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Endogenous and Dietary Indoles: A Class of Antioxidants and Radical Scavengers in the ABTS Assay

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Indoles are very common in the body and diet and participate in many biochemical processes. A total of twenty-nine indoles and analogs were examined for their properties as antioxidants and radical scavengers against 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) $ABTS^{\bullet+}$ radical cation. With only a few exceptions, indoles reacted nonspecifically and quenched this radical at physiological pH affording ABTS. Indoleamines like tryptamine, serotonin and methoxytryptamine, neurohormones (melatonin), phytohormones (indoleacetic acid and indolepropionic acid), indoleamino acids like L-tryptophan and derivatives (N-acetyltryptophan, L-abrine, tryptophan ethyl ester), indolealcohols (tryptophol and indole-3-carbinol), short peptides containing tryptophan, and tetrahydro-β-carboline (pyridoindole) alkaloids like the pineal gland compound pinoline, acted as radical scavengers and antioxidants in an ABTS assay-measuring total antioxidant activity. Their trolox equivalent antioxidant capacity (TEAC) values ranged from 0.66 to 3.9 mM, usually higher than that for Trolox and ascorbic acid (1 mM). The highest antioxidant values were determined for melatonin, 5-hydroxytryptophan, trp-trp and 5-methoxytryptamine. Active indole compounds were consumed during the reaction with $ABTS^{\bullet+}$ and some tetrahydropyrido indoles (e.g. harmaline and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester) afforded the corresponding fully aromatic β-carbolines (pyridoindoles), that did not scavenge $ABTS^{\bullet+}$. Radical scavenger activity of indoles against $ABTS^{\bullet+}$ was higher at physiological pH than at low pH. These results point out to structural compounds with an indole moiety as a class of radical scavengers and antioxidants. This activity could be of biological significance given the physiological concentrations and body distribution of some indoles.

Keywords: Indoles; Antioxidants; Radical scavengers; ABTS; Indoleamines; Indoleamino acids, β-carbolines, Indole derivatives, Melatonin

INTRODUCTION

Oxidative stress caused by free radicals play an important role in many human diseases such as cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration and diabetes.[1,2] Consequently, a great effort has dealt with the study of endogenous, dietary and drug antioxidants as potential agents against oxidative stress related diseases in order to prevent and improve human health. Dietary antioxidants are able to remove free radicals in animal studies and there is a growing interest on the beneficial health effects provided by certain foods and beverages from vegetable origin. This beneficial effect is attributed to vitamins C and E, carotenoids and polyphenols or flavonoids.[3–7] All these compounds have in common the ability to protect against reactive oxygen and nitrogen species involved in pathological states.

Besides the above-mentioned antioxidants (i.e. vitamins, carotenoids and flavonoids) there are many other endogenous and/or dietary compounds that may exhibit significant antioxidant and related biological actions. Many natural products from plants such as indoles and glucosinolates inhibit chemical carcinogenesis, and are often described as chemopreventive agents.[8] Indole-3-carbinol (I3C) has been reported as a potential radical scavenger,[9,10] and the intake of vegetables rich in I3C is usually related to a reduced risk of cancer development.[11] In the last few years, increasingly attention has focused on melatonin, an indole neurohormone

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(N-acetyl-5-methoxytryptamine) occurring in the pineal gland and reported to act as a good radical scavenger and antioxidant *in vivo*.[12] The pharmacology of some particular indoles such as melatonin and I3C is now being considered in view of its potential applications in various therapeutic areas.[13,14] Another pharmacologically active indole analog is stobadine, a tetrahydropyridoindole derivative with cardio- and neuroprotectant actions attributed to its antioxidant properties.[15,16] Other class of pyridoindoles such as tetrahydro-β-carboline alkaloids occur in biological systems and foodstuffs,[17–22] exhibit a wide range of biological and pharmacological properties,[21–24] and may act as radical scavengers.[25]

The present work studies the antioxidant capacity of many indole compounds, that occur naturally in the diet and are endogenously present or formed in the body including simple indoles, indoleamino acids, indoleamines, alkaloids and short peptides containing tryptophan. Some are biogenic amines and classical neurotransmitters such as serotonin, or neurohormones such as melatonin while others are phytohormones (indole-3-acetic acid) and essential amino acids (L-tryptophan) or their derivatives. The total antioxidant capacity of these compounds is measured by following the disappearance of one radical in the presence of indoles. Interestingly, the results reported below show that almost every compound with an indole moiety was able to donate electrons in some degree and to scavenge the free radical cation $ABTS^{\bullet+}$ (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonate), a well established method to measure total antioxidant activity.[7] These results suggest that indoles in general, and not exclusively a few remarkable indoles such as melatonin, may constitute a structural family of radical scavengers (electron donors).

