Effect of Dietary Sweet Basil and Fennel on Utilization of Calcium in Adult Rats.

Mona S. El-kutry, Hoda S. Ibrahim, Effat A. Affifi, Farouk M. El-Tellawy and Dalia A. Zidan

1Home Economics Dept. - Faculty of Education - Ain Shams University
2Nutrition and Food Sciences Dept., Faculty of Home Economics, Helwan University
3National Nutrition Institute

ABSTRACT

The present study aimed to investigate the effect of feeding sweet basil leaves (BL) and fennel seeds (FS) on calcium (Ca) utilization in experimental animals. Forty two adult albino rats weighing 120±5gm were divided into seven groups: 1. Basal diet, 2. Basal diet + 0.5% BL, 3. Basal diet + 0.5% FS, 4. Ca deficient diet, 5. Ca def + 0.5% BL, 6. Ca def +0.5% FS, 7. Ca def + BL[(0.25%) +FS(0.25%)]. Body weight and food intake were recorded twice a week, feces were collected at the middle and the end of the experiment. Results showed that, feeding on BL as FS led to increase body weight gain and food intake (FI), however Ca concentration in feces, and both AST and ALT activities as well as urea in all deficient Ca groups were decreased. Addition of FS to the diet lead to significant increase in Ca concentration in serum, heart and tibia as well as Ca/P ratio in Ca def. groups. While feeding animals basil leaves revealed significant increase in and phosphorus conc. in serum as well as Feed efficiency ratio (FER). Mixing of basil and fennel led to significant increase in FI, FER, and AST & ALT activities. Liver showed normal structure in rats fed on Ca def + BL, Ca def + FS diets. It is concluded that both tested herbs have beneficial effect on minerals utilization especially fennel seeds but the ingestion must be under precaution.

Key words: Calcium utilization - Basil leaves - Fennel seeds - Liver & Kidney functions.

Introduction

The use of herbs in medicine is ancient in its origins, however recently systematic attempts have been made to codify them into acceptable regulations (Mazza and Oomah, 2000). Culinary herbs as foods flavor items have also been grown and used antiquity, although it consumed in small amounts. It is interesting to note that it contains similar health-promoting photochemical as do fruits and vegetables (Watson, 2001). Some studies were carried out to determine the minerals content of plants (Zengin et al., 2008) concluded that aromatic plants are important sources of nutrients and essential elements, while (Zeghichi et al., 2003) showed that some plants contained appreciable amounts of calcium, iron, zinc and potassium.

Minerals such as Calcium is required for normal growth and skeleton development, aids in transmission of nerve impulses throughout the body; it is also required in association with phosphorus, sodium and potassium for water balance (Wardlaw and Kessel, 2002) and (Vasudevan and Sreekumari, 2007). On the other hand, basil and fennel contain appreciable amounts of minerals and trace elements (Ansari et al., 2004) and (Zeghichi et al., 2003). The role of minerals in our general health is now well demonstrated, on the other hand, the demand for herbs and spices is increasing nowadays for their safety and culinary appeal.

Few studies have been done concerning utilization of minerals from herbs and spices, so that this study aimed to illustrate the effect of feeding on sweet basil and fennel on calcium utilization in rats.

Corresponding Author: Mona S. El-kutry, Home Economics Dept. - Faculty of Education - Ain Shams University
E-mail: monaelkutry@yahoo.com
Materials and Methods

Materials

Dried sweet basil leaves (*Ocimum basilicum*) as well as fennel seeds (*Foeniculum vulgare*) were purchased from the local herbs' stores in Cairo, and then grounded to get powder using the blender.

B) Animals

Forty two adult male albino healthy rats of Sprague Dawley strain weighing 120±5gm, were obtained from National Nutrition Institute, Cairo, Egypt.

Methods:

All rats were housed individually in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation; diet and water were available ad-libitum. The basal diet was prepared according to Reeves *et al.*, (1993).

Experimental design:

After adaptation period, rats were divided into 7 groups, six rats each as following:

- **Group 1:** Fed the basal diet (Control negative) (-)
- **Group 2:** Basal diet + Sweet basil leaves (0.5%) (Control positive) (+)
- **Group 3:** Basal diet + Fennel seeds (0.5%)
- **Group 4:** 50% Calcium deficient diet (50% Ca def)
- **Group 5:** 50% Ca def + Sweet basil leave (0.5%)
- **Group 6:** 50% Ca def + Fennel seeds (0.5%)
- **Group 7:** 50% Ca def + Sweet basil leaves (0.25%) + Fennel seeds (0.25%)

The experiment lasted for six weeks, food intake and body wt were recorded twice a week. Feces were collected twice after 3 weeks and 6 weeks from the beginning of the experiment. At the end of the experimental period; rats were fasted overnight before sacrificing. Blood samples were collected from the orbital sinus veins in dry clean centrifuge tubes (for serum) and Ethylene diamine tetraacetic acid (EDTA) tubes (for whole blood).

