MODULATION OF CARCINOGENICITY BY *OCIMUM SANCTUM* EXTRACT IN *SWISS ALBINO* AND *C57BL* MICE

Neha Rai[1] R.C.Agrawal[1] and Ashfaq Khan[2]

1. **Department of Research Jawaharlal Nehru Cancer Hospital** Bhopal,M. P. (INDIA)
2. Department of Botany Govt.Science and commerce college Benazeer Bhopal M. P. (INDIA)

ABSTRACT

The anticarcinogenic activity of *Ocimum* extract was evaluated using two stage protocol in skin papiloma model in swiss albino mice and melanomas model in C57Bl tumor bearing mice. Significant prevention in number of papilomas was observed in DMBA+ Ocimum extract +Croton oil group as compared to DMBA+Croton oil. The first appearance of papilloma was also delayed in DMBA *Ocimum* extract + Croton oil group (52 days) as compared to DMBA+ Croton oil group (29 days).The cumulative numbers of papillomas was also reduced in DMBA+ *Ocimum* extract + Croton oil group (7) as compared to DMBA+ Croton oil group (40). In another experiment the anticarcinogenicity of *Ocimum* extract was also studied using melanoma tumour model in the C57 B1 mice. The mice which received extract of *Ocimum* at the dose of 500 mg/Kg body weight for 30 days showed increase in life span of animals and tumour size was significantly reduced in *Ocimum* extract treated mice as compared to control. The tumor volume was significantly reduced to 69% and 73% in *Ocimum* extract treated mice as compared to untreated control animals. These results suggest the anticarcinogenic activity of *Ocimum* extract and it may be an alternative medicine for chemotherapy of cancer treatment.

Key words- Anticarcinogenecity, Melonomas, Skin Papilloma, *Ocimum* extract, and Croton oil

INTRODUCTION

Plant and herbal preparation have been used as medicine since ancient time. Tulsi is sacred plant not only in India but throughout the world because of the outstanding medicinal and purifying properties. Tulsi (*Ocimum scantum*) belongs to family Labiatae and it is one of the important herbal drugs used in various system of traditional medicine for several ailments. The essential oil of *Ocimum sanctum* showed potent anthelminitic activity in the caenorhabditis elegans model (Asha et al.2001) Antibacterial activity against S.aureus, Bacillus pumilus and Pseudomonas (Singhet al. 1999) aeruginosaantiinflammatory activity against carrageenan and different other mediator induced pawedma in rates (Singh etal.1996) where as aqueous extract of *Ocimum sanctum* posses potential ant cataract activity against selenite induced experimental carcinogenesis (Singh S,et al 2005).Treatment of albino rats with a benzene extract of *Ocimum* sanctum leaves for 48 days decrease total sperm count sperm motility and forward velocity.4 Two weeks treatment of diabetic rabbits with the *Ocimum sanctum* Linn. Seed oil showed no significant hypoglycemic effect 5 Aqueous extract of *Ocimum santum* inhibited hypercholesterolemia –induced erythrocyte lipid peroxidation activity (Geetha et al. 2004), Administration of ethanolic extract of *Ocimum santum* attenuated the alteration induced by noise exposure. Hydro alcoholic extract of *Ocimum sanctum* protects the rats from chronic restraints stress induced changes in the myocardium.(Sood S, 2006) the reduction in corticosterone level

