MODULATORY EFFECT OF GREEN TEA ON LIPID METABOLISM AND BRAIN NEUROTRANSMITTERS OF OBESE MICE MODEL

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Abstract

Objective. *The main goal of the present work was to study the relationship between the weight-reducing effect of green tea and its effect on GABA and 5-HT neurotransmitters in obese mice. Furthermore, the antilipidemic activity of green tea was also evaluated.*

Methods. *Adult male albino mice weighing 20 to 35 gm (both obese and non-obese) were used. The newborn mice were injected SC with MSG for the first 5 postnatal days. A control group was injected SC with physiological saline solution. The animals were weaned at the age of 4 weeks and kept on a commercial chow and tap water. At the age of 8 weeks, female mice were excluded from the study. At age of 12 weeks, each group was further subdivided into two different groups: group (a) that was not allowed to take green tea and group (b) that was allowed to take green tea (1% in diet for 2 weeks). Now, mice groups are four: control group without tea intake (Ia), control group with tea intake (Ib), obesity group without tea intake (IIa) and obesity group with tea intake (IIb). Serum cholesterol, triglyceride, HDL cholesterol and LDL cholesterol were measured by enzymatic calorimetric method using kits. Brain tissue GABA and 5-HT were determined by spectrophoto-fluorometric method.*

Results. *The obtained data revealed that; green tea caused a significant decrease in body weight, Lee index and food intake of obese mice group (IIb). There was also a significant decreased level of serum cholesterol, TAG and LDL and increased level of HDL in group (Ib). The study showed a significant increased level of serum cholesterol, TAG and LDL and decreased level of HDL in group (IIa). There was no significant difference between GABA concentration in group (Ia) and group (IIa). Treatment with green tea caused non-significant changes in brain GABA and significant elevation of the brain levels 5-HT. The present work clearly demonstrated that; obese mice have been shown reduced 5-HT brain levels compared to non-obese animals.*

Conclusion. *It is concluded that the green tea has antiobesity effect might be mediated by modulation of serum lipid profiles and brain 5-HT neurotransmitter.*

Keywords: green tea, mice, antioxidant, lipid metabolism, neurotransmitters.

EFECTUL MODULATOR AL CEAIULUI VERDE ASUPRA METABOLISMULUI LIPIDIC ȘI NEUROTRANSMIȚĂTORILOR ÎNTR-UN MODEL EXPERIMENTAL PE ȘOARECI OBEZI

Rezumat

Obiectivul *principal al studiului a fost investigarea relației dintre efectul de scădere în greutate a ceaiului verde și efectul său asupra neurotransmițătorilor GABA și 5-HT la șoarecii obezi. S-a evaluat, de asemenea, și activitatea antilipemiantă a ceaiului verde.*

Metode. *S-au utilizat șoareci albi masculi, cu greutatea între 20-35 g (obezi*

și non-obezi). Șoarecii nou născuți au fost injectați subcutan cu MSG timp de 5 zile postnatal. Un grup martor a fost injectat cu ser fiziologic. Animalele au fost înțărcate la 4 săptămâni și ținute cu hrană standard din comerț și apă de robinet. La vârsta de 8 săptămâni, femelele au fost excluse din studiu. La 12 săptămâni, fiecare grup a fost împărțit în 2 subgrupe: grupa (a) fără ceai verde, grupa (b) la care s-a administrat ceai verde 1% în dietă timp de 2 săptămâni. Au rezultat 4 grupe: Ia – grup martor fără ceai verde, Ib – grup martor cu ceai verde, IIa – șoareci obezi fără ceai verde, IIb – șoareci obezi cu ceai verde. Colesterolul seric, trigliceridele, HDL și LDL colesterolul s-au determinat prin metoda de calorimetrie enzimatică, utilizand kituri prefabricate. GABA și 5-HT din țesutul cerebral au fost determinate prin metoda spectrofoto-fluorimetrică.

Rezultate. *Datele obținute au evidențiat următoarele: ceaiul verde a determinat o scădere semnificativă a greutății corporale, indexului Lee și cantității de hrană ingerată la șoarecii obezi (IIb). O scădere semnificativă s-a înregistrat și la nivelele de colesterol seric, TAG și LDL, precum și creșterea HDL la grupul IIb. S-a evidențiat de asemenea o creștere semnificativă a nivelelor de colesterol seric, TAG și LDL, precum și creșterea HDL la grupul IIa. Nu s-au găsit diferențe semnificative în privința GABA între grupele Ia și IIa. Tratamentul cu ceai verde a dus la modificări nesemnificative a GABA și creșteri semnificative a 5-HT cerebral. Studiul demonstrează clar că șoarecii obezi au prezentat nivele reduse de 5-HT față de șoarecii non-obezi.*

Concluzie. *Ceaiul verde are un efect anti-obezitate, care poate fi mediat de modularea profilelor lipidelor serice și neurotransmițătorului 5-HT din creier.*

Cuvinte cheie: ceai verde, șoareci, antioxidanți, metabolism lipidic, neurotransmițători.

