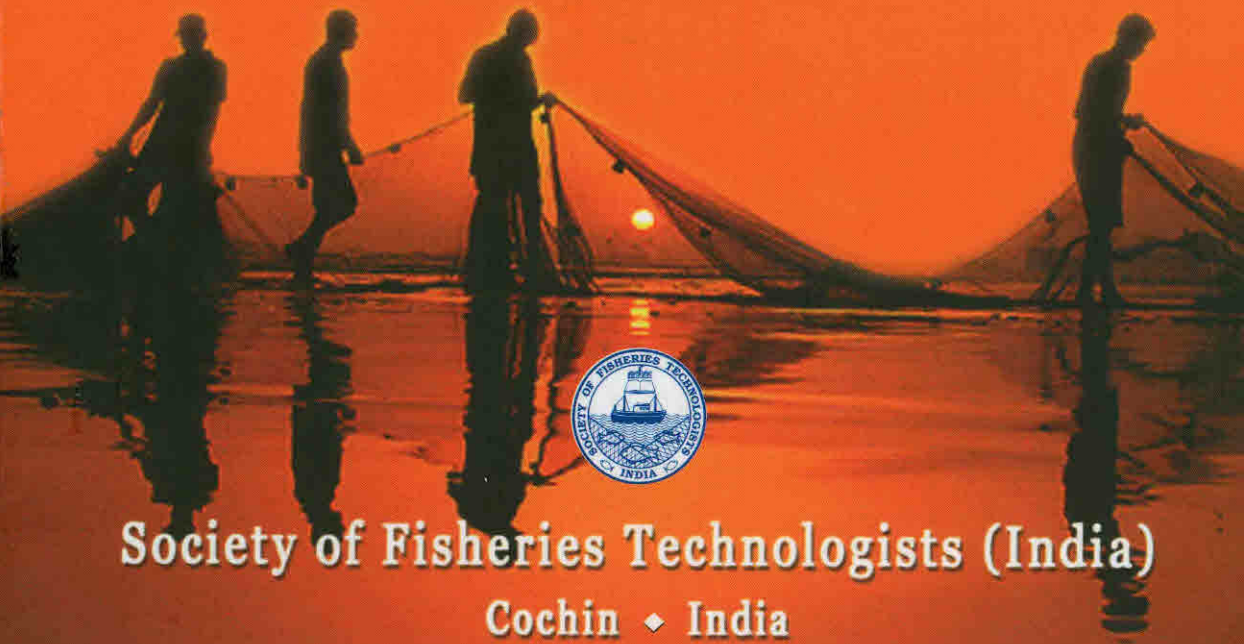
Coastal Fishery Resources of India

Conservation and Sustainable Utilisation



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Coastal Fishery Resources of India: Conservation and Sustainable Utilisation

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2010

Effect of Red Mangrove (*Rhizophora apiculata*) Root Extract on Sodium Nitrite-induced Oxidative Stress in Rats

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Introduction

Oxidative stress arising from an imbalance in the antioxidant system is responsible for the ailments of the heart, brain dysfunction, immune system decline and also for aging. Thus there has been an increasing interest in recent years in healthy life styles and interest in antioxidants and food supplements has grown remarkably. The role of natural products as a source for remedies has been recognized since ancient times (de Pasquale, 1984). An analysis of a number of chemotherapeutic agents and their sources indicates that over 60% of approved drugs are derived from natural compounds (Cragg *et al.*, 1997). With a proven record of natural products in drug discovery, there is a compelling need for expanding exploration of nature as a source of novel healing agents. Besides the endogenous antioxidant defenses that combat the oxidative stress, consumption of antioxidants appears to be important. Antioxidants can be either synthetic or of natural origin. The use of synthetic antioxidants in food is being discouraged by many health regulating agencies in recent times. Plants are good sources of natural antioxidants (Shahidi, 2000), which has been the basis of numerous studies in the last decade. Among these, flavonoids and related phenolics have gained importance. Unpublished data on mangrove plant, *Rhizophora apiculata* or red mangrove, from CIFT, Cochin and earlier studies have established that the root of this plant is a rich source of flavonoids and has several low molecular weight compounds like glucosides, fatty acids, sterols and hydrocarbons (Sharaf *et al.*, 2000). Perera *et al.* (2008) extracted polyphenolic compounds and flavonoids from root and bark of *Rhizophora mangle*, a species closely related to *R. apiculata*. Several investigators have established that mangroves are also rich in polyphenols, among

which flavonoids are a significant group (Kathiresan and Ravi, 1990; Ravi and Kathiresan, 1990; Achmadi *et al.*, 1994). The aim of the present study was to examine the effect of the ethanolic root extract of the mangrove plant *Rhizophora apiculata* on experimentally induced oxidative stress in brain tissue of male albino rats.

