

# Preventive and curative effects of aqueous extracts of *Descurainia sophia* L. on nephrolithiasis induced in rats

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## Abstract

**Purpose:** Kidney stone is the most common diseases. The aim of this study was to evaluate the effects of aqueous extracts of *Descurainia sophia* L. (Ds) on the prevention and treatment of kidney stones induced by ethylene glycol (EG) and ammonium chloride (AC) in rats. **Materials and Methods:** Sixty-four male Wistar rats were randomly assigned into 8 groups of 8: control, sham (received only EG 1%+AC 0.25%), experimental 1 and 2 received only Ds (200 and 400 mg/kg/4 weeks), experimental 3 and 4 received EG+AC+Ds (200 and 400 mg/kg/4 weeks), and experimental of 5 and 6 received EG+AC+Ds (200 and 400 mg/kg) from day 14 until the end of the experiment period. The kidneys were isolated on the day 29 of the test and the number of calcium oxalate crystal and tissue changes was examined and was analyzed by ANOVA test. **Results:** No significant change was observed in any of the parameters in experimental 1 and 2 compared to control. All of parameters except of renal corpuscle and glomeruli showed a significant increase in EG group compared to control, and the diameter of renal corpuscle and glomeruli showed a significant decrease. In groups receiving EG with extract of Ds, there was a significant decrease in parameters of urinary space, collecting duct, tissue damage, and deposits of calcium oxalate crystals and significant increase in renal corpuscle and glomeruli compared to the sham. Experimental 4 having the most impact of improvement. **Conclusion:** Extract of Ds has an effect on the prevention and treatment of kidney stones, which can be attributed to antioxidant and anti-inflammatory properties.

**Key words:** Calcium oxalate, *Descurainia sophia* L., kidney calculi, urolithiasis

## INTRODUCTION

Urinary stones are the third most common affliction of the urinary tract,<sup>[1]</sup> and the incidence of urolithiasis has increased in both the developed and the developing countries during 1980–2012.<sup>[2]</sup> In USA, the prevalence of nephrolithiasis was 10.6% in men and 7.1% in women.<sup>[3]</sup> In Iran, recurrence rate was 16% after 1 year, 32% after 5 years, and 53% after 10 years.<sup>[4]</sup> Approximately 80% of stones are composed of calcium oxalate.<sup>[1]</sup> Most people are diagnosed with kidney stones suffering from colic pains which usually do not relieve with common analgesic drugs, so opiate drugs are used to relieve pain. These drugs have side effects. On the other hand, the obstruction caused by these stones can reduce the output of kidneys, and if not treated in the short term, it can cause chronic renal failure

(4%) and end-stage renal disease (0.2%).<sup>[5]</sup> Treatment of urolithiasis involves the use of oral medicines, stone removal by arthroscopy (transurethral lithotripsy), use of stone crusher with shock waves from outside the body (extracorporeal shock wave lithotripsy), extraction through the skin (percutaneous nephrostolithotomy), and open surgery.<sup>[1]</sup> Nowadays, with the clarification of the side effects of chemical drugs, the complications of surgery, the high cost of treatment, and the possibility of recurrence of urinary stones, there has been a

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**Received:** 25-02-2018

**Revised:** 19-03-2018

**Accepted:** 27-03-2018

rapidly growing use of herbal medicines among the modern researchers and the effects of a variety of herbal medicines on the treatment of kidney stones have been studied in numerous studies.<sup>[6,7]</sup> Ds, scientifically known as Ds, is a herbaceous and annual (rarely biennial) plant and a genus in the mustard family (Brassicaceae). *Seeds* of Ds which have been traditionally used for the treatment of various diseases have a diuretic, anticancer,<sup>[8]</sup> anti-inflammatory, analgesic,<sup>[9]</sup> antioxidant,<sup>[8,9]</sup> and antibacterial properties<sup>[10]</sup> and contain compounds such as mucilage,<sup>[11]</sup> amino acids, fatty acids, cholesterol, flavonoids, glucosinolates, heart glucosides, and phenolic compounds. Therefore, given to the composition and properties of Ds and since there has been no study done on the effect of aqueous extract of Ds on the formation of calcium oxalate stones, we decided to investigate the effects of aqueous extract of Ds on kidney stone induced by ethylene glycol (EG) and ammonium chloride (AC) in male Wistar rats.

