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Anticonvulsant potential of callus cultures of *Convolvulus microphyllus* Sieb.

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SUMMARY

Callus cultures of *Convolvulus microphyllus* Sieb. was induced on Murashige and Skoog's medium supplemented with 2,4-dichloro phenoxy acetic acid, 6-benzyl adenine, indole acetic acid and kinetin (1 ppm each). Methanolic extracts of whole plant, leaf, stem and leaf and stem calli were tested for anticonvulsant activity against standard drug phenytoin using maximal electroshock model on mice. It was observed that the animals treated with methanolic extracts of stem callus, leaf callus and whole plant (200 mg/kg, oral) showed significant protection against tonic convulsions induced by transcorneal electroshock. Anticonvulsant activity of methanolic extract of stem callus was comparable to that of standard drug phenytoin.

Key words: Anticonvulsant activity; MES; *Convolvulus microphyllus*; Callus culture

INTRODUCTION

Convolvulus (C.) microphyllus Sieb. (Family: Convolvulaceae) commonly known as "Shankhpushpi" in India, distributed in temperate regions like Sindh, Sudan and Egypt etc (Shah and Bole, 1961). *C. microphyllus* has been utilized as brain tonic (Singh *et al.*, 1979) and reported to be useful in CNS disorders (Pawar *et al.*, 2001), hypertension (Chaturvedi *et al.*, 1966), thyrotoxicosis (Gupta *et al.*, 1981), ulcer (Sairam *et al.*, 2001) and also as antibacterial and antifungal agent (Kapoor *et al.*, 1981). But till date no anticonvulsant activity has been carried out on callus cultures developed from *C. microphyllus* and leaf and stem part of parent plant. Though water-soluble portion of alcoholic extract of whole plant was found to antagonize the electrically induced convulsive seizures and tremorine induced tremors (Sharma *et al.*, 1985). Hence in the present investigation an attempt has been made to develop *in vitro* cultures of *C. microphyllus* as an alternative source and to compare the anticonvulsant activity of developed cultures with that of natural drug.

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MATERIALS AND METHODS

Plant material

The plant was collected during the month of December - January from Herbal garden of Hamdard University, New Delhi and authenticated from the Department of Botany of University. Voucher specimen was deposited in the Herbarium of University and HPTLC fingerprints of methanolic extracts were recorded.

Development of leaf and stem callus

The immature leaf and stem portion was surface sterilized by-

1. Washing with running tap water
2. Scrubbing clean with dilute detergent (1 - 2%

cdepol soln.) for 2 - 4 min., washing with tap water and finally with distilled water.

3. Sterilization with 0.6% w/v mercuric chloride for 8 - 10 min and washing with sterile distilled water (5 - 6 times).

Surface sterilized leaf and stem explants (10 - 15 mm) were inoculated under sterilized condition on agar solidified 'Murashige and Skoog's (MS) medium (Murashige and Skoog, 1962) supplemented with growth regulators: 2,4-dichloro phenoxy acetic acid (2, 4-D), 6-benzyl adenine (6-BA), indole acetic acid (IAA) and kinetin (1 ppm each). The pH of the medium was adjusted to 5.6 - 5.8 and cultures were maintained at 25 ± 2°C for 16 h and 8 h light dark cycle with light intensity 1,600 lux in BOD incubator.

Preparation of extracts

Dried and coarse powdered (# 10) whole plant, leaf and stem (10 g each) were soxhlet extracted with 100 ml methanol for 2 h. Six-month-old leaf and stem calli were dried in oven at 40°C for 24 h and reduced to moderately coarse powder. Powdered leaf and stem calli (10 g each) were then soxhlet extracted with 100 ml methanol for 2 h. All the extracts were filtered separately and filtrates were concentrated to dryness on water bath. The dried marcs left were then weighed and suspended in double distilled water with the help of Tween 80 (0.01% w/v) to get 20 mg/ml uniform aqueous suspension, which was administered orally 30 min prior to study.

