Comparative Study between a Combination of Plants Extracts and Drugs on Thyroid Hormones and Lipid Profile in Experimental Animals

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Abstract
The thyroid gland is a vital part of the overall endocrine system, which is regulated some of body function as oxygen use, basal metabolic rate, growth, cellular metabolism and development This study shed light on a number of extracts that have been shown to have beneficial effects on the thyroid and its function, as well as the various factors linked to thyroid dysfunction. The experiment was conducted to determine the effect of a mixture of two extracts of "*Fucus vesiculosus* (150 mg/ kg) with *Coleus forskohlii* (1000 mg/ kg) and *Rosmarinus officinalis* (220 mg/ kg) with *Camellia sinensis* (1.25 mg/ kg)" on thyroid hormones as well as lipids profile and tested the effectiveness of two drugs one of them stimulates the hormones of the thyroid (Levothyroxine) and the other is an inhibitor (Carbimazole). Nine female albino rats, aged 8-10 weeks and weighing 60-120g, and nine albino female mice, aged 8-10 weeks and weighing 23-25 g, were divided into 3 groups, each with 3 animals, and were given three different doses orally, with the exception of the control group, which received only normal saline. The result showed that the combination of *F. vesiculosus* with *C. forskohlii* was better than Levothyroxine against hypothyroidism, while the combination of *R. officinalis* with *C. sinensis* was better than Carbimazole against hyperthyroidism. The results of this study found that the extracts may have the ability to regulate hormone levels of thyroid gland in humans.

Keyword: Thyroid gland, Hyperthyroidism, Hypothyroidism, Levothyroxine, Carbimazol

INTRODUCTION

In humans, the thyroid is the first endocrine gland to mature. The thyroid gland arises from a diverticulum in the pharyngeal median ventral wall known as the thyroid diverticulum (Fancy et al., 2010). The thyroid's function is to make thyroid hormones, which are released into the blood and then carried to all of the body's tissues. Thyroid hormone aids the body use energy, remain warm and keep the brain, heart, muscles, and other organs working as they should. Thyroxine, also known as T4 because it contains four iodine molecules, is the thyroid's main hormone. The thyroid gland also produces small amounts of Triiodothyronine (T3), a more potent thyroid hormone that contains three iodine molecules. The majority of T3 in the blood, on the other hand, comes from T4 in other body tissues. American Thyroid Association (ATA), 2013.

An increased in circulating free thyroid hormones is defined as hyperthyroidism. According to Fumarola et al. (2010), the prevalence is around 1.3 percent, with a male/female ratio of 1 to 5–7 (2 percent in women and 0.2 percent in men). Carbimazol is one of the drugs used to treat hyperthyroidism (CBZ). CBZ, a pro-drug of TMZ, is quickly converted into TMZ by

splitting of the ethoxycarbonyl residue (Cooper, 2003). TMZ is regarded as the drug of choice due to its longer half-life, improved effectiveness, and more favorable side effect profile (Cooper and Rivkees, 2009).

One type of thyroid disease is hypothyroidism. Hypothyroidism is a condition in which the thyroid gland is inactive (hypo-means under or below normal). The thyroid does not produce enough thyroid hormone in people with hypothyroidism to keep the body functioning normally (American Thyroid Association (ATA) 2013). The standard treatment for hypothyroidism is levothyroxine or "LT4" (Hennessey, 2017). LT4 can be taken orally in a variety of forms (including tablet, soft gel capsule, and liquid formulations) (Virili et al., 2019), and LT4 tablets are accessible in several of branded and generic forms (Hennessey, 2017).

The target of this study was to comparative between a combination of two extracts of *F. vesiculosus* (150 mg/kg) with *C. forskohlii* (1000 mg/kg) and *R. officinalis* (220 mg/kg) with *C. sinensis* (1.25 mg/kg) against two drugs (Levothyroxine) and (Carbimazole) on thyroid hormones as well as lipids profile in rats.

MATERIAL AND METHOD

Plants collection

Dry leaves of Rosmarinus officinalis, Camellia sinensis, Melissa officinalis, Curcuma longa rhizome, Coleus forskohlii roots, and Fucus vesiculosus thallus were collected from local markets in Baghdad, Iraq. Each sample was air dried in the shade and ground in a blender to produce small (2 mm) pieces, which were then saved in glass containers at room temperature in a dry, dark location.