## MATERIALS AND METHODS

### Plant materials

The Ds seeds were purchased in the spring from Attari and after confirmation by the botanical expert (Dr. Amir Barjian from Islamic Azad University, Jahrom), they were soaked at 30°C with distilled water and completely crushed by a blender after one day. The extract was then separated using a rotary machine and placed in an incubator at 25°C to be purified and dried.<sup>[12]</sup> In this study, according to the previous study,<sup>[9]</sup> two doses of 200 mg/kg and 400 mg/kg were used.

### Animals

This is an empirical and completely randomized study that was performed on 64 male Wistar rats with an average weight of 180 up to 200 g after obtaining ethical permission from the Ethics Committee of Jahrom University of Medical Sciences (IR.JUMS.REC.1394.156) and with compliance of ethical issues. During the whole experiment, the animals were kept under standard conditions (12 h of darkness and 12 h of light) and temperatures of 23 ± 2°C. These studies were conducted at the Animal Hospital of Jahrom University of Medical Sciences.

### Experimental protocol

To develop kidney stones in animals, AC (0.25%) with EG (1%) was used in drinking water throughout the test (28 days).<sup>[7,13]</sup> In experimental groups, Ds extracts were administered by gavage. Animals were selected randomly and divided into 8 groups of 8 (14), as follows:

Control group: This group did not receive any treatment during the trial period.

Sham group (EG [1%] + AC [0.25%]): During the experiment, EG 1% and AC 0.25% were added to the drinking water of this group.

Experimental group 1 (Ds [200 mg/kg/4 weeks]) and 2 (Ds [400 mg/kg/4 weeks]) received aqueous extract of Ds (200 mg/kg and 400 mg/kg) for 4 weeks, respectively.

Experimental group 3 (EG+AC+Ds [200 mg/kg/4w]) and experimental group 4 (EG+AC+Ds [400 mg/kg/4 weeks]) received EG and AC as well as aqueous extract of Ds (200 mg/kg and 400 mg/kg) for 4 weeks from the 1<sup>st</sup> day to the end of the experiment, respectively.

Experimental group 5 (EG+AC+Ds [200 mg/kg/2 weeks]) and experimental group 6 (EG+AC+Ds [400 mg/kg/2 weeks]) received EG and AC in drinking water and aqueous extract of Ds in a dose of 200 mg/kg and 400 mg/kg from day 14 to the end of the experiment.

### Pathology study

After 28 days of treatment, the animals were anesthetized after using the standard method on the day 29 and the kidneys were rapidly isolated, and each pair of kidneys was placed within a container that containing 10% formalin, and after 24 h, the kidneys were removed of solution. After the usual stages of the tissue passage, paraffin sections were prepared in a thickness of 5 µ<sup>[7]</sup> in serial series. From each kidney, 10 slides containing 5 sections were prepared and then stained with H and E method and examined by Nikon light microscope. The number of calcium oxalate crystals was measured in 10 microscopic fields (magnification of 10 \* 10). The number of oxalate crystals was reported as ± standard error mean. The mean diameter of different parts of the nephron was calculated, and tubulointerstitial changes such as tubular cell necrosis, atrophy, dilation, and interstitial inflammation were analyzed semi-quantitatively.

0 = none, 1 = trace (<10%), 2 = mild (10–25%), 3 = moderate (26–50%) and 4 = marked (>50%).<sup>[13,14]</sup>

### Statistical analysis

The data were analyzed by SPSS software. To analyze the results and compare the mean, one-way ANOVA test was used for general comparison, followed by Duncan's test for intragroup comparison. *P* < 0.05 was considered meaningful.

