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Effect of a 2-month treatment with Klamin®, a Klamath algae extract, on the general well-being, antioxidant profile and oxidative status of postmenopausal women

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Abstract

Background and aim. Because of a growing demand for alternative treatments of the psychological and somatic/vasomotor symptoms related to menopausal transition, in this study we aimed to investigate the effect of a 2-month supplementation period with the Klamath algae extract (Klamin®, Nutratec Srl, Urbino, Italy) on the general and psychological well-being of a group of 21 menopausal women not treated with hormonal therapy, as well as on their oxidative stress status and level of antioxidants. Klamin is an extract naturally rich in powerful algal antioxidant molecules (AFA-phycocyanins) and concentrated with Klamath algae's natural neuromodulators (phenylethylamine as well as natural selective MAO-B inhibitors).

Conclusions. At the end of the Klamin supplementation period, plasma lipid peroxidation significantly decreased (as proven by a significant lowering of plasma MDA levels), while the overall antioxidant system improved thanks to the significant increase in the plasma levels of carotenoids, tocopherols and retinol. Furthermore, the average Green Scale score, which evaluates menopausal symptoms and thus by contrast the overall and psychological well-being of menopausal women, was significantly reduced. As it did not show the steroid-like effects on the hormonal parameters, Klamin could be proposed both as a valid natural remedy for women seeking an alternative to hormonal therapy, as well as as a complementary treatment for many climacteric symptoms.

Keywords: *Menopause, oxidative stress, Klamin®, Klamath algae extract, vitamins, malondialdehyde*

Introduction

Hormone replacement therapy (HRT) has been considered the most effective treatment for menopausal symptoms; however, as not all women might be well suited for HRT and/or because of the possible contraindications or side-effects of HRT, there is a growing demand for alternative treatments of the psychological and somatic/vasomotor symptoms related to menopausal transition. When HRT is not administered, the psychological component(s) during climateric period may be addressed only with specific neuroactive molecules, possibly of natural origin and acting physiologically; the somatic/vasomotor component may be more directly related to a combination of hypoestrogenic state, inflammation and oxidative stress, and probably may be prevented and/or treated by increasing the antioxidant and anti-inflammatory defenses of the ageing menopausal and postmenopausal women [1]. Indeed, because aging is accompanied by a progressive oxidation of the physiological sulphur pool, the role of the B vitamins in helping to maintain glutathione in its normal reduced state (GSH) has been emphasised; while other known antioxidant minerals and vitamins, such as vitamin E and carotenoids, may also play a role in this context [2].

Therefore, we focussed our attention on a Klamath microalgae extract, Klamin®, which besides

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maintaining some of the nutrients found abundantly in the whole algae (*Aphanizomenon flos-aquae* or AFA) (such as B vitamins, chlorophyll and β-carotene), effectively increases the concentration of a key anti-inflammatory, antioxidant and neuroprotective molecule naturally present in the algae, namely the specific AFA-phycocyanins, as well as the powerful endogenous neuromodulator phenylethylamine (PEA), whose activity is significantly supported by some physiological selective MAO-B inhibitors naturally found in this algae.

The phycocyanin (PC) typical of AFA algae has a peculiar structure relative to other microalgae [3], and this seems to explain its significantly higher antioxidant power, which in *in vitro* studies have shown to be up to 200 times more effective than ordinary PCs [4,5]. The presence in Klamin of a strongly antioxidant pool, whose activity is due in part to some antioxidant nutrients, and for the most part to the powerful AFA-PCs, justifies the expectation that Klamin could effectively improve the antioxidant status of the menopausal women, and through this, their general feeling of well-being.

In fact, PCs have been demonstrated to be powerful neuroprotectants, and showed a positive effect on psychological stress by protecting the hypothalamic–pituitary–adrenal (HPA) axis, directly involved in the management of stress [6]. Similar or even stronger effects can be expected of AFA-PCs, as preliminary studies found that AFA-PCs have a very high neuroprotective activity at very low dosages. [7].

Even more direct effects on the psychological well being might be related to Klamin's relevant content of PEA, a neuroactive endogenous molecule tightly linked to dopaminergic and serotonergic pathways so as to positively modulate mood and mental energy [8–10]. What is specific of this microalgae is the fact that it also contains some molecules (especially MAAs – mycosporine-like aminoacids, and a complex molecule unique to this algae called AFA-phytochrome) that act as powerful natural selective MAO-B inhibitors, a fact that is essential to overcome the problem of the rapid destruction by MAO-B enzymes of PEA, when the latter is ingested alone.

