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Anti-Cancer Activity of *Vinca difformis* against DMBA Induced Skin Papilloma in Mice

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ABSTRACT

The aim of this study is to evaluate the chemopreventive potential of *Vinca difformis* in an experimental skin carcinogenesis mice model system. Skin tumor was induced by topical application of 7,12-dimethyl benz(a) anthracene (DMBA) and promoted by croton oil in Swiss albino mice. To assess the chemopreventive potential of *Vinca difformis*, it was orally administered at a concentration of (200 mg/kg and 400 mg/kg body weight) continued for 8 weeks. The development of skin carcinogenesis was assessed by histopathological analysis. Reductions in tumor size and cumulative number of papillomas were seen due to extract treatment. Average latent period was significantly increased as compared to carcinogen treated control. They significantly increased the levels of enzyme involved in oxidative stress glutathione (GSH), Lipid Peroxidation (LPO) and catalase. The elevated level of lipid peroxidase in the control group was significantly inhibited by V.D.E. administration. The results from the present study suggest the chemopreventive effect of *Vinca difformis* in DMBA and croton oil induced skin carcinogenesis in swiss albino mice and one of the probable reasons would be its antioxidant potential.

INTRODUCTION

Cancer is a term used for diseases in which abnormal cells divide without control and are able to invade other tissues, cancer cells can spread to other parts of the body through the blood and lymph system. There are more than 100 different types of cancers. Most cancers are named for the organ or type of cell in which they start. Cancer that forms in tissues of the skin. Most skin cancers form in older people on parts of the body exposed to the sun or in people who have weakened immune systems[1].

In recent years, plant sources have made great contribution towards cancer research. Reported that 60% of the approved chemotherapeutic agents and their sources were all plant derived and 11% of the 252 drugs that were considered novel by World Health Organization(WHO). The search for novel drugs in the treatment of cancer, cardiovascular diseases, infectious diseases and viral diseases has become evidently important[2].

The genus Vinca (Apocyanaceae) is an evergreen shrubs or herbaceous perennials, native to western Europe. *Vinca difformis* known commonly as intermediate periwinkle. There are at least 86 alkaloids extracted from plants in the *Vinca* genus.[12] The chemotherapy agent vincristine is extracted from *Vinca rosea* (current name *Catharanthus roseus*), and is used to treat some leukemias, lymphomas, and childhood cancers, as well as several other types of cancer and some non-cancerous conditions. Vinblastine is a chemical analogue of vincristine and is also used to treat various forms of cancer. Dimeric alkaloids such as vincristine and vinblastine are produced by the coupling of smaller indole alkaloids such as vindoline and catharanthine. In addition, the nootropic agent Vincamine is derived from *Vinca minor*[11].

In the present study, attempts have been made to understand the chemopreventive potential of *Vinca difformis* in DMBA croton oil induced skin carcinogenesis mice model system.

MATERIALS AND METHODS

Plant Material

Collection of Plant Material

The aerial part of *Vinca difformis* was collected from garden of Cagliari, Italy and powdered using grinder mill. The powdered drug was packed in paper bags and stored in air tight container until use.

Authentification

The botanical identity was confirmed by Universita degli studi di Cagliari, Dipartimento di scienze botaniche, Italy, the voucher specimen number is 925/B.

Preparation of extracts

The powdered drug was extracted with ethanol:acetone 1:1 by maceration. After completion of extraction, solvent was recovered and the saturated solvent was dried over water bath at 40-50°C. The semisolid paste formed is transferred to petri plates and kept in hot air oven at 60°C for further drying and stored in air tight container and kept at 2-8°C for further use.

PHYTOCHEMICAL SCREENING[3]

The freshly prepared crude extracts of the aerial parts of *Vinca difformis* were qualitatively tested for the presences of alkaloids, Tannins and Phenolic compounds, Flavanoids, Steroids, Glycosides, Saponins, Proteins and Amino acids. Total alkaloidal and Phenolic content was also determined.