Blood was centrifuged for 20 min. at 2500 r.p.m to separate serum then transferred carefully into dry clean well stopped plastic vials and kept frozen till analysis. Liver, kidney, heart, spleen and tibia were removed, washed with saline and plotted over a piece of filter paper, then weighed to calculate the relative weight (wt). Specimens from liver and kidney were taken and fixed in 10% buffered neutral formalin solution for histopathological examination according to Bancroft *et al.*, (1996).

Biological evaluation:

The total food consumption of the experimental period (6 weeks) was calculated. Body weight gain (BWG) and food efficiency ratio (FER) were determined according to Hsu *et al.*, (1978).

Biochemical analysis:

A) Determination of serum calcium and phosphorus:

Calcium and Phosphorus were determined colorimetrically in serum according to Tietz, (1970) and El-Merzabani *et al.*, (1977), respectively.

B) Determination of calcium in organs and feces:

Calcium in kidney and heart were determined by Atomic Absorption Spectrophotometer Py – Unicam SP 192 using wet ash according to Lorenz *et al.*, (1980). Fecal calcium were determined using flame atomic absorption spectroscopy.
C) Calcium concentration in tibia:

Right tibia from each rat was removed and then dried at 80 °C for 18 h in a ceramic pot to evaluate the dry bone weight, then ashed at 550 °C until light grey color to determine the ash weight. The amount of Ca in the tibia was determined according to Willis (1960).

D) Liver functions:

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were measured according to the method described by Reitman and Frankel (1957).

E) Kidney functions:

Serum urea and creatinine were determined according to the method described by Fawcett and Scott (1960) and Bartles et al., (1972), respectively.

f) Serum Albumin

Determination of albumin in serum was carried out according to Doumas and Biggs (1976).

Statistical analysis

One – way analysis of variance (ANOVA) test and Duncan’s multiple range tests were used to show the differences among means. T- Test was used to show the significance in Ca concentration in feces at the two period (middle and the end of the experiment) significance was considered at P ≤ 0.05 (SAS,1996).

Results:

4.1 Effect of dietary sweet basil leaves and fennel seeds on body weight gain (BWG), food intake (FI) , feed efficiency ratio (FER) and relative organs weight in rats

Table (1) shows the effect of sweet basil leaves and fennel seeds on body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) of rats. Data revealed that addition of basil leaves increased BWG of rats fed on calcium deficient (Ca def.) diet unsignificantly.

Table 1: Effect of dietary sweet basil leaves and fennel seeds on body weight gain (BWG), food intake (FI) and feed efficiency ratio (FER) in rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>BWG (gm) Mean ± SE</th>
<th>FI (gm) * Mean ± SE</th>
<th>FER Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + (-)</td>
<td>Control (+) + BL</td>
<td>1.23 ± 0.14a</td>
<td>9.07 ± 0.33c</td>
<td>0.136 ± 0.01a</td>
</tr>
<tr>
<td>Control (+) + FS</td>
<td>Control (+) + FS</td>
<td>1.42 ± 0.13a</td>
<td>10.15 ± 0.44abc</td>
<td>0.140 ± 0.01a</td>
</tr>
<tr>
<td>Calcium deficient + (BL + FS)</td>
<td>1.48 ± 0.29a</td>
<td>11.34 ± 0.52a</td>
<td>0.131 ± 0.01a</td>
<td></td>
</tr>
</tbody>
</table>

All data represented as mean ± standard error
* Values in given column which have different superscript letters are significantly different (P<0.05).

Concerning food intake, results showed that rats fed basil leaves or fennel seeds with the basal diet had increased food intake with mean values of 10.15±0.44 and 9.89±0.23 gm respectively, compared to the control group (9.07±0.33gm), the differences were not significant.

As regards to feed efficiency ratio (FER), there were no significant differences among groups; but it was noticed that addition of basal leaves to the diet increased FER in control and Ca def groups with mean values of 0.140±0.01 and 0.134±0.01, respectively, compared with the other supplements in the same groups (G.3,G.6 and G.7). Table (2) represents the relative organs weight (liver, kidney, spleen and heart) of rats fed sweet basil leaves and fennel seeds. It could be seen that all the experimental groups showed a slight reduction in relative liver wt compared to the negative control group. Concerning relative kidney weight, results showed that the lowest one was in seen in the control group fed basil leaves with a mean value of 0.73±0.05. Relative spleen wt was increased in both control groups fed basil or fennel with means of 0.36±0.01 and 0.37±0.03 respectively compared to the negative control group 0.34±0.01 , while there were a reduction in Ca def groups fed BL or FS or its mixture compared to the Ca def only. No significant differences were seen among groups...
concerning relative spleen wt and heart wt.

