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Anticonvulsant potential of holy basil, *Ocimum sanctum* Linn., and its cultures

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Callus cultures from stem of *O. sanctum* were induced on slightly modified Murashige and Skoog's (MS) medium and supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D, 1-2 ppm) and kinetin (kn, 1 ppm). Different extractives of stem, leaf and stem callus of *O. sanctum* were tested for anticonvulsant activity against standard drug phenytoin using maximal electroshock (MES) model. Ethanol and chloroform extractives of stem, leaf and stem calli were effective in preventing tonic convulsions induced by transcorneal electroshock.

Keywords: Anticonvulsant activity, *Ocimum sanctum*

Ocimum sanctum Linn. (Family Labiatae) commonly known as 'Sacred Basil' or 'Holy Basil' (*Tulsi* in Hindi) is a herbaceous annual plant indigenous to India. *O. sanctum* has been utilised as a general promotor for health in herbal medicine[1] and most of its properties like antistress[2], adaptogenic[3], anticancer[4], anti-inflammatory[5,6], antihyperlipidemic[7], antihypercholesteremic[8], hepatoprotective[9], radioprotective[10] and antimicrobial[11] have been examined scientifically. But till date no anticonvulsant activity has been carried out on tissue cultures developed from *O. sanctum* and stem part of the parent plant, though in its leaf anticonvulsant activity has been observed[12]. Hence in the present study, an attempt was made to determine the anticonvulsant effect of cultured tissue and stem part of *O. sanctum* and to compare it with that of leaf portion.

Plant material — *Ocimum sanctum* Linn. herb was collected from cultivated plants grown in the Medicinal Plants Garden of the University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh.

Development of stem callus - The stem portion was surface-sterilized by:

(a) Washing with running tap water.
(b) Scrubbing clean with dilute detergent (1-2% Cleansol solution) for 2-4 min, washing with tap water and finally with distilled water.
(c) Sterilizing with 0.1% w/v mercuric chloride for 3-5 min and washing with sterile distilled water (3-4 times).

Surface-sterilized stem explants (10-15 mm) were inoculated under sterilized condition on agar-solidified 'Murashige and Skoog's (MS) medium[13] with some modifications (ferric citrate and manganese sulphate monohydrate were used in place of ferrous sulphate and manganese sulphate tetrahydrate, respectively, and edamine was not used). The medium was supplemented with 2% sucrose and growth regulators: 2,4-dichlorophenoxy acetic acid (2,4-D, 1-2 ppm) in combination with 6-furfuryl aminopurine (kinetin, Kn (1 ppm). The *p*H of the medium was adjusted to 5.7-5.8. The cultures were maintained at 25° ± 2°C for 12hr a day using white fluorescent tubes (0.6 m long, 20 W each).

Preparation of extractives — Stem callus (SC) tissue (6 months old) developed on MS + 2,4-D (2 ppm) + Kn (1ppm) and oven-dried at 40°C for about 24 hr was reduced to moderately coarse powder and was extracted by refluxing with chloroform and ethanol (95% v/v) for 4 and 5 hr, respectively. The dried marc was shaken with warm distilled water for 20 hr and filtered.

Stem (St) and leaf (Lf) portions of parent plant (4 months old) dried separately in shade were also reduced to moderately coarse powder (#10) and soxhlet extracted separately for about 22hr with petroleum ether (60°-80°C), chloroform and ethanol (95% v/v). The dried marc was left in contact with distilled water for 48 hr and filtered.

Phytochemical screening of various extractives obtained indicated positive tests for saponins[14], sterols[14], triterpenoids[14], carbohydrates[15], tannins[16] and proteins[16] in Sc, St and Lf, while flavonoids[14] were detected in St and Lf only.

Animals — Albino mice (Laca strain) weighing 20-28g procured from the Central Animal House of Panjab University, Chandigarh were used. The animals received a standard pelleted diet (M/s Hindustan Lever Foods, Calcutta, India) and water *ad libitum*, and were maintained under standard environmental conditions (22°±5°C with 12hr of light/dark cycle). The experimental protocols were approved by the Institutional Animal Ethical Committee.