EXPERIMENTAL ANIMALS

Male Swiss albino mice 25-30gm of avg.wt. have been used. The animals maintained under standard environmental conditions had free access to standard diet and water *ad libitum*. Mice were housed in groups of six per cage. All the animals were maintained under standard conditions; that is room temperature 26±1°C, relative humidity 45-55% and 12:12 hrs light-dark cycle. The cages were maintained clean, and all experiments were conducted between 9 am and 4 pm.

Acute Toxicity Study[4]

Swiss Albino mice (25 - 30 gm weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD 425 and animals were observed for mortality and behavioral changes.

Ethical Approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC). All the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

PHARMACOLOGICAL SCREENING

The animals were divided into four different groups. Each group contains five number of animals. Wistar albino mice of either sex carried out. Ethanolic : acetone [1:1] *vinca* extract-200mg/kg and 400mg/kg body weight was given orally[5].

Group 1 (vehicle treated) 10 mice were selected randomly and hair of the dorsal skin shaved. Initially 0.5% DMBA in acetone (200µl) was applied topically to the mice. The animals were treated with control (0.5% PEG) orally on croton oil application. Then after 1hr 10% croton oil in acetone (200µl) was applied topically. Croton oil applied after 14 days of DMBA application. The treatment with croton oil and vehicle was continued for 8 weeks at alternate days.

Group 2 (extract treated 200mg/kg body weight) 10 mice were selected randomly and hair of the dorsal skin shaved. Initially 0.5% DMBA in acetone (200µl) was applied topically to the mice. The animals were treated with control (0.5% PEG) orally on croton oil application. Then after 1hr 10% croton oil in acetone (200µl) was applied topically. Croton oil applied after 14 days of DMBA application. The treatment with croton oil and vehicle was continued for 8 weeks at alternate days.

Group 3 (extract treated 400mg/kg) 10 mice were selected randomly and hair of the dorsal skin shaved. Initially 0.5% DMBA in acetone (200µl) was applied topically to the mice. The animals were treated with vehicle (0.5% PEG) orally on croton oil application. Then after 1hr 10% croton oil in acetone (200µl) was applied topically. Croton oil applied after 14 days of DMBA

application. The treatment with croton oil and vehicle was continued for 8 weeks at alternate days.

Group 4 (standard 5-flurourasil) 10 mice were selected randomly and hair of the dorsal skin shaved. Initially 0.5% DMBA in acetone (200µl) was applied topically to the mice. The animals were treated with vehicle (0.5% PEG) orally on croton oil application. Then after 1hr 10% croton oil in acetone (200µl) was applied topically. Croton oil applied after 14 days of DMBA application. The treatment with croton oil and vehicle was continued for 8 weeks at alternate days.

PARAMETERS[6]:

- Tumor incidence: The number of mice carrying at least one tumor, expressed as % incidence.
- Tumor yield: The average number of papillomas per mouse.
- Tumor burden: The average number of tumors per tumor bearing mouse.
- Diameter: The diameter of each tumor was measured.
- Weight: The weight of the tumors of each animal at the termination of each experiment was measured.
- Body Weight: The weight of the mice was measured weekly.

BIOCHEMICAL PARAMETERS:

Biochemical alterations will be studied in animals of all groups at the time of the termination of the experiment. The animals will be killed by cervical dislocation. Dorsal skin affected by tumors will be quickly excised and washed thoroughly with chilled saline (Ph7.4) and then weighed and blotted dry. 10% tissue homogenate will be prepared from part of the sample (skin) in 0.15M Tris-KCl (pH7.4) and the homogenate will be then centrifuged at 12000rpm for 15 min. The supernatant thus obtained will take for estimation of catalase, reduced glutathione (GSH) and lipid peroxidation (LPO).

LPO ESTIMATION

Add thiobarbituric acid (0.8%), sodium dodecyl sulphate (0.1%) and acetic acid (20%) into 100µl of the tissue homogenate 10%. This mixture heated for 60 min, cool and extracted with n-butanol-pyridine and the optical density (OD) of LPO will record at 532nm[7].

GSH ESTIMATION

Taken 0.05ml of supernatant and added 0.2ml of DTNB stock, 0.4ml of 1M Tris (pH8). Then added 3.350ml distilled water and kept it for incubation of 5 min and absorbance at 412nm with appropriate blank[8].