Preparation of an Aqueous Plants Extracts

Each plant's aqueous extract was prepared in a different way depending on the parts of the plant (Harborne, 1984). The extracts were then kept in the refrigerator until they were needed. Various concentrations of each plant were made by dissolving a specific weight of each plant extract powder in distal water according to the concentration required. Then, by using the equation below, different concentrations of plant extracts (mg/ml) were prepared (Al-Naqqash, 2013).

(Concentration mg/ml = weight/volume x 1000)

Laboratory Animals

Nine female albino rats, aged 8-10 weeks and weighing 60-120 grams, and nine albino female mice, aged 8-10 weeks and weighing 23-25 grams, were bought from the National Center for Drug Control and Research, housed at a temperature of 27 ±2 degrees Celsius, and subjected to the OCED-designed experimental protocol (OECD, 2001). The experiments were carried out at the Veterinary Drug Research and Production Center, which ethically approved all of the experimental protocols. The animals were divided into groups at random and stored in separate plastic cages. The animals were kept in standard ventilation, temperature, and humidity conditions. There was food and water available. At the equator, all animals were slaughtered.

Study Design

Experiment -1-

The purpose of this experiment was to see how mixed doses of two plants aqueous extract affected rats. Rats were given mixed doses of two plants orally (mg/kg) and were divided into three groups, each with three rats:

Group 1: Rats were treated with mixed dose of an aqueous extract of *F. vesiculosus* (150mg/kg) and *C. forskohlii* (1000 mg/kg).

Group 2: Rats were treated with mixed dose of an aqueous extract of *R. officinalis* (220 mg/kg) and *C. sinensis* (1.25 mg/kg).

Group 3: contained control rats which received normal saline only.

Experiment -2-

This experiment was designed to show the effect of two drugs on mice; one is used to treat hyperthyroidism (Carbimazole 5mg / AMDIPHARM/ UK) and the other is used to treat hypothyroidism (Levothyroxine 25 µg/ Merck serono/ Germany); 9 mice were divided into three groups, each group with three mice as the following:

Group 1: Mice were treated with Carbimazole at 0.6 mg/kg body weight orally.

Group 2: Mice were treated with levothyroxine at 2µg/ml orally.

Group 3: Control mice which received Normal Saline only.

Blood Samples and Organs Collection

Five milliliters (ml) of blood were collected by drawn from the heart of the animals directly by cardiac puncture using a medical syringe. Each blood sample was put in a tube containing a gel free anticoagulant to get the serum and centrifuged at 3000 rpm for 15 minutes to ensure an adequate amount of serum free of red blood cells. After that, the serum was then drawn and put in a special plastic tube, which was kept clean and stored at -20 ° C until used (Cheng, 2002).

Biochemical Analysis

Thyroid stimulating hormone (TSH) and triiodothyronine (T3) (O'Neil, 2001) levels, as well as thyroxine (T4) (Wagner et al., 2008), were tested using the previously mentioned methods (CHO) Triglyceride, Meiattini et al., 1978 (TG) High Density Lipoprotein, Fossati and Prencipe, 1982 (HDL) Low Density Lipoprotein, Burstein et al., 1980 (LDL) Very Low Density (VLD) and Nauck et al., 2002.

Statistical Analysis

All data is presented as a mean with standard deviation (standard deviation). The data were analyzed using the SPSS software package version 17.0 and a one-way ANOVA followed by a student t-test (SPSS Inc., Chicago, IL, USA). P 0.05 was used to determine whether the differences were significant. SAS (SAS, 2012).

RESULTS AND DISCUSSION

Thyroid Profile Assay for the Mixture of Two Plant Extracts

The results of thyroid profile assay for the mixture of *F. vesiculosus* with *C. forskohlii* extracts and *R. officinalis* with *C. sinensis* extracts were shown in (Table 1). The current-study found-that there was a significant increase in serum T3 and T4 levels were increased by 33% and 4% respectively, as well as a significant decrease in serum TSH level which reduced

by 86% in rats treated with mixture of two aqueous extracts of *F. vesiculosus* with *C. forskohlii* as compared with control, while, the mixture of *R. officinalis* with *C. sinensis* reducing the level of T3 and T4 by 25% and 32% respectively,, however the level of TSH was increased by 24% as compared with control.

