Biosci. Biotech. Res. Asia Vol. **3,** No.(**1**) 99-102 (2005)

IN VITRO ANALYSIS OF ANTI-CANCER ACTIVITY OF *Oroxylum indicum* WITH THE COMBINATION OF *Catharanthus roseus*, *Commiphora mukul*, AND *Cynodon dactylon* IN DLA TRANSPLANTED SWISS ALBINO MICE

Smitha Sam and N. Ganesh

Jawaharlal Nehru Cancer Hospital & Research Centre, Bhopal - 462 001 (India)

(Received May 07, 2005; Accepted June 13, 2005)

ABSTRACT

A comparative study of anticancer efficacy of *Oroxylum indicum* with the combination of *Catharanthus roseus*, *Commiphora mukul* and *Cynodon dactylon* in DLA (Daltons Lymphoma Ascities tumor cell lines) transplanted Swiss albino (*Mus muculus)* mice by *in vitro* methods were done. Swiss albino male mice (32 in number) treated with DLA cell lines were used in the present investigation. Along with this, sweet potatoes, which were transformed with *Agrobacterium tumifacienc*e and treated with different concentrations of medicinal plants like *Oroxylum indicum, Catharanthus roseus, Commiphora mukul* and *Cynodon dactylon* and were developed with tumors, after 21 days was also used for the study. The total number of tumors per disc was counted and DNA was isolated from both the transformed sweet potatoes and tumor cells of the cancerous mice. They were loaded in agarose gel and checked the percentage of genotoxicity by 'DNA laddering method' by comparing the molecular weight with EcoR1 Hind III Double Digested marker. While studying the results of each experiment, it was found that *Oroxylum indicum*, the unexplored ayurvedic medicinal plant, is a real promise in the cancer treatment in near future with low cost and high mode of activity.

Keywords: *Oroxylum indicum*, *Catharanthus roseus*, *Commiphora mukul*, *Cynodon dactylon*, DLA cell lines, DNA laddering method and EcoR1 Hind III Double Digested marker.

INTRUDOCTION

Cancer is a disorder of the genetic make-up of somatic cells which results in a clone of cells with an abnormal pattern of growth control[1]. Now most developing countries are viewing traditional medicinal practice as an integral part of their culture. Because of this sudden awareness, the international trade in plants of medicinal importance is growing phenomenally[2]. Various plants have got potential role in cancer therapy as either a direct anticancer agent, chemopreventive agent, and immunomodulator or as radiosensitizer[3]. Here comes the importance of, one of the medicinal plants, which is widely using in ayurveda *Oroxylum indicum* (Bignoniaceae family) with its anti- cancer property. Lapachol (2-hydroxy-3-(3methyl-2 butenil)-1,4 and Beta Lapachone are the two very important naphthaquinone, which inhibit the action of the DNA-Topoisomerase I enzyme, which unwinds the DNA and helps in the replication of the cell.The plant can use as an anticancer, antiviral, antileukemic drug and can be used against skin ulceration, cough and as a purgative[4]. In 1960, a cancer patient from Savopolo (Brazil) was treated with water extraction of the bark, for two months, got high percentage of reveal from pain[5]. Few other medicinal plants like, *Catharanthus roseus, Commiphora mukul* and *Cynodon dactylon* is also selected for comparing the anti-cancer effect in Swiss albino mice inoculated with Daltons Lymphoma Ascites (DLA) cell line[6]*. Catharanthus roseus*'s alkaloids like Vincristine and Vinblastine are the first isolated chemically useful plant compounds and are widely using in toxicological, pharmacological and chemical studies. In human being 'Leurocrystine' is highly effective against Wilm's tumor, breast cancer, primary brain tumor, carcinoma in cervix, prostrate and kidney. By producing 'microtubule crystals', these alkaloids are competing with tubulin, the protein component of microtubule and mitotic spindle[7] and these alkaloids can use as an 'Oral Insulin Substrate'. *Commiphora mukul* (The medicinal plants of India, 2002) having wide spectrum of action, it can use against arthritis, cancer, cholesterol reduction. *Cynodon dactylon* is a very good anticancer, antibacterial medicine, which significantly elevate the activities of cytochrome - P450, Cytochrome -

b5, aryl - hydrocarbon, hydroxilase; all are important for esterification of carcinogens.

