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Effect of green tea (*Camellia sinensis* L.) aqueous extract and L-carnitine supplements on some hormonal levels and some haematological parameters in obese male rats

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This work was designed to study the effect of daily oral administration for two groups of male albino rats suffering from weight gain (obese rats) with both 10% aqueous extract of green tea and 21 mg L-Carnitine/kg body weight for 9 weeks. Green tea (GT) and L-Carnitine (LC) cause significant body weight reduction in overweight rats with low thyroid hormone levels (triiodothyronin T_3 and thyroxin T_4) as well as testosterone. Treatment of obese male rats with GT caused decrease in count of red blood cells and blood haemoglobin and elevate in both count of white blood cells WBCs and haematocrit PCV%, while LC caused elevate in blood haemoglobin levels Hb, RBCs, WBCs and (PCV) % compared with both negative and positive groups of control while haematocrit PCV% in rats of positive contrl group was reduced compared with rats of (*C*-ve) group and all treatment groups.

Keywords: Green tea, L-Carnitine, Obesity, Thyroxin, Triiodothyronin, Testosterone.

INTRODUCTION

Obesity has reached pandemic proportions around the world, and now poses one of the greatest public health challenges. One billion out of the approximately 6.5 billion people in the world are estimated to be overweight (body mass index, bmi >25kg/m^2) and of these at least 300 million are obese bmi >30kg/m^2 (WHO, 2009), these numbers are predicted to more than double to reach 2.3 billion overweight and 700 millions obese by 2015 (WHO, 2010). Rats injected with pure green tea intra-pretonialy were studied for acute effects on endocrine systems, significantly reduction in food intake, body weight gain, testosterone blood level as well as there has been a growth in the prostate and uterus in lean and obese male zucker rats (Yung et al., 2000). Green tea extract at dose level of 5.0% has the potential to alter the thyroid gland physiology and architecture that is an enlargement of thyroid gland as well as hypertrophy and/or hyperplasia of the thyroid follicles and significant decrease in serum T_3 and T_4 and a parallel increase in serum thyroid stimulating hormone (TSH) (Chandra et al., 2010). The level of estrogen, progesterone and testosterone in the serum was not affected by the treatment of L-Carnitine as dairy supplementation in cats following ovariohysterectomy, since, long-term study is recommended (Tavakoli et al., 2017). L-Carnitine is a peripheral antagonist of thyroid hormone action at least in some tissues. L-Carnitine was

able to both reverse and prevent/minimize nine hyperthyroidism related symptoms (Salvatore et al., 2001), triiodothyronin T_3 and thyroxin T_4 were increased in obese rats treated with L-Carnitine (Ridha and Kocher, 2015).

This study is concerned with the evaluation of the serum testosterone hormone thyroid hormones T_3 and T_4 and blood picture effects of green tea and L-Carnitine supplementation in male obese rats which were treated using L-Carnitine supplementation and aqueous extract of green tea.

MATERIALS AND METHODS

Plant Material used

Green tea*, Camellia sinensis* (Ericales: Camellia), green tea package made in China was purchased from the local market in Cairo Governorate. Green tea extract (GT) was orally administered to rats at a dose of 1 ml/100g body weight daily.

Drug used

L-Carnitine (LC) (dietary supplement): 1 ml containing 250 mg carnitine was purchased from the Arab Company for Pharmaceuticals and Medicinal Plants (MEPACO-MEDIFOOD) Enshas El-Raml, Sharkeya, Egypt. Oral administration of a dose of 300 mg/kg body weight was done, daily.

Experimental Animals

Mature male albino rats (130g±10) were used in the present study. They were kindly supplied by the Egyptian Organization of Biological Products and Vaccines. The animals were bred in appropriate conditions for two weeks for acclimatization before treatments.

Experimental Design

A total of forty male rats, were randomly assigned into two groups, normal 10 male rats, and thirty male rats, which were divided into three groups after induction of obesity. In the experiment 10 rats were used in each group. Our goal is to achieve obesity model in 8 weeks by high fat diet feeding, followed by treatment period for nine weeks. This model provides a reliable method and resembles the clinical cases of obesity and its treatments; also, this period of treatment is safe and recommended in previous researches (OECD, 2018).

Normal Diet:

Protein 21% - Fats 3.4% - Fibers 3.3%- concentration 10% Carbohydrate. Yellow maize – soybeans – bone dust-diet complementary.

High Fat Diet (HFD)

The HFD contained 20g of fat/100 g of diet (19g of butter oil and 1 g of soybean oil to provide essential fatty acids) and provided 19.34 kJ/g of diet, including 7.74 kJ/g as fat (Stephen et al., 2002).

Treatment

Green tea aqueous solution 10% (El-Sayed and Islam, 2014), and L–Carnitine 21mg/kg body weight (Meky et al., 2016), to be used for treated animals are shown in (Table 1) for long term (9 weeks). The administration solutions were prepared daily.