On such basis, we aimed to investigate the effect of a 2-month treatment with the Klamath algae extract on the general and psychological well-being of menopausal women as well as on their oxidative stress status and level of antioxidants.

Methods

Subjects

Twenty-one females, either volunteers or outpatients of the Department of Obstetrics and Gynecology, University of Modena (Modena, Italy) were included in the present study after giving their written informed consent. All participants fulfilled the following eligibility criteria: (1) age 47–54 years; (2) amenorrhoea of at least 12 months with climateric symptoms of at least 4 months; (3) no hormonal treatments; (4) absence of organic problems and uterus dysfunctions after gynecological control and transvaginal ultrasound; (5) no cardiovascular diseases or hypertension and diabetes. Routine haematological and hormonal laboratory tests were performed for each patient before the beginning of the study and after the supplementation period. The Green Scale, a 12-question interview covering both the psychological as well as the somatic/vasomotor components of menopause, was performed for each participant before and after the treatment interval.

Study design and blood sampling

Participants were treated for a period of 2 months with Klamin, a supplement constituted by a Klamath microalgae extract (Nutratec Srl, Urbino, Italy). All subjects received two 0.8 g tablets of Klamin a day per os.

Blood samples were collected from each patient in heparinised tubes both before (T0) and after 2 months (T1) treatment. Blood samples were immediately centrifuged at 2500 rpm for 10 min and plasma aliquots were stored at (80°C until assayed. On each sample, hormonal parameters were determined [luteinising and follicle stimulating hormones (LH and FSH, respectively), estradiol, progesterone, prolactin, cortisol, 17-hydroxyprogesterone (17-OHP), insulin and C-peptide] and the following biochemical parameters were monitored during the study: malondialdehyde (MDA) as marker of oxidative stress; liposoluble vitamins (α-, δ- and γ-tocopherol, retinol, lutein, lycopene, α- and β-carotene) as non-enzymatic antioxidants.

Hormonal determinations

All samples from each subject were assayed in duplicate in the same assay. Plasma LH and FSH concentrations were determined using a previously described immunofluorimetric assay (IFMA) [11]. The sensitivity of the assay expressed as the minimal detectable dose was 0.1 IU/mL. The cross reactivities with free α- and β- subunits of LH, FSH and TSH were less than 2% [11]. Intra-assay and inter-assay coefficients of variation were 5.1 and 7.3%, respectively.

Plasma E2, 17-OHP, cortisol, PRL and T were determined by radioimmunoassay (Radim, Pomezia, Rome, Italy) as previously described [12]. Based on two quality control samples the average within- and between-assay coefficients of variation were 4.1% and 9.5%, respectively.

Plasma insulin was determined using an immunoradiometric assay (Biosource Europa S.A.,

Nivelles, Belgium). Based on two quality control samples the average within- and between-assay coefficients of variation were 4.5% and 11.7%, respectively.

Plasma C-peptide concentrations were determined using a chemiluminescence assay (DBC immulite one, Los Angeles, CA). Based on two quality control samples the average within- and between-assay coefficients of variation were 4.5% and 8.2%, respectively.

MDA determination

MDA plasmatic levels were evaluated by reverse-phase HPLC as previously described [13]. Briefly, sample derivatisation was carried out by adding 50 μL 0.05% butylated hydroxytoluene solution, 400 μL 0.44 mol/L H_3PO_4 solution and 100 μL 42 mmol/L thiobarbituric acid (TBA) to 50 μL plasma. Tubes were vortexed and then heated for 1 h at 100°C. Following derivatisation, samples were placed on ice for 5 min and 250 μL butanol were added for extraction of the MDA–TBA complex. Tubes were vortexed and then centrifuged at 10,000*g* to separate the two phases. 20 μL were removed from the butanol layer and placed into HPLC injector for analysis without evaporation. The assay was performed using a Alltima C18 column (4.6 × 250 mm^2, 5 μm, from Alltech, Milan, Italy) equipped with a guard column Alltima C18 (4.6 × 7.5 mm^2, 5 μm). The eluent phase was methanol:buffer (40:60, v/v), buffer consisting of 50 mmol/L KH_2PO_4 pH 6.8. The flow rate was 1 mL/min. UV detection was carried out at 532 nm, the fluorescence detector was set at an excitation wavelength of 515 nm and emission wavelength of 553 nm. All the organic solvents were pure HPLC-grade from VWR International, Milan, Italy. The HPLC instrumentation was from Jasco Corporation, Tokyo, Japan.

