Influence of *Melissa officinalis* methanolic extract on hyperthyroidism in a rat model

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Received: 27 October 2020
Revised: 16 January 2021
Accepted: 17 February 2021
Published: 9 June 2021

Egyptian Pharmaceutical Journal 2021, 20:134–144

Background and objective
Thyroid disease represents the most common endocrine abnormality in recent years. This study was conducted to evaluate the effect of *Melissa officinalis* methanolic extract (MME) on hyperthyroidism in a rat model.
Materials and methods
Hyperthyroidism was induced by daily subcutaneous injection of L-thyroxine (250 μm/kg body weight) for 14 days. Total phenolic compounds in extract and the in-vitro antioxidant activity of extract were determined. Moreover, identification of methanolic extract component of *Melissa officinalis* leaves (MME) was done using liquid chromatography–mass spectrometry. After 30 days of MME treatments, blood samples were collected for further biochemical determinations. Liver and kidney were excised for the determination of oxidative stress markers. Thyroid gland was also removed for histopathological examination.
Results
Various thyroid hormones (total and free triiodothyronine, as well as total and free thyroxine) were seriously affected and increased significantly with hyperthyroidism induction. Significant increases in serum glucose, interleukin-6, and interleukin-8 were detected in hyperthyroid group compared with control values, whereas hemoglobin level has not changed. Compared with control group, hyperthyroidism-induced glutathione depletion and reduction in glutathione peroxidase activity in the liver and kidney tissues, with significant increase in the lipid peroxidation and nitric oxide levels. Upon treatment with MME, significant improvements in thyroid hormones and the other aforementioned parameters were achieved. MME succeeded also in ameliorating the histological picture of the thyroid gland.
Conclusions
Current results indicate that MME treatment counteracts the oxidative stress induced by L-thyroxine and protects the liver and kidney and regulates blood glucose in hyperthyroidism state. We suggest that MME treatment may be considered for therapeutic use for hyperthyroidism.

Keywords:
antioxidant, histopathology, hyperthyroidism, kidney, liver, *Melissa officinalis*

Egypt Pharmaceut J 20:134–144

1687-4315

Introduction

In the human body, the thyroid gland is one of the most important organs as it regulates majority of the body's physiological actions [1]. It produces triiodothyronine (T3) and thyroxine (T4) hormones that are essential for growth, development, and regulation of energy metabolism through their effect on protein, carbohydrate, and lipid metabolism [2,3]. Thyroid hormones regulate also many other important regulatory hormones such as insulin and catecholamines [4]. Any dysfunction in the thyroid gland can affect the production of thyroid hormones leading to various pathological conditions throughout the body [1]. Hyperthyroidism is an endocrine disorder characterized by excessive secretion of thyroid hormones (T3 and T4) [5], which lead to an increase in basal metabolic rate and oxygen consumption in numerous tissues [2,3]. The toxic diffuse goiter and Graves' disease are the most common causes of hyperthyroidism. Less common causes of hyperthyroidism are toxic nodular goiter or autonomous functioning thyroid nodules [6]. Hypermetabolic state resulting from hyperthyroidism is associated with various degrees of oxidative stress in the organism. Oxidative damages to proteins, lipids, and DNA, which is indicative of oxidative stress, have been found in hyperthyroid rats [3,7].

In recent years, plants rich in natural compounds such as polyphenols, tocopherols, flavonoids, alkaloids, tannins, carotenoids, and terpenoids, were intensely studied owing to their potent immunomodulatory, anticarcinogenic, and antioxidant properties. *Melissa officinalis* L (lemon balm, from the Lamiaceae family) is a well-known medicinal plant species used in the treatment of several diseases; it is widely used as a vegetable, adding flavor to dishes [8]. *M. officinalis* is a rich source of natural antioxidant; its leaves contain many phytochemicals, including polyphenolic compounds, such as caffeic acid derivatives [9], imeric compounds [10], flavonoids [11], essential oil, and abundant citral [12]. In addition, leaves contain also vitamins E and C, which have important activity as free radical inactivators [9]. *M. officinalis* has antispasmodic, anti-histaminic, and antibacterial properties [13]. It is used also in cases of anxiety, neurosis, nervous excitability, palpitation, and headache [14]. *M. officinalis* is also thought to have beneficial effects for individuals with Alzheimer's disease [15] and has therapeutic potency in modulation of mood and cognitive performance [16].

Although several reports have been published about the role of *M. officinalis* in the treatment of several diseases, there is no reported information, to our knowledge, regarding the effect of methanolic extract of *M. officinalis* on hyperthyroidism state. Therefore, this study was designed to evaluate the effect of *M. officinalis* methanolic extract (MME) on hyperthyroidism state in a rat model. The antihyperthyroidism effect of this extract was detected by assessment of thyroid hormones. We studied also the effect of hyperthyroidism induction on liver and kidney functions, as well as body weight gain, blood glucose, and hemoglobin. We hypothesized that MME would attenuate hyperthyroidism through antioxidant effect; therefore, we analyzed the composition of MME and assessed its in-vitro antioxidant effects. We evaluated also the in-vivo antioxidant effect of the extract by determining the end product of lipid peroxidation [malondialdehyde (MDA)], nitric oxide (NO), glutathione (GSH), and the activity of glutathione peroxidase (GPx) in liver and kidney tissues, as well as the changes in serum interleukin-6 (IL-6) and IL-8 levels.

