ANTI-OXIDANTS IN HEALTH AND DISEASE

THESIS

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BY

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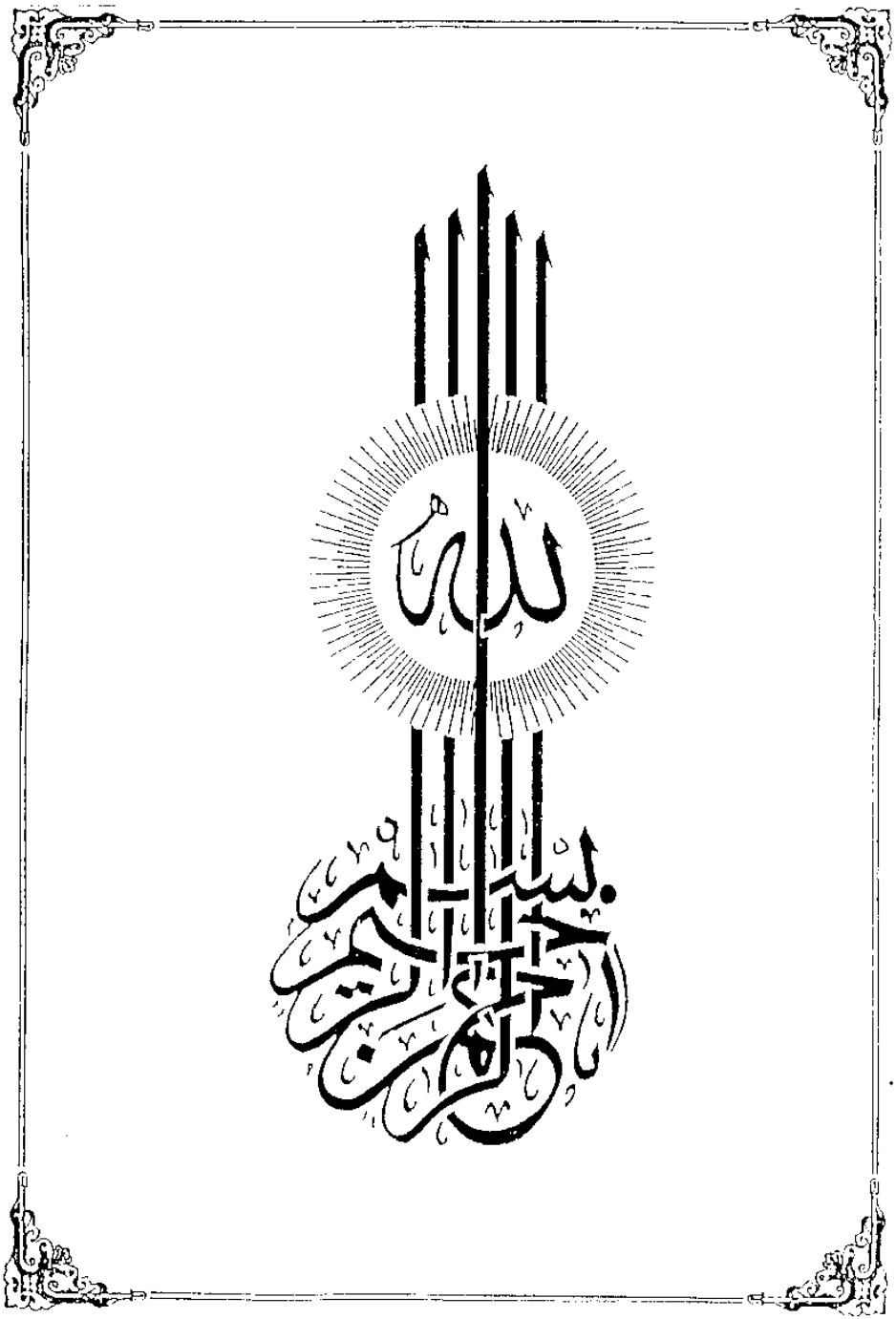

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INTRODUCTION

The principal antioxidants present in human blood, serum or plasma are urate, vitamins C and E, superoxidase dismutase (SOD), and proteins particularly ceruloplasmin, albumin and lactoferrin. Intracellularly there are in addition the enzymes catalase, SOD, glutathion peroxidase, and methionine sulfoxide reductase *(Halliwell, 1989 and Lunec, 1990).*

