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Alteration in the Antioxidant Potential of *Aloe vera* Due to Fungal Infection

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Abstract: Under most pathological conditions there is generation of reactive oxygen species and other free radicals. These, in turn, alter the structure and functions of bimolecules, the accumulation of which is responsible for reversible or irreversible damage to the tissue. Ageing too involves identical oxidative damage to the cells and tissues. Though the cells have an inherent ability to counter the oxidative stress, a variety of herbs are employed in strengthening this. *Aloe vera* is one of these herbs capable of enhancing the antioxidant defenses of the subject and is commonly employed as general detoxifier and in the treatment of surface wounds skin infection, arthritis, asthma, liver disorders, kidney infection and many more ailments. Undesirable consequences may, however, result from use of herbs infected by certain fungi. *Aloe vera* infected with *Alternaria alternata*, a deuteromycete fungus which is known to cause allergy and asthma in man, was encountered. To ascertain the effect of infection of leaves of *Aloe vera* by *Alternaria alternate*, the current study was planned. The free radicals were generated using chemical system and the ability of the healthy plant, infected plant and fungal biomass in scavenging them was assayed. It was noted that the antioxidant potential of the herb diminished due to the infection by *A. alternate*. This emphasizes the need to screen the medicinal plants before they are used for therapeutic preparation.

Key words: *Aloe vera*, pro-oxidant, antioxidant, fungus, reactive oxygen species

INTRODUCTION

A vast majority of diseases seem to result from a shift in the balance between the pro-oxidant and antioxidant potential of the tissues. The pro-oxidant conditions arise from an increased generation of free radicals under oxidative stress, due to inadequate scavenging of free radicals or due to the depletion of the dietary supply of antioxidants is a common cause of pro-oxidant conditions (Govindarajan *et al*., 2004; Ashok, 2001). Accumulation of oxidative damages results in aging and the related ailments such as Alzheimer's disease, atherosclerosis, liver cirrhosis, diabetes and even cancer (Dong-Jiann *et al*., 2004). Reactive Oxygen Species (ROS), a collective term that encompasses oxygen free radical, superoxide radicals, hydroxyl radicals and other non-radical derivatives of molecular oxygen inflict injuries to the tissues through lipid peroxidation and covalent binding with a variety of biomolecules (Govindarajan *et al*., 2004).

A variety of plants with their natural antioxidant capacities provide a great potential for developing novel preparations against various pathologies and ailments related to aging. A number of herbal remedies with anti-inflammatory, digestive, anti-narcotic, neuroprotective and hepatoprotective properties have been shown to possess antioxidant and radical scavenging capacity as the mechanism of their action (Dong-Jiann *et al*., 2004). Some of these herbs have been extensively investigated for their antioxidant and radical scavenging constituents. One among them is *Aloe vera*.

Aloe vera a native from North Africa and Spain has emerged as a wonder herb in the system of alternative medicine. Aloe species have been used for centuries for their laxative, anti-inflammatory, immuno-stimulant, anti-septic effect (Capasso *et al*., 1998) wounds and burn healing capacities (Chitra *et al*., 1998) and anti-ulcer (Koo, 1994) and anti-tumor (Saito, 1993) as well as anti-diabetic (Ajabnoor, 1990) properties. The therapeutic applications of *A. vera* are mostly due to the ability of this plant to enhance antioxidant defenses of the consumer.

The collection of the plant material for medicinal preparation is however indiscriminate without any attention paid to the origin, purity, safety, efficacy, botanical identity and method of cultivation. Hence the objective of this work has been to investigate the effect of infection of *A. vera* by *A. alternata* so as to emphasize the need of screening the plant material to be employed as medicine.

MATERIALS AND METHODS

Chemicals: Trichloroacetic Acid (TCA), Thiobarbituric acid (TBA) Loba chemicals, sodium nitropusside,

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sulphanilamide, napthylethelenediamine Sd. Fine Chemicals, Nitro-blue Tetrozolium salt (NBT), Nicotinamide Adenine Dinucleotide Reduced Disodium salt (NADH) Sisco Res. Lab. All chemicals used were of analytical grade.

Plant material: *A. vera* leaves were washed and sliced to separate the gel by scratching. Thus gel was then blended and stored in the refrigerator for further use.

Infection of *A. vera* by *A. alternata*: Infection of *A. vera* by the *A. alternata* was first reported by Gupta and Masood (2003). But for the purpose of this study *A. vera* was infected by the fungus and its pathogenicity was established by Koch's Postulate as described by Aneja (1993).

Radical scavenging activity: Various *in vitro* models have been used to generate the free radicals and the ability of antioxidants to reduce them has been measured.

Superoxide radical scavenging activity: The superoxide radical scavenging capacity was studied using the method of Flohe and Otting (1984). The free radicals were generated in a chemical system containing PMS-NADH and superoxide scavenging capacity was assayed by the reduction of NBT, in presence and absence of the *A. vera* gel.

Nitric oxide radical scavenging activity: Nitric oxide radical scavenging capacity of the herb was determined using the method of Shreejayan and Rao (1997). This method is based on the inhibition of nitric oxide radicals generated from sodium nitroprusside in buffered saline and measured by Griess reagent.