caused by chronic exposure to noise stress was prevented by the treatment of animals with *Ocimum sanctum* extract(Sembulingam et al. 1997). The effect of *Ocium santum* leaf extract on the changes in the concentration of serum T3 T4 were investigated in the male mouse. *Ocium sanctum* leaf exhibited anti-thyroidicand antioxidative properties. Oral administration of *Ocimum sanctum* extract provided protection against $HgCl_2$ induced toxicity in Swiss albino ice (Sharmaetal.2002) The fixed oil of *Ocium sanctum* was found to posses significant antiulcer activity against aspirin, indomethacin, alcohol, Histamine , reserpine serotonin and stress induced ulceration in experimental animals' models. Chronic oral administration of *Ocium sanctum Linn* augments cardiac endogenous antioxidant and prevents isoproterenol induced myocardial necrosis in rats. (Sood etal.2005). *Ocimum sanctum* plant extract has been shown to protect against chemically induced oral cancer and the development of the skin papillomas in rodents (Karthikeyan, et al) feeding tulsi leaves along with the normal diet in adult male wister rates decreased in sexual behavioral score (Kantak, etal 1992) Hepatoprotective activity of *Ocimum sanctum* leaf extract against paracetmol induced hepatic damage in rats has been reported (Chattopadhyay, et al.1992).Tulsi leaf powder was fed at 1% level in normal and diabetic rats for a period of one month shown hypoglycemic and hypolipidimic effect in diabetic rats (Rai, et al1997) *Ocimum sanctum* modulates the humoral immune response by acting at various levels in the immune mechanism such as antibody production release of mediators of hypersensitivity reaction and tissues responses to these mediators on the target organs (Mediratta, et.al 1988) Aqueous extract of *Ocimum sanctum* used for ameliorating 131 Iodine – induced damage to the salivary glands Aqueous extract of *Ocimum sanctum* posses significant wound healing and antioxidant activities which may be useful in the management of abnormal healing such as keloids and hypertrophic scars. (Jena, et al. 2003).A significant two fold elevation of reduced glutathione content in the skin increased glutathione S-transferees activity was also observed. Rat hepatocytes pretreated with the *Ocimum* and then with DMBA showed significant reduction in DMBA-DNA adducts. Similar effects were also noted with DMBA – induced hamster buccal pouch carcinogenesis. Another study showed increased activity of cytochrom p-450, cytochrome b5, aryl hydrocarbon hydroxylanse and glutathione S-transferees all of which are important in the detoxification of carcinogens and mutagens *Ocimum sanctum* leaf aqueous extract posses protective effects against chromium and mercury induced genotoxicity with the lower doses of the leaf extract found to be more effective then the higher doses. There are lacks of information about the anticarcinogenecity of *Ocimum sanctum* extract; it is therefore we have undertaken to study the anticarcinogenecity potential of *Ocimum sanctum* extract in Skin papillomas and melanomas model in C57BL mice.

MATERIALS AND METHODS

Chemicals: *Ocimum* leaves were obtained from the local garden in September 2007 and identified by the botanist of local laboratory at Bhopal. Aqueous extract was prepared using separating funnel, cyclophophamide and croton oil was purchased from the Sigma chemicals Co. USA and others chemicals were reagents grade and were procured locally for the study.

Skin Bioassay protocol: Male swiss albino mice of 15-20 gm body weight used in the study. There were kept in the synthetic pellet diet and water ad libitum. The animals were randomly divided into the 6 groups. Each group comprises of 6 animals. Mice were shaved in 2 cm^2 area with the help of hair removing cream to interscapular region initially and after every two 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 ml acetone 2 times/week was given up to 8 weeks

Group 3 (DMBA alone) 104 mg DMBA was dissolved in 100 ml acetone and signel application was given.

Group 4 (DMBA+ Croton oil) 104 mg DMBA was dissolved in 100 ml of acetone and single application was given afterwards 1% croton oil was applied on skin 2 times a week up to 8 weeks.

Group 5(DMBA+ *Ocimum* ext. + Croton oil) 104 mg DMBA was dissolved in 100 ml of acetone and single application was given after one week the 100 ml of Tulsi extract at the dose of 500 mg/kg b.wt. was given one hour before the each application of 1% croton oil two times a week up to the 8 weeks.

Group 6 Croton oil alone 1% croton oil was applied on skin two times in a week up to 8 weeks

The animals of all groups were kept under observation and tissues were fixed in neutral forlain for gross and microscopic changes.

Antitumor activities:

Melanoma cell line were obtained from National cell science center Pune and maintained in our laboratory. The C57 B1 hybrid mice of both sexes of the mean weight of 25 gm and 6-7 gm weeks old were obtained from the animal colony of our institute. They were housed in good laboratory condition and given slandered mouse pellet diet and water ad Libitim. All the mice were kept at controlled light and temperature condition. Cell suspension having total 5 lacks cell/ animal were injected. After implantation of the melanoma cell line animal were kept under observation and experiment was started after 10 days when the tumors were seen. The treatment was given orally for 30 days and tumor volume and survival time of each animal was recorded. The following groups were maintained.

