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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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

Lamiaa A. Ahmed, Reham S. Ramadan and Reham A. Mohamed
Department of Nutrition and Food Science, Faculty of Home Economics, Helwan University, Egypt

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 fork 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 add to distilled water (1:5 wt/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:

$$BWG\ (g) = final\ weight\ (g) - initial\ weight\ (g)$$

$$FER = weight\ gain\ (g)/food\ intake\ (g)$$

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 ether 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 inquinal adipose pads were removed and weighed. The sum of adipose pads to body weight, multiplied by 100, yielded adiposity index (Jeyakumar *et al.*, 2006). 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).

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

$$VLDL\text{-}c = Triglycerides/5$$

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 Patrono 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,

Table 1: Relative concentrations of determined isoflavone derivatives isolated from 5% marjoram and chicory water extracts

	Relative concentrations of isoflavone derivatives (mg/ml)					
	Apginine	Diadizein	Glystine	Genstine	Cinammic acid	Total
Marjoram	0.034	-	15.90	23.53	9.75	49.21
Chicory	0.37	15.41	25.91	54.72	2.66	99.07

Table 2: Effect of water extracts of marjoram, chicory and mixture of both herbs on Food Intake (FI), Body Weight Gain (BWG), Food Efficiency Ratio (FER) and adiposity index in obese rats

Groups	FI (g/day)	BWG (g/day)	FER	Adiposity index
Negative control	28.97±0.99^{c}	2.42±0.49^{b}	0.08±0.004^{a}	0.46±0.031^{a}
Positive control	21.75±1.87^{ab}	2.77±.093^{c}	0.13±0.014^{d}	1.26±0.07^{e}
Marjoram at 5%	20.95±1.57^{ab}	2.30±0.13^{b}	0.11±0.002^{c}	1.10±0.10^{d}
Marjoram at 10%	22.60±0.67^{b}	2.29±0.22^{b}	0.10±0.012^{bc}	0.99±0.10^{c}
Chicory at 5%	19.96±1.77^{a}	1.97±0.05^{a}	0.10±0.008^{bc}	0.88±0.03^{bc}
Chicory at 10%	19.88±0.77^{a}	1.82±0.04^{a}	0.09±0.003^{ab}	0.81±0.35^{b}
Mixture at 5%	19.69±1.33^{a}	1.79±0.11^{a}	0.09±0.004^{ab}	0.82±0.05^{b}
Mixture at 10%	19.59±1.05^{a}	1.80±0.14^{a}	0.09±0.006^{ab}	0.78±0.03^{b}

Values are mean±SD. Values in the same column sharing the same superscript letters are not statistically significantly different

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 apginine, glystine, genstine and cinammic 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 vacuolar degeneration of hepatocytes 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

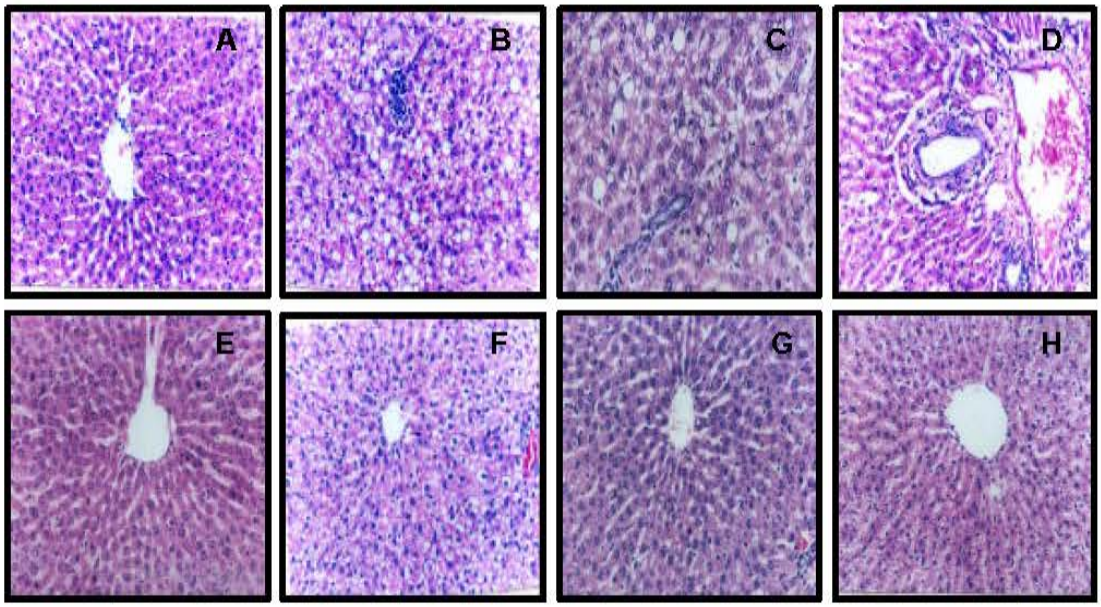

Fig. 1: Histopathological changes detected in the liver of (A) negative control, (B) positive control, (C) 5% marjoram, (D) 10% marjoram, (E) 5% chicory (F) 10% chicory, (G) 5% mixture of marjoram and chicory and (H) 10% mixture of marjoram and chicory. (H and E X 100)