4.2 Effect of dietary sweet basil leaves and fennel seeds on calcium and phosphorus concentration in serum and organs of rats

Results in table (3) shows the effect of ingesting basil and fennel on Ca and P concentrations as well as Ca/P ratio in the serum of tested rats. It was clear that rats fed BL diet control (+) had significant decrease in Ca conc. in serum; which was $10.95\pm0.16\text{mg/dl}$ compared to $12.12\pm0.17\text{mg/dl}$ for the negative control. On the other hand, there were significant increases (at $P\leq0.01$) in both Ca def fed on fennel and Ca def fed (BL+FS) with mean values of $11.07\pm0.07\text{mg/dl}$ and $11.17\pm0.28\text{mg/dl}$; respectively, compared to $10.17\pm0.06\text{mg/dl}$, for Ca def group. Regarding phosphorus conc. in serum, there were significant increases in both control groups fed basil and  fennel at $P\leq0.01$ with means $10.1\pm0.07$ and $10.1\pm0.37\text{mg/dl}$, respectively compared to the negative control ($7.5\pm0.03\text{mg/dl}$).

The highest P concentration in serum was seen significantly in Ca def + BL group ($14.9\pm0.17\text{mg/dl}$), followed by Ca def+(BL+FS) group with mean value of $12.3\pm0.09\text{mg/dl}$. Concerning serum Ca/P animals had Ca def + FS group showed the highest Ca/P ratio ($1.2\pm0.07$) compared to the other positive control groups and Ca deficient groups. Table (4) shows the Ca concentration in kidney, heart and tibia of rats fed sweet basil leaves & fennel seeds. The data of kidney indicated that the control group having basil had significant increase ($P\leq0.01$) in Ca in kidney with mean value of $0.37\pm0.02\text{mg/g}$ compared to the negative control and fennel control groups ($0.2\pm0.01\text{mg/g}$ and $0.25\pm0.02\text{mg/g}$), respectively. While no significant differences were found in Ca conc. in kidney in all Ca def groups but they had significant decreases compared to the basil control group. On the other hand, Ca conc. in heart didn't differ significantly in all groups except Ca def + FS group which was higher with a mean of $0.46\pm0.03\text{mg/g}$ compared with both negative control group ($0.37\pm0.01\text{mg/g}$) and fennel control group ($0.35\pm0.03\text{mg/g}$). Data of tibia revealed that adding of both basil and fennel led to an increase in Ca concentration in Ca def groups but the significancy was noticed in the fennel group only. Ca def + FS group with a mean value of $14.87\pm0.37\text{mg/g}$ compared to $11.08\pm0.8\text{mg/g}$ and $11.96\pm0.7\text{mg/g}$ for Ca def group and Ca def + BL groups; respectively. These results were emphasized with the high significant increase in the Ca def. + (BL & FS) with a mean value of $24.50\pm1.55\text{mg/g}$.

4.3 The differences between calcium concentration in feces at the middle and the end of the experiment

Table(5) summarizes the differences between Ca concentration in feces collected at the middle and the end of the experiment. It could be noticed that Ca concentration in feces decreased significantly at the end of the experiment ($P\leq0.05$) in both negative and basil (BL) control groups ($1.69\pm0.07\text{mg/g}$ and $1.93\pm0.25\text{mg/g}$) respectively, compared to $2.00\pm0.24$ and $2.19\pm0.1\text{mg/g}$; respectively at the middle of the experiment.

4.4 The effect of basil and fennel feeding on liver and kidney functions

The effect of dietary basil and fennel on the activity of AST and ALT enzymes in rats fed. Ca deficient diet is recorded in Table (6). Results showed that Ca deficient groups, fed on basil, fennel and their mixture had significantly decreased in both AST and ALT concentration in serum at $(P\leq0.01)$ compared to Ca def. and negative control groups. Table (6) also, illustrates the effect of feeding on basil leaves and fennel seeds on albumin levels and kidney function of rats. Concerning albumin concentrations, it increased significantly $(P\leq0.01)$ in Ca deficient groups fed on basil, fennel as well as its mixture with means $5.33\pm0.33\text{g/dl}$, $5.38\pm0.22\text{g/dl}$ and $7.98\pm0.43\text{g/dl}$, respectively, compared to the negative control ($3.73\pm0.08\text{g/dl}$) and Ca def. group ($4.18\pm0.3\text{g/dl}$).