HPTLC fingerprint and preliminary phytochemical screening

Methanolic extract of whole plant, leaf, stem, leaf callus and stem callus (1 g/ml each) was applied (2 µl each) on silica gel G F_{254} HPTLC plate in duplicate with band width of 6 mm using Linomat V applicating device. The chromatogram was developed in Twin trough chamber using solvent system Toluene: Ether (1 : 1) saturated with 10% acetic acid and scanned in Scanner III at 366 nm wavelength using mercury lamp in fluorescence mode. The preliminary phytochemical screening of natural plant material and *in vitro* cultures were carried out using general chemical tests as described by Zafar and Mujeeb (2002).

Animals

Swiss albino mice of either sex (22 - 32 g) supplied by central animal house facility of Jamia Hamadard, New Delhi (Registration No. 173/CPCSEA) were used. All animals were housed in cages in-group of 6, at 23 - 30°C with a natural light dark cycle. They had free access to standard Pellet diet (Amrut laboratory rat & mice feed, Navamaharashtra Chakal oil mills Ltd., Pune) and tap water. The study has been approved by ethics committee CPCSEA (Project no. 70/2001). Ethical norms were strictly followed during all experimental procedures.

Drugs and dosing schedules

Phenytoin (Dilantin suspension, Park Davis) was given p.o. in a volume of 10 ml/kg and dose of 22 mg/kg body weight 2 h prior to each observation (Shahid *et al.*, 2004). All other test samples were given p.o. in a volume of 10 ml/kg and doses of 200 mg/kg body weight 30 min prior to each observation. Control group was given distilled water in a volume of 10 ml/kg body weight.

Increasing current electroshock seizures (ICES)

The ICES, a relatively new and sensitive method for studying drugs affecting seizure threshold in animals was proposed by Kitano *et al.* (1996) and modified by Marwah *et al.* (1999) was used to evaluate the anticonvulsant effect of drugs. To start with a current of 2 mA electroshock to each mouse via ear electrodes as a single train of pulse (for 0.2 s) was given with linearly increasing intensity of 2 mA/2 s using an electro-convulsometer (INCO, Ambala, India). The current at which tonic hind limb extension (HLE) occurred was recorded as the seizure threshold current (STC). When no tonic HLE was observed by a current of 30 mA,

Table 1. Anticonvulsant activity of methanolic extracts of *C. microphyllus* Sieb. and its callus cultures

S. No.	Treatment group (n = 6)	Dose (Oral route)	Seizure protection (%)	Mortality protection (%)	Seizure threshold current (mA) mean ± S.E.M.
1	Control	10 ml/kg	00	00	16.33 ± 0.61
2	Phenytoin	12 mg/kg	100	100	30.00 ± 0.00***
3	Whole plant extract	200 mg/kg	66.66	83.33	26.00 ± 2.52*
4	Leaf extract	200 mg/kg	33.33	50.00	18.00 ± 1.26
5	Stem extract	200 mg/kg	50.00	66.66	22.33 ± 2.70
6	Leaf callus extract	200 mg/kg	83.33	100	28.66 ± 1.33**
7	Stem callus extract	200 mg/kg	83.33	100	29.00 ± 1.00***

n = no. of animals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ Vs vehicle treated group (student's '*t*' test).

electroshock was terminated. Duration of tonic convulsions (tonic hind limb extension) and the percentage of seizure protection and mortality were recorded.

Statistical analysis

Each group consisted of a minimum of six animals. Results were expressed as mean ± S.E. and all the extracts and standard drug phenytoin were compared with vehicle treated control group separately using one way analysis of variance (ANOVA) followed by students '*t*' test. *P* value < 0.05 was considered statistically significant.

RESULTS

The leaf and stem callus cultures were successfully induced on MS medium supplemented with 2,4-D, 6BA, IAA and Kin (1 ppm). Callus developed was greenish white in colour and soft in texture.

HPTLC fingerprints of methanolic extracts of whole plant, leaf, stem, leaf callus and stem callus showed presence of 8, 6, 8, 8 and 7 spots respectively. The spots having Rf value 0.24, 0.33, 0.46, 0.71 and 0.83 were found present in all samples while the spots having Rf value 0.14 and 0.57 were found present in all samples except stem callus and leaf respectively. One compound having Rf value 0.02 was found present in leaf callus and stem callus while absent in others. Moreover, the compounds having Rf value 0.11, 0.37 and 0.85 were specific only in root, stem and whole plant respectively.

