BIOCELL
2006, 30(1): 9-13

ISSN 0327 - 9545
PRINTED IN ARGENTINA

Evaluation of cytotoxic potential of latex of *Calotropis procera* and Podophyllotoxin in *Allum cepa* root model

R. Sehgal, S. Roy, and V.L. Kumar*

Department of Pharmacology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110 029, INDIA.

Keywords: *Calotropis procera,* Podophyllotoxin, cyclophosphamide, cytotoxic, *Allium cepa*

ABSTRACT: In the present study we have utilized the *Allium cepa* root tip meristem model to evaluate the cytotoxic and anti-mitotic activities of latex of *Calotropis procera* (DL) and podophyllotoxin. Standard cytotoxic drug cyclophosphamide and non-cytotoxic drugs cyproheptadine and aspirin served as controls. Like cyclophosphamide, both DL and podophyllotoxin significantly inhibited the growth of roots and mitotic activity in a dose-dependent manner. However, podophyllotoxin was more potent in this regard and produced root decay. Cyproheptadine and aspirin, on the other hand, showed a marginal effect on the root growth and mitotic activity at much higher concentrations.

Introduction

A wide variety of anti-cancer drugs exhibit cytotoxic effect by interfering with cell-cycle kinetics. These drugs are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase (Gali-Muhtasib and Bakkar, 2002). An alkylating agent, cyclophosphamide, interferes with DNA integrity and thereby exhibits strong anti-mitotic activity both *in vivo* and *in vitro* (Crook *et al*., 1986; Misra and Bloom, 1991; Gereis *et al*., 1987). Most of the plant derived anti-cancer drugs affect the microtubule dynamics of the cell and induce persistent modification of biological processes and signaling pathways that ultimately lead to apoptotic death (Mollinedo and Gajate, 2003). Podophyllotoxin is one of such plant derived anti-mitotic agent that has been shown to produce mitotic arrest by changing the organization of mitotic spindle (Jordan *et al.*, 1992). However, most of the cytotoxic drugs exhibit serious side effects (Powis, 1983). Hence, there is a need for drugs that are equally efficacious but have lesser side effects.

Calotropis procera, a wild growing plant is well known for its medicinal uses in traditional system of medicine for the treatment of variety of disease conditions that include leprosy, ulcers, tumors and piles (Kirtikar and Basu, 1935). The milky white latex obtained from the plant exhibits potent anti-inflammatory activity in various animal models that is comparable to standard anti-inflammatory drugs (Sangraula *et al.*, 2002). It has been well established through various experimental and clinical studies that drugs possessing

Address correspondence to: Dr. Vijay L. Kumar. Department of Pharmacology. All India Institute of Medical Sciences. Ansari Nagar, New Delhi-110 029, INDIA.
Fax: (+91-11) 26588663. E-mail: kumarv198@hotmail.com
Received on December 21, 2004. Accepted on December 13, 2005.

anti-inflammatory activity also exhibit anti-cancer properties (Umar *et al.*, 2003). Earlier *in vitro* studies demonstrate potent cytotoxic activity in the flower extracts of *Calotropis procera* that is comparable to standard anticancer drug cisplatin (Smit *et al.*, 1995). The present study was carried out to evaluate the cytotoxic and antimitotic potential of latex of *C. procera* and podophyllotoxin by standard assay method using *Allium cepa* root meristem model (Sharma, 1983). The effect was compared with standard anticancer drug cyclophosphamide and non-cytotoxic drugs cyproheptadine and aspirin.

Materials and Methods

Growing Allium cepa meristems

Locally available *Allium cepa* bulbs (50 ± 10 g) were grown in the dark over 100 ml tap water at ambient temperature until the roots have grown to approximately 2-3 cm. The water was changed daily.

Calotropis procera latex collection

The latex was collected from the aerial parts of *C. procera* growing in the wild. The plant was identified by the Raw Materials, Herbarium and Museum Division, National Institute of Science Communication, CSIR, New Delhi where a voucher specimen is preserved (Voucher No. PID 1739). The latex was dried under shade at ambient temperature (DL).

