SHORT COMMUNICATION

Chloramphenicol-Induced *in vitro* Bioproduction of Hyoscyamine from *Datura Stramonium Linn.* and *Datura Innoxia* Mill

M. K. TAILANG*, R. K. RAY AND M. D. KHARYA
Biotechnology Laboratory, Dept. of Pharmaceutical Sciences,
Dr. Hari Singh Gour Vishwavidyalaya, SAGAR (M.P) - 470 003

Received 31st January 1995

The present work is aimed at enhancing hyoscyamine production in *Datura stramonium* and *Datura innoxia* through chloramphenicol supplementation of culture medium. One to 2 ppm chloramphenicol in medium enhanced the callus growth as well as hyoscyamine content up to 112% W/W compared to callus grown on control medium. Supplementation of medium with higher concentrations of chloramphenicol caused no further increase in hyoscyamine production. On the contrary callus growth as well as hyoscyamine content reduced markedly.

ALKALOIDS are the most extensively investigated compounds among the secondary products because of their therapeutic importance. *Datura stramonium* and *Datura innoxia* (Farm. - Solanaceae) were extensively explored and found to contain two major alkaloids, hyoscyamine, and hyoscine[1,2].

Chloramphenicol, a well known protein synthesis inhibitor affects several physiological processes in plants.[3,4,5] According to Parr et al.[6] putrescine (a major intermediate in hyosyamine biosynthesis) primarily and preferentially is utilized in the formation of proteins followed by relatively small production of hyoscyamine in *Datura* spp. If the pathway leading to protein formation is inhibited, maximum of putrescine may be enrouted to enhance hyoscyamine production. Chloramphenicol interferes the interaction between substrates and inhibits the peptide bonding and thus inhibiting the protein synthesis[7,8]. Nasser[9] observed increased amino acid pool in plants following chloramphenicol treatment.

*** For Correspondence**
B.N. College of Pharmacy
Udaipur - 313 001 (Raj.)

Leaves from *Datura stramonium* and *Datura innoxia*, after through surface sterilization, were cut into explants and inoculated on MS medium supplemented with various concentration of Benzylaminopurine (BAP), α-Naphthalene acetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D) and Indole-3- butyric acid (IBA). After preliminary studies MS (1) [MS media supplemented with NAA (1 ppm), 2,4-D (1 ppm) and BAP (0.5 ppm)] and MS - (2) [MS media supplemented with NAA (1 ppm), BAP (0.5 ppm) and IBA (0.2 ppm)] were selected for further investigations. Calli obtained from MS (1) and MS (2) media were subcultured on the same media containing 1 to 10 ppm of chloramphenicol and culture tubes were exposed to fluorescent light (1600-2000 Lux for 16 h) at 25±2° for four weeks. Calli were withdrawn from culture tubes for their fresh and dry weights and growth indices were determined. The dried mass so obtained was estimated for hyoscyamine content.

Number of methods have been proposed for the determination and estimation of hyoscyamine in pharmaceutical preparations, plant extracts and plant cell culture extracts.[10] For the present study spectro-

Table - 1

Chloramphenicol induced in-vitro **Hyoscyamine bioproduction from** D.stramonium

S. No.	Medium	Growth Index* (after 4 weeks)	Percent variation in growth compared to control	Hyoscyamine content (% w/w)	Percent Hyoscyamine variation compared to control
1.	MS-(1) (Control)	5.2	-	0.76	-
2.	MS-1+Chloramphenicol (1 ppm)	5.2	**(+) 1.7	0.14	(+) 88.56
3.	MS-1+Chloramphenicol (2 ppm)	5.0	***(-) 2.5	0.16	(+) 105.26
4.	MS-1+Chloramphenicol (4 ppm)	4.4	(-) 15.3	0.17	(+) 94.74
5.	MS-1+Chloramphenicol (6 ppm)	3.1	(-) 39.9	0.12	(+) 60.53
6.	MS-1+Chloramphenicol (8 ppm)	1.4	(-) 72.6	0.08	(+) 8.68
7.	MS-1+Chloramphenicol (10 ppm)	Inhibition in Growth			
8.	MS-(2) (Control)	4.6	-	0.12	-
9.	MS-2+Chloramphenicol (1 ppm)	4.8	(+) 3.7	0.19	(+) 52.69
10.	MS-2+Chloramphenicol(2 ppm)	4.4	(-) 10.6	0.23	(+) 87.60
11.	MS-2+Chloramphenicol (4 ppm)	3.5	(-) 24.2	0.22	(+) 82.64
12.	MS-2+Chloramphenicol (6 ppm)	2.5	(-) 46.9	0.19	(+) 55.38
13.	MS-2+Chloramphenicol (8 ppm)	1.0	(-) 78.0	0.14	(+) 17.35
14.	MS-2+Chloramphenicol (10 ppm)	Inhibition in Growth			

