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Influence of a green tea extract product on oxidative stress and muscle fatigue sensation in sedentary people subjected to physical exercise
Influența unui produs conținând extract de ceai verde asupra stresului oxidativ și senzației de oboseală musculară la sedentari supuși efortului fizic

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Abstract

Background. The effect of green tea extract (GTE) on oxidative stress and on the fatigue state is a constant concern of research.

Aims. The aim is to assess the influence of GTE on oxidative stress and muscle fatigue perception in moderate training and intense physical exercise stress in sedentary subjects, through the comparative investigation of two indicators, malondialdehyde (MDA) and muscle fatigue sensation (MFS).

Methods. The chosen subjects (n = 24) were selected based on the requirements of the study. Training consisted of running on an Excite + Run MD treadmill, at 30 watts, daily, for a week. Stress was represented by acute, short duration physical exercise, carried out on the same treadmill. The analyzed indicators were MDA and MFS. The chosen phytotherapeutic preparation contained GTE. Statistical evaluation was done using the Student test.

Results. Following the administration of GTE, MDA and MFS were reduced, more intensely pre- and post-exercise stress than during physical training, compared with subjects who did not receive any treatment.

Conclusions. 1) Under the influence of GTE, oxidative stress and muscle fatigue sensation were significantly reduced in the case of moderate physical training as well as of acute exercise stress in sedentary subjects. 2) It was demonstrated that the GTE effect was significantly higher on stress induced by intense physical exercise than over the physical training period. 3) There were differences between the GTE treated group and the untreated control group regarding malondialdehyde and muscle fatigue sensation evolution. 4) We suggest the utility of GTE in the modulation of oxidative stress and muscle fatigue sensation, both for moderate physical training and stress caused by intense exercise, in sedentary people.

Key words: green tea extract, physical exercise, physical stress, oxidative stress, malondialdehyde, muscle fatigue sensation.

Rezumat

Premize. Efectul pe care extractul de ceai verde (ECV) îl are asupra stresului oxidativ și a stării de oboseală este o preocupare constantă în cercetarea de specialitate.

Obiective. Sudiul urmărește evaluarea influenţei unui ECV asupra stresului oxidativ şi percepţiei oboselii musculare, în antrenamentul moderat și în stresul din efortul fizic intens, la subiecţi sedentari, prin investigarea comparativă a doi indicatori, malondialdehida (MDA) şi senzația de oboseală musculară (SOM).

Metode. Subiecţii aleşi (n=24) au fost selectaţi conform cerinţelor studiului. Antrenamentul a constat în alergarea pe o bandă rulantă Excite+ Run MD, la 30 watt, zilnic, timp de o săptămână. Stresul a fost reprezentat de un efort fizic acut şi de scurtă durată, realizat pe aceeași bandă rulantă. Indicatorii analizaţi au fost MDA şi SOM. Preparatul fitoterapic ales conține ECV. Evaluarea statistică s-a făcut pe baza testului Student.

Rezultate. În urma administrării ECV, MDA şi SOM au fost diminuate, mai intens pre- şi poststres de efort fizic, decât pe durata antrenamentului fizic, comparativ cu subiecţii care nu au urmat nici un tratament.

Concluzii. 1) Sub influenţa ECV, stresul oxidativ și senzația de oboseală musculară au fost semnificativ reduse, atât în cazul antrenamentului fizic moderat, cât și a stresului din efortul fizic acut, la subiecţi sedentari. 2) S-a dovedit că efectul ECV este semnificativ mai mare asupra stresului produs de efortul fizic acut, decât asupra perioadei de antrenament fizic. 3) Există diferenţe între lotul supus tratamentului cu ECV şi lotul netratat, martor, atât pentru evoluţia dinamică a MDA, cât şi pentru cea a senzației de oboseală musculară. 4) Sugerăm continuarea acestui studiu cu alte investigații, pentru a putea argumenta suplimentar utilitatea ECV în modularea stresului oxidativ și a senzației de oboseală musculară, atât pentru antrenamentul fizic moderat, cât și pentru stresul cauzat de efortul fizic acut, la persoane sedentare.

Cuvinte cheie: extract de ceai verde, efort fizic, stres fizic, stres oxidativ, malondialdehida, senzația de oboseală musculară.

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Introduction

Under physiological conditions, a wide range of antioxidant defenses protect against the adverse effects of free radical production in vivo (Halliwell, 1989). Tea drinking, by providing antioxidants, may become valuable in several oxidative stress conditions (Coimbra et al., 2006). Tea is grown in about 30 countries, but is consumed worldwide, although at greatly varying levels (Graham, 1992). Green tea (leaves of *Camellia sinensis*, Theaceae) is a popular beverage in East Asia and is also used as a herbal remedy in Europe and North America (Abolfathi et al., 2012).