MATERIALS AND METHODS

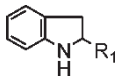
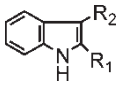
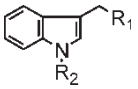
Reference Compounds and Standards

Indole compounds, including amino acids, amines, short peptides, hormones and alkaloids were obtained of the highest purity commercially available: indole-2-carboxylic acid, indoline-2-carboxylic, indole-3-acetic acid, N-methylindole-3-acetic acid, L-abrine, indole-3-propionic acid, 5-methoxytryptamine and pinoline (6-methoxy-1,2,3,4-tetrahydro-β-carboline) were obtained from Aldrich (Germany); indoline, tryptophol, 5-hydroxytryptophan were from Fluka; gramine (3-(dimethylaminomethyl)-indole), indole-3-carbinol, indole-3-aldehyde, tryptamine, tryptophan ethyl ester, N-acetyltryptophan, harmine hydrochloride, harmaline, serotonin oxalate, tryptophan, trp-trp, trp-gly-gly, β-carboline-3-carboxylic acid methyl ester were purchased from Sigma (Sigma Chemicals, St Louis, MO, USA); indole from Merck (Darmstadt, Germany); carbazole from Sharlau (Spain), and melatonin from Acros (Spain). 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester (MTCA-EE) was synthesized from L-tryptophan ethyl ester and acetaldehyde through a Pictet-Spengler condensation. 2,2′-Azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and potassium persulfate were obtained from Sigma, ascorbic acid was obtained from Merck, Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), 2,2′-azobis(2-amidinopropane) dihydrochloride and quercetin from Aldrich, (+)-catechin from Sigma and Kaempferol from Fluka.

Antioxidant Activity of Indole Compounds

The ABTS assay developed by Re *et al.*[7] that has proved to work well to measure total antioxidant activity[26,27] was used to measure the radical scavenger activity. 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) was dissolved in water to 7 mM concentration and the ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with potassium persulfate (2.45 mM final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. $ABTS^{\bullet+}$ radical cation was diluted with 5 mM phosphate buffered saline (PBS), pH 7.2 to give an absorbance value of 0.7 at 734 nm. Solutions of indole compounds, Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) and ascorbic acid were freshly prepared in deionized water, water-alcohol or acidified water, and then used for radical scavenger assay in several concentrations (0, 1.66, 3.33, 6.6 and 10 μM, final concentration) (total volume 3 ml), by measuring the percentage of the inhibition at room temperature of the absorbance at 734 nm (quenching of the radical cation $ABTS^{\bullet+}$) as a function of time. The antioxidant capacity was measured in comparison with Trolox, the water soluble vitamin E analog, as standard. The Trolox equivalent antioxidant capacity (TEAC) is defined as the concentration of Trolox with equivalent antioxidant activity (percentage inhibition of absorbance of the radical cation) to a 1 mM concentration of the substance under investigation. To measure the effect of pH on the antioxidant activity, the assay of ABTS was accomplished in 5 mM PBS buffer solutions at different pHs and compared with Trolox. On the other hand, some 1 mM indole solutions (tryptamine, abrine, tryptophol, melatonin, pinoline, indole-3-propionic acid) were incubated for a period of time in eppendorf tubes with 6.5 mM or 30 mM 2,2′-azobis-2-(amidinopropane) (AAPH) at 37 or 45°C, respectively, and then examined in the ABTS assay and also analyzed by HPLC, to see the effect of the oxidation of indoles on the activity against $ABTS^{\bullet+}$.

TABLE I Trolox equivalent antioxidant capacity (TEAC) values calculated for indoles and analogues. From quadruplicate experiments (concentration 0–3.3 μM, 5 min time-point)

	R_1	R_2	Name	TEAC (mM)
	H		Indoline	1.39 ± 0.02
	COOH		Indoline-2-carboxylic acid	1.04 ± 0.02
	H	H	Indole	0.66 ± 0.03
	COOH	H	Indole-2-carboxylic acid	0.08 ± 0.01
	H	COH	Indole-3-aldehyde	0.03 ± 0.01
	$N(CH_3)_2$	H	Gramine	0.12 ± 0.01
	COOH	H	Indole-3-acetic acid	1.64 ± 0.03
	CH_2COOH	H	Indole-3-propionic acid	1.62 ± 0.01
	COOH	CH_3	N-methylindoleacetic acid	1.30 ± 0.01
	CH_2OH	H	Tryptophol	2.05 ± 0.02
	OH	H	Indole-3-carbinol	2.31 ± 0.06

