Biochemical and Histopathological Studies on the Water Extracts of Marjoram and Chicory Herbs and Their Mixture In Obese Rats

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Abstract: The effects of water extract of marjoram, chicory or mixture of both on some biological, biochemical and histological factors of hyperlipidemic obese rats were studied. Forty adult female rats were distributed into eight groups, the first one was kept as negative control, while the other seven groups were fed on high fat diet for induction of obesity. One of these groups was kept as positive control while the left 6 groups were orally given daily by stomach tube the 1 ml of water extract of marjoram, chicory or mixture of both at 5% and 10%, for 4 weeks. Results showed that isoflavones content in chicory water extract was higher than that of marjoram water extract. Body weight gain was markedly lower especially in the chicory and in mixture of marjoram and chicory groups (5% and 10%) compared to high fat diet control group. Oral administration of marjoram, chicory or their mixture water extracts at 5% and 10% lowered concentrations of total cholesterol, triglycerides, LDL-c, VLDL-c and decreased activity of AST and ALT enzymes. Furthermore, all extracts caused significant increase in HDL-c and T3 hormone concentrations except marjoram groups. Histopathological examination showed amelioration of histopathological lesions seen in liver of obese rats received the water extract of chicory or mixture of both. This study recommends that intake of water extract the mixture of marjoram and chicory especially at 10% because it may be useful for treating obesity accompanied by hyperlipidemia.

Key words: Obese rats, marjoram, chicory, isoflavones, serum lipids, histopathology

INTRODUCTION

Obesity is a complex problem resulting from an imbalance between energy intake and energy expenditure with genetic, metabolic and behavioral components. Obesity is defined as an excessive fat accumulation in the body that presents a risk to health. Despite a major contribution of genetic susceptibility, the rapid development of the obesity must reflect substantial changes in other factors such as diet (Archer et al., 2007). In particular, increases in the amount of fat in the diet have been shown to be associated with the risk of obesity and hyperlipidemia in human and rodents by altering cholesterol and triglyceride levels in plasma and tissues. Hyperlipidemia is known to enhance the risk of coronary heart disease, fatty liver disease and carcinogenesis, which is associated with reactive oxygen species formation (Roberts et al., 2006). In recent years, many studies have focused on the bioavailability of phenolic compounds in the prevention and treatment of obesity. Phenolic compounds and flavonoids have pharmacological properties such as antioxidant, antimutagenic, antithrombotic, anti-inflammatory, anticancer and antihyperlipidemic. They are widely distributed in plants and form part of the human diet (Son and Lewis, 2002).

Marjoram is one of the most familiar kitchen herbs. It is cultivated for use of its aromatic leaves for flavouring and other culinary purposes. Sweet marjoram leaves are also excellent in salads. The medicinal effects of marjoram are gastrointestinal tract stimulant, tonic, carminative, diaphoretic, hypoglycemic, diuretic as well as antibacterial (Leeja and Thoppil, 2007) and as antioxidant (Handl et al., 2008).

Chicory is a root vegetable whose green leafy part is often used in cooking or in salads. It has a long history of herbal use for its tonic effect upon the liver and digestive tract. Previous studies on chicory extracts and formulations containing its roots or leaves revealed that they produce hepatoprotective (Mitra et al., 2001; Ahmed et al., 2003 and Krylova et al., 2006), antihyperglycemic (Petlevski et al., 2003) and antioxidant effects (Sarawathy and Devi, 2001; Rossetto et al., 2005). Furthermore, Urias-Silvas et al. (2007) concluded that inulin-type fructans extracted from chicory regulate appetite and lipid/glucose metabolism. It has also promising effects on the body weight and fat mass development.

There are no scientific studies available on the effects of both chicory and marjoram extracts in obesity although these plants are widely used as a folk remedy for the treatment of obesity. Therefore the present work was designed to study the effects of water extracts of marjoram and chicory herbs and the mixture of both on some biological, biochemical parameters and histopathological changes of obese rats.