The present article is a continuation of previous researches of the authors on the relationships between sport and stress (Jurcău, 2012a; Jurcău et al., 2012a; Jurcău et al., 2012b), sport and oxidative stress (Jurcău et al., 2011), and sport and polyphenols (Jurcău, 2012b).

Hypothesis

The influence of green tea polyphenols on the oxidative process has represented a growing concern in recent years. The use of polyphenols in exercise is also a point of interest in research. The influence of green tea on physical exertion during physical training compared to stress induced by acute, short duration exercise has less been explored.

Objectives

We propose the evaluation of the influence of green tea extract (GTE) on oxidative stress and muscle fatigue sensation both in physical training and stress induced by acute physical exercise, through the comparative investigation of two indicators, malondialdehyde and muscle fatigue sensation, in sedentary subjects treated and untreated with this product.

Material and methods

The study and measurements were carried out in May 2013, in the Medical Family Office 122 in Cluj-Napoca.

The study was approved by the Ethics Committee in accordance with the Good Practice Guide by approval. It complied with the conditions of the Helsinki Declaration, the protocol of Amsterdam, the Directive 86/609/EEC and Bioethics Commission regulations of the The Physicians College Cluj-Napoca.

a) *Groups*

The participation of all subjects in the study was voluntary. Subjects were tested on the Excite + Run MD Inclusive treadmill. The selection of subjects was performed based on the STAI X 1 questionnaire for the detection of the state of anxiety. All the selected participants were sedentary subjects. Persons with mental disorders, cortisonic therapies and toxic addictions - alcohol, tobacco, drugs, coffee were excluded from the trials.

Two groups were investigated: the control group (C), which received no therapy, and the experimental group (E), which was administered GTE. Both groups were subjected to the same type of physical exercise on the treadmill.

b) *Subjects*

The number of subjects in a group was 12 men, for both E and C. The mean age was 28.2 ± 3 for E and 31.4 ± 4 for C (Table I). The participants were asked not to consume alcohol, coffee, not to smoke and not to use any medication or antioxidant on the day before physical stress.

Table I

Number and type of subjects by groups.

Group	Experimental stress (E)	Control (C)
No. of subjects	12	12
Mean age	28.2 ± 3	31.4 ± 4
Gender	Men	Men

c) *Study design*

Training consisted of running on an Excite + Run MD treadmill, at 30 watts, daily, for a week. The running time was increased gradually and evenly, from the first day - 4 min, to the last day - 28 min. Stress represented by physical exercise was conducted at the end of the one week training period, on the eighth day. For stress caused by physical exercise, a model of intense, short duration physical exercise on the same treadmill was chosen. Before physical testing, the participants had a 4-min muscle heating session on the treadmill set at 30 watts. After an 8-min break, followed the testing session carried out on an Excite + Run MD treadmill. The exercise test was performed at a treadmill rate starting with a power of 30 watts, for four minutes, followed by a gradual increase of power up to 30 W more every four minutes, and continued until the onset of the feeling of fatigue.

The chosen phytotherapeutic preparation has a particular content of green tea extract (GTE), is called „Antioxidant" and is produced by the Fares Orăștie company (1). GTE was given the E, daily, for 3 weeks, in a dose of 3 capsules a day, at 8.00-14.00-20.00, prior to the period of physical training on the treadmill.

d) *The indicator determination program* was the same for C and E, beeing carried out as follows:

For *physical training*: time 1 = first time determination, basal (T1) - in the morning of the day before the initiation of the one week training - for both parameters; times 2-5 = immediately after completing training on each of the days 1, 3, 5, 7 (T2-T5) - only for the muscle fatigue sensation.

For *stress represented by acute physical exercise*: time 6 = sixth time determination (T6) - in the morning of the stress test, 30 min before the initiation of testing - just for malondialdehyde; time 7 = seventh time determination (T7) - 15 min after the exercise stress; and time 8 = eighth determination (T8) - 24 hours after the exercise stress - for both parameters.

e) *Explorations*

The examinations consisted of measuring malondialdehyde and the muscle fatigue sensation.

- *Oxidative exploration*

For the evaluation of lipid peroxidation, venous blood malondialdehyde (MDA) was measured at the Synevo laboratory of Cluj-Napoca (de Zwart et al., 1999, Janero, 1990). In order to determine MDA, the high performance liquid chromatography method (HPLC) with fluorescence detection was used (3, 4).

