New Insights on Dimethylaminoethanol (DMAE) Features as a Free Radical Scavenger

Gabriela Malanga[1], María Belén Aguiar[1], Hugo D. Martinez[2] and Susana Puntarulo[1,*]

[1]*Physical Chemistry-PRALIB, School of Pharmacy and Biochemistry, University of Buenos Aires-CONICET, Buenos Aires, Argentina;* [2]*Pharmaceutical Technology Department, School of Pharmacy and Biochemistry, University of Buenos Aires-CONICET, Buenos Aires, Argentina*

Abstract: Recently, a number of synthetic drugs used in a variety of therapeutic indications have been reported to have antiaging effects. Among them, Dimethylaminoethanol (DMAE), an anologue of dietylaminoethanol, is a precursor of choline, which in turn allows the brain to optimize the production of acetylcholine that is a primary neurotransmitter involved in learning and memory. The data presented here includes new information on the ability of the compound to scavenge specific free radicals, assessed by Electron Spectroscopic Resonance (EPR), to further analyze the role of DMAE as an antioxidant. DMAE ability to directly react with hydroxyl, ascorbyl and lipid radicals was tested employing *in vitro* assays, and related to the supplemented dose of the compound.

Keywords: Dimethylaminoethanol (DMAE), antioxidant, ascorbyl radical, hydroxyl radical, lipid radicals.

INTRODUCTION

Recently, a number of synthetic drugs used in a variety of therapeutic indications have been reported to have antiaging effects. Among them, Dimethylaminoethanol (DMAE), an anologue of dietylaminoethanol, allows the brain to optimize learning and memory [1]. DMAE, that is a naturally occurring nutrient found in food such as anchovies and sardines, is used in the treatment of panic attack, in problems related to behavior and learning, mainly in children with hiperactivity and attention deficit, in chronic fatigue, in light depression syndrome, to improve the dream quality [2], on periorbital oedema (swelling of the eyelids) and on skin tightness [3]. It was also reported that DMAE administration to rats inhibits the formation of the aging pigment (lipofuscin) and flushes it from the body. Lipofuscin is believed to be formed by free radical reactions on the inefficient metabolism of fatty acids, and it accumulates with age in all body tissues. DMAE also seems to limit other aging symptoms in brain and heart muscle [4]. Free radical scavengers and antioxidants can reduce lipid peroxidation and the generation of reactive oxygen species (ROS). Live cells continuously produce low amounts of ROS like superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) as by-products of aerobic metabolism. The H_2O_2 is further involved with transition metals through the Fenton reaction forming highly reactive hydroxyl radicals ($^{\bullet}OH$) [5]. Moreover, some cellular constituents like ascorbate (AH^-) can reduce oxidized Fe^{3+} to Fe^{2+} generating catalytically active Fe that fuels $^{\bullet}OH$ and ascorbyl radical ($A^{\bullet}$) formation. Also, unsaturated membrane lipids can generate radical species like peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$) and alkyl ($R^{\bullet}$) radicals by reactions catalyzed by Fe.

*Address correspondence to this author at the Fisicoquímica, Facultad de Farmacia y Bioquímica, Junín 956, 1113 Buenos Aires, Argentina;
Tel: (54-11) 4964-8244; Fax: (54-11) 4508-3646;
E-mail: susanap@ffyb.uba.ar

Electron Paramagnetic Resonance **(**EPR), also known as Electron Spin Resonance (ESR) is, at present, the only analytical approach that permits the direct detection of free radicals. This technique reports on the magnetic properties of unpaired electrons and their molecular environment. The electron spin resonance spectral lines have shape, width, intensity and position (g-value), and hyperfine spectral line splitting from the interaction of unpaired electrons with magnetic nuclei that can determine the structure or positions of free radical components. Biologically important paramagnetic species include free radicals and many transition elements. In view of the interest in the use and characterization of natural and synthetic compounds, the antioxidant properties of many products, including commercially available dietary supplements from wheat bran, gingko biloba (EGb) and alfalfa extracts [6, 7] were compared. Therefore, EPR techniques allow the specific identification of the antioxidant potential of a compound, or a mix of compounds under analysis. Moreover, over the past decade the interest in $A^{\bullet}$ metabolism in biological systems has been growing and the EPR detectable concentration of $A^{\bullet}$ has been interpreted as a reflection of the ongoing free radical flux in the studied system [8]. Thus, EPR techniques might be used for the determination of free radical generation rate and content in biological and chemical systems, besides analyzing the capacity of a product to scavenge an individual reactive species.

