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Research Paper

Preventive treatment of calcium oxalate crystal deposition with immortal flowers

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ABSTRACT

Ethnopharmacological relevance: A number of medicinal plants are used for their diuretic, urolithiatic and anti-inflammatory effects on urinary system problems in Turkey and the most common traditional remedy for kidney stones is the tea of immortal flowers. The aim of this study is to evaluate the preventive effect of infusions prepared from capitulums of *Helichrysum graveolens* (M.Bieb.) Sweet (*HG*) and *Helichrysum stoechas* ssp. *barellieri* (Ten.) Nyman (*HS*) on formation of kidney stones.

Materials and method: Sodium oxalate (Ox-70 mg/kg intraperitoneally) was used to induce kidney stones on Wistar albino rats. At the same time, two different doses of the plant extracts (*HG*: 62.5 and 125 mg/kg; *HS*: 78 and 156 mg/kg) were dissolved in the drinking water and administered to animals for 5 days. Potassium citrate was used as positive control in the experiments. During the experiment, water intake, urine volume and body weights of the animals were recorded. At the end of the experiments, liver, kidney and body weights of the animals were determined; biochemical analysis were conducted on urine, blood and plasma samples. Histopathological changes in kidney tissues were examined and statistical analysis were evaluated.

Results: *HS* extract showed the highest preventive effect at 156 mg/kg dose (stone formation score: 1.16), whereas a number of kidney stones were maximum in sodium oxalate group (stone formation score: 2.66). *Helichrysum* extracts decreased urine oxalate and uric acid levels and increased citrate levels significantly. In addition, *Helichrysum* extracts regulated the negative changes in biochemical and hematological parameters occurred after Ox injection.

Conclusions: We conclude that *Helichrysum* extracts could reduce the formation and growth of kidney stones in Ox-induced urolithiasis and can be beneficial for patients with recurrent stones. In addition, this is the first study on the preventive effect of immortal flowers.

1. Introduction

Urolithiasis is a growing health problem in industrialized countries and often correlated with habits such as hypertension, high purine intakes, diabetes, obesity, and metabolic syndrome (Rosa et al., 2013). Unexpectedly, 12% of the world's population is affected by urinary system stone disease (Pak, 1998; Basavaraj et al., 2007). Urolithiasis is formation of kidney stones in urine-collecting spaces of the kidneys. Under certain conditions, substances normally dissolved in the urine can separate out as crystals and accumulate to form a solid mass called kidney stone. Stones can migrate into the ureters, the bladder and finally be evacuated in the urine. Even surgical procedures, drug treatment, extracorporeal shock wave lithotripsy and endoscopic techniques are commonly used to treat stones; none of these options inhibit formation of new kidney stones. It is established than the recurrence rate of calcium oxalate stones is 10.5% in the first year; but it reaches 50% at the tenth year (Menon et al., 1998). Thus, after treatment, there is a great demand for nonsurgical methods to prevent the recurrence of urolithiasis.

In recent years, beside drug treatment, herbal medicines and their components have been widely investigated (Rosa et al., 2013). Data from *in-vitro*, *in-vivo* and clinical trials revealed that phytotherapeutic agents could be usefull as either an alternative or a complementary therapy in the management of urolithiasis (Butterweck and Khan, 2009). Therewithal, various plants and herbal preparations have been used for treatment of kidney stones since ancient times. Among these, infusions prepared from capitulums of *Helichrysum* species are

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used all around Turkey (Baytop, 1999). Many ethnobotanical studies revealed that different species of *Helichrysum* genus such as *Helichrysum arenarium* (Sargın et al., 2013), *armenium* (Özüdoğru et al., 2011), *compactum* (Kargıoğlu et al., 2010), *orientale* (Gürdal and Kültür, 2013), *pallasi* (Altundağ and Öztürk, 2011), *plicatum* (Honda et al., 1996), and *stoechas* (Tuzlacı and Sadıkoğlu, 2007) have been used to pass kidney stones (Tabata et al., 1993). *Helichrysum stoechas* and *Helichrysum graveolens,* most common and widely grown species in Turkey, are sold for their medicinal purposes including diuretic and anti-urolithiatic effects in herbal shops called aktar.

Nineteen *Helichrysum* species (Asteraceae) grow in Turkey (Sümbül et al., 2003), and many of them have been widely used for stomachache, as anti-asthmatic, anti-diabetic, diuretic, lithagogue, to pass kidney stones, and as herbal tea in Turkish folk medicine (Sezik et al., 1991; Sezik et al., 2001). According to traditional usage, number of research works have been worked on different biological activities of *Helichrysum* species (Süzgeç et al., 2005; Aslan et al., 2007a, 2007b; Sezik et al., 2010; Orhan et al., 2014).

In vitro models of experimental urolithiasis relate to only one event and aspect of the kidney stone disease. Thus, animal models are widely preferred in order to understand all aspects of the pathogenesis, including the anatomic and pathological role of kidneys. The objective of this study is to evaluate the preventive and beneficial effects of *Helichrysum* species on experimental calcium oxalate crystallization in rats.

