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In vitro effect of hydroalcoholic extract of *Peganum harmala* on human ureteric contractions

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ABSTRACT

Introduction: Ureteral obstruction by stones is one of the most common urological problems. **Objectives:** This study aimed to investigate the in vitro effects of hydroalcoholic extract of *Peganum harmala* L. on the contraction of the human ureter.**Materials and Methods:** In this study, 28 samples of human ureter tissue were studied. Six tissue samples were examined for the evaluation of *P. harmala* extract at concentrations of 1, 2, and 4 mg/mL. Moreover, five tissue samples were examined for checking NaCl solution since, six pieces of tissue for the examination of prazosin 10⁻⁸M solution.**Results:** There was a significant difference between the second contraction of *P. harmala* group with concentration of 4 mg/mL and NaCl group ($P < 0.001$). Additionally, a significant difference between the prazosin and *P. harmala* groups with a concentration of 4 mg/mL ($P = 0.048$) was observed. There is a marginal difference between the secondary contractions in two groups. A significant decrease in secondary contraction (15 minutes after adding the solution) was observed between the prazosin group and the *P. harmala* group at a concentration of 1 mg/mL ($P < 0.001$). Moreover, the secondary contraction of the prazosin group showed a significant decrease compared with the NaCl group ($P < 0.001$).**Conclusion:** Administration of *P. harmala* extract can be useful in reducing urinary human ureteric contractions while the best effect was achieved at 4 mg/mL of *P. harmala* extract.

Implication for health policy/practice/research/medical education:

Administration of *Peganum harmala* extract can be useful in reducing urinary ureteric contractions as a supplementary drug.**Please cite this paper as:** Shirani M, Lorigooini Z, Pouriamofrad A, Keivani Hafshejani Z. In vitro effect of hydroalcoholic extract of *Peganum harmala* on human ureteric contractions. J Renal Inj Prev. 2019;8(3):235-239. DOI: 10.15171/jrip.2019.44.

Introduction

Renal colic is one of the most commonly referred cases of emergency ward seen among the people aged 30 to 40 years with prevalence of 3 in 1000 every year, which is usually described as a severe pain in the flanks that is spread to the inguinal region and is associated with nausea and vomiting in 50% of people. In the United States, 40% of the people refer to the emergency ward for the first time due to the renal colic, which has been doubled over the past decade (1-3). The prevalence of this disease is in both genders and all races, although it is seen more in the African-American and Caucasian breeds. Most of the causes of renal colic are due to the presence of upper urinary tract stones and urinary tract obstruction, and about 80% of the urinary tract stones are of calcium oxalate type (2). One of the most common causes of ureteral obstruction is the movement of stones from the kidneys and their capture in the ureter.

This discomfort causes severe pain, which is characterized by sudden acute pain, nausea, vomiting, pallor, hematuria, and change in the color of the urine and, in those who have a single kidney, anuria can be mentioned as a sign of strangulation of the stone in the ureter (1-3). Although the ureteric tissue is without extensive nerve tissue, there are several receptors including cholinergic and adrenergic receptors on the ureteric tissue, while 70% of which is alpha-adrenergic. Alpha-receptors are stimulated by the epinephrine and phenylephrine increased contractions, and alpha receptor blockers, such as prazosin reduces tissue contractions (1-3).

One of the plants that have been considered in traditional medicine in Iran for a long time is *Peganum harmala*. The alkaloids of this plant have extensive pharmacological effects in various fields such as antispasmodics, anticancer effects (4,5), antihistaminic monoamine oxidase inhibitors

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(6), agonist to receptors such as 5-TH and binding to benzodiazepine receptors (7), anti-platelet aggregation (8), immunomodulatory effect (9), and also the effect of decreasing blood pressure (10). Based on previous studies and the similarity of the receptors on the ureter and muscle tissue of the prostate, drugs that affect the reduction of ureteric contractions can be expected to have a similar effect on the contraction of the prostate tissue and to reduce the symptoms of the lower urinary system.

