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A comparative study of analgesic activity of *Plumbago zeylanica* Linn. callus and root extracts in experimental mice

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ABSTRACT

Plumbago zeylanica Linn. (Plumbaginaceae) commonly known as 'Chitrak', is a perennial subscandant shrub and is listed as threatened medicinal plant. The roots of the plant were used traditionally as an abortifacient, germicidal and in treatment of liver disease, cancer, body pain, fever and inflammation. Since it is an threatened and potential medicinal plant therefore it is of great interest to evaluate the analgesic effect of callus developed by nodal explant and to compare its action with respect to root extract of parent plant. Dried callus and roots from parent plant were powdered and extracted with ethanol. The callus extract (PCE) and root extract (PRE) at dose 100,200 and 400 mg/kg were evaluated for peripheral and central analgesic activity by glacial acetic acid induced writhing and tail immersion model respectively. PRE significantly ($P<0.01$) reduced the writhing count at 200 mg/kg whereas PCE alters the pain threshold significantly at 400 mg/kg. In tail immersion model, PRE increase the reaction time significantly ($P<0.01$) at 400 mg/kg. while PCE failed to alters the reaction time significantly throughout the observation period (upto 3 hrs). In conclusion, a massive light creamish brown and granular callus formed with MS M\medium supplemented with naphthalene acetic acid (1.5 ppm) and kinetin (0.25 ppm) and it possess a significant peripheral analgesic activity,

Keywords: *Plumbago zeylanica* Linn., Plumbaginaceae, tissue culture, analgesic

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INTRODUCTION

Plumbago zeylanica Linn. (Plumbaginaceae) commonly called chitrak, is an perennial, subscandant shrub found wild in South India and West Bengal. It is also cultivated in gardens throughout India [1-2]. Its roots are used in traditional system of medicine to cure various ailments like body pain, headache, fever and inflammation [3]. *Plumbago zeylanica* roots were reported to possess antioxidant, hypolipidemic, anti artherosclerotic, central nervous system stimulant and anti fertility properties [4-7]. It is a threatened plant [8] with potential medicinal value hence it was considered worthwhile to evaluate the analgesic activity of callus obtained from nodal explant and to compare its activity with respect to root extract from parent plant.

MATERIAL AND METHOD

Identification of Plant material

Plumbago zeylanica roots were procured from kharibawri market, Delhi. They were identified and authenticated by the Dr. H.B. Singh, Head, Raw material, Herbarium and Museum division, National Institute of Science Communication And Information Resources (NISCAIR), New Delhi. The stem twigs for tissue culture study were collected from Medicinal and Aromatic Plant Garden, CCS Haryana Agricultural University and were identified by Dr. C. S. Tyagi, Head, Medicinal Aromatic and Under Utilized Plant Section, Department of Plant Breeding, CCS HAU, Hisar. A voucher specimen is preserved in the Department for the ready reference.

Tissue Culture Study

Plant material collected was thoroughly washed in running tap water followed by treatment with Teepol solution 2% (v/v) to remove the adhere dust particles. The stem segments were further cut into 3-4 cm pieces with sterile blade having one node, used as the explant. The explants were treated with fungicide 'Tagstin' 2% (w/v) for 7-8 minutes. The explant were surface sterilized with 0.1% (w/v) aqueous mercuric chloride solution for 10-15 minutes followed by three to four washing with sterile water to remove last traces of sterilizing agent [9]. Nodal explants were inoculated in culture bottles with sterile murashige and skoog nutrient medium [10] with different concentration of phytoharmones naphthalene acetic acid (0.25-2.00 ppm) and Kinetin (0.05-0.50 ppm) in five batches. Each batch of the experiment was started with 20 cultures and follow strict aseptic conditions. All the cultures were maintained at 26°±2°C under 16 hours photoperiod per day provided by white fluorescent tubes.