Table 3: Effect of water extracts of marjoram, chicory and mixture of both herbs on serum lipids parameters (mg/dl) in obese rats

	TC	TG	HDL-c	LDL-c	VLDL-c
Negative Control	100.67±2.08^{a}	69.33±3.06^{a}	50.27±6.81^{d}	36.53±4.37^{a}	13.86±0.61^{a}
Positive Control	160.20±9.23^{e}	112.60±6.95^{d}	28.36±5.57^{a}	109.32±9.83^{e}	22.52±1.39^{d}
Marjoram at 5%	138.33±2.88^{d}	91.67±4.51^{c}	34.17±1.93^{ab}	85.83±4.03^{d}	18.33±0.90^{c}
Marjoram at 10%	135.00±4.08^{d}	90.00±4.08^{bc}	34.85±3.06^{ab}	82.15±6.84^{d}	18.00±0.82^{bc}
Chicory at 5%	120.60±4.39^{c}	83.60±4.67^{bc}	37.40±1.82^{b}	66.48±6.22^{c}	16.72±0.93^{bc}
Chicory at 10%	116.01±6.92^{bc}	83.67±5.13^{bc}	38.07±4.47^{b}	61.20±5.39^{c}	16.73±1.03^{bc}
Mixture at 5%	110.33±5.03^{b}	82.67±2.52^{b}	40.67±5.51^{bc}	53.13±5.76^{bc}	16.53±0.50^{b}
Mixture at 10%	111.00±5.48^{b}	84.00±6.28^{bc}	47.72±6.03^{cd}	46.48±6.06^{ab}	16.80±1.26^{bc}

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

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 (58.1 mg/g), flavonoids (7.23 mg/g) and carotenoids (0.52 mg/g). Furthermore, Vági *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 ß-(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

Table 4: Effect of water extracts of marjoram, chicory and mixture of both herbs on serum levels of liver function enzymes in obese rats

Groups	AST (U/L)	ALT (U/L)
Negative Control	77.33±2.52^{a}	23.67±4.81^{a}
Positive Control	128.2± 4.76^{f}	33.80±4.43^{c}
Marjoram at 5%	110.00±1.00^{e}	32.67±2.51^{bc}
Marjoram at 10%	94.00±3.91^{d}	27.75±1.89^{ab}
Chicory at 5%	94.20±3.89^{d}	26.60±2.51^{a}
Chicory at 10%	93.33±5.50^{cd}	27.00±2.65^{a}
Mixture at 5%	86.00±1.01^{b}	26.01±1.01^{a}
Mixture at 10%	88.20±2.39^{bc}	23.80±1.64^{a}

Values are mean±SD. Values 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, chicory and mixture of both herbs on serum levels of thyroid hormones and Thyroid Stimulating Hormone (TSH) in obese rats

Groups	Free T3 (ng/dl)	Free T4 (ng/dl)	TSH (µg/L)
Negative Control	75.73±5.96^{c}	3.90±0.09^{c}	0.005±0.002^{a}
Positive Control	65.75±5.55^{a}	2.05±0.03^{a}	0.004±0.002^{a}
Marjoram at 5%	69.80±4.35^{b}	2.15±0.03^{a}	0.004±0.001^{a}
Marjoram at 10%	68.54±5.74^{b}	2.18±0.01^{a}	0.003±0.002^{a}
Chicory at 5%	69.01±4.96^{b}	2.22±0.06^{a}	0.005±0.003^{a}
Chicory at 10%	71.38±3.63^{b}	2.82±0.05^{b}	0.003±0.001^{a}
Mixture at 5%	72.76±4.35^{bc}	3.12±0.03^{b}	0.004±0.002^{a}
Mixture at 10%	71.80±3.63^{b}	3.17±0.02^{b}	0.005±0.001^{a}

Values are mean±SD. Values 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 chicory 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 (1991).

The potent hypercholesterolemic and hypotriglyceridemic effects of chicory 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 chicory or inulin feeding. The difference in the cholesterolemic 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 fooding rats on diets containing 1%, 5% chicory 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 chicory 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 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 microsome 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 chicory has antihepatotoxic effect 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 Kuroshim *et al.* (1971) who study the effects of a HFD for 4-5 weeks on thyroid activity and found that HFD caused a marked hypertrophy 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 chicory herbs on thyroid gland hormones but its effect on increasing thyroid hormones could be indirect result of their effect on lipids metabolism.

