ANTIOXIDANT EFFECTS OF Convolvulus pluricalus IN RAT BRAIN MITOCHONDRIA AGAINST OXIDATIVE DAMAGE INDUCED BY GAMMA RADIATION AND PHOTOSENSITIZATION

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Abstract

Aqueous and methanolic extracts of natural herb, Convolvulus pluricalus (CP) was monitored for their antioxidant ability against photosensitization and radiation induced damages in rat brain mitochondria. Both the extracts of CP (50 µg/ml assay) showed significant protection against the formation of the lipid peroxidation product, TBARS. Protein oxidation and degradation of proteins was shown to be significantly high following photosensitization, while CP extracts could effectively modify these damages. Exposure of plasmid DNA pBR322 to gamma radiation resulted in enhanced formation of single strand breaks (SSB). Simultaneous treatment of pBR322 DNA with CP extracts during radiation effectively prevented the damage. Further, these extracts also showed inhibition against stable radicals, 2, 2’- azino-bis (3- ethylbenzthiazoline-6- sulfonylic acid (ABTS) and 1, 1-diphenyl-2-picryl hydrazyl (DPPH). The scavenging capacity of the extracts was quantified by pulse radiolysis experiments. Hence, Convolvulus pluricalus may emerge as potent antioxidant/radioprotector, capable of scavenging singlet oxygen, hydroxyl radical, as well as other stable radicals, ABTS and DPPH.

Introduction

Oxygen derived reactive oxygen species (ROS) and their adverse effects are inevitable for aerobic organisms. Cellular constituents, lipids, proteins and DNA are the crucial targets for ROS attack. Overexpression of ROS is being implicated in several degenerative diseases [1,2]. Therefore intake of antioxidants from natural origin with less toxicity (compared to synthetic antioxidant) in relation to human health is useful. Plants have several phytochemicals/micronutrients with creditable medicinal values useful in ayurveda [3]. Since photosensitization, as well as radiation exposure can result in oxidative stress through reactive oxygen species (ROS) [4,5], the aim of the present study was to evaluate the effect of phytoconstituents of the medicinal plant, Convolvulus pluricalus (Family-
Convolvulaceae, Fig.1) The plant occurring in the plains of North India is a known intellect-promoter, psycho-stimulator and an excellent brain/ cardio-tonic [6].

**Materials and Methods**

**Assessment of oxidative damage**

Oxidative damage to lipids was studied by monitoring formation of TBARS while protein oxidation and degradation of mitochondrial proteins were assessed by measuring carbonyl contents [4,5] and SDS PAGE [7] respectively. Plasmid pBR322 was exposed to γ radiation at 6 Gy with and without CP extracts and protection by CP was assessed by gel electrophoresis and quantification. Assays of DPPH and ABTS were carried out by standard methods [8,9].

**Results and Discussion**

**Membrane peroxidation and protein oxidation**

Due to the high contents of polyunsaturated fatty acids, membranes are prone to the attack by ROS forming several peroxidation products which are toxic to cells. One of the stable products is thiobarbituric acid reactive substances (TBARS). Using photosensitization system the antioxidant potential of CP extracts was monitored using the TBARS assay. A significant increase in TBARS was demonstrated when mitochondria were exposed to photosensitization for 15 min. Simultaneous addition of methanolic or aqueous extracts of CP (50 µg/ml) in independent experiments during photosensitization could prevent TBARS formation effectively. The results demonstrated about 40% decrease in TBARS levels by both the extracts of CP (Fig 2).

Oxidation of protein due to ROS is one of the important and deleterious reactions of membrane proteins implicated in several diseases including aging [10]. Photosensitization showed enhanced formation of protein carbonyls, the index of protein oxidation. The extent of formation of protein carbonyls was high at 15 min photo exposure. Presence of CP extracts, however,
exerted protective effects against this damage (Fig 3).

Photosensitization also induced time dependent degradation of the mitochondrial proteins. The effect of CP on photosensitization-induced degradation of brain mitochondrial proteins was assessed by SDS PAGE (Fig 4). The intensity of protein bands was less in the lanes containing photosensitized samples (exposure time 30 and 60 mins). The intensity was considerably less at 60 min exposure compared to that at 30 min. Photosensitization of mitochondria in presence of CP (50 µg /ml) prevented protein degradation as seen from the higher intensity of bands.

**Radiation exposure to plasmid DNA pBR322**

Generation of ROS especially through low LET ionizing radiations and its profound damaging effects on cellular system is well known. In the present studies expression of radiation damage and protection of DNA repair mechanism is explored by studying a simple cell free system i.e. plasmid pBR322. The pBR322 DNA was exposed to radiation (6 Gy) with and without CP extracts. Radiation exposure induced single strand breaks (SSB) in closed circular form (CC-Form I) of pBR322 increasing the open circular form (Form II). It is evident from the gel image that CP extracts reversed the effect of radiation by preventing the formation of SSB. The results are compared with unexposed control (Fig 5).
These extracts also showed inhibition against stable radicals, 2, 2’- azino-bis (3-ethylbenzthiazoline-6- sulfonic acid (ABTS)) and 1, 1- diphenyl-2-picryl hydrazyl (DPPH). Scavenging capacity was compared with standard antioxidants (Fig 6). The rate constant of CP by pulse radiolysis technique in presence of ABTS was evaluated as $4 \times 10^{-4} \text{S}^{-1}$. Thus based on the above results, preparations from *Convolvulus pluricalus* appear to be potent antioxidants, capable of scavenging singlet oxygen, hydroxyl radical, as well as other radicals, ABTS and DPPH.

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