The hypothesis of this work was that the commercially available DMAE has antioxidant properties. To analyze the mechanisms of DMAE to exert its capacity as scavenger, spin trapping and direct EPR spectroscopy were used to get evidence for reduction of radical intermediates of lipid peroxidation and hydrophilic radicals. The ability of DMAE to inhibit rat liver microsomal NADPH-dependent lipid peroxidation (lipid radicals), and $^{\bullet}OH$, and chemical generation of $A^{\bullet}$ radicals was studied.

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MATERIALS AND METHODS

Material

DMAE Bitartrate salt (2-dimethylaminoethanol), a synthetic drug with therapeutic use (Fig. **1**), was obtained from Saporiti Chemical Co S.A.C.I.F.I.A., Argentina with Analytical Certification Number 2654 (set DB 909). DMAE is not an approved additive in the USA nor is an orphan drug.

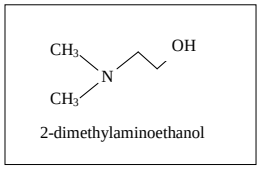

Fig. (1). DMAE chemical structure.

Thiobarbituric Acid Reactive Substances (TBARS) Content

TBARS was measured using a modified fluorescence method [6]. Rat liver microsomes were supplemented with 0.1 mM NADPH, 50 μM Fe-EDTA (1:2), in 100 mM potassium phosphate buffer (pH 7.4) and incubated during 20 min at 37ºC in the presence or absence of aliquots of DMAE. To 0.5 ml of reaction medium, 0.05 ml of 4% (w/v) BHT and 0.2 ml of 3% (w/v) sodium dodecyl sulfate were added. After mixing, 2 ml of 0.1 N HCl, 0.3 ml of 10% (w/v) phosphotungstic acid, and 1 ml of 0.7% (w/v) 2-thiobarbituric acid was added. The mixture was heated for 45 min in boiling water, and TBARS were extracted into 5 ml of n-butanol. After a brief centrifugation, the fluorescence of the butanol layer was measured at λ_{excit}=515 nm and λ_{emis}=555 nm. The values were expressed as nmol TBARS (malondialdehyde equivalents) per mg of protein. Malondialdehyde standards were prepared from 1,1,3,3-tetramethoxypropane.

Lipid Radicals Generation Determined by EPR-spin Trapping

Rat liver microsomes were prepared in α-phenyl-tert-N-butyl-nitrone (PBN). EPR spectra were obtained at room temperature using a Bruker Espectrometer ECS 106, operating at 9.75 GHz with 50 kHz modulation frequency. EPR instrument settings for the spin trapping experiments were: microwave power, 20 mW; modulation amplitude, 1.232 G; time constant, 81.92 ms; receiver gain, 2 x 10^4 [8]. Quantitation of the spin adduct was performed using an aqueous solution of 2,2,5,5-tetramethyl piperidine 1-oxyl (TEMPO) introduced into the same sample cell used for spin trapping. EPR spectra for both sample and TEMPO solutions were recorded at exactly the same spectrometer settings and the first derivative EPR spectra were double-integrated to obtain the area intensity, then the concentration of spin adduct was calculated according to Kotake *et al.* [9].