Table 2: Effect of dietary sweet basil leaves and fennel seeds on relative organs weight in rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Liver * Mean ± SE</th>
<th>Kidney * Mean ± SE</th>
<th>Spleen Mean ± SE</th>
<th>Heart Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control (+)</td>
<td>3.42 ± 0.07a</td>
<td>0.8 ± 0.04abc</td>
<td>0.34 ± 0.01a</td>
<td>0.37 ± 0.01a</td>
</tr>
<tr>
<td>Control + BL</td>
<td>Control + BL</td>
<td>3.23 ± 0.13ab</td>
<td>0.73 ± 0.05c</td>
<td>0.36 ± 0.02a</td>
<td>0.35 ± 0.02a</td>
</tr>
<tr>
<td>Control + FS</td>
<td>Control + FS</td>
<td>3.14 ± 0.06abc</td>
<td>0.78 ± 0.04bc</td>
<td>0.37 ± 0.03a</td>
<td>0.36 ± 0.02a</td>
</tr>
<tr>
<td>Calcium deficient</td>
<td>Ca def.</td>
<td>3.25 ± 0.07ab</td>
<td>0.92 ± 0.05a</td>
<td>0.4 ± 0.04a</td>
<td>0.38 ± 0.02a</td>
</tr>
<tr>
<td>Calcium deficient</td>
<td>Ca def. + BL</td>
<td>3.13 ± 0.03abc</td>
<td>0.84 ± 0.03abc</td>
<td>0.36 ± 0.03a</td>
<td>0.36 ± 0.01a</td>
</tr>
<tr>
<td>Calcium deficient</td>
<td>Ca def. + FS</td>
<td>3.25 ± 0.11ab</td>
<td>0.89 ± 0.05ab</td>
<td>0.35 ± 0.04a</td>
<td>0.38 ± 0.02a</td>
</tr>
<tr>
<td>Calcium deficient</td>
<td>Ca def. + (BL+FS)</td>
<td>3.43 ± 0.05a</td>
<td>0.91 ± 0.04a</td>
<td>0.33 ± 0.02a</td>
<td>0.38 ± 0.02a</td>
</tr>
</tbody>
</table>

All data presented as mean ± standard error
Values in a given column which have different superscript letters are significantly different ($P\leq0.05$).
Table 3: Effect of dietary sweet basil leaves and fennel seeds on calcium and phosphorous concentration in serum as well as Ca/P ratio of rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Serum Ca (mg/dl)*</th>
<th>Serum P (mg/dl)*</th>
<th>Ca/P ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td>Control (-)</td>
<td>12.12±0.17b</td>
<td>7.5±0.03e</td>
<td>1.6±0.02c</td>
</tr>
<tr>
<td></td>
<td>Control + BL</td>
<td>10.95±0.16cd</td>
<td>10.1±0.07c</td>
<td>1.09±0.02e</td>
</tr>
<tr>
<td></td>
<td>Control + FS</td>
<td>11.50±0.30bc</td>
<td>10.1±0.37c</td>
<td>1.1±0.06e</td>
</tr>
<tr>
<td>Calcium deficient</td>
<td>Ca def.</td>
<td>10.17±0.06e</td>
<td>9.2±0.06d</td>
<td>1.1±0.01e</td>
</tr>
<tr>
<td></td>
<td>Ca def. + BL</td>
<td>10.48±0.25de</td>
<td>14.9±0.17a</td>
<td>0.7±0.02f</td>
</tr>
<tr>
<td></td>
<td>Ca def. + FS</td>
<td>11.07±0.07cd</td>
<td>9.5±0.52cd</td>
<td>1.2±0.07e</td>
</tr>
<tr>
<td></td>
<td>Ca def. + (BL + FS)</td>
<td>11.17±0.28cd</td>
<td>12.3±0.09b</td>
<td>0.9±0.03c</td>
</tr>
</tbody>
</table>

All data presented as mean ± standard error
* Values in a given column which have different superscript letters are significantly different (P < 0.01).