$$*\text{Growth Index} = \frac{\text{Final dry wt.} - \text{Initial dry wt.}}{\text{Initial dry wt.}}$$

**(+) = Increase.

***(-) = Decrease.

photometric method developed by Chan and Stabs[11] was selected for the estimation of hyoscyamine in callus.

The data of Tables (1) and (2) reveal that 1 ppm chloramphenicol supplemented modified MS medium enhanced the callus growth in leaf derived *Datura stramonium* and *D. innoxia* callus culture. Increase in chloramphenicol concentration did not favour the callus growth but retarded it gradually.

One to four ppm chloramphenicol supplemented modified MS medium enhanced the hyoscyamine content upto 112% in *Datura innoxia* and 105% in *D. stramonium* as compared to control. Supplementation of medium with higher concentrations of chloramphenicol caused no further increase in hyoscyamine content.

The overall results reveal that chloramphenicol supplementation in the medium enhances the hyoscyamine level in *D. stramonium* and *D. innoxia* which justifies the hypothesis of inhibition of conjugate process leading to protein synthesis and thus making more putrescine available for hyoscyamine

Table - 2

Chloramphenicol induced in-vitro Hyoscyamine bioproduction from D.innoxia

S. No.	Medium	Growth Index* (after 4 weeks)	Percent variation in growth compared to control	Hyoscyamine content (% w/w)	Percent Hyoscyamine variation compared to control
1.	MS-(1) (Control)	5.9	-	0.10	-
2.	MS-1+Chloramphenicol (1 ppm)	6.1	(+) 13.7	0.17	(+) 77.32
3.	MS-1+Chloramphenicol (2 ppm)	5.3	(-) 10.4	0.21	(+) 112.37
4.	MS-1+Chloramphenicol (4 ppm)	4.3	(-) 28.1.	0.18	(+) 89.62
5.	MS-1+Chloramphenicol (6 ppm)	2.6	(-) 56.4	0.14	(+) 48.45
6.	MS-1+Chloramphenicol (8 ppm)	1.6	(-) 73.6	0.10	(+) 23.71
7.	MS-1+Chloramphenicol (10 ppm)	Inhibition in Growth			
8.	MS-(2) (Control)	5.1	-	0.18	-
9.	MS-2+Chloramphenicol (1 ppm)	5.1	(+) 1.4	0.31	(+) 72.22
10.	MS-2+Chloramphenicol (2 ppm)	5.0	(-) 0.4	0.36	(+) 101.11
11.	MS-2+Chloramphenicol (4 ppm)	3.5	(-) 30.4	0.32	(+) 76.67
12.	MS-2+Chloramphenicol (6 ppm)	1.8	(-) 64.3	0.28	(+) 55.56
13.	MS-2+Chloramphenicol (8 ppm)	1.3	(-) 74.9	0.20	(+) 13.32
14.	MS-2+Chloramphenicol (10 ppm)	Inhibition in Growth			

$$*\text{Growth Index} = \frac{\text{Final dry wt.} - \text{Initial dry wt.}}{\text{Initial dry wt.}}$$

(+) = Increase

(-) = Decrease

biosynthesis. Observations pertaining to 3 ppm and above concentration of chloramphenicol in the medium are not worthy as these have detrimental effect on callus growth. In the light of Parr **et al**[6] hypothesis, higher concentration of any protein synthesis inhibitor may retard the protein synthesis to a considerably extent disturbing the normal physiological functioning and thus ultimately threatening the very existence of plant cells which was true with 10 ppm chloramphenicol supplementation of medium inhibiting the noticed callus growth completely.

