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Effects of *Equisetumarvense* Plant Extracts on the Kidney Stones and its Diuretic Action

HuseyinTurker[1*] and MesureTurkay[2]
[1]Department of Biology, Science Faculty, Ankara University, 06500, Ankara, Turkey
[2]Ankara Science High School, Balgat, 06520, Ankara, Turkey

[*]**Correspondingauthor:** HuseyinTurker, Department of Biology, Science Faculty,Ankara University,06500, Ankara, Turkey, E-mail: hturker14@hotmail.com

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Abstract

In this study, the effect of *Equisetum arvense* L. extracts on kidney stones and diuretic action was investigated. The plant drogs were prepared from aerial parts of the plant by infusion and decoctium methods. In the first part of the study, different concentrations of extracts (0.1 and 10.0 mg/mL) were orally administrated to Guinea pigs (rats) and their urine outputs were examined for 10 days. As a result, it was found that *E. arvense*had a diuretic action since the urine collected from the treated Guinea pigswas less dense than that of the urine collected from the specimen given only tap water (Control group). In the second part, it was found that secretory organ was more affected by the drog. Electric currents were applied to the ureter and the bladder in isolated organ baths with various drog concentrations and it was found that the drog was more affective on the bladder muscles. It wasdetermined that Herbaequiseti (the aerial parts of the plant) was affective on kidney stones. This result was obtained after keeping the kidney stones in drogs with various concentrations and pH values for about 45 days. The amount of the calcium ions that impregnated to the drogs were also measured in 22 days at 37°C; as ppm. It was obvious that the kidney stones were dissolved during the treatment. This results show that *E. arvense* extracts exhibit safe and strong diuretic activity as claimed in traditional medicine. It is thought that this action may be mediated via silicon activity and lead to an increase in glomerular filtration rate.

Keywords: *Equisetum arvense* (Field horsetail); Extracts; Kidney stones;Diuretic effect;Guinea pig.

Introduction

A kidney stone is a solid piece of material that forms in the kidney out of the substances in the urine. Stones can form in any part of the urinary system, from the kidney to the bladder. They may be small or large and one or many. Kidney stones are most common in middle-aged people and are three times more common in men than in women. They tend to recur. These stones may remain in the kidney; also degrade to the ureter and bladder [1, 2].

There are several types of kidney stones, but most stones are inorganic or calcium stones. They occur when there is too much calcium in the urine. If the kidneys don't work properly or too much calcium is absorbed from the intestines, there might be an excess of calcium in the urine. Kidney stones can also be formed from uric acid and cystine [3-7].

It was suggested that geographical distribution, race, occupation, climate, heredity, diet, vitamin deficiency, excess vitamin D metabolism and secretion disorders play an important role in the occurrence of kidney stones[1, 7, 8].

Diagnosis of kidney stones are made by urine tests, kidney function tests, blood tests, x-ray findings, renal scintigraphy and ultrasonographic methods, whereas, their treatments are usually made by surgery or instrumental ways [9-12].

Besides these treatments, the roots, stems, leaves and flowers of some plants are used by boiling and drinking to supply a diuretic effect and remove the kidney stones. Among these plants, *Equisetum arvense* (horsetail) is the most popular one[13 - 15].

Substances that increase the volume of urine are referred as diuretics. These substances increase the amount of urine extracts from the kidney by affecting the nephrons and collecting channels. These substances diminish the reabsorption of water by reducing the sodium and chloride ions from the kidney tubules. As a result, the amount of water and sodium are thrown out from the body excessively [15 - 17].

The genus Equisetum, used in the current study,consists of 20 species in the world and 7 species in Turkey. It is commonly known among people as the field horsetail, small ponytail, kırkkilit grass and liaison grass etc. Throughout its life cycle, it is an herbaceous perennial plant that exists as a pale yellowish non-photosynthetic spore-bearing fertile stem, produced in early spring. It grows spontaneously in damp and wet areas generally near the streams, rivers and wetlands of northern hemisphere [18]. *E. arvense*'s inefficient green shoots above the soil is known as Herbaequiseti and used by people to remove the kidney stones and bladder stones [19].

This plant contains abundant minerals such as calcium, potassium, phosphate, iron, manganese and silica [20, 21], as well as small amounts of pharmacologically active compounds (tannin, resin, cellulose, pectic substances, fatty acids, alkaloids, glycosides), and flavonoids (Galiteolin, izokersitrozit and Equisetrozit) [22, 23]. This plant has been used as a traditional medicine in respiratory tract infections, bone tissue regeneration [24, 25], skin conditions [26] and kidney diseases [27].