$^{\bullet}$OH Production Determined by EPR-spin Trapping

Prior to use, DMPO was purified, following the method of Green and Hill [10], to remove contaminants that contribute to EPR background signal. In this procedure, 10 ml of 1 mM DMPO in doubly distilled water are mixed with 1.25 g activated charcoal for 1 min, allowed to stand for 1 h, and then filtered. This purification procedure was repeated twice. The basic microsomal incubation system consisted of 100 mM DMPO, microsomes (1 mg protein/ml), 0.5 mM sodium azide, 0.6 mM DTPA, and 50 mM potassium phosphate (pH 7.4). Reactions were started by addition of 0.5 mM NADPH. The microsomal reaction system was transferred to a Pasteur pipette for direct observation of the reaction in a Bruker ECS 106 EPR spectrometer at room temperature. The EPR spectrometer settings were as follows: microwave power, 20 mW; modulation amplitude, 0.490 G; time constant, 655.36 ms; field scan, 100 G; scan time, 167.772 s; and modulation frequency 50 kHz [11].

Detection of A$^{\bullet}$

A Bruker ECS 106 spectrometer was used for A$^{\bullet}$ measurements. Ascorbic acid (60 μM) in the presence of 50 μM Fe-EDTA (1:2) was supplemented with dimethylsulfoxide (DMSO) and the spectra were immediately scanned in the following conditions: 50 kHz field modulation, room temperature, microwave power 10 mW, modulation amplitude 1 G, time constant 655 ms, receiver gain 1 x 10^5, microwave frequency 9.81 GHz, and scan rate 0.18 G/s [12]. Quantification was performed as previously described, according to Kotake *et al.* [9].

Statistical Analysis

Data in the text, figures and tables are expressed as means ± SEM of 3 to 6 independent experiments. Statistical tests were carried out using Statview for Windows, ANOVA, SAS Institute Inc., version 5.0.

RESULTS

Peroxidation of rat liver microsomes was studied in the presence of Fe-EDTA as the Fe catalyst and NADPH as the reductant for the microsomal electron transfer system. Production of TBARS exhibited a linear time course for 20-25 min (data not shown). Supplementation of DMAE in the concentration range of 0 to 4.2 M, did not show any significant inhibitory effect on TBARS production by rat liver microsomes. Since lipid peroxidation seems as the biochemical process leading to the appearance of lipofuscin that was reported as decreased in the tissues supplemented with DMAE, a more sensitive and specific method, EPR, was used to study this effect. The decomposition of hydroperoxides formed during NADPH-dependent peroxidation in rat liver microsomes supplemented with Fe led to the generation of lipid radicals, such as ROO$^{\bullet}$, RO$^{\bullet}$ and R$^{\bullet}$ radicals, that combined with the spin trap PBN resulted in adducts that gave a characteristic EPR spectrum with hyperfine coupling constants of a_N=15.8 G and a_H=2.6 G (Fig. **2A c**), in agreement with computer spectral simulated signals obtained using those parameters (Fig. **2A a**). Even though these constants could be assigned to lipid radicals, spin trapping studies cannot readily distinguish between ROO$^{\bullet}$, RO$^{\bullet}$ and R$^{\bullet}$ adducts, owing to the similarity of the corresponding coupling constants [13]. In the absence of

microsomes no EPR signal was observed (Fig. **2A b**). The addition of the tested drug exhibited a maxima scavenging activity, reducing the PBN-adduct signal by 44% (Fig. **2A d**) in the presence of 2 M of the substance in comparison with the control sample without the drug addition, which represents 100% PBN-lipid radical adduct (Fig. **2A c**). The results presented here are consistent with the hypothesis that indicates that lipid radicals are quenched by DMAE supplementation.