Determination of tocopherols, retinol and carotenoids

Plasma levels of liposoluble antioxidants were measured by reversed-phase HPLC following deproteinisation with ethanol and extraction with hexane [14]. After centrifugation, the organic layer was removed and evaporated; the residue was dissolved in 400 μL of a mixture of acetonitrile:tetrahydrofuran:methanol (68:22:7 v/v) and 100 μL were injected into HPLC system. The assay was performed using a Alltima C18 column (4.6 × 250 mm^2, 5 μm, from Alltech, Milan, Italy) equipped with a guard column Alltima C18 (4.6 × 7.5 mm^2, 5 μm). The eluent phase was acetonitrile:tetrahydrofuran:methanol (68:22:7 v/v) adjusted to 100 v/v with 1% ammonium acetate; the flow rate was 1.5 mL/min. UV and fluorescent detectors were programmed according to absorption, excitation and emission wavelengths of each molecule. All the organic solvents were pure HPLC-grade from VWR International, Milan, Italy. The HPLC instrumentation was from Jasco Corporation, Tokyo, Japan.

Statistics and data processing

Results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using *t*-test for paired data. Probability values of < 0.05 were accepted. Statistics and graphs were obtained using the software Microcal™ Origin 6.0 (Microcal Software, Northampton, USA).

Results

Lipid oxidation and antioxidant profile

Plasmatic levels of MDA, carotenoids and liposoluble vitamins were evaluated for each patient both before (T0) and after (T1) Klamin supplementation. As reported in Table I, a significant decrement ($p < 0.01$) of MDA as index of lipid peroxidation was found at T1 with respect to T0 (−38%). Concomitantly, a significant increment of lutein (+43%), lycopene (+38%), α-carotene (+27%), β-carotene (+29%), retinol (+18%), γ-tocopherol (+28%) and α-tocopherol (+32%) was evidenced at the end of the study when compared with the basal evaluation. Plasma levels of δ-tocopherol were also increased (+33%), though the data did not reach statistical significance (Table I). Data linear regression analysis revealed a negative correlation between MDA, carotenoid and vitamin levels; in particular, MDA negatively correlated with lutein ($R = -0.670$, $p < 0.0001$), lycopene ($R = -0.755$, $p < 0.0001$), α-carotene ($R = -0.440$, $p = 0.0149$), β-carotene ($R = -0.643$, $p < 0.0001$) and α-tocopherol ($R = -0.570$, $p = 0.0003$).

Menopausal symptoms

Menopausal symptoms and psychological well-being were evaluated before and after treatment by the use

Table I. Plasmatic levels of MDA, carotenoids and liposoluble vitamins before (T0) and after (T1) treatment with Klamin.

Parameter (μmol/L)	T0	T1	T1 *vs.* T0 (*p*-value)
MDA	3.36 ± 2.26	2.09 ± 1.13	0.0069
Lutein	0.30 ± 0.14	0.43 ± 0.17	0.0025
Lycopene	0.37 ± 0.27	0.51 ± 0.28	0.0011
α-Carotene	0.15 ± 0.11	0.19 ± 0.08	0.0472
β-Carotene	0.41 ± 0.26	0.53 ± 0.28	0.0167
Retinol	2.89 ± 0.72	3.41 ± 0.64	0.04261
δ-Tocopherol	0.09 ± 0.05	0.12 ± 0.08	0.1829
γ-Tocopherol	0.81 ± 0.45	1.04 ± 0.58	0.0444
α-Tocopherol	15.8 ± 7.3	20.8 ± 8.8	0.0023

of a visual analogic scale (Green Scale). A highly significant reduction of the scale score was found at the end of the supplementation period (T1) with respect to the basal value (T0) (21.8 ± 2.7 *vs.* 28.1 ± 2.7, $p = 0.0008$) (Figure 1). Interestingly (Table II), although the results in the psychological well-being are likely to be referable to the synergy of PEA and MAO-B inhibitors, the plasmatic levels of MDA were positively correlated with the Green scale score ($p < 0.05$); on the contrary, a significant negative correlation was observed between the scale score and some vitamin levels, namely lutein, lycopene, α-carotene, β-carotene and α-tocopherol. Such data suggest a clear connection of the improvements in well-being with the antioxidant activity.