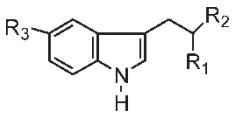
RP-HPLC Analysis of Indole Reaction with $ABTS^{\bullet+}$

The analysis of indole compounds by RP-HPLC and *uv-DAD* and fluorescence detection was carried out as previously[25] using an HPLC 1050 (Hewlett Packard) provided with a diode array detector (DAD) and a 1046A-fluorescence detector. A 150 × 3.9 mm, 4 μm, Nova-pak C18 column (Waters, Milford, MA, USA) was used for separation. Chromatographic conditions were as follows: 50 mM ammonium phosphate buffer (pH 3) (buffer A) and 20% of A in acetonitrile (buffer B). Gradient programmed from 0% (100% A) to 32% B in 8 min and then 90% B at 18 min. The flow rate was 1 ml/min, the column temperature was 40°C and the injection volume was 20 μl. Fluorescent detection was set at 270 nm (excitation) and 343 nm (emission) for indoles and tetrahydro-β-carbolines, and 300 nm (excitation) and 433 nm (emission) for fully aromatic β-carbolines.

RESULTS

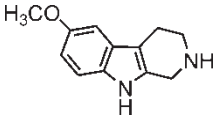
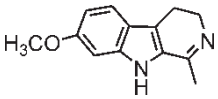
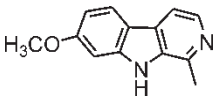
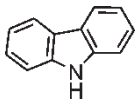
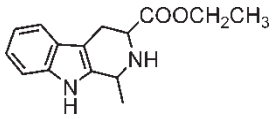
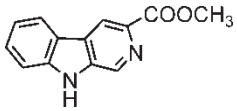
The activity as radical scavengers and antioxidants of 29 indole compounds (Tables I–III) was measured with the ABTS assay that measures the suppression of the radical cation $ABTS^{\bullet+}$ produced by an antioxidant (hydrogen or electron donor).[7] It is based on the one-electron reduction of the relatively stable cation radical $ABTS^{\bullet+}$ previously formed by an oxidation reaction. When active indole compounds were added to PBS medium (pH 7.2) containing $ABTS^{\bullet+}$, this cation radical (absorbance maxima at 414 nm, and 645, 734 and 815 nm) rapidly disappeared giving the ABTS

TABLE II TEAC values for indoleamino acids, indoleamines and derivatives. From quadruplicate experiments (concentration 0–3.3 μM, 5 min time-point)



R_1	R_2	R_3	Name	TEAC (mM)
NH_2	H	H	Tryptamine	2.61 ± 0.03
NH_2	H	OCH_3	Methoxytryptamine	3.14 ± 0.04
$NHCOCH_3$	H	OCH_3	Melatonin	3.96 ± 0.11
NH_2	H	OH	Serotonin	2.88 ± 0.03
NH_2	COOH	H	Tryptophan	2.32 ± 0.05
$NHCOCH_3$	COOH	H	N-acetyltryptophan	1.73 ± 0.03
$NHCH_3$	COOH	H	Abrine	2.70 ± 0.04
NH_2	COOH	OH	5-Hydroxytryptophan	3.45 ± 0.02
NH_2	$COOCH_2CH_3$	H	Ethyl tryptophanate	2.56 ± 0.02
NH_2	Trp-COOH	H	Trp-Trp	3.31 ± 0.03
NH_2	Gly-Gly-COOH	H	Trp-Gly-Gly	2.49 ± 0.04
Phe	$COOCH_3$	H	Phe-Trp-OCH_3	1.32 ± 0.02

TABLE III TEAC values for β-carbolines and carbazole. From quadruplicate experiments (concentration 0–3.3 μM, 5 min time-point)

		TEAC (mM)
	Pinoline	2.40 ± 0.04
	Harmaline	0.92 ± 0.01
	Harmine	0.02 ± 0.02
	Carbazole	0.02 ± 0.01
	MTCA-EE	1.62 ± 0.02
	βCME	0.03 ± 0.02

non-radical (absorbance maximum at 340 nm) (Fig. 1). The $ABTS^{\bullet+}$ was suppressed to an extent and on a time scale dependent on the antioxidant activity or the reducing properties of the substance. The elimination of $ABTS^{\bullet+}$ was accompanied with the consumption of the indole compound as determined by RP-HPLC