Conditions for drug Incubation

Working dilutions of all the drugs were made in tap water. DL was triturated in water to obtain 1 mg/ml and 10 mg/ml concentrations. Podophyllotoxin was initially dissolved in 100 µl ethanol and then diluted to required concentrations (0.05 mg/ml and 0.5 mg/ml) with water. Cyclophosphamide was used at 1 mg/ml and 10 mg/ml concentration while cyproheptadine and aspirin both were used at 5 mg/ml concentration. The bulbs with root tips grown upto 2-3 cms were placed over drug solutions and incubation was carried out at ambient temperature. The length of roots grown in drug solution (newly appearing roots not included), root number and the mitotic index were recorded at 0, 48 and 96 h and compared with that of control bulbs placed over tap water.

Microscopic examination and determination of mitotic index

The root tips (2-3 mms) were collected, placed in 1 N HCl for 5 minutes, squashed and finally stained with 2% aceto-orceine. For each root tip the number of mitotic and total meristematic cells were counted in 5-8 fields using high power (100X) light microscope (Badria *et al*., 2001). In all 400-500 cells were counted and cells manifesting different stages of mitosis i.e., interphase and prophase (P), metaphase (M), anaphase (A) and

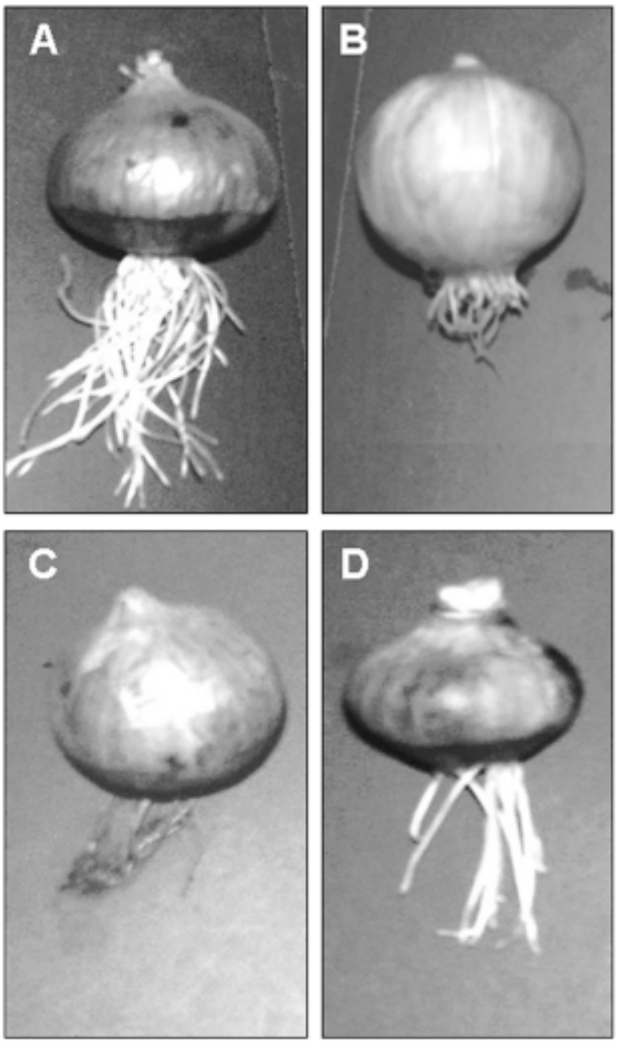

FIGURE 1. *Allium cepa* bulbs showing the effect of various cytotoxic agents on root length following 96 h of incubation. A: control; B: DL (10 mg/ml); C: Podophyllotoxin (0.5 mg/ml); D: cyclophosphamide (10 mg/ml)

telophase (T) were recorded. The mitotic index was calculated using the following formula:

$$\text{Mitotic index} = \frac{P + M + A + T}{\text{Total cells}}$$

Statistical analysis

The values are given as mean ± SEM and the data was analyzed by Student's t-test.

