Assessment of some serum and urine biochemical constituents and renal tubular architecture in Sprague-Dawley rats supplemented with parsley(\textit{petroselinum crispum}) leaves

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Abstract
The current study has been designed to investigate the physiological effect of parsley (\textit{petroselinum crispum}) leaves on serum and urine concentrations of some biochemical constituents and renal tubules architecture in albino rats. Twenty albino rats were assigned in two groups: control and treatment group, fed on standard and parsley leaves-supplemented feed, respectively for ten days. At the end of the experiment, blood and urine samples were collected for serum and urine analysis. Kidneys were removed and transferred to the tissue processing. The results revealed an acidic urine, proteinuria, hypokalemia, hyponatremia, significant decrease in BUN and significant increase in serum uric acid level in treatment group. The histological study revealed a hydropic changes in convoluted tubules epithelium, sloughing of some epithelium to the lumen, the renal tubules was dilated and contained colloid casts.

Introduction:
The use of herbal medicinal product continues to increase and there is a growing evidence that herbal medicines are used broadly by all groups of the society including children, pregnant, and breast feeding (1). A number of different botanical medicines has been used in treatment of a variety of urologic disorders, of these plants is parsley (\textit{Petroselinum crispum}) (2) a plant that originates from Mediterranean, it had been used as a diuretic, carminative, or agent that relieves intestinal spasms in traditional medicine (2,3). Major compounds of parsley are flavonoids particularly apigenin, furanocmaris:
bergapten and oxypeucednin are major contents and volatile oils that found in seed and leaf which involve apiol and myristicin as a major volatile oils (1). (4) found that apigenin from an orally administrated parsley extract associated with antioxidant activity, while (5) found that apigenin exerts antinflammatory effects in vitro. Parsley considers as one of a dietary sources for vanadium, so it may serve a beneficial role in normal glucose metabolism (6). An aqueous parsley extract is able to lower blood pressure (7). Because parsley is a member of Apiaceae family like celery and lovage, high lightly have the tendency of this family members to be diuretic (2) we deals in this study to evaluate the physiological effects of parsley on renal tubules in rats.

Materials and Methods

- **Plant collection and preparation:** The herbal plant parsley (*Petroselinum crispum*) has been used in this experiment. The whole plant was brought from the local market. Leaves were removed from other parts of plant to be introduce to rats as fresh herb.

- **Experiment Design:** twenty Wister albino rats were used in this study. After acclimatization, animals were divided into two equal groups and treated as follow:
  A. control group (c): Rats were fed on standard feed.
  B. treatment group (t): Rats were fed on standard feed plus fresh parsley leaves at a dose of 1 g/rat daily for ten days (8).

- **Samples Collection:** after anesthesia, blood samples were obtained by direct cardiac puncture for estimation Na⁺, K⁺, serum uric acid and blood urea nitrogen (BUN). Direct catheterization of urinary bladder with a syringe to collect single random urine samples for protein, glucose and pH determination. Kidneys were removed and preserved in formalin for the histological study.

- **Parameters used in the experiment:**
  A. **Serum examination:** serum sodium and potassium levels were estimated by using a flame photometer (Elicou Co. LTD, England) in the Central Lab for researches in Al-Qadisiya University according to the method of Sood (9), BUN and serum uric acid had been estimated according the colorimetric method of Sood (9), (Cromatent, Spain) and (Spinreact, Spain) sticks were used for BUN and serum uric acid respectively.
  B. **Urine Analysis:** urine samples examined with a special urine reagent strips (Cybow, DFICO., LTD, Korea) for urine analysis in order to detect protein, glucose and pH, the results readed after exactly 60 second according to the colorimetric method.
  C. **Histological Study:** the preserved kidneys were transferred for tissue processing, the tissue sections were (5) micron in thickness and prepared according to Samuelson method (10) then the sections had been stained with H. & E. and PAS to observe the histological change in these sections using the microscope (BEL phonotics microscope, India) at 100 × and 400 × powers.

Results

The results of the present study revealed that the treated group which received fresh parsley leaves at a dose of 1 g/ rat daily for ten days lead to a significant decrease (p<0.05) in urine pH (acidic urine), the pH of treatment with standard error was 6.29±0.15 in comparison to that of control group 7.18±0.18 for control group. Urine protein (mg/ dl) was significantly increased (p<0.05) 108.6±33.95 which indicates proteinuria in treatment group versus 65±9.04 for control group. While the results of glucose in urine (mg/dl) showed no significant differences between the two groups, which revealed no glycosuria (Table 1). The results of serum examination (Table 2) showed significant decrease (p<0.05) in sodium ion concentration (mEq/L) in serum (hyponatrema) in treatment group 168 ± 28.35 versus 422.48 ± 9.25 for control group.
There was a significant decrease (p<0.05) in serum potassium level (mEq/L) in treatment group 13.3±1.54 in comparison with 17.1±1.86 for control group. The blood urea nitrogen BUN (mg/dL) was significant decreased (p<0.05) which was 41.5±0.33 and 42.5±0.64 for treatment and control group, respectively. While Serum uric acid levels (mg/dl) in treatment group was significantly increased (p<0.05) 5.6±0.26 versus 3.7±0.05 for control group. The results of histological study revealed a destruction of renal tubules, the epithelium of the convoluted tubules is swollen and granular (hydropic change). In places, the lining cells are necrotic, also some of the epithelium lining of the collecting tubules has died and sloughed into the lumen, the tubules are dilated and contain eosinophilic (colloid) casts (Fig. 2). The control group showed normal renal tubules (Fig. 1).

