Original Article

Protective Effect of *Curculigo Orchioides* Extract on Cyclophosphamide-Induced Neurotoxicity in Murine Model

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ABSTRACT

Free radicals are one of the frequent products of normal cellular metabolism. Disparity of metabolism and excessive generation of free radicals predisposes to disorders like Parkinson's disease, Alzheimer's disease, and aging phenomenon. *Curculigo orchioides Gaertn.* is known for "adaptogen" and "aphrodisiac" activity and has been proved for antiasthmatic, estrogenic, antiosteoporotic activity along with protection from cisplatin-induced cell damage. *C. orchioides* was powdered and subjected to soxhlet extraction using methanol. Phytochemical studies and estimation of polyphenols and flavonoids was performed. Acute toxicity studies were performed by Organization for Economic Cooperation and Development OECD guidelines. Animals were treated with cyclophosphamide to induce neurotoxicity. *Curculigo orchioides* was powdered and subjected to soxhlet extraction using methanol. Catalase, superoxide dismutase, glutathione peroxidase, and lipid peroxidation were estimated by reported methods. C. *orchioides* (400 mg/kg) significantly promoted restoration of catalase ($P < 0.005$), superoxide dismutase ($P < 0.005$), and glutathion ($P < 0.05$) levels. Similarly, a very significant decrease ($P < 0.005$) in the levels of malondialdehyde was observed. In all cases as mentioned previously, *C. orchioides* at dose 200 mg/kg promoted significant ($P < 0.05$) restoration of enzyme levels. *C. orchioides* (Kali Musli) is rich source of phytochemicals like flavonoids and polyphenols. Flavonoids and polyphenols are reputed to demonstrate neuroprotective effect. These phytochemicals in the present study might be responsible to demonstrate neuroprotective effect.

Key words: Antioxidant enzymes, brain, *Curculigo orchioides*, neuroprotective

INTRODUCTION

Free radicals are one of the frequent products of normal cellular metabolism. Imbalance in antioxidants defense mechanism and overproduction of free radicals due to environmental stress is ultimately responsible for neurodegeneration. Imbalance of metabolism and excessive generation of free radicals predisposes to disorders like Parkinson's disease, Alzheimer's disease, and aging phenomenon.[1] Free-radicals-mediated lipid peroxidation is known to change structure cell membrane and its activity. Increase in production of free radicals stimulates lipid peroxidation which leads to increased levels of malondialdehyde.[2,3] Plant sources could be considered as novel source of phytochemicals out of which flavonoids and polyphenols are of great importance that may be helpful to cope up with such oxidative stress.

Curculigo orchioides Gaertn. (family Amaryllidaceae), one of the jeopardized Indian "rasayan" herb, is commonly known as "Kali Musli." The plant is known for "adaptogen" and

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"aphrodisiac" activity. The plant also finds use in Kampo and Chinese medicines.[4] Scientifically, plant has proved for antiasthmatic[5], estrogenic[6], antiosteoporotic[7] activity. It also protects from cisplatin-induced cell damage.[8]

The current work was undertaken to evaluate neuroprotective potential of *C. orchioides* against cyclophosphamide-induced neurotoxicity.

EXPERIMENTAL

Plant collection, identification, and extraction

Curculigo orchioides was purchased from Bhopal region and was authenticated at Pinnacle Biomedical Research Institute, Bhopal. Dried *Curculigo orchioides* was powdered and subjected to soxhlet extraction using methanol.

Phytochemical screening

Phytochemical testing was performed to identify presence of different phytoconstituents.[9]

Phytoanalytical studies

Determination of total phenolic compounds

Total soluble phenolic compounds in the methanol extract were determined with Folin-Ciocalteu reagent according to reposted method[10] using pyrocatechol as a standard phenolic compound. Briefly, 1 ml of HEE (1000 µg/ml) in a volumetric flask was diluted with distilled water (46 ml). One milliliter of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 minutes, Na_2CO_3 (3 ml, 2% w/v) was added and then allowed to stand for 2 hours with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer (Shimadzu-1700). The total concentration of phenolic compounds in the extract determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph:

Absorbance = 0.0053 × total phenols (pyrocatechol equivalent [µg]) −0.0059

Assay for total flavonoids content

Total flavonoid content was determined using the method given elsewhere.[10] Briefly, aluminium trichloride (1 ml, 2% w/v) in methanol was mixed with the same volume of the HEE (1 ml, 2000 µg/ml). Absorption readings at 415 nm were taken after 10 minutes against a blank sample consisting of a methanol extract (1 ml, 2000 µg/ml) with methanol (1 ml) without $AlCl_3$. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph:

Absorbance = 0.0338 (quercetin [µg]) −0.0002; R^2 = 0.9969

Acute oral toxicity studies

Acute oral toxicity study was carried out in mice as per OECD-423 guidelines. The four fixed dose levels were selected as 5, 50, 300, 2000 mg/kg body weight. The mice were continuously observed for their mortality and behavioral response for 24 hours and thereafter once in a day for 14 days.[11] Hence, a dose of 200 mg/kg and 400 mg/kg was used in this study.

Animal grouping and treatment

Animals were divided into four groups containing five animals each. Animals were dosed as

Group I : Normal control
Group II: Toxic control [Cyclophosphamide (50 mg/kg i.p.)]
Group III: Extract treated [Cyclophosphamide (50 mg/kg i.p.) +200 mg/kg extract]
Group IV: Extract treated [Cyclophosphamide (50 mg/kg i.p.) +400 mg/kg extract]

Cyclophosphamide[12] was administered once on the first day; extract was administered for 5 consecutive days.