Table 4: Effect of dietary sweet basil leaves and fennel seeds on calcium concentration in kidney, heart and tibia in rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Kidney Ca (mg/g)*</th>
<th>Heart Ca (mg/g)*</th>
<th>Tibia Ca (mg/g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td>Control (-)</td>
<td>0.2±0.01bc</td>
<td>0.37±0.01bc</td>
<td>16.77±0.59b</td>
</tr>
<tr>
<td></td>
<td>Control + BL</td>
<td>0.37±0.02a</td>
<td>0.42±0.03abc</td>
<td>15.09±0.67b</td>
</tr>
<tr>
<td></td>
<td>Control + FS</td>
<td>0.25±0.02b</td>
<td>0.35±0.03c</td>
<td>16.43±0.54b</td>
</tr>
<tr>
<td>Calcium deficient</td>
<td>Ca def.</td>
<td>0.23±0.02bc</td>
<td>0.41±0.02abc</td>
<td>11.08±0.80c</td>
</tr>
<tr>
<td></td>
<td>Ca def. + BL</td>
<td>0.21±0.01bc</td>
<td>0.39±0.02abc</td>
<td>11.96±0.73c</td>
</tr>
<tr>
<td></td>
<td>Ca def. + FS</td>
<td>0.19±0.01c</td>
<td>0.46±0.03a</td>
<td>14.87±0.37b</td>
</tr>
<tr>
<td></td>
<td>Ca def. + (BL + FS)</td>
<td>0.21±0.03bc</td>
<td>0.45±0.04ab</td>
<td>24.67±1.56a</td>
</tr>
</tbody>
</table>

All data presented as mean ± standard error
* Values in a given column which have different superscript letters are significantly different (P < 0.01).

Table 5: The differences between calcium concentration in feces at the middle and the end of the experiment:

<table>
<thead>
<tr>
<th>Parameters (A) Feces Ca (mg/g)</th>
<th>Parameters (B) Feces Ca (mg/g)</th>
<th>T test</th>
<th>Sign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (-)</td>
<td>2.00 ± 0.24</td>
<td>1.69 ± 0.07</td>
<td>3.75*</td>
</tr>
<tr>
<td>Control + BL</td>
<td>2.19 ± 0.10</td>
<td>1.93 ± 0.25</td>
<td>2.73*</td>
</tr>
<tr>
<td>Control + FS</td>
<td>2.03 ± 0.46</td>
<td>1.75 ± 0.16</td>
<td>1.35</td>
</tr>
<tr>
<td>Ca def.</td>
<td>2.76 ± 0.39</td>
<td>2.9 ± 0.14</td>
<td>-0.78</td>
</tr>
<tr>
<td>Ca def. + BL</td>
<td>2.86 ± 0.42</td>
<td>2.62 ± 0.14</td>
<td>1.49</td>
</tr>
<tr>
<td>Ca def. + FS</td>
<td>2.36 ± 0.12</td>
<td>2.29 ± 0.19</td>
<td>0.52</td>
</tr>
<tr>
<td>Ca def. + (BL + FS)</td>
<td>2.56 ± 0.29</td>
<td>2.55 ± 0.19</td>
<td>0.19</td>
</tr>
</tbody>
</table>

All data presented as mean ± standard deviation.
* P < 0.05  S = Significant  NS = Non significant

Table 6: Effect of dietary sweet basil leaves and fennel seeds on Liver functions, Albumin and Kidney function in rats:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>AST (U/L)*</th>
<th>ALT (U/L)*</th>
<th>Albumin (g/dl)*</th>
<th>Creatinine (mg/dl)*</th>
<th>Urea (mg/dl)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Control</td>
<td>Control (-)</td>
<td>49.33a ± 0.42</td>
<td>42.67a ± 1.75</td>
<td>3.73a ± 0.8</td>
<td>0.52e ± 0.03</td>
<td>51.00b ± 0.68</td>
</tr>
<tr>
<td></td>
<td>Control + BL</td>
<td>50.17a ± 1.4</td>
<td>40.75b ± 2.03</td>
<td>3.65d ± 0.32</td>
<td>0.87cd ± 0.03</td>
<td>37.67 ± 1.84</td>
</tr>
<tr>
<td></td>
<td>Control + FS</td>
<td>48.5a ± 2.01</td>
<td>44.92a ± 0.78</td>
<td>4.1cd ± 0.14</td>
<td>0.67de ± 0.08</td>
<td>42.00c ± 1.29</td>
</tr>
<tr>
<td>Calcium deficient</td>
<td>Ca def.</td>
<td>48a ± 2.02</td>
<td>42.27a ± 1.53</td>
<td>4.18cd ± 0.3</td>
<td>0.98bc ± 0.07</td>
<td>52.17b ± 1.11</td>
</tr>
<tr>
<td></td>
<td>Ca def. + BL</td>
<td>39.67b ± 1.67</td>
<td>31.5e ± 1.43</td>
<td>5.33b ± 0.33</td>
<td>1.08abc ± 0.13</td>
<td>44.17c ± 1.35</td>
</tr>
<tr>
<td></td>
<td>Ca def. + FS</td>
<td>35.72c ± 1.35</td>
<td>27.83e ± 1.33</td>
<td>5.33b ± 0.22</td>
<td>1.40a ± 0.06</td>
<td>59.00a ± 0.45</td>
</tr>
<tr>
<td></td>
<td>Ca def. + (BL + FS)</td>
<td>30.17d ± 2.06</td>
<td>21.67d ± 1.56</td>
<td>7.98a ± 0.43</td>
<td>1.22ab ± 0.14</td>
<td>29.00f ± 2.31</td>
</tr>
</tbody>
</table>

All data presented as mean ± standard error
* Values in a given column which have different superscript letters are significantly different (P < 0.01).

