ISSN 2250-3153

Evaluation of Anticarcinogenic Activity of herbal Medicinal Plant *Aloe vera in Swiss albino mice*

R.C.Agrawal*,Sonam Pandey**

Associate Professor*
Research Associate**
Priyamvada Birla Cancer Research Institute
Satna – 485005 Madhya Pradesh India

Abstract- Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. There exists a plethora of knowledge, information and benefits of herbal drugs in our ancient literature of Ayurvedic, Siddha, Unani and Chinese medicine. *Aloe vera* (L.) is commonly used plant for burn, cosmetic etc.. It belongs to the family Liliaceae.
The present study reports antitumour activity of this plant in two stage skin carcinogenesis tumour model and antimutagenic activity using chromosomal aberration assay in the experimental animals. In present investigation, the comparative antitumour effect of *Aloe vera* extract has been undertaken by topical/oral application of 7, 12-dimethyabenz (a) anthracene followed by 1% croton oil till the end of the experiment (16 weeks).GSH level were also measured during carcinogenicity studies. In another set of experiment the antimutagenicity activity was performed using chromosomal aberration assay in bone marrow cells of *Swiss albino* mice.
The results have indicated that there was a delayed in the first appearance of tumour and significant reduction in incidence and cumulative numbers of papillomas which were observed in the *Aloe vera*extract treated groups (by topical and oral route) as compared to control. The GSH levels were restored in *Aloe vera* extracts along with DMBA + croton oil treated groups whereas DMBA + croton oil treated group depleted the GSH levels.

In chromosomal aberrations assay, single application of *Aloe vera* extract at the dose of 250, 500, and 750 mg/kg b.wt.,24 hours prior the i.p. administration of Cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the chromosomal aberrations in bone marrow cells of mice as compared to Cyclophosphamide group. The above studies indicate that Aloe vera may be used as an alternative medicine for chemoprevention of cancer. Therefore, the present study is immensely important in future drug development programs for the cancer treatment.

I. Introduction

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year [1]. An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or combination) to block the development of cancer in humans. Plants, vegetables and herbs used in the folk and traditional medicine have been accepted currently as one of the main source of cancer chemoprevention drug discovery and development [2]. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine. *Aloe vera* (*Sottrukattalai*, Tamil) is a perennial succulent belonging to the Liliaceae family and is called the healing plant or the silent healer. *Aloe vera* contains 75 potentially active constituents:
vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids [3].Aloe gel has demonstrated wound healing [4], anti-inflammatory [5], antiviral [6], spermicidal [7], gastroprotective [8] and immune-stimulating [9] properties. Plant derived natural products such as flavonoids, terpenoids, and steroids *etc* have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [10, 11]. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection against infection and degenerative diseases. The preventive effect of Aloe vera extract was also reported in Skin papillomagenesis in mice [12]There are insufficient report suggesting the anticarcinogenicity and antimutagenicity of *Aloe vera* extract. It is therefore we have undertaken to see if any preventive effect of *Aloe vera* extract using skin papillioma model and antimutagenicity using chromosomal aberrations test in *Swiss albino* mice.

II. Material and Methods

I. Animals

The random breed, 6-7 weeks old male *Swiss albino* mice of weight 25 ± 2 gm body were used in the study. These mice were maintained under controlled conditions of temperature (22±1°C) and light (12 light: 12 dark) and water was given *ad libitum*.

II. Chemicals:

The DMBA. Croton oil and cyclophosphamide were purchased from sigma chemical Co., U.S.A. and other chemical were procured from CDH, Ranchem and Hi Media Ltd., India.

III. ***Preparation of Aloe vera extract***.

Aloe vera leaves were collected from the local herbal garden and identification of the plant *Aloe vera* (family*: Liliaceae*) was done by botanist Dr. S.S. Khan (Voucher Specimen No: SP/AV /2010), Department of Botany, Safia Science College, Bhopal, Madhya Pradesh (India). Pulps were taken out from leaves and mixed in distilled water and kept in separating funnel for 6 hours. The supernatant was collected and this process was repeated until clear solution of supernatant was

ISSN 2250-3153

obtained. All supernatant was pooled together and dried to the powder at 40^0 C water bath. The powder was dissolved in double distilled water before the each treatment at required concentrations.

IV. Experimental Protocol:

Three days before the commencement of the experiment, hair on the interscapular region of the mice were shaved. Only the mice showing no hair growth were selected for the study. The animals (male *Swiss albino*) were randomly divided into 8 groups comprising six mice each. The treatment was provided topically on shaved area using the following protocol Berenblum, 1975. (13)

V. Treatment Groups

Group 1 (Untreated control): No treatment

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

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

Group 4 (Croton Oil Alone): - 1 % Croton oil was applied on skin 2 times a week up to 16 weeks.

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

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

Group 7 (DMBA + *A. vera* Extract. + Croton Oil): - 104 µg DMBA was dissolved in 100 µl acetone and single application was given afterwards the 100 µl dose of *A. vera* extract at the dose of 500 mg/kg b. wt.dose (Tropical) was given one hour before the each application of 1 % croton oil 2 times a week up to 16 weeks.

