

Clinical study of three medicinal plants (*Foeniculum vulgare*, *Zea mays* and *Petroselinum sativum*) against urinary tract infection and stones

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Abstract

This clinical study aimed for the evaluation of three medicinal plants which are parsley seeds, fennel fruits and corn silk against kidney stone and infections. The study's methods were included preliminary phytochemical investigation of all plant parts used and the study was done by using cold maceration with 90% ethanol. Different reagents were used for testing different active compounds such as alkaline reagent test for flavonoids, foam test for saponins, terpenoids test for terpenoids, Fehling's reagent for reducing sugar and Dragendroff's reagent for alkaloids. The clinical study carried out in an outpatient clinic in Imam Al-Hussein medical city, Karbala, Iraq. The 300 patients divided into two groups, the first group includes 150 patients for urinary tract infection for ten days and the same number of patients for stones. The dosage form of plant extracts in this study was 500 mg as soft gelatin capsules three times a day before meal. Each capsule contained 200 mg of fennel fruit extract, 200 mg of corn silk extract and 100 mg of parsley seed extract. All results analyzed by Spss and compare with T-test value at 0.001 probability. The preliminary investigation revealed that flavonoids, saponins, alkaloids, reducing sugar, and terpenoids were presented in plants extracts which used in this study. The result of urinary tract infection study gives indication that these three plant extracts were very effective in the treatment of infection which caused by bacteria especially E-coli. The treatment of stones also effective, but need more time to get rid of all types of stones.

Keywords: Urinary tract infection, Stone, clinical study, *Foeniculum vulgare*, *Zea mays* and *Petroselinum sativum*

INTRODUCTION:

Kidneys are important organ in the body of human and animals because they have a basic role in filtration process of waste products from blood, regulation of blood pressure, balancing the electrolyte in the body, and increasing the production of red blood cells.⁽¹⁾ Urinary tract infection (UTI) caused by microbes such as bacteria that overcome the body's defenses in the urinary tract, they are one of the most common types of infection that account around 8.1 million visit to doctors each year.⁽²⁾

The symptoms of UTI depend mainly on gender, age, presence of infected tract. Common symptoms of UTI include: nausea and vomiting, abdominal pain, muscle ache, strong and frequent urgency of urination, pain or burning sensation on urination, and cloudy, bloody or strong smell urine.⁽³⁾

Approximately about 18% of Iraqi people suffer from urinary tract stones. Stones prevalence increase with age in both genders males and females, this study has suggested that diet and life behavior may play a more role in stone formation than ethnicity.⁽⁴⁾

Most stones form due to a combination of genetic and environmental factors. Risk factors include: obesity, high calcium level in urine, some medication, certain foods, hyperparathyroidism, calcium supplement, gout, and drinking of little fluids.⁽⁵⁾

Today, medicinal plants had been widely used for centuries by the ancient Greek. In Ayurvedic medicine, the herbs had been utilized to maintain the efficient function of the urinary system. Certain herbal remedies have long been used in treating kidney problems and other problems associated with urinary tract.⁽⁶⁾

Recently the world health organization (WHO) referred to about 80% of people in the world used a herbal agent in primary health care and treatment several disease. In herbal medicine the management of UTI was high necessitate due to increase the resistance of antibiotics among bacterial pathogens.⁽⁷⁾ The aim of this study was conducted to select three medicinal plants with investigation of their general active compounds and evaluation of the clinical activity for urinary tract infection and stone, these plants were included corn silk (*Zea mays*), parsley seeds (*Petroselinum sativum*), and fennel fruits (*Foeniculum vulgare* Mill.).

MATERIAL AND METHODS

Plant samples preparation:

Samples of corn silk, fennel fruits and parsley seeds were obtained from Al- Waady al-akhter office for herbs and medicinal plants in Baghdad – Iraq. All plant samples were identified and authenticated by national herbarium of Agriculture Ministry of Iraq. After drying the plant samples were converted to powder by mechanical grinder.

Plant sample extraction:

The powders of samples were macerated in 90% ethanol overnight, then filtered and drying by using rotatory evaporator.^{(8),(9)}

Preliminary phytochemical investigation:

The preliminary investigation was done by using alkaline reagent test for flavonoids, foam test for saponins, terpenoids test for terpenoids, Fehling's reagent for reducing sugar and Dragendroff's reagent for alkaloids.