Flavonoids are compounds with a diuretic and diaphoretic effect which pass into water easily and their structures are not distorted by boiling in water. Since alkaloids do not easily pass into water and do not have any harmful effects on the organisms when the plants extracts are drunk [28, 29].

Because of the fact that the structure of *E.arvense*in flavonoid do not change during metabolism, it has a diuretic effect when taken orally or given on organ, *E. arvense* has been preferred as a sample for experimental study in this plant. Furthermore, even though the effects of *E. arvense* extracts over the muscles of excretory system, its effects on kidney activities as sucking and filtering characteristics are known very well; any scientific studies about the fragmentation effects of kidney stones have not been encountered.

Material and Methods

In this study, *E. arvense* extracts, 40 Guinea pigs and kidney stones taken from hospitals which removed from human beings by operations were used.Rats (300-325 g) of either sex were obtained from the Experimental Research Centre. The animals were housed in standard metal cages before and during the experiment. The experiments were conducted in Ankara University Experimental Research Centre and in the labs of this university.

Plant Material

*E. arvense*plant was collected from the nature and purchased from plant markets and then identified by Ankara University Herbarium[30]. After collection, the plant was sun-dried for 10 days and made into a coarse powder by grinding. 500 g of *E. arvense* was extracted by boiling with 1000 ml of water (100°C) for 30 minutes in an apparatus. After the filtration of the mixture, the extract was obtained by centrifugation of 5000 cycles/min. Then, 1% and 10% concentration of extracts were obtained by using tap water.

Experimental Animals

Twenty-four hours prior to the experiment, the Guinea pigs were placed into metabolic cages with withdrawal of food and water. Then, the animals were divided into three groups, containing ten rats in each group. Group I was provided as the control group and was given only tap water along the experiment. Group II and III were separated as the experimental groups and the plant extracts of 1% and 10% doses were given orally for ten days, respectively.

Screening of Diuretic Activity

For this process, prior to the experiment, collecting containers were placed under the cages to collect the urine. While tap water was given to control group, 1% and 10% doses of extracts were given to the experimental groups orally (by gavage) for 10 days. Urine samples were collected from the cages and their concentrations were analysed by densitometer and/or refractometer.

*Fr*agmentation Effects of *E. arvense* Extracts on the Kidney Stones

In this study, *E. arvense* extracts and kidney stones were placed into beakers which include acidic and basic extracts and tap waters. The kidney stones were examined for 22 days. The PH values of the extracts were arranged as 5.8 and 8.5 and the temperature was kept constant at 37°C throughout the experiment. The extracts PH values were stabilised at the same level along the experiment. Before and at the end of the experiment, the kidney stones were weighed and then the amount of calcium passed into extract was measured by atomic absorption methods.

In the second study, the kidney stones and *E. arvense* extracts (1% and 10%) were placed into other 10 beakers which include acidic and basic extracts and tap waters. These experiments were lasted for 45 days. The extracts were changed every three days during the experiment. PHs were arranged as 5.8 and 8.5 and the temperature of the extracts were kept constant at 37°C throughout the experiment. At the end of the experiment, the fragmentation and softening of the kidney stones were examined.

Effects of E.arvense Extracts*on the*Some Tissues

In this study, the effect of *E. arvense* extracts on the contraction of ureter and bladder muscles was observed with electrical stimulation methods created outside the body. Under the anaesthetic process, the Guinea pigs' abdominal cavities were opened; bladder and ureter parts were taken out and then put into Locke solution which was prepared previously [31]. Because, the Locke solution is an organ bath holding the organs just like in the body.

Two organ baths (pH=7.4) with 20 cc Locke solution each were prepared for the ureter and bladder muscles. The temperature of these baths was maintained constant at 37°C by a thermostat. The tissue parts were made striped and hanged. During the experiment, 5% CO_2 and 95% O_2 were given to organ baths repeatedly. A 5g weight was connected to the bladder, and a 2g weight was connected to the ureter. Taking the durability of the ureter and bladder muscles into consideration, a 60 volt electricity currency was applied to ureter and 50 volt to the bladder. The measurements were plotted with the aid polygraph. After the organs were hung in the isolation bath, they were washed four times in an hour and the organs were made to adopt to the environment and distract regularly.

After the contractions of the organs were being regular, 3 cc of 1% and l0% concentration of extracts were put into organ bats, and then a 60 volt electric current was applied to the bladder, a 50 volt electric current was applied to ureter striped by the stimulators. After that, the responses of the bladder and ureter muscles were observed and their graphs were taken. Experiments were repeated once again.

All experiments were carried out in accordance with the Ankara University guidelines for the care of experimental animals. Also, guiding principles and procedures found in Declaration of Helsinki of the World Medical Association regarding animal experimentation were followed in the study.

