

Short Communication

Melatonin Scavenges Phenylglyoxylic Ketyl Radicals

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Abstract. The antioxidant properties of melatonin were tested in this work by EPR technique. It was found that melatonin scavenges phenylglyoxylic ketyl radicals. Its effectiveness was 10-times lower than that of vitamin C. A new method of generation of phenylglyoxylic ketyl radicals by spontaneous decomposition of D,L-2,3-diphenyltartaric acid in propan-2-ol was used.

Key words: Antioxidant activity — Melatonin — Spin trapping

Melatonin is a principal hormone of pineal gland which exhibits several physiological functions: regulation of sleep-wake cycles, thermoregulation, reproduction, circadian rhythm, immuno-modulation (Skwarlo-Sonta 1996; Hilton 2002). Melatonin is also known as a free radical scavenger and broad spectrum antioxidant (Reiter 1996; Tan et al. 2000). It scavenges a variety of free radicals and reactive oxygen species reacting with hydroxyl, alkoxy, peroxy and nitric oxide radicals (Matuszak et al. 2003). Melatonin interacts also with singlet oxygen (Matuszak et al. 2003) and hydrogen peroxide (Tan et al. 2000).

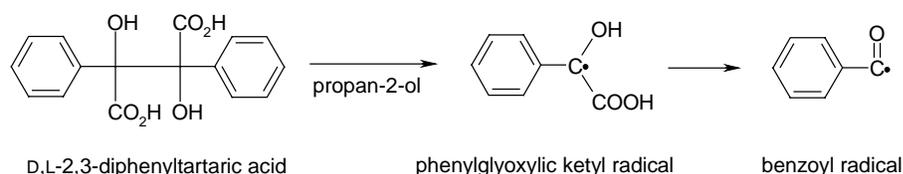
The aim of this work was to determine effectiveness of melatonin to scavenge phenylglyoxylic ketyl radicals.

Melatonin, *N-tert*-butyl- α -phenylnitrone (PBN), L-ascorbic acid and propan-2-ol were purchased from Sigma-Aldrich Chemie GmbH. D,L-2,3-diphenyltartaric acid (DPTA) was prepared by the procedure described previously (Šeršeň et al. 2004; Vencel et al. 2004).

DPTA was used as a source of phenylglyoxylic ketyl radicals undergoing spontaneous decomposition in the propan-2-ol solution according to the reaction shown in Scheme 1 (Šeršeň et al. 2004; Vencel et al. 2004). This spontaneous decomposition was carried out at laboratory temperature. During the first phase of this decomposition, phenylglyoxylic ketyl radicals appeared and were transformed into

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benzoyl radicals during the second phase. The lifetime of these radicals was relatively short, therefore, they were stabilized as their spin adducts with the spin trap (PBN). The lifetime of such spin adducts was longer (some tens of minutes) so that they could be easily recorded by a continual wave EPR spectrometer.



Scheme 1

The EPR spectra were recorded by an instrument ERS 230 (ZWG Akademie der Wissenschaften, Berlin, Germany), which operates in X-band (~ 9.3 GHz) at modulation amplitude 0.1 mT and microwave power 5 mW. UV-VIS spectra were measured using diode array spectrophotometer HP 8452A. All experiments were carried out at 25 °C.

The EPR spectra of propan-2-ol solution containing $0.025 \text{ mol}\cdot\text{dm}^{-3}$ DPTA, $0.05 \text{ mol}\cdot\text{dm}^{-3}$ PBN (control sample) and desired concentrations (0.001 – $0.05 \text{ mol}\cdot\text{dm}^{-3}$) of melatonin or vitamin C were recorded. The ability of compounds tested for scavenging phenylglyoxylic ketyl radicals was determined by the lowest melatonin or vitamin C concentration at which no EPR signal was registered.