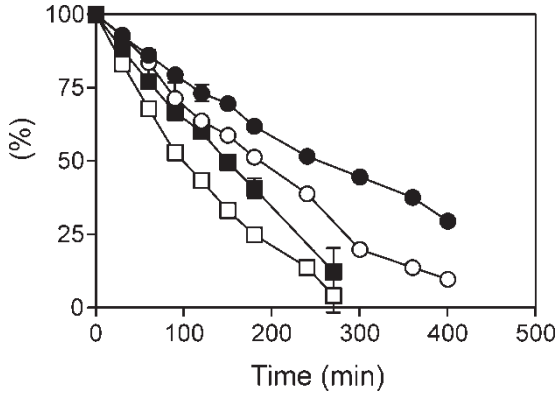


FIGURE 2 Relative antioxidant activity (%) against $ABTS^{\bullet+}$ (closed symbols) and relative amount (%) (open symbols) of melatonin (squares) and pinoline (circles) that remain following incubation with 2,2′-azobis-(2-amidinopropane) (AAPH). Indoles (1 mM) were incubated (45°C) with AAPH 30 mM in 5 mM PBS buffer, pH 7.2. An aliquout (30 μl) diluted in 3 ml PBS buffer and $ABTS^{\bullet+}$-PBS buffer was used for HPLC and absorbance (734 nm) measurement, respectively. Initial values ($t = 0$) taken as 100%. Values are average of duplicate experiments.

(inset of Fig. 1). This suggests a reaction between the $ABTS^{\bullet+}$ cation radical and active indoles, which acted as electron donors. No changes in the absorbance or chromatographic profile were observed for those indole compounds with no activity against $ABTS^{\bullet+}$ (e.g. gramine, harmine, etc.) (results not shown). On the other hand, the activity against $ABTS^{\bullet+}$ cation radical was progressively lost when indoles (melatonin and pinoline) were incubated (oxidized) with the peroxyl radical generator 2,2′-azobis(2-amidinopropane), and it correlated with the degradation of the same indoles as measured by HPLC (Fig. 2). A loss of the antioxidant activity following oxidation of indoles was also evident when active indoles were incubated for 20 h in 6.5 mM 2,2′-azobis(2-amidinopropane) and 37°C.

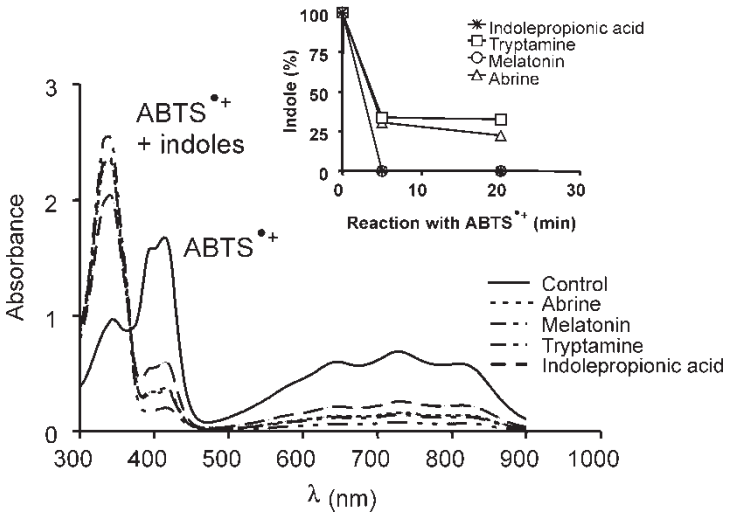


FIGURE 1 UV-visible spectra of $ABTS^{\bullet+}$ cation radical and $ABTS^{\bullet+}$ reacted with indoles (10 μM) in PBS buffer, pH 7.2. Consumption of indoles as determined by RP-HPLC during the reaction with $ABTS^{\bullet+}$ cation radical (inset).

