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Protective effect of rhizome extracts of the herb, vacha (*Acorus calamus*) against oxidative damage: An *in vivo* and *in vitro* study

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Abstract

The rhizome of *Acorus calamus*, an herb widely used in Indian system of medicine for many ailments including epilepsy, mental illness and rheumatism, was subjected to soxhlet extraction to elucidate antioxidant property of different solvent extracts using *in vitro* assays. The benzene extract was most potent in scavenging hydroxyl and superoxide radicals and in reducing 1,1-diphenyl-2-picryl hydrazyl and ferric reducing antioxidant power. In addition the benzene extract prevented oxidative damage to DNA and mitochondria. It was also effective in preventing stress -induced decrease in total plasma anti-oxidant activity as determined *in vivo* using rat model wherein stress was induced by exposing to restraint and forced swimming. The minimum effective dose of the benzene extract was 5 mg/kg body weight (oral), and at this dose, its effect was similar to the same dose of a standard anti-oxidant, ascorbic acid. The study for the first time, clearly demonstrates a potent anti-oxidant activity of *A. calamus* combining *in vitro* and *in vivo* results. Hence, the therapeutic value of this herb maybe due to its anti-oxidant property.

Keywords: *Acorus calamus*; Antioxidant; Forced swimming; Radical scavenging; DNA protection assay

1. Introduction

The reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damage to lipids, proteins, enzymes, and nucleic acids leading to cell or tissue injury and are implicated in the processes of aging as well as in wide range of degenerative diseases *viz.* cancer, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson, and coronary heart pathologies, *etc.* [1,2]. Antioxidant based drugs and formulations have appeared during last three decades [3]. Currently available synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroquinon and gallic acid esters have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been imposed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity [4]. The search for natural antioxidants as alternatives is therefore of great interest.

Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exert an antioxidant action [5,6]. Our present study focuses on one of the most potent Indian medicinal herb *Acorus calamus* (vacha or sweet flag or buch plant) for its antioxidant property. It belongs to Acoraceae family and has been used in the Indian and Chinese system of medicine for several decades to treat diseases, especially the central nervous system (CNS) abnormalities [7,8]. The rhizome of this plant is widely used in the treatment of several ailments like epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers glandular, abdominal tumors, kidney and liver troubles, rheumatism, sinusitis and eczema [9]. However, there is scanty information on antioxidant properties of *A. calamus* and it is not known whether it has DNA protective activity. Hence, the aim of this study was to determine the antioxidant potential of *A. calamus* using different solvent extracts of rhizome using *in vitro* assays including DNA protection.

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Stress leads to hyper secretion of glucocorticoids [10] and an increased secretion of glucocorticoids (GCs) results in overproduction of reactive oxygen species [11] and leads to oxidative damage [12]. Hence there is a need to prevent stress induced oxidative damage. Several studies demonstrate suppression of long term stress-induced oxidative damage following administration of herbal extracts [13,14]. Thus far there is no information as to whether the altered antioxidant status due to short term stress exposure is prevented by herbal extracts. Earlier we have reported that acute stress *i.e.* 1 h exposure of adult male rats to restraint followed by forced swimming for 15 min after a gap of 4 h alters the antioxidant status [15]. Hence this is a good model for speedy assessment of total antioxidant activity *in vivo*. The present study using this model investigates whether benzene extract of rhizome prevents stress induced changes in antioxidant status using total antioxidant assay.

2. Materials and methods

2.1. Chemicals

L-ascorbic acid, gallic acid, 1,1-diphenyl-2-picryl hydrazyl (DPPH), ethylene diamine tetra acetic acid (EDTA), nitro blue tetrazolium (NBT), β-nicotinamide adenine dinucleotide (β-NADH), ferric chloride ($FeCl_3$), ferrozine, trichloroacetic acid (TCA), ferric chloride, guanidine hydrochloride, 5-methylphenazinium methosulphate (PMS), 2,4-dinitrophenylhydrazine (2,4-DNPH), hydrogen peroxide (H_2O_2), dichlorofluorescein (DCF), 2′,7′ dichlorofluorescein diacetate (DCF-DA) and 2,4,6 tripyridyl-s-triazine (TPTZ) were purchased from E. Merck India.