Materials and Methods

Standards like glutathione, epinephrine, glutathione reductase, NADPH and tetraethoxy propane were obtained from M/s. Sigma Chemical Company, St. Louis. MO, USA. All the other chemicals used were of analytical grade. Adult male Wistar strain albino rats, weighing 100-120 g were selected for the study. The animals were housed individually in polyurethane cages under hygienic conditions and maintained at normal room temperature. The animals were allowed food and water *ad libitum.* The experiment was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee.

Oxidative stress in the brain of the experimental groups of rats was induced by injecting a single subcutaneous dose of sodium nitrite (75 mg.kg body wt^{-1}. day^{-1}) (Naik *et al.*, 2006). Experimental protocol: Four days after acclimatization, the animals were divided into three groups of 6 rats each. Group I rats were fed commercial diet and taken as control. Group II rats were also fed commercial diet and administered a single dose of sodium nitrite (s.c.) on day 10 of the study. Group III rats were given the mangrove plant root extract orally for a period of 10 days. 30 min after giving the last dose of the extract, sodium nitrite was injected. One hour after the injection the rats were sacrificed and brain tissue was excised immediately and washed with chilled isotonic saline. Accurately weighed brain tissue was homogenized in ice-cold 0.1 M Tris-HCl buffer, pH 7.2 and centrifuged. The supernatant was used for further biochemical analyses. Total reduced glutathione (Ellman, 1959), lipid peroxides (Ohkawa *et al.*, 1979) and antiperoxidative enzymes, superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (CAT) (Takahara *et al.*, 1960) and glutathione peroxidase (Paglia and Valentine, 1967) were assayed. Protein was estimated by the method of Lowry (Lowry *et al.*, 1951). Results were expressed as mean±SD, and Student's t-test was used to assess statistical significance.

Results and Discussion

Sodium nitrite combines with haemoglobin in the blood to form methaemoglobin, which has a much higher (up to 20 times) affinity for oxygen and therefore cannot be exchanged with CO_2 in the tissues, thus causing hypoxia. Brain is one of the most important organs of the living organism. Glucose is the chief source of energy that the brain utilizes and in the process generates considerable levels of free radicals. The brain is uniquely vulnerable to oxidative injury, due to its high metabolic rate and elevated levels of polyunsaturated lipids, the target of lipid peroxidation (Reiter 1995). Endogenous oxygen- and nitrogen-centered free radicals are considered to play a decisive role in a variety of neurodegenerative diseases. In the present study oxidative stress in the brain is induced by injection of a single dose of sodium nitrite (s.c.). The levels of lipid peroxides were significantly ($p<0.001$) elevated (Fig. 1) in sodium nitrite-injected Group II rats when compared to Group I control rats. This is in agreement with other published reports (Tsoi *et al.*, 2008). Sodium nitrite is reported to stimulate free radical generation (Naik *et al.*, 2006). It combines with biological amines to form nitrosoamines which in turn react with biomolecules like DNA and enzymes disrupting their structure and function. The content of lipid peroxides was considerably ($p<0.001$) decreased in mangrove root extract-supplemented Group III (Fig. 1) rats