To determine the preventive effect of the extracts on renal calculi formation, *Helichrysum graveolens* and *Helichrysum stoechas* ssp. *barellieri* capitulum extracts were administered to rats with sodium oxalate (Ox-70 mg/kg i.p.) simultaneously for 5 days. Metabolic cages were used to measure water intake and urine volumes for 24 h. Plasma and urine biochemical parameters were measured and histopathological studies were waged in the experiments.

2. Material and methods

2.1. Plant materials

Helichrysum graveolens (M.Bieb.) Sweet *(HG)* was collected from Ilgaz Mountain, Kastamonu/Turkey (July 2010) and *Helichrysum stoechas* ssp. *barellieri* (Ten.) Nyman *(HS)* was collected from the open field near Saint Pierre Church, Antakya/Turkey (May 2011). Plant species were identified by Prof. Dr. Mustafa Aslan and herbarium specimens are deposited in the Herbarium of Gazi University Faculty of Pharmacy (GUE 2490, 2491).

2.2. Preparation of the extracts

Infusions [3% (w/v)] of dried capitulums of *Helichrysum* species was prepared in water on a water bath by mixing for 30 min. The extract was filtered using filter paper and concentrated under *vacuum* using rotary evaporator at 45 °C and extracts were dried in a freeze dryer. The yields of the extracts were: *HG*: 18.0%, and *HS*: 22.5%.

The doses of the plant extracts were calculated according to folkloric knowledge and yield of the extracts. In Turkey, a cup of infusion or decoction (3%) of the *Helichrysum* capitulums is drunk three times a day for 10 days to pass kidney stones (Baytop, 1999). Before the experiments, daily water intake of the rats in metabolic cages were monitored. The quantity of the dried extract of *Helichrysum* capitulums to be weighed was calculated pursuant to the doses and dissolved in drinking water according to the mean of daily water consumption.

2.3. Animals

Wistar Albino male rats (200–250 g) were used in this study with the approval of Animal Experiments Local Ethical Committee (GUET-09.079). Experiments were performed according to the international rules considering the animal experiments and biodiversity rights. Animals were purchased from the animal breeding laboratories of Experimental Animal Research Center of Gazi University (GUDAM) (Ankara, Turkey) and the experiments were carried on at the same research center. The animals were maintained on standard pellet diet and water *ad libitum* throughout the experiment.

2.4. Experimental procedures

Animals were divided into seven groups of six rats each. Sodium oxalate (70 mg/kg) dissolved in physiological saline was administered intraperitoneally (i.p.) for 5 days to induce urolithiasis (Bouanani et al., 2010). To evaluate the effect of *Helichrysum* extracts on stone formation, extracts were given to animals by mixing to drinking water during Ox administration. Only daily diet and water was given to control group. Potassium citrate was administered to positive control (4 meq/daily per rat) by adding to drinking water. *HG* (62.5 and 125 mg/kg) and *HS* extracts (78 and 156 mg/kg) were given to rats at two different doses by adding to drinking water for 5 days.

On the 5^{th} day, body weights of the animals were recorded then rats were anesthetized with ketamine/xylazine and the blood samples were withdrawn by cardiac puncture. The liver and kidneys were removed from the abdominal cavity quickly, cleaned with ice cold physiological saline and weighted. In order to compare the changes in the organ weights, relative organ weights were calculated after the experiment according to the formula

Relative Organ Weight = [Organ Weight/Body Weight (at the last day)] × 100

Kidneys were used to examine histopathological changes and calcium oxalate crystals in tissues.

2.5. Hematological and biochemical analysis

Blood samples were collected from the heart into two tubes for biochemical and hematological analysis (a heparinized tube and a tube with EDTA). In order to determine biochemical parameters, blood samples collected in heparinized tubes were centrifuged at 3000*g* for 10 min to obtain plasma. For the hepatic functions, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were determined from plasma samples. In addition, some lipit parameter such as cholesterol, triglyceride, high density lipoprotein (HDL), very low density protein (VLDL) and low density lipoprotein (LDL), blood urine nitrogen (BUN), creatinine, uric acid, total protein, albumin and levels of some elements (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, and Cl^{-} ions) were evaluated. Also hematological analysis were carried out by a Beckman Coulter LH780 blood autoanalyzer.

Urine samples of each group were collected from metabolic cages after 24 h. Urine citrate, oxalate, uric acid, BUN, creatinine, total protein, albumin, and levels of some elements (Ca^{2+}, Na^{+}, K^{+}, and Cl^{-} ions) were measured from the urine samples of the last day. All biochemical parameters were determined by Beckman Coulter AU2700, oxalate and citrate levels were measured by a Hitachi U2700 spectrophotometer.

2.6. Histopathological analysis

For the histopathological studies, the kidney tissue samples from both kidneys of the animal were fixed in 10% formalin for a period