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MEASUREMENT OF TOTAL ANTIOXIDANT ACTIVITY WITH CHLORPROMAZINE RADICAL CATION

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MEASUREMENT OF TOTAL ANTIOXIDANT ACTIVITY WITH CHLORPROMAZINE RADICAL CATION (Abstract): In the present study there is described a simple and sensitive method for the evaluation of antioxidant activity in chlorpromazine-Fe (III) system using ascorbic acid as standard. **Material and method**: Chlorpromazine [2-chloro-N-(3-dimethyl-aminopropyl)-phenothiazine] is oxidized in acidic media by Fe (III) with the formation of a stable radical cation characterized by an intense visible absorbtion band (λ = 540 nm). The optimum parameters for the stability of the radical cation have been studied: molar ratio chlorpromazine-Fe (III), pH, solvents. Spectrophotometric methods have been used in these studies. **Results**: The chlorpromazine radical cation is stable in the acidic media (pH = 3) at a molar ratio chlorpromazine : Fe (III) of 2 : 1. Ascorbic acid reduces these radicals and induces a decrease of absorbance. Percent inhibition was calculated and plotted as a function of the concentration of standard antioxidant solutions. The results show that percent inhibition varies in a linear manner with the ascorbic acid concentration. Percent inhibition is higher when the antioxidant solution is added after generation of radical cation. **Conclusions**: It has been developed a method for evaluating antioxidant activity in the chlorpromazine : Fe (III) system using ascorbic acid as a standard. The method is fast, simple and sensitive; it can be applied for the detection and evaluation of the antioxidant activity of simple or complex systems. **Key words:** CHLORPROMAZINE RADICAL CATION, ANTIOXIDANT ACTIVITY, SPECTROPHOTOMETRIC METHODS

Oxidative stress created by in excess production of free radicals is involved in many degenerative diseases: coronary heart disease, stroke, diabetes mellitus, cancer, rheumatism, aging.

Free radicals derived from oxygen (reactive oxygen species – ROS) or nitrogen (reactive nitrogen species – RNS) are unstable, highly reactive and energized molecules having unpaired electrons. They play an important role in cellular injuries and also initiate the peroxidation of polyunsaturated fatty acids in biological membranes (1,2,3,4).

Aerobic organisms are protected from the radicals effects by a natural antioxidant defence system involving enzymatic and non-enzymatic mechanisms.

At the same time, the exogenous antioxidants from fruits and vegetables have protective effects against cell oxidation (5,6,7,8,9).

Different methods for the evaluation of antioxidant activity have been reported. Such methods often use stable free radicals for studying radical scavenging abilities (10,11, 12,13,14).

This study describes a simple and sensitive decolorization assay based on the generation of chromogenic chlorpromazine radical cation and on its scavenging by ascorbic acid.

MATERIAL AND METHOD

The following reagents were used :

- L (+)-ascorbic acid (Merck) as standard antioxidant ; 0.05 M stock solution was prepared in twice-distilled water ; the solution was diluted just before use ;
- iron (III)-chloride hexahydrate puriss. p.a. (Fluka Chemie Buchs), 0.05 M solution ;
- 37% hydrochloric acid (Farmitalia), 0.1 M solution ;
- chlorpromazine maleate, 0.01 M solution ; the solution was prepared in acidulated water, at light heating.

Experiments were performed using a Hewlett Packard 8453 UV–VIS spectrophotometer.

Preparation of chlorpromazine radical cation solution

Chlorpromazine solution (20 mL, 0.01 M) was added to 10 mL of 1.0 M HCl. Chlorpromazine acidic solution was then treated with 5 mL of 0.005 M $FeCl_3$. Radical cation solution had a maximum absorbance at λ = 540 nm and became stable after 10 min.

In order to study antioxidant activity, different volumes of ascorbic acid solution were added ; the final concentration of ascorbic acid in reaction mixture ranged between 0.125 μmol and 0.750 μmol. The absorbance was determined after 10 min.

Percent inhibition was calculated as : % inhibition = $(1-A_s/A_0)$ x 100, where A_0 is the absorbance of the unscavenged radical cation solution and A_s is the absorbance of the sample after the addition of antioxidant solution.

Percent inhibition was plotted as a function of the concentration of standard antioxidant.

RESULTS AND DISCUSSIONS

The most important property of phenothiazine and its derivatives is their susceptibility to oxidation. They are easily oxidized by different chemical, electrochemical, photochemical and enzymatic agents with the formation of a coloured oxidation product-intermediate radical cation (15).

Chlorpromazine reacts with $FeCl_3$ in acidic media yielding a red coloured radical cation as a result of univalent oxidation of phenothiazine ring.

In the present study, this chromogenic reaction was used in order to develop a decolorization assay for the estimation of antioxidant activity of some natural antioxidants. For this purpose, the optimum parameters for the stability of the radical have been studied.

The stability of oxidation products depends on acidity, concentration of oxidizing agents, time, temperature and the presence of some salts.

The stability of the cation radical colour depends mainly on the oxidizing agent. In the case of strong oxidants, the colour of radical disappears quickly due to the second step of reaction which leads to the formation of a colourless sulphoxid.

The effect of different concentrations of an oxidizing agent on the colour intensity and stability of the radical cation was studied (fig. 1). At a molar ratio chlorpromazine : Fe (III) of 2 : 1 the radical is stable for almost 1 h.