- *Evaluation of muscle fatigue sensation* (MFS)

It was performed using a 5-point Likert scale questionnaire. This was applied as follows: the participant placed a check mark in the specific box that correlated with their perceived mood level for fatigue. The numbers ranged from one (not feeling that particular mood) to five (highest level of mood). There was an even number of ”disagree” and ”agree” and a neutral answer in the middle: 1 – Strongly disagree, 2 – Disagree, 3 – Neutral, 4 – Agree, 5 – Strongly Agree (Likert, 1932, Norman, 2010).

f) *Statistical evaluation*

- The results obtained were analyzed using the SPSS 13.0. statistical package.
- For continuous data examination, Student’s t test was used.
- The differences were considered significant at $p<0.05$.

Results

Note that the *reference values* were those of C and the *reference times* were considered to be: 1) in the case of *physical training* - T_6 for MDA and T_5 for MFS; 2) for *stress represented by physical exercise* - T_7.

a) *Influence of moderate physical training on the evolution of the evaluated parameters, MDA and MFS*: 1) In the case of MDA, the influence was the following (Table II): for C, MDA significantly increased from T_1 to T_6 ($p<0.005$); for E, MDA increased from T_1 to T_6, but insignificantly. 2) In the case of MFS, the influence was the following (Table III): the fatigue sensation was perceived by C as significantly more intense at T_5 than at T_1 ($p<0.001$), T_2 ($p<0.002$), T_3 ($p<0.005$), T_4 ($p<0.01$); the fatigue sensation was perceived by E as insignificantly more intense at T_5 than at T_1, T_2, T_3 and T_4.

Table II

Influence of moderate physical training on the evolution of MDA

Evaluation time	C - MDA		E - MDA	
	Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation
T_1	1.821	±1.518	1.842	±1.745
T_6	3.118	±2.784	1.905	±1.713

Table III

Influence of moderate physical training on the evolution of MFS.

Evaluation time	C - MFS		E - MFS	
	Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation
T_1	1.021	±0.886	1.214	±1.167
T_2	2.121	±2.002	1.102	±1.083
T_3	3.108	±2.409	1.023	±0.921
T_4	4.022	±3.351	2.101	±2.094
T_5	4.152	±3.458	2.281	±2.145

b) *Influence of acute physical exercise on the evolution of the evaluated parameters, MDA and MFS* (Table IV): 1) In the case of MDA, the influence was the following: for C, MDA significantly increased from T_1 to T_7 ($p < 0.001$) and significantly decreased from T_7 to T_8 ($p < 0.002$); for E, MDA significantly increased from T_1 to T_7 ($p < 0.05$) and insignificantly decreased from T_7 to T_8. 2) In the case of MFS, the influence was the following: the fatigue sensation was perceived by C as significantly more intense at T_7 than T_1 ($p<0.002$) and it significantly decreased from T_7 to T_8 ($p<0.003$); the fatigue sensation for E significantly increased from T_1 to T_7 ($p<0.05$) and significantly decreased from T_7 to T_8 ($p<0.05$).

c) *Comparative evaluation of the parameters’ evolution*: 1) MDA (Fig. 1): at all assessment times, C values were higher than E values, significant differences being at T_6 ($p<0.02$) and T_7 ($p<0.004$). 2) MFS (Fig. 2): it was perceived by E as significantly more intense, both during moderate physical training ($p<0.05$ - T_2; $p<0.02$ - T_3; $p<0.005$ - T_4; $p<0.004$ - T_5) and after stress caused by acute physical exercise ($p<0.001$ - T_7; $p<0.03$ - T_8).

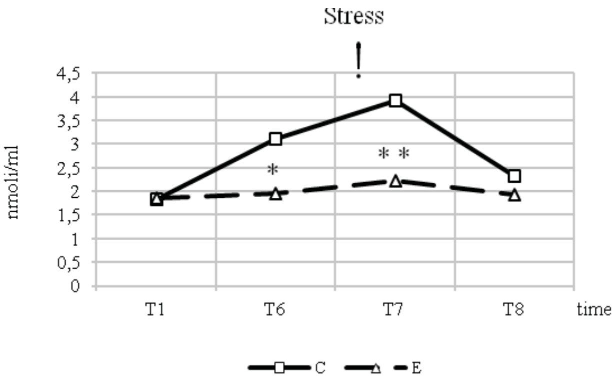

Fig. 1 – MDA changes in physical exercise.
*p<0.02 forT_6C-T_6E, **p<0.004 for T_7C-T_7E
”stress” = time of acute, short duration physical exercise.

