The renal toxicity of hydroalcoholic extract of Stachys lavandulifolia Vahl in Wistar rats

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ABSTRACT: Stachys lavandulifolia is used as the herbal tea in gastrointestinal disorders. It is believed that this plant has beneficial curative properties. However, more studies are needed to determine the toxic effects of plant. The aim of this study was to evaluate the nephrotoxicity of hydro-alcoholic extract of *Stachys lavandulifolia* Vahl on male Wistar rats. In this experimental study, 100 adult male Wistar rats (200-250 g) were divided into 5 groups of 20; including one control and 4 experimental groups, and injected i.p saline or *Stachys lavandulifolia* Vahl extract (50,100,150 and 200 mg/kg) for 1 month. Then sampling was done from half of the animas of each group. The left animals in each group were held without injection for one more month and then sampling was done. In the groups that *Stachys lavandulifolia* Vahl extract were used for one month, a mild degeneration of renal tubular epithelial cell was observable. However, in the second month of the study, the histologic lesions were significantly more ($P<0.05$). *Stachys lavandulifolia* Vahl extract has renal tubular toxicity and this toxicity may continue even following drug discontinuation. However, further studies need to evaluate renal complications of this drug in human.

[Taghikhani M, Nasri H, Asgari A, Afrough H, Namjoo AR, Ansari-Samani R, Shahinfard N and Rafieian-kopaei. **The renal toxicity of hydroalcoholic extract of Stachys lavandulifolia Vahl in Wistar rats**. Life Sci J 2012;9(4):3025-3031] (ISSN:1097-8135). http://www.lifesciencesite.com. 444
Keywords: Lamiaceae family, Therapeutic properties, Renal toxicity, Tubular degeneration

Introduction

For thousands of years people have used plants and herbs as curative elements (1,2). Stachys lavandulifolia (Lamiaceae) is widely used in various parts of the world as herbal tea. It is used for the treatment of gastrointestinal and respiratory disorders. The genus Stachys, which belongs to the Lamiaceae family, consists of about 280 species (3-6). Plants of this genus also exhibit dose-dependent antibacterial activities against different bacteria. The extracts are more active against Gram-positive microorganisms compared to Gram-negative bacteria (7). The plant has also some anti-tumor activity (8). This effect is attributed to flavonoids, phenylpropanoids or terpenoids of the aerial parts of this plant (9-11). Germacrene-D, betaphellandrene, beta-pinene, myrcene and alpha-pinenehave have been reported to be the main components of the essential oil of S. lavandulifolia (9-11).

A phenylethanoid glycoside, lavandulifolioside A, lavandulifolioside B, verbascoside, leucosceptoside A, and an iridoid glycoside, 5-O-β-allopyranosyloxy-aucubin have also been isolated from the flowering aerial parts of the

plant (10). The existence of flavonoids such as apigenin and luteolin are also demonstrated in aerial parts of S. lavandulifolia (11).
In spite of widespread use in Iran, the pharmacological characteristics of the Stachys lavanduifolia and its probable toxicities have not been studied in detail. Therefore, the aim of this study was to evaluate the renal effect of hydroalcoholic extract of *Stachys lavandulifolia* Vahl in male Wistar rats.

Materials and methods

Extraction method

Aerial part of *Stachys lavandulifolia* Vahl was gathered from Chaharmahal & Bakhtiyari province in Iran, in July 2011 and authenticated at the Medical Plants Research Center, Shahrekord University of Medical Sciences (Voucher no 78).
The *Stachys lavandulifolia* Vahl leaves were dried and powdered. Then, 500 grams of the powder were macerated with ethanol (70%) at 28°C for 24 hours and filtered. The extraction process continued two times and then was concentrated in a rotary evaporator under low pressure to give one third of the primary volume. The solution was then dried by oven at 40°C. The dried extract was reconstructed with distilled water to make 50, 100,150 and 200 mg/kg doses.

Experimental studies

In this experimental study, 100 adult male Wistar rats (200-250 g) were used. The animals were divided randomly into 5 groups of 20; including one control and 4 experimental groups. Five groups of animals were injected i.p saline or *Stachys lavandulifolia* Vahl extract (50,100,150 and 200 mg/kg) for 1 month. Then sampling was done from half of the animas of each group (13-16). The left animals in each group were held without injection for one more month and then sampling was done.

Histology

After the rats were anesthetized with ether, systematic method of dissection was done. Sterile incision was made in the specific location. Kidneys were removed and examined. Then a longitudinal incision was made on kidneys. One half of kidney for staining with hematoxylin and Eosin (H&E) was placed in 10% buffered formalin solution for 24 hours .The Staining routine method with H&E was done and histopathology slides were prepared. Using optical microscopy the toxicity was evaluated qualitatively (12-20). Statistical analysis was done using Chi-square test.

