In vitro scavenging capacity of Organic Silicium (oral anti-aging) against reactive oxygen and nitrogen species

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Reactive oxygen and nitrogen species can cause oxidative damage to biomolecules, eventually leading to many chronic diseases, aging, and other degenerative diseases in humans. In this context, Exsynutriment® is an oral anti-aging supplement based on organic silicium, which, according to the company, promotes the renovation of the conjunctive tissue, restructuring the skin sustainment fibers. The aim of this study was therefore to evaluate the redox properties of Exsynutriment®. It was evaluated *in vitro* redox tests as TRAP/TAR (non-enzymatic antioxidant potential), TBARS (lipoperoxidation), hydroxyl (OH^-) and nitric oxide ($^{\cdot}NO^{\cdot}$) scavenging and SOD-like and CAT-like activity. The TRAP/TAR was determined using a method based on the chemiluminescence induced by the peroxyl radical generation initiated by AAPH. The Exsynutriment ® solution in the concentration 1 mg/mL showed a significant reduction in the effectiveness of CL induced by AAPH. Thiobarbituric acid reactive species (TBARS) assay was employed to measure the antioxidant capacity of Exsynutriment® in lipids incubated with AAPH and 2-deoxyribose degradation assay was employed to assesses the hydroxyl scavenging activity. Both the assays all doses tested had no effect against lipoperoxidation and did not reduce deoxyribose oxidative damage. However, nitric oxide radical generated from sodium nitroprusside was inhibited by all concentrations tested. The capacity of Exsynutriment® to interact with and/or scavenge/quench H_2O_2 and superoxide radicals *in vitro* was evaluated, respectively, by the catalase-like and the superoxide dismutase-like reaction assays. Our results did not show a significant variation to CAT and SOD-like activity. Results of the study showed no significant variation in such trials. Through the results we conclude that the organic silicium used as oral anti-aging showed antioxidant activity in vitro tests TRAP / TAR and nitric oxide (NO) scavenging, suggesting that future studies may elucidate chemical mechanisms.

Keywords: antioxidant, organic silicium, oral anti-aging.

Avaliação *in vitro* da capacidade *scavenging* do silício orgânico (*anti-aging oral*) contra espécies reativas de oxigênio e nitrogênio

Espécies reativas de oxigênio e nitrogênio podem causar dano oxidativo a biomoléculas, eventualmente conduzir a varias doenças crônicas, envelhecimento, e outras doenças degenerativas em humanos. Nesse contexto, Exsynutriment® é um suplemento oral anti-idade à base de silício orgânico que, segundo a empresa produtora, promove a renovação do tecido conjuntivo, reestruturando as fibras de sustentação da pele. O objetivo desse estudo foi, portanto, avaliar a atividade redox do Exsynutriment®. Utilizou-se testes redox *in vitro* como TRAP/TAR (potencial antioxidante não enzimático), TBARS (lipoperoxidação), hidroxil (OH^-) e óxido nítrico ($NO^{\cdot}$) *scavenging*, atividade SOD-*like* e CAT-*like*. O TRAP/TAR foi determinado usando-se o método baseado na quimiluminescência (CL) induzida por geração de radicais peroxil pelo AAPH. A solução de Exsynutriment® na concentração 1 mg/mL apresentou efetividade significativa na redução da CL induzida pelo AAPH. O método de espécies

reativas ao ácido tiobarbitúrico (TBARS) foi utilizado para mensurar a capacidade antioxidante do Exsynutriment® em lipídios incubados com AAPH e a atividade *scavenging* do radical hidroxil foi testada pela degradação da 2-deoxirribose. Em ambos os ensaios, nenhuma concentração utilizada teve efeito contra a lipoperoxidação e não reduziu o dano causado pela desoxirribose. No entanto, o radical óxido nítrico gerado a partir de nitroprussiato de sódio foi inibido por todas as concentrações testadas. A capacidade do Exsynutriment® em interagir com e/ou sequestrar/quelar H_2O_2 e radicais superóxido *in vitro* foi testada, respectivamente, pelos métodos CAT e SOD-*like*. Os resultados do estudo não mostraram nenhuma variação significativa para tais ensaios. Através dos resultados conclui-se que o silício orgânico usado como anti-aging oral apresentou atividade antioxidante nos testes *in vitro* TRAP/TAR e óxido nítrico (NO) *scavenging*, sugerindo que trabalhos futuros poderão elucidar tais mecanismos químicos.
Palavras-chave**:** *antioxidante, silício orgânico, antienvelhecimento oral*.