Objectives

The present study was aimed to determine the in vitro effect of *Peganum harmala* extract on human ureteric contractions.

Patients and Methods

Preparation of the herbal extract

Peganum harmala was prepared and authenticated by expert botanists. A voucher specimen was deposited in Herbarium of Medical Plants Research center, Shahrekord University of Medical Sciences. The seeds were ground by a mechanical grinder. The 70% hydroalcoholic extract was prepared using maceration method at room temperature. The extract was then filtered and evaporated using vacuum rotary evaporator.

The present study was an experimental-interventional study. The studied ureteral tissue was separated from total nephrectomy. Uterine tissues have not been involved with cancer cells, tissue infection, and abnormal dilatation of the ureters. In this experimental study, 28 samples of human ureter tissue were studied. Six tissue samples were examined for evaluation at concentration of 1 mg/mL, 6 tissue samples at concentration of 2 mg/mL, and 5 tissues at 4 mg/mL concentration of *P. harmala* extract. Also, 5 tissue samples were examined for checking normal saline solution and 6 pieces of tissue for checking prazosin 10^{-8} M solution. The ureter tissue was removed by nephrectomy with any reason, placed in a UW solution at 0°C, and immediately transferred to the lab. The ureter was separated with the size of one to 2 cm, put in a bath of oxygenated Krebs solution at 37°C (pH = 4.7), and then placed vertically between two hooks of stainless steel. The upper hook was connected to an isometric transducer by a thread and then to the physiography device. One gram weight on the opposite side of the transducer lever hangs on the tissue to make a primitive stretch. Then, by adding potassium chloride, the tissue was contracted and, after reaching to maximum contraction to examine the mechanism of the effect of the extract, was incubated with *P. harmala*, normal saline, and prazosin extracts, and the percentage of contraction force changes on paper was recorded and calculated.

Ethical issues

The research followed the tenets of the Declaration

of Helsinki. This research was approved by the Ethics Committee of Shahrekord University of Medical Sciences. (Ethics Committee reference number: IR.SKUMS.REC.1395.233). Additionally, Shahrekord University of Medical Sciences supported this research financially (Grant#1462). This study was conducted as the M.D., thesis of Arsham Pouriamofrad at this university.

Statistical analysis

Data were analyzed by the Prism using descriptive, ANOVA, and Tukey tests. *P* value less than 0.05 was considered statistically significant.

Results

In this study, 28 samples of human ureter tissue were studied. Six tissue samples were examined for evaluation of *P. harmala* solution at concentration of 1 mg/mL, 6 tissue samples at concentration of 2 mg/mL, and 5 pieces of tissue for evaluation of *P. harmala* solution at 4 mg/mL concentration. Also, 5 tissue samples were examined for checking NaCl solution and 6 pieces of tissue for examination of prazosin solution.

As shown in Table 1, distribution of data using the Shapiro-Wilk test, as global, and in terms of the examined groups was normal ($P < 0.05$). ANOVA was used to compare contraction between the groups. There was no significant difference between the initial contraction after adding KCl ($P = 0.21$), but there was a significant difference between the secondary contraction (15 minutes after the addition of NaCl, prazosin and *P. harmala* extracts) ($P < 0.05$).

As shown in Figure 1, two-way ANOVA was used to compare the contraction between the groups. Bonferroni post analysis showed that there was no significant difference between initial tension after adding KCl ($P = 0.21$), but there was a significant difference between secondary contraction (15 minutes after adding NaCl, prazosin and *P. harmala*) ($P < 0.05$).