-Alkaline reagent test for flavonoids:

The test of flavonoids was done by adding 10 ml. of ethyl acetate to 0.2g of the powdered plant material then heat it in a water bath for 3 minutes, cooled and filtered, then the filtrate was treated with 1 ml of diluted ammonia.⁽¹⁰⁾

-Foam test for saponins:

The detection of saponins was done by shake 0.2gm of crude extract with 2ml of distilled water.⁽¹¹⁾

-Reducing sugar test (Fehling's test):

Fehling's reagent was added to the extract and the presence of reddish brown precipitate of cuprous oxide would indicate the presence of reducing sugar.⁽¹²⁾

-Terpenoids test:

By adding 2 ml chloroform with 3 ml of concentrated sulfuric acid to plant extracts and the presence of reddish brown color interface would indicate the presence of terpenoids.⁽¹³⁾

-Alkaloids test:

Mayer's and Dragendroff's reagent were used for detection of alkaloids.⁽¹¹⁾

Clinical study:

The prospective randomized clinical trial was carried out on 300 patients were divided into two groups. The first group was included 150 patients for urinary tract infection for ten days and the same number of patients for stones .

The clinical trial was carried out at Imam Al-Hussein Medical City at Karbala, Iraq, over a period from February 2017 – March 2017; three capsules per day, each one weigh 500 mg of which 200 mg of fennel, 200g of corn silk and 100 mg of parsley seeds powder, this dose was taken according to the British pharmacopeia. The study was approved by scientific and ethical committee in Imam Al-Hussein Medical City; an informed consent was taken from all patients. The inclusion criteria were; adult patients with chronic recurrent UTI who develop at least one UTI in the last 6 months. The exclusion criteria were: patient with chronic diseases like ischemic heart disease, diabetes, and hypertension problems. For all patients, UTI is confirmed by defining symptoms together with laboratory results. Routine examination of urine is utilized, the microscopic examination is done for the presence of RBCs, pus cells, and crystals; a 10 ml sample is sufficient for conducting the test.⁽¹⁴⁾ Manifestation of UTI were checked clinically include supra pubic pain, urgency, frequency and dysuria. ⁽¹⁵⁾All of the patients were followed up after 10 days for UTI and 20 days for stones and crystals from starting a course of treatment. ⁽¹⁶⁾

RESULTS AND DISCUSSION:

Preliminary phytochemical investigation:

preliminary investigation revealed the presence of different active compounds according to the medicinal plants used in this study and these results were recorded in table (1).

Table 1 : General active compounds of corn silk, parsley seeds and fennel fruits.

| Active compounds | Corn silk | Parsley seeds | Fennel fruits |
|------------------|-----------|---------------|---------------|
| Flavonoids | + | +++ | ++ |
| Saponins | +++ | ++ | + |
| Reducing sugar | + | +++ | ++ |
| Terpenoids | + | + | + |
| Alkaloids | + | + | + |

Clinical Study:

UTI (Urinary Tract Infection)

The group that taken to study the effect of plants on UTI consist from 150 patients, from which 37 patients were male which the other 111 patients were female. The percent of gender was shown in the table (2).

Table (2) : Gender frequency and percentage of UTI patients

| Gender | Frequency | Percent |
|--------|-----------|---------|
| Male | 39 | 26 |
| Female | 111 | 74 |
| Total | 150 | 100 |

The patients suffer from many diseases in addition to the UTI symptoms, about 30 patients from the origin group 150 patients suffer from hypertension while the other not as shown in table (3).

Table (3) suffering of patients from hypertension.

| HT | Frequency | Percent |
|-------|-----------|---------|
| No | 120 | 80 |
| Yes | 30 | 20 |
| Total | 150 | 100 |

Also during the study we found 15 of the patients suffer from diabetes mellitus as shown in table (4)

Table (4) Suffering of patient from diabetes Mellitus .Types 2

| DM | Frequency | Percent |
|-------|-----------|---------|
| No | 135 | 90 |
| Yes | 15 | 10 |
| Total | 150 | 100 |

There are many factors could affect on the UTI disease and its treatment in the previous group like: smoking, recurrence of disease and symptoms and occupation so they take as attention factors and listed in table 5 , 6 and 7.