While Ca def. + BL & FS showed a significant increase in albumin conc. compared to all other experimental groups with a mean value of 7.98 ± 0.43 g/dl.

Creatinine conc. in serum showed significant increase in control + BL group at P < 0.01 with a mean value of 0.87 ± 0.03 mg/dl while no significant increase was seen in control + FS group (0.67 ± 0.08 mg/dl) compared to the negative control group (0.52 ± 0.03 mg/dl).

However, both Ca def. + BL and Ca + BL + FS groups showed no significant increase (1.08 ± 0.13 mg/dl) & (1.22 ± 0.14 mg/dl); respectively, while a significant increase (P < 0.01) was observed in Ca def. + FS group (1.4 ± 0.06 mg/dl) compared to Ca def. group (0.98 ± 0.07 mg/dl). No difference was noticed between basil and fennel groups concerning creatinine level in serum, but all means value were higher than the control groups.

Regarding urea, results indicated high significant decreases in urea conc. in serum of both control groups fed basil and fennel with means value of 37.67 ± 1.84 and 42 ± 1.29 mg/dl, respectively compared to 51 ± 0.68 mg / dl for the negative control one. A similar result was noticed in Ca def. + BL group which had a significant decrease in urea conc. in serum compared to the Ca def. group, while Ca def. + FS group gave
higher concentrations of urea with a mean of $59 \pm 0.45$ mg/dl compared to the Ca def. group. However, a deficient Ca group fed a mixture of basil and fennel had the lowest mean of urea conc. ($29 \pm 2.31$ mg/dl) at $P < 0.01$.

4.5 Histopathological Results

4.5.a) Liver

Microscopically, liver of rat from the negative control group showed no histopathological changes as shown in Fig. (1). While, when rats received basil leaves, examination of liver showed activation of kupffer cells and vacuolar degeneration of some hepatocytes as shown in Fig. (2). However, liver of rats fed on basal diet plus fennel seeds revealed congestion of hepatoportal blood vessels and portal infiltration with mononuclear leucocytic cells Fig. (3). Examined liver of rats given deficient calcium diet only, showed vacuolar degeneration of some hepatocytes, dilatation of bile duct and few leucocytic cells infiltration in portal triad as shown in Fig. (4). However, liver sections of rats fed the same diet with basil leaves showed apparent normal hepatocytes in Fig. (5). In addition, when adding fennel seeds to the Ca deficient diet, the examined sections of liver from rats revealed no histopathological changes as illustrated in Fig. (6). Microscopically, liver of rats fed Ca def. + BL + FS showed vacuolar degeneration of hepatocytes as shown in Fig. (7).

![Fig. 1](image1.png)

**Fig. 1:** Liver of rats fed the basal diet (-ve control) showing no histopathological changes (H & E $\times$ 200)

![Fig. 2](image2.png)

**Fig. 2:** Liver of rats fed sweet basil leaves (+ve control) showing vacuolar degeneration of some hepatocytes (H & E $\times$ 200).

4.5.b) Kidney

Histopathologically, kidneys of rats from the negative control group showed no histopathological changes as clear in Fig. (8), but kidneys of rats from the control + BL group revealed vacuolar degeneration of epithelial lining renal tubules and congestion of glomerular tufts as shown in Fig. (9).

![Fig. 3](image3.png)
Fig. 3: Liver of rats fed on fennel seeds (+ve control) showing congestion of hepatoporal blood vessel (small arrow) and portal infiltration with mononuclear leucocytic cells (H & E × 200).

Fig. 4: Liver of rats fed calcium deficient diet showing dilatation of bile duct (small arrow) and few leucocytic cells infiltration in portal triad (large arrow) (H & E × 200).

Fig. 5: Liver sections of rats fed calcium deficient diet plus sweet basil leaves showing apparent normal hepatocytes (H & E × 200).

Fig. 6: Liver sections of rats fed calcium deficient diet with fennel seeds showing no histopathological changes (H & E × 200).