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 subgroups. Each group consisted of four animals. The melanomas cell line was injected by S.C. route. The tumor bearing mice were orally given doses of 500mg/kg body weight of *Ocimum sanctum.*

RESULT AND DISCUSSION

The anticarcinogenic activity of *Ocimum* extract was evaluated using two stage protocol in skin papilloma model in swiss albino mice and melanoma model in C57Bl tomour bearing mice. Significant prevention in no. of papilloma was observed in DMBA+ Croton oil group (100 % tumour). The first appearance of papilloma was also delayed DMBA + *Ocimum* extract + Croton oil group in 29 days. The cumulative no. of papilloma was also reduced in DMBA + *Ocimum*

extract + Croton oil group (7) as compare to DMBA+ Croton oil group (40). In another experiment the anticarcinogenecity of *Ocimum* extract was also studied using melanoma tumour model in the C57Bl mice. The mice which received extract of *Ocimum* at the dose of 500mg/kg weight for 30days showed increase in life span of animals and tumour size was significantly reduced in *Ocimum* extract treated mice as compare to control. The tumour volume was significantly reduced in *Ocimum* extract treated mice as compare to control. The tumour volume was significantly reduced to 69% and73% in *Ocimum* extract treated mice as compared to untreated control animals. These results suggest the anticarcinogenic activity of *Ocimum* extract and it may be an alternative medicine for chemotherapy of cancer treatment.

In our experiment, we have observed the anticarcinogenic effect of *Ocimum* extract using skin papilloma and melanoma models. There few reports about the effect of *Ocimum* in the anticarcinogenecity (Karthikeyan etal.) Hepatoprotective activity(Chattopadhyayet al.1992). Hypoglycemic and hypolipidemic effect in diabetic rats.18 Immunomodulatory effect(Medirattaet al.1988), radioprotective effect (Bartiyaet al. 2006) wound healing and antioxidant activity(Shetty et al. 2006), elevation of reduced glutathione content in the skin increased glutathione S- transferase activity, significant reduction in DMBA and DNA adducts. Protective effects against chromium and mercury induced genotoxicity with lower doses of the leaf extract found to be more effective than the higher doses(Jena et al 2003).These results are important because tulasi is being used in various disease and alternative medicine for chemotherapy of cancer.

TABLE 1
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

No in brackets denotes the cumulative no. of papillomas
***** 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.

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

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

TABLE 2

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

Group	Dose	Days of 1st appearance 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.

