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Research Article

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Centella asiatica Extract Exhibit Anticancer Activity Against Different Types of Tumours

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ABSTRACT

The aim of the present study is to evaluate the effect of aqueous extract of plant Centella asiatica against papilloma and melanoma tumours in Swiss Albino mice. Topical application of the Centella asiatica extract at the dose of 500 and 1000 mg/kg body weight at the pre promotion phase showed a significant reduction in tumor incidence, tumor burden, tumor weight, tumor size, cumulative number of papillomas. Aqueous extract of Centella against melanoma cells in experimental mice two groups of C57BL hybrid mice were maintained and orally pretreated 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 cell number, packed cell volume, decrease in tumour weight of the mice, increase in life span .These observations are suggestive of the protective effect of Centella extract against papilloma and melanoma tumours.

Keywords: *Centella asiatica , papilloma ,melanoma, Anticancer agents.*

INTRODUCTION

Cancer is one of the ailments which cannot be completely subdued by chemotherapy. The chemotherapeutic agents though effective against various types of tumour are not totally free from side effects[1]. This fostered our attempts to evaluate some plant products against cancer, as they are less likely to cause serious side effects (Christina). Many Indian plants like black pepper, asafoetida, pippali and garlic are quoted to be useful in different types of cancer[2,3]. One such plant is "*Jivanti*" (*Leptadenia reticulata*), belonging to family Asclepiadaceae, , well known for its tonic, restorative and stimulant property in the Indian system of medicine. This plant is distributed in the Southern parts of India. The main constituents reported are stigmasterol, β – sitosterol, flavonoids, pregnane glycosides and proteins[4]. Presence of triterpenes and steroids were also reported[4]. Aerial parts of *Leptadenia reticulata* is reported to contain tocopherol and possess several pharmacological activities such as galactogogue, antimicrobial and anti-inflammatory activity. Simiarenol (3 β –hydroxy-E: B-friedo-hop-5-ene), a rare triterpene alcohol was isolated from the leaves of *L.reticulata*. Seeds of *L.reticulata* are reported to contain hyperoside, a flavonoid glycoside. *L. reticulata* is claimed to have hypotensive effect in dogs[4] Antioxidant principles derived from plants are reported to have antitumour activity[5]. Hence plantscontaining flavonoids are constantly being screened for antitumour activity[6]. Some of the active principles present in this plant are reported to be flavonoids[4]. It is also used by the tribals of Kolli Hills, Tamil Nadu, India for various types of tumors and by practitioners of traditional systems of medicine against acute tumours.

Hence it was decided to illustrate the ethnobotanical use of the plant and the study was planned to evaluate the effect of lplant extract of *Centella asiatica* against papilloma and melanoma tumours.

MATERIALS & METHODS

Animals: Male Swiss Albino mice (Mus- musculus) of 15-20 gm body weight were used in the study. They were kept on synthetic pellet diet and water *ad libitum*. The animals were randomly divided in to 8 groups. Each group comprises of 6 animals. Mice were shaved in 2 cm^2 area with the help of hair removing cream in interscapular region initially and after every 2 weeks hair were removed with the help of scissors. The treatment was provided topically on shaved area using the following protocol. The study protocol is approved by the Departmental Animal Ethical Committee. (IAEC, Ref. no,- 670/225/2008).

Chemicals: The initiator DMBA and croton oil (used as promoter) were procured from sigma chemical Co (St Louis, MO).DMBA was dissolved at a concentration of 100µg/100µl in acetone. Croton oil was mixed in acetone to give a solution of 1%dilution.

Cell line and Culture: 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.

Preparation of plant extract

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

Identification - The identification *Centella asiatica* plants wasdone by botanist Dr. S.S.Khan (Voucher specimen NoNR/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 at60ºC. After the complete extraction, the extract was kept it inwater bath for removing the solvent and the dry powder was obtained.