Materials and methods

Animals

Male albino rats (150–200 g) were obtained from the animal colony, National Research Centre. These animals were maintained on free access to food and water for a week before starting the experiment; they received humane care according to ethical committee of National Research Centre (FWA 0001 4747).

Plant materials

Lemon balm (*M. officinalis*) herb was obtained from Abd El-Rahman Harraz (Bab El-Khalk Zone, Cairo, Egypt). The herb was identified in the Botany Department, Faculty of Science, Cairo University.

Chemicals and drugs

Eltroxin 100 mg (Thyroxin 100 mg tablets) is commercially available and provided by GlaxoSmithKline Co. (New Cairo City, Cairo, Egypt). All other chemicals were of the highest analytical grade available and obtained from national research center stores.

Herb extraction

The herbal leaves were air-dried, grinded, and soaked in methanol (1 : 10 w/v) for 3 days for extraction process under continuous shaking. After filtration, the solvent was evaporated using rotary evaporator under reduced pressure until dryness was achieved, and then the yield percentage was calculated as gram (extract)/100 g (crude powdered herb). We examined, then, the in-vitro antioxidant activity of the extract, and then the extract was stored at −20°C until further use.

Determination of total phenolic compounds

Overall, 5 mg of the extract was dissolved in a 10-ml mixture of acetone and water (6 : 4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of 10-fold diluted Folin-Ciocalteu reagent and 0.8 ml of sodium carbonate solution (7.5%). After 30 min at room temperature, the absorbance was measured at 765 nm using V-530 UV/visible spectrophotometer. Estimation of phenolic compounds as catechin equivalents was carried out using standard curve of catechin [17].

Determination of radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl assay

Certain crude extracts were dissolved in methanol to obtain a concentration of 200 ppm. A volume of 0.2 ml of this solution was completed to 4 ml by methanol, and 1 ml 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (6.09×10^{-5} mol/l), in the same solvent, was then added. The absorbance of the mixture was measured at 516 nm after 10-min standing. The reference sample (blank) was 1 ml of DPPH solution and 4 ml of methanol. Triplicate measurements were made, and the antioxidant activity was calculated by the percentage of DPPH that was scavenged [18].

Chemical composition identification of the methanolic extract

Liquid chromatography–mass spectrometry (LC/MS)/MS,4000 Qtrap Applied Biosystems, Waters Corporation, Milford, MA, USA, (Quadrupole/linear ion trap mass spectrometer), and the liquid chromatography (Agilent Technologies, Palo Alto, California, USA) system coupled with an electrospray ionization–MS detector were employed to identify the chemical composition of the methanolic extract [19,20].

Experimental design

Hyperthyroidism was induced according to the method of Carageorgiou *et al.* [21] by daily subcutaneous injection of L-thyroxine (dissolved in 0.01 N NaOH, the final solution was prepared with saline) in a dose of 250 µm/kg body weight, for 14 days. After induction of hyperthyroidism models, rats (hyperthyroidism modeled and normal ones) were weighed and treated with the extract and classified into the following groups (eight rats each): group I included normal animals that were fed free of any treatments and acted as a normal control; group II included normal animals that were subjected to oral administration of MME (450 mg/kg/day) [22] for a period of 1 month to study its physiological effects; group III included hyperthyroidism modeled animals without any treatments; and group IV included hyperthyroid rats that were treated with the extract (450 mg/kg/day) for 1 month, after the 14-day induction period.

At the end of the experimental period, the animals were weighed, and then fasted overnight, and following inhalation of diethyl ether anesthesia, blood samples were withdrawn from the retro-orbital venous plexus. Each blood sample was divided into two portions: the smaller portion was taken on heparin for the determination of hemoglobin, whereas the other portion was centrifuged at 3000 rpm for 15 min at 4°C where the clear serum was separated and stored at −70 until biochemical determinations.

After blood collection, each animal was rapidly killed, the liver and kidney were dissected out and washed with saline, dried on filter paper, and then a part from each liver and kidney was weighed and homogenized in 50 mmol/l phosphate buffer (ice cold) solution (pH 7.4) to give 10% w/v homogenate. The homogenate was centrifuged at 3000 rpm for 15 min at 4°C. The clear supernatant was separated for further determination of the oxidative stress markers. Moreover, thyroid gland was dissected out and immediately kept in 10% buffered formalin? saline solution for a later histopathological examination, which was carried out by cutting 5-µm-thick paraffin sections and then stained with hematoxylin and eosin [23] and investigated by light microscope.

Biochemical determinations

Serum thyroid-stimulating hormone (TSH), total T3, and total T4 levels were determined by enzyme-linked immunosorbent assay (ELISA) using rat ELISA kits purchased from MyBiosource Co. (San Diego, California, USA). Serum free triiodothyronine (FT3) and free T4 levels were determined by ELISA technique using rat ELISA Kits Cat. 1650 for quantitative determination purchased from Alpha Diagnostic International (San Antonio, Texas, USA). Serum IL-6 and IL-8 levels were determined by ELISA technique using rat kits manufactured by Assaypro Co., Saint Charles, MO, USA. Colorimetric determinations of serum glucose and blood hemoglobin concentrations were carried out using kits produced by Biodiagnostic Co. (Giza, Egypt).