Hydroxyl radical scavenging activity: Capacity of the *A. vera* gel to scavenge hydroxyl radicals was assessed by the method described by Mualik *et al.* (1999). Hydroxyl radicals were generated *in vitro* using Fe^{+3}/H_2O_2/EDTA/ ascorbate system based on Fenton reaction. Scavenging of these hydroxyl radicals in presence and absence of the *A. vera* gel was measured.

Lipid peroxidation assay: For *in vitro* studies, liver of normal rats was dissected and homogenized in ice-cold phosphate buffer (20 mM, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 14, 000 rpm for 15 min. One milliliter aliquot of the supernatant was incubated separately with the gel extracted from healthy *A. vera* leaves, fungal infected *A. vera* leaves and fungal extract in the presence of 15 mM $K_2Cr_2O_7$ at 37°C for 1 h. The reaction was stopped and MDA levels were estimated by the method of Okhawa *et al.* (1979). The capacity of *A. vera* gel from healthy and infected leaves as well as of the fungal biomass in inducing inhibition of lipid peroxidation by rat liver was calculated ad expressed as % inhibition.

Evaluation of iron-chelating activity: Ability to chelate iron was studied by the method described by Iwasa and Torri (1962).

Determination of total flavonoid content and phenolic content: The two complementary colorimetric methods, using aluminum chloride ($AlCl_3$) and 2, 4-dinitrophenylhydrazine (2, 4 DNPH) as described by Chang *et al.* (2002) were used for determining total flavonoids content. Total phenolic compounds were determined using Folin-Ciocalteu method as described by Singleton and Rossi (1965).

RESULTS AND DISCUSSION

Formation of free radicals during normal cell metabolism is well known (Shiow and Jiao, 2000). Scavenging of these free radicals involves donation of electrons and protons to ROS by the antioxidants. The antioxidant quenches ROS and converts them into more stable and less damaging species. Antioxidant potential owes to the radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing capacity of a tissue or drug (Salma *et al.*, 2004).

Nitric oxide is a free radical produced in mammalian cells during various physiological processes. However, excess production of NO is implicated in inflammation, cancer and other pathological conditions (Yaoward *et al.*, 2004). Figure 1 shows the nitric oxide radical scavenging ability of gel extracted from healthy and fungal infected *A. vera* leaves and of the fungal biomass. Radical scavenging activity is expressed as IC50, which is concentration of the drug required to achieve 50% inhibition of free radicals. The IC50 value for healthy *A. vera* is 0.1 mg. The antioxidant principle is the gel from healthy *A. vera* leaves converts the nitric oxide free radicals into nitrite radicals by donating electrons and protons, (which are otherwise donated to molecular oxygen to form water accompanied by release of water). The IC50 value for gel extracted from infected *A. vera* leaves is 0.25 mg suggesting reduction in the antioxidant principle. The fungal biomass exhibited infinitesimal IC50 value since the radical scavenging capacity is absent.

Superoxide and hydroxyl radicals are the most significant free radicals in the damaging the cells. In the cellular oxidation reaction, superoxide radical is normally

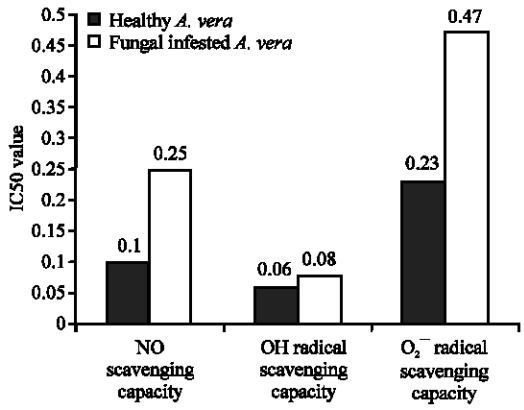

Fig. 1: Free radical scavenging capacity of gel extracted from healthy and fungal infected *A. vera* leaves

formed first and its effect gets amplified as it produces other kinds of cell damaging oxidizing agents.

Among these free radicals the damaging action of hydroxyl radicals is the strongest. Superoxide and hydroxyl radicals actively initiate the lipid peroxidation reaction (Liu and Ng, 2000) Fig. 1 also records the superoxide and hydroxyl radical scavenging activity of the gel from healthy *A. vera* leaves, gel from fungal infected *A. vera* leaves and of the fungal biomass. The IC50 value was found to be less for healthy *A. vera* indicating higher radical scavenging capacity. The IC50 value for the gel extracted from *A. vera* leaves infected by *A. alternata* was more so that the capacity to scavenge hydroxyl and superoxide free radicals is lesser. Since the IC50 value for fungal biomass of *A. alternata* was infinitesimal, it is evident that the reduction in antioxidant capacity of the herbs is due to the fungal infection.