Test materials

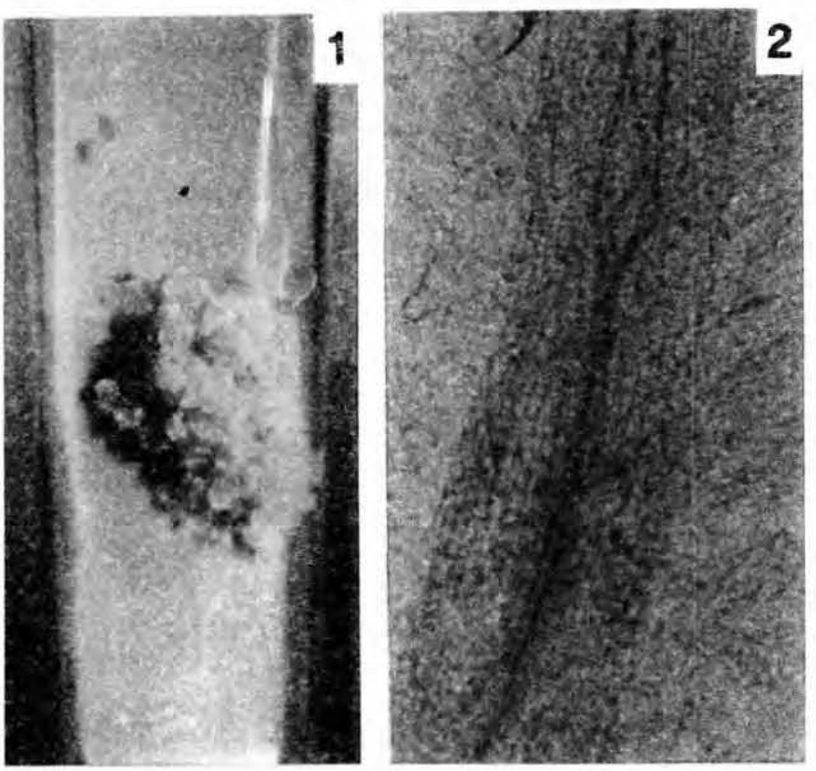
I. Chloroform, ethanol and water extractives of callus.
II. Petroleum ether, chloroform, ethanol and water extractives of stem.
III. Petroleum ether, chloroform, ethanol and water extractives of leaves.

Anticonvulsant activity — The anticonvulsant activity of the extractives (100, 200, 400 and 800 mg/kg, orally) was tested against standard drug, phenytoin (25 mg/kg) (Parke-Davis, Bombay, India) using maximal electroshock model[17].

Statistical analysis — Each group consisted of a minimum of five animals. Results were expressed as mean ± SE and all the extractives were compared with phenytoin (standard) and control separately using one way analysis of variance (ANOVA) followed by Dunnett's test. $P<0.05$ was considered statistically significant.

Callus cultures were successfully induced on the medium MS+2,4-D (2 ppm)+Kn (1 ppm) (Fig. 1). MS medium, because of high salt concentrations[18] and combination of 2, 4-D and Kn is preferred by many authors[19]. Callus developed was of white, green and brown colour and of nodular but soft texture. Organogenesis was observed in the form of root primordia in the callus (Fig. 2) and outermost layer was comparable to epidermis. Root hairs arising profusely from the epidermis were prominent. On MS+2,4-D (1 ppm)+Kn (1 ppm) medium the callus growth was slow and the callus formed was of green color with brown and white patches. Its texture was hard and nodular. Phytochemical screening of extractives showed that except flavonoids all the major constitutents present in the stem callus extractives were the same as those present in the stem and leaf extractives.

In the present investigations both reduction and mortality (% age recovery) as well as total time spent



Figs 1-2 — (1) — Seven-week-old callus of *Ocimum sanctum* on MS + 2,4-D (2ppm) + Kn (1ppm) and (2) — Root primordia in stem callus on MS + 2,4-D (2ppm) + Kn (1ppm), x 25.

in various convulsive phases were observed. The various extractives of chloroform and ethanol, viz. stem, leaf and stem calli exhibited significant anticonvulsant activity in maximal electroconvulsive shock induced convulsion. Chloroform extractive of stem callus tissue exhibited significant decrease in the time spent in the extensor phase at doses of 400 mg/kg and 800 mg/kg indicating protective effect against MES-induced convulsions (Fig. 3). Stem chloroform extractive was also effective at a dose of 200 mg/kg besides doses of 200, 400 and 800 mg/kg (Fig. 3). Leaves were found to be more effective as compared to stem and stem calli (Fig. 3).

Ethanol extractive of stem callus tissue resulted in a significant decrease in the time spent in extensor phase in a dose-dependent manner as compared to control (Fig. 4) and it showed reduction in mortality rate. Moreover, its doses 400 mg/kg and 800 mg/kg exhibited activity comparable to phenytoin. Ethanol extractive of stem exhibited dose-dependent activity as compared to that of control (Fig. 4). Leaf extractive at doses of 400 and 800 mg/kg was found to be as potent as standard drug phenytoin.