Results

Effect of *E. arvense* extracts on urine density:

At the experiment, urine samples were collected from the cages and their densities were analysed by densitometer and/or refractometer for every day. The results were shown in Table 1 and Figure 1.

Upon the examination of the data, while the average level of urine density in the control group (drunk tap water) was 1038.25, the urine density decreased to 1010-1011 for the first group (1% extract); and it decreased to 1006-1007 for the second group (10% extract).The results indicated the diuretic effects of *E. arvense* plant.

Table 1: The mean urine density of control group and experimental groups drinking *E.arvense* extracts.

	Experimental time (days)										**Mean**
	1 d	**2 d**	**3 d**	**4 d**	**5 d**	**6 d**	**7 d**	**8 d**	**9 d**	**10 d**	
Control group (Tap water)	1038.25	1039.32	1038.15	1040.16	1037.02	1040.10	1036.30	1036.10	1038.10	1037.25	**1038.05**
1st group (1% Extract) (n=10)	1011.30 ±54	1011.60 ±39	1010.33±73	1011.33 ±93	1012.67±57	1011.33±1,27	1011.10±82	1011.67±79	1011.68±54	1011.67±73	**1011.50**
2nd group (10% Extracts) (n=10)	1007.67±70	1006.60 ±33	1006.60±33	1008.0 ±00	1006.60±33	1007.33±66	1006.66±66	1007.00±57	1007.33±88	1007.00±1.00	**1007.10**

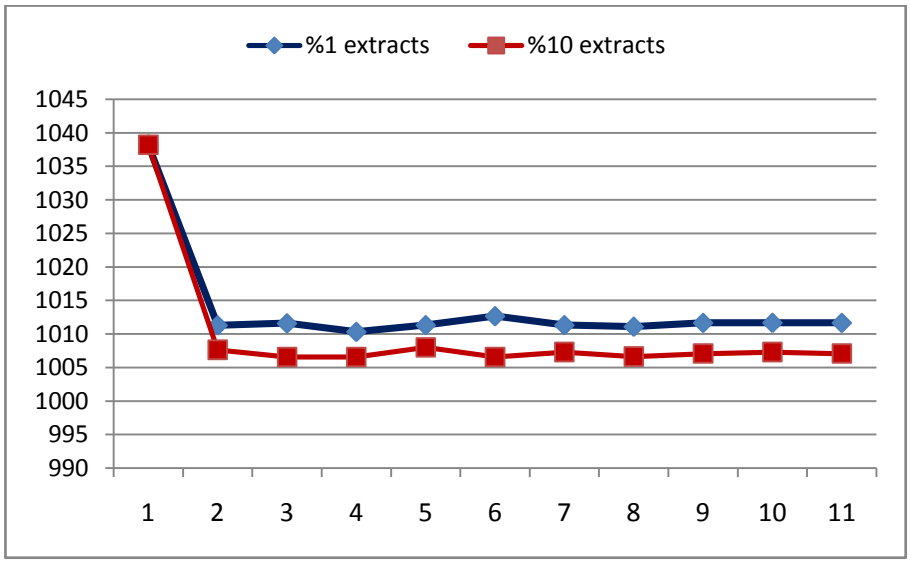

Figure 1: Changes in urine densities of Guinea pigs during the experimental period for 10 days.

TheFragmentation Effects of 10%*E. arvense* Extracts on the Kidney Stones

In this experiment, before and at the end of the experiment lasting 22 days, the kidney stones were weighed and the amount of calcium passing into extracts and water were measured and analysed by atomic absorption techniques (ppm). While serious fragmentations occurred on kidney stones in acidic extracts, no significant fragmentation occurred on the stones in basic extracts and tap waters. Similarly, more calcium was seen in acidic extracts than in the basic extracts and tap water. These results revealed the melting effects of *E. arvense* on the kidney stones and losing their weights by the application of time. The results were shown in Table 2.

Table 2: The changes in kidney stones' weights and calcium (Ca) amounts during the experiment. The extract density is 10%.

	Kidney stones in acidic extracts (PH=5.8)				**Kidney stones in basic extracts (PH=8.5)**			**Kidney stones in tap water (PH=7**		
Kidney stones	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Weight of the kidney stones before the experiment	2.55	0.62	2.92	7.11	0.50	0.51	0.50	0.65	0.45	1.65
Weight of the kidney stones after the experiment	2.45	0.54	2.82	6.82	0.48	0.47	0.47	0.73	0.45	1.7
Amount of the kidney stones in the extracts	-10	-8	-10	-30	-2	-4	-3	+8	+0	+5
Amount of Ca in the extracts (ppm)	240	264	185	205	34	47	35	16	13	2

Correspondingly, a weight increase was incubated on some kidney stones in tap water, although there was no significant increase in the calcium mineral. Although there was no chemical reaction or modification, there might be a possible crystallization in water and therefore there might be an increase in the weight of the kidney stones.