We preferred a molar ratio chlorpromazine : Fe (III) of 8 : 1 in order to avoid an excess of Fe (III) which might interfere with the reaction between antioxidant and radi-

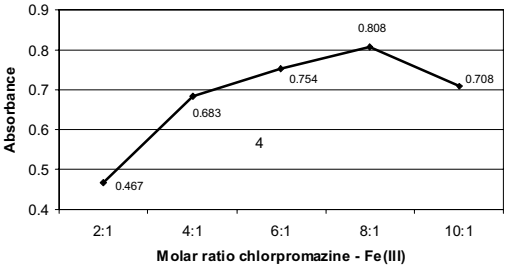

Fig. 1. The influence of molar ratio chlorpromazine : Fe (III) on the stability of the coloured radical cation

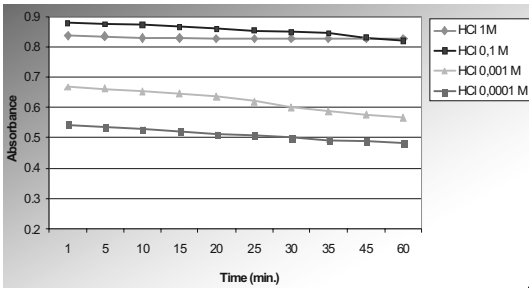

Fig. 2. Time course of chlorpromazine cation formation at different acid strengths (aqueous solutions of 0.001 M–1.0 M HCl)

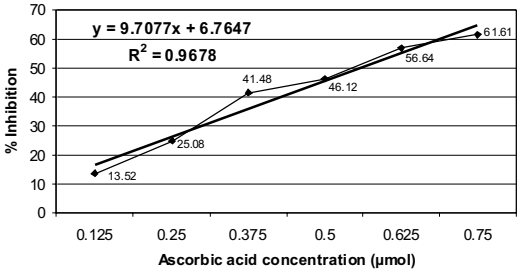

Fig. 3. Effects of ascorbic acid concentration on the inhibition of chlorpromazine radical cation (ascorbic acid was added after generation of chlorpromazine radical cation)

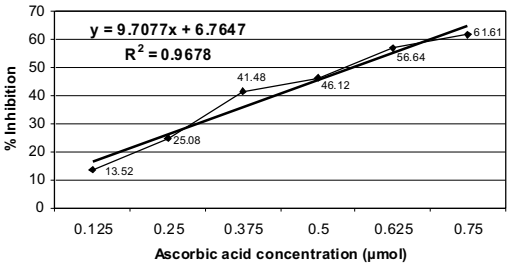

Fig. 4. Effects of ascorbic acid concentration on the inhibition of chlorpromazine radical cation (ascorbic acid was added before generation of chlorpromazine radical cation)

cal cation leading to erroneous results.

The effect of acid strength on the stability of chlorpromazine radical cation was also monitored (fig. 2).

The stability of the radical cation increases in an acid medium.

The inhibition or scavenging of the radical cation by ascorbic acid as standard antioxidant has been studied.

The reaction of ascorbic acid with chlorpromazine radical cation has been found to be fast, sensitive and reproducible.

Percent inhibition was calculated and plotted as a function of the concentration of standard antioxidant solutions in two series of determinations :

- ascorbic acid has been added to reaction mixture after generation of chlorpromazine radical cation (fig. 3) ;
- ascorbic acid has been added to reaction mixture before generation of chlorpromazine radical cation (fig. 4).

The results show that percent inhibition varies in a linear manner in both cases but % inhibition is higher when the antioxidant solution has been added after generation of radical cation.

CONCLUSIONS

The proposed assay for the evaluation of antioxidant activity in the chlorpromazine--Fe (III) system using the ascorbic acid as standard is fast, simple and sensitive ; it can be applied for the detection and evaluation of antioxidant activity of simple or complex systems.

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NOUTĂŢI

KERATINA 17 PROMOVEAZĂ PROLIFERAREA EPITELIULUI ŞI CREŞTEREA TUMORALĂ PRIN POLARIZAREA RĂSPUNSULUI IMUN ÎN PIELE

Tumorile cutanate bazocelulare, incluzând carcinomul bazocelular (BCC) şi hamartomul folicular bazocelular, sunt asociate cu un semnal aberant al genelor Hedgehog (Hh) şi, în cazul BCC, cu un set de multiple variante genetice ale keratinei 5 (codificată de gena KRT5) componenta a filamentului de keratina. Autorii au demonstrat că absenţa /lipsa în tumorile cutanate bazocelulare din cauze genetice a keratinei 17 şi deficitul de co-polimerizare cu keratina 5 întârzie in vivo iniţierea şi creşterea hamartoamelor foliculare bazocelulare la şoarecii care exprimă Hh în epiderm. Aceasta întârziere este precedată de reducerea inflamaţiei şi de polarizarea citokinelor inflamatorii de la Th1 si Th17 cu profil dominant spre un profil dominant Th2. Absenţa keratinei 17 atenuează de asemenea hiperplazia şi inflamaţia la modelele cu dermatită acută. Re-expresia keratinei 17 în *Gli2*^{tg} ; keratinocitele *Krt17*$^{-/-}$ induc selecţia Th1 cu rol deja stabilit în BCC. Se pare că keratina 17 are un rol modulator în expresia Hh în tumorile cutanate bazocelulare ceea ce ar putea avea importanţă în înţelegerea evoluţiei tumorilor cutanate, a psoriazisului şi a reparării leziunilor cutanate (DePianto D, Kerns ML, Dlugosz AA, Coulombe PA. Keratin 17 promotes epithelial proliferation and tumor growth by polarizing the immune response in skin. *Nat Gen* 2010 ; 42 : 910–914).

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