On the other hand, kidneys of rats from control + FS group showed congestion of glomerular tufts as well as perivascular leucocytic cells infiltration as shown in Fig. (10).

For the calcium deficient groups, kidneys of rats from group that fed no additives revealed presence of eosinophilic proteinaceous cast in the lumen of renal tubules as in Fig. (11). Moreover, examined sections from Ca def. + BL group revealed congestion of renal blood vessels and presence of eosinophilic cast in the lumen of some renal tubules Fig. (12). Also, kidneys of rats from Ca def. + FS group showed protein cast in the lumen of some renal tubules as illustrated in Fig. (13). Meanwhile, kidneys of rats from Ca def. + BL + FS group showed eosinophilic protein cast in the lumen of renal tubules as shown in Fig. (14).
Discussion

The human body normally absorbs 25 to 75 percent of dietary calcium, depending on a variety of factors including age, presence of adequate vitamin D, the body's need for calcium and calcium intake (Insel et al., 2004).
Fig. 11: Kidneys of rats fed calcium deficient diet (50%) only showing eosinophilic proteinaceous material in the lumen of renal tubules (H & E × 200).

Fig. 12: Kidneys of rats fed calcium deficient diet plus basil leaves showing congestion of renal blood vessels and presence of eosinophilic cast in the lumen of some venal tubules (H & E × 200).

Fig. 13: The kidneys of rats fed calcium deficient diet plus fennel seeds showing protein cast in the lumen of some venal tubules (H & E × 200).

Fig. 14: Kidneys of rats fed calcium deficient diet and a mixture of basil leaves & fennel seeds (50/50%) showing eosinophilic protein cast in the lumen of renal tubules (H & E × 200).

This study presented utilization of two plants as basal, fennel and their mixture as calcium supplement and their effect on many nutritional and biochemical parameters in rats. Results indicated that rats fed on basil leaves increased in BWG compared to the other groups in the same section. These results are supported with those reported by Ibrahim et al.,(2000) who found that rabbits received sweet basil increased significantly in averages of final body wt and body gain. However, Dasgupta et al., (2004) showed no significant differences in either weight gain profile or terminal body wt of 8-9 weeks old Swiss albino mice fed hydroalcoholic
extract of fresh leaves of basil compared to the control. It was noticed from the results, that both Ca def. group had the lowest mean values of BWG. Paradis and Cabanac (2005) reported that the low Ca diet had induced Ca deficiency and led to a decrease body wt. Moreover, Sebastion (2005) showed that rats deprived of Ca for 21 days had body weights of 3% less than those of the control. The increase in BWG in groups fed basil compared with fennel may be due to the fibers content in fennel which had been found to decrease BWG. Concerning FER, results of this study were supported with those of Abd El Ghany et al., (2006) who showed that the daily FI and FER of all experimental rats groups showed no significant differences as the values were increased or decreased without statistical significance except low protein low Ca group which showed a significant decrease in FER compared with the control. It was noticed from this study that FER was lower in fennel groups compared to the other groups; Hur et al., (2006) found that the FER was significantly lower in the fennel group compared with the control group in growing rats during inhalation of essential oils on. The results of this study also agreed with those of Okoye et al., (2006) who found that the BWG and FI of rabbits fed ginger spice diet were significantly higher than of those fed the control diet with no spices; this indicated that the spices made the diets more palatable.

Our results showed that both basil and fennel had no or little effects on organs weights, these findings were supported by Dasgupta et al., (2004) who showed that the administration of basil leaf extract induced slight reduction in relative liver wt of mice but it was not significant. In addition, no significant differences were seen concerning relative heart and spleen wt, the means were nearly the same in all groups.

Results of Ca conc. in kidney showed that an inverse relationship, between serum Ca and kidney Ca content, where the lowest mean value of serum Ca was shown in the control + BL group which had the highest value of Ca content in kidney. However the opposite was shown in the Ca def + FS group. Insel et al., (2004) reported that when blood calcium is low, PTH and calcitriol increases Ca reabsorption through the kidney. When blood Ca levels are low, both PTH and vit. D act on the kidney to decrease Ca loss in urine (Mc Guire and Beerman, 2007).

Concerning Ca conc. results in heart our study showed that Ca def. + FS group had the highest mean value compared with the control groups. It has been shown in the literature that calcium plays a critical role in muscle contraction (Wardlaw and Kessel, 2002). There is also growing evidence that adequate calcium consumption can help reducing the risk for cardiovascular disease (Mc Guire and Beerman, 2007), but very high concentrations of Ca in the blood can lead to cardiac arrest and coma (Insel et al., 2004).