INTRODUCTION

Tea (Camellia sinensis, Theaceace) is the second most popular beverage in the world next to water and has been extensively studied for its putative disease preventive effects [1]. A large part of the research on green tea has focused on its effects related to the prevention of cancer, and encouraging data regarding efficacy, safety and potential mechanisms of action have accumulated in this area [2]. Furthermore, the anti-inflammatory [3], anti-arthritic [4], anti-angiogenic [5], anti-oxidative [6], anti-bacterial, anti-viral [7] and neuroprotective effects [8]of green tea and isolated green tea constituents have been investigated.

Many of the putative health benefits of tea are attributed to the high polyphenol (also called catechins) content of this beverage. There are four kinds of catechins in green tea: epicatechin, epigallocatechin, epicatechin-3- gallate and epigallocatechin-3-gallate (EGCG). EGCG is the most abundant catechin present in green tea and accounts for approximately 30–50% of the catechin content [9].

Obesity is caused by the chronic imbalance between energy intake and energy expenditure. Discrete areas of the hypothalamus act as regulatory centers for obesity where energy homeostasis is regulated through food intake (hunger and satiety), energy expenditure and the secretion of hormones that regulate the use and storage of substrates [10].

It is currently well established that neurotransmitters play a key role in regulation of appetite and food intake. Norepinephrine, serotonin (5-HT) and gamma aminobutyric acid (GABA) are among the most important mediators in the brain that have an important role in appetite and food intake control, as demonstrated by numerous investigators [11]. The modern pharmacological approach concentrates on drugs that interfere with monoamine neurotransmitter effects (e.g. sibutramine) and act as appetite suppressants, drugs which increase thermogenesis (e.g. β_3-adrenoreceptor agonists), decrease fat absorption by inhibiting the pancreatic lipase (orlistat) or drugs which act on brain peptides [10]. The sympathetic nervous system (SNS) plays a major role in the regulation of energy expenditure and lipolysis. Therefore, there are a number of approaches to increase SNS activity either directly (by β-adrenergic agonists) or indirectly (by norepinephrine releasers and reuptake inhibitors) [12].

The dearth of information on the possible role of GABA on the appetite and food intake control has tempted us to carry out the present work. The main goal of the present work was to investigate the relationship between the weight reducing effect of green tea and its effect on GABA and 5-HT neurotransmitters in obese mice. Furthermore, the antilipidemic activity of green tea was also evaluated. There are no reports especially with neurotransmitters after green tea intake in obese animals up to date.

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METHODS

A - Materials

Green tea was obtained from a Saudi Arabia local market in the form of granules and stored at room temperature. Monosodium glutamate (MSG) was brought from Fluka Chemie, Switzerland. MSG was dissolved in 0.9% saline and kept in dark glasses. It was used in volume of 0.1 ml/mouse [13]. GABA and 5-HT was obtained from the MP Biomedicals, Eschwege, Germany. Lipid profiles kits were obtained from Boehringer-Mannheim, Germany.

B - Experimental Animals

Adult male albino mice were obtained from the animal house of the Faculty of Medicine in Assiut University. The body weight range of both obese and non-obese mice was 20 to 35 gm and their age was 12 weeks. Mice were housed in boxes, 6 animals each, under standard conditions of temperature and humidity and a 12-hours light/dark cycle. The mice were fed a standard diet of commercial chow and tap water and left to acclimatize to environment for two weeks prior to inclusion in the experiment. All experiments were performed during the same time of day, between 8 a.m and 2 p.m to avoid variations due to diurnal rhythms. Manipulations throughout the test were carried out by the same person [14].

C - Procedures

1 - Induction of Obesity

The newborn mice were injected subcutaneously with MSG 0.1 ml/mouse (3 mg/gm body weight), per day for the first 5 postnatal days [13]. A control group of newborn mice was injected subcutaneously with physiological saline solution (0.1 ml /mouse) per day for the first 5 postnatal days. The animals were weaned at the age of 4 weeks and kept on a commercial chow and tap water. At the age of 8 weeks, female mice were identified and excluded from the study. A mouse is considered obese when the Lee index is more than 340.

$$\text{Lee index} = \sqrt[3]{\text{body weight(gm)}} \div \text{nasoanal length(cm)} \times 1000$$

At age of 12 weeks, each group was further subdivided into two different groups: group (a) that was not allowed to take green tea and group (b) that was allowed to take green tea (1% in diet for 2 weeks). Four groups resulted: control group without tea intake (Ia), control group with tea intake (Ib), obesity group without tea intake (IIa) and obesity group with tea intake (IIb).