1. INTRODUCTION

The actions of antioxidants have been attributed to their ability to scavenge free radicals, thereby reducing oxidative damage of cellular biomolecules such as lipids, proteins, and DNA [1,2].

Oxidative damage is related to the continuous production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the organism. These substances can attack membrane lipids by reducing double bonds in a process called peroxidation, which can lead to membrane destabilization and damage to the cell metabolism [3, 4].

Few drugs currently are marketed for the treatment and prevention of skin aging, whereas many cosmeceuticals and herbal remedies are touted in the lay press [5]. A Brazilian distributor of international supplying brands is introducing on the market Exsynutriment® beauty capsules, an anti-age oral supplement based on organic silicium, whose stability, according to the company, promotes the renovation of the conjunctive tissue, restructuring the skin sustainment fibers (collagen and elastin). It contains a rejuvenator and photoprotector combination: Exsynutriment® 150 mg, Vitamin C 300 mg, β-carotene 25 mg and excipient q.s.p. However, there are no published studies on their antioxidant activity. Thus, the aim of the present study was to evaluate the antioxidant activity of this product.

2. MATERIAL AND METHODS

2.1 Total antioxidant potential (TRAP) and total antioxidant reactivity (TAR)

TRAP was determined by measuring the chemiluminescence (CL) intensity of luminol induced by 2,2′-azobis(2-amidinopropan) dihydrochloride (AAPH) according to the method of Lissi and coworkers [6]. The background CL was measured by adding 4 mL of AAPH (10 mM) dissolved in glycine buffer (0.1 M, pH 8.6) to a glass scintillation vial. Then 10 μL of luminol (4 mM) was added to each vial and the CL was measured until constant light intensity. After this stabilization time, 10 μL of sample was added, and the CL was measured in a liquid scintillator counter working in the out of coincidence mode. The last count prior to the addition of samples was considered as 100%. The count time was 10 s, and the CL emission was monitored for 3000 s after the addition of samples. Graphs were obtained by plotting percentage of counts per minute (% cpm) versus time (s). The AUC was calculated using GraphPad Prism software.

2.2 TBARS (thiobarbituric acid reactive species)

TBARS assay was employed to quantify lipid peroxidation [7] and an adapted TBARS method was used to measure the antioxidant capacity of Exsynutriment® using egg yolk homogenate as rich lipid substrate. Briefly, egg yolk was homogenized (1% w/v) in phosphate buffer (pH 7.4), 1 mL of homogenate was sonicated and then homogenized with 0.1 mL of

Exsynutriment® at different concentrations or 0.1 mL of Trolox solution (water-soluble vitamin E analogue). Lipid peroxidation was induced by addition of 0.1 mL of AAPH solution (0.12 M). Samples were centrifuged with trichloroacetic acid at 1200 g for 10 min. An aliquot of 0.5 mL from supernatant was mixed with 0.5 mL TBA and heated at 95 ºC for 30 min. After cooling, samples absorbance was measured using a spectrophotometer at 532 nm. The results were expressed as percentage of TBARS formed by AAPH alone (induced control).

2.3 Hydroxyl radical ($^{\cdot}OH^{-}$) scavenging assay

Hydroxyl radicals were generated by a Fenton system ($FeSO_2$-H_2O_2). The method for determining the scavenging on hydroxyl radicals was performed according to a previously described procedure [8].

2.4 Nitric oxide ($NO^{\cdot}$) scavenging assay

Nitric oxide was generated from sodium nitroprusside (SNP) and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete with oxygen to reduce production of nitric oxide. Sodium nitroprusside (SNP, 5 mM) in phosphate buffer saline (PBS) was mixed with 3.0 mL of different concentrations (1, 10, 100 ηg/mL, 1, 10, 100 μg/mL, and 1 mg/mL) of the Exsynutriment and incubated at 25 ºC for 60 min. The samples were added to Griess reagent (1% sulphanilamide, 2% H_3PO4, and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was measured at 546 nm [9].

2.5 Superoxide and hydrogen peroxide-scavenging activities (SOD/CAT-like activities)

The ability of Exsynutriment to mimic the activity of catalase (CAT-*like* activity) was measured. CAT-*like* activity was assayed by measuring the rate of decrease in H_2O_2 absorbance at 240 nm [10]. Superoxide dismutase-like (SOD-*like*) activity was assayed by measuring spectrophotometrically by the inhibition of adrenaline auto-oxidation at 480 nm, as previously described [11].