Table (1) Effect of Parsley (*Petoselinum crispum*) leaves on urine pH, protein, and glucose levels (means ± standard error) in Wister Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.18±0.18a</td>
<td>6.29±0.15b</td>
</tr>
<tr>
<td>Protein (mg/dl)</td>
<td>65±9.04a</td>
<td>108.6±33.95b</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>Negative</td>
<td>Trace a</td>
</tr>
</tbody>
</table>

*The different small letters refer to significant differences between two groups (p<0.05)  
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Table (2) Effect of Parsley (*Petoselinum crispum*) leaves on serum Na, K⁺, BUN, and uric acid levels (mean ± standard error) in Wister Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium(mEq/L)</td>
<td>422.48±9.25 a</td>
<td>168.64±28.35 b</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>17.1±1.86 a</td>
<td>13.3±1.54 b</td>
</tr>
<tr>
<td>BUN(mg/dL)</td>
<td>42.5±0.64 a</td>
<td>41.5±0.33 b</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>3.7±0.05 a</td>
<td>5.6±0.26 b</td>
</tr>
</tbody>
</table>

The different small letters refer to significant differences between two groups (P<0.05).

Fig. (1) Kidney- treated group, the epithelium of convoluted tubules is swollen and granular (hydropic changes) (C) with necrosis of some other tubule (N). 400 ×, H & E stain.

Fig. (2) Kidney- Control group, normal renal tubules. 400 ×, H & E stain.
Discussion

In this study we tend to evaluate the effect of parsley leaves on some hematological and biochemical constituents as well as renal tubules in rats. The study based on some parameters for renal tubule assaying these parameters varies between urine, serum and histological examinations. Urine analysis is one of the most frequent tested, its provides information's about metabolic, electrolytes dehydration, urinary and endocrine systems status (11). Our urine analysis results revealed a proteinuria, acidic urine with no glycosuria in treatment group. Normally, protein must be not detectable in urine, although a minute or trace amount is excreted by the normal kidney (12, 13), but (14) in 2002 found that the administration of some natural medications such as parsley leaf, root and seed were associated with proteinuria. Glucose usually does not occur in urine, but it will appear in urine if blood glucose levels exceeds the reabsorption capacity of renal tubules (11,13) this indicates that rats of treatment group were have and healthy glucose metabolism, this may be due to vanadium mimics actions of insulin (15) which found in parsley and it promotes healthy glucose metabolism (6). The urine pH express hydrogen ion concentration in urine, which measures the renal degree of acidity or alkalinity of urine (11). It is an indicator of renal tubules ability to maintain normal hydrogen ion concentration in the plasma and extracellular fluids (13). Although the normal urine pH in rats is about 7.8-8.5 (16), the acidity of the treatment group urine appeared in the present study may be due to increase tubular secretion of hydrogen ion to maintain acid-base balance leading to decrease urine pH, or the parsley may act as a chlorothiazide diuretics which cause acid urine to be exerted (13). The significant increase in uric acid and significant decrease in BUN and both electrolytes Na+ and K+ as metabolic by product of nitrogenous catabolism may considered an additional cause for the acidity of urine (11) as well as the diuresis found (13). BUN is a metabolite of protein and amino acids breakdown (17). BUN levels are used to evaluate kidney functions (11). The significant decrease in BUN in treatment group indicates the effect of parsley increasing the efficiency of kidney to remove nitrogenous waste urea from blood. Sodium levels are used to detect mine electrolytes, water balance, water intoxication and dehydration (13). Potassium and sodium levels are used to diagnose acid-base balance (13). Our results revealed hyponatremia and hypokalemia. It has been shown that hypokalemia associated with diuretic administration (12,13,18) noted that hyponatremia associated with diuretics also. Diuretics are substances that act within kidney and promote loss of fluid from the body (19) to be clinically effective it must be increase the excretion of urine from the body (20) as well as the amount of Na+ in urine (20, 21) this achieved by interfering with reabsorption of water and ions through the walls of kidney tubules (19, 22). Parsley is one of the herbal plants have a traditional diuretic uses (20). (23) suggested that parsley may promotes diuresis by blocking K+ absorption or secretion or may be both, also parsley cause inhibition of Na+-K+ pump activity (21). The results of histological study revealed a colloid casts in the dilated renal tubules, this casts may be an accumulation of Na+ and K+ in the lumen of the kidney tubules due to decrease K+ secretion and of Na+-K+ absorption (23). The dilation of renal tubules occur in proximal convoluted tubules, such effect in this segments of renal tubules had been reported to be caused by osmotic diuretics as mannitol, this dilation may be attributed to remaining of the diuretic substance in the lumen of proximal convoluted tubules as an unreabsorbed osmolyte and thereby reduces fluid reabsorption, thus lead to proximal convoluted tubules dilation (24). The cell lining of convoluted tubules were swollen due to hydropic change, this change can be caused by administration of osmotic diuretics or hyponatremia for various causes, and it resulted from swelling of the lysosomes forming.
numerous vacuoles within the cells (25). Our results of histological study revealed necrosis and sloughing of renal tubules lining epithelium. (14) found that the administration of some types of Chinese herbs has been associated with pathologic features of nephropathy, one of these herbs was parsley (leaf, root, and seed). From all previous results we can conclude that parsley appear to have a strong diuretic effects. The diuretic effect is associated with the pharmacological activities of myristicin (sympathomimetic action) which complete for monoamine oxidize enzymes. There by exhibiting a monoamine oxidize enzyme-like action, myristicin composed 85% of parsley leaves, and activites of apiole (irritant effect) (1).

**References**