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Antioxidant and genotoxic properties of South African herbal extracts

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Abstract

This study investigated the antioxidant and genotoxic properties of 13 South African herbal extracts. Results from the single-cell gel electrophoresis (Comet) assay indicated that there were profound differences between the plant extracts in their ability to produce DNA damage, which varied from highly genotoxic to protective. Similarly, water and methanol extracts of all the herbal preparations showed variable potencies in scavenging hydroxyl radicals, as measured by means of electron spin resonance spectrometery (ESR) with the spin trap α-phenyl-*N*-tert-butylnitrone (PBN). In general, methanol extracts were better scavengers of hydroxyl radicals than the corresponding water extracts. This was also true of the ability of these extracts to inhibit membrane lipid peroxidation, assessed with diphenyl-1-pyrenylphosphine (DPPP). However, neither methanol nor water extracts had the ability to protect against DNA damage. The results show that further research on South African traditional herbal extracts is imperative to gain understanding of the mechanisms involved in their pharmacological effects. The tests implemented in the present investigation are recommended for screening other herbal extracts.

Keywords: DNA damage; Comet assay; Lipid peroxidation; Hydroxyl radical; Traditional herbal remedies; South Africa

1. Introduction

The use of traditional herbal remedies as alternative medicine plays a significant role in South Africa, since it forms part of the culture and beliefs of the indigenous population and also features significantly in

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primary health care [1]. However, objective evidence is lacking that these remedies are effective and, more importantly, not harmful. Work conducted on traditional herbal medicine in South Africa has mainly concentrated on the collection, identification and the structural elucidation of their chemical constituents [2,3]. In addition, the use of herbal products for the treatment of different ailments has been compiled [4], while the toxicity of some herbal medicines has been documented [5]. Little information is available on the antioxidant or pro-oxidant properties of these South African herbal preparations. Reactive oxygen species (ROS), generated through normal metabolic processes or from toxic insult, lead to a state of oxidative stress that contributes to the pathogenesis of a number of human diseases by damaging lipids, protein and DNA [6]. In this study, 13 medicinal plant extracts used in South Africa were screened for their antioxidant or pro-oxidant potential. Hot water extracts of these plants were used since they are prescribed as infusions. These infusions and the methanol extracts of the same plant material were tested for their propensity to scavenge the hydroxyl radical ($HO^{\bullet}$), generated via a Fenton-type reaction, as well as for their ability to protect normal human peripheral blood mononuclear cells against lipid peroxidation and damage to DNA.

2. Materials and methods

2.1. Chemicals and solutions

PBN was purchased from the Council for Scientific and Industrial Research, Modderfontein, South Africa. DPPP was obtained from Molecular Probes Inc., The Netherlands and RPMI from Highveld Biological, South Africa. All reagents used were of high-grade analytical quality. Glassware was washed in 30% nitric acid to eliminate background production of the $HO^{\bullet}$ radical.

2.2. Traditional herbal plants

Thirteen herbal plants, which are regularly used to treat several ailments, were identified for analysis. The vernacular and scientific names, their medicinal use and the plant parts utilized are listed in Table 1. These specimens were obtained from the Adler Museum (Medical School, the University of the Witwatersrand, Johannesburg) and their identification was verified by a botanist, Dr. V. Williams (School of Animal, Plant & Environmental Sciences, University of the Witwatersrand, Johannesburg).

2.3. Water and methanol plant extracts

Plant material (0.3 g) was suspended in 3 ml deionised water and was then brewed as tea by boiling for 15 min. In addition, plant material (1 g) was extracted in 10 ml methanol by incubating the mixture overnight at room temperature. All the supernatants were then passed through 0.45-μm acetate filters (Osmonics Inc., USA) and subjected to analyses.

2.4. Electron spin resonance (ESR) spectrometry

ESR measurements were carried out on a Bruker EMX, fitted with an AquaX cell. The settings were as follows: 25 mW, scan time 40 s, scan width 10 Gauss. The ESR software was the Bruker WinAcquisition (Version 3.04) with integrated auto-sampler controlling software. Data analysis and construction of graphs were carried out with Statistica (Version 5.0). The $HO^{\bullet}$ radical was generated by a Fenton-type reaction and measured by the spin trapping method using PBN [7]. Scavenging activity was determined as the percent inhibition of the peak intensity of the control. Experiments were carried out in quadruplicate.

2.5. Lymphocyte isolation

Peripheral blood mononuclear cells (PMNC) were isolated from 10-ml samples of whole human blood, collected into lithium heparin tubes as previously described [8]. Briefly, blood was centrifuged at $275 \times g$ at 4 °C for 10 min. Serum was aspirated and the buffy coat layer was transferred to clean tubes, where an equal volume of cold PBS (pH 7.4) was added and mixed. The buffy coat/PBS mixture was carefully layered onto 3 ml of FICOL histopaque 1077, and after centrifugation at $275 \times g$ and 4 °C for 20 min, the serum/PBS was aspirated and the MNC transferred into a clean tube and washed twice with PBS (pH 7.4) by spinning at $180 \times g$ for 10 min. Cold PBS was added to the pellet, to a final concentration of 1×10^7 cells/ml.