## RESULTS

Pathologic examination was performed to detect possible tissue damage and counting of calcium oxalate crystals as well as measurement of the diameter of the nephron. Calcium oxalate deposits were observed as crystal forms in the renal tubules using

optical microscopy. No calcium oxalate deposits and tissue damage were observed in the control, Ds (200 mg/kg/4 weeks), and Ds (400 mg/kg/4 weeks) groups. Furthermore, none of the measured parameters of nephron diameter showed a significant difference in the Ds (200 mg/kg/4 weeks) and Ds (400 mg/kg/4 weeks) groups compared to the control group [Table 1 and 2 and Figure 1]. Diameter of collecting duct, urinary space, loop of Henle, tissue damage, and deposit of calcium oxalate crystals in the sham group showed a significant increase compared to the control group and diameter of the renal corpuscle and glomeruli showed a significant decrease [Tables 1 and 2]. There was a significant decrease in renal corpuscle and glomerular diameter in the EG+AC+Ds (200 mg/kg/4 w) group compared to control as well as a significant increase was observed in tissue damage and calcium oxalate crystals deposit [Tables 1 and 2]. No significant changes were observed in EG+AC+Ds (400 mg/kg/4 weeks) compared to controls in any of the parameters [Tables 1 and 2]. Significant increase was found in urinary space, collecting duct, tissue damage, and deposit of calcium oxalate crystals in the EG+AC+Ds (200 mg/kg/2 weeks) group compared to control and a significant decrease in renal corpuscle and glomeruli [Tables 1 and 2]. In addition, there was a significant decrease in renal corpuscle and glomeruli in

the EG+AC+Ds (400 mg/kg/2 weeks) compared to control and also a significant increase in tissue damage and precipitation of calcium oxalate crystals [Tables 1 and 2]. In all groups receiving EG together with aqueous extract Ds, there was a significant decrease in parameters of urinary space, collecting duct, tissue damage, and deposits of calcium oxalate crystals compared to the group receiving only EG as well as a significant increase was observed in renal corpuscle and glomeruli [Tables 1 and 2]. EG+AC+Ds (400 mg/kg/4 weeks) group having the most impact of improvement [Tables 1 and 2].

Data were represented based on mean  $\pm$  SE. ( $N = 8$ ). Based on the Duncan test, if there is at least one common line in each row, those groups do not have a significant difference with each other.  $P < 0.05$  is considered as a statistically significant level.

## DISCUSSION

The results of this study indicated that in the groups that received only Ds no calcium oxalate crystals, tissue damage, and significant change were found in the diameter of the different parts of the nephron similar to the control group. In