Results

The inhibitory effect of podophyllotoxin and DL was evaluated on the growth and mitotic activity of *Allium cepa* root meristems and the effect was compared with standard anti-cancer drug cyclophosphamide. A progressive increase in root number and root length was observed in control group. The root length in control group at 0, 48 and 96 h was 2.80±0.28 cm (n=12), 3.09±0.22 cm (n=24) and 3.82±0.19 cm (n=34). Incubation of bulbs in different concentrations of cytotoxic agents produced a growth retarding effect that was associated with a decrease in the root number (Fig. 1). Both DL and cyclophosphamide arrested the root growth. However, the root number did not increase any further at 10 mg/ml concentration. Podophyllotoxin, on the other hand, produced root decay and decreased the root length and root number significantly at 48 h and 96 h as compared to that at 0 h ($p<0.001$). The root length at 0.5 mg/ml of podophyllotoxin was 2.72±0.18 cm (n=13), 1.46±0.22 cm (n=8) and 1.00±0.05 cm (n=3) at 0 h, 48 h and 96 h, respectively. Non-cytotoxic drugs, cyproheptadine and aspirin, did not produce a change in the root growth and root number (Table 1).

The mitotic cells were counted in the root meristems in above groups at 0, 48 and 96 h of incubation with each drug. The mitotic index ranged between 60.7±0.7 and 63.0±2.3 in the control group over a period of 96 h.

TABLE 1.

***Allium cepa* root length attained following incubation with various drugs**

Groups	Root length (cms)		
	0	48	96
Control	2.80±0.28 (n = 12)	3.09±0.22 (n = 24)	3.82±0.19* (n = 34)
DL 1 mg/ml	2.38±0.28 (n = 17)	2.59±0.18 (n = 32)	2.51±0.17 (n = 42)
DL 10 mg/ml	2.14±0.27 (n = 15)	2.61±0.09 (n = 12)	2.34±0.09 (n = 12)
Pod 0.05 mg/ml	3.48±0.45 (n = 8)	2.35±0.29 (n = 16)	1.96±0.23** (n = 13)
Pod 0.5 mg/ml	2.72±0.18 (n = 13)	1.46±0.22** (n = 8)	1.00±0.05** (n = 3)
Cyc 1 mg/ml	2.21±0.30 (n = 15)	2.81±0.18 (n = 35)	3.20±0.20* (n = 43)
Cyc 10 mg/ml	2.36±0.30 (n = 13)	2.46±0.29 (n = 12)	2.46±0.27 (n = 12)
Cyp 5 mg/ml	2.04±0.26 (n = 5)	2.24±0.27 (n = 9)	3.06±0.20* (n = 27)
Asp 5mg/ml	2.40±0.12 (n = 16)	2.58±0.19 (n = 19)	3.09±0.20* (n = 34)

Statistical significance is given for comparison of root length attained at 48 and 96 h with respect to the 0 h control *$p<0.05$; **$p< 0.001$; Pod: Podophyllotoxin; Cyc:cyclophosphamide; Cyp: cyproheptadine; Asp: aspirin

DL produced a significant decrease in mitotic index that was dose and time dependent. The mitotic index at 10 mg/ml concentration of DL was 32.7±0.8 at 48 h as compared to 57.6±0.4 at 0 h while at 96 h the cellular morphology was lost. Treatment with 0.05 mg/ml of podophyllotoxin significantly reduced the mitotic index to 43.9±2.5 at 96 h ($p<0.01$) while at higher concentration the cellular morphology was lost and the roots decayed. Treatment with cyclophosphamide at 1 mg/ml and 10 mg/ml concentration brought down the mitotic index to 40.6±1.3 and 37.3±1.0 at 96 h ($p<0.0001$). Cyproheptadine and aspirin produced only a marginal decrease in mitotic index at 5 mg/ml concentration (Table 2).