Our histopathological results showed that obese rats supplemented with chicory 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 chicory extract had antihepatotoxic 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 chicory 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.

REFERENCES

Ahmed, B., T.A. Al-Howiriny and A.B. Siddiqui, 2003. Antihepatotoxic activity of seeds of *Cichorium intybus*. J. Ethnopharmacol., 87: 237-240.

Allain, C.C., L.S. Poon and C.S. Chan, 1974. Enzymatic determination of serum total cholesterol. Clin. Chem., 20: 470-475.

Amarowicz, R., Z. Zegarska, R. Rafalowski, R.B. Pegg, M. Karamac and A. Kosin´ska, 2008. Antioxidant activity and free radical-scavenging capacity of ethanolic extracts of thyme, oregano and marjoram. Eur. J. Lipid Sci. Technol., pp: 110.

Angelico, F., M. Del-Ben, R. Conti, S. Francioso, K. Feole, D. Maccioni, T.M. Antonini and C. Alessandri, 2003. Non-alcoholic fatty liver syndrome: a hepatic consequence of common metabolic diseases. J. Gastroenterol Hepatol, 18: 588-594.

Angulo, P., 2002. Non-alcoholic fatty liver disease. N. Engl. J. Med., 346: 1221-31.

Archer, Z.A., J. Corneloup, D.V. Rayner, P. Barrett, K.M. Moar and J.G. Mercer, 2007. Solid and liquid obesogenic diets induce obesity and counter-regulatory changes in hypothalamic gene expression in juvenile Sprague Dawley rats. J. Nutr., 137: 1483-1490.

Bergmeyer, H.U., P. Schreiber and A.W. Wahlefeld, 1978. Optimization of methods for aspartate and alanine aminotransferases. Clin. Chem., 24: 58-61.

Bhatt, B.A., J.J. Dube, N. Dedousis, J.A. Reider and R.M. Doherty, 2006. Diet-induced obesity and acute hyperlipidemia reduce IκBα level in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol., 290: 233-240.

Bird, I.M., 1989. High performance liquid chromatography: Principles and clinical application. Br. Med. J., 299: 783-787.

Cani, P.D., A.N. Neyrinck, N. Maton and N.M. Delzenne, 2005. Oligofructose Promotes satiety in rats fed a high-fat diet: Involvement of glucagon-like peptide-1. Obesity Res., 13: 1000-1007.

Carleton, H., 1979. Histological Techniques, The 4th Edn., London, Oxford University Press, New York, USA.

Clark, J.M. and A.M. Diehl, 2003. Nonalcoholic fatty liver disease: an under recognized cause of cryptogenic cirrhosis. JAMA., 289: 3000-3004.

Clark, J.M., F.L. Brancati and A.M. Diehl, 2003. The prevalence and etiology of elevated aminotransferase levels in the United States. Am. J. Gastroenterol., 98: 960-967.

Friedewald, W.T., R.I. Levy and D.S. Friedrickson, 1972. Estimation of plasma or erum low density lipoprotein cholesterol concentration without use of preparaline ultracentrifuge. Clin. Chem., 18: 499-502.

Gazzani, G., M. Daglia, A. Papetti and C. Gregotti, 2000. *In vitro* and *ex vivo* anti and prooxidant components of *cichorium intybus*. J. Pharm. Biomed. Anal., 3: 127-133.

Handl, S., P. Hellweg, A. Khol-Parisini, B. Rossmann, K. Thurner, W. Luf, J. Novak and J. Zentek, 2008. Effect of oregano (*O. majorana*) on performance and antioxidative capacity of quails fed a diet rich in omega3 fatty acids. J. Anim. Physiol. Anim. Nut., 92: 242-245.

Huang, W., N. Dedousis, B.A. Bhatt and R.M. O'Doherty, 2004. Impaired activation of phosphatidylinositol 3-kinase by leptin is a novel mechanism of hepatic leptin resistance in diet-induced obesity. J. Biol. Chem., 279: 21695-21700.

Jeyakumar, S.M., A. Vajreswari and N.V. Giridharan, 2006. Chronic dietary vitamin A supplementation regulates obesity in an obese mutant WNIN/Ob rat model. Obesity; 14: 52-59. doi: 10.1038/oby.2006.7.

Kim, M. and H.K. Shin, 1998. The water soluble extract of chicory influences serum and liver lipid concentrations, short chain fatty acids and fecal lipid excretion in rats. J. Nutr., 128: 1731-1736.

Kocsis, I., K. Hagymàsi, Á. Kéry, E. Szôke and A.A. Blàzovics, 2003. Effects of chicory on pancreas status of rats in experimental dislipidemia. Acta Biologica Szegediensis, 47: 143-146.