These results revealed the diuretic effects of *E. arvense* plants. In addition, when the kidney stones were held in the acidic extracts, the kidney stones were losing their weights, but calcium minerals increased in the solutions.

In a different study, the kidney stones were placed into tap water and *E. arvense* extracts (1% and 10%) and the extracts PH values were arranged as 5.8 and 8.5 and temperature of these extracts were kept constant at 37°C for 45 days. The extracts and tap water were renewed for every three days. The PH values of the extracts were stabilised during the experiment. At the end of the experiment, while the kidney stones were softened and their outer layers peeled out in acidic extracts, but no fragmentation was seen on the kidney stones in basic extracts and tap waters.

Effects of *E. arvense* Extracts on the Contraction of Ureter and Bladder Muscles

In this study, the results indicated that there were no significant impacts on the contraction of ureter muscles in 1% and 10% concentration of extracts, but a significant influence on the contraction occurred on the bladder muscles in 10% concentration of extracts. These results were shown in Figure 2 and 3.

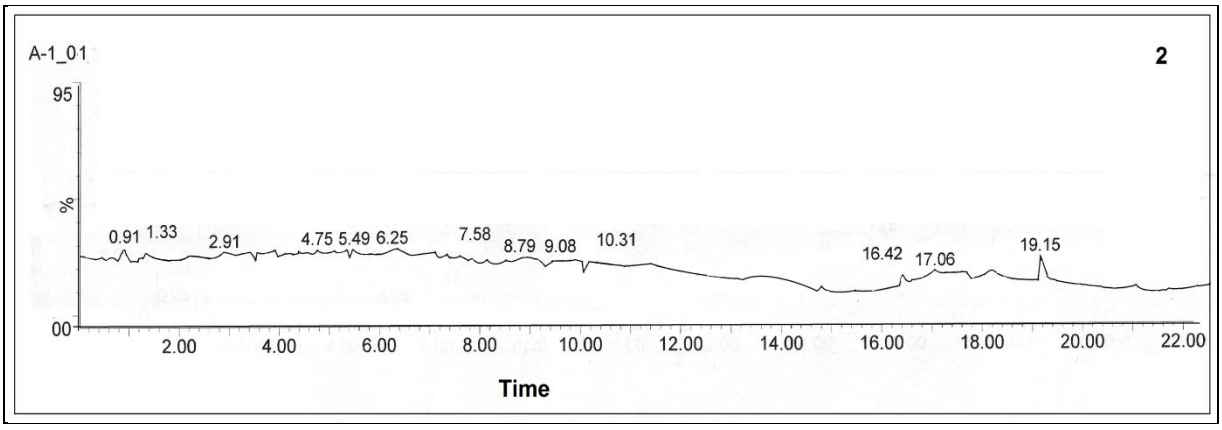

Figure 2: The response of 10% extracts on the ureter muscles.

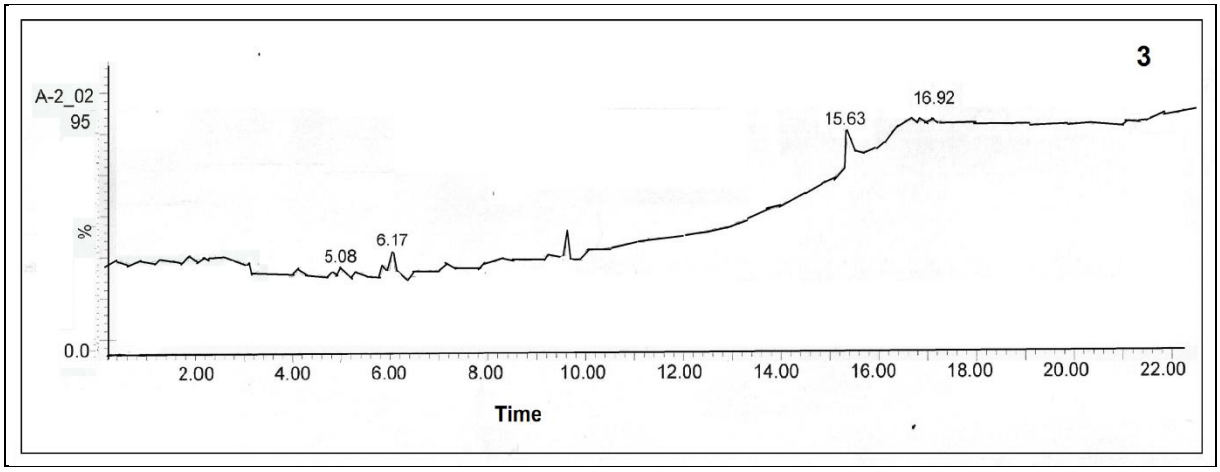

Figure 3: The response of 10% extracts on the bladder muscles.