An enzymatic procedure was used to determine serum urea level using a kit obtained from Biodiagnostic Co.. Serum creatinine concentration was kinetically evaluated using a kit obtained from Biodiagnostic Co.. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined using reagent kits purchased from Human Gesell Schaft fur Biochemical und Diagnostica mbH (Hessen, Germany). GPx activity, and also the levels of GSH and NO in the supernatant of hepatic and renal homogenates were determined using reagent kits obtained from Biodiagnostic Co. However, lipid peroxidation level in hepatic and renal homogenates was estimated chemically according to the method described by Ruiz-Larnea *et al.* [24] on the base of MDA reaction with thiobarbituric acid which forms a pink complex that can be measured photometrically.

Statistical analysis

The obtained data were subjected to analysis of variance followed by Duncan multiple post hoc test at level of *P* vale less than or equal to 0.05 according to Steel and Torrie [25] using a statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc. (Cary, North Carolina, USA).

Results

In-vitro results

As shown in Table 1, it can be clearly noticed that the methanolic extract of lemon balm leaves (*M. officinalis*) possesses considerable values of total phenolic compounds (TPC) and radical scavenging activity (RSA) (one of the antioxidant mechanism).

Table 2 shows 21 compounds that were identified in the methanolic extract of *M. officinalis* leaves using LC/MS. The identified compounds were found to include high amounts of polyphenols, tocopherols, flavonoids, alkaloids, and nitrogenous compounds.

Administration of MME alone to normal group did not affect the hormonal profile of thyroid gland, reflecting its thyroid safety in normal case at the used level herein. Interestingly, the results of the current study revealed that the measured thyroid hormones were significantly increased after hyperthyroidism induction, whereas upon treatment of the hyperthyroidism modeled animals with MME, significant improvement in thyroid hormones was achieved, whereas TSH level insignificantly changed (Table 3).

There was no change in the body weight gain in the group of normal rats that received MME, whereas hyperthyroidism modeled group recorded a significant decrease in body weight gain when compared with the control group. Administration of MME to hyperthyroidism modeled rats produced a significant improvement in body weight gain compared with hyperthyroidism group (Fig. 1a).

Compared with the control group, hyperthyroidism-induced GSH depletion and reduction in GPx activity in liver and kidney tissues, with significant increases in

Table 1 The yield, total phenolic compounds and radical scavenging activity of *Melissa officinalis* methanolic extract

Yield (g %)	13.1±0.42
TPC (mg/g)	1.39±0.25
RSA (%)	62.4±2.55

Values are the mean±SE for three replicates measurements. RSA radical scavenging activity; TPC, total phenolics content.

Table 2 Liquid chromatography–mass spectrometry analysis of methanolic extract of *Melissa officinalis* leaves (lemon balm)

