Chemopreventive Potential of *Centella asiatica* on B6F10 Melanoma Cell Lines in Experimental Mice

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Summary

The present study demonstrates the potential of aqueous extract of *Centella* against melanoma cells in experimental mice. Two groups of C57BL hybrid mice were maintained and orally pretreated with aqueous extract of *Centella asiatica* at the doses of 500 and 1000mg/kg body weight for 30 days showed increased activity in life span of animals and tumour volume was significantly reduced as compare to control. Histopathological studies revealed that control group of hybrid mice shows majority of viable tumour tissue compare to *Centella* extract treated section shows few areas of loci of viable tumour tissue with large area of necrosis with apoptotic bodies. The present finding showed chemopreventive potential of extract on melanoma tumour model.

Key words : *Centella asiatica*, Melanoma,Chemopreventive

Introduction

Medicinal plants have been a useful source for the research of new biologically active compounds. Different approaches are used to select a plant for research, specially the ethno-medical data approach. Unfortunately, the ethno-medical data is not always completely reliable, since it is difficult to diagnosed cancer well. Apart from the medicinal effects of traditional herbs, exploratory researches have been made and a wide variety of new biological activities from traditional medicinal plants have recently been reported, including anticancer activity (1).

Centella asiatica (L) Urb., popularly known in Brazil as cairuçu-asiático, centelha, codagem and pata-de-mula (2) is a cosmopolitan member of the Umbelliferae family that presents pantropical distribution. It is a perennial herb that has been used for centuries in Ayurvedic medicine to treat several disorders, such as insanity, asthma, leprosy, ulcers and eczema and for wound healing (3)(4). *Centella asiatica* contains triterpene glycosides such as centellasaponin, asiaticoside, madecassoside and sceffoleoside and also asiatic acid and madecassic acid (5)(6). Asiaticoside is the most abundant triterpene glycoside in the water extract and it is transformed into asiatic acid *in vivo* by hydrolysis. Although the asiatic acid has shown cytotoxic activity on fibroblast cells (7) and induces apoptosis in different sorts of cancer(8)(9) to date no scientific report related the presence of phenolic compounds of *Centella asiatica* to cytotoxic activity. Consequently, we have focused on establishing a relationship between the total phenolic content

and antioxidant activity with cytotoxic activity, evaluating the activity against cancer cell lines using aqueous extract (AE) obtained from *Centella asiatica* leaves.

The present investigation indicates that *Centella* extract provide clue for its antitumour activity in B6F10 melanoma cancer cells. We have focused on the activity against cancer cell lines using aqueous extract obtained from *Centella asiatica* plant.

Material and Method

Preparation of plant extract:

Collection of Plants - *Centella asiatica* was obtained from the local garden in September 2007.

Identification - The identification *Centella asiatica* plants was done by botanist Dr. S.S.Khan (Voucher specimen No NR/O1/LGOB/2006) Department of Botany Safia Science College Bhopal M.P India. Plant powder was taken for aqueous extraction through soxhlet apparatus and refluxed for 2-3 days at 60ºC. After the complete extraction, the extract was kept it in water bath for removing the solvent and the dry powder was obtained.

Antitumour activity:

Cell line and culture- Melanoma model: Melanoma cell line were obtained from National Cell science centre, Pune and maintained in our laboratory. The C57 BL hybrid mice of both sexes of the mean weight of 25 gm and 6-7 weeks old were

obtained from the animal colony of our institute. They were kept on controlled temperature (22˚C) and 12: 12 hours light and dark cycle and given standard mouse pellet diet and water ad Libitum. Cell suspensions having total 5x105 cells/ animal were injected. After implantation of the melanoma cell line, animal were kept under observation and experiment was started after 10 days when the tumours were seen. The treatment was given orally for 30 days and tumour volume and survival time of each animal was recorded. The following groups were maintained.

Melanoma Skin Bioassay

Control Group: This group consisted of four mice. The melanoma cell line (B6F10) were injected subcutaneously (S.C.) in all four mice.