Rat liver microsomes in the presence of DMPO, NADPH and Fe-EDTA generate an EPR spectra (Fig. **3A c**) with the parameters characteristics of the DMPO-OH spin adduct (a_N=15 G and a_H=15 G), according to computer simulation records [14] (Fig. **3A a**). In the absence of microsomes no EPR signal was observed (Fig. **3A b**). The basic system, without the addition of any scavenger, which represents 100% DMPO-OH radical adduct showed an steady state concentration of 4.5 ± 0.1 μM. The addition of the tested

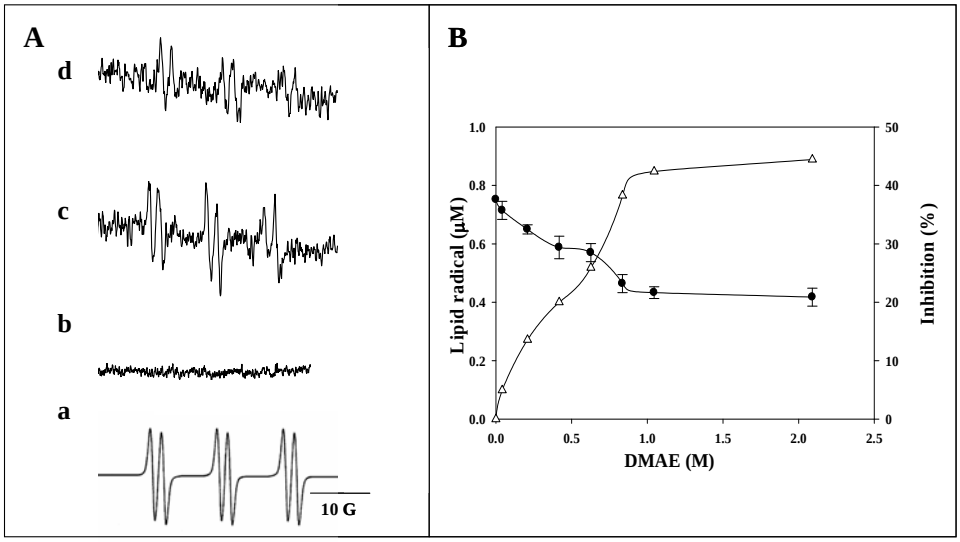

Fig. (2). Scavenging ability of DMAE against lipid radicals. **A**. EPR spectra of the lipid radical-PBN spin adduct, (**a**) computer simulated spectrum employing as spectral parameters a_N=15.8 G and a_H=2.6 G and g= 2.005; (**b**) and basal system (in the absence of microsomes); (**c**) lipid radical-PBN spin adduct generated in rat liver microsomes; (**d**) lipid radical-PBN spin adduct generated in rat liver microsomes in the presence of 1 M DMAE. **B**. Dose-dependent effect of DMAE on lipid radical content (●), and percentages of inhibition shown by DMAE supplementation on lipid radical steady state concentration (Δ).
PBN stands for α-phenyl-tert-N-butyl-nitrone.