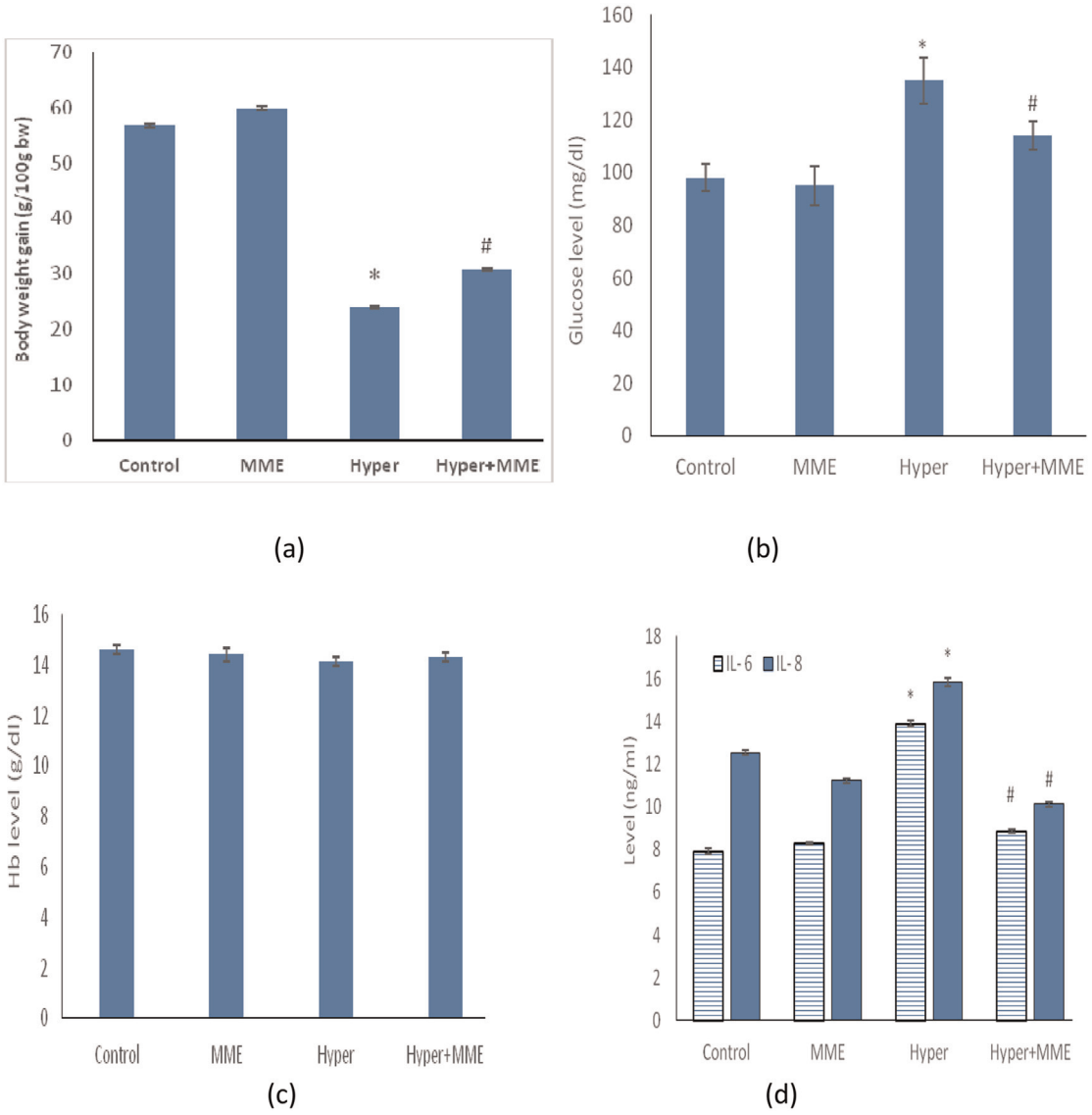
	Retention (time/min)	M-H$^-$	Area %	Compound	Fragments
1	0.78	377.1257	1.72	Caffeic acid derivative	341, 245, 225, 217, 215, 179, 165, 160
2	7.09	355.1075	1.87	Feruloyl Glycoside	311, 173, 265, 247, 223, 205, 164
3	7.40	519.2194	1.39	Dihydrosinapic acid hexoside-pentoside	355, 387, 431
4	7.56	433.2791	2.28	Quercetin-3-arabinoside	423, 351, 301
5	7.81	639.2576	1.36	Isorhamnetin 3,4′ diglucoside	567, 395, 301, 293, 225, 179,163
6	9.21	623.2678	8.38	Isorhamnetin-3-O, 7-O-rhamnoside	503, 461, 315, 297, 179, 101
7	9.94	685.2842	9.06	6-Hydroxy 7,3,4–trimethoxyflavanol –di-O-hexose	624, 623, 377
8	10.11	635.2820	1.51	Kaempferol-3-O- (4′′-O-acetyl) rutinoside	607, 433, 345, 311, 659
9	11.51	719.2656	1.80	Ellagic acid derivative	697, 652, 651, 577, 555, 301, 243, 183
10	12.46	711.4164	0.90	Quercetin-7-O- hexoside-3-O- malonylhexoside	654, 627, 592, 591, 343, 301, 271, 197
11	12.66	315.0956	1.37	Isorhamnetin	285, 315, 316, 344, 459, 607, 631, 632
12	13.71	383.2184	2.39	Rosmarinic acid	359, 330, 329, 299, 269
13	13.79	329.1166	3.08	4′, 5, 7 trihydroxy- 3, 6 methoxy flavone	329, 299
14	14.78	343.1245	4.84	5, 6 dihydroxy, 3′, 4′, 7 trimethoxy flavone	343, 313
15	16.54	641.3920	0.63	6-Hydroxy quercetin-3-O-di- hexose	599, 329, 307, 301
16	20.33	609.5366	4.93	Luteolin-di-O- glucoside	361, 294, 301
17	21.43	311.2762	5.15	Caftaric acid	296, 295, 293, 179, 149
18	23.49	595.3819	2.51	Quercetin pentoside hexoside	481, 393, 327, 325, 301, 293
19	24.17	623.3601	1.36	Isorhamnetin-3-O-glucoside-7-O-rhamnoside	556, 503, 461, 433, 337, 315
20	24.55	463.3847	1.72	Quercetin-3-O-glucoside (Hirsutrin)	571, 484, 483, 463, 377, 345, 339, 301, 278, 178, 130
21	30.46	669.5769	7.75	6,3′-dimethoxy quercetin-7-O-ß-O- glucopyranosyl (6→1) O-glucopyranoside	531, 507, 423, 330, 301, 287, 176

Table 3 Effect of *Melissa officinalis* methanolic extract on serum thyroid hormones in different studied groups

	TSH (ng/ml)	Total T3 (ng/ml)	Free T3 (pg/ml)	Total T4 (μg/dl)	Free T4 (ng/dl)
Control	1.26±0.12	65.5±2.9	2.22±0.11	5.62±0.15	1.49±0.12
MME	0.82±0.03*	60±1.37	2.91±0.05	6.03±0.18	1.51±0.04
Hyper	0.14±0.03*	173±1.94*	4.77±0.09*	15.61±0.18*	3.66±.09*
Hyper+MME	0.154±0.03	83.9±3.1$^\#$	3.13±0.09$^\#$	11.08±0.14$^\#$	

Data are presented as mean±SE for eight rats. Hyper, hyperthyroidism model; MME, *Melissa officinalis* methanolic extract; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone. *Statistically significant from control group while. $^\#$Statistically significant from hyperthyroid group at *P* value less than r equal to 0.05.