Since the different extractives suppressed tonic convulsions it suggested that leaf, stem and stem calli of *O. sanctum* contain the active compounds which inhibited the convulsive seizure activity. Phytochemical investigations demonstrated the presence of saponins, triterpenoids, flavonoids, tannins, proteins and carbohydrates. Anticonvulsant activity may be because of

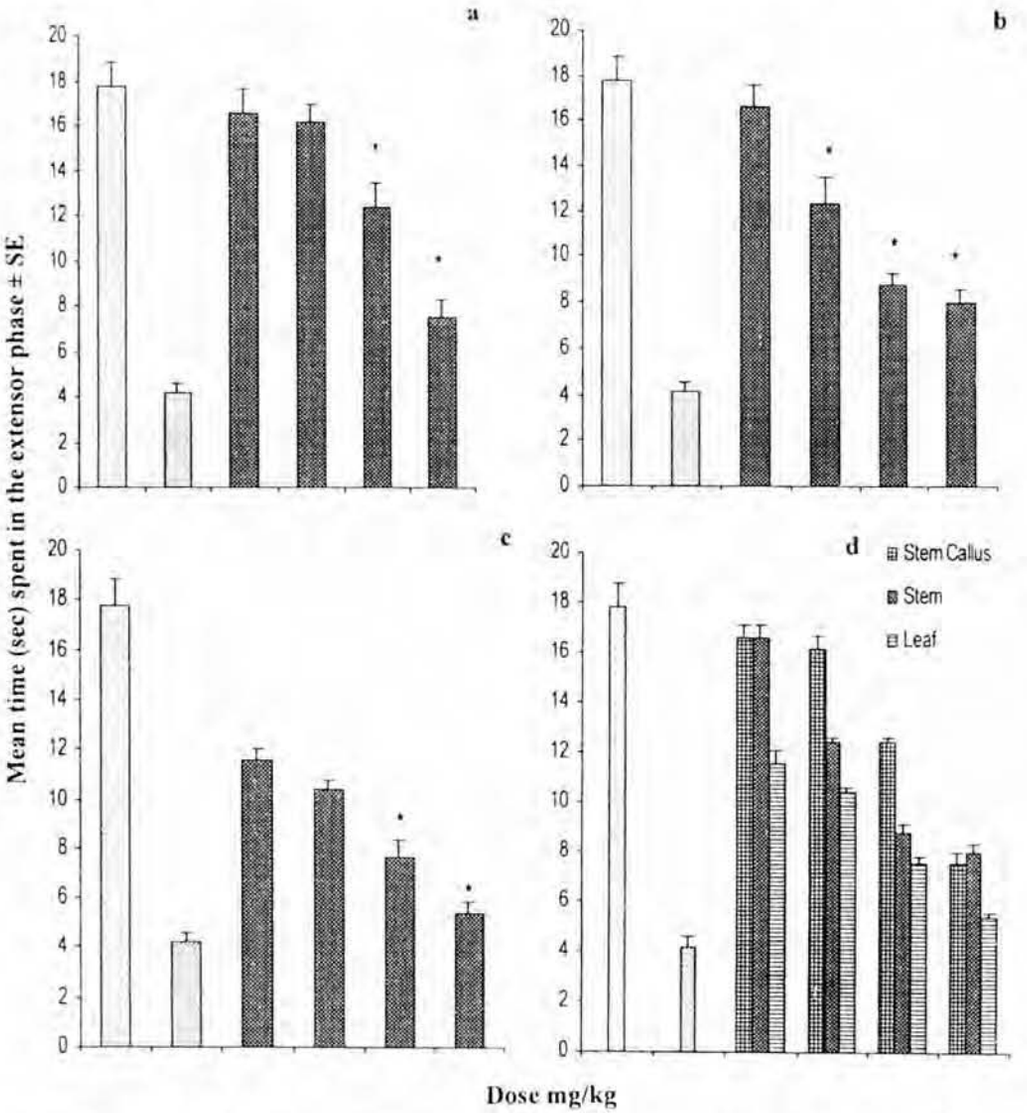


Fig. 3 — Anticonvulsant effect of chloroform extractives of (a) stem callus, (b) stem, (c) leaf and (d) comparison of anticonvulsant effect of stem callus, stem and leaf extractives $^{*}P < 0.05$ vs control (data are analysed by one way ANOVA followed by Dunnett's test)