2.6 Statistical analysis

Data are expressed as mean ± S.E.M. The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test. All tests were performed in triplicate. Data analyses were performed using the GraphPad Prism 5.0 software. Differences were considered significant if $p < 0.05$.

3. RESULTS AND DISCUSSION

The field of antioxidants and free radicals is often perceived as focusing around the use of antioxidant supplements to prevent human disease. In fact, antioxidants/free radicals permeate the whole of life, creating the field of redox biology [12].

3.1 Total antioxidant potential (TRAP) and total antioxidant reactivity (TAR)

The TRAP and TAR methods are widely employed to estimate the general antioxidant capacity of samples *in vitro* [2,17]. These methods were determined using a technique based on the quenching of luminol enhanced chemiluminescence derived from the thermolysis of a water-

soluble azo compound, AAPH, used as a reliable and quantifiable source of alkyl peroxyl radicals. At the TRAP and TAR assays, Exsynutriment® at concentration of 1 mg.mL^{-1} showed significant anti-oxidant effects (Figure 1A and 1B).

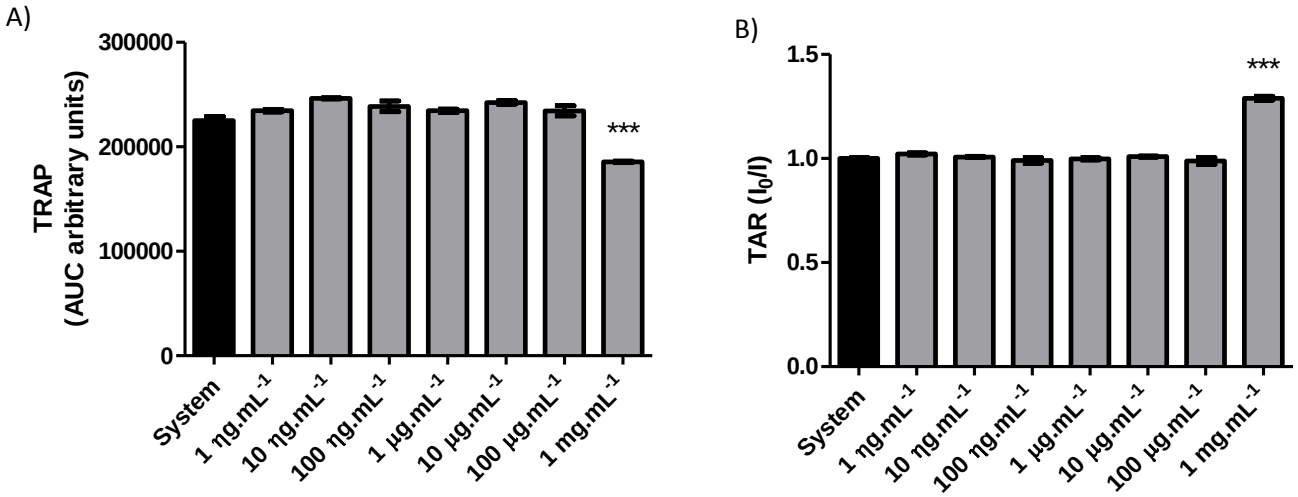

*Figure 1. A- Total Radical-Trapping Antioxidant Parameter (TRAP) at different concentrations from Exsynutriment®. B- Total antioxidant reactivity (TAR) was calculated as the ratio of light intensity in absence of samples expressed as percent of inhibition (I_0/I) at different concentrations from Exsynutriment®. Bars represent mean ± SEM. *** $p < 0.001$ different from system (1-way ANOVA followed by Tukey's multiple comparison post test).*

3.2 TBARS (thiobarbituric acid reactive species)

Lipid peroxidation (LPO) has been defined as the biological damage caused by free radicals which are formed under oxidative stress [13]. The ability of the Exsynutriment® to inhibit microsomal lipid peroxidation induced by Fe^{3+}/ascorbate and the water soluble AAPH were also evaluated by measuring TBARS. All doses of Exsynutriment® tested had no effect lipid peroxidation induced by the system (Figure 2).