Figure 1



Body weight gain values (a), blood glucose level (b), blood hemoglobin (Hb) level (c), and serum cytokines levels (d) in different studied groups. Data are presented as mean±SE for eight rats. *Statistically significant from control group while #statistically significant from hyperthyroid group at *P* value less than or equal to 0.05. Hyper, hyperthyroidism model; IL-6, interleukin-6; IL-8: interleukin-8; MME, *Melissa officinalis* methanolic extract.

the lipid peroxidation (represented by MDA formation) and NO level. The hyperthyroidism animals that received MME showed significant decreases in hepatic and renal MDA and NO levels coupled with significant increases in the values of GSH and GPx as compared with the hyperthyroidism group (Tables 4 and 5).

Table 6 illustrates the effect of MME on liver and kidney functions of normal and hyperthyroidism modeled rats. Compared with control rats, hyperthyroidism significantly increased the activities of serum AST and ALT, with insignificant changes in serum urea and creatinine levels. The treatment with MME alone resulted in no significant effects on these parameters as compared with normal rats; this indicates that MME had no adverse effects on liver and kidney at the used dose. Administration of MME to hyperthyroidism group ameliorated significantly AST, with a little effect on ALT activity.

Significant increases in serum glucose, IL-6, and IL-8 levels were detected after hyperthyroidism induction when compared with the control values (Fig. 1b, d), whereas hyperthyroid animals that received MME showed significant decreases in the aforementioned parameters toward the values of the controls. These findings indicate the hypoglycemic and anti-inflammatory effects of MME. On the contrary, blood hemoglobin level showed insignificant change among the different studied groups (Fig. 1c).

Table 4 Hepatic oxidant–antioxidant markers in different studied groups

	GPx (U/g tissue)	GSH (mg/g tissue)	NO (μmol/g tissue)	MDA (nmol/g tissue)
Control	3277±132	1.88±0.066	9.9±1.05	55.1±2.47
MME	3339±142	2.11±0.073	10.4±0.86	51.8±2.24
Hyper	2266±112*	0.99±0.045*	27.7±1.33*	149.1±4.51*
Hyper+MME	3123±132#	1.71±0.062#	10.5±0.94#	65.9±3.11#

Data are presented as mean±SE for eight rats. GPx, glutathione peroxidase; GSH, glutathione; Hyper, hyperthyroidism model; MDA, malondialdehyde; MME, *Melissa officinalis* methanolic extract; NO, nitric oxide. *Statistically significant from control group while. #Statistically significant from hyperthyroid group at *P* value less than or equal to 0.05.

Table 5 Renal oxidant–antioxidant markers in different studied groups

	GPx (U/g tissue)	GSH (mg/g tissue)	NO (μmol/g tissue)	MDA (nmol/g tissue)
Control	3187±63 A	2.19±0.075 A	12.2±1.41D	93±6.72D
MME	3200±78 A	2.24±0.073 A	11.3±1.55D	89±6.22D
Hyper	2032±86*	1.17±0.064*	28.4±2.02*	184±8.65*
Hyper+MME	3039±94#	1.87±0.077#	17.7±1.69#	118±5.71#

Data are presented as mean±SE for eight rats. GPx, glutathione peroxidase; GSH, glutathione; Hyper, hyperthyroidism model; MDA, malondialdehyde; MME, *Melissa officinalis* methanolic extract; NO, nitric oxide. *Statistically significant from control group while. #Statistically significant from hyperthyroid group at *P* value less than or equal to 0.05.

Table 6 Markers of liver (aspartate aminotransferase and alanine aminotransferase) and kidney (urea and creatinine) functions in different studied groups

	AST (U/l)	ALT (U/l)	Urea (mg/dl)	Creatinine (mg/dl)
Control	25.6±3.6	26.2±1.6	36.1±2.8	0.98±0.05
MME	27±2.47	26±2.24	30.4±1.97	0.85±0.03
Hyper	34.3±2.13*	34.1±1.85*	33.9±1.27	0.92±0.05
Hyper+MME	26±1.47#	30.2±1.57	32.5±2.16	0.86±0.05

Data are presented as mean±SE for eight rats. ALT, alanine aminotransferase; AST, aspartate aminotransferase; *Hyper*: hyperthyroidism model; *MME*, *Melissa officinalis* methanolic extract. *Statistically significant from control group while. #Statistically significant from hyperthyroid group at *P* value less than or equal to 0.05.

Histological examination of thyroid sections from control rat detected thyroid follicles of different sizes; their cavities contained acidophilic colloid. Thyroid follicles were lined by cubical follicular cells that exhibited rounded nuclei. The follicular epithelium exhibited cubical cells. These cells showed spherical vesicular nuclei (Fig. 2a). The examination of hematoxylin and eosin-stained sections of thyroid glands treated with MME showed the thyroid follicles more or less appeared normal, but some vacuoles were observed in peripheral colloid (Fig. 2b).