Preparation of extracts

Dried roots and callus were mechanically pulverized to a coarse powder and extracted with ethanol 95% in soxhlet extractor for 72 hours. After exhaustive extraction, the root extract

(PRE) and callus extract (PCE) were filtered and concentrated over boiling water bath to recover the solvent.

Experimental animals

Swiss albino male mice weighing around 20-30 g. procured from disease free small animal house, CCS Haryana Agriculture University, Hisar. The animals had free access to food and clean water. The animals were housed in natural light dark cycle (12 hour each).The care of experimental animals was taken as per the guidance of CPCSEA, Ministry of Forest and Environment, Govt. of India.

Drugs and chemicals

The drugs and chemicals used in the present study was obtained from following sources. Murashige and Skoog nutrient medium (Hi-media Laboratories Pvt. Ltd., Mumbai), indomethacin (Jagsonpal Pharmaceutical Ltd., Haryana), pentazocine (Vardhman labs, Haryana), glacial acetic acid (Qualigens Fine Chemicals, Mumbai) and carboxy methyl cellulose (Hi-Media Laboratories Pvt. Ltd., Mumbai).

Dose

Test compounds: Root extract (ethanol) - 100, 200 and 400 mg/kg
Callus extract (ethanol) - 100, 200 and 400 mg/kg
Standard drug: pentazocine 5 mg/kg and indomethacin 10 mg/kg

Vehicle

The extracts and indomethacin were suspended in 0.5% carboxy methyl cellulose and given per orally. pentazocine was dissolved in normal saline and were injected intraperitoneally.

Screening of analgesic activity

The peripheral analgesic activity of extracts were investigated by glacial acetic acid (0.6%) induced writhing test in mice [11,12]. The abdominal stretching were observed and counted for 20 minutes after administration of glacial acetic acid.

The central analgesic activity was assessed by tail immersion test in which tail of mice was entirely immersed in water at temperature about 58°C and time until withdrawl of tail was measured by stop watch [13].

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Statistical Analysis

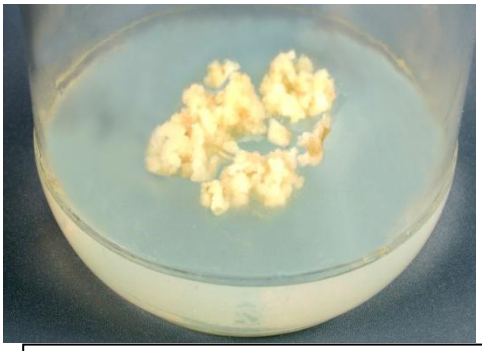
The results are expressed as mean ± SEM. Statistical analysis of data was performed using unpaired student's *t* test following one way ANOVA. P<0.05 was considered significant [14].

RESULTS

A massive, light creamish brown and granular callus formed in 90 percent cultures in MS medium supplemented with NAA (1.5 ppm) and Kinetin (0.25 ppm) (Table 1) (Figure 1,2). In acetic acid induced writhing test, Ethanol extract of roots (PRE) significantly (P<0.01) reduced the writhing count at 200 mg/kg while callus extract (PCE) alters the pain threshold significantly (P<0.05) at 400 mg/kg (Table 2). In tail immersion test, PRE increase the reaction time significantly, at dose 400 mg/kg indicates the significant peak reaction time at 2 hour (P<0.01), whereas Pentazocine, reference drug, has significant peak reaction time at 1hour (P<0.001). The callus extract (PCE) failed to alter the reaction time significantly throughout the observation period upto 3hr (Table 3).