Group 8 (*A. vera* Extract Alone): - was applied on skin 2 times a week up to 16 week.

The animals of all groups were kept under observation for gross and microscopic changes in skin.

VI. Biochemical Study:

Biochemical alterations were studied in all the groups at the time of termination of the experiment (i.e., at 16th week). The hepatic level of glutathione (GSH) was determined by the method of Moron *et al.* [14]. The GSH content in blood was measured spectrophotometrically using Ellman's reagent with 5-5, dithiobis-2-nitrobenzoic acid (DTNB) as a coloring reagent, according to the method of Beutler *et al.* [15].

Data Analysis:

The differences in the incidence of tumors among different groups were considered to besignificant at 5% significance level ($p<0.05$) when evaluated by Student's 't' test.

VII. Chromosomal Aberration Assay

For chromosomal assay, different doses of *Aloe vera extract* i.e. 250, 500 and 750mg/kg b. wt. were administered. *Aloe vera* extract were dissolved in double distilled water and administered as single dose in 0.2ml per mouse i.p. to 6 animals, 24 hours before the treatment of cyclophosphamide. Control mice were administered an equal volume of vehicle alone. The positive control group also received a single i. p. injection of 50 mg/kg CP in 0.9% saline. The animals were sacrificed by cervical dislocations and bone marrow cells were harvested. Colchicine (4 mg/kg b. wt.) was administered intraperitoneally 2 hrs. before the harvest of the cells. The slides prepared essentially as per modified method of Preston *et al* (1987) [16]. Briefly, femur bones were excised and the bone marrow extracted in 0.56% KCl. The harvested cells were incubated at 37°C for 20 minutes. and then centrifuged for 10 mins. at 1000 rpm. Cells were fixed in Carnoy's fixative (methanol: acetic acid = 3:1) and bursed opened on clean slides to release chromosome. The slides were stained with 5% Giemsa solution for 15 mins and then put in xylene and mounted with DPX. A total of 100 well spread metaphase plates were scored for chromosomal aberrations at a magnification of 1000 X (100 X 10) for each group. Different types of chromosomal aberration such as chromatid breaks, gaps, centromeric association, etc. were scored and expressed as % of chromosomal aberrations. The statistical significance was determined using Student's 't' test.

RESULTS

The results of the present investigation have been summarized in Table 1 Single topical application of DMBA followed by croton oil, produced skin papillomas, which started appearing from the sixth week onward. The tumor incidence in the DMBA + croton oil treated mice (carcinogen control) reached 100% by the end of the experiment (16 weeks). The cumulative number of papillomas was recorded 20 and average number of papillomas per mouse (tumor yield) as well as the papillomas per papilloma-bearing mice (tumor burden) was found 3.33 in carcinogen control group. A significant reduction was observed in tumor incidence, tumor burden, tumor weight, tumor size, and cumulative number of papillomas in *Aloe vera* treated groups (oral) relative to the carcinogen treated control.No papilloma was observed in *Aloe vera* (Topical) extract along with DMBA +Croton oil treated group.

A significant fall in GSH level was noticed in blood and liver in the carcinogen control animals as compared to *Aloe vera* extract treated groups (groups VI- VII), at the time of termination of the experiment (i.e., 16 weeks). Treatment of *Aloe vera* resulted in an enhanced level of GSH ($p<0.05$) in such groups as compared to carcinogen control group (Table 2).

In chromosomal aberrations assay, single application of *Aloe vera* extract at the dose of 250, 500, and 750 mg/kg b.wt.,24 hours prior the i.p. administration of Cyclophosphamide (at the dose of 50 mg/kg have significantly prevented the chromosomal aberrations in bone marrow cells of mice as compared to Cyclophosphamide group .(Table 3).

III. Discussion

The present study was carried out to evaluate the antitumor activity of aqueous extract of *Aloe vera* on papilloma bearing mice. The extract treatment at the dose (topical and oral) of 500 mg/kg inhibited the Cumulative no. papillomas and tumour incidence as compared to carcinogen control group and also restore the level of GSH in extract treated groups (i.e. topical and oral).*Aloe vera* was also showed chromosomal protection against cyclophasphamide positive control group.

Chemoprevention is currently an important strategy for controlling the process of cancer induction. Therefore, there is a need to explore medicinal plants or other natural agents that can work as chemo preventive agents. The present study demonstrates the chemopreventive potentialof *Aloe vera* extract on DMBA-induced skin tumorigensis in male *Swiss albino*. Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumour cells [16] and antitumor activity in experimental animals [17]. The lowering of lipid peroxidation and increase in levels of GSH, SOD and catalase in *Aloe vera* -treated group indicates its potential as an inhibitor of skin papilloma and chromosomal aberretion induced intracellular oxidative stress. *A vera*, used in the tumor study, is a well-known natural substance. It is a source of polysaccharides, selenium, calcium, copper, zinc, chromium, and vitamins A, E, and C, and it has been found to have anti-inflammation, wound-healing, antihepatitis, antigastric ulcer. Thus, the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, triterpenoids, steroids, *etc*, present in *Aloe vera* could be responsible for its potent antitumor activity and antimutagenic activity.