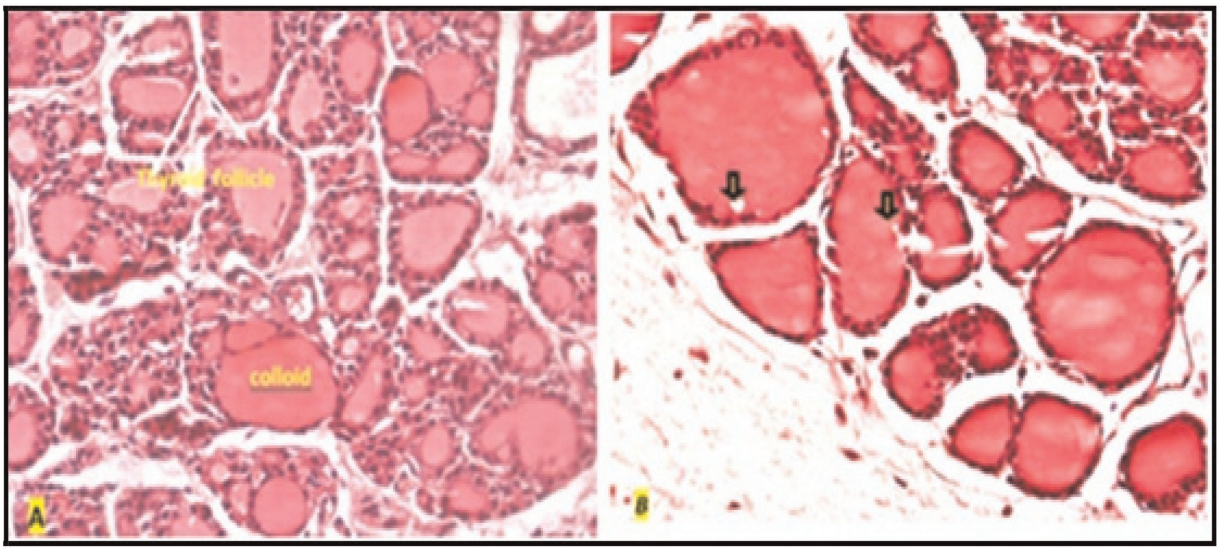
Histological alterations in the thyroid glands of rats of the hyperthyroid group revealed architecture distortion and increase in follicles size with vacuolated colloid. Some follicles showed absence of colloid and others filled with partially of colloid. Marked epithelial hyperplasia of follicular cells, vacuolar degeneration, as well as dilatation and congestion of capillaries were clearly noted. In addition, fibrous tissue appeared to be increased in the stroma between thyroid follicles (Fig. 3a and b). In the case of thyroid follicles in the group of hyperthyroidism rates subjected to MME, some improvement in pathological changes was seen, although some changes were recorded in the form of scalloping thyroid colloid and some empty follicles. Proliferation or multiple layers of some follicular cells were clearly observed (Fig. 3c).

Discussion

Hyperthyroidism induction, in the present study, caused a significant increase in serum total T3, FT3, total T4, and FT3 levels associated with a reduction in serum TSH level; these results go with the results of Ourique *et al.* [26] and Asker *et al.* [27].

Hyperthyroidism state, in the present study, was asserted also by a decreased body weight gain that was associated with the increase in catabolic activity described in hyperthyroidism [28,29]. In hyperthyroidism, the high T4 levels induce a catabolic condition in which there is increased energy expenditure allied to lipolysis and protein degradation [30]. Upon treatment of the hyperthyroidism modeled animals with MME, a significant improvement in the thyroid hormones was achieved, indicating the antihyperthyroidism potential

Figure 2



Photomicrograph of a section of the thyroid gland of rat (a) control showing thyroid follicles (white arrow) of different sizes; their cavities contain acidophilic colloid. Thyroid follicles are lined by cubical follicular cells (hematoxylin and eosin ×200), (b) a rat treated with MME only demonstrating thyroid follicles of variable sizes and normal colloid with some vacuoles in colloid (black arrow) (hematoxylin and eosin ×200). MME, *Melissa officinalis* methanolic extracts.

of MME as it counteracted the levels of T3 and T4 and prevented the associated loss in body weight.