Skin papilloma bioassay protocol: The animals were randomly divided in to 8 groups. Each group comprises of 6 animals. Mice were shaved in 2cm 2 area with the help of hair removing cream in interscapular region initially and after every 2 weeks hair were removed with the help of scissors.

The treatment was provided topically on shaved area using the following protocol.

Group 1: (Untreated control) No treatment was given.

Group 2: (Vehicle control) 100 µl acetone 2 times /week was applied topically up to 16weeks

Group 3: (DMBA Alone) 104 µg DMBA was dissolved in 100 µl acetone and single topical application was given.

Group 4: (Croton oil Alone) 1% croton oil was applied topically 2 days / week for 16 weeks.

Group 5: (DMBA + Croton Oil) 104 µg DMBA was dissolved in 100 µl acetone and single topical application was given. Afterwards 1 % Croton oil was applied on skin 2 times a week up to 16weeks.

Group 6: (DMBA + *Centella asiatica* + Croton Oil) 104 µg DMBA was dissolved in 100 µl acetone and single topical application was given. After one week, the 100 µl of *Centella* extract, at the dose of 500 mg/kg b.wt. was given one hour before the each application of 1 % croton oil 2 times a week up to 16weeks.

Group 7: (DMBA + *Centella asiatica* + Croton Oil) 104 µg DMBA was dissolved in 100 µl acetone and single topical application was given. After one week, the 100 µl of *Centella* extract, at the dose of 1000 mg/kg b. wt. was given afterwards the each application of 1 % croton oil 2 times a week up to 16weeks.

Group 8: (*Centella asiatica***)** 100µl of *Centella* extract at a dose of 500mg/kg b.wt was given topically two times a week up to 16 weeks.

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) [7].

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.

RESULTS

Papilloma Model: The anticarcinogenicity study of *Centella* extract shows that Single topical application of DMBA followed by croton oil 2days/week for 16 week produced skin Papillomas which started appearing from 7 th week (53 days) onwards. The incidence of tumors reached 100% and the cumulative number and mean no. of papillomas in DMBA+ Croton oil were recorded as 14 and 2.33.respectively

The papilloma was delayed and observed after 74 days in the group which received the treatment of DMBA + Croton oil + *Centella asiatica.* When *Centella* extract was topically applied to animals along with DMBA + Croton oil, the tumor incidence was found to be 66%.and 20 % in dose of 500 and 1000 mg/kg b.wt. respectively and mean number of Papillomas were recorded as 0.83 and 0.20 respectively, these difference were observed to be significantly decreased than DMBA + croton oil. DMBA, Croton oil, solvent (vehicle control) induced no tumour till the end of the experiment.

Melanoma: 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.1: Effects of extract from *Centella asiatica* on B16 F10 melanoma cell lines

Group	Dose	Mean time of survival	Tumour volume (mm)	% IR	%ILS
Control		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

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

IR - inhibition rate, ILS - Increase in life span

CA- *Centella asiatica*, Control- Untreated

Table.2: Effect of *Centella* Extract on DMBA induced Papillomas in Swiss albino mice

	No. of Papillomas				
Groups	**4th week**	**8th week**	**12th week**	**16th week**	**Mean no.of papillomas**
Untreated	-	-	-	-	-
Vehicle control	-	-	-	-	-
Croton oil** alone	-	-	-	-	-
DMBA* alone	-	-	-	-	-
Centella *** ext. alone	-	-	-	-	-
DMBA* + Croton oil**	-	2/5 (6)	3/5 (8)	4/5 (19)	3.8
DMBA* + *Centella ext*** +croton oil**	-	1/6(1)	2/6(3)	4/6(5)	0.8
DMBA* + *Centella ext.*** ** + croton oil**	-	1/5	1/5(1)	1/5(1)	0.20

***** Single application of DMBA was given at the dose of 104 µg/animal (4 mg/kg b.wt.)

******1 % croton oil was given after each application of *Centella extract.*

****Centella* extract at the dose of 500 mg/kg body weight was given one hour before the each application of croton oil.

*****Centella* extract at the dose of 1000 mg/kg body weight was given one hour before the each application of croton oil.