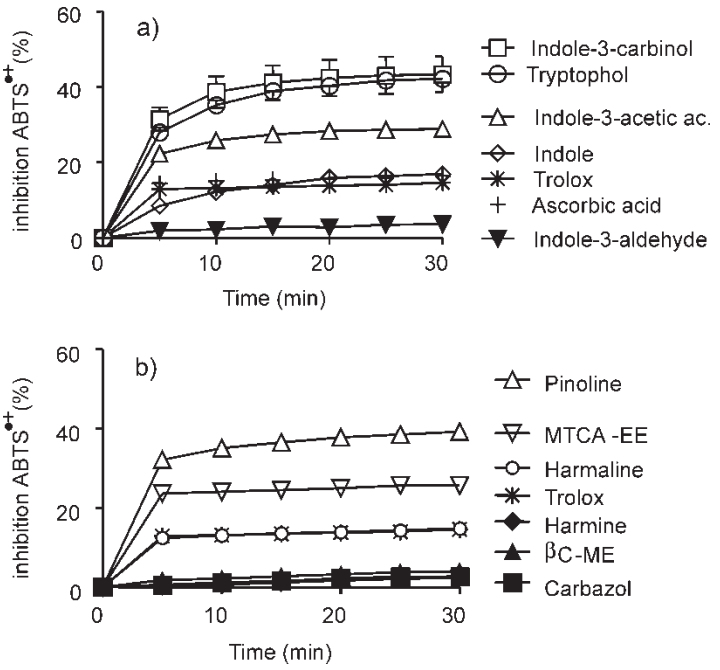


FIGURE 3 Inhibition (%) of $ABTS^{\bullet+}$ radical cation (absorbance at 734 nm) during the course of the reaction with various indoles (a), β-carbolines (b) and ascorbic acid and Trolox (concentration 3.33 μM). Values are average of quadruplicates.

Upon the addition of indoles the inhibition of $ABTS^{\bullet+}$ was initially rapid and reached a high percentage within 5 min (minimum time taken for reading absorbance) (Figs. 3 and 4). This rapid reaction agrees with results for other antioxidants reacting with $ABTS^{\bullet+}$ cation radical.[7] For some indoles (e.g. indole-3-carbinol, indole, tryptophol, indoleacids, abrine and melatonin) the reaction was still progressing after 5 min, probably due to different reaction rates for these compounds, or/and the formation of secondary products still reacting with $ABTS^{\bullet+}$. Most indoles, including alcohols (tryptophol and I3C), acids (indole-3-acetic acid), amino acids (tryptophan and its derivatives), amines and derivatives (tryptamine, serotonin, melatonin), tetrahydro-β-carboline alkaloids (pinoline, MTCA-EE), and short peptides containing tryptophan exhibited greater reactivity with $ABTS^{\bullet+}$ than the classical antioxidants ascorbic acid and Trolox. In contrast, no scavenging reaction of $ABTS^{\bullet+}$ was observed for the β-carbolines harmine

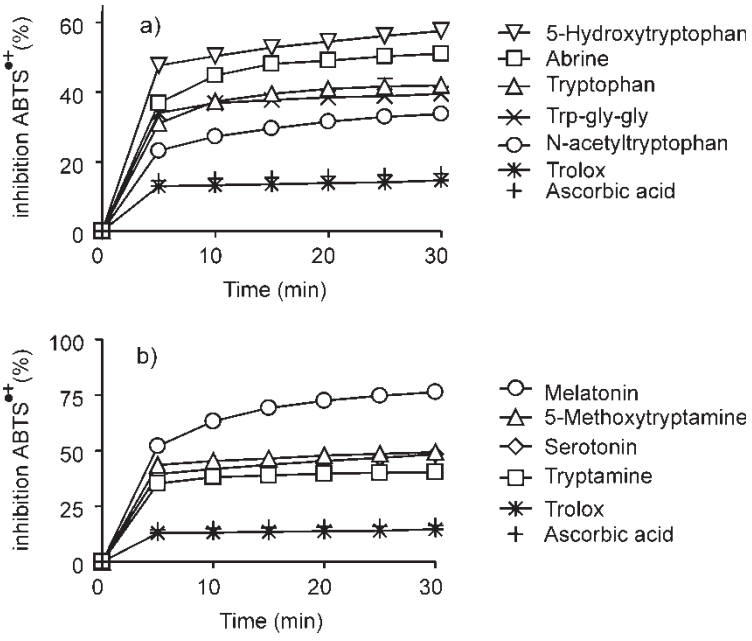


FIGURE 4 Inhibition (%) of $ABTS^{\bullet+}$ radical cation (absorbance at 734 nm) during the course of the reaction with various indoleamino acids and derivatives (a), indoleamines (b) and ascorbic acid and Trolox (concentration 3.33 μM). Values are average of quadruplicates.

and β-carboline-3-carboxylic acid methyl ester, carbazole and the indoles indole-3-aldehyde, gramine (3-(dimethylaminomethyl)indole), and indole-2-carboxylic acid.