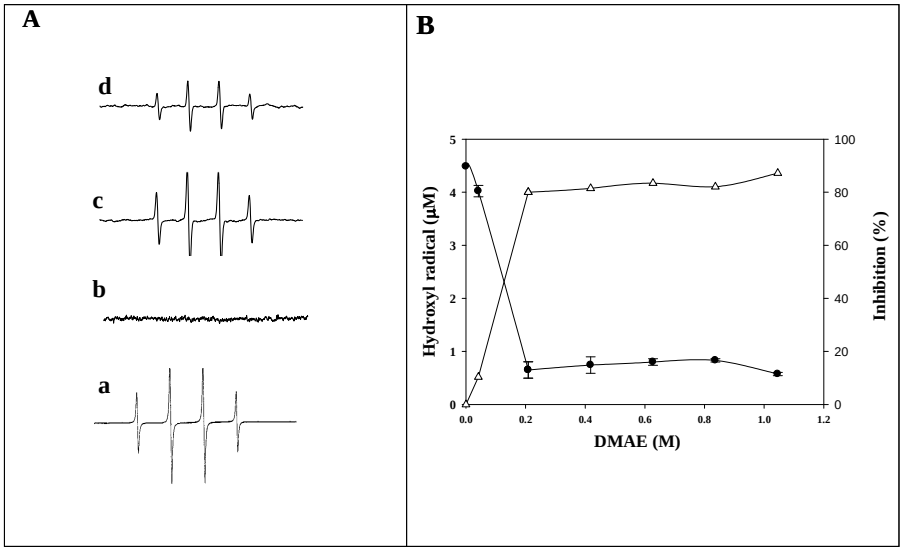

Fig. (3). Scavenging ability of DMAE against ˙OH. **A**. EPR spectra of DMPO-OH spin adduct, (**a**) computer simulated spectrum employing as spectral parameters a_N=15 G and a_H=15 G; (**b**) and basal system (in the absence of microsomes); (**c**) DMPO-OH spin adduct generated in rat liver microsomes; (**d**) DMPO-OH spin adduct generated in rat liver microsomes in the presence of 1 M DMAE. **B**. Dose-dependent effect of DMAE on ˙OH steady state concentration (●), and percentages of inhibition by DMAE supplementation on ˙OH steady state concentration (Δ).
DMPO stands for 5,5-dimethyl-1-pyrroline n-oxide.

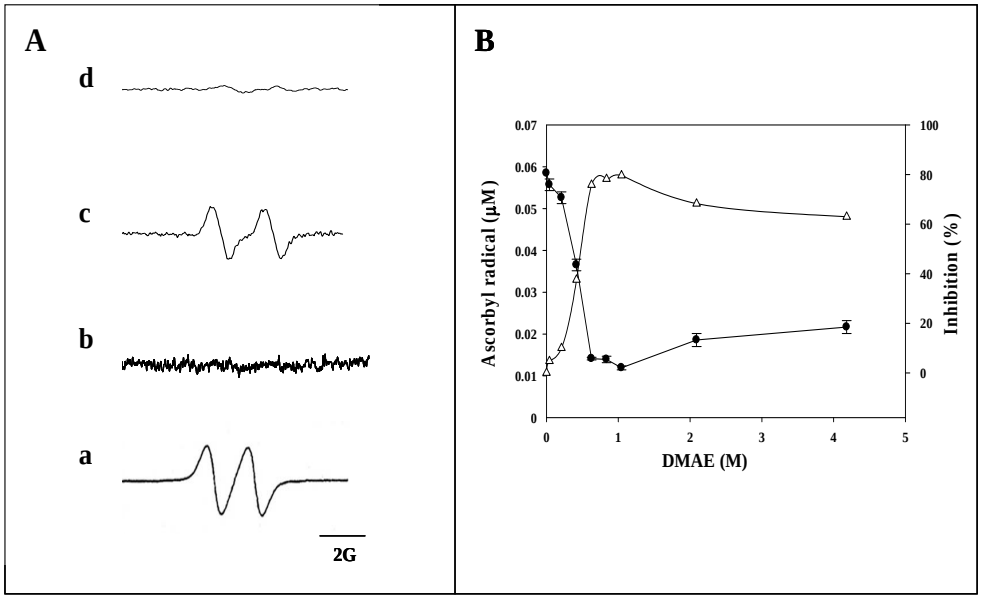

Fig. (4). Scavenging ability of DMAE against A•. **A**. Electron paramagnetic resonance (EPR) spectra from A•, (**a**) computer simulated spectrum employing as spectral parameters a_H=1.88 G and g=2.0054; (**b**) basal system (in the absence of ascorbic acid), (**c**) ascorbic acid 60 μM in DMSO (**b**); (**d**) ascorbic acid 60 μM in DMSO and 1 M DMAE. **B**. Dose-dependent effect of DMAE on A• steady state concentration (●), and percentages of inhibition by DMAE supplementation on A• steady state concentration (Δ).
DMSO stands for dimethylsulfoxide.

drug exhibited a maximum in the scavenging activity, reducing the adduct signal by 87% (Fig. **3A d**) in the presence of 2 M of the substance in comparison with the control sample without the drug addition, which represents 100% DMPO-OH adduct (Fig. **2A c**). Moreover, radical concentration was significantly decreased as the DMAE concentration added increased (Fig. **3B**). The results presented here are consistent with the hypothesis that indicates that •OH was efficiently quenched by DMAE addition, under these experimental conditions.