2.2. Experimental animals

Adult male Wistar rats (25) weighing 180–200 g were obtained from the inbred colony of the central animal facility of the University of Mysore and were maintained (2 or 3 rats/cage) under 12 h:12 h light and dark cycle. The animals were provided standard rat chow and water *ad libitum*. The experimental protocols were approved by the Institutional animal ethics committee.

2.3. Plant material and preparation of the extracts

The rhizome of the *A. calamus* was shade dried and a coarse powder was prepared (particle size ~0.25 mm) and was subjected to successive extraction using solvents with increasing polarity *viz.* petroleum ether, benzene, chloroform, ethanol, cold water, hot water and 0.2 N sodium hydroxide (NaOH) by continuous percolation process in a soxhlet apparatus. The aqueous extract was prepared by maceration with water. Each extract was concentrated by distilling off the solvent and evaporating to dryness followed by dissolution in rectified spirit.

2.4. Determination of percentage of inhibition

Efficacy of *A. calamus* extracts at different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL) was measured as percentage of inhibition of free radicals generation *in vitro* assays involving superoxide, hydroxyl, and DPPH radicals and ferrous ion chelating activity. The inhibition percentage (*I*%) was calculated using the formula (Eq. (1)),

$$I\% = \frac{(\boldsymbol{A}\mathrm{c} - \boldsymbol{A}\mathrm{s})}{\boldsymbol{A}\mathrm{c}} \times 100 \quad (1)$$

where Ac is the absorbance of the control; As is the absorbance of the sample containing plant extract.

2.5. In vitro anti-oxidant assays: 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity

Reduction of DPPH radical by an antioxidant results in the loss of absorption at 517 nm [16]. Each extract (0.3 mg/mL) of *A. calamus* was mixed with 5 mL of 0.1 mmol/L methanolic solution of DPPH and incubated at 20° C for 20 min in darkness. The control was prepared without plant extract and methanol was used for the base line correction. Then the absorbance of the sample was measured at 517 nm. The difference in absorbance between control and plant extract mixed samples indicated reducing activity of the extracts.

2.6. Superoxide anion radical scavenging activity

The superoxide anion scavenging activity of the extracts was determined by using Liu [17] method. Methylphenazinium methosulphate and β-nicotinamide adenine dinucleotide were allowed to react to generate superoxide anions which were reduced by NBT. Reaction mixture contained 3 mL Tris HCl buffer (100 mmol/L, pH 7.4), 0.75 mL of NBT (300 μmol/L) solution, 0.75 mL of NADH (936 μmol/L) and 0.3 mL of different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL) of each *A. calamus* extract. Reaction was started by adding 0.75 mL of PMS (120 μmol/L). The mixture was allowed to stand for 5 min at room temperature. The absorbance was read at 560 nm. The difference in absorbance between control and plant extract mixed samples indicated scavenging activity of the extracts.

2.7. Hydroxyl radical scavenging activity

Inhibition of hydroxyl radical ($OH^{\bullet}$) mediated peroxidation was carried out by deoxyribose assay [18]. The reaction mixture contained different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL) of each extract, 50 μl of deoxyribose (2.8 mmol/L) in KH_2PO_4 NaOH buffer (50 mmol/L pH 7.5), 200 μl of $FeCl_3$ (100 mmol/L), 104 mmol/L EDTA, ascorbic acid (100 mmol/L) and 50 μl H_2O_2 (4 mmol/L). The final reaction volume was made up to 1 mL with distilled water and incubated at 37° C for 1 h. After incubation 1 mL each of TCA and TBA was added and again incubated at 100° C for 20 min and control was prepared without plant extract. The mixture was allowed to cool and the absorbance was read at 532 nm. The difference in absorbance between control and extract mixed samples indicated reducing activity of the extracts.