MATERIAL AND METHODS

Swiss albino (*Mus muculus)* male mice (32 in number, 25-30 g), were used for the studies and animals were maintained under standardized, environmental conditions (22- 28°C, 60-70 % relative humidity, 12 hours dark/light cycle and water *ad libitum.* All the experiments were conducted following the guidelines of Institutional Animal Ethical Committee.

Methods for inducing cancer in mice

The Swiss Albino male mice used for the study were taken. The Dalton's Lymphoma Ascites (DLA) cell lines from a cancerous mice were taken (1x 10^6 cells) in 1ml syringe, containing 0.1ml of P.B.S (pH 7.3) and injected in to the mice intraperitonially. Tumor was developed in the abdomen of mice, after 15 days of inoculation. The cell lines were maintained by subculturing within the mice (3 x 10^6 DLA cells/ml were taken in 300µl PBS and inoculated in the intraperitonial cavity of the mice) itself.

Dosage of medicine

The normal mice is treated as vehicle (Group-I) and the tumor alone, (group II) without any treatment. The third group was treated with the extract of *O. indicum* first, after one month DLA cell lines (10ml / Kg body weight). So 0.75ml three times daily. The fourth group mice were treated with *O. indicum*, in the same day (After DLA induction), 0.75ml , three times daily. Group V was, mice with DLA, treated with mixture, 0.75 ml, three times daily. The duration of the study was 7 weeks.

Potato Disc Bio-assay

This is one of the best, cheap *in vitro* methods for checking the anti-cancer effect of medicinal plant. Cylindrical core of sweet potato were made and placed on 1.5% water agar and were treated with 50µl of 48hrs old *Agrobacterium tumifascienc*e culture. Various concentrations like, 20, 40, 60, 80 and 100 ìl of, 'Mixture' (group II), *O. indicum* (group III) extracts were added on different sets of discs with, sterile water as control(group I).The inoculated discs were maintained at 25°C for 21 days. Succeedingly the tumor no: were counted after staining with lugol's solution and ethanol.

Experimental Design - Grouping of Animals:

S. No.	Group	Description	No of Mice
1	I	Normal Swiss Albino male mice (vehicle)	6
2	II	Swiss Albino male mice with DLA cell lines	6
3	III	Swiss Albino male mice treated with *O. indicum* extract (before the inoculation of DLA)	6
4	IV	Swiss Albino male mice treated with *O. indicum* extract (after the inoculation of DLA)	6
5	V	Swiss Albino male mice treated with the 'Mixture' of *O. indicum, C. alba*, *C. mukul* and *C. dactylon*	6

$$\% \text{ Inhibition (N)} = \frac{\text{Average No:tumors on control discs + Average No: tumors on treated discs}}{\text{Average No: tumors on control discs}} \times 100$$

Isolation of Genomic DNA from transformed potato discs - DNA Laddering Method

The *A. tumefascience* affected sweet potatoes were taken for checking its damage in DNA level after tumor induction. The transformed sweet potato from different groups, treated with different concentrations of extracts medicinal plants were collected and homogenized with the extraction buffer. Kept it at 55°C and centrifuged and added sodium acetate, double the volume of chloroform: iso-amyl alcohol to the supernatant. After treating it with sodium acetate and ethyl alcohol, washed the pellet with alcohol and Tris EDTA buffer. The DNA were runned in 0.7% agarose with EcoR1 - Hind III-Double digested marker with DNA from potato disc,which treated with different concentrations of extracts like 100,80,60,40 µls, 'control' and 'mixture' in well number, 1,2,3,4 ,5,6and 7 respectively. Later observed under ultraviolet chamber and studied.

Isolation of DLA cell lines of tumor affected mice - DNA Laddering Method

2ml of DLA cell lines were collected from group II, III, IV and V animals and treated with 2ml of 5% EDTA and kept in deep freezer followed by 1x SSC treatment. To the supernatant added with 18ml of TEN -A buffer, 10% SDS and 85µl of proteinase-K for 2 hours at 50°C. Supernatant were precipitated after centrifugation at room temperature. The pellets were washed with ethanol and dissolved in TE buffer, runned in 0.7% agarose with EcoR1 - Hind III - Double digested marker with DNA from, group III, IV, V and II in well number, 1,2,3,4 and 6 respectively. Later DNA were observed under ultra violet chamber.