Discussion

Allium cepa root tip meristems have been widely used for the evaluation of cytotoxic and anti-mitotic activity of various compounds (Shehab, 1980; Williams and Omoh, 1996; Al-Meshal, 1987). In the present study we have tested the cytotoxic and anti-mitotic effect of DL, standard anticancer drugs, podophyllotoxin and cyclophosphamide in *Allium cepa* root tip meristem model and compared it with two non-cytotoxic drugs cyproheptadine and aspirin. Podophyllotoxin, an inhibitor of microtubule assembly was most effective in inhibiting mitosis in *Allium cepa* root tip meristems. In this model, its cytotoxic effect was evident in the form of shortening and decaying of roots. Anti-tumor drugs that interact with microtubules and tubulin are known to block mitosis and induce cell death by apoptosis (Jordan, 2002). The cytotoxic effect of DL was comparable to that of cyclophosphamide and both agents inhibited root growth and mitosis to a significant extent. Earlier the extracts prepared from the dried flowers, roots and leaves of *C. procera* have been reported to exhibit potent cytotoxic activity (Smit *et al.*, 1995; Hussein Ayoub and Kingstonb, 1981). This activity has been attributed to the cardinolide calotropin that is present in the latex as well (Kupchan *et al*., 1964; Kiuchi *et al*., 1998). Further, the DL might be inhibiting growth factor mediated mitogenic signaling pathways. On the other hand, cyclophosphamide alkylates DNA and proteins after it has been metabolized by cytochrome P450 to yield phosphoramide mustard and acrolein. It induces plasma membrane blebbing, DNA fragmentation and cleavage of poly (ADP-ribose) polymerase (PARP) and produces cell death by apoptosis (Schwartz and Waxman, 2001).

It is important to note that non-cytotoxic drugs, cyproheptadine and aspirin did not affect the root length

TABLE 2.

Mitotic index in *Allium cepa* meristems following incubation with various drugs.

Groups	Mitotic index		
	0 h	48 h	96 h
Control	62.9±0.3	60.7±0.7	63.0±2.3
DL 1 mg/ml	61.3±0.5	40.8±1.2**	34.4±2.2**
DL 10 mg/ml	57.6±0.4	32.7±0.8**	ND
Pod 0.05 mg/ml	61.0±1.9	53.8±1.5*	44.0±2.5*
Pod 0.5 mg/ml	62.7±0.3	41.7±4.2*	ND
Cyc 1 mg/ml	62.7±1.4	47.3±0.3**	40.6±1.3**
Cyc 10 mg/ml	63.3±0.5	43.5±0.4**	37.3±1.0**
Cyp 5 mg/ml	58.0±2.1	56.6±0.5	55.7±0.3
Asp 5mg/ml	59.4±0.1	57.6±1.2	55.8±0.4**

Statistical significance is given for comparison of mitotic index obtained at 48 and 96 h with respect to the 0 h control *$p<0.01$; **$p< 0.0001$; ND: not detectable; Pod: Podophyllotoxin; Cyc:cyclophosphamide; Cyp: cyproheptadine; Asp: aspirin

and root number. Both drugs also produced marginal anti-mitotic effect at a dose that was much higher than that of anti-cancer drugs. At higher concentration cyproheptadine has been shown to suppress cell division and exhibit cytotoxic effect of non-specific nature (Zang *et al.*, 1975). The cytotoxic effects of aspirin, an anti-inflammatory drug, are mainly due to its ability to induce DNA fragmentation and proteolytic cleavage of PARP. It is also known to activate caspases through cyclooxygenase-independent mechanism (Bellosillo *et al.*, 1998).

Thus, our study demonstrates that latex of *C. procera* exhibits cytotoxic properties like standard anti-cancer drugs podophyllotoxin and cyclophosphamide. However, the mechanism for such an effect needs further evaluation.

References

Al-Meshal IA (1987). Mitodepressive effect of (-)-cathinone, from Catha edulis (khat), on the meristematic region of *Allium cepa* root tips. Toxicon. 25: 451-454.

Badria FA, Houssein WE, Zaghloul MG, Halim AF (2001). Anti-mitotic activity of gossypol and gossypolone. Pharm Biol. 39: 120-126.

Bellosillo B, Pique M, Barragam M, Castano E, Villamor N, Colomer D, Montserrat E, Pons G, Gil J (1998). Aspirin and salicylate induce apoptosis and activation of caspases in B-cell chronic lymphocytic leukemia cells. Blood. 92: 1406-1414.

