubule arranged well and microvilli vanished once a while; mitochondria decreased in number individual mitochondrial membrane is incomplete; Hyperplasia and fibrosis could be observed in renal interstitium. Glomerular did not have abnormal changes. Obvious abnormality was not found in Low dosage group and control group.

DISCUSSION

A. debilis Sieb.et Zucc. is also named as roots of *A. debilis*, *A. debilis* Sieb. et Zucc, roots of aristolochic, Elecampane, *Crispateleaf ardisia* root and rhizome, etc (Arlt et al., 2002). The main components of *A. debilis* Sieb.et Zucc. are aristolone, Aristolochic Acid, Allantoin, acid of *A. debilis* Sieb.et Zucc., magnoflorine, etc (Tomita, 1962). Its action is to relieve pain by subduing hyperactivity of the liver and to counteract toxicity and cause subsidence of swelling. It is used to treat dizziness, headache and distending pain in the chest and abdomen, carbuncles, boils, snake bite and insect bite (Wang et al., 2007). Ma YZ reported a case that a patient who once took 250 g Aristolochia debilis Sieb.et Zucc suffered from renal failure (Ma, 1997). However, the usual dose of Aristolochia debilis Sieb.et Zucc. is 4.5 - 9 g. Therefore, it has practical significance to evaluate the toxicity and the relationship between toxicity and dosage of *A. debilis* Sieb. et Zuccc. The results showed that urine NAG in the High dosage group was significantly increased than that in Control group after two months ($P < 0.05$). After administrated three months continuously, urine NAG was also increased in moderate dosage group. there were significant differences among high dosage group, moderate dosage group and low dosage group. It was shown that high dosage of *A. debilis* Sieb.et Zucc could lead to renal damage. Furthermore, renal damage was progressively injured with time prolonged. Morphological examination showed that lesions of High dosage group mostly located in renal tubulointerstitial. Light microscope observation showed that the lumen of renal tubule in the boundary between cortex and medulla dilated significantly. Hyperplasia and renal interstitial fibrosis could be observed in renal interstitium. Glomerular did not have abnormal changes. It is similar to the characteristics of renal damage caused by traditional Chinese medicine which contains aristolochic acid reported in literature. The main manifestation is the damage of function of renal tubule. Therefore, we consider that aristolochic acid is the main toxicity composition which caused renal damage in Aristolochia debilis Sieb.et Zucc. In the present study, serum creatinine and BUN that reflect glomerular filtration function did not increase obviously. It also proved that renal damage in rats caused by Aristolochia debilis Sieb.et Zucc. is mainly at renal tubulointerstitial. There are no disadvantages to glomerular. Maybe it is due to our shorter test time and lower dose. It showed that taking Aristolochia debilis Sieb.et Zucc. at a moderate dose for three months also could cause damage of renal tubule. It is found that the damage is similar to High dosage group in histopathology examination, mainly focus on renal tubulointerstitial, but those were slighter and more limited than that of High dosage group. Low dosage group: during the experimental period, all the monitoring indexes and histopathology examination did not have significant abnormal changes. Every drug should have the safe dose range. There are strict regulations about administration methods and dosage in herbs use. Ye (1999) took advantage of compound prescription which contains 15 g Aristolochia debilis Sieb.et Zucc. to treat 65 cases of acute renal failure caused by bitten by poisonous snakes and have a remarkable effect (Ye, 1999). However, when dose increased to 250 g it could lead to renal failure. Our experiment also proved that high dose Aristolochia debilis Sieb.et Zucc could lead to renal tubulointerstitial to a certain degree, but the low dose should have no significant side effect. The strength of the present study is presenting that low dosage of aristolochia debilis Sieb.et Zucc is relatively safety and does not cause the renal abnormalities, but high and moderate dosage of *A. debilis* Sieb.et Zucc may induce renal tubular-interstitial injury. The limitation is that this study was carried out in animal not in human body.

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