In Fig. (**4A c**) is shown the typical ESR spectrum of A• generated in the presence of ascorbic acid and Fe-EDTA, with the characteristic two lines at g=2.005 and a_{H+}=1.8 G, in accordance with computer spectral simulated signals (Fig. **4A a**), obtained using the parameters stated in the Materials and Methods section. DMSO itself was examined and no DMSO spin adduct was observed (Fig. **4A b**). A• content, assessed by quantification of EPR signals, was significantly decreased by the supplementation of increasing amounts of DMAE (Fig. **4A d**). The basic system which represents 100% A• content, showed a steady state concentration of A• of (5.9 ± 0.1) 10^{-2} μM, which was significantly decreased by DMAE supplementation (Fig. **4B**). The results presented here are consistent with the hypothesis that indicates that A• was efficiently quenched by DMAE, under these experimental conditions.

The half-inhibition concentration (IC50) of DMAE, was calculated from the respective concentration-activity curves and represents the concentration that gives 50% of the maximum inhibition of the microsomal lipid radical content, the •OH production rate and the A• generation rate. The data are summarized in Table **1**. The relative scavenging capacity (RSC) represents the number of IC50 per g of DMAE, and would allow the comparison with the scavenger ability of other compounds [6]. Data in Table **1** showed the RSC for each radical species, and strongly suggest that •OH is the most efficiently scavenged species by DMAE among the tested ones.

Table 1. The IC50 and RSC of DMAE for to the Studied Radical Species

Radical Species	IC50 (M)	RSC (IC50/g DMAE)
Lipid radicals	0.4 ± 0.2	8.3
•OH	0.26 ± 0.07	41.8
A•	0.55 ± 0.06	8.3

IC50 represents the concentration that gives 50% of the maximum inhibition of the microsomal lipid radical content, or •OH production rate, or the A• generation rate in the chemical system.

RSC represents the number of IC50 per g of DMAE.

DISCUSSION

DMAE, that is available as a nutritional supplement, is a chemical that have been used to treat a number of conditions affecting the brain and the central nervous system. It has been postulated the therapeutic use of this compound for two major groups of pathologies: i) brain disorders, and ii) aging-related effects. Preliminary evidence suggests that DMAE may be helpful for attention deficit hyperactivity disorder (ADHD) [15, 16]. Unclear results on the effectiveness of DMAE in the treatment for either Tardive dyskinesia [15-20], or for Huntington's chorea disease [15, 21, 22] and Alzheimer's disease [17], have been obtained. Thus, widely marketed as a memory and mood enhancer, and as an agent to improve intellectual functioning, there is not complete

agreement among the clinical studies that support its use for these purposes.

In 1954, Denham Harman proposed a free radical theory of aging [23]. Today a huge body of evidence confirms that oxidative stress promotes aging and many seemingly diverse age-associated diseases [24, 25]. In 1977, the Hungarian physician Imre Nagy proposed the membrane hypothesis of aging which posited the cell membrane as the key target of free radical activity and which has been confirmed experimentally [26-29]. Also it was shown that higher levels of oxygen predispose membranes to produce ROS that attack and easily oxidized the polyunsaturated fatty acids (PUFA) in the lipid bi-layers, producing an inflammatory cascade that causes cellular damage and senescence [30, 31]. Although aging is a natural phenomenon and bodily decay is an inexorable process, aging can at least be postponed or prevented by certain approaches [1]. DMAE has powerful anti-inflammatory effects when applied to skin, and with the proper carrier it increases underlying muscle tone showing acute and cumulative effects [32].