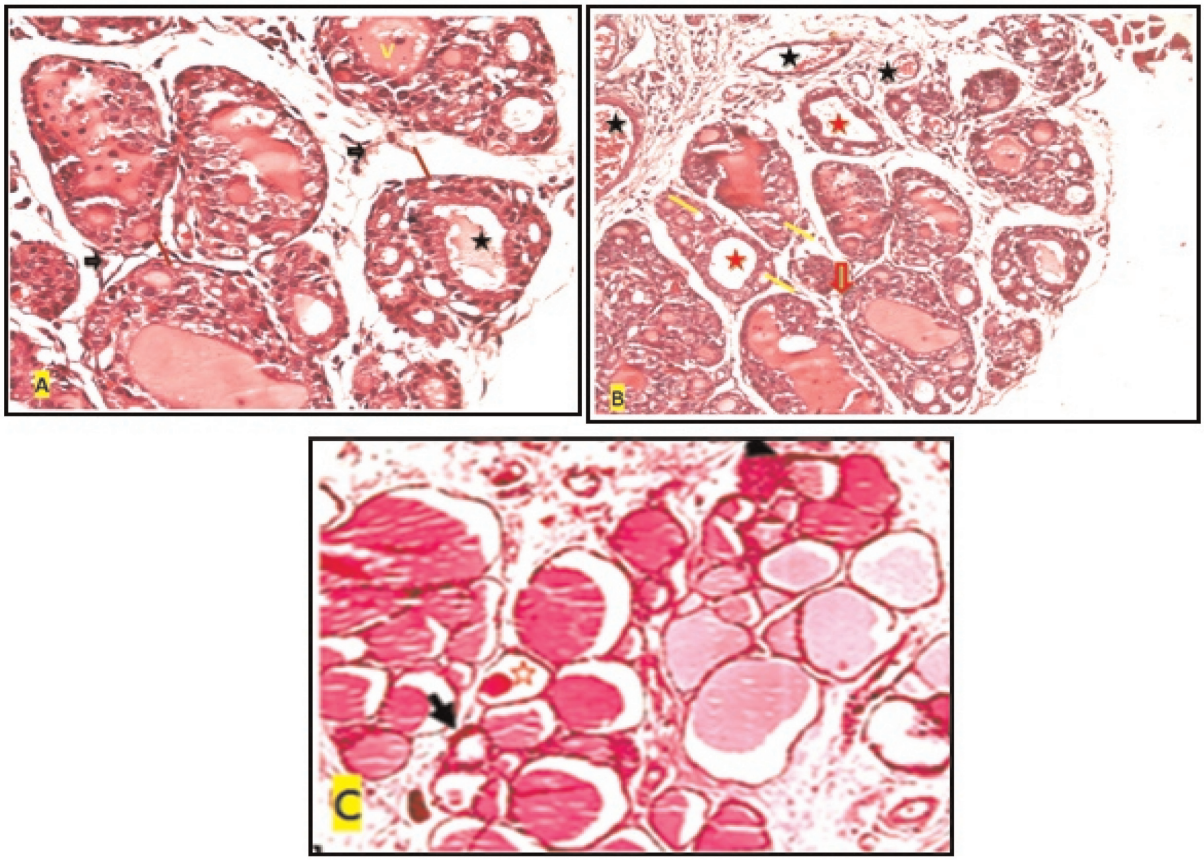
Hyperthyroidism rat model in the current study showed an increase in hepatic MDA and NO levels and a reduction in the antioxidants GSH and GPx, associated with increased activities of ALT and AST in serum. Previous studies mentioned that the hyperthyroidism induces apoptosis in liver, resulting in hepatic dysfunction [31,32], which is associated with the elevation of liver enzymes such as ALT, AST, and alkaline phosphatase in serum [31]. GSH, superoxide dismutase, and GPx are the principal components of the antioxidant defense system, and a deficiency in these components can cause oxidative stress [33]. Hypermetabolic condition caused by thyroid hormones creates an increase in the amount of free radicals by increasing mitochondrial electron transport [1]. In biologic membranes, the active free radicals, such as superoxide anions and hydroxyls, are capable of removing hydrogen atoms from the peripheral chains of saturated fatty acids and result in lipid peroxidation [34,35]. Treatment with MME suppressed oxidative stress and ameliorated liver enzymes. As MME has antioxidant compounds such as phenolic compounds which can inhibit lipid peroxidation and stabilize cell membrane and prevents the oxidation of membrane lipids, hence there is decreased leakage of hepatic AST and ALT to bloodstream.

Thyroid hormones influence also kidney structure, glomerular filtration rate, renal hemodynamics, membrane transport, and also sodium and water homeostasis [36]. Increased mitochondrial energy metabolism along with reduction of antioxidant enzymes which occurs in hyperthyroidism, contributes to an increased free radical generation that causes renal injury [1]. Hyperthyroidism induction, in the present study, enhanced renal lipid peroxidation and NO along with a decrease in the values of GSH and GPx. These observations agree with previous reports that showed reduced antioxidants and increased lipid peroxidation in the kidney of hyperthyroid rats [33,37]. Administration of MME was potentially effective in reducing lipid peroxidation and improving the antioxidant status in kidney tissue; this effect was evidenced by the decrease in the elevated levels of MDA and NO toward the corresponding values of normal group, and significant improvement of the antioxidant defense system.

However, in the present study, L-thyroxin administration did not cause a change in serum urea and creatinine levels, although there was an increase in lipid peroxidation. The oxidative stress occurred on kidney at the used dose may be not enough to affect the nephron function. As opposed to our findings, it was reported that 500-μg/kg body weight L-thyroxin administration enhanced urea level in serum [37]. This contradictory finding may have resulted from the difference in the dose of L-thyroxin and the period of administration.

Our results concerning glucose level showed the elevation of blood glucose in hyperthyroid rats, and

Figure 3



Photomicrograph of a section of the thyroid gland of rat (a) thyroid from the group of hyperthyroidisms showing architecture distortion and increase in follicular size. Fibrous tissue appears to be increased in the stroma between thyroid follicles (black arrow). The thyroid follicles are filled with vacuolated colloid (v), some follicles are filled with partially colloid (black star) (hematoxylin and eosin ×200), (b) another field of previous group showing absence of colloid of some follicles (red star), epithelial hyperplasia of follicular cells (red arrow) and vacuolated colloid (yellow arrow) are seen. Atrophied of some thyroid gland follicles and congested blood capillaries are also noticed in this section (black star) (hematoxylin and eosin ×100) and (c) thyroid of hyperthyroidism rat treated with MME showing scalloping of thyroid colloid and some empty follicles (red star). Proliferation or multiple layers of some follicular cells are seen (black arrow) (hematoxylin and eosin ×400). MME, *Melissa officinalis* methanolic extracts.

this elevation was counteracted by MME. Hyperthyroidism leads to reduced insulin secretion and insulin content caused by reduced β-cell number and volume [38].