*The percentages of differences were estimated according to a previously described equation (Al-Jamal and Alqadi, 2011):

C1 – C2/C1*100

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Table 1: The-Effect of The Mixture of Two Extracts of *F. vesiculosus* with *C. forskohlii* and *R. officinalis* with *C. sinensis* on Thyroid Hormones

Plants	Mean ± SE		
	T3 (nmol/L)	T4 /(nmol/L)	TSH/ (µIU/ml)
Control	2.1 ± 0.09	124 ± 6.21	2.3 ± 0.05
F. vesiculosus 150mg/kg+ *C. forskohlii* 1000mg/kg	2.8 ±0.08	129.5 ± 6.19	0.33 ± 0.03
R. officinalis 220mg/kg + *C. sinensis* 1.25mg/kg	1.57 ± 0.08	84.5 ± 3.71	2.85 ± 0.08
LSD value	0.408	17.74	0.669

Synergism can take place between the constituents of a single extract and in a mixture of herbs. Medical herbalists have always insisted that better results are obtained with whole plant-extracts and combinations of these rather than with isolated compounds (Phillipson, 1994).

Lipid Profile Assay for the Mixture of Two Plants Extracts

Several plant preparations like a mixture of aqueous extracts of "*F. vesiculosus* at 150 mg/kg with *C. forskohlii* at 1000mg/kg and *R. officinalis* at 220 mg/kg with *C. sinensis* at 1.25mg/kg" were currently utilized to manage lipid profile (Table 2). These results showed that the mixture of *F. vesiculosus* with *C. forskohlii* extracts reduced the level of CHO, TG, LDL and VLDL by 9, 30, 26 and 30 % respectively,, In comparison to the control group, the level of HDL increased by 38%. However, when a mixture of *R. officinalis* and *C. sinensis* was given, the levels of CHO, TG, LDL, and VLDL increased by 5, 16, and 4%, respectively, while the level of HDL decreased by 8%, as compared to the control.

Table 2: The Effect of The Mixture of Two Extracts of *F. vesiculosus* with *C. forskohlii* and *R. officinalis* with *C. sinensis* on Lipid Profile

Plants	Mean ± SE				
	CHO/ (mg/dl)	**TG/ (mg/dl)**	**HDL (mg/dl)**	**LDL/ (mg//dl)**	**VLDL/ (mg/dl)**
Control	140 ± 6.82	161 ± 8.61	20 ± 0.76	87.8 ± 3.62	32.2 ± 1.44
F. vesiculosus 150mg/kg+ *C. forskohlii* 1000mg/kg	127 ± 6.17	113 ± 4.96	27.5 ± 149	65.3 ± 2.07	22.6 ± 1.02
R. officinalis 220mg/kg+ *C. sinensis* 1.25mg/kg	147 ± 7.61	167 ± 8.94	18.5 ± 0.81	102.1 ± 5.14	33.4 ± 1.33
LSD value	23.82	29.52	5.08	16.81	4.95

The mixture of *F. vesiculosus* with *C. forskohlii* extracts have hypolipidemic properties and can be used for the controlling of hypolipidimic activities. These results are due to the synergist interactions which are of vital importance in phytomedicines. These interactions are documented for constituents within a whole extract of a single herb, as well as between different herbs in a preparation. Rats treated with mixture of *F. vesiculosus* and *C. forskohlii* showed an increase of HDL-cholesterol and a reduction of LDL, TG and CHO (El-Newary, 2016). The hypolipidimic activity of the mixture of *F. vesiculosus* with *C. forskohlii* showed here could be attributed-to the presence of active hypolipidimic agents found in the mixture. But, the mixture of *R. officinalis* with *C. sinensis* showed hyperlipidimic activity in this experiment due to interaction between the hyperlipidimic agents present in it which may be caused this status. The analysis of phytochemical composition of aqueous extracts of *F. vesiculosus* with *C. forskohlii* demonstrated the presence of phenolic acids. Decreasing of serum lipid levels through food or drug therapy seems to be associated with a lowering in the risk of vascular disease and related complications (Yin *et al.*, 2011).