Phytochemical screening of extracts of whole plant, leaf, stem and their calli showed positive test for alkaloids, amino acids, ascorbic acid coumarin, carbohydrates, proteins, saponins and steroids in all samples, while flavonoids were only present in natural plant leaf and stem but found absent in *in vitro* cultures.

The results of the ICES showed that the animals treated with methanolic extracts of stem callus, leaf callus and whole plant showed significant protection against electroshock induced convulsion by increasing seizure threshold current (29.00, 28.66 and 26.00 respectively) Table 1.

The methanolic extracts of stem callus and leaf callus (200 mg/kg p.o.) were found to possess 83.33% protection against ICES seizure and 100% protection against mortality, which is some what comparable to that of oral administration (22 mg/kg) of standard drug phenytoin. Methanolic extracts of leaf and stem showed protection against seizures and mortality but results are not significant.

DISCUSSION

C. microphyllus Sieb. have been used for treatment of various disorders in Indian System of Medicine including seizures of epilepsy (Shastri, 1952). In the present investigation the anticonvulsant activity of methanolic extracts of whole plant leaf stem and

their cultures of *C. microphyllus* has been evaluated against seizure induced maximal electroshock seizures (MES).

Sine the different extracts suppressed tonic convulsions it suggested that whole plant, leaf and stem calli of plant contains the active compounds which inhibited the convulsive seizure activity. Phytochemical investigations demonstrated the presence of alkaloids, amino acids, ascorbic acids, carbohydrates, coumarins, proteins, saponins and steroids whereas flavonoids were only present in *in vitro* cultures. The mechanisms of absence of flavonoids in *in vitro* cultures may be associated with the lack of amounts of essential enzyme and coenzyme systems in the biosynthetic pathways or it may be associated with the supply of enzyme-synthesized precursors but the possibility of specialized storage cells which tend to occur more in certain tissues within the organ than others as suggested by Kodama *et al.* (1980) and influence of environmental conditions cannot be ruled out.

As earlier reports on various plant species the anticonvulsant activity may be because of alkaloids (Ameri *et al.*, 1997) protein (De Lucia *et al.*, 1997), saponins (Chadha, 1966), flavonoids (Nghyen and Tarr, 1993), and coumarins (Chaturvedi *et al.*, 1974). Anticonvulsant activity have also observed in protein solution of sea weed *Himanthalia elongata* (Anca *et al.*, 1990) in saponins and flavonoids of *Centella asiatica* (De Lucia *et al.*, 1997) and in chloroform extracts of callus cultures of *Ocimum sanctum* (Jaggi *et al.*, 2003).

The alkaloids and coumarins reported in plant found present in all extracts (Zafar *et al.*, 2005) might be responsible for this activity of plant. The variation in potency of different extracts for its anticonvulsant activity may be because of the amount of the above constituents present.

C. microphyllus has been reported to have some behavioral effect on CNS in which the methanolic extract of whole plant decreases motor activity and prolonged sleeping time induced by pentobarbitone (Pawar *et al.*, 2001). This study suggests that methanolic extract possess a sedative and CNS depressant effect, which is probably responsible for anticonvulsant effect of extracts.

The antiepileptic drugs that block the MES induced tonic extension acts by blocking seizure spread (Rogawski and Porter, 1990). Furthermore, MES induced tonic extension can be prevented either by drugs that inhibit voltage dependant Na^+ channels, such as phenytoin, valproate (Rogawski and Porter, 1990; Macdonald and Kelly, 1995; White, 1997) or by drugs that block glutamatergic excitation mediated by N-methyl-D-aspartate receptor, such as falbamate (McCabe *et al.*, 1993; Subramaniam *et al.*, 1995).

C. microphyllus Sieb. and its *in vitro* cultures exhibited anticonvulsant activity in MES induced seizures comparable to those of classical anticonvulsant drugs but the exact mechanism and the active compounds involved in this effect need to be clarified in further studies.

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