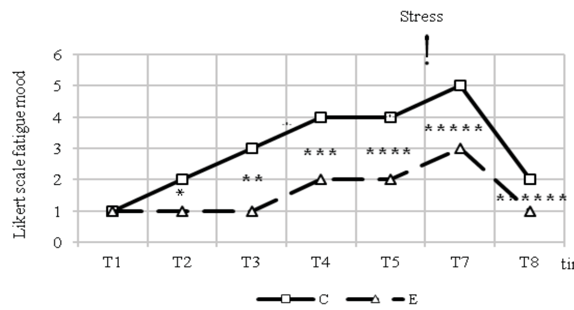

Fig. 2 – MFS changes in physical exercise.
*p<0.05 for T_2C-T_2E, **p<0.02 for T_3C-T_3E,
***p<0.005 for T_4C-T_4E, ****p<0.004 for T_5C-T_5E,
*****p<0.001 for T_7C-T_7E, ******p<0.03 for T_8C-T_8E,
”stress” = time of acute, short duration physical exercise.

Table IV

Influence of acute physical exercise on the evolution of MDA and MFS.

Evaluation time	C-MDA		E-MDA		C-MFS		E-MFS	
	Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation
T_1	1.821	±1.518	1.842	±1.745	1.021	±0.886	1.214	±1.167
T_7	3.903	±3.361	2.121	±1.912	5.019	±4.481	3.104	±2.544
T_8	2.301	±1.948	1.912	±1.269	2.031	±1.813	1.214	±1.011

d) *Analysis of the T_7/T_7 ratio for C and E groups*: the ratio was higher for C-MFS than for C-MDA and lower for E-MDA than for E-MFS, and the difference of the C-E ratio values was significantly higher (p<0.005) for MDA (1.03) than for MFS (0.5).

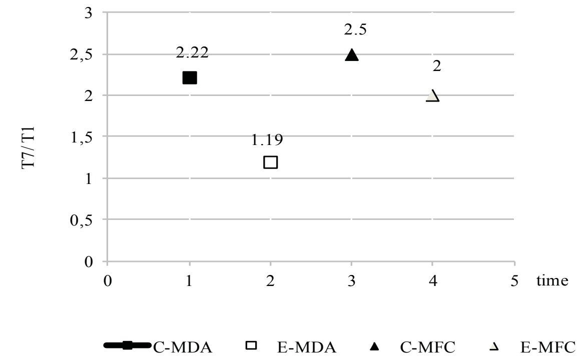

Fig. 3 – Analysis of T_7/T_7 ratio for C and E groups.

Discussion

Green tea polyphenols

Flavonoids are a large group of polyphenolic antioxidants that are present in fruits, vegetables and beverages such as tea and wine. It has been reported that tea polyphenols protect unsaturated phospholipids from oxidation by directly reacting with radicals *in vitro* (Chen et al., 2000). The most widely known health benefits of tea relate to polyphenols as the principal active ingredients in protection against oxidative damage (Hininger-Favier et al., 2009). Green tea is consumed primarily in China, Japan, and a few countries in North Africa and the Middle East and is prepared in such a way as to preclude the oxidation of green leaf polyphenols (Graham, 1992). So, an infusion prepared with the leaves of Camellia sinensis is particularly rich in flavonoids, which are strong antioxidants (Coimbra et al., 2006) and lead to protective effects against oxidative stress (Hininger-Favier et al., 2009).

The mechanism of action of green tea

A series of polyphenols known as catechins are abundant in green tea (Murase et al., 2006). Catechins have various physiological effects (Nagao et al., 2005). The ingestion of tea extract or catechins induces antioxidant activities (Yoshino et al., 1994). The main catechins in green tea are epicatechin; epicatechin gallate (ECG); epigallocatechin (EGC); epigallocatechin gallate (EGCG). EGCG is the most active polyphenol in green tea (Guo et al., 1996), having antioxidative effects (Zhao et al., 2001, Kuriyama et al., 2006). Normally, 10–20% of the catechins in green tea leaves are epigallocatechin and epigallocatechin gallate (Graham, 1992). A portion of ingested EGCG is absorbed and widely distributed throughout the body (Nakagawa, Miyazawa, 1997). Epigallocatechin gallate, a major component of green tea polyphenols, protects against the oxidation of fat-soluble antioxidants including lutein (Li et al., 2010, Aldini et al., 2003).

The antioxidant effect of green tea – chronological Pubmed evidence

The effect of GTE on oxidative stress has been a constant concern of research. Thus, it was proven that “tea extracts have antioxidant properties and that green tea extract is more potent” (Ojo et al., 2007) and that “superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) are involved in the intracellular defense against ROS” (Reddy, Labhasetwar, 2009). In 2010, it was found that the ”polyphenol-rich antioxidant supplement containing green tea extract has important antagonizing effects on oxidative stress” (Fenercioglu et al., 2010) and that ”limited clinical trials have shown green tea intervention to lower oxidative stress in smokers and healthy subjects” (Basu et al., 2010).