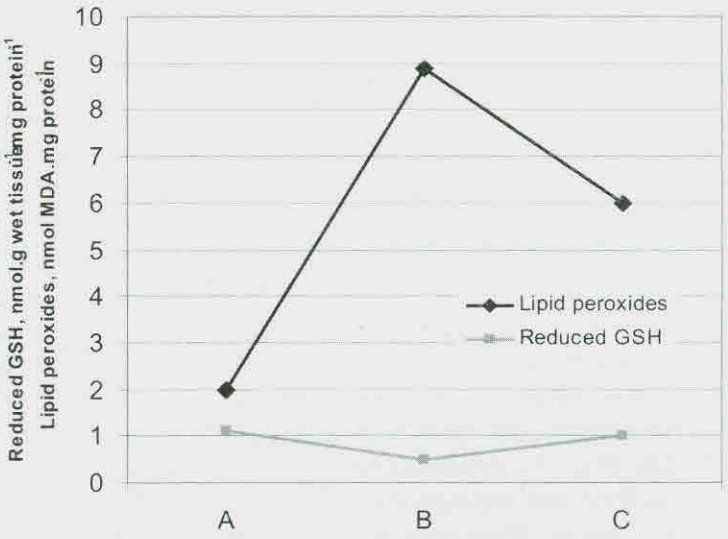


Fig. 1: Levels of reduced glutathione and lipid peroxides in control (A), Sodium nitrite administered (B) and Mangrove root extract administered (C) rats

when compared to Group II rats. Several reports support this observation. Shutenko *et al.* (1999) describe that flavonoids attenuate free radical-induced peroxidation during ischemia in rat brain. Teselkin *et al.* (1998) report that flavonoids reduce lipid peroxidation in rats following radiation exposure.

Glutathione is a small non-protein tripeptide (L-glutamyl cysteinyl glycine) thiol antioxidant molecule that plays a significant role in thwarting oxidative damage in all types of cells and organs. It plays a central role in coordinating the body's antioxidant defense processes and detoxification. Glutathione is a component of a pathway that uses NADPH to provide cells with their reducing milieu. This is essential for (a) maintenance of the thiols of proteins and of antioxidants (e.g. ascorbate, alpha-tocopherol), (b) reduction of ribonucleotides to form the deoxyribonucleotide precursors of DNA, and (c) protection against oxidative free radical damage, and other types of toxicity (Meister, 1991). Perturbation of GSH status of a biological system reflects defunct oxidant defense system. In the present study, a significant ($p<0.001$) decrease was observed in the levels of brain GSH content in sodium nitrite-treated Group II rats when compared to Group I normal control rats. Similar observations were reported by other investigators which establish the fact that GSH depletion occurs following sodium nitrite administration. Depletion in GSH was thought to be due to enhanced consumption in the ROS (Reactive Oxygen Species) ravaged brain cells. In a cell under attack from ROS, there is enhanced utilization of antioxidant molecules like GSH and antioxidant enzymes (Comporti, 1985). The reduction in GSH may be also due to increased degradation, reduced synthesis and reduced rate of reformation from oxidized state GSH. Glutathione being a potent cellular reductant with a broad redox potential, acts as a scavenger of peroxides and ROS and serves as a storage and transport form of reduced sulphur. It has been shown also that glutathione also acts as a regulator of gene expression (Alscher 1989, Baier and Dietz 1997). In view of the numerous physiological roles that GSH plays in a living cell, a decline in GSH levels would deprive the cells of many of its specific roles exacerbating the oxidative damage. In the present study the brain content of GSH was significantly ($p<.0.001$) increased in mangrove root extract-treated Group III rats when compared to Group II rats (Fig. 1). Antioxidants have the ability to scavenge free radicals and restore the endogenous antioxidant system thus protecting the cells from damage. Flavonoid rich mangrove root extract was shown to augment GSH and exert cytoprotective effect in experimental conditions that produce oxidative stress. Myhrstad *et al.* (2002) had demonstrated

that flavonoids increased intracellular GSH levels by activating gamma glutamyl synthetase activity. In a study reported by Ishige *et al.* (2001), one of the mechanisms by which flavonoids protected neuronal cells from oxidative damage was by elevating the cellular GSH content. The prior administration of flavonoid rich root extract to rats intoxicated with sodium nitrite prevented the decline in GSH content in the brain tissue of Group III rats. This reveals the antioxidant capacity of mangrove root extract. Explanations of the possible mechanism underlying the protective action include the prevention of GSH depletion and destruction of free radicals. The extract strengthens the endogenous antioxidant defenses to fight ROS damage and restore the healthy state of the cell by neutralizing the reactive species.