2 - Assessment of the Antiobesity Effect

The weight (gm), nasoanal length (mm) and Lee index of the animals were measured on the first and last day of the experiment. The food intake (gm) per day was determined for each rat (total food at beginning of day – remaining food after 24 hours divided on 6) [15].

3 - Collection of Blood and Brain Samples

The animal was anaesthetized with i.p. sodium pentobarbital (20 mg/kg body weight). Blood was collected (5ml of blood for each) from the retro-orbital plexus, using heparinized capillary tube inserted in the medial canthus of the eye globe. To obtain serum, the blood was collected into a dry clean graduated centrifuge tube and left for clotting. Then it was set to centrifuge at 3000 r.p.m. for 15 minutes. Serum was sucked out into eppendorf tubes and stored frozen at -20°C until required.

Brains were rapidly removed and dissected. Then each brain was put in a test tube containing 5 mL of acidified butanol. The mixture was homogenized in a conical tube immersed in ice. The homogenate was centrifuged at 1000 r.p.m. for 5 min. then 2.5 mL of the supernatant was transferred to a test tube for determination of GABA and 5-HT by spectrophoto-fluorometric method.

4 - Lipid Profiles Measurements

Serum cholesterol, triglyceride, HDL cholesterol and LDL cholesterol were measured by enzymatic calorimetric method using kits [16,17].

5 - Determination of Brain GABA by Spectrophoto-Fluorometric Method

The method is based on the reaction of ninhydrin with GABA and glutamic acid in the octanolic milieu, a fluorescent product, presumably a copper-II–chelate–complex is formed [18].

6 - Determination of Brain 5-HT Spectrophoto-Fluorometric Method

The method is based on the the fact that 5-HT form highly fluorescent complexes with O-phthalaldehyde. The addition of L-cysteine to the reaction mixture improves the sensitivity of the method [19].

7 - Statistical Analysis

Statistical analysis was performed using the computer software program prism (Comshare's version of a decision support system = DSS) version 3.3. Data are expressed as means ± SEM. Statistical significance for data was determined using a one-way analysis of variance (ANOVA) with Bonferroni's Multiple Comparison Test to find inter-group significance. $P<0.05$ was considered statistically significant.

RESULTS

1 - Assessment of the Antiobesity Effect of Green Tea

The obtained data revealed that; the induction of obesity in newborn mice caused growth of mice which became obese adult animals. The Lee index was significantly increased in obesity mice as compared with control mice. Green tea caused a significant decrease in body weight, Lee index and food intake of obese mice group (IIb) compared with group (IIa). The nasoanal length was not significantly changed after intake of green tea in group (IIb) compared with group (IIa) as shown in table I.

2 - Lipid profiles levels in the studied groups

The obtained data showed a significant decreased level of serum cholesterol, triglycerides (TAG) and LDL and increased level of HDL in group (Ib) as compared to

Table I. Effect of green tea intake on body weight, nasoanal length, Lee index and food intake in obese mice (14 weeks/old).

Parameters / Groups	Body weight (gm)	Nasoanal length (mm)	Lee index	Food intake (gm)
Ia	30.7±1	90.8±1*	330±2.3*	4.3
Ib	28.2±1.5	89.0±0.5*	387±5.9*	3.9
IIa	30.3±1.05	80.7±3.41	414±9.9	7.1
IIb	18.2±0.79*	78.0±1.44	379±4.8*	4.8

Data represent mean ± S.E. of 6 cases.
* Significant difference at P<0.05 from group IIa.

Table II. Effect of green tea intake on lipid profiles in obese mice (14 weeks/old).

Parameters / Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Ia	228.8±19.04*	14.05±0.79*	48.4±6.5*	234.25±10.8*
Ib	154.2±15.04*	10.8±1.1*	41.9±2.5*	218.25±10.8*
IIa	262.8±22.4	22.62±2.09	70.9±10.5	292.82±14.4
IIb	235.3±17.0*	16.6±0.09*	42.1±8.8*	170±5.4*

Data represent mean ± S.E. of 6 cases.
* Significant difference at P<0.05 from group IIa.

(Ia) group. The obtained data showed a significant increased level of serum cholesterol, TAG and LDL and decreased level of HDL in group (IIa) as compared to groups (Ia) and (IIb), table II.