Discussion

Plants have been used for medicinal purposes as long as history has been recorded. Herbal medicine is a complementary therapy that uses plants to treat some disorders. In various countries, a large number of plants have been used as therapeutic agents in the traditional medicine [32, 33].

In reviewing the literature, a lot of scientific studies were encountered about the diuretic effects of medicinal plants [34, 35, 19], but there has been no study about the fragmentation or melting effects of these plants on the kidney stones.

Since being at the top of diuretic herbs and widely used for removing the kidney stones by the public, *E. arvense* has been an important factor for that's why it was chosen as the experimental material in this experiment [36, 17].

After the application, *E. arvense* extracts showed a decrease in urine density that appeared to vary with dose and time as well as the nature of the extracts. Compared to the tap water, the 1% and 10% extracts produced diuretic effects, particularly with their application after the first day. The lower dose of the plant extract (1%) was able to produce significant effect beginning from the 1st day until the end of the experimental period. This could probably suggest that the lower doses might represent sub-threshold doses. But, the higher dose of plant extract (10%) produced more effect than that of the lower dose of extract (1%) and tap water. The results show that the decrease in urine concentration is an indicator of urine gravity decrease. This can be interpreted as the filtration of water and throwing more urine from the kidneys. This result indicates the diuretic effect of *E. arvense* plant by this experiment. If you remove plenty of urine from the kidneys and if there are small stones and sands in the urinary system, this means that you throw them from the body by movement [37].

The exact principles responsible for the diuretic effects of the extracts of the plant are not known. But, according to some phytochemical analysis carried out with the extracts, there are several compounds which could be responsible for the plants diuretic effects such as flavonoids, saponins or organic acids [34]. This effect might be produced by stimulating blood flow or initial vasodilatation or by producing inhibition of tubular reabsorption of water and ions [38].

Upon examining the fragmentation effects of *E. arvense* extracts on the kidney stones, a significant fragmentation occurred on the kidney stones in acidic extracts, but no significant fragmentation was seen on the kidney stones in basic extracts and tap waters. Besides, a lot of dissolutions of Ca minerals were determined (as ppm) in acidic extracts by atomic absorption method, but a little or no dissolution occurred in basic extracts and tap waters. When the kidney stones were put into other 1% and 10% *E. arvense* extracts and waited for a long time (45 days), by checking manually, the kidney stones were softened and their outer layers were peeled off. These features occurred more in 10% extracts than in the 1% extracts. This result showed the melting effects of *E. arvense* extracts on the kidney stones. Although a detailed investigation was carried out in the literature, no scientific information was found about the melting effects of this plant on the kidney stones.

It was found in the investigations that these kidney stones were found to have an inorganic structure (Ca stones). This result indicated that inorganic stones occurred in the basic character of urine [4, 7]. Therefore, there had not been any softening or melting in the kidney stones in the basic extracts and tap water.

Kidney stones, in their nature, are not always homogenous and their structures change from one person to another person. The structure of kidney stones can vary depending upon the metabolism and nutrition. While the stones are formed, a core material and some crystalloid debris are combined to make the kidney stones. Furthermore, different salts such as magnesium and phosphates are added on them [1, 15, 7]. Therefore, as a result of dissolution of the kidney stones in the extracts, the amount of calcium (as ppm) also changes in extracts.

When the effects of *E. arvense* extracts on the contraction of the bladder and ureter muscles are examined, it is thought that they have more effects on the bladder muscles than the ureter muscles and therefore it might have increased the urinary excretion. All diuretic drugs and plants have a direct impact on the nephrons in the excretory system of kidney. Although, it was investigated widely in the literature, no scientific information was found about the effects of diuretic plants on the ureter and bladder system.

This result showed that *E. arvense* extracts actas diuretic effects and remove the kidney stones in Guinea pigs. When the plant extracts drunk by people, this extracts cause strong diuretic actions in terms of cumulative urine output and melting effects. It will also be effective for the fragmentation of large kidney stones and remove small stones and sands from the kidney. Advanced studies are needed to understand the effects of this plant extracts on the ureter and bladder muscles.

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