Hormone plasma levels

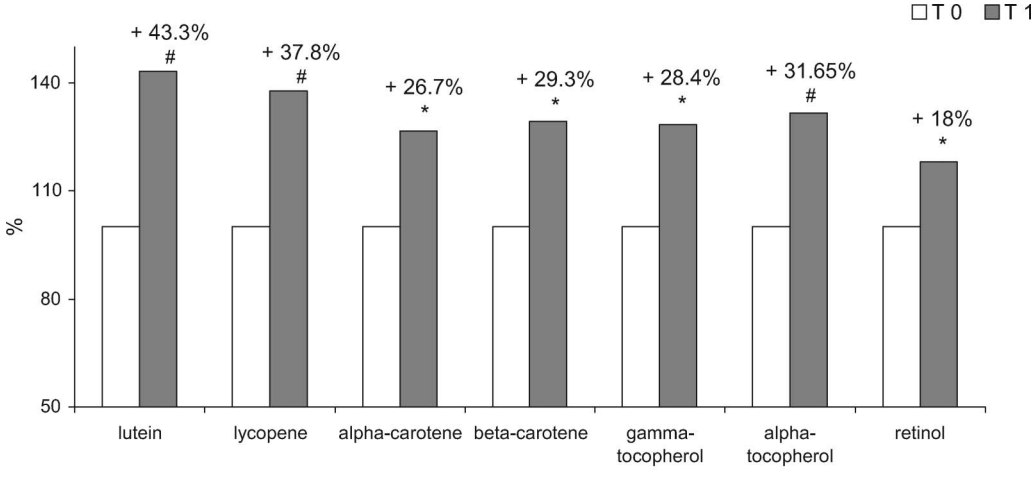
No significant changes were observed at the end of the supplementation period (T1) with respect to the basal evaluation (T0) for the hormonal parameters studied (Table III).

Discussion

The present study reported that the supplementation with the Klamath algae extract determined a significant change of several biochemical markers of the oxidative processes as well as a significant change in the subjective well-being of the patients. In fact, symptoms and processes typical of menopausal and postmenopausal women (such as hot flashes, acceleration of arteriosclerosis and immune dysfunctions) are linked not only to estrogen loss but also to high levels of oxidative stress, arising through the increased production of reactive species and/or a deficiency of antioxidant defences [1,2]. Previous studies demonstrated that menopause is associated with a significant decrease in plasmatic antioxidants such as ascorbic acid, α-tocopherol and total thiols, and a significant increase in lipid peroxides and lipoprotein oxidation [15,16]. Some synthetic steroids, such as tibolone, are effective in inhibiting oxidative stress related to menopause by reducing MDA and increasing α-tocopherol plasma levels [16]; however, despite these favourable effects, not all women are well suited for HRT administration, while others might desire less iatrogenic treatments to alleviate menopausal symptoms and psychological discomfort.

Our data demonstrated for the first time that supplementation with Klamin, a Klamath microalgae extract, successfully reduced the oxidative condition in postmenopausal women not treated with HRT. In fact, plasma lipid peroxidation (expressed as MDA concentration) significantly decreased after the 2-month period of supplementation, while the antioxidant defence system improved since a significant increase of carotenoid, tocopherol and retinol plasma levels. Accordingly, a significant inverse correlation between lipid peroxidation and antioxidant concentrations was evidenced throughout the study. These results can be explained by Klamin's rich content of natural antioxidant molecules, in particular AFA-phycocyanins, which generate a twofold type of effect. On the one hand, they perform their own independently strong radical scavenging activity, thus diminishing free radical and MDA accumulation; on the other hand, by thus reducing the need for increased antioxidant consumption in menopausal women characterised by higher levels of oxidative stress, they help preserving, and thus increasing, endogenous vitamin concentrations.

In any case, both mechanisms can be thought of as responsible for the significant reduction of plasmatic MDA, a by-product of lipoprotein oxidation which is significantly increased during menopause. In fact, it



significantly different when compared with T0 (* p<0.05, # p< 0.01).

Figure 1. Statistically significant increase in plasma carotenoids and tocopherols after 2 months of Klamin supplementation.

Table II. Correlation index (R) and p values between Green Scale score and plasmatic levels of MDA, carotenoids and liposoluble vitamins.