**Table 1: Comparative measurement results in different groups**

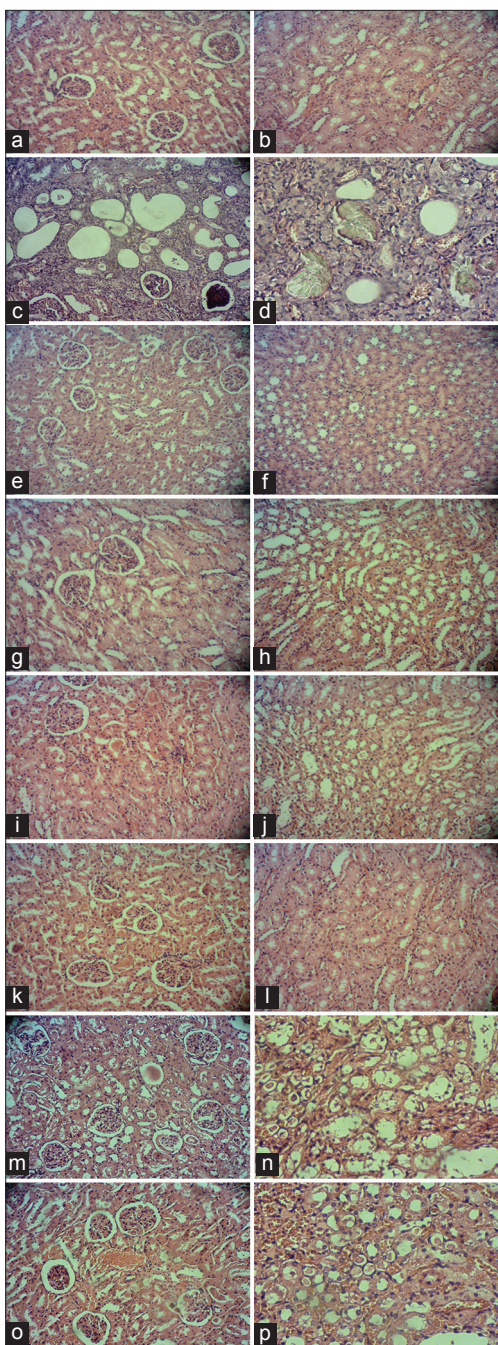
Groups / Parameters	Renal corpuscle diameter ( $\mu\text{m}$ )	Urinary space diameter ( $\mu\text{m}$ )	Glomerulus diameter ( $\mu\text{m}$ )	Collecting duct diameter ( $\mu\text{m}$ )	Henle diameter ( $\mu\text{m}$ )
Control	120.01 $\pm$ 4.73 <sup>ab</sup>	11.64 $\pm$ 0.64 <sup>a</sup>	99.03 $\pm$ 2.40 <sup>ab</sup>	27.48 $\pm$ 2.75 <sup>a</sup>	16.20 $\pm$ 0.51 <sup>c</sup>
EG (1%)+AC (0.25%)	95.28 $\pm$ 5.47 <sup>e</sup>	16.94 $\pm$ 1.07 <sup>c</sup>	78.18 $\pm$ 3.25 <sup>f</sup>	74.78 $\pm$ 9.86 <sup>e</sup>	21.79 $\pm$ 3.96 <sup>a</sup>
Ds (200 mg/kg/4 week)	122.9 $\pm$ 3.53 <sup>ab</sup>	11.85 $\pm$ 0.52 <sup>a</sup>	101.20 $\pm$ 3.40 <sup>a</sup>	30.57 $\pm$ 3.12 <sup>a</sup>	16.93 $\pm$ 0.72 <sup>c</sup>
Ds (400 mg/kg/4 week)	126.15 $\pm$ 2.38 <sup>a</sup>	11.63 $\pm$ 0.71 <sup>a</sup>	102.9 $\pm$ 2.68 <sup>a</sup>	29.77 $\pm$ 3.16 <sup>a</sup>	16.79 $\pm$ 1.32 <sup>c</sup>
EG+AC+Ds (200 mg/kg/4 week)	109.75 $\pm$ 2.49 <sup>c</sup>	11.35 $\pm$ 0.66 <sup>a</sup>	88.05 $\pm$ 2.02 <sup>cd</sup>	31.56 $\pm$ 3.02 <sup>a</sup>	17.27 $\pm$ 0.68 <sup>c</sup>
EG+AC+Ds (400 mg/kg/4 week)	116.95 $\pm$ 2.52 <sup>b</sup>	12.05 $\pm$ 0.79 <sup>a</sup>	94.35 $\pm$ 3.29 <sup>b</sup>	30.43 $\pm$ 2.04 <sup>a</sup>	17.06 $\pm$ 0.63 <sup>c</sup>
EG+AC+Ds (200 mg/kg/2 week)	103.57 $\pm$ 2.72 <sup>d</sup>	14.78 $\pm$ 0.88 <sup>b</sup>	83.46 $\pm$ 1.09 <sup>e</sup>	48.77 $\pm$ 2.30 <sup>b</sup>	19.83 $\pm$ 0.71 <sup>b</sup>
EG+AC+Ds (400 mg/kg/2 week)	111.1 $\pm$ 2.39 <sup>c</sup>	12.35 $\pm$ 0.54 <sup>a</sup>	87.10 $\pm$ 1.97 <sup>d</sup>	32.00 $\pm$ 2.29 <sup>a</sup>	17.71 $\pm$ 0.96 <sup>c</sup>

The means are presented in the form of mean $\pm$ SEM.  $P < 0.05$  is considered statistically significant. According to the Duncan test, if there is at least one common letter in each column, those groups do not differ significantly. EG: Ethylene glycol, AC: Ammonium chloride, Ds: *Descurainia sophia*, SEM: Standard error of the mean