DNA from Agrobacterium transformed potato discs and DLA cell lines: DNA Laddering Method

The DNA isolated from *A.tumefascience* transformed potato discs and DLA cell lines were showing characteristic difference in their molecular weight. The DNA bands of cells, shows 'A ladder like' arrangement in agarose gel, which was one of the most significant, proof for the abnormal chromosomal break down in cancer cells. So it got its name as 'DNA laddering method'. The DNA bands of Hind III marker shows different molecular weight varying from 564 KDaltons to 21,226 KDaltons. DNA of DLA cell lines of different groups of animals' shows a wide range of fragmentation with molecular weight as – line two (Groups -III) near to 2,027 and 3,530KD. Line 3 (Group IV) shows, DNA with molecular weight near to 1, 904, 2,027, 2,830 and 4,920 KD. Line 4 (Group IV) shows the bands near to 2,027, 3,530 and 10,125 KD. Line 6 (group-II), is showing a clear, sheared pattern of DNA with some clear bands in between with molecular weight ranging from 831 KD to 21,226 KDL with 3,530 and 4,268 KDs.

In case of DNA bands of transformed cells, line number 2,3,4,5,6, and 7, corresponding to the cells of different concentrations like 100, 80, 60, 40, control and mixture respectively is showing a DNA band with same molecular weight as 21,226 KDs. But in case of DNA of control discs, at line no: 6 is showing two marked, fragmented DNA bands with molecular weight near to 5,148 and 3,680KDs.

RESULTS AND DISCUSSION

Oroxylum indicum, the future promise of Ayurvedic medicine, were used for the *in vitro* studies by using the methods like 'Potato Disc Assay' and 'D.N.A laddering method'. From the datas in the table- 1, the results obtained as the medicinal plants *O.indicum* shows a much effective tumor suppression activity on the other groups, one is treated with 'Mixture' and the other with 'Control'.

The number of discs at the maximum concentration of *A. tumefascience*, *A. tumefascience* and Mixture', *A. tumefascience* and *O. indicum* shows 12, 5 and 2 respectively. Same time at higher concentration (100 µl), the drug shows maximum percentage of inhibition as 83.3%, where as mixture shows it as 66%. This shows the effectiveness of the drug against tumor in plants also.

Table - 1 : Anticancer effect of *O. indicum* and 'mixture' in *Agrobacterium* transformed sweet potato

	Concentration of drug (µl)	No. tumors / disc	Percentage of Inhibition (%)
Control (*A. tumefascience* alone)	20	8	-
	40	8	-
	60	9	-
	80	11	-
	100	12	-
A. tumefascience + *'Mixture' of medicinal plants*	20	8	33.3
	40	7	41.66
	60	6	50
	80	5	58.33
	100	5	58.33
A. tumefascience + *O. indicum*	20	4	66
	40	4	66
	60	3	75
	80	3	75
	100	2	83.3

In DNA laddering method, DNA from control animals shows an abnormal, uncontrolled breakdown of DNA from cancer cells, shows a large number of bands, but the animals treated with *O.indicum* shows significant reduction in chromosomal damage and thus show only few DNA fragments. DNA laddering method in case of transformed sweet potatoes shows a comparative change in their molecular damage. Potatoes treated with different concentrations of *O.indicum* and 'Mixture', shows similar pattern of arrangement (The Mol.Wt = 5,145KDs). But the untreated potato, shows another band (line-6, Figure -2) with molecular weight = 3,680KDs.

REFERENCES

1. Sikkora**,** *Proceedings of ayurveda seminar* (1993)
2. Sahai,S., Medicinal plants - I, *The Hindu,* Jan 12 & 13, (1997)
3. Dahankar. S. A., Kulkarni. R. A., and Rege, M. N., Pharmacology of medicinal plants and natural products, *In.Jrn.Pharmacology*, **32,** s81 - s118 (2002)
4. Santana.M.J., Pannikkar.D.R., Cytotoxic action of *O. indicum* , *Lab. Inav*, **44,** 44 - 48, (1968)
5. Arrora. R. B., Beneficial effects of fraction 'A' of gum guggul in arthritic syndrome of liver function in clinical and experimental arthritis- *Rheumatism,* **18(1)**, 9 -16, (1982)
6. Chakrabarthi, S., Chromosome analysis of Dalton's Lymphoma adapted to the Swiss albino mouse, clonal evaluation and C- heterochromatin distribution, *Cancer - Genet -Cytogenet*, **11(4),** 417-423, (1984)
7. Gordon,S.D.,Kamingcha,J.K., A flurometric assay of peroxidase activity utilizing 2,7- dichlorofluresce in with thiocynate: application to the study of salivary secretion, *Jrn. Bioche. Biophys. Methods*, **28:** 69-76 (1993)