The extent of $ABTS^{\bullet+}$ quenching was determined as a function of the concentration of indoles (0–10 μM). Generally, indoles quenched a high percentage of the radical at 6.6 μM and above (final concentration). There was a progressive loss of the radical with increasing concentration of indoles with linear curves generally obtained at concentrations lower than 6.6 μM. The antioxidant capacity of each indole in relation to Trolox measured as TEAC (mM) at a fixed time point of 5 min is given in Tables I–III. Under the conditions of this assay, TEAC values ranging from 0.66 to 3.9 mM were calculated for indoles and were usually higher than those for ascorbic acid and trolox (1.01 mM). A good antioxidant capacity was observed for indoleamines and indoleamino acids such as melatonin, 5-hydroxytryptophan, 5-methoxytryptamine tryptamine, serotonin, tryptophan and several tryptophan derivatives and short peptides such as Trp-Trp and Trp-Gly-Gly (Table II). Simple indole, indoline and phytohormones such as indole-3-acetic and indole-3-propionic acid, or tryptophol exhibited slight antioxidant properties, as well (Table I). Tetrahydro-β-carboline alkaloids like pinoline, a pineal gland compound, and MTCA-EE scavenged the $ABTS^{\bullet+}$ in contrast with the absence of radical scavenger capacity for structurally related β-carbolines (harmine) and carbazole (Table III). Under the specific conditions of this ABTS assay, the TEAC (mM) value measured for some indoles is somehow comparable to that of some phenolics and carotenoids, widely recognized as antioxidant.[7] Indeed, the TEAC determined here in ethanol-$ABTS^{\bullet+}$ for quercetin, a known phenolic antioxidant, was 3.0 ± 0.06. Nonetheless, it should be noticed that the TEAC calculated for indoles depended on the concentration range and the reaction time used. It decreased for the highest indole concentrations (within a range from 0 to 32% lower at 6.6 μM), but increased with time (0–35% higher at a reaction time of 20 min) owing to a slow progression of the reaction with $ABTS^{\bullet+}$ compared to Trolox after 5 min.

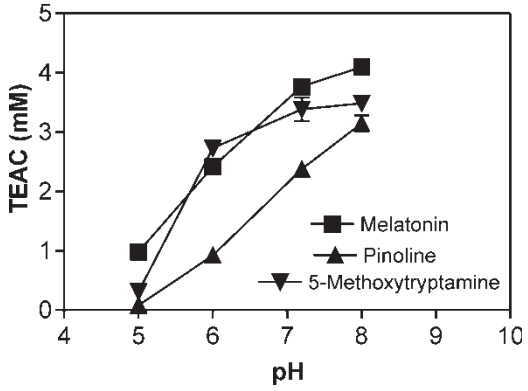


FIGURE 5 Values of TEAC measured at different pHs for some active indoles in the assay of ABTS. Values are average from quadruplicate experiments (concentration 3.33 μM, 5 min time-point).

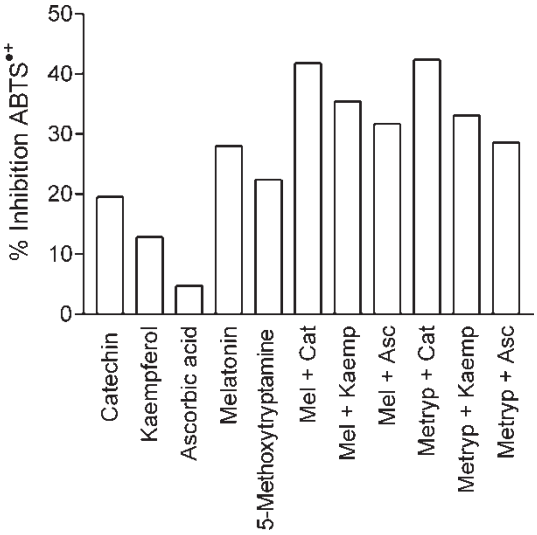


FIGURE 6 Inhibition (%) of $ABTS^{\bullet+}$ radical cation (absorbance at 734 nm) produced individually by polyphenols catechin (Cat) and kaempferol (Kaemp), ascorbic acid (Asc), melatonin (Mel) and 5-methoxytryptamine (Metryp) at 1.66 μM and by a combination of the compounds with indoles (1.66 μM compound plus 1.66 μM indole). $ABTS^{\bullet+}$ in PBS buffer pH 7.2, 5 min time-point. Values are average of duplicates.

TEAC values in Tables I–III were obtained at physiological pH; however, the antioxidant activity of indoles against $ABTS^{\bullet+}$ radical cation was very much dependent on the pH. Thus, at pH 7.2 and 8 the activity of melatonin, pinoline and 5-methoxytryptamine was higher than trolox but it decreased quickly at lower pH values (Fig. 5). On the other hand, in order to study a possible additive or synergistic effect of indoles with other antioxidants in their antioxidant action against the radical cation $ABTS^{\bullet+}$, several combinations were carried out which included an indole, and a flavonoid, ascorbic acid or Trolox. The antioxidant effect against $ABTS^{\bullet+}$ was mainly additive as shown in Fig. 6 for the indoles melatonin and 5-methoxytryptamine alone and in combination with the flavonoids catechin and kaempferol and ascorbic acid. Similar results were obtained for other compounds such as 5-hydroxytryptophan, quercetin and Trolox.