**Table 2: Impact of aqueous extract of Ds on tissue changes and calcium oxalate deposits**

Groups / Parameters	Calcium oxalate deposition	Tubulointerstitial damage
Control	0 $\pm$ 0 <sup>a</sup>	0 $\pm$ 0 <sup>a</sup>
EG (1%)+AC (0.25%)	21.26 $\pm$ 5.42 <sup>d</sup>	3.62 $\pm$ 0.18 <sup>d</sup>
Ds (200 mg/kg/4 week)	0 $\pm$ 0 <sup>a</sup>	0 $\pm$ 0 <sup>a</sup>
Ds (400 mg/kg/4 week)	0 $\pm$ 0 <sup>a</sup>	0 $\pm$ 0 <sup>a</sup>
EG+AC+Ds (200 mg/kg/4 week)	1.86 $\pm$ 0.71 <sup>b</sup>	0.62 $\pm$ 0.17 <sup>b</sup>
EG+AC+Ds (400 mg/kg/4 week)	0.68 $\pm$ 0.81 <sup>a</sup>	0.17 $\pm$ 0.16 <sup>ab</sup>
EG+AC+Ds (200 mg/kg/2 week)	3.87 $\pm$ 0.59 <sup>c</sup>	1.87 $\pm$ 0.35 <sup>c</sup>
EG+AC+Ds (400 mg/kg/2 week)	2.45 $\pm$ 0.66 <sup>b</sup>	1.50 $\pm$ 0.19 <sup>c</sup>

The means are presented in the form of mean $\pm$ SEM.  $P < 0.05$  is considered statistically significant. According to the Duncan test, if there is at least one common letter in each column, those groups do not differ significantly. EG: Ethylene glycol, AC: Ammonium chloride, Ds: *Descurainia sophia*, SEM: Standard error of the mean



**Figure 1:** Microscopic views of the kidney tissue in study groups. (a) Control group (H and E,  $\times 100$ ). (b) Control group (H and E,  $\times 100$ ). (c) Sham group (H and E,  $\times 100$ ). (d) Sham group (H and E,  $\times 100$ ). (e) Ds (200 mg/kg/4 weeks) (H and E,  $\times 100$ ). (f) Ds (200 mg/kg/4 weeks) (H and E,  $\times 100$ ). (g) Ds (400 mg/kg/4 weeks) (H and E,  $\times 100$ ). (h) Ds (400 mg/kg/4 weeks) (H and E,  $\times 100$ ). (i) EG+AC+Ds (200 mg/kg/4 weeks) (H and E,  $\times 100$ ). (j) EG+AC+Ds (200 mg/kg/4 weeks) (H and E,  $\times 100$ ). (k) EG+AC+Ds (400 mg/kg/4 weeks) (H and E,  $\times 100$ ). (l) EG+AC+Ds (400 mg/kg/4 weeks) (H and E,  $\times 100$ ). (m) EG+AC+Ds (200 mg/kg/2 weeks) (H and E,  $\times 100$ ). (n) EG+AC+Ds (200 mg/kg/2 weeks) (H and E,  $\times 100$ ). (o) EG+AC+Ds (400 mg/kg/2 weeks) (H and E,  $\times 100$ ). (p) EG+AC+Ds (400 mg/kg/2 weeks) (H and E,  $\times 100$ ). EG: Ethylene glycol, AC: Ammonium chloride, Ds: *Descurainia sophia*

the group receiving only EG, the diameter of the collecting duct, urinary space, loop of Henle, tissue damage, and deposit of calcium oxalate crystals showed a significant increase compared to other groups and the diameter of the renal corpuscle and glomeruli decreased significantly. In groups that received both EG and Ds, the amount of tissue damage and deposition of calcium oxalate crystals were decreased. The highest rate of improvement was observed in the group EG+AC+Ds (400 mg/kg/4 weeks). The most common kidney stones are composed of calcium oxalate. Calcium stones occur because of high concentration of calcium salts in the supersaturation level. In addition, a number of metabolic abnormalities such as hyperoxaluria, hypercalciuria, and hypocitraturia can change the composition and concentration of urine and increase the tendency of the kidney to form stone as well.<sup>[15]</sup> So far, a lot of laboratory models have been reviewed to investigate the precise mechanism of the formation of kidney stones and to identify the factors affecting the prevention and treatment of stones.<sup>[13,14,16-19]</sup> Due to their similarity to humans in the formation of calcium oxalate deposit, rats were used to test kidney stones.<sup>[20]</sup> Induced kidney stone in the laboratory mouse model is often carried out by EG alone or in combination with other substances, including AC.<sup>[17,20]</sup> The likelihood of formation of calcium stones increases with elevating levels of oxalate in the urine.<sup>[21]</sup> Both EG and ammonium chloride increase urinary oxalate and calcium oxalate crystalline deposits in the kidney.<sup>[20]</sup> Crystals can damage the renal cells, leading to the inflammatory response and secretion of cytokines, chemokines, and increased ECM synthesis, resulting in fibrosis.<sup>[22]</sup> In this study, tissue damage and deposit of calcium oxalate crystals were observed in all groups receiving EG and AC, especially in the group receiving only EG and AC, higher levels of infiltration of lymphocytes, fibrosis, and dilatation of collecting ducts, and loop of Henle and tissue damage were observed. It was found that these damages are probably due to the formation of calcium oxalate crystals. Kidney stones can cause obstruction in the urinary tract. The obstruction of the urethra can reduce the glomerular filtration rate, and consequently, the contraction of afferent arterioles and urine flow in the kidneys decreases.<sup>[23]</sup> Furthermore, kidney stones can cause a decrease in kidney function,<sup>[24]</sup> and if not treated, it can lead to chronic renal failure.<sup>[5]</sup> In the group that only received EG and AC, there was a significant increase in the diameter of the collecting duct, the loop of Henle and the urinary space, as well as the diameter of the renal corpuscle and glomeruli compared to other groups. These effects can be due to obstruction of the urethra as a result of calcium oxalate deposit. Free radicals produced by oxalate can damage the membrane and consequently accumulate crystals. By neutralizing free radicals, antioxidants protect from the membrane and prevent oxalate accumulation and formation of stones.<sup>[25,26]</sup> Furthermore, flavonoids which possess antioxidant effects are able to impair the free radical production system and prevent tissue damage through direct immobilization of free radicals, xanthine oxidase inhibition, nitric oxide inhibition, and decreased adhesion of