A significant decrease in secondary contraction (15 minutes after adding the solution) was observed between the prazosin group and the *P. harmala* group at a concentration of 1 mg/mL ($P < 0.001$). Besides, the secondary contraction of the prazosin group showed a significant decrease compared with that of the NaCl group

Table 1. Total values of initial pull (after adding KCl) and secondary contraction (after adding NaCl, prazosin and *Peganum harmala* extracts)

Group	Contraction after adding KCL	Contraction after adding NaCl, Prazosin and <i>Peganum harmala</i> extracts
Prazosin	3.9±0.6	1.08±0.4
NaCl	4.3±0.7	3.9±0.7
<i>Peganum harmala</i>	4.4±0.5	3.05±0.8
<i>P</i>	0.21	<0.05

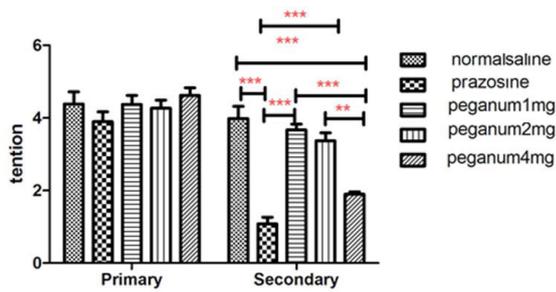


Figure 1. Comparison tension in the primary and secondary groups.

($P < 0.001$). However, there was no significant difference in the secondary contraction reduction between the *P. harmala* group treated with concentration of 1 mg/mL and NaCl group ($P = 0.1$).

By comparing the secondary contraction (15 minutes after addition of KCl solution), there was a significant decrease between the prazosin group and the *P. harmala* group at a concentration of 2 mg/mL ($P < 0.001$). However, there was no significant difference in secondary pulling reduction between the *P. harmala* group treated with concentration of 2 mg/mL and NaCl group ($P = 0.31$).

In addition, there was a significant difference in the second contraction between the *P. harmala* group treated with concentration of 4 mg/mL and NaCl group ($P < 0.001$). Additionally, a significant difference was observed between the prazosin and *P. harmala* groups with a concentration of 4 mg/mL ($P = 0.048$), which is borderline and there is little difference in the secondary contraction between the two groups.

Comparing the three groups receiving *P. harmala* extract with concentrations of 1.2 and 4 mg/mL, the analysis showed that the response was dose-dependent with increasing concentration. There was no significant difference between *P. harmala* group with concentration of 1 mg/mL compared with *P. harmala* group with concentration of 2 mg/mL ($P < 0.05$). A significant decrease was observed between *P. harmala* group with concentration of 1 mg/mL compared with *P. harmala* group with concentration of 4 mg/mL ($P < 0.001$). Meanwhile, there was a significant decrease between *P. harmala* group with concentration of 2 mg/mL compared with *P. harmala* group with concentration of 4 mg/mL ($P < 0.01$). The highest reduction in the secondary contraction is related to the *P. harmala* group with a concentration of 4 mg/mL, which is very close to the secondary contraction of the prazosin group.

Discussion

In the present study, the effects of hydroalcoholic extract of Peganum harmala on human ureteric contractions in vitro were investigated.

Peganum harmala extract treatment showed a significant

difference at concentration of 4 mg/mL compared with NaCl treatment. The difference in mean secondary stretch at 4 mg/mL concentration was significantly lower than that of prazosin treatment compared with lower concentrations of *P. harmala* (1 and 2 mg/mL). In fact, the effect of *P. harmala* extract at the concentration of 4 mg/mL was closer to the level of prazosin effect. Depending on the dose, the alkaloid derivatives of *P. harmala* seeds (harmine and harmaline) reduce the aortic contractions induced by noradrenaline and KCl (11). The aqueous extract of *P. harmala* caused the elimination of the jejunum contractions of rabbits and pigs produced by acetylcholine (12). Furthermore, another study by Nakada et al on smooth muscle of the ureter wall showed that the herbal drug doxazosin can reduce the contraction of the smooth muscle of the ureter wall, which was also dose-dependent. Also, the use of doxazosin reduced the effects of epinephrine contraction on these muscles (13).

Sukwan et al reported that the ginseng root extract (*Talinum paniculatum*) could significantly prevent self-contraction of the ureter. This extract also significantly inhibited contractions caused by KCl and Oxytocin (14). The results of this study were consistent with those of our present study.