DISCUSSION

The results presented above show the ability of indoles to undergo single-electron transfer reactions in the $ABTS^{\bullet+}$ reduction assay.[7] This assay has worked fairly well to measure total antioxidant activity of dietary antioxidants and foods.[7,26,27]

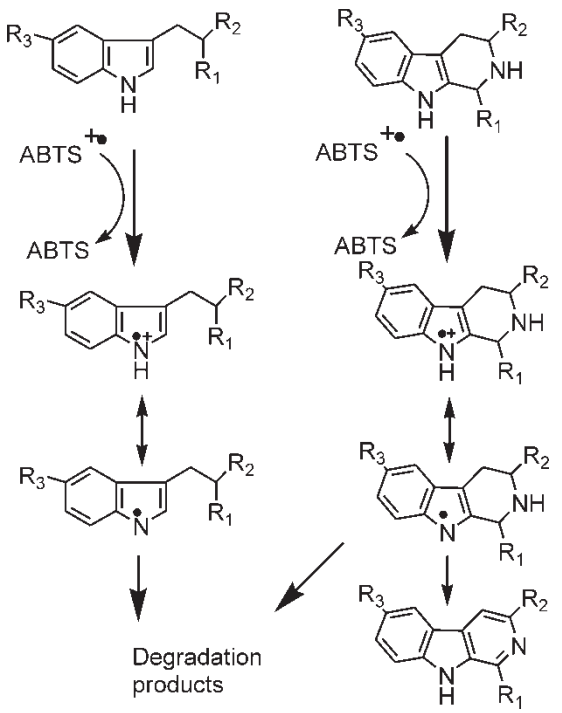


FIGURE 7 Proposed scheme for indoles and tetrahydro-β-carbolines in the process of acting as radical scavengers against free radicals such as $ABTS^{\bullet+}$.

Previously, an ABTS enzymatic assay was employed to determine the total antioxidant activity of plasma and naturally occurring antioxidants.[10,28] The results obtained on the scavenging of $ABTS^{\bullet+}$ may be expected to be in line with the scavenging of other type of radicals.[29] The evaluation of a total of 29 indoles has shown that the majority of compounds with an indole ring moiety exhibited antioxidant activity against $ABTS^{\bullet+}$ at physiological pH. Indole compounds reacted nonspecifically with this radical cation, and indole itself was a weak scavenger of $ABTS^{\bullet+}$. Indole lost this property when the pyrrole is closely modified to give indole-2-carboxylic acid, indole-3-aldehyde, 3-(dimethylaminomethyl)indole (gramine), or further aromatized to afford tricyclic pyridoindoles such as β-carbolines or carbazole. This agrees with the fact that the aromatic β-carboline alkaloids norharman and harman were unable to give electrons to $ABTS^{\bullet+}$.[25] N-methylation of indolic nitrogen ($N-CH_3$) slightly decreased but did not suppress the activity as compared with protonated indolic nitrogen (NH) (see N-methylindoleacetic acid vs. indoleacetic acid). As the antioxidant activity of indoles or tetrahydropyrido indoles against $ABTS^{\bullet+}$ appears to be mediated via the indolic nitrogen, chemical modifications in the side chain of the indole did not eliminate the reaction with $ABTS^{\bullet+}$. However, such changes may affect this activity as seen from the different values of TEAC among indoles. The inclusion of substituents in the indolic ring (i.e. methoxy, hydroxy) in methoxytryptamine, melatonin or 5-hydroxytryptophan increased the activity against the $ABTS^{\bullet+}$. On the other hand, indole structural analogs such as indoline also exhibited antioxidant activity that was even more potent than indole, itself. This is in agreement with the antioxidant activity of the pharmacologically active stobadine,[16] a compound related to this structure.