Krylova, S.G., L.A. Efimova and E.P. Zueva, 2006. The effect of Cichorium root extract on the morphofunctional state of liver in rats with CCL4 induced hepatitis model. Eksp. Klin. Farmacol., 69: 34-36.

Kuroshim, A., K. Doi and S. Itoh, 1971. Effects of a high fat diet on thyroid activity, with special reference to thyroidal responses to cold. Int. J. Biometeorol., 15: 55-64.

Lairon, D., 1996. Dietary fibers: effects on lipid metabolism and mechanisms of action. Eur. J. Clin. Nutr., 50: 125-133.

Leeja, L. and L.E. Thoppil, 2007. Antimicrobial activity of methanol extract of *Origanum majorana L.* J. Envriron. Biol., 28: 145-146.

Mitra, S.K., M.V. Venkataranganna, S. Gopumadhavn, S.D. Anturlikar, S. Seshadri and U.V. Udupa, 2001. The protective effect of HD-03 in CCL4-induced hepatic encephalopathy in rats. Phytotherp. Res., 15: 493-496.

Ninfali, P., G. Mea, S. Giogini, M. Rocchi and M. Bacchiocca, 2005. Anti-oxidant capacity of vegetables, spices and dressings relevant to nutrition. Br. J. Nutr., 93: 257-266.

Patrono, C. and B.A. Peskar, 1987. Radioimmunoassay in basic and clinical pharmacology. handbook of experimental pharmacology, springer-verlag, Berlin, Germany.

Petlevski, R., M. Hadzija, M. Slijepcevic, D. Juretic and J. Petrik, 2003. Glutathione S-transferase and malondialdehyde in the liver of mice after short term treatment with plant mixture extract. Phytotherp. Res., 17: 311-314.

Rang, H.P. and M.M. Dale, 1991. Pharmacology, 2nd Edn., Ch. 15, pp: 376.

Reeves, P.G., F.H. Nielson and G.C. Fahey, 1993. AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.

Richmond, N., 1973. Colorimetric determination of total cholesterol and high density lipoprotein cholesterol (HDL-c). Clin. Chem., 19: 1350-1356.

Roberts, C.K., R.J. Barnard, R.K. Sindhu, M. Jurczak, A. Ehdaie and N.D. Vaziri, 2006. Oxidative stress and dysregulation of NAD(P)H oxidase and antioxidant enzymes in diet-induced metabolic syndrome. Metabolism, 55: 928-934.

Rossetto, M., A. Lante, P. Vanzani, P. Spettoli, M. Scarpa and A. Rigo, 2005. Red chicory as potent scavenger of highly reactive radicals. A study on their phenolics composition and peroxyl radical trapping capacity and Efficiency. J. Agric. Food Chem., 53: 8169-8175.

Sarawathy, S.D. and C.S. Devi, 2001. Modulating effect of an Ayurvedic formulation on antituberculosis drug-induced alterations in rat liver microsomes. Phytotherp. Res., 15: 501-505.

Snedecor, G.W. and W.G. Cochran, 1986. Statistical Methods. 4th Ed. Iowa State University Press, Ames, Iowa, USA, pp: 91.

Son, S. and B.A. Lewis, 2002. Free radical scavenging and antioxidative activity of caffeic acid amide and ester analogues: structure-activity relationship. J. Agric. Food Chem., 30: 468-472.

Urias-Silvas, J.E., P.D. Cani, E. Delmee, A. Neyrinck, M.G. Lopez and M.N. Delzenne, 2007. Physiological effects of dietary fructans extracted from Agaves tequilana and *Dasylirion* spp. Br. J. Nutr., 99: 254-261.

Vági, E. , E. Rapavi, M. Hadolin, K. Vásárhelyiné-Perédi, A. Balázs, A. Blázovics and B. Simándi, 2005. Phenolic and triterpenoid antioxidants from *origanum majorana* L. herb and extracts obtained with different solvents. J. Agri. Food Chem., 53: 17-21.

Wahlefeld, A.W., 1974. Methods of enzymatic analysis, Academic Press, Chapter 5, pp: 1831-1835.

Wight, W.A. and J.V. Niekerk, 1983. Determination of reducing sugars, sucrose and insulin. J. Agric. and Food Chem., 31: 282-285.

Yassin, M.N., I.S. Ashoush and E.M. El-Hadidy, 2007. Antioxidants content of chicory leaves extract and its effect as hypolipidemic agent in experimental rats. Ann. Agric. Sci. ,52: 177-186.

Zafar, R. and A.S. Mujahid, 1998. Antihepatotoxic effects of roots of *Cichorium intybus* L., J. Ethnopharmacol., 63: 227-231.