Table (5) Suffering of patients from smoking

| Smoking | Frequency | Percent |
|---------|-----------|---------|
| No | 111 | 74 |
| Yes | 39 | 26 |
| Total | 150 | 100 |

Table (6) Suffering of patients from occupation

| Occupation | Frequency | Percent |
|------------|-----------|---------|
| Housewife | 83 | 55.34 |
| Employee | 45 | 30 |
| Student | 15 | 10 |
| Others | 7 | 4.66 |
| Total | 150 | 100 |

Table (7) Suffering of patients from recurrence

| Recurrence | Frequency | Percent |
|------------|-----------|---------|
| No | 98 | 65.34 |
| Yes | 52 | 34.60 |
| Total | 150 | 100 |

After taking all the previous factors in consideration the data of UTI pus in the above 150 patients were as follows in table 8.

Table (8) The valid percent and frequency of UTI pus (+) before treatment (Btx)

| UTI pus (+) Btx | Frequency | Valid Percent |
|-----------------|-----------|---------------|
| 0 | 17 | 11.58 |
| 1 + | 46 | 31.0 |
| 2 ++ | 39 | 26.34 |
| 3+++ | 23 | 15.54 |
| 4 ++++ | 23 | 15.54 |
| Total | 148 | 100.0 |
| Missing system | | 2 |
| Total | 150 | |

Table (9) The frequency and valid percent of UTI pus (+) after treatment (Atx)

| UTI pus (+) Atx | Frequency | Percent |
|-----------------|-----------|---------|
| 0 | 78 | 52.7 |
| 1 + | 62 | 41.9 |
| 2 ++ | 8 | 5.4 |
| Total | 148 | 100.0 |
| Missing System | | 2 |
| Total | 150 | |

Table (10) The frequency and valid percent of Bacteria (+) before treatment (Btx)

| Bacteria (+) Btx | Frequency | Valid percent |
|------------------|-----------|---------------|
| 0 | 69 | 46.6 |
| 1 | 62 | 41.9 |
| 3 | 17 | 11.5 |
| Total | 148 | 100.0 |
| Missing System | 2 | |
| Total | 150 | |

Table (11) The frequency and valid percent of Bacteria (+) after treatment (Atx)

| Bacteria (+) Atx | Frequency | Valid Percent |
|------------------|-----------|---------------|
| 0 - | 111 | 75 |
| 1 + | 37 | 25 |
| Total | 148 | 100.0 |
| Missing system | 2 | |
| Total | 150 | |

The valid percent and frequency of crystals (Btx) and (ATx) were occurring in table 12 and 13 .

Table (12) The valid percent and frequency of crystals before treatment (Btx)

| Crystals Btx | Frequency | Valid Percent |
|----------------|-----------|---------------|
| 0 | 116 | 78.9 |
| 1 | 16 | 10.5 |
| 2 | 8 | 5.3 |
| 3 | 8 | 5.3 |
| Total | 148 | 100.0 |
| Missing system | 2 | |
| Total | 150 | |

Table (13) The valid percent and frequency of crystals after treatment (Atx).

| Crystals Atx | Frequency | Valid Percent |
|----------------|-----------|---------------|
| 0 | 140 | 94.6 |
| 1 | 8 | 5.4 |
| Total | 148 | 100.0 |
| Missing system | 2 | |
| Total | 150 | |

Stone

The group that taken to study the effect of medicinal plants used in this study one stone consist from 150 patients, from which 110 patients were male while the other 40 patient were female. The percent of gender was shown in the table (14).

Table (14) The patients were suffering from kidney stone

| Gender | Frequency | Percent |
|--------------|-----------|---------|
| Male | 110 | 73.3 |
| Female | 40 | 26.7 |
| Total | 150 | 100 |

The patients suffer from many diseases in addition to the stone symptoms, about 120 patients from the origin group 150 patients suffer from hypertension while the other not as shown in the following table (15) .

Also during the study we found 25 of the patients suffer from diabetes mellitus type 2 while the other not as shown in table (16)

Table (15) : The frequency and percent of patient suffer from hypertension.

| HT | Frequency | Percent |
|-------|-----------|---------|
| No | 120 | 80 |
| Yes | 30 | 20 |
| Total | 150 | 100 |

Table (16) patient frequency and percent of patients suffer from diabetes mellitus type 2.

| DM | Frequency | Percent |
|-------|-----------|---------|
| No | 125 | 83.3 |
| Yes | 25 | 16.7 |
| total | 150 | 100 |

There are many factors could calculated in patients with stone in the previous group like smoking , recurrence of disease and symptoms and occupation so they take as attention factors and listed as in tables (17), (18) and (19) .