MATERIALS AND METHODS

Marjoram dry leaves (Origanum majorana, Family Lamiaceae) and chicory dry leaves (Cichorium intybus,
Family *Asteraceae*) were obtained from the local market of herbs and medicinal plants, Cairo, Egypt and scientifically identified at Horticultural Research Institute, Agriculture Research Center, Egypt. All chemicals and diagnostic kits were purchased from El-Gomhoria Co., Cairo, Egypt.

**Preparation of the herbs extract:** The dried leaves of marjoram or chicory was added to distilled water (1:5 w/v) and mixed for 10 min at 100°C. The water extracts of each herb was filtered and a hand refractometer was used to measure the concentration by determining the refractive index of solutions that were adjusted to either 5% or 10% for each herb in the final solution.

**Experimental animals:** This study was carried out on forty adult female Sprague Dawley albino rats weighing 155-165 g body weight. The rats were obtained from Laboratory Animal Colony, Helwan, Egypt. Before their use in the experiment, the rats were kept for one week for acclimatization to the laboratory conditions. They were fed on basal diet and provided with water and food ad-libitum.

**Preparation of experimental diets and induction of obesity:** Basal diet (AIN-93M) was prepared according to Reeves et al. (1993) which provide about 9.5% of its energy from fat (40 g corn oil/kg diet). In order to induce obesity, High Fat Diet (HFD) was used in which at least 45% of its energy comes from fat as reported by Bhatt et al. (2006). Basal diet was modified to contain 40 g corn oil + 200 g ghee/kg diet and the amount of add saturated fat was substituted from the amount of corn starch.

**Experimental procedure:** Rats were divided into eight groups consisting of five rats each. The first group was fed on the basal diet and kept as a control negative, while from the second to the eighth group, they were fed on HFD during the experimental period. After 6 weeks that was required to induce obesity as stated by Huang et al. (2004) and Bhatt et al. (2006) the second group was left as a control positive, while the rest were given daily by stomach tube for 4 weeks 1 ml of one of the following extracts: 5% marjoram, 10% marjoram, 5% chicory, 10% chicory, 5% mixture of both herbs and 10% mixture of both herbs. During the experiment period, the food intake and body weight were weighed daily and twice a week, respectively. Body Weight Gain (BWG) and Food Efficiency Ratio (FER) were calculated at the end of the experimental period according to the following equations:

\[
\text{BWG} = \text{final weight (g)} - \text{initial weight (g)}
\]

\[
\text{FER} = \frac{\text{weight gain (g)}}{\text{food intake (g)}}
\]

**Collection of blood samples and organs:** At the end of the experimental period, rats were sacrificed following a 12 h fast. The rats were lightly anaesthetized by eether and about 7 ml of blood was withdrawn from the hepatic portal vein into dry centrifuge plastic tubes. Blood samples were centrifuged for 20 min at 3000 rpm to separate the serum samples which were kept in tubes at -20°C till biochemical analysis. Left and right inguinal adipose pads were removed and weighed. The sum of adipose pads to body weight, multiplied by 100, yielded adiposity index (Jeyakumar et al., 2008). In addition, livers of the sacrificed rats were removed for histopathological study.

**Biochemical analysis:** Isoflavones were isolated from the water extracts of marjoram and chicory herbs and estimated using High Performance Liquid Chromatography (HPLC) method with UV detection at 260 nm according to Bird (1989).

Serum total cholesterol was calorimetrically determined according to Allain et al. (1974) and triglyceride was determined calorimetrically according to Wahlefeld (1974). High Density Lipoprotein cholesterol (HDL-c) was determined calorimetrically according to Richmond (1973). Low Density Lipoprotein cholesterol (LDL-c) and Very Low Density Lipoprotein cholesterol (VLDL-c) were calculated mathematically according to Friedewald et al. (1972).

\[
\text{LDL-c} = \text{TC} - [\text{HDL-c} + (\text{TG}/5)]
\]

\[
\text{VLDL-c} = \frac{\text{Triglycerides}}{5}
\]

The activity of Aspartate Aminotransferases (AST) and Alanine Aminotransferases (ALT) enzymes were assigned by the method of Bergmeyer et al. (1978). Thyroid hormones (free T4 and free T3) and thyrotrophin or Thyroid Stimulating Hormone (TSH) were estimated in serum using Radioimmunoassay (RIA) as described by Patrano and Peskar (1987).