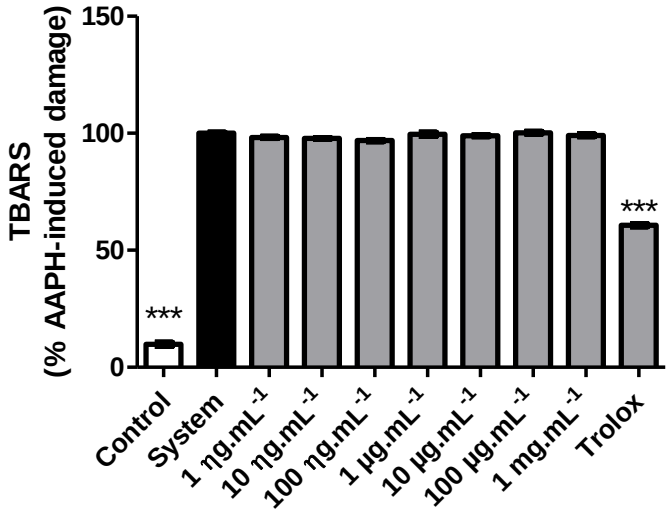

*Figure 2. Thiobarbituric acid-reactive substances (TBARS) in vitro. A lipid-rich system was incubated with a free radical source (AAPH) and the effect of different concentrations of Exsynutriment® on the lipoperoxidation was measured by quantifying TBARS. Control is incubation medium without AAPH; other groups contained AAPH alone or in the presence of different concentrations of Exsynutriment® or its vehicle alone. Bars represent mean ± SEM. *** $p < 0.001$ different from system (1-way ANOVA followed by Tukey's multiple comparison post test).*

3.3 Hydroxyl radical ($^{\cdot}OH^{-}$) scavenging assay

The ability of the Exsynutriment® to scavenge hydroxyl radicals was assessed using the deoxyribose (DR) assay [14,15]. Hydroxyl radicals were generated by a Fenton system (ascorbic acid/$FeCl_3$-EDTA/H_2O_2). When exposed to hydroxyl radicals, the sugar deoxyribose (DR) is degraded to malonaldehyde, which generates a pink chromogen on heating with thiobarbituric acid (TBA), at low pH. The DR method for determining the scavenging effect of the potential scavenger on hydroxyl radicals was performed according to a described procedure [8].

Hydroxyl radicals have been formed in biological systems and have been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [16]. This radical has the capacity to join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity [17]. Hydroxyl radical scavenging capacity of a sample is directly related to its antioxidant activity [9].

All the concentrations did not prevented $^{\cdot}HO^{-}$ induced degradation of deoxyribose into malonaldehyde (Figure 3A).

3.4 Nitric oxide ($NO^{\cdot}$) scavenging assay

Nitric oxide plays an important role in various types of inflammatory processes. Figure 3B shows the results obtained in the $NO^{\cdot}$ scavenging assay. All the assayed concentrations prevented the $NO^{\cdot}$ formation.

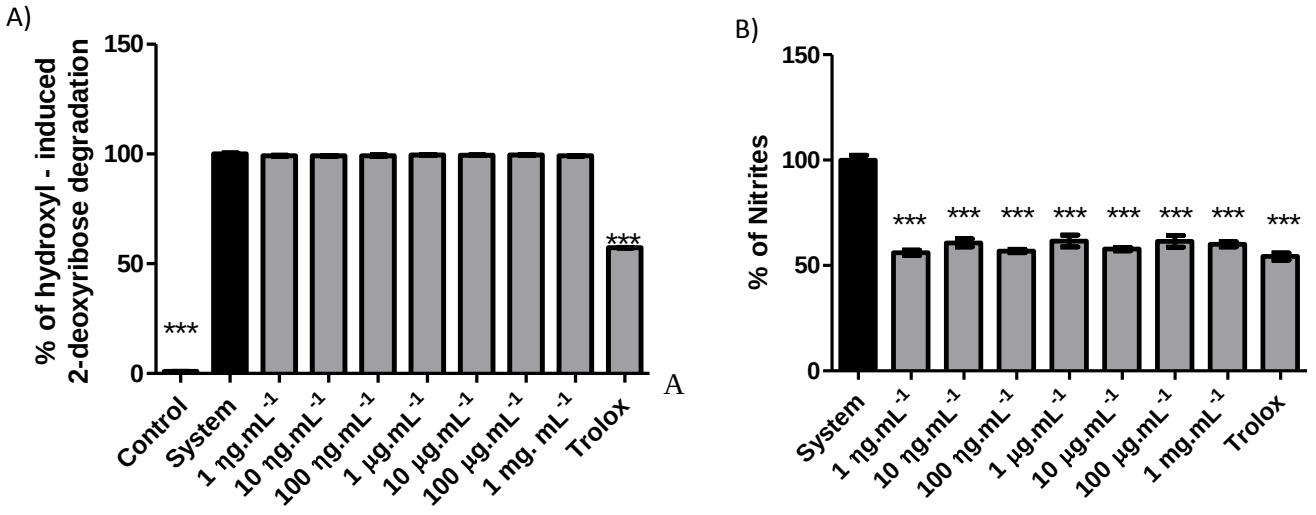

*Figure 3. A- Hydroxyl radical–scavenging activity of Exsynutriment®. B- Nitric oxide (NO) radical scavenging activity of Exsynutriment®. Values represent mean± standard error; experiments were performed in triplicate. Bars represent mean ± SEM. *** p < 0.001 different from system (1-way ANOVA followed by Tukey's multiple comparison post test).*