Antioxidants play an important role in protecting the body from an oxidative insult by peroxides, hydroxyl radicals, and superoxidase anion radicals. Oxidizing agents have been implicated in the pathological changes seen in a wide range of diseases, including alcoholic liver diseases, autoimmune diseases (e.g. rheumatoid arthritis and Chron's disease), emphysema and Parkinson's disease, *(Halliwell, 1989 and Lunec, 1990).*

Currently measurement of antioxidants concentrations in biological fluids has no established place in the laboratory based diagnosis and management of patients. This is due to the unavailability of a routine simple laboratory test and a poor understanding of a likely clinical significance of the results of such tests *(Wayner and Burton, 1990).*

Current assays for antioxidants (the total radical - trapping antioxidant parameter "TRAP") rely on Oxygen Electrode and Pressure Transducer techniques. The Oxygen Electrode Mode has several disadvantages. Only plasma could be used for analysis. Plasma must be both separated and analysed (or stored at - 80°C) immediately. The apparatus (dissolved oxygen analyzer) should warm-up for at least 15 minutes. The temperature needs always to be set-up at 37°C. The experiment takes 60 to 90 minutes. Thorough cleaning of cells after each experiment is necessary. The electrode membranes should be changed regularly (e.g. every other day). The silver anode requires meticulous cleaning and polishing, to prevent any build-up, before a new membrane is attached. The whole electrode needs to be replaced every few month. Room light may photodecompose the initiator, so it is necessary to protect the cell from light exposure. The Pressure Transducer Method is even more cumbersome and inconvenient *(Wayner and Burton, 1990)*.

The Enhanced Chemiluminescent Assay (ECL) for antioxidants can be used for rapid and simple determination of the total antioxidant capacity of serum, as well as a range of other biological fluids including saliva and cerebrospinal fluid *(Thorpe and Whitehead, 1991)*.

Aim of Work

The study has two proposed phases :

1) Enhanced Chemiluminescence antioxidant assay development, with optimization of the assay conditions, choice of quality control materials, measurement of individual antioxidants and testing stability of serum samples.

2) Evaluation of the clinical utility and values of the ECL assay in healthy subjects, as well as in two groups of patients (Diabetics and patients in a nutritional support program).

REVIEW OF LITERATURE

REVIEW OF LITERATURE

I. Oxidative Tissue Injury :

The survival of aerobic organisms in an oxygen environment involves a complicated interplay between the prelogical generation of very reactive chemical species, called free radicals, and the ability of the organism to control these substances *(Royston, 1988).*

A) Free Radicals :

A "free radical" is defined as any chemical species, capable of independent existence, that contains an unpaired electron occupying an outer orbital by itself. Free radicals are usually unstable and highly reactive being capable of abstracting electrons from (i.e. oxidizing) surrounding molecules *(Halliwell and Gutteridge, 1990).*

The common free radicals encountered by cells are listed in table (1). Among others, oxygen derived free radicals (ODFR) have gained particular attention since much of the endogenously derived oxidant stress arises intracellularly from the incomplete reduction of molecular oxygen *(Bast et al., 1991).*

The protonated and unprotonated intermediate forms occurring during reduction of oxygen are shown in Fig. (1) The single electron

Table (1): Types of free radicals with biological relevance

Type of radical	Examples	Comments
Hydrogen-centered	H atom (1 proton, 1 electron)	H atom abstraction from carbon often initiates radical chain reactions, e.g., HO. can initiate lipid peroxidation by abstracting H from the fatty acid side chains of membrane lipids : L–H + HO. → L. + H_2O
Carbon-centered	Tricholoromethyl radical, CCl_3.; carbon-centered radicals in membrane lipids formed by H abstraction (L.)	Major agent in CCl_4 toxicity
Sulphur-centered	Thiyl radical, R–S.	Reactive radical produced during oxidation of thiol compounds (accelerated by transition metals).
Nitrogen-centered	Phenyldiazine radical, C_2H_2H = N.	Involved in phenylhydrazine toxicity to erythrocytes
Oxygen-centered*	Inorganic Superoxide ($O^{\cdot}_2$) Hydroxyl radical (HO.)	Important agents in oxidative stress:hydroxyl very reactive, superoxide poorly so
	Organic Alkoxy radicals (LO.) Peroxy radicals (LO_2.)	Produced during peroxidation by reaction of L. with O_2 (LO_2.) and by metal-dependent decomposition of lipid peroxides (LO. and LO_2); any carbon-centered radical usually reacts quickly with O_2 to yield peroxy radicals (4); e.g., CCl_3. + O_2 → O_2CCl_3 . (trichloromethylperoxy radical).
Transition metal ions	Cu^{+}/Cu^{2+} Fe^{2+}/Fe^{3+} Ti(III) / Ti(IV)	Ability to accept and donate single electrons makes them important catalysts of free radical reactions.