**Histopathological study:** Livers of the scarified rats were dissected, removed, washed with normal saline and put in 10% formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. The tissue specimens were cleared in xylene, embedded in paraffin, sectioned at 4-6 microns thickness, stained with Hematoxylen and Eosin (H and E) and then studied under an electronic microscope according to Carleton (1979).

**Statistical analysis:** Results are expressed as mean values with their standard deviation of the mean. Statistical differences between groups were evaluated using one-way ANOVA followed by Duncan post hoc test using SPSS version 11.0 for Windows (SPSS, Chicago,
IL, USA). Differences were considered significant at (p<0.05) according to Snedecor and Cochran (1986).

RESULTS
Total isoflavones isolated from the 5% water extracts of marjoram and chicory herbs were presented in (Table 1). Water extracts of marjoram and chicory contain 49.21 mg/ml and 99.07 mg/ml of total isoflavones respectively. Marjoram water extract was devoid of diadizein isoflavone which is present in chicory water extract (15.41 mg/ml). It is noticed that the isoflavones content in chicory water extract from apgvine, glycyne, genistone and cinammin acid were higher than that of marjoram.

Food intake was lower in the HFD fed rats compared to normal diet fed rats and lower in the rats given oral administration of either marjoram, chicory or their mixture water extract at 5% and 10% but the difference was not significant as shown in (Table 2). Body weight gain was markedly lower in the chicory groups (5% and 10%) and in mixture of marjoram and chicory groups (5% and 10%) compared to positive control group. At the end of the study, body weight gain of chicory groups(at 5% and 10%) and in mixture of marjoram and chicory groups (5% and 10%) was about 29%:35% lower compared to positive control group. Thus Food Efficiency Ratio (FER) was significantly lowered. FER of positive control group was higher than normal diet fed rats and lowered by giving marjoram, chicory and mixture of both at 5% and 10% to obese rats. Adiposity index of marjoram, chicory or their mixture water extract at 5% and 10% supplemented groups was significantly lower than that of positive control group.

Oral administration of marjoram or chicory water extracts or their mixture at 5% and 10% caused significant decreases in serum levels of total cholesterol, triglycerides, LDL-c and VLDL-c compared to positive control group (Table 3). Serum HDL-c levels increased but not significantly by the administration of marjoram water extract at 5% or 10%. Obese rats that were given chicory water extracts at 5% or their mixture at 5% or 10% showed significantly higher levels of HDL-c compared to positive control. These values resembled to that of negative control group.
The administration of water extracts of marjoram at 5% significantly reduced AST level but it did not affect ALT level. On the other hand, administration of marjoram at 10%, chicory at 5% and 10% and mixture of both herbs at the same levels significantly reduced serum levels of AST and ALT enzymes in obese rats compared to positive control group (Table 4). More reduction in ALT enzyme was observed, that were not significant compared to negative control groups.

From Table 5 it could be noticed that oral administration of the water extracts of marjoram at 5% and 10% did not affect free T4 activity. On the other hand, water extracts of chicory (5% and 10%), mixture of both herbs (5% and 10%) induced significant increases in serum levels of free T4 and T3 hormones compared to positive control group. All tested water extracts caused non significant changes in serum level of Thyroid Stimulating Hormone (TSH).

Histopathological examination of liver of the negative control rats fed on basal diet revealed normal histological picture of hepatic lobule which consists of central vein surrounded by normal hepatocytes as shown in (Fig. 1-A). Examination of liver of positive control obese rats showed fatty degeneration of hepatocytes and infiltration of leucocytes in hepatic sinusoid (Fig. 1-B). Liver of rats given orally 5% marjoram water extract showed little vacular degeneration of hepatocyes and some improvement in fatty degeneration (Fig. 1-C). In addition, portal edema and few leucocytes infiltration in hepatic lobule were observed in marjoram 10% water extract (Fig. 1-D). Liver of rats given orally chicory water extract (5% and 10%) and mixture of marjoram and chicory water extracts at
the same concentrations showed marked improvements with no observed pathological lesions (Fig. 1-E-H)

**DISCUSSION**

Several studies have shown that each of chicory and marjoram extract contains considerable amounts of important compounds which may serve as antioxidants. For example, Yassin et al. (2007) reported that chicory extract had high content of phenolics compounds (56.1 mg/g), flavonoids (7.23 mg/g) and carotenoids (0.52 mg/g). Furthermore, Vagi et al. (2005) and Amarowicz et al. (2008) found that marjoram ethanolic extract contain considerable amounts of total phenolics compounds and have antioxidant activity and free radical-scavenging capacity.