Blood samples

By the end of the experimental period, venous blood samples were collected from the orbital sinus of normal, obese control, obese treated rats via glass capillaries at fasting state. Blood sample were prepared by two methods as follows:-

Serum

Blood samples were collected in a dry tube, allowed to coagulate at room temperature and centrifuged at 3500 rpm for 15 minutes for separation of serum. The clear, non-haemolysed supernatant sera were separated and stored at -20°C for subsequent biochemical measurements

Table (1) Green tea (GT) and L-Carnitine (LC) concentrations to be used for treated obese animals.

Group No.	Treatment	Concentration of GT or LC
Group 1	Control (normal diet)	-
Group 2	Positive Control HFD	-
Group 3	GT administration + HFD	1ml/100g of B.wt. of 10% aqueous solution of GT
Group 4	LC administration + HFD	21 mg/kg of B.wt of LC.

as follows: thyroid hormones levels and testosterone level (Henry, 1979).

Whole blood

Whole blood sample were collected in EDTA (1.5 mg/ml blood), then used as fresh as possible for determination of blood cells count, haematocrit (PCV%), and haemoglobin content (Miller and Dubos, 1937).

Haematological Estimation

The total count of red blood cells (RBCs) and white blood cells (WBCs), the haemoglobin (Hb) content and the haematocrit (PCV%) of the blood were estimated as described by (Dacie and Lewis, 1984).

Statistical analysis

Statistical analysis for all data was carried out using analysis of variance (ANOVA) using general linear model program of SAS (SAS, 2000). Statistically significant difference among means were set at $p \leq 0.05$ level by using Duncan's Multiple Ranges Test (DMRT) procedure (Duncan, 1955).

RESULTS

Reduction of body weight% in obese rats treated with (GT and LC)

Depicts the progression of body weight percentage in the four groups of animals. Effect of aqueous water extract prepared from green tea and L-Carnitine on body weight gain % of obese rats presented in Table (2). The mean of body weight gain% of the positive control group increased as compared to the negative control group, 74.1% and 72.3%, respectively. The progression of body weight% of two treated groups with green tea aqueous extracts and treated group treated with L-Carnitine showed decreased in the progression of body weight% compared with positive and negative control groups, 74.1% and 72.3%, respectively. While the progression of body weight% in group which treated with L-Carnitine is 53.2%, was more decreased than those treated with green tea extract being 61.3% (Table 2).

Effects of GT and LC on testosterone hormone levels

Rats treated with each of GT and LC, had significant decreases in testosterone hormone level. After nine weeks of treatments their values were 1.08 and1.47 ng/l, respectively, compared with negative and positive control which were 3.13 and 2.32 ng/l, respectively (Table 3).

Effects of GT and LC on thyroid hormone levels

Rats treated with GT and LC had significant changes in thyroid hormones T_3 and T_4 levels. After nine weeks of treatments Triiodothyronin (T_3) were 46.64 and 73.00 ng/l in rats treated with GT and LC, respectively, compared with either positive or negative control, which were 77.17 and 57.03 ng/l, respectively. Thyroxin hormone (T_4) were 3.58 and 4.87 µg/dl, respectively, compared with each of positive or negative control, which were 6.25 and 4.37 µg/dl, respectively. Rats which treated with GT revealed that Triiodothyronin and Thyroxine (T_3 and T_4), were decreased compared with each of negative or positive control, while rats which treated with LC showed that Triiodothyronin (T_4) and Thyroxine (T_3) were increased compared with control negative, on contrast they were decreased compared with control positive (Table 3).

Effect of GT extract and LC treatments on Haematological parameters

RBC's, WBC's, Hb, and PCV% values increased after green tea and L-Carnitine treatments; 6.948×10^6, 8.87×10^3, 19.02 g/dl and 40.9%, respectively. All parameters increased after treatment with green tea which were for each of WBC's is 8.02×10^3 and PCV% is 42.3%, on the other hand value of RBC's is 4.778×10^6 and Hb content is 16.4 g/dl, were decreased, respectively, compared with either their negative or positive control values (Table 4).

Table (2): **Percentage of body weight (BW) gain of male albino rats administrated with Green Tea extract (GT) and L-Carnitine (LC) with high fat diet (HFD) for 9 weeks.**

Week Group	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
G1 Control (-)	11.6	17.9	27.5	38.1	46.8	57.1	63.4	69.6	72.3
G2 Control (+)	14.3	20.8	30.9	42.7	53.4	59.8	65.8	72.8	74.1
G3 GT Treatment	14.2	20.3	29.7	40.1	49.6	54.3	56.9	59.8	61.3
G4 LC Treatment	10.1	12.9	21.8	30.4	36.4	39.9	45.9	50.1	53.2

Table (3): Effect of GT (aqueous extract) and L- Carnitine on serum Testosterone, T_3 and T_4 in male albino rats.