The spontaneous decomposition of DPTA ($0.025 \text{ mol}\cdot\text{dm}^{-3}$) in propan-2-ol was monitored by EPR. EPR spectra of spin adducts of PBN with above mentioned radical intermediates described in Scheme 1 were recorded. In the first phase of measurement, the EPR spectrum of the PBN adducts with phenylglyoxylic ketyl radicals was registered (Fig. 1A). This EPR spectrum consisted of three doublets as a result of the hyperfine interaction of unpaired electron with the nitrogen (^{14}N) nucleus spin $I = 1$ (triplet, $A_{\text{N}} = 1.4$ mT) and with the hydrogen (^1H) nucleus spin $I = 1/2$ (doublets, $A_{\text{H}} = 0.24$ mT). The intensity of this signal increased in time and reached maximum after 35 min. After about 15 min, a new signal appeared in EPR spectra which was manifested as an unequal line intensity in doublets and as a shoulder on the first and the last doublets (Fig. 1B). The line intensity of the first sextet began to decrease after 35 min whereas the intensity of the new signal increased (Fig. 1C). After 50 min, only new sextet of lines was observed with following splitting constants: $A_{\text{N}} = 1.4$ mT, $A_{\text{H}} = 0.36$ mT, which has been attributed to the spin adduct of benzoyl radical with PBN (Fig. 1D). After 120 min, no lines in EPR spectra could be observed. It indicated that no radicals were present in the tested solution.

After adding $0.05 \text{ mol}\cdot\text{dm}^{-3}$ of melatonin or vitamin C into the propan-2-ol solution with $0.025 \text{ mol}\cdot\text{dm}^{-3}$ of DPTA and $0.05 \text{ mol}\cdot\text{dm}^{-3}$ of PBN, no EPR signals were observed. In the next experiment we were looking for the minimal

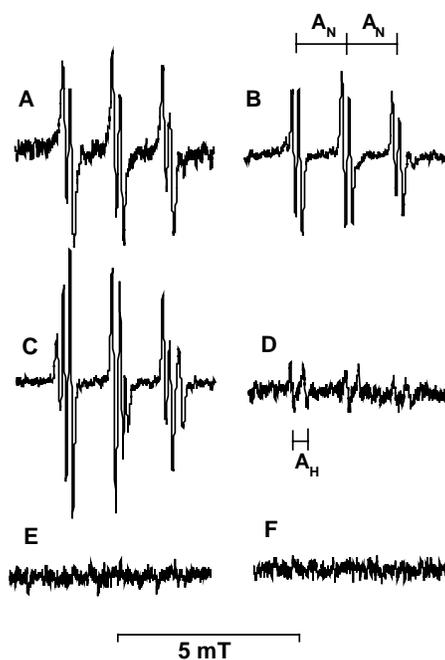


Figure 1. The EPR spectra of the control sample (solution $0.025 \text{ mol}\cdot\text{dm}^{-3}$ DPTA and $0.05 \text{ mol}\cdot\text{dm}^{-3}$ PBN in propan-2-ol) measured 10 min (A), 20 min (B), 40 min (C) and 60 min (D) after mixing. The lines E and F are spectra of the control sample after adding $12.5 \text{ mmol}\cdot\text{dm}^{-3}$ of melatonin or $1.25 \text{ mmol}\cdot\text{dm}^{-3}$ of vitamin C recorded 10 min after mixing. Spectra of B and C were registered at 0.5 amplification.

concentration of melatonin or vitamin C needed for registering of EPR signal in measured samples. It was found that the lowest melatonin concentration was $12.5 \text{ mmol}\cdot\text{dm}^{-3}$ (Fig. 1E). Similar effect was obtained after adding $1.25 \text{ mmol}\cdot\text{dm}^{-3}$ of vitamin C (Fig. 1F). Comparing the antioxidant effectiveness of melatonin to that of vitamin C, it is evident that vitamin C is 10-times more effective than melatonin.

In order to find if melatonin inhibits the spontaneous decomposition of DPTA in propan-2-ol or if it interacts with radical intermediates of this decomposition, an experiment by UV-VIS spectroscopy was carried out. In our previous work (Vencel et al. 2004) it was found that spontaneous decomposition of DPTA is initiated by hydrogen radical which is separated from the central carbon atom of propan-2-ol and it reacts with DPTA. The spontaneous DPTA decomposition is accompanied by changes in the UV-VIS spectra which are displayed by increasing the absorption at 290 nm (Vencel et al. 2004). The increase in this absorption can be observed also in the presence of melatonin (not documented). This result allows us to assume that the spontaneous DPTA decomposition in propan-2-ol takes place also in the presence of melatonin and, consequently, melatonin scavenges phenylglyoxylic ketyl radicals.

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