3 - Effect of Green Tea on Brain GABA and 5-HT in Obese Mice

There was no significant difference between GABA concentration in group (Ia) and group (IIa). Induction of obesity did not significantly change the levels of brain GABA in mice. However, obesity caused a highly significant decrease in brain 5-HT in mice in group (IIa) compared with group (Ia). Treatment with green tea (group IIb) caused non-significant changes in brain GABA and significant elevation of the brain levels 5-HT compared with group (IIa), table III.

Table III. Effect of green tea intake on GABA and 5-HT in obese mice (14 weeks/old).

Parameters / Groups	GABA concentration (µg/gm brain tissue)	5-HT concentration (ng/gm brain tissue)
Ia	800±41.2	502±30.22*
Ib	920±50.1	609±28.1
IIa	850±31.5	310±26.97
IIb	996±71.3	650±68.89*

Data represent mean ± S.E. of 6 cases.
* Significant difference at P< 0.05 from group IIa.

DISCUSSION

Various studies suggested different mechanisms responsible for MSG-induced obesity. Fernandez–Tresguerres [20] indicated that MSG injection caused degeneration and necrosis of several nuclei of the hypothalamus which may be responsible for obesity of animals after puberty.

In the present work, intake of green tea leads to significant decrease in the Lee index of obesity in obese mice. A number of mechanisms have been proposed to explain the antiobesity effects of green tea. One reported by several groups is related to modulation of dietary lipid digestion and absorption by green tea treatment. Catechins reduced gastric and intestinal fat digestion-mediated by direct inhibition of gastric and pancreatic lipases as well as a reduction of lipid emulsification process [21]. Also, green tea increases fecal lipid content in high fat-fed rats. Similar findings have been observed in high fat-fed mice [22].

Other possible mechanisms including; increased tissue thermogenesis, increased fat oxidation and decreased appetite, may also play roles in the antiobesity effects of green tea. It can inhibit catechol *O*-methyltransferase (COMT), an enzyme involved in the degradation of norepinephrine. As a consequence, once released, norepinephrine remains in the synaptic cleft longer and provides a prolonged stimulation of adrenergic receptors [23]. Caffeine also inhibits the phosphodiesterase, an "enzyme that induces degradation of intracellular cyclic AMP (cAMP)" [24]. After consumption of green tea, cAMP concentration rises and SNS activity will be increased and inactive hormone-sensitive lipase (HSL) will be activated, which promotes lipolysis that results in higher energy expenditure and fat oxidation [25].

Stimulation of thermogenesis and fat oxidation by the green tea was not accompanied by an increase in heart rate [26]. In this respect, the green tea extract is distinct from sympathomimetic drugs; whose use as antiobesity thermogenic agents is limited by their adverse cardiovascular effects, and hence, are particularly inappropriate for obese individuals with hypertension and other cardiovascular complications [12].

The mechanism of hyperlipidemia in obesity could either be an increase in splanchnic production of very low density lipoprotein or a defect in the removal of the very low density lipoproteins and/or chylomicron. The decreasing of HDL in obesity may be a result of an increased triglyceride load in the HDL particle that is acted upon by hepatic lipase, which hydrolyzes the triglycerides. The loss of the triglyceride results in a small HDL particle that is filtered by the kidney, resulting in a decrease in apolipoprotein A and HDL concentrations. The improving effect of green tea on obesity is mostly due to decreasing lipogenesis [27]. In addition, its polyphenols were found to modulate the hypercholesterolaemia in the experimental animals by upregulating the liver LDL receptor, inhibiting cholesterol synthesis and increasing fecal excretion of cholesterol, total fatty acids and bile acid [28]. Catechins were reducing triglyceride absorption, although the mechanisms of these reductions are not yet clear. A possible explanation is that tea polyphenols may modify dietary fat emulsification in the gastrointestinal tract [29]. In particular, green tea catechins might affect lipid metabolism by interfering with the micellar solubilization of cholesterol in the digestive tract, which in turn, decreases cholesterol absorption [30].

Brain levels of GABA are similar in brain of obese compared to non-obese animals. However, the present work clearly demonstrated that; obese mice show reduced 5-HT brain levels compared to non-obese animals. The antiobesity effect of the green tea was remarkably associated with a significant increase in the brain levels of 5-HT. This suggests that the antiobesity effect of the green tea might be mediated, in part, by increasing levels of 5-HT in certain brain regions.

The possible mechanism through which 5-HT exerts its body weight losing effect could be a result of an action on the hypothalamus that causes anorexia, weight loss and increased thermogenesis. This serotonergic activity is possibly mediated via an inhibition of hypothalamic neurons that express the powerful appetite-stimulating peptide NPY (neuropeptide Y) [31].

CONCLUSION

It is concluded that the green tea has antiobesity effect might be mediated by modulation of serum lipid profiles and brain 5-HT neurotransmitter.

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