Figure 1,2: Callus initiation and proliferation after different time period of inoculation on MS medium with NAA-1.50ppm & Kinetin-0.25ppm

Callus after 08 days of inoculation



Callus after 25 days of inoculation

DISCUSSION

A massive, light creamish brown and granular callus formed with higher concentration of auxin i.e. naphthalene acetic acid and comparatively lower concentration of cytokinin i.e. kinetin. Analgesic effect of *P. zeylanica* root extract (PRE) and callus extract (PCE) was studied for central (opiod) and peripheral (non opiod) activity. Results of study reveals that analgesic effect of PRE was comparable to that of Indomethacin. Relief of pain by Indomethacin is due to suppressing the formation of pain substances in the peripheral tissues, where prostaglandins and bradykinin were suggested to play an important role in pain perception [15]. Therefore it is likely that extracts might suppress the formation of these substances and show analgesic

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Table 1. Effect of hormones (alone and in combination) on callus induction

Batch No.	NAA (ppm)	Kinetin (ppm)	Callus initiation time (days)	Explant showing callusing (%)	Nature of callus
1	0.25	-	-	-	-
2	0.50	0.05	09-12	30	GY, G
3	1.00	0.10	08-10	60	LB, N
4	1.50	0.25	08-11	90	LCB, G, C
5	2.00	0.50	10-12	50	LB, C

C- Compact; N- Nodular; GY- Greenish Yellow; LCB- Light Creamish Brown; G- Granular

Table 2. % inhibition of no. of writhing by ethanolic extract of root and callus

Groups	Extract	Dose (mg/kg)	No. of writhings in 20 minutes	% inhibition
1.	Control	-	33.8±0.43	-
2.	Indomethacin	10	9.2±0.28**	72.78
3.	Roots	100	21.4±0.76	36.69
4.		200	14.6±0.38**	56.80
5.		400	23.4±1.07	30.76
6.	Callus	100	26.4±0.91	21.89
7.		200	22.6±0.45	33.13
8.		400	19.2±0.31*	43.19

Table 3. Reaction time of ethanolic extract of roots and callus using tail immersion model

Groups	Extracts/ Drugs	Dose mg/kg	Reaction time after				
			0 hr	1/2 hr	1 hr	2 hr	3 hr
1.	Root extract	100	0.88 ± 0.32*	1.86 ± 0.69**	2.48 ± 0.89**	2.94± 1.31**	2.34± 1.21**
2.	Root extract	200	0.97 ± 0.53	1.75 ± 0.93*	2.82 ± 1.01**	3.58 ± 1.71**	2.85 ± 1.91**
3.	Root extract	400	1.18 ± 0.63	1.96 ± 0.71**	2.15 ± 0.98*	2.71 ± 1.12**	2.32 ± 0.91**
4.	Callus extract	100	0.88 ± 0.41	1.10 ± 0.58	1.17 ± 0.37	1.29 ± 0.41	1.19 ± 0.31
5.	Callus extract	200	1.03 ± 0.45	1.21 ± 0.37	1.37 ± 0.33	1.45 ± 0.41	1.32 ± 0.39
6.	Callus extract	400	0.99 ± 0.29	1.14 ± 0.31	1.38 ± 0.39	1.78 ± 0.49	1.58 ± 0.51
7.	Pentazocine	5	0.95 ± 0.21	2.04 ± 0.91**	4.07 ± 1.16**	3.68 ± 0.99**	3.30 ± 0.78**
8.	Control	–	0.98 ± 0.34	0.94 ± 0.19	1.01 ± 0.41	1.05 ± 0.26	0.98 ± 0.31

Values are expressed as mean ± SEM, n= 5* P < 0.05 as compared to control group

** P < 0.01 as compared to control group

activity in peripheral pain model. In tail immersion model, PRE (400 mg/kg *p.o.*) significantly increase the reaction time suggesting its central analgesic activity. *Plumbago zeylanica* was reported to possess the flavonoids, and flavonoids were known to inhibits the prostaglandins synthetase [16]. Since prostaglandins are involved in pain perception, it could be suggested that nonavailability of prostaglandin by flavonoids of PRE, might be possible for its analgesic effect.

CONCLUSION

In conclusion, present attempt revealed that the ethanolic extract of root and callus possess significant peripheral analgesic activity whereas as the root extract possess significant central analgesic activity as compared to callus extract.

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