Table (17): The frequency and percent of smoker patients suffer from stone.

| Smoking | Frequency | Percent |
|---------|-----------|---------|
| No | 65 | 43.4 |
| Yes | 85 | 56.3 |
| Total | 150 | 100 |

Table (18): The frequency and percent of occupation patients suffer from stone.

| Occupation | Frequency | Percent |
|------------|-----------|---------|
| Housewife | 25 | 16.7 |
| Employee | 63 | 42.0 |
| Student | 12 | 8.0 |
| Others | 50 | 33.3 |
| Total | 150 | 100 |

Table (19): The frequency and recurrence percent of patients suffer from stone.

| Recurrence | Frequency | Percent |
|------------|-----------|---------|
| No | 50 | 33.3 |
| Yes | 100 | 66.7 |
| Total | 150 | 100 |

After taking all the previous factors in consideration the data of stone in the above 150 patients were as follows in the table (20) and table (21).

Table (20): The frequency, valid and cumulative of crystals before treatment (Btx).

| Crystals Btx | Frequency | Valid percent | Cumulative |
|--------------|-----------|---------------|------------|
| 0 | 100 | 66.7 | 66.7 |
| 2 mil | 12 | 8.05 | 75.0 |
| 3 mil | 12 | 8.05 | 83.3 |
| 4 mil | 13 | 8.60 | 41.7 |
| 10 mil | 13 | 8.60 | 100.0 |

Table (21): The frequency, valid and cumulative of crystals after treatment (Atx) .

| Crystals | Frequency | Valid | Cumulative |
|----------|-----------|-------|------------|
| 0 | 125 | 83.3 | 83.3 |
| 1 | 25 | 16.7 | 100 |
| Total | 150 | 100 | |

The usage of plants in the treatment of diseases in general and especially UTI and stone is very important. Urinary tract

infection (UTI) can be effectively treated with corn silk, parsley and fennel. The utilization of herbal medicine that utilized to treat disease depend upon first : their action, and second : the appropriate therapeutic dose strategies than can determine the effectiveness of the herbal treatment and prevent the any need to use the antibiotics.⁽¹⁷⁾ The three plant we used clinically has antibacterial activity so they are efficient in the treatment of UTI . Its believe now that some of medicinal plants can prevent the recurrence of UTI.

The mechanism of action of parsley involve an inhibition of Na⁺/ K⁺ pump that will lead to reduction in sodium and potassium re-absorption, thus, to an osmotic water flow in to lumen and diuresis.⁽¹⁸⁾

The mechanism of action of fennel has soothing effect on the muscles and tissue of urinary tract, it promote elimination of waste from urinary tract, it relieve pain, swelling, infection and congestion in the urinary tract.^{(9), (19)}

The mechanism of action of CS has diuretic activity due to soothes and relaxes the lining of the urinary tract and bladder, relieving irritation and improve the urine flow and elimination.⁽²⁰⁾

The first group (150) patients in the study of plant effect on UTI there was noticeable effect on the number of plus (indication of inflammation) in table (8) before treatment and (9) after treatment, first there was no four plus and second the patient number cured increase to 78 from the origin 150 during 10 days, but it is important to mention there was one patient can not follow up for unclear reasons.

The table of results of bacteria study in the previous patients give indication that those three plants were very effective in the treatment of bacteria that cause UTI especially E. coli in the table (10) before treatment and (11) after treatment, the number of plus decrease from three plus to one plus and the number of cured patients was increase from 69 to 111, also there were two missing patients. Table (12) crystals before treatment and able (13) after treatment indicate that there is improvement in the patients, the plus number decrease from three to one plus and only 8 patient has that one plus, also there were 2 missing patients.

Table (20) crystals before treatment and table (21) after treatment. The incidence of crystals decrease from ten to one after treatment and the number of curing patients increase from 100 to 125 from the origin of 150 this makes imagination if the number of infected patients increase the numbers of curing patient also increase. The above results mean there were 25 patients treated completely from origin of 50 and crystals decrease from ten to one.

CONCLUSION:

The combination of the three medicinal plant extracts (parsley, corn silk, and fennel) that used in this clinical study has strong effect in the treatment of urinary tract infection and stones more than the individual extracts. The Combination of the anti-oxidative stress, and antibacterial activity of these three medicinal plants related to the mechanism of treatment of UTI and kidney stones.

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