IV. Conclusions

From the present study, it has been observed that the *A vera* is a source of many anticarcinogenic agents and antioxidants, which may be useful for the prevention of cancer treatment.

Acknowledgements:

The authors are thankful to Indian Council of Medical Research (ICMR), New Delhi for providing financial assistance to carry out the above project work. The authors are also thankful to Dr. S.K. Maheshwari, Medical Director ,Priyamvada Birla Cancer Research Institute, Satna for providing facility to carry out the above work.

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AUTHORS

First Author – Dr.R.C.Agrawal, Ph.D., Priyamvada Birla Cancer Research Institute
rcagrawal60@yahoo.com
Second Author – Dr.Sonam Pandey, Ph.D., ., Priyamvada Birla Cancer Research Institute
Satna

CORRESPONDENCE AUTHOR – – DR.R.C.AGRAWAL, PH.D., rcagrawal60@yahoo.com Contact No.91 9826949427

Table 1: Effect of *Aloe vera* extract on DMBA-induced papillomas in *Swiss albino* micegroup

S.N.	Groups	1st Appearance of papilloma(in Days)	Cumulative No. of Papillomas	% Tumour Incidence	Tumour yield	Tumour Burden
1.	Vehicle alone	-	00	0/6	-	
2.	DMBA alone (1 application)	-	00	0/6	-	
3.	Croton oil alone	-	00	0/6	-	
4.	*Aloe vera* extract Alone(oral and tropical)	-	00	0/6	-	
5.	DMBA+ Croton oil	50	20	6/6 (100%)	3.33	3.33
6.	DMBA + *Aloe vera*(oral) extract (500 mg/kg)+ CO	63	09	4/6 (66.6%)	1.5 *	2.25*

ISSN 2250-3153

7.	DMBA + *Aloe vera*(Topical) extract (500 mg/kg)+ CO	-	0	0	0	0

*Denotes statistical significance in Student's `t' test (p<0.05) as compared to carcinogen control group.

Table 2 Effect of Aloe vera Extract on % incidence of tumours bearing mice

Tretment	8 Weeks	10 Weeks	12 Weeks	14 weeks	16 weeks
No treatment	-	-	-	-	-
Vehicle alone	-	-	-	-	-
Croton oil alone	-	-	-	-	-
DMBA alone	-	-	-	-	-
Aloe vera Ext. alone	-	-	-	-	-
DMBA + Croton oil	33	50 %	50 %	66.6 %	66.6 %
DMBA Aloe vera Ext. (Topical)+ croton oil	-	0 %	0 %	0 %	0 %
DMBA + Aloe vera. (oral) + croton oil	0	16	32	32	32

Table 3: Effect of *Aloe vera* extract on GSH level in blood (µg/ml) and Liverin DMBA induced papillomas model

S.No.	Treatments	GHS Level in Liver (In µmole/gm)	GSH Level in Blood (In µmole/ml)
1.	Normal mice	54.02 ± 0.577	3.50 ± 0.03
2.	Carcinogen (DMBA + Croton oil)	41.46 ± 0.48	1.80 ± 0.06
3.	DMBA + AVE (500/kgbt)Oral + Croton oil	50.1 ± 0.45*	2.45 ± 0.14*
4.	DMBA + AVE (500/kgbt)Tropical + Croton oil	52.0 ± 0.58*	3.11 ± 0.19*

*Denotes statistical significance in Student's `t' test (p<0.05) as compared to carcinogen control group.

Table 4: Protection against Cyclophosphamide Induced Chromosomal Aberrations By *Aloe vera* Extract

S.N	Groups	Mean ± S.E	Different types of chromosomal aberration (%)				% Protection
			CB	**CG**	**CA**	**RF**	
	Cyclophosphamide (50mg/kg)	62.83 ±9.19	31	18	12	4	**-**
	A. vera Ext.+CP (250mg/kg)	55.387 ±3.20	20	2	8	1	**11.86**
	A. vera Ext.+CP (500mg/kg)	38.02 ±4.50 *	19	4	4	4	**39.52**
	A. vera Ext.+CP (750mg/kg)	37.22 ±1.79 *	13	7	11	3	**40.00**
	A. vera Ext alone(250mg/kg)	13.4±5.89	8	1	3	-	**-**
	Solvent (DDW)	10.00 ± 2.9	5	3	-	2	**-**

- Denotes statistical significance in Student's `t' test ($p<0.05$) as compared to Cyclophosphamide treated group.
- 6 animals in each groups.
- CB- Chromatid Break, CG- Chromosome Gap, CA- Centromaric Association, RF=Ring Formation