In the present study, the use of *P. harmala* extracts, especially at a concentration of 4 mg/mL, reduced the effect of KCl-induced contraction. Since the main factor of contraction of smooth muscle is the presence of calcium ions, they can enter the cell through the active calcium channels and cause smooth muscle contraction. The contraction process continues as long as these calcium channels are open. Therefore, the contraction of the ureters caused by potassium chloride can be due to these channels (15-16).

In the study of Aqel et al, the aqueous extract of *P. harmala* seed had an anti-contraction effect on smooth muscles of the intestine, lung, and arteries of rabbits and pigs. The researchers said the aqueous extract of *P. harmala* seed had anti-spastic, anticholinergic, antihistamine, and anti-adrenergic effects. In their study, the researchers reported that the *P. harmala* seed extract may block the calcium ion transfer through voltage-dependent channels and receptor-dependent canals and cause anticonvulsant effects (12).

It has also been reported that the active compounds of *P. harmala*, such as harmine, harmaline, and harmine, showed vasodilatory effects that appear to be related to the ability to increase NO production. In addition, quinazoline alkaloid and vasicinone isolated from *P. harmala* seed showed relaxant and anti-contraction effects on smooth muscle of arteries in mice (12, 17). In the present study, the use of *P. harmala* extract on smooth muscle of the ureter had a severe contraction.

In a previous study by Mirzaie et al, hydroalcoholic extract of *P. harmala* at low concentrations increased

spontaneous contraction of the uterus muscles and decreased the contraction at high concentration of 400 µg/mL. Reducing muscle contractility at high concentration in the present study may be associated with an increase in the concentration of inhibitory compounds present in the extract which compensate the stimulatory effects at low concentrations (18).

Ureteral obstruction induces induction of apoptosis in the kidney tissue. It may also lead in the rats to interstitial tuberculosis, glomerulosclerosis, infiltration of inflammatory cells, and inflammation of the interstitial tissue (4); oxidative stress is likely to play a key role in the onset and continuation of post-obstruction inflammation, resulting in renal tubule injury, interstitial fibrosis, necrosis, and apoptosis. Besides, DNA damage increases and the mechanisms for its repair decrease. It has also been shown that after using antioxidants such as bioflavonoids, the damage and oxidative stress as well as the expression of apoptotic genes in the obstructed kidney decrease (4). Different mechanisms for the protective effect of *P. harmala* and other bioactive compounds in different tissues have been proposed (4). On the other hand, the antioxidant effects of *P. harmala* have been proven in previous studies. Therefore, it could be suggested that *P. harmala* may also have a protective role against the side effects of ureteral obstruction as well as other prostate disorders through anti-oxidant effects.

Active compounds of *P. harmala* are mainly composed of alkaloids such as tetrahydroharmine, harmine and harmaline (4,19). In addition, Astulla et al observed that the *P. harmala* alkaloids (including harmaline and harmine) had anti-plasmodium effects and reduced the contractions in the aorta of the mouse (20).

Other alkaloids in *P. harmala*, such as vasicinone, have anti-proliferative properties and can produce cell cytotoxicity in tumor cells in a laboratory environment (21). Of course, it should not be overlooked that the β-carboline alkaloids contained in the *P. harmala* can cause poisoning at high doses (22); hence the use of this plant should be done in an effective and non-toxic treatment.

Conclusion

In this study, we found the administration of *P. harmala* extract can be useful in reducing urinary human ureteric contractions while the best effect was achieved at 4 mg/mL of *P. harmala* extract.

Authors' contribution

ZK provided study concept and design, acquisition of data, analysis, and interpretation of data. MS prepared study concept, drafting of the manuscript. ZL supervised the final revision of the manuscript for important intellectual content, and conducted administrative, technical, and material supports. AP conducted administrative,

technical, and material supports.

Conflicts of interest

The authors declared no conflict of interest

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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