Effect of Drugs on Thyroid-Hormones

The findings of thyroid profile-assay for the Carbimazole and levothyroxine are shown in (Table 3) indicated stimulatory effect of levothyroxine on T3 and T4 were increased by 111% and 49% respectively, While TSH levels were reduced by 91 % when compared to control, Carbimazole had an inhibitory effect on T3 and T4 levels, which were reduced by 61 % and 59 %, respectively, while TSH levels were raised by 121 %.

Drugs	Mean ± SE		
	T3/ (nmol//L)	T4/(nmol//L)	TSH (µIU/ml)
Control	2.01 ± 0.08	114 ± 5.59	2.31 ± 0.09
Carbimazole	0.775 ± 0.04	46.5 ± 2.26	5.11 ± 0.59
Levothyroxine	4.25 ± 0.20	170 ± 8.84	0.20 ± 0.05
LSD value	1.025	26.88	1.008

Table 3: The Effect of Two Drugs on Thyroid Hormones

The orally treatment of rats with Carbimazole (5mg) for four weeks causes reduce in the level of T3 and T4 hormones by 61 and 59% respectively compared with control. These findings are agreed with the results of (Debasish et al., 2013; Ilyas et al., 2015) were they found that carbimazole decreases thyroid hormones (T3, T4) compared with their normal range level in the control group, that maybe due to known mechanism of carbimazole to inhibit peroxidase enzyme TPO action which is an important enzyme in the synthesis of thyroid hormones. While, the administration of Levothyroxine (25µg) was increase the level of T3 and T4 hormone by 111% and 49% respectively as compared with control. These findings similar to the results of Bolk et al., (2007) who found a decrease in mean TSH and an elevated in free thyroid hormone levels when a stable dose of morning levothyroxine"LT4" are given. The mechanism of action of levothyroxine is that it raises the basal metabolic rate and regulates growth and development. Bolk et al. observed the same investigation (2010).

Effect of Drugs on Lipid Profile

The effect of carbimazole increased the levels of CHO, TG, LDL, and VLDL by 8, 68, 61, and 68 %, respectively, while the level of HDL was reduced by 35 %, according to the results in (Table 4). However, when compared to the control, Levothyroxine reduced the levels of CHO, TG, LDL, and VLDL by 3, 5, 2, and 5%, respectively, while increasing the level of HDL by 28%.

Table 4: The Effect of Two Drugs on Lipid Profile

Drugs	Mean ± SE				
	CHO (mg/dl)	**TG (mg/dl)**	**HDL (mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
Control	118 ± 5.08	109 ± 5.02	34 ± 1.96	105.2 ± 4.37	21.8 ± 1.08
Carbimazole	127.5 ± 5.89	183 ± 8.72	22 ± 1.37	169.4 ± 8.21	36.7 ± 2.33
Levothyroxine	115 ± 4.70	104 ± 4.73	43.5 ± 2.79	103.3 ± 4.19	20.8 ± 1.22
LSD value	25.92	33.51	7.44	29.53	7.39

The present study indicates that-the level of CHO, TG, LDL, and VLDL were increased, while, the level of HDL was decreased after the administration with Carbimazole (5mg). These results are assented with previous investigation of Hennemann *et al.*, (2001) who found that Carbimazole action cause decrease in thyroid hormones and these hormones can regulate fat metabolism and affect level of lipids, so decrease thyroid hormone lead to high cholesterol level. The reduction in the level of thyroid hormones leads to imbalance in fat digestion, absorption, synthesis or prevent activation of their receptors (Santi *et al.*, 2010). Hypothyroidism causes decrease of lipoprotein lipase in fatty tissues and liver cells this lead to accumulation of high concentration of TG, LDL and VLDL (Saleh, 2015).

Levothyroxine, on the other hand, decreased the levels of CHO, TG, LDL, and VLDL, while increasing the level of HDL. These findings are agreed with the results of Danese *et al.*, (2000) who demonstrated that levothyroxine decreased serum total cholesterol as well as low-density lipoprotein (LDL) cholesterol. Further study of Tanis *et al.*, (1996) found that levothyroxine at 15mg/dl was reduced the level of cholesterol by irrespective of the initial level.

CONCLUSION

In these experiments both mixture were effect on thyroid hormones and lipids profile and causes hypothyroidism and hyperthyroidism, but the combination of *F. vesiculosus* with *C. forskohlii* was better than Levothyroxine against hypothyroidism.

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