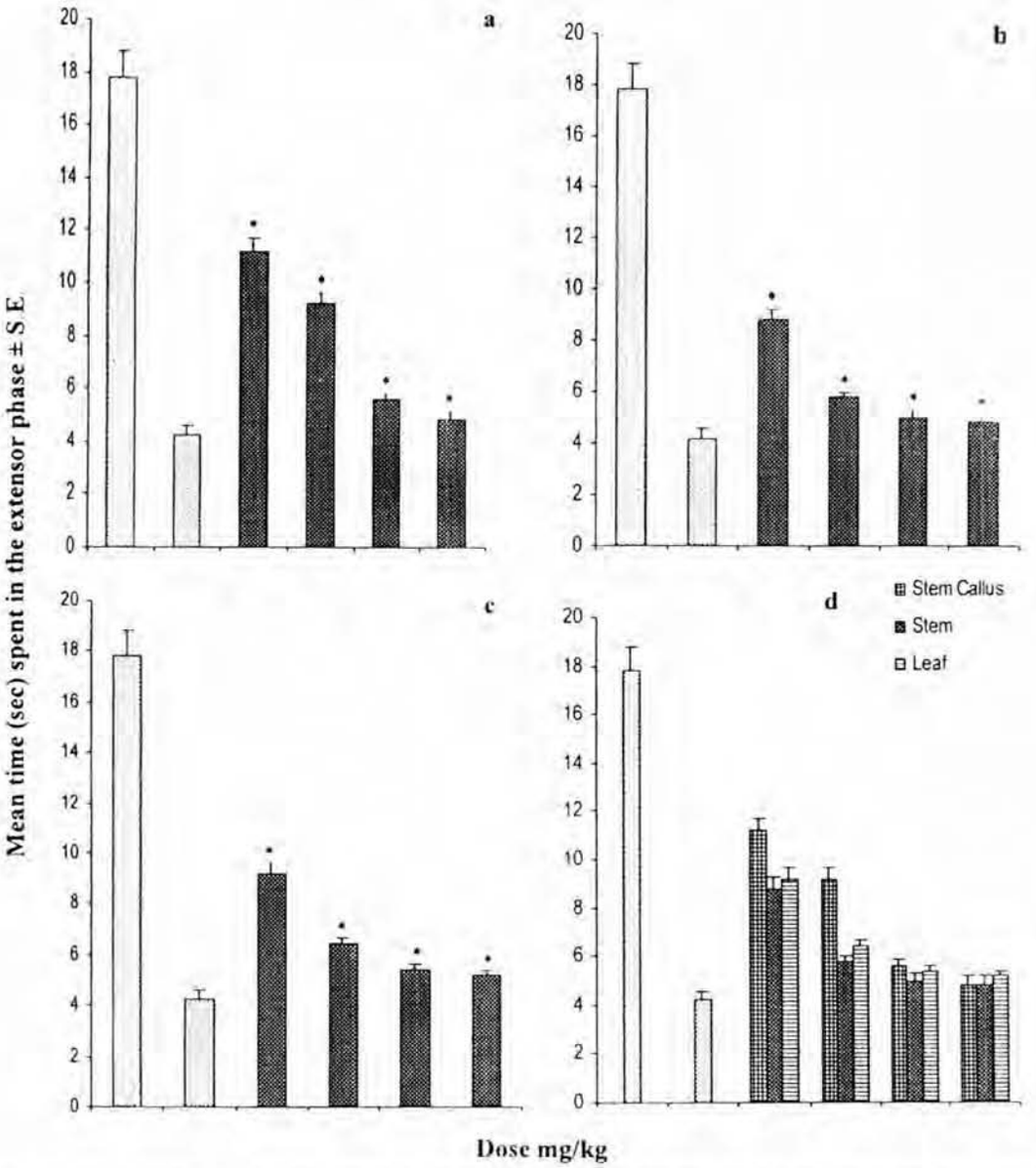


Fig. 4 — Anticonvulsant effect of ethanol extractives of (a) stem callus, (b) stem, (c) leaf and (d) comparision of anticonvulsant effect of stem callus, stem and leaf extractives $^{*}P < 0.05$ vs control (data were analysed by one way ANOVA followed by Dunnett's test)

saponins[20], flavonoids[21], proteins[22]. The anticonvulsant activity has also been observed by De Lucia *et al*[22]. in saponins and flavonoids of *Centella asiatica*[23] and by Anca *et al.*[23] in proteins solutions obtained from the seaweed *Himanthalia elongata*[24]. The potency of a particular extract for its anticonvulsant activity may be because of the amount of the above constituents present.

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