It is well-known from the literature that the main active compounds of chicory extract are inulin and fructooligosaccharides (Kocsis et al., 2003). Inulin is a polymer of fructose with β-(2-1) glycosidic linkages (Wight and Niekerk, 1983). As it is water soluble and not hydrolysed by human digestive enzymes, it behaves like a soluble fiber. It may increase the viscosity of the stomach content, which can slow down the rate of gastric emptying of water, nutrients and lipids, or it can cause alterations in hormone secretions, which affect lipid metabolism. The observed effect of chicory extract on food intake and body weight in this study was agreed with that reported by Cani et al. (2005) and Urias-Silvas et al. (2007) that the addition of oligofructose; a short-chain fructans obtained from chicory inulin; might enhance satiety, thereby resulting in greater reductions in energy intake and protects against the body weight gain, fat mass development in normal and obese rats. The effect of herbal mixture of both marjoram and chicory on food intake and body weight could be attributed to the presence of inulin-type fructans of chicory herb in that mixture.

In accordance with the present results, Yassin et al. (2007) reported that chicory extract improve lipid
The incidence of fatty liver which is a metabolic consequence of obesity (Angulo, 2002; Angelico et al., 2003). Moreover, Clark et al. (2003) and Clark and Diehl (2003) reported that fatty liver is commonly associated with long term elevations in liver enzymes. The reduction in the serum levels of aminotransferases as a result of herbal administration during the present study might probably be due in part to the presence of isoflavones, polyphenols and other antioxidants as mentioned before which aided in reducing the liver injury induced by HFD. For example, the water soluble antioxidant properties of *Cichorium intybus* was investigated by (Gazzani et al., 2000) and evaluated in vitro and in ex vivo as protective activity against rat liver cell microsomal lipid peroxidation. Moreover, reduced fat cells in the liver as a result of reducing body weight may also improve liver function. In accordance with the present study, Zafar and Mujahid (1998); Mitra et al. (2001) and Ahmed et al. (2003) concluded that chlorella has antihyperlipidemic and significantly lowers serum levels of AST and ALT enzymes even in CCL4 intoxicated rats. High fat fed animals showed significant increase in thyroid hormones when compared to normal diet fed animals. This result was agreed with Kuroshima et al. (1971) who study the effects of a HFD for 4-5 weeks on thyroid activity and found that HFD caused a marked hypertrrophy of brown and white adipose tissue, but no change in the weight of thyroid, while there was a significant decrease in the thyroid hormones. On the other hand, no available literature could be obtained concerning the effect of marjoram and chlorella herbs on thyroid gland hormones but its effect on increasing thyroid hormones could be indirect result of their effect on lipids metabolism.

The potency of chlorella fed rats supplemented with chlorella or mixture of both can prevent/reduce diet induce fatty liver. This fat reduction in the liver was confirmed by serum lipid analysis and by measurement of liver specific marker enzymes as mentioned before. Zafar and Mujahid (1998) and Ahmed et al. (2003) reported that chlorella extract had antihyperlipidemic activity and rats given it showed almost complete normalization of liver tissues, no fatty degeneration and no necrosis. The observed improvements may be revealed to the presence of many antioxidant components found in both herbs. On the basis of the present results, we could conclude that herbal mixture of marjoram and chlorella especially at 10% water extract may have synergistic effect and its intake of be useful for treating obesity accompanied by hyperlipidemia as it reduces food intake and body weight, improves serum lipid profile, liver function and thyroid activity in obese rats. Moreover, this mixture has a promising effect on the liver tissues as it ameliorates the histopathological lesions seen in this organ of obese rats.

