Effect of Ancymidol on the Production of Forskolin from Cell Cultures of *Coleus forskohlii*

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The treatment of cell cultures of *Coleus forskohlii* Briq with 50 μM of ancymidol a inhibitor of gibberellin biosynthesis enhanced the bioproduction of forskohlin by 150%, using production medium in six well plate as culture vessel.

Coleus forskohlii, Lamiaceae is a perennial herb with tuberous roots. The active constituent of the plant was found to be forskolin, a diterpene. Forskolin has a unique property of activating almost all hormone-sensitive adenylate cyclase enzymes in a biological syster[1]. Forskolin is reported to be useful in the treatment of congestive heart failure, glaucoma, asthma and certain type of cancers[2]. The root of the plant is used as condiment in pickles[3]. Forskolin is a chemically complex molecule with no commercially viable method of synthesis. The best alternative for the production of forskolin is through plant tissue culture. There are several reports on the production of forskolin from the callus, and cell cultures of *Coleus forskohlii*[4-6]. However, the yields are poor. Ancymidol, a growth retardant, is known to inhibit the conversion of geranylgeranyl pyrophosphate GGPP to gibberllins[7]. In the present paper we describe the effect of ancymidol on the production of forskolin in cell cultures of *C. forskohlii* using six-well plate technique. The callus was induced from the hypocotyl portions of aseptically germinated seeds of *C. forskohlii* on B5 medium supplemented with sucrose (3%w/v), casein hydrolysate (600mg/l), 2,4-D (1 mg/l), kinetin (0.2 mg/l). Suspension cultures were established after 4 mo on the same medium. After 3 passages, the cell cultures were transferred onto production medium [B5 medium with Indole-3-Butyric acid (0.4 mg/l), kinetin (0.2 mg/l), sucrose (5% w/v) and with 50% v/v as inoculum[8]. Cell cultures (5 ml) were transferred into each well of pre-sterilized six-well plate (Corning, USA) separately under laminar flow. Ancymidol (Sigma Chemical Company, St. Louis, Mo) (5 μM and 50 μM), was added to the cell cultures in each well of six-well plates under laminar flow. For each concentration, one six-well plate was used, while running suitable control and incubated at 25±2° at 120 rpm.

The cell cultures of *C. forskohlii*, after 7 d of incubation at 25±2° and 120 rpm with different concentrations of ancymidol, and control cultures were extracted with three volumes of dichloromethane (15 ml) and stored in deep freezer for 24 h. Then, it was allowed to stand at room temperature, and the dichloromethane layer of each culture was separated, filtered and the process was repeated thrice to make sure the complete extraction of forskolin takes place. The extracts were pooled together and evaporated to dryness[9]. The residue was dissolved in HPLC grade methanol (1 ml) and subjected for TLC/HPLC analysis.

The TLC of the extracts was done using silica gel G plate (precoated aluminum backed UV_{254} (E. Merck, Mumbai) with a solvent system of toluene:ethylacetate (85:15). The detection of the spots was detected by spraying ansialdehyde-sulfuric acid reagent followed by heating at 140° for 3 min[10].

The HPLC analysis of the extracts was performed with a Shimadzu LC-10AT model, by injecting 10 μl of the each standard solution and the extract with a Hamilton syringe, using RP C18 column (Tracer Analitica, Nucleosil-100, 25x0.4 cm, 5 μm) with Shimadzu photodiode array (SPD-

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M10A VP Model) detector. The mobile phase (acetonitrile-water 65:35) adjusted to a pH of 2.5 by adding orthophosphoric acid was pumped isocratically at a flow rate of 1 ml/min, and forskolin was detected by its absorption at 202 nm[11]. All data represent the mean values and standard deviation (from three individual wells of six-well plate the experiment was repeated thrice). The present investigations were aimed to enhance the production of forskolin in cell cultures of *C. forskohlii* by ancymidol. The extracts derived from the cell cultures of *C. forskohlii* and ancymidol treated cultures showed the presence of forskolin upon co-chromatogarphy with authentic sample. The extracts gave greenish black spots with R_f value of 0.9 that is same as authentic sample. HPLC analysis of the extracts had the same UV absorption maxima as the authentic sample. The peak purity (peak height and peak area) of the extracts of the cell cultures was same as that of standard sample. The retention time (R_t) of all the extracts of the cell cultures were coincident with that of authentic sample (5.2 min). The addition of ancymidol to the cell cultures of *C. forskohlii* significantly improved the production of forskolin over the control cultures. The effect of ancymidol on forskolin production showed dose response relation. The 5 μM of ancymidol showed less effect as compared to that of 50 pM of ancymidol. There was 1.5 fold increase in forskolin production by supplementation of ancymidol (5 μM) and 2 fold increase with addition of 50 μM of ancymidol over the control cell cultures repeated twice (fig. 1). Ancymidol is a compound known to inhibit gibberellin biogenesis. This overall improvement in forskolin content may be due to increased availability of GGPP, as forskolin is produced through acetate-mevalonate pathway[12]. This is the first report on the enhancement of production of forskolin by ancymidol. However, the other growth retardants such as chloro choline chloride, 2-chloroethyl phosphoric acid were ineffective in enhancing the yields of forskolin.