Test Group: This group was divided into two sub groups. Each group consisted of four animals. The melanoma cell line was injected by S.C. route. The tumour bearing mice were orally given dose of 500 mg/kg, 1000mg/kg body weight in aqueous extract of *Centella asiatica* as standardized by us in earlier experiments (10).

Statistical analysis: The experimental data were mean ± standard deviation of three measurements (n = 3).

Results & Discussion

The metastasis ability of B6F10 was determined by *C.asiatica* extract treated with different dose. The C57 BL mice which received extract of *Centella* at the dose of 500 and 1000 mg/ kg

body weight for 30 days showed increase in life span of animals and tumour size was significantly reduced in *Centella* extract treated mice as compared to control. The tumour volume was significantly reduced to 61 % and 66 % in *Centella* extract treated mice as compared to untreated control animals. Survival time was also increased in *Centella asiatica* treated mice as compared with untreated tumour bearing mice.

Table No 01 Effects of extract from *Centella asiatica* on B6 F10 melanoma cell lines

* Denotes statistically significant in student't' test at $p<0.05$

IR - inhibition rate, ILS - Increase in life span

CA- *Centella asiatica* Control- Untreated

The metastasis ability of B6F10 was determined by *C.asiatica*

Group	**Dose**	**Mean time of survival**	**Tumour volume**	**% IR**	**%ILS**
Control	Untreated	17.5 days	1638±34	_	
CA treated	500mg/kg	25 days	643±202*	61	42.8
CA treated	1000mg/kg	26.5 days	571±283*	66	51.4

extract treated with different dose. Each value in the table was obtained by calculating the average of three experiments ±standard deviation (n = 3).

Aqueous extract of *Centella asiatica* was investigated at different dose rates 500 and 1000mg/kg body weight against B6F10 melanoma cell lines in C57BL hybrid mice. The inhibition of growth of the cell lines by the extract was concentration dependent.

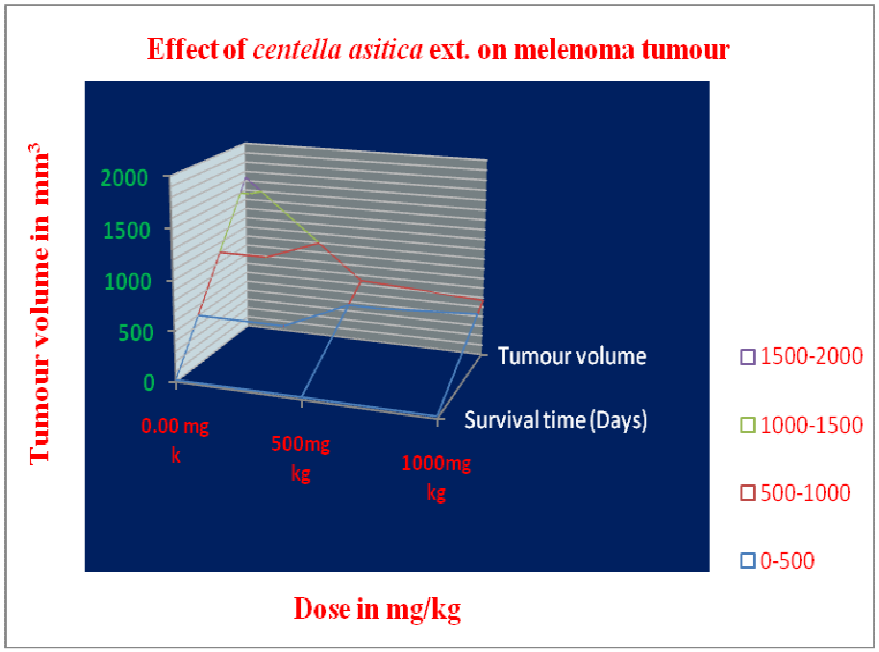

Fig 01 – Effect of *Centella asiatica* on melanoma tumour bearing mice C57BL

Control-untreated C57BL

Test group1- Centella asiatica extract (500mg/kg)

Test group 2- *Centella asiatica* extract (1000mg/kg)

Photograph for histopathological analysis.