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### Table 4: Effect of water extracts of marjoram, chlorella and mixture of both herbs on serum levels of liver enzyme activities in obese rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>77.3±2.52</td>
<td>23.6±4.91</td>
</tr>
<tr>
<td>Positive Control</td>
<td>128.2±4.76</td>
<td>33.8±4.43</td>
</tr>
<tr>
<td>Marjoram at 5%</td>
<td>110.0±4.1</td>
<td>27.7±1.89</td>
</tr>
<tr>
<td>Marjoram at 10%</td>
<td>94.0±3.91</td>
<td>26.8±2.51</td>
</tr>
<tr>
<td>Chlorella at 5%</td>
<td>90.2±3.89</td>
<td>27.0±2.66</td>
</tr>
<tr>
<td>Chlorella at 10%</td>
<td>86.0±1.01</td>
<td>26.0±1.01</td>
</tr>
<tr>
<td>Mixture at 5%</td>
<td>89.2±3.96</td>
<td>25.8±1.64</td>
</tr>
<tr>
<td>Mixture at 10%</td>
<td>89.2±3.96</td>
<td>25.8±1.64</td>
</tr>
</tbody>
</table>

Values are means±SD. In the same column sharing the same superscript letters are not statistically significantly different at (p<0.05)

### Table 5: Effect of water extracts of marjoram, chlorella and mixture of both herbs on serum levels of thyroid hormones and Thyroid Stimulating Hormone (TSH) in obese rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Free T3 (ng/dl)</th>
<th>Free T4 (ng/dl)</th>
<th>TSH (ug/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>75.7±5.96</td>
<td>3.90±0.09</td>
<td>0.005±0.02</td>
</tr>
<tr>
<td>Positive Control</td>
<td>65.7±5.65</td>
<td>2.06±0.03</td>
<td>0.004±0.02</td>
</tr>
<tr>
<td>Marjoram at 5%</td>
<td>69.8±4.35</td>
<td>2.15±0.03</td>
<td>0.004±0.01</td>
</tr>
<tr>
<td>Marjoram at 10%</td>
<td>69.5±4.74</td>
<td>2.18±0.01</td>
<td>0.003±0.00</td>
</tr>
<tr>
<td>Chlorella at 5%</td>
<td>69.0±4.06</td>
<td>2.22±0.06</td>
<td>0.005±0.03</td>
</tr>
<tr>
<td>Chlorella at 10%</td>
<td>71.3±3.63</td>
<td>2.82±0.05</td>
<td>0.004±0.01</td>
</tr>
<tr>
<td>Mixture at 5%</td>
<td>72.7±4.35</td>
<td>3.12±0.03</td>
<td>0.004±0.02</td>
</tr>
<tr>
<td>Mixture at 10%</td>
<td>71.8±3.63</td>
<td>3.17±0.02</td>
<td>0.004±0.01</td>
</tr>
</tbody>
</table>

Values are means±SD. In the same column sharing the same superscript letters are not statistically significantly different at (p<0.05)

profiles by lowering plasma total cholesterol and triglyceride concentrations while Ninfali et al. (2005) reported similar results for marjoram. The hypocholesterolemic effect of marjoram and chlorella water extract could be attributed to presence of isoflavones in both herbs which prevent intestinal absorption of cholesterol by competition for its absorption sites as mentioned by Rang and Dale (1981). The potent hypercholesterolemic and hypotriglyceridemic effects of chlorella extract could be due to the presence of inulin which behaves like a soluble fiber and possesses hypolipidemic effect (Lairon, 1996). On the other hand, in Kim and Shin (1998) study, serum total cholesterol and triglyceride concentrations were not significantly affected by chlorella or inulin feeding. The difference in the chlorella effect of similar dietary fibers among different studies may be due to the percentage of added dietary cholesterol, the presence or absence of cholic acid, the level of dietary fiber and species. The results of serum lipoproteins were coincide with that of Kim and Shin (1998) who reported that feeding rats on diets containing 1%, 5% chlorella extract or 5% inulin for 4 weeks resulted in higher serum concentration of HDL-c and lower serum concentration of LDL-c. In addition, Yassin et al. (2007) stated that HDL-c concentration was significantly elevated in chlorella extract group than in normal control or high fat group.

The observed elevation of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) in high fat fed groups (obese groups) may be attributed to the
REFERENCES