* O_2 itself is a radical; the diatomic oxygen molecule has two unpaired electrons. Hence one-electron reduction of oxygen gives $O^{\cdot-}_2$ (one unpaired electron) and two-electron reduction gives H_2O_2 (no unpaired electrons). Thus H_2O_2 does not qualify as a radical, although its ability to generate HO. makes it an important oxidant.

(Quoted from Halliwell, 1987).

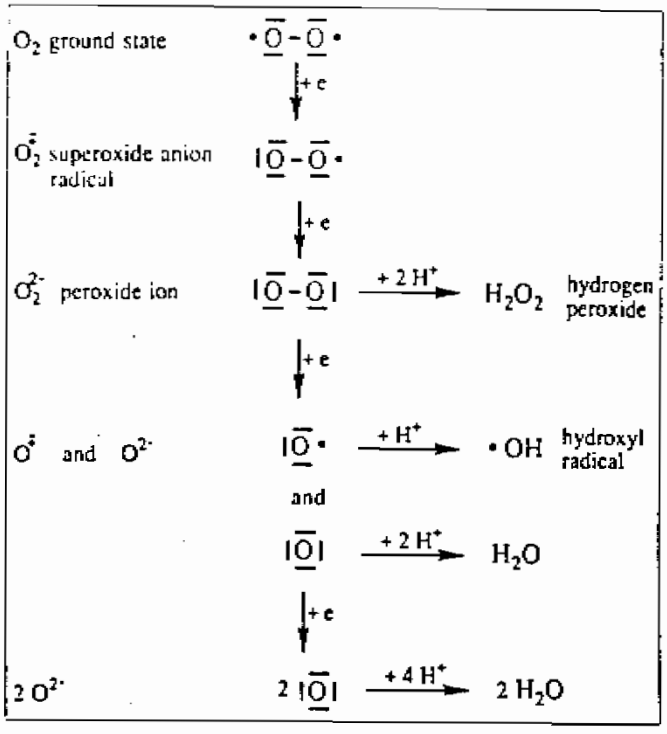

Fig. (1): The univalent reduction of oxygen

(Quoted from Bast et al., 1991)

reduction creates the superoxide anion (O_2^-) which plays a role in initiating the lipid peroxidation that disrupts biological membranes and renders lipoproteins more atherogenic. The two electron reduction of oxygen creates the peroxide ion (O_2^-) which can damage a number of intracellular enzymes. Transition metal ions, as iron and copper, can greatly amplify the threat from the peroxide ion. This is because they can easily undergo single valency changes thus providing an ideal template for electron transfers involving free radicals. This is most clearly illustrated by the interaction of

hydrogen peroxide (H_2O_2) and ferrous ions which react to form a ferric ion and the highly reactive hydroxyl radical (•OH) (The Fenton reaction):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^-$$

The extremely reactive hydroxyl radical may then be capable of widespread damage to local structures including DNA. Furthermore, several forms of singlet oxygen ($^1O_2$) are produced intracellularly, *(Halliwell, 1987; Royston, 1988).*

B) Sources of Free Radicals :

During the course of lifetime, the human body is continuously exposed to potentially harmful oxidative stresses. These may arise from exogenous as well as from endogenous sources *(Bast et al., 1991).*

Exogenous sources of oxidants include drugs, environmental pollutants and irradiation.

Many of the anthracyclic antineoplastic agents and other antibiotics that depend on guinoid groups or bound metals for activity are able to generate oxygen radicals. Many of the chemotherapeutic effects and cytotoxic side effects of these drugs