Relationship between green tea and exercise - chronological Pubmed evidence

In 2006, it was found that ”running times to exhaustion in mice fed 0.5% GTE were 30% higher than in Ex-cont mice. These results suggest that the endurance-improving effects of GTE were mediated, at least partly, by increased metabolic capacity and utilization of fatty acid as a source of energy in skeletal muscle during exercise” (Murase et al., 2006). Several years later, Jowko reported that ”in previously untrained men, dietary supplementation with green tea extract GTE (in combination with strength training) enhances the antioxidant defense system in plasma at rest and, in turn, may give protection against oxidative damage induced by both short-term muscular endurance test and long-term strength training” (Jówko et al., 2011). In the same year, it was found that ”habitual GTE ingestion, in combination with moderate-intense exercise, was beneficial to increase the proportion of whole-body fat utilization during exercise” (Ichinose et al., 2011).

Relationship between green tea and malondialdehyde - chronological Pubmed evidence

The MDA - GTE relationship has been analyzed in literature studies.

In 2009, it was found that ”EGCG, as a major component of green tea catechins, may lower mean levels of MDA” (Ramesh et al., 2009). Three years later, it was proven that “on administration of Green tea extract (Gtex), the MDA levels have decreased and the GSH levels have increased. This indicates that in the presence of Green tea extract there is an improvement in the oxidative stress” (Abolfathi et al., 2012). Three years later, it was demonstrated that “MDA levels were decreased significantly after treatment with Gtex” (Wu et al., 2012).

The results obtained by MDA testing under GTE action are consistent with the data provided by the latest studies related to MDA changes under the influence of GTE. The difference compared to the cited studies is the fact that, while they show the action of GTE on MDA in oxidative processes in general, our study proves the oxidative stress-decreasing effect of GTE in sedentary subjects undergoing physical training and stress induced by intense, short duration physical exercise.

Relationship between green tea and muscle fatigue sensation - chronological Pubmed evidence

The link between fatigue and polyphenols as well as that between fatigue and GTE has been evidenced by literature studies. Integration, a journal with 0.646 IF (2), mentioned in 1993 the following: ”fatigue is relieved when gazing upon the color green; examples are given. The spirit of the Way of Tea is described as based on the principles of harmony, respect, purity, and tranquility by Sen Rikyu" (Sen, 1993). 2005 brought the finding that ”Trichopus zeylanicus contains NADH, polyphenols and sulfhydryl compounds, which have the ability to scavenge reactive oxygen species suggesting that the antioxidant activity may be an important mechanism of action of Trichopus zeylanicus to combat fatigue" (Tharakan et al., 2005). In the same year, ”the pivotal role of oxidative stress in the pathophysiology of Chronic fatigue syndrome CFS" was evidenced, as well as the fact that ”green tea extract GTE and catechin could be used as potential agents in the management of CFS and warrant the inclusion of GTE and catechin in the treatment regimen of CFS patients" (Singal et al., 2005). Two years later, it was demonstrated that ”applephenon attenuates physical fatigue" (Ataka et al., 2007). In 2009, epigallocatechin-3-gallate, EGCG from GTE ”has been shown to improve endurance capacity in mice" (Dean et al., 2009). The year 2010 provided the information that ”chocolate may improve symptoms in subjects with chronic fatigue syndrome" (Sathyapalan et al., 2010).

The results obtained by muscle fatigue sensation testing under GTE action are consistent with the data provided by the latest studies related to changes of fatigue under the action of polyphenols. The difference compared to the cited studies is the fact that, while they show the action of polyphenols on fatigue in general, our study demonstrates the effect of GTE on the reduction of muscle fatigue sensation in sedentary subjects undergoing physical training and stress induced by intense, short duration physical exercise.

Conclusions

1. Under the influence of GTE, oxidative stress and muscle fatigue sensation were significantly reduced in the case of moderate physical training as well as intense exercise stress, in sedentary subjects.
2. It was proven that the effect of GTE was significantly higher on stress induced by intense physical exertion than over the physical training period.
3. There were differences between the GTE treated group and the untreated control group regarding both malondialdehyde and muscle fatigue sensation evolution.
4. We suggest the utility of GTE in the modulation of oxidative stress and muscle fatigue sensation, both for moderate physical training and stress caused by intense exercise, in sedentary people.

Conflicts of interest
Nothing to declare.

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