TABLE 3

Effect of *Centella asiatica* extract in melanoma skin Bio assay

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

References

1. Ahmed M., Ahamed, R.N., Aladakatti, R.H. and Ghosesawar, M. G, Reversible anti-fertility effect of benzene extract of Ocimum sanctum leaves on sperm parameters and fructose content in rats. J. Basic Clin Physiol Pharmacol (2002); 13 (1): 51-59
2. Asha MK, Prashad D, Murli B, P admaja R,Amit A. Anthelmintic activity of essential oil of *Ocimum Sanctum* and eugenol. Fitoterapia 2001:72(6):669-70
3. Bartiya, U.S, Raut, Y.S. and Joseph L.J, Protective effect of *Ocimum sanctum* L after high-dose 131iodine exposure in mice: an in vivo study. Indian J Exp Biol; (2006).44(8): 647-52.
4. Chattopadhyay, R.R., Sarkar, S.K., Ganguly, S., Medda, C. and Basu, T.K, Hepatoprotective activity of *Ocimum sanctum* leaf extract against paracetamol induced hepatic damage in rats. Indian J Pharmacol(1992); 24: 163-5.
5. Geetha, R.K. and Vasudevan, D.M. Inhibition of lipid peroxidation by botanical extracts of *Ocimum sanctum* in vivo and in vitro studies. Life Sci(2004); 76(1): 21-8.
6. Gupta, S., Mediratta, P.K., Singh, S., Sharma, K.K. and Shukla, R. Antidiabetic, anti hypercholesterolaemic and antioxidant effect of *Ocimum sanctum* (Linn) seed oil. Indian J Exp Biol(2006).; 44(4): 300-4
7. Jena, G.B., Nemmani, K.V., Kaul, C.L and Ramarao, P, Protective effect of a polyherbal formulation (Immu-21) against cyclophosphamide-induced mutagenicity in mice. Phytother Res (2003).; 17(4): 306-10
8. Kantak, N.M. and Gogate, M.G.).Effect of short term administration of Tulsi (*Ocimum sanctum* Linn.) on reproductive behavior of adult male rats. Indian J Physiol Pharmacol. (1992) 36(2): 109-11
9. Karthikeyan, K., Ravichandran, S. and Govindasamy, S, Chemopreventive effect of *Ocimum sanctum* on DMBA-induced hamster buccal pouch carcinogenesis. Oral Oncol; 35: 112-9.
10. Mediratta, P.K., Dewan, V., Bhattacharya, S.K., Gupta, V.S., Maiti, P.C.and Sen, P, Effect of *Ocimum sanctum* Linn on humoral immune responses. Indian J Med Res(1988).; 87: 384-6.
11. Panda, S. and Kar, A, *Ocimum sanctum* leaf extract in the regulation of thyroid function in the male mouse. Pharmacol Res (1998); 38(2): 107-110

12. Rai, U.V., Iyer, M. and Mani.U, Effect of Tulasi (*Ocimum sanctum*) leaf powder supplementation on blood sugar levels, serum lipids and tissue lipids in diabetic rats. Plant Foods Hum Nutr(1997); 50: 9-16.
13. Samjon, J., Sheeladevi, R. And Ravindran R. Oxidative stress in brain and antioxidant activity of *Ocimum sanctum* in noise exposure. Neurotoxicology (2007).; 28: 679-685.
14. Sembulingam K, Sembulingam P, Namasivayam A, Effect of *Ocimum sanctum* Linn on noise induced changes in plasma corticosterol level. Indian J Physiol Pharmacol; 41(2) (1997):139-43.
15. Sharma MK, Kumar M, Kumar A, *Ocimum sanctum* aqueous leaf extract provides protection against mercury induced toxicity in Swiss albino mice. Indian J Exp Biol (2002). 40(9): 1079-82.
16. Shetty S, Udupa S, Udupa L, Somayaji N, Wound healing activity of *Ocimum sanctum* Linn with supportive role of antioxidant enzymes. Indian J Physiol Pharmaco(2006).l; 50(2):163.
17. Singh S, Majumdar DK, Evaluation of the gastric antiulcer activity of fixed oil of *Ocimum sanctum* (Holy Basil). J Ethnopharmacol(1999); 65:13-9
18. Singh S, Majumdar DK, Rehan HMS, Evaluation of anti-inflammatory potential of fixed oil of *Ocimum sanctum (*Holy basil) and its possible mechanism of action. J Ethnopharmacol (1996).; 54: 19-26.
19. Singh S, Majumdar DK., Evaluation of the gastric antiulcer activity of fixed oil of *Ocimum sanctum* (Holy Basil). J Ethnopharmacol(1999); 65:13-9
20. Singh S, Malhotra M, Majumdar DK, Antibacterial activity of *Ocimum sanctum* L. fixed oil. Indian J Exp Biol(2005).; 43(9):835-7.
21. Sood S, Narang D, Dinda AK, Maulik SK, .Chronic oral administration of *Ocimum sanctum* Linn. augments cardiac endogenous antioxidants and prevents isoproterenol-induced myocardial necrosis in rats. J Pharm Pharmacol.(2005); 57(1): 127-33.
22. Sood S, Narang D, Thomas MK, Gupta YK, Maulik SK, Effect of *Ocimum sanctum* Linn. on cardiac changes in rats subjected to chronic restraint stress. J Ethnopharmacol (2006); 108: 4237.