Results

Effects of Stachys lavandulifolia Vahl extract on renal tubular epithelial cells after first month is shown in table 1. Following one month drug usage the degeneration of renal tubular epithelial cell was mild. In the second month (one month after drug cessation) there was a significant increase in renal tubular epithelial cells degeneration compared to the first month. The results indicate that the degeneration of renal tubular epithelial cells was increased with time, even after drug cessation ($P<0.05$). Tabe 2 also shows that the necrosis of epithelial cells in the second month have been more than first month ($P <0.05$).
The result of interstitial mononuclear cell infiltration in kidneys of rats showed that the amount of infiltration in the first and the second months was almost identical (Table 3). The amounts of fibrous tissue in the medulla of the kidney tissue sections as well as the mononuclear cell interstitial tissue of rats in the first and the second month were also almost similar.
In table 4, the frequency distribution of fibrous tissue in the medulla is shown. The results of this table show that the amounts of fibrous tissue in the medulla in the first and the second months are not different.

Discussion

The results showed that injection of *Stachys lavandulifolia* Vahl with different concentrations

had toxic effects on renal tubule cells. The toxicity was substantiated after cessation of drugs for 1 month. The results also showed that the toxicity was dose dependent.

The safety profile of this plant in acute, subacute and subchronic tests was determined in Monji et al. (21). To assess the toxicity profile of this extract, female mice were administered the extract by oral gavages in acute (24 hrs), subacute (14 days) and subchronic (45 days) models. All clinical, hematological, biochemical and histopathological changes were assessed in appropriate mid points and end points and compared with control group. Doses up to 140 mg⁄kg were recognized as maximum tolerated dose in subchronic model. Abnormal changes in kidney and liver weight in treatment groups as well as the significant elevation of biochemical parameters in 45 study days has suggested the possible hepatic and renal toxicity potentials of S. lavandulifolia extract with doses upper than 140mg⁄kg. Doses up 70 mg⁄kg had no observable adverse effect. Therefore, it was concluded that low doses could be used in clinical trials on the possible therapeutic effects (21).

Phenylpropanoids belonging to the largest group of secondary metabolites is produced by plants, in response to biotic or abiotic stresses such as infections. It is thought that the molecular basis for the protective effect of phenylpropanoids in plants is their antioxidant and free radical scavenging properties. It was determined from other studies that potential safety issues exist if suitable doses of flavonoids and isoflavones were consumed daily. Since the protective effects of Phenylpropanoids on the liver and kidney (22-24). and nephroprotection by flavonoid, epigallocatechin gallate and phenolics, propyl gallate and nordihydroguaiaretic acid, have been demonsrated in mice. (25-27), presumably other compounds are toxic. Phenol ring-containing flavonoids, upon oxidation by peroxidases, yield phenoxyl radicals which are cytotoxic (28-30). Other specific researches are necessary to find out the exact toxic component in this plant. In this regard and based on the results of this study the consumption of this plant should be with caution.

Conclusion

It must be kept in mind that clinicians should remain cautious until more definitive studies demonstrate the safety, quality and efficacy of S. lavandulifolia (31). For these reasons, extensive pharmacological and chemical experiments, together with human metabolism will be a focus for future studies. In this study, we also found that the use of extract can cause side effects in some cases, such as damage to the kidneys, even in some cases, the damage goes far necrosis of renal tissue (32-33).

Conflict of interest

The author declared no competing interests.

Acknowledgements

This study was extracted from M.D thesis and supported by the Research Deputy of Shahrekord University of Medical Sciences (grant #813).

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11/24/2012

Table1. Frequency of renal tubular epithelial cell degeneration in rats studied

Dose mg/kg/day	Degeneration of renal tubular epithelial cells					
	First Month			Second Month		
	moderate	mild	No lesion	moderate	mild	No lesion
50	0	6	0	0	0	3
100	0	5	1	0	0	6
150	1	6	0	0	0	9
200	0	7	0	0	2	3
Total	1	24	1	0	2	21

Table2. Frequency of necrotic epithelial cells in kidney slices of rats

Dose mg/kg/day	Necrosis of epithelial cells					
	First Month			Second Month		
	moderate	mild	No lesion	moderate	mild	No lesion
50	-	2	6	-	1	2
100	-	2	4	-	4	2
150	-	1	3	-	8	1
200	-	0	2	-	5	0
Total	-	5	15	-	18	5

Table.3: Distribution of mononuclear cells in the interstitial tissue slices of rat kidney

Dose mg/kg/day	mononuclear cells in the interstitial tissue					
	First Month			Second Month		
	moderate	mild	No lesion	moderate	mild	No lesion
50	-	0	6	-	-	3
100	-	1	5	-	-	6
150	-	2	5	-	-	9
200	-	0	7	-	-	5
Total	-	3	23	-	-	23

Table.4: The frequency distribution of fibrous tissue in the medulla

Dose mg/kg/day	fibrous tissue in the medulla					
	First Month			Second Month		
	moderate	mild	No lesion	moderate	mild	No lesion
50	-	0	3	1	2	0
100	-	2	5	0	3	2
150	-	7	5	0	2	7
200	-	5	5	0	0	5
Total	-	14	18	1	7	14