the leukocytes to the endothelial wall. Moreover, flavonoids have antiatherosclerotic, anti-inflammatory, antitumor, antiviral, and antithrombogenic effects.<sup>[27]</sup> Ds contains flavonoids and phenolic compounds and have antioxidant and anti-inflammatory properties.<sup>[8,9]</sup> In a study by Mirzaei *et al.*, properties and compositions of antioxidant of Ds were evaluated using various tests, and in all of the studied models, it was shown that the hydroalcoholic extract of Ds had miscellaneous levels of antioxidant activity.<sup>[28]</sup> In this study, there was a decrease in tissue damage and inflammation in the groups receiving Ds which could be due to flavonoids and antioxidants present in this plant. With their specific nature, nanobacteria can cause calcium and mineral phosphate deposition, resulting in tissue damage and stone formation.<sup>[29]</sup> In a study by Aghaabbasi *et al.*, the antibacterial properties of the Ds were tested and showed that the plant has an antibacterial effect.<sup>[10]</sup> In this study, a decrease in the reduction in the stone formation in the treated group can be attributed to antibacterial effect of the plant. Glycosaminoglycan plays an important role as a preventive agent in kidney stones.<sup>[30]</sup> In their study, Erturk *et al.* showed that patients with kidney stones had a lower level of urine glycosaminoglycan.<sup>[31]</sup> Ds contains mucilaginous compounds.<sup>[11]</sup> It seems that because of mucilage compounds, Ds has a contribution in preventing stone formation and contributing to the removal of stones. Today, a variety of invasive and non-invasive methods are used to treat kidney stones; however, there is still no effective medication for the prevention and treatment of urinary tract stones. Herbal remedies can be a good alternative to prevent and treat kidney stones. Nevertheless, plant compounds may also have side effects, and consequently, further studies are required on the efficacy and safety of these compounds.

## CONCLUSION

The results of this study showed that both EG and AC cause calcium oxalate crystals formation, tissue damage, increased diameter of the collecting duct, urinary space, loop of Henle, and decrease in the diameter of the kidney and glomeruli in rat. Furthermore, aqueous extract of Ds can be used to reduce the tissue damage and prevent formation, crushing and removal of calcium oxalate stones in the kidney, both in the prevention and the treatment groups; the higher the dose, the better the effect. Although the exact mechanism and function of this plant are not known, these effects seem to be due to the presence of compounds such as flavonoids, fatty acids, mucilages, and antioxidant, anti-inflammatory, antibacterial, and diuretic properties of this plant.

## ACKNOWLEDGMENTS

The authors of this paper would like to thank Jahrom University of Medical Sciences for providing financial support of this research.

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**Source of Support:** Nil. **Conflict of Interest:** None declared.