A significant ($p<0.001$) decrease was observed in the activities of the antiperoxidative enzymes SOD and CAT (Fig. 2) in the brain of sodium nitrite-treated Group II rats when compared to normal Group I rats. These results are in concurrence with other previous studies. SOD and CAT along with GPX form the first line of defense against ROS and are referred to as primary antioxidants. SOD essential to catalyze the dismutation of superoxide, protects cells from oxygen free radicals (Kojda and Harrison, 1999; Anand *et al.*, 1998). Catalase, which decomposes H_2O_2 to water and O_2, is a widely distributed enzyme and is an important member of

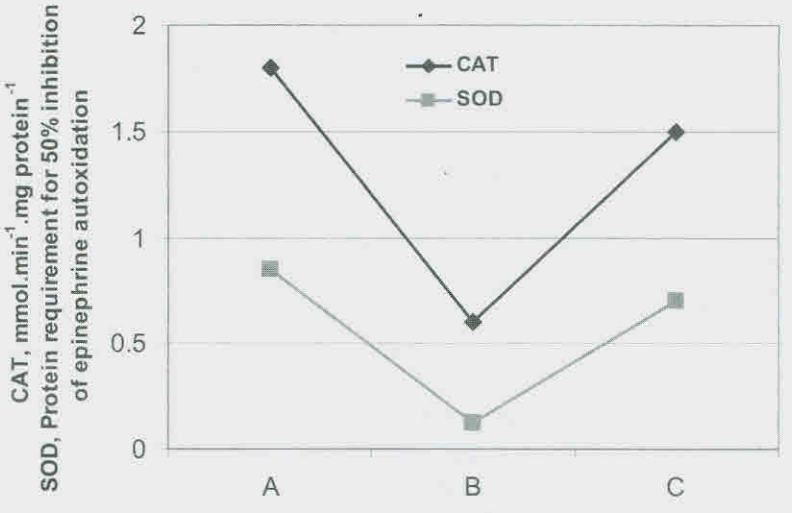
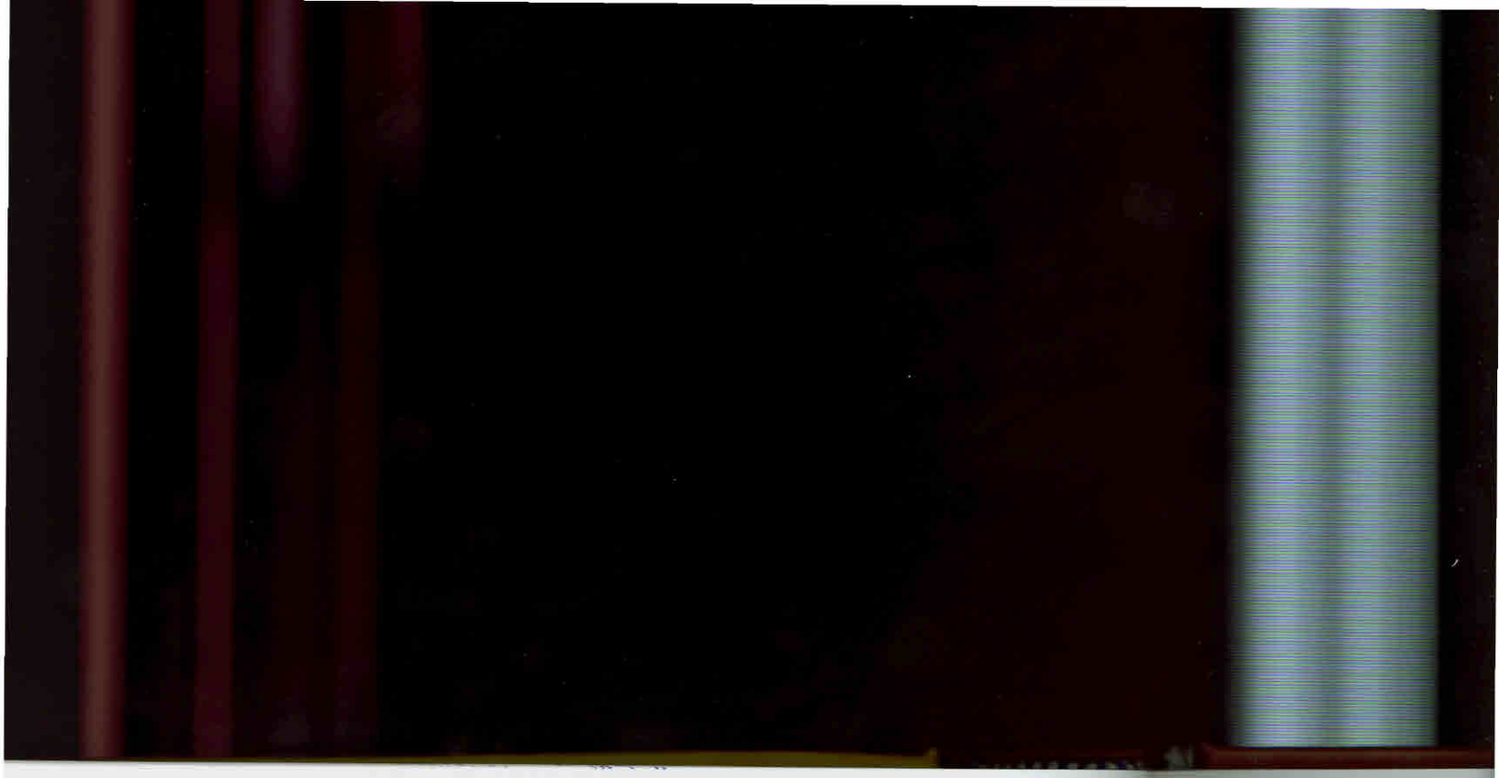