There is some controversy about the action mechanisms proposed for DMAE effects [2-8, 15, 17, 33]. Moreover, Nagy and Floyd (1984) [32] have shown in an *in vitro* study that DMAE was a competitive $^{\bullet}OH$ scavenger, supporting a molecular mechanism for the anti-aging effects of DMAE in terms of the membrane hypothesis of aging. More recently, Gragnani *et al.* [34] have shown that DMAE reduced the proliferation of fibroblasts, increased cytosolic Ca and changed the cell cycle, causing an increase in apoptosis (cellular death associated to free radical production) in human fibroblasts. Thus, a basic analysis employing a specific technique such as EPR, was employed to assess the ability of the compound to scavenge radicals responsible for producing damage in the water phase (such as $^{\bullet}OH$ and $A^{\bullet}$) and in the lipid phase (such as lipid radicals), comparatively. The data presented here clearly showed that DMAE efficiently scavenges all the radical species tested. However, the comparison of the IC50 indicated that the efficiency is not identical in the lipo- and hydrophilic phases. By the direct comparison of the IC50 (Table **1**) for the radical species tested it is concluded that DMAE the best scavenger capacity towards $^{\bullet}OH$ (87% inhibition) and $A^{\bullet}$ (79% inhibition) as compared to lipid radicals (44% inhibition). Thus, DMAE seems as a better scavenger for radicals generated in the hydrophilic milieu, with a relative lower ability for the scavenging of lipid radicals.

It was suggested that EGb is a scavenger of peroxyl radicals generated in both lipid and aqueous environments [35], through indirect measurements, and EPR studies [6]. Kose and Dogan [36] have shown that EGb extracts have more antioxidant potential than water-soluble antioxidants (ascorbic acid, glutathione and uric acid); and was as effective as lipid-soluble antioxidants (alpha-tocopherol and retinol acetate) in protecting red-cell suspensions against lipid peroxidation induced by H_2O_2. The RSC of EGb towards lipid radicals was 862 AU [6], being almost two orders of magnitude higher than the RSC reported here for DMAE. This observation is consistent with the lack of effect on TBARS content in the microsomes after the addition of DMAE, under the tested experimental conditions. The RSC of EGb towards $^{\bullet}OH$ was 260 AU [6], being six-fold higher than the RSC reported here for DMAE. As EGb is a mixture of different chemical constituents, its scavenging activity could be due to a particular component as well as to the interactions of different antioxidant molecules, and the component responsible for its scavenging properties could not be specifically identified. Further, the membrane-stabilizing action of EGb 761 has been previously demonstrated, since it decreased the osmotic fragility of rat erythrocytes and penetrated into membrane phospholipid domain [14]. Besides Ginkgoflavone glycosides and terpenoids, the extract also contains other substances of minor interest, such as organic acids, which would play a role in its water solubility. Thus, EGb extracts would be able to show both lipophilic and hydrophilic characteristics. Moreover, Deby and Pincemail [37] have suggested that polyphenolic substances in EGb extract play a protective role at another level, since during their transformation into quinone, they can give up two hydrogen atoms and their electron to lipoperoxides. DMAE, that shares with EGb the characteristic of improving brain alert and focus [38], has only one hydroxyl group with possible antioxidant ability suggesting that other mechanism should contribute to explain the observed effects of DMAE. However, the role of DMAE in dermatology including a potential anti-inflammatory effect and a documented increase in skin firmness with possible improvement in underlying facial muscle tone [39] could be a conjunction of many actions including its antioxidant activity. On the other hand, the incorporation of chemical compounds into the cell is a function of their lipophilicity. Thus, the antioxidant activity of extracts appears to be dictated not only by the structural features but also by their location in the membranes. The small size of this molecule could be a positive factor to get access to protect cellular targets against free radical damage. The results presents here might be taken into consideration for further biotechnological developments of protective antioxidants, which could have important applications in human diseases accompanied by free radical injury. Any biologically active compound should appear in the target tissues in significant amounts to elicit bioprotective effects. Future studies should consider interactions of the supplemented compound with endogenous antioxidants, as well as tissue specificity, compartmentalization and concentration levels of the active compound/s in target organs, to appropriately assess effectiveness *in vivo*.

CONFLICT OF INTEREST

Declared none.

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ABBREVIATIONS

BHT = Butylated hydroxytoluene

DMPO = 5,5-dimethyl-1-pyrroline n-oxide

DTPA = Diethylenetriaminepentaacetic acid

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