Control- slide shows majority of viable tumour tissue containing of sheets of round to polygonal shaped pleomorphic tumour cells containing large round nuclei, Coarsed clumped chromatin. 1-2 prominent nuclei and abundant cytoplasmic fragment with mitotic activity noted. It is suggestive of malignant melanoma of skin.

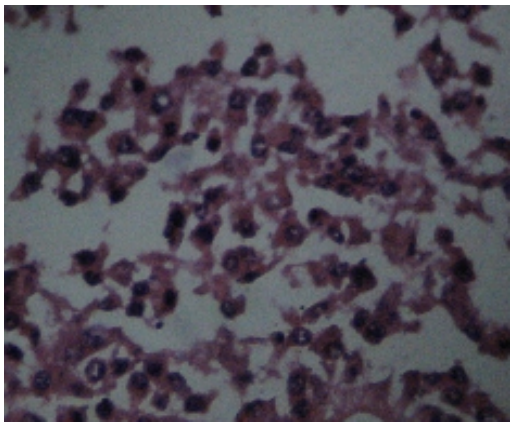

Fig.02 Shows majority of viable tumour cell suggestive of malignant melanoma. (H&Ex10x) untreated melanoma

***Centella* extract treated-** section shows few areas of loci of viable tumour tissue with large area of necrosis with apoptotic bodies. It is suggestive of suppressing of melanoma.

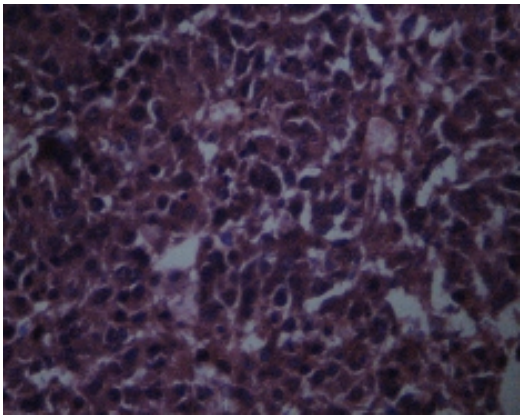

Fig03. Shows few area of viable tumour cells (H&E10x) treated with *Centella* extract

Discussion: *Centella* extract was studied for the inhibition of B6F10 melanoma tumour bearing mice. The inhibition rate was increased *centella* extract group. The life span was also increased in *centella* extract alone as compared to control group. Studies have been reported that several naturally occurring compound exhibited anitumour promoting activity in B6F10 melanoma. *Withania somnifera*, and its bioactive fraction-Withanolide D were studied for their anti-metastatic activity using B6F10 melanoma cells in C57BL/6 mice. Prophylactic administrations of both extract as well as Withanolide were ineffective in inhibiting the metastasis of B16 F10 melanoma cells (11). Keishi-ka-kei-to is a traditional Chinese herbal medicine which is reported inhibits pulmonary metastasis in mice bearing B6F10 melanoma cells through the stimulation of CD8+ T cells (12)(Suzuki *et al,* 1995). Histopathlogical analysis shows C57 BL mice which received extract of *Centella* at the dose of 500 and 1000 mg/ Kg body weight for 30 days showed

increase in life span of animals and tumour size was significantly reduced in *Centella* extract treated mice as compared to control.

These results suggest a possible selectivity of the AE of *Centella asiatica* against some cancer cell lines, as observed for the cisplatin compounds that are preferentially used for testicular and ovarian cancer (13). The selectivity of action could be related to the differences in morphology and physiology between tested cell lines, although this is not yet proven. These results are very encouraging, considering that most chemotherapeutic agents found on the market act both on tumor and normal cells (14) and cannot promote a specific treatment for the cancer without causing side effects as a result of damage

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