Table.3: Effect of *Centella asiatica* in skin papilloma model

Group	**Dose**	**% of Papiloma in weeks**				**Total papillomas/No. of animals**
		4th	**8th**	**12th**	**16th**	
DMBA* + Croton oil	104µg/animal+1%	0	40	60	80	19/5
DMBA* + *Centella* *** +Croton oil **	104µg/animal+1% +500mg/kg	0	16	33	66	5/6
DMBA*+*Centella* **** + Croton oil **	104µg/animal+1% +1000mg/kg	0	20	20	20	1/5
DMBA* alone	104µg/animal	0	0	0	0	0/6
Croton oil alone **	1%	0	0	0	0	0/6
Solvent (acetone)	100µl	0	0	0	0	0/6

***** Single application of DMBA was given at the dose of 104 µg/animal (4 mg/kg b.wt.)

******1 % croton oil was given after each application of centella extract.

****Centella* extract at the dose of 500 mg/kg body weight was given one hour before the each application of croton oil.

Table.4: Effect of *Centella asiatica* on cumulative no. and appearance of Papillomas in mice

Group	**Dose**	**Days of 1st appearence of papilloma**	**Cumulative no.of papilloma**
DMBA*+ Croton oil **+CA***	104µg/animal+1% +500mg/kg	74 days	5/6
DMBA*+ Croton oil **+CA****	104µg/animal+1% +1000mg/kg	83 days	1/5
DMBA*+ Croton oil **	104µg/animal+1%	53 days	19/6
DMBA * alone	104µg/animal	0 days	0
Croton oil ** alone	1%	0 days	0
Solvent	100µl	0 days	0

***** Single application of DMBA was given at the dose of 104 µg/animal (4 mg/kg b.wt.)

******1 % croton oil was given after each application of *Centella* extract.

****Centella* extract at the dose of 500 mg/kg body weight was given one hour before the each application of croton oil.

*****Centella* extract at the dose of 1000 mg/kg body weight was given one hour before the each application of croton oil.

DISCUSSION AND CONCLUSION

Cancer is a group of more than 100 different diseases characterized by uncontrolled cellular growth local tissue invasion and distant metastases [Chabner BA, 199011] and the free radicals have been implicated in carcinogenesis[Player T. 198212]. Supportive to this, many plant extracts containing antioxidant principles have been reported to possess antitumour activity . Based on this, it was contemplated to carry out this study. In the present study We examined single topical application of DMBA followed by 2days/week for 16 week croton oil produced skin papilloma which started appearing from 7 th week (53 days) onwards.

The incidence of tumors reached 100% and the cumulative number and mean no. of papillomas in DMBA + Croton oil were recorded as 14 and 2.33.respectively.The papilloma was delayed and observed after 74 days in the group which received the treatment of DMBA+ Croton oil +*Centella asiatica*. When *Centella* extract was topically applied to animals along with DMBA +Croton oil, the tumor incidence was found to be 66%.and 20 % in dose of 500 and 1000 mg/kg b.wt. respectively and mean number of papillomas, were recorded as 0.83 and 0.20 respectively,these difference were observed to be significantly decreased than DMBA + croton oil. evaluated protective effects of CA on antioxidant tissue defense system against adriamycin against cardiomyopathy in rats. Prophylactic administrations of both extract as well as Withanolide were ineffective in inhibiting the metastasis of B16 F10 melanoma cells[5]. 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[8]. *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. These observations on the effect of *centella* extract on various parameters studied to evaluate the antitumour activity enabled us to conclude that it has significant antitumour activity. However, further investigations are essential for the isolation of the active principles of *centella* extract .

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