3.5 Superoxide and hydrogen peroxide-scavenging activities (SOD/CAT-*like* activities)

The capacity of Exsynutriment® to interact with and/or scavenge/quench H_2O_2 and superoxide radicals and *in vitro* was evaluated, respectively, by the catalase-*like* and the superoxide dismutase-like reaction assays. Figure 4A and Figure 4B shows the results obtained in the SOD and CAT–*like* activities, respectively. No activity was observed for both assays.

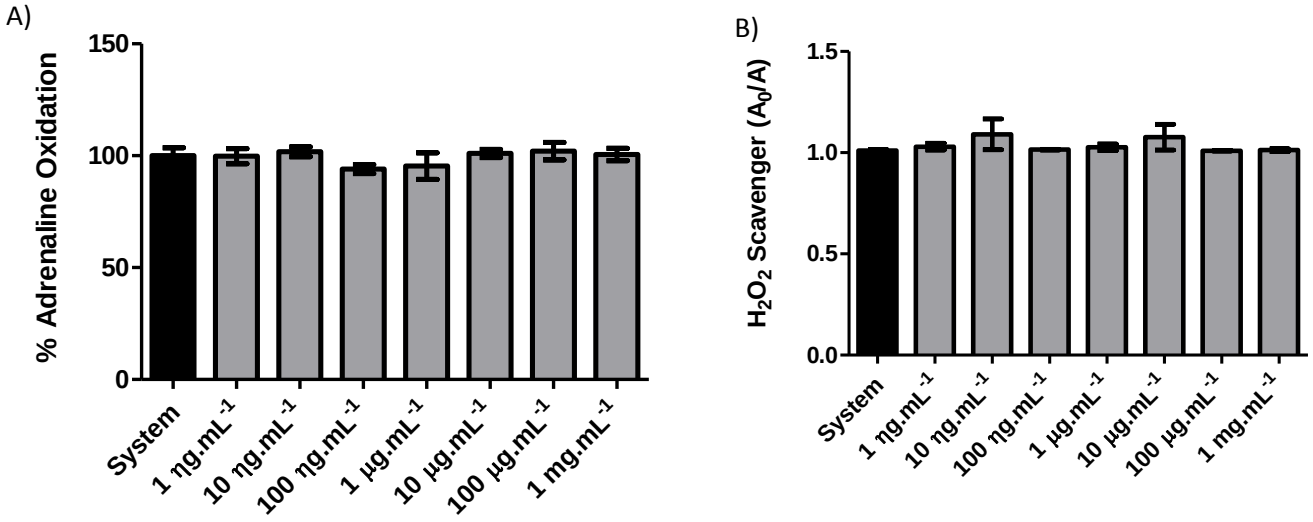

*Figure 4. A- SOD-like activity was determined by following formation of adrenochrome in a SOD reaction buffer containing native purified catalase and adrenaline (O_2^- generator group). B- CAT-like activity was measured in a catalase reaction buffer with H_2O_2. Bars represent mean ± SEM. *** p < 0.001 different from system (1-way ANOVA followed by Tukey's multiple comparison post test).*

SOD activity leads to the production of hydrogen peroxide, which can react with iron via the Fenton reaction to generate hydroxyl radicals, which are thought to be the most toxic oxygen

molecules *in vivo*. CAT could scavenge an excess of hydrogen peroxide, avoiding its potential role as an oxidative stress-facilitating molecule [18, 19].

4. CONCLUSION

Results obtained in this study showed that Exsynutriment®, which is widely recognized to exert antioxidant properties, may act as antioxidant depending on the assay and radicals scavenging. The organic silicium used as oral anti-aging showed antioxidant activity in vitro tests TRAP/TAR and nitric oxide ($NO^{\cdot}$) scavenging. More complete screenings of redox properties of novel compounds are needed, for this reason we must perform detailed investigations on the chemical properties of such compounds.

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