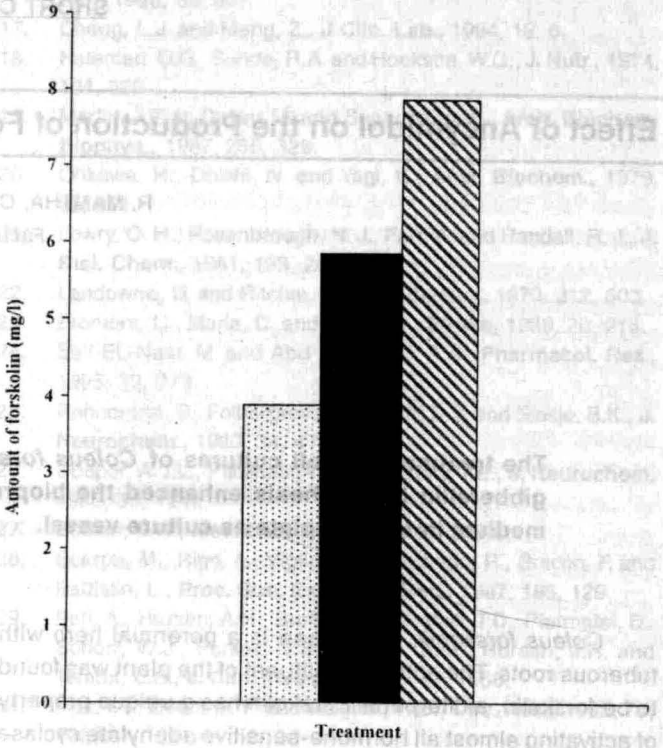

Fig. 1: Effect of ancymidol on production of forskolin in cell cultures of *C. forskohlii*.

The effect of ancumidol on production of forskolin in cell cultures of *C. forskohlii* showed dose response relation the 5μM (■) of ancymidol showed 1.5 fold increase in forskolin production and 2 fold increase with addition of 50 μM (▨)ancymidol over the control (▤) cell cultures.

REFERENCES

1. DeSouza, N.J. and Shah, V., In; Wagner, H., Hikino, H. and Farnsworth, N.R., Eds., Economic and Medicinal Plant Research, Vol. 2, Academic Press, New York, 1988, 1.
2. Bhat, S.V., Minale, L., Riccio, R. and Zollo, F., In; Herz, W., Kirby, G.W., Moore, R.E., Steglich, and Tamm,C.H., Eds., Progress in the Chemistry of Organic Natural Product, Vol. 62, Springer-Verlag, New York, 1993, 1.
3. Bhat, S.V., Bajwa, B.S., Dornauer, H. and DeSouza, N.J., **Tetrahedron Lett.,** 1977, 1669.
4. Krombholz, R., Mersinger, R., Kreis, W. and Reinhard, E., **Planta Med.,** 1992, 58, 328.
5. Kreis, W. and Reinhard, E., **Planta Med.,** 1989, 55, 409.
6. Tripathi, C.K.M., Basu, S.K., Jain, S. and Tandon, J.S., **Biotechnol. Lett.,** 1995, 17, 423.
7. Baochum, L.I. and David, J.W., **Plant Cell Reports,** 1995, 14, 529.
8. Ciddi, V., Srinivasan, V. and Shuler, M.L., **Biotech. Lett.,** 1995, 17, 1343.
9. Inamdar, P.K., Kanitkar, P.V., Reden, J., and DeSouza, N.J., **Planta Med.,** 1984, 50, 30.
10. Mersinger, R., Dornauer, H., and Reinhard, E., **Planta Med.,** 1988, 54, 200.
11. Mukherjee, S., Ghosh, B. and Jha, S., **Plant Cell Reports,** 1996, 15, 691.
12. Akhila, A., Rani, K., and Thakur, R., **Phytochemistry,** 1990, 29, 821.