Groups of treatments	Testosterone ng/l	T_3 ng/l	T_4 µg/dl
Control negative	3.13	57.03	4.37
Control positive	2.32*	77.17*	6.25*
Green tea treatment	1.08*	46.64*	3.58*
L-Carnitine	1.47*	73.00*	4.87*

Table (4): Effect of GT aqueous and L-Carnitine on blood picture of male albino rats.

Treatments	RBC'S Count		WBC'S Count		HB concentration		HCT value	
	X(10) 6 ML	% of Control	X(10) 3 ML	% of Control	g/dl	% of Control	%	% of Control
Control (-)	4.978±0.098	100.00	7.35±0.367	100.00	17.3±0.6	100.00	41.8±1.41	100.00
Control (+)	5.325±0.3	106.97	7.98±.186	108.57	18.02±0.3	104.16	41.9±2.3	100.20
Green tea	4.778±0.44	95.98	8.02±0.768	109.00	16.4±0.37	94.80	42.3±1.3	101.12
L-Carnitin	6.948±0.29	139.57	8.87±0.126	120.68	19.02±0.9	109.94	43.3±0.89	103.59

All values = mean ± SE, Control (with normal diet) values are referred to 100%.

DISCUSSION

Green tea is containing polyphenols and flavonoid. in common with synthetic antioxidants several flavonoids can interfere with thyroid hormone biosynthesis free radical iodination (Ferreira et al., 2002; Doerge and Sheehan, 2002), most studies of polyphenols aimed to determine the protective effects of polyphenols against diseases or toxic drugs and relatively few investigators have examined their possible toxicity. No acute toxicity was observed after oral administration of green tea aqueous extract. The present study describes that green tea aqueous extract (GT) and L-Carnitine (LC) exposure at the high dose with 10% GT and recommended dose of 21 mg LC/kg of body weight as used in our investigation had reduced the gain in body weight in adult obese male albino rats in both treatments GT and LC compared with both control groups *(C*+ve and *C*–ve) even though the average daily food intake did not differ between the groups during study period. These findings are in line with previous work which showed that green tea and L-Carnitine have potential role in body weight control. But decrease in percentage of body weight gain of male albino rats which administrated with GT was less than decrease in percentage of body weight gain of male albino rats which administrated with LC. The increase in weight loss in L-Carnitine-treated animals may be higher than the weight loss in fat animals treated with green tea. This is explained by the resulting increase in thyroid hormone secretion in the case of L-Carnitine treatment than in case of green tea treatment which increases the rate of body fat metabolism. In addition LC decreased of body weight in obese rats because it is increase mitochondrial β-oxidation of fatty acids. L-Carnitine supplementation increase fat mobilization from adipose tissue, while the effect of endocrine system in the rats was different when treated with green tea than in the case of treatment with L-Carnitine. The circulating levels of testosterone were lowered GT at the high level intake may have anti-androgenic effects thus influencing male fertility. Peripheral thyroid hormones T_3 and T_4 are moderately increased in control positive group. When rats were administered with GT or LC, weight reduction leads to a long term nine weeks decrease in the peripheral thyroid hormones. Triiodothyronin and thyroxin, T_3 and T_4, in serum levels (Doerge and Chang 2002; Benvenga et al., 2004; El Mgeed et al., 2009; Chandra and Neela 2014). LC is a peripheral antagonist of thyroid hormone action. In particular, L-Carnitine inhibits both T_3 and T_4 entry into the cell so leading to their accumulation in the blood. Also, (Elgazzar et al., 2012) mentioned that, L-Carnitine was acting thyroid hormone target tissue and not at the level of thyroid gland as an inhibitor of thyroid hormone synthesis [23]. Treatment with GT reduced RBCs count and haemoglobin content. Green tea is containing polyphenols may also have anti-nutritional effects. The inhibition of non-haem absorption attributable to simultaneous tea consumption is well known; high consumption of polyphenols may increase the risk of iron depletion in populations of individuals with marginal iron status [24,25]. It is important to consider that green tea black tea wine and coffee contain many polyphenols may

be dangerous to human health especially causing anemia.

CONCLUSION

Based on the results of this study, green tea aqueous extract with moderate dose is neither low nor high and L-Carnitine supplement with permissible dose were able to reduce body weight in male obese rats but with some negative effects. The production of testosteron hormone decreased when treated with both green tea and L-Carnitine, and thyroid hormones was also decreased in rats which treated green tea. Conversely, thyroid hormones (T_3-T_4) production increased in rats which treated with L-Carnitine supplementation

In addition, the presence of poly phenol in green tea is one of the main causes of anemia, which was demonstrated by the reduction in the count of red blood cells and blood haemoglobin content compared to the control groups.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally in all parts of this study.

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