REFERENCES

1. Prabhakar V.S., Sarin Y.K. and Atal C.K. **Indian J. Pharm.** 1971, 33, 35.
2. Shah C.A. and Saji, A.N. **Indian J. Pharm.**, 1976, 29, 315.
3. Rao P.G., Reddy A.N. and Kumar N.R. **Z. Pflanten Physiol.** 1976, 80, 279.
4. Srivastava, H.S., **Indian J. Plant Physiol.**, 1976, 19, 53.

5. Hadi S.M. and Krishnamurthy, C.R. **Indian J. Biochem.**, 1976, 4, 1.

6. Parr A.J., Robins R.J., Hamil J.D. and Rhodes M.J.R. **Planta Medica**, 1990, 56, 602.

7. Sande M.A. and Mandell, GL., In; Gilman, A.G., Goodman I.S. and Gilman, A. Eds. The Pharmacological Basis of Therapeutics, 6th Ed. MC Millan Publishing Company New York, 1980, 1179.

8. Ellis, R.J., **Science.**, 1969, 163, 477.

9. Nassar, A.R., **Egypt, J. Hortic.**, 1976, 3, 231.

10. Peigen X. and Liyi H., **Planta Medica.**, 1982, 45, 112.

11. Chan W.N. and Staba E.J. **Lloydia** 1965, 28, 57.

Simultaneous Estimation of Captopril and Hydrochlorothiazide in two Component Tablets by Ultra Violet Absorption Spectrophotometry

A. SACHAN, D. K. JAIN AND P. TRIVEDI*
Dept. of pharmacy, S.G.S.I.T.S., 23 Park Road, Indore - 452 003.

Received 24th october 1995

A simple, rapid and economical procedure for simultaneous estimation of captopril and hydrochlorothiazide in two component tablet formulation has been developed. Captopril is quantitated utilising two wave lengths, 238 and 260 nm. A minor absorbance maxima at 322 nm of hydrochlorothiazide, where captopril had no absorbance, was used for estimation of hydrochlorothiazide. Beer Lambert's law was obeyed in the concentration ranges employed for the analysis. The results of analysis have been statistically validated and were found to be satisfactory.

CAPTOPRIL is an antihypertensive, used for treatment of hypertension and chronic congestive heart failure. Hydrochlorothaizide is a well known diuretic. Captopril is official in the U.S.P.[1]. Hydrochlorothiazide is official in I.P.[2], B.P.[3] and U.S.P.[4]. The U.S.P. describes a titrimetric method for analysis of captopril. Further literature survey revealed few HPLC[5] and Spectrophotometric[6,7] methods for analysis of captopril and also another HPLC[8] method for simultaneous analysis of captopril and hydrochlorothiazide. I.P.,B.P. and U.S.P. suggest a titrimetric method for analysis of hydrochlorothiazide. Other methods for estimation of hydrochlorothiazide include spectrophotometry[9], phosphorimetry[10], fluorimetry[11] and HPLC[8,12]. Shimadzu UV-160A recording spectrophotometer was employed for this work. Stock solutions of strength 100 mcg/ml each of captopril and hydrochlorothiazide in 0.05 M NaOH were used for analysis and the two wavelengths were selected for captopril using the two wave length data processing programme in the quantitative mode of analysis of the instrument. These wave lengths were found to be 238 nm and 260 nm. Hydrochlorothiazide was determined at 322 nm, where captopril had no absorbance. The overlain spectra of captopril and hydrochlorothiazide is given in Fig. -1.

The mixed standards of captopril and hydrochlorothiazide were prepared as per the concentrations cited in Table -1. All the mixed standard solutions

* For correspondence