In presence of certain flavoenzymes such as glutathione reductase Cr (VI) is reduced to Cr (V) with a coupled oxidation of molecular oxygen to superoxide radical. The superoxide radical being a more potent oxidizing agent, it is dismutated to hydrogen peroxide. The hydrogen peroxide thus formed reacts with Cr (V) and in the Fenton like reaction hydrogen free radicals are generated. Thus during thee one-electron reduction of Cr (VI), a whole spectrum of ROS is generated (Jianping *et al*., 1999) and his is responsible for lipid peroxidation.

Fig. 2, shows that the gel extracted from healthy *A. vera* leaves inhibits Cr (VI) induced lipid peroxidation by 95 %.

The inhibition could be due to scavenging of hydroxyl and superoxide radicals. The gel extracted from fungal infected *A. vera* induced 30 times increase in the lipid peroxidation while the fungal biomass caused a

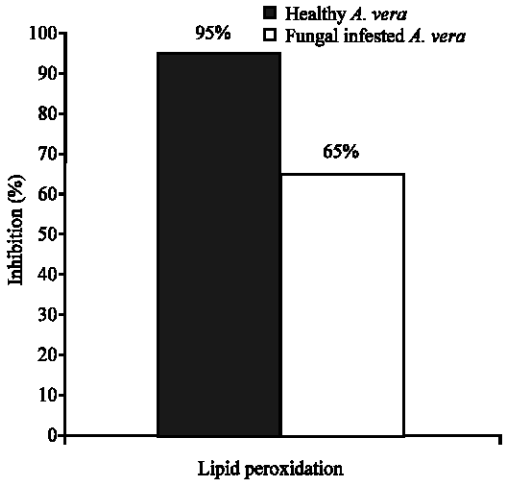

Fig. 2: Inhibition of lipid peroxidation in tissue homogenate incubated with healthy and fungal infected *A. vera* leaves

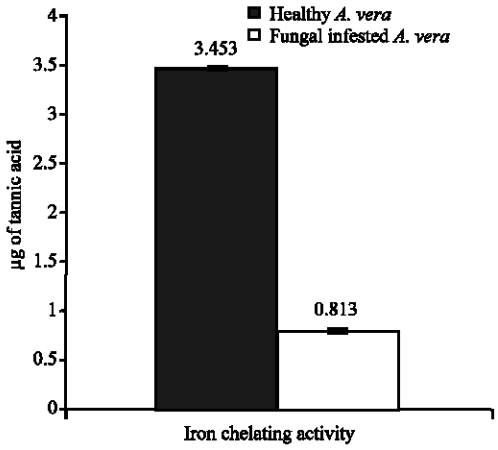

Fig. 3: Iron chelating activity in gel extracted from healthy and fungal infected *A. vera* leaves

further 100 times increase in lipid peroxidation. This indicates that the fungus *A. alternata* is responsible for the increase in peroxidation.

The Cr (VI) induced lipid peroxidation could be also inhibited by chelating iron and thus altering the ration of Fe^{+3}: Fe^{+2}. The iron chelating capacity can thus be equated to antioxidant potential.

The gel extract of healthy *A. vera* leaves was found to have a higher iron chelating capacity as compared to the gel obtained from *A. vera* leaves infected by *A. alternata*. Moreover the fungal extract of *A. alternata* exhibited no iron chelating ability (Fig. 3) so that there is room for the assumption that the fungal infection diminishes this iron chelating ability.

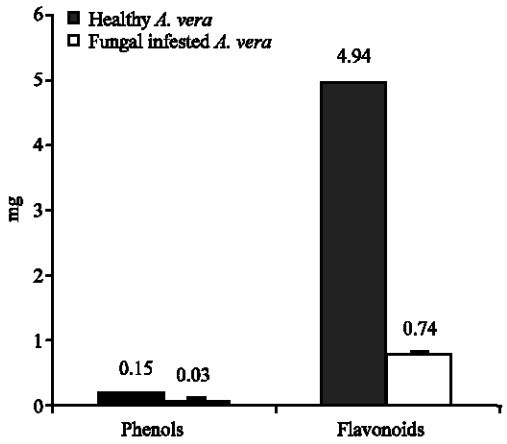

Fig. 4: Total phenolic and flavonoid content in the gel extracted from healthy and fungal infected *A. vera* leaves

Govindarajan *et al.* (2003) have recorded similar observation related to iron chelating activity while working on *Picrorhiza kurrora* royle ex. Benth.

The most common antioxidant principles in plants are the polyphenols including flavonoids. Polyphenols have an important role in stabilizing lipid peroxidation and are associated with antioxidant activity. They may contribute directly to antioxidant action (Dong-Jiann *et al*., 2004). Phenols and flavonoids content of the gel from healthy *A. vera* leaves is 5 and 6 times higher respectively than in the gel from *A. vera* leaves infected with *A. alternata*, which in itself has no phenolic or flavonoids content (Fig. 4).

CONCLUSIONS

A. vera extract can strengthen the antioxidant defenses of the consumer though care must be taken in ensuring healthy state of the herb. Infection of *A. vera* leaves by *A. alternata* not only reduces the efficacy of the herb but also nullifies its effect and may even exert an undesirable influence on the consumer. Standardization norms should be defined and enforced for therapeutic herbal preparation from *A. vera*.

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