	Parameter	R	p-value
Green Scale score *versus*	MDA	0.373	0.02511
	Lutein	−0.391	0.0185
	Lycopene	−0.409	0.0119
	α-Carotene	−0.350	0.0367
	β-Carotene	−0.454	0.0054
	Retinol	−0.093	0.5827
	δ-Tocopherol	−0.005	0.9793
	γ-Tocopherol	−0.170	0.3287
	α-Tocopherol	−0.354	0.0367

Table III. Hormone plasma levels before (T0) and after (T1) treatment with Klamin.

	T0	T1
LH (U/L)	22.6 ± 12.8	20.9 ± 14.7
FSH (U/L)	42.9 ± 21.6	39.8 ± 27.7
Estradiol (pg/mL)	13.9 ± 4.3	13.3 ± 4.9
Progesterone (ng/mL)	0.4 ± 0.3	0.4 ± 0.2
Prolactin (ng/mL)	9.4 ± 8.7	8.9 ± 8.2
Cortisol (μg/dl)	13.5 ± 3.3	14.9 ± 4.5
17-OHP (ng/mL)	0.6 ± 0.4	0.6 ± 0.4
Insulin (mU/L)	7.3 ± 4.9	7.6 ± 5.6
C-peptide (ng/mL)	1.5 ± 0.6	1.5 ± 0.6

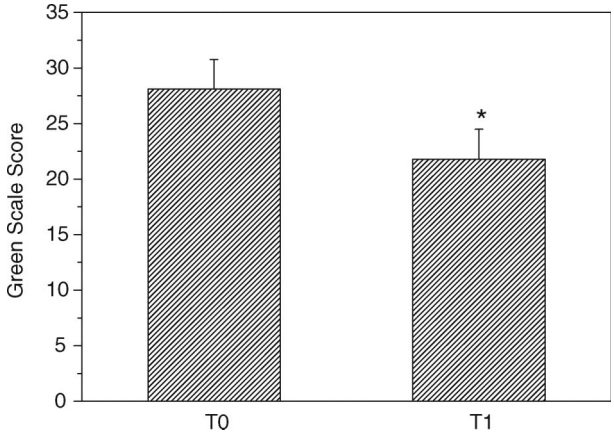


Figure 2. Green Scale score reduced significantly after Klamin supplementation (*$p < 0.01$).

is well known that lipid and lipoprotein metabolisms are markedly impaired in postmenopausal women, with total cholesterol and lipoprotein values significantly increased [17]. At the same time, lipoprotein oxidation, due to excessive free oxygen radical production, plays a crucial role in the pathogenesis of atherosclerosis, and it is this very combination of excess oxidation and hypo-estrogenism that exposes menopausal women to a greater cardiovascular risk [18]. In this context, the ability of Klamin to lower MDA concentrations in just 2 months of treatment may be of great clinical relevance, cardiovascular prevention being one of the main targets in relation to menopausal women, especially considering that cardiovascular diseases greatly increase in the first years after the onset of menopause.

Further favourable effects on the health and quality of life of the participants were also found after Klamin supplementation, particularly in terms of a significant reduction of the visual analogic Green Scale score, which evaluates menopausal symptoms and overall psychological well-being. Such improvement may be directly related on the one hand to the antioxidant effect, because the scale score was positively correlated with MDA plasmatic values and inversely correlated with plasma vitamin levels; and on the other hand, in relation to the psychological well-being, to the significant Klamin's content both PEA and synergic molecules such as the neuroprotectant AFA-PCs and the natural MAO-B inhibitors (MAAs and 'AFA-phytochrome'). Being PEA deeply involved in the amine modulation inside the nervous system [8–10], the increase of PEA levels might explain the higher feeling of psychological well-being in the participants. Indeed, PEA physiologically decreases during menopause, thus altering both dopaminergic and serotoninergic pathways, which are so essential to many aspects of health and well-being, and significantly affected by Klamin administration.

Finally, Klamin did not induce any hormonal change, as LH, FSH and estradiol plasma levels remained unchanged, thus confirming its lack of steroid action. However, further studies are needed to better evaluate whether the alga Klamath extract might affect some of the neuroendocrine pathways impaired by ageing and during menopausal transition.

In conclusion, our study supports the efficacy of Klamin as a valid non-steroid natural extract capable of reducing oxidative processes and improving subjective feeling of well-being in menopausal women. As such, it could be proposed both as a complementary treatment to HRT, and as a potential response to those women searching for an alternative to HRT as a way to overcome climateric symptoms.

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