Fig. 2: Activities of Catalase (CAT) and Superoxide dismutase (SOD) in brain tissue of control (A), Sodium nitrite administered (B) and Mangrove root extract administered (C) rats



the cellular defense system against oxidative stress. Even if it is not strictly essential, the lack or malfunction of catalases may lead to severe defects, such as an increased susceptibility to thermal injury (Leff, 1993), high rates of mutations (Halliwell and Aruoma, 1991) and, in higher organisms, inflammation (Halliwell and Gutteridge, 1990) with other antioxidant molecules in the cell.

Reduction in the activities of these enzymes leads to the accumulation of O^{2-} and H_2O_2, which in turn can form hydroxyl radical ($OH^{\cdot}$) and bring about a number of reactions harmful to the cellular and subcellular membranes (Kalra *et al.*, 1988). Free radical damage of the active sites of SOD and CAT might be a possible cause of the decline in their activity in sodium nitrite-intoxicated rats (Datta *et al.*, 2000). The enzymes have amino acids arginine and histidine in their active sites that have an unpaired electron each and are susceptible to free radical damage. Group III rats that were on mangrove root extract-supplemented diet showed a significant ($p<0.001$) rise in the levels of CAT and SOD (Fig 2) that implies the protective effect of the extract in oxidative stress in brain. Similar findings were reported by other researchers. Choi *et al.* (2002) had shown that plant extracts rich in flavonoids reduced antioxidant damage by elevating the levels of antiperoxidative enzymes.

The flavonoid-rich extract supplementation could have reduced lipid peroxidation by directly scavenging the ROS which protected the antiperoxidative enzymes from the oxidative destruction. Other investigators have shown the beneficial effects of the ROS-quenching capacity of flavonoids, specifically in relation to attenuation of lipid peroxidation, reduction of membrane damage and permeability, and inhibition of intracellular oxidation in different cells (Moridani *et al.*, 2003, Galisteo *et al.*, 2004 and Kostyuk *et al.*, 2004). Mangroves inhabit a very hostile environment that is characterized by high salinity, low nutrition and high radiation. Exposure to stressful conditions causes the production of ROS in these plants. Interestingly, the concentration and activity of the antioxidative enzymes is high in these species (Das *et al.*, 2001) to neutralize the ROS. Also mangroves are generously endowed with polyphenolic compounds (Naskar *et al.*, 1995) that help to fight the oxidative stress by acting as potent antioxidants (Banerjee *et al.*, 2008, Chandini *et al.*, 2008). The antioxidant property of the flavonoids and other polyphenolic compounds is attribiuted to the free radical scavenging ability mediated by their hydroxyl groups (Hatano *et al.*, 1980).

Conclusion

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Conclusion

The beneficial effect of the mangrove root extract as an antioxidant in ameliorating oxidative stress in brain in this study is attributed to the presence of flavonoid and polyphenolic compounds.

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