Crook TR, Souhami RL, McLean AE (1986). Cytotoxicity, DNA cross-linking and single strand breaks induced by activated cyclophosphamide and acrolein in human leukemia cells. Cancer Res. 46: 5029-5034.

Gali-Muhtasib H, Bakkar N (2002). Modulating cell cycle: current applications and prospects for future drug development. Curr Cancer Drug Targets. 2: 309-336.

Gereis M, Burford Mason AP, Watkins SM (1987). Suppression of *in vitro* peripheral blood lymphocyte mitogenesis by cytotoxic drugs commonly used in the treatment of breast cancer: a comparative study. Agents Actions. 22: 324-9.

Hussein Ayoub SM, Kingstonb DGI (1981). Screening of plants used in Sudan folk medicine for anticancer activity. Fitoterapia. 52: 281-284.

Jordan MA, Thrower D, Wilson L (1992). Effects of vinblastine, Podophyllotoxin and nocodazole on mitotic spindles - Implications for the role of microtubule dynamics in mitosis. J Cell Sci. 102: 401-416.

Jordan MA (2002). Mechanism of action of antitumor drugs that interact with microtubules and tubulin. Curr Med Chem Anti-Canc Agents. 2: 1-17.

Kirtikar and Basu (1935). Indian Medicinal plants, Vol. 1-8. Bishen Singh Mahendra Pal Singh, Dehradun, India.

Kiuchi F, Fukao Y, Maruyama T, Obata T, Tanaka M, Sasaki T, Mikage M, Haque ME, Tsuda Y (1998). Cytotoxic principles of a Bangladeshi crude drug, akond mul (roots of Calotropis gigantea L.). Chem Pharm Bull (Tokyo). 46: 528-30.

Kupchan SM, Knox JR, Kelsey JE, Saenzrenauld JA (1964). Calotropin, a cytotoxic principle isolated from *Asclepias curassavica* L. Science. 146: 1685-1686.

Misra RR, Bloom SE (1991). Roles of dosage, pharmacokinetics, and cellular sensitivity to damage in the selective toxicity of cyclophosphamide towards B and T cells in development. Toxicology. 66: 239-256.

Mollinedo F, Gajate C (2003). Microtubules, microtubule-interfering agents and apoptosis. Apoptosis.8: 413-450.

Powis G (1983). Dose-dependent metabolism, therapeutic effect, and toxicity of anticancer drugs in man. Drug Metab Rev.14: 1145-1163.

Sangraula H, Dewan S, Kumar VL (2002). Evaluation of anti-inflammatory activity of latex of *Calotropis procera* in different models of inflammation. Inflammopharmacology. 9: 257-264.

Schwartz PS and Waxman DJ (2001). Cyclophosphamide induces caspase 9-dependent apoptosis in 9L tumor cells. Mol Pharmacol. 60: 1268- 1279.

Sharma CBSR (1983). Plant meristems as monitors of genetic toxicity of environmental chemicals. Curr Sci. 52: 1000-1002.

Shehab AS (1980). Cytological effects of medicinal plants in Qatar II. Mitotic effect of water extract of Teucrium pilosum on Allium cepa. Cytologia (Tokyo) 45: 57-64.

Smit HF, Woerdenbag HJ, Singh RH, Meulenbeld GJ, Labadie RP, Zwaving JH (1995). Ayurvedic herbal drugs with possible cytostatic activity. J Ethnopharmacol 47: 75-84.

Williams GO, Omoh LE (1996). Mitotic effects of the aqueous leaf extract of Cymbopogon citratus in *Allium cepa* root tips. Cytobios. 87:161-168.

Umar A, Viner JL, Anderson WF, Hawk ET (2003). Development of COX inhibitors in cancer prevention and therapy. Am J Clin Oncol. 26: S48-57.

Zang KD, Eicke J, Stengel Rutkowski S, Zankl H (1975). Chromosome studies on human fibroblast cultures following exposure to cyproheptadin. Z Kinderheilkd. 118: 303-313.