Data concerning Ca conc. in tibia revealed that addition of fennel seeds led to increase Ca conc. in tibia comparing to the other groups fed basil leaves with or without any additives with significant differences. These results are parallel to that of the blood. On the other hand, Muhlbaier et al., (2003) found that feeding rats 1gm/day of dry fennel significantly inhibited bone resorption.Medeiros et al., (2004) concluded that female weanling rats fed calcium restricted diet had lower bone mineral density (BMD) and bone mineral content (BMC) than control rats. Muhlbaier et al., (2002) reported that the effect of vegetables and herbs which inhibit bone resorption in rats is not mediated by their lose excess but possibly by a pharmacologically active compound.

Muhlbaier et al., (2003) suggested that foodstuffs of vegetable origin offer a variety of active compounds, which their nature is still unknown. Recently, dietary phytoestrogens are considered to be an effective alternative estrogenic substance in preventing bone loss caused by the deficiency of either estrogen and/or androgen (Urasopon et al., 2007). Moreover, Fugh-Berman (2003), investigated that fennel is among herbs that are hormonally active as a potent estrogen.

Fecal calcium is derived from the diet and that portion of the large amount of intestinal secretions that has not been reabsorbed and therefore lost from the body (Mayne, 1994). In other words, the unabsorbed amount of Ca is excreted in feces; therefore the excreted portion in the feces represents the unabsorbed Ca from the diet (Barasi, 1997). It is obvious that all mean values of Ca conc. in feces in control and Ca def. groups decreased at the end of the experiment except Ca def. group. This finding illustrates that Ca from the diets contained basil leaves and fennel seeds; was well absorbed especially when Ca deficiency is existed. Results of this study indicated that addition of both basil and fennel to the basal diets of the normal rats didn't show any significant difference. On the other hand, addition of basil, fennel and their mixture led to a significant decrease in both AST and ALT concentrations in serum of rats fed Ca def. diet. Our finding, was agreed with the histopathological results, that's indicated the liver of rats fed basal or fennel and their mixture had some abnormality in liver tissue. In contrast, rats fed the basal, fennel in Ca def. diet their liver apparent was normal.

These results agreed with those of Chaturvedi et al., (2007) who found that ALT and AST levels declined in all experimental groups fed wild basil leaves (10, 20, 40 and 80 mg / kg body wt). However, Seung Cheol et al., (2007) reported a significant decrease in serum ALT and AST activities in all tested herbal diets, compared with the control diet group of red sea bream. However, Souza et al., (1999) reported that many
plants constituents as flavonoids, lignans and spice principles as eugenol prevent liver damage. The results concerning fennel seeds were in accordance with those reported by Kalantari (1998) and Ozbek et al., (2004), who reported that mice received fennel extracts at 400 mg / kg one hour after CCl4 administration showed reduced serum aminotransferase activities and liver wt.

Increases in serum albumin levels are seen in dehydration (Doumas and Biggs, 1976). This finding could interpret the high level of albumin concentrations shown by the rats fed Ca def and mixture of basil and fennel. The lowest mean of albumin was in the basil fed control group which had also low mean value of Ca conc. in serum. About half of total serum calcium is bound mostly to albumin, Gaw et al., (1999) explained that if albumin concentration falls, total serum Ca is low because the bound fraction is decreased.

Creatinine was reported not to be influenced by diet (Coles, 1974). The increases in Ca def. groups may be due to some changes in glomerular filtration rate (GFR) especially in Ca def. + FS group which showed high significant increase in urea. Ruilope et al., (1994) referred to that the kidney is the principal target organ of changes in Ca concentration. These results agree with that found in kidney histopathologically that’s showed eosinophilic protein cast in the lumen of renal tubules and approximate all kidney tissue of rats fed basal ,fennel and their mixture appeared abnormal features.

Akdogan et al., (2003) noticed significant increases in urea and creatinine levels in plasma of rats fed spearmint Labiatae, while peppermint didn’t show nephrotoxicity. Similarly, Ibrahim et al., (2000) found significant increase in both urea and creatinine levels in rabbit groups received sweet basil. Fennel roots as an aqueous extract had pronounced a great diuretic activity. Caceres et al., (1987) and El Bardai et al., (2001) also reported that fennel extract increased water, sodium and potassium excretion and appeared to act mainly as a diuretic and a natriuretic. The same observation was obtained by Beaux et al., (1997) who found that hydroalcohol extract of fennel roots increased urine flow and urinary sodium excretion indicating diuretic activity in rats.

This study recommends using of herbs in low dosages followed by consumption of large amounts of liquids to avoid any deleterious effects in kidney. Further researches with different doses are recommended.

References