The ability of indoles to act as radical scavengers in the reaction with $ABTS^{\bullet+}$ might occur through a single electron transfer mechanism (SET). This reaction would initially start at the indole ring affording by a single electron transfer mechanism an indolyl cation radical that might occur in different isoforms depending on the pH (i.e. cation radical losses a proton and become neutral radical in physiological pH) (Fig. 7). This type of mechanism has been proposed for tryptophan oxidation, and melatonin or stobadine while acting as radical traps.[30–32] The indolyl radical could be oxidized further to new compounds that may act as possible electron donors. These compounds may break down to give kynurenines such as N-acetyl-N-formyl-5-methoxy-kynuramine and related compounds as reported in the case of melatonin.[12,33] The course of the reduction of $ABTS^{\bullet+}$ in the presence of indoles followed by HPLC evidenced the consumption of those active indoles such as indole acids, amines, amino acids, peptides and tetrahydro-β-carbolines. With a few exceptions no single major breakdown products or adducts were apparent in the chromatograms. However, some tetrahydro-β-carbolines such as harmaline and MTCA-EE seemed to oxidize to the fully aromatic pyridoindoles (β-carbolines) harmine and 1-methyl-β-carboline-3-carboxylic acid ethyl ester, respectively, as determined by chromatographic and *uv-DAD* and fluorescence data. Previously, it was reported that tetrahydro-β-carboline-3-carboxylic acids afford the corresponding fully aromatic β-carbolines norharman and harman upon oxidation with $ABTS^{\bullet+}$[25]. When indoles are oxidized by reacting with peroxyl radicals, they lost the activity as radical scavengers against $ABTS^{\bullet+}$ as shown in Fig. 2.

These results suggest that indoles in general, may conform a family of radical scavengers and antioxidants that might act as potential protectants and free radical sinks at physiological pH. A few remarkable indoles such as melatonin and indole-3-carbinol are known antioxidants,[9,10,12] but many others, both naturally occurring in the diet and present in biological tissues and fluids, might act similarly. Therefore, a role of indoles as radical scavengers deserves further consideration. Lissi *et al.*[34] reported that some indoles showed a high reactivity toward oxygenated radicals and also decreased oxidation. Various endogenous indoles may provide an antioxidant defense in the brain,[35] and indole-3-propionic acid was a potent radical

scavenger in the brain and protected hepatic microsomal membranes from iron-induced oxidative damage.[36,37] Indoles might participate in the scavenging process of radicals such as $^{\bullet}OH$, peroxyl, and in the protection of proteins[38–42] and there is still the possibility of additive or synergistic actions *in vivo* with other physiological and dietary antioxidants. It should be noticed that among indoles considered here, melatonin exhibited the highest antioxidant activity against $ABTS^{\bullet+}$. Endogenous concentrations of pineal melatonin are in the low nanomolar range, although it might reach higher concentrations in tissues and at intracellular level.[43] Melatonin distribution in tissues is not homogenous and in some places it can reach a physiological level up to two order of magnitude higher than in plasma. This indole is currently being considered for its potential pharmacological actions likely related to its good antioxidant properties.[12] It is noticeable that many other indoles may behave in the same way as melatonin in the process of trapping and detoxification of free radicals. Indeed, some of the indoles studied here may reach higher physiological concentrations in tissues than melatonin, itself. This is especially relevant for CNS indoleamines and amino acids, and for dietary indoles (e.g. the essential amino acid tryptophan, indoleamines, phytohormones, alcohols and tetrahydro-β-carbolines). The essential amino acid tryptophan may reach the high μM level in plasma[44] and is converted to serotonin that reaches from low μM to nM concentrations, and other indoles such as 5-hydroxytryptophan, tryptamine, 5-methoxytryptamine, indoleacetic acid, 5-hydroxyindolacetic acid, tryptophol and pinoline that may appear in the nM range. These levels may vary substantially in different tissues and increase after exogenous intake as occur with indole-3-carbinol.[9] This endogenous distribution of indoles along with a dietary intake is noticeable if compared with polyphenol antioxidants that might need a relatively high amount ingested for a significant presence in plasma.[45] This is, however, compensated with the abundance of polyphenols in the diet.

In summary, endogenous and dietary indoles might act as electron donors and free radical sinks providing antioxidant protection. Nonetheless, this purported antioxidant action of indoles should be checked further on an individual basis for each indole by using various oxidant systems and under different conditions, both *in vitro* and *in vivo*. This action might be limited by the spectrum of intracellular and physiological distribution of each indole, as shown for melatonin which exhibits a very good *in vivo* antioxidant potential owing to its facility to cross the cellular membranes.[12] On the other hand, although indole compounds may act as electron donors as seen here with $ABTS^{\bullet+}$, the indole moiety does not guarantee the absence of undesirable side effects. Following are few examples: hydroxyindoles such as 5-hydroxytryptophan and serotonin may behave as prooxidant in the presence of metals,[42] upon oxidation and formation of new radicals, indoles could provide further toxic or neurotoxic compounds,[46,47] and tetrahydro-β-carboline alkaloids could afford potential neurotoxic N-methyl-β-carbolines following N-methyl bioactivation and oxidation.[48]

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