Both decreased peripheral insulin sensitivity and impaired insulin secretion are the factors contributing to the development of abnormal glucose tolerance in the hyperthyroid state [39]. The clinical and experimental hyperthyroidism is often accompanied by abnormal glucose tolerance, which is attributed to mitochondrial oxidative damage caused by the enhanced production of reactive nitrogen and oxygen species resulting from the overproduction of the thyroid hormones [40]. It was reported by Asadi *et al.* [41] that the hydroalcoholic (70%) solvent of *M. officinalis* was safe and effective in reduction of inflammation, improvement of lipid profile, and glycemic control when ingested by type 2 diabetic patients.

Hyperthyroidism can lead to the activation of proteins that are related with inflammation, apoptosis, and hypertrophy [29]. Hyperthyroidism causes the activation of NF-κB, a ubiquitous family of transcription factors [42], which have a central role in regulating genetic transcription and encoding of inflammatory cytokines, such as IL-8, tumor necrosis factor (TNF-α), and IL-6 [43]. These cytokines influence NO [44] that induces lipid peroxidation and free radical production. We found in our study an increase in IL-6 and IL-8 levels in hyperthyroid group. The presence of hyperthyroidism has been associated with cytokines secretion such as IL-1 beta, IL-6, and TNF-α in rats [45]. Additionally, Senturk *et al* [46] found increases in levels of IL-8, TNF-α, IL-1-beta, and IL-6 in patients with hyperthyroidism. MME treatment in our study suppressed hyperthyroidism-induced oxidative damage as well as the related increase in IL-6 and IL-8 levels, indicating the anti-inflammatory potential of MME.

Our results showed insignificant change in blood hemoglobin among the different studied groups. However, this result is in conflict with other study which found that hemoglobin level increased in hyperthyroid erythrocytes of rats that received in drinking water, L-thyroxine sodium salt (0.0012%) [47]. On the contrary, Messarah *et al.* [48] observed a marked decrease in red blood cell number, hemoglobin concentration, and hematocrit in rats that received L-thyroxine sodium salt (0.0012%) in drinking water. The noticeable variability in the reports about blood hemoglobin might be referred to differences in study design, species, and difference in the dose of L-thyroxine and the period of administration.

Our biochemical results were confirmed by the histopathological investigations of the thyroid gland tissue. The follicles of thyroid gland of hyperthyroid rats were filled with vacuolated colloid. Proliferation or multiple layers of follicular cells and bands of fibrous tissue, and increase in interstitial tissue were also seen. In basal conditions, thyroid epithelial cells produce moderate amounts of reactive oxygen species that are physiologically required for thyroid hormone synthesis which are not toxic because they are continuously detoxified either in the process of hormone synthesis or by endogenous antioxidant systems [49]. However, in case of hyperthyroidism, the excess of thyroid hormones leads to increased oxidant production and mitochondrial oxidative damage [40]. However, animals treated with MME resulted in an improvement in thyroid gland tissue as compared with the group of hyperthyroidisms, as MME demonstrated improvement in the pathological changes. This improvement occurred probably by preventing oxidative stress and by ameliorating thyroid hormones.

MME possesses strong antioxidant properties that are proved from the data of DPPH radical scavenging activity and further confirmed by the increased values of GPx and GSH in the kidney and liver tissues. MME contains a variety of nutrients and phytochemicals. As shown by LC/MS analysis in the current study, MME contains 21 compounds that include polyphenolic compounds, tocopherols, flavonoids, alkaloids, and nitrogenous compounds. Polyphenolic compounds are among the most important antioxidants [50]. These compounds, especially flavonoids, have protective effects on liver damage induced by free radicals and liver toxins [51,52]. Flavonoids and polyphenolic compounds can also revive the cells against GSH depletion and protect them by increasing the capacity of antioxidant enzymes (GPx, GSH reductase, and catalase) [53].

Other compounds that may also play a role in the activity of MME are cinnamic acid derivatives including caffeic acid, as well as the oleanolic acid and the triterpenoids ursolic acid. Ursolic acid and oleanolic acid are known for their hepatoprotective, antioxidant, anti-inflammatory, antiviral, antitumor, and antimicrobial effects [54]. MME contains also monoterpene aldehydes and ketones which were found to be the most powerful scavenging compounds in *M. officinalis* extract [55,56]. Furthermore, MME contains rosmarinic acid which was described as having antimicrobial, antinociceptive, anti-inflammatory, and anticancer activities [57,58]. Rosmarinic acid shows also an important antioxidant activity as a reactive oxygen species scavenger and lipid peroxidation inhibitor [59]. Accordingly, the ameliorative effect of MME on liver and kidney could be attributed to either an increase in the antioxidant enzymes or a decrease in MDA as a biochemical indicator of cell damage and lipid peroxidation, or its potent natural antioxidant components, such as caffeic acid derivative, rosmarinic acid, and flavonoids quercetin-3-arabinoside.

Conclusion

The results obtained demonstrated that oxidative stress in liver and kidney tissues and the disturbance in blood glucose increased in hyperthyroidism. Besides, it is seen that the treatment with MME counteracted thyroid hormones and consequently improved liver and kidney functions and ameliorated glucose level. The beneficial effect of this extract in correcting hyperthyroidism and associated complications could be attributed to its high contents of antioxidant components. Further studies are required for supporting the role of MME in improving the hyperthyroidism in human.

Acknowledgements

The authors are thankful to National Research Center, El Dokki, Cairo, Egypt, for providing the necessary facilities to carry out this research project.

Source(s) of support: This work was supported by the National Research Centre (project numbers 11010331: 2016/2019).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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