Brain isolation and brain antioxidants study

After euthanasia, brains from animals were isolated and rinsed with ice-cold normal saline, followed by ice-cold 0.15 M tris HCl (pH 7.4). For estimation of antioxidant markers, viz., superoxide dismutase, catalase, glutathione, and malondialdehyde, a 10% w/v tissue homogenate in 0.15 M tris HCl and centrifuged at 15,000 rpm for 15 minutes at 4°C, supernatant was used for analysis.

Catalase[13], superoxide dismutase[14], glutathione peroxidase[15], and lipid peroxidation[16] were estimated by reported methods.

Statistical analysis

All results were analyzed by one-way analysis of variance (ANOVA), and post-hoc analysis was performed with Bonferroni's test. Value of $P < 0.05$ was considered to be statistically significant in all the cases.

RESULTS

Phytochemical screening and phytoanalytical studies

Phytochemical screening of extract demonstrated presence of flavonoids, polyphenolics, and alkaloids. The total amount of phenolic content present in extract was found to be 752.23 ± 5.78 mg pyrocatechol equivalent (PE)/100 g. By using the standard curve of quercetin (R^2 = 0.9998), the total flavonoid content of extract was found to be 203.52 ± 4.56 mg quercetin equivalent (QE)/100 g.

Acute toxicity studies

C. orchioides extract did not showed any toxicity at a dose of 2000 mg/kg as evidenced by observations. No signs of abnormal behavior or mortality were observed during the study period. Thus, dose of 200 mg/kg and 400 mg/kg of extract were selected for further studies.

Brain antioxidants study

As evident by Figure 1, *C. orchioides* (400 mg/kg) significantly promoted the restoration of catalase ($P < 0.005$), superoxide dismutase ($P < 0.005$), and glutathion ($P < 0.05$) levels. Similarly, a very significant decrease ($P < 0.005$) in the levels of malondialdehyde was observed. In all cases as mentioned previously, *C. orchioides* at dose 200 mg/kg promoted significant ($P < 0.05$) restoration of enzyme levels.

DISCUSSION

Etiopathogenesis of various nerve-racking situations lead to generation of several "psychotic disorders" where normal physiological functions of neurotransmitters are altered. Cyclophosphamide, an anticancer agent is reported to produce adverse effects on brain[17] which is possibly due to development of oxidative stress. Antioxidant is any molecule that is capable of alleviating effects of free radicals prior to attack to cell. Human body contains a variety of antioxidant systems and enzymes that continuously protect body from harm full effects of free radicals and oxidative stress. Supplementation with antioxidant-rich food could be useful to provide adequate protection.[18]

Superoxide dismutase, an antioxidant enzyme, catalyzes translation of superoxide free radical to hydrogen peroxide and water, and serves to be useful in inhibiting oxidative stress.[19] Catalase is an important enzyme accountable for disposition of H_2O_2. Diminution of activity of this enzyme is associated with increased activity of free radicals, which

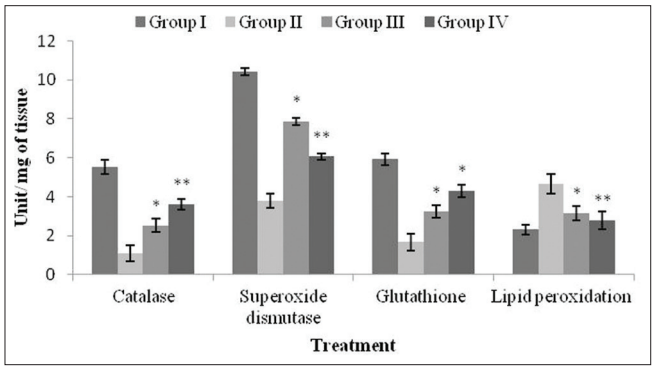

Figure 1: Protective effect of Curculigo orchioides extract on brain antioxidants in rats.Values are mean ± S.E.M. ($n = 5$), *$P < 0.05$ (significant); ** $P < 0.01$ (very significant) when compared to toxic control group

may lead to alter the activity of cell membranes.[20] In the present study, restoration in the levels of antioxidant enzymes by extract demonstrated protective effect.

Basically, there exists equilibrium between production of reactive oxygen species and antioxidant defense system which regulates homeostasis towards oxidative stress of cell. Due to low levels of antioxidant enzymes, hippocampal neurons are predominantly vulnerable to oxidative stress. Glutathione can be regarded as a chief "intracellular non-protein thiol compound" and act as scavenger of free radicals. Thus, glutathione could be regarded as a first line of antioxidant defense.[21] Glutathione depletion therefore may cause death of nerve cells after ischemia of forebrain.[22] Flavonoids from plants are widely recognized to prevent membrane damage and thus protect the integrity of cell.[23]

Curculigo orchioides (Kali Musli) is rich source of phytochemicals like flavonoids and polyphenols. Flavonoids[24,25] and polyphenols[26,27] are reputed to demonstrate neuroprotective effect. These phytochemicals in the present study might be responsible to demonstrate neuroprotective effect. However, more systematic studies along isolation and characterization of phytoconstituents responsible for activity seem to be necessary.

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