CATALASE ASSAY

The change in absorbance will be followed spectrophotometrically at 240nm after the addition of Hydrogen peroxide (30mM) to 100µl of the supernatant in 50mM phosphate buffer (pH7). The activity of the enzyme is expressed as µmol of Hydrogen peroxide reduced/mg protein/min [9,10].

STATISTICAL ANALYSIS

Statistical analysis was carried out using primer of Bio-statistical software. All results were expressed as mean ±standard error mean (SEM). Data was analyzed using one-way ANOVA followed by Bonferroni t-Test. In the entire tests, the criterion for statistical significance was $P < 0.05$.

RESULTS

PHYTOCHEMICAL ANALYSIS

The results of the chemical tests performed in the screening revealed the presence of flavonoids, alkaloids, tannins, glycosides, saponins, terpenoid in the extract of aerial parts of *Vinca difformis*.

Sl.No.	Tests	Inferences
1.	Carbohydrates	+
2.	Alkaloids	+
3.	Glycosides	+
4.	Flavanoids	+
5.	Tannins and Phenolic compounds	+
6.	Steroid	+

Acute oral toxicity

Acute oral toxicity studies revealed the non-toxic nature of the *Vinca difformis*. The extract of *Vinca difformis* did not show any sign and symptoms of toxicity and mortality up to 2000 mg/kg

dose after fourteen days of study. This indicates that the extracts were found to be safe up to the dose levels studied. Since all the animals survived at a dose of 2000 mg/kg body weight, the LD_{50} of the extract will be >2000 mg/kg body weight. No major behavioral changes were observed during the period of study. Therefore, $1/10^{th}$ and $1/5^{th}$ of the maximum tolerated safe dose was selected for further pharmacological activity.

Anticancer activity of *Vinca difformis* against DMBA induced skin papilloma in mice.

(A) Animal study parameter in relation to skin papilloma.

Animal study parameter in relation to skin papilloma									
Groups	Body Weight		Tumor Incide nce %	% Inhibition of Tumor Incidence	Mortality Index %	Cumulative No.of Tumors	Tumor Yield	Tumor Burden	Diameter of Papilloma (mm)
	Initial	Final							
Control	24.65	22.32	100		50	51	5.1	10.2	1.672
Standard	30	33.25	20	80	10	3	0.3	1.5	1
V.D.E 200mg/kg	25	26.5	60	40	40	26	2.6	4.33	1.51
V.D.E 400mg/kg	28	32.15	40	60	20	1.3	1.3	3.25	1.07

(B) Estimation of biological parameter of mice skin after applied DMBA application.

Groups	**Biological Parameter**		
	LPO (nM/mg protein)	**GSH (nmg/mg)**	**Catalase (unit/mg protein)**
Control	4470.4±62.83	217.12±4.295	1.57±0.2108
Standard	1386±47.9**	401.03±4.232**	30.77±1.826**
V.D.E (200mg/kg)	1843.3±164*	231.28±27.44*	12.08±1.028*
V.D.E (400mg/kg)	1234.3±75.14*	288.39±25.38*	20.40±1.092*

Values are expressed as Mean ± SEM, for 5 animals, **P<0.05, significantly when compared with control group.

MORPHOLOGICAL EVALUATION OF MICE SKIN

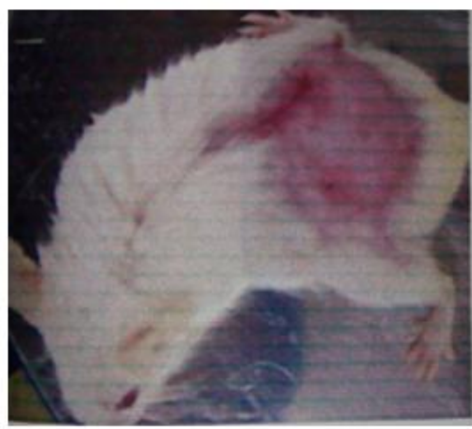

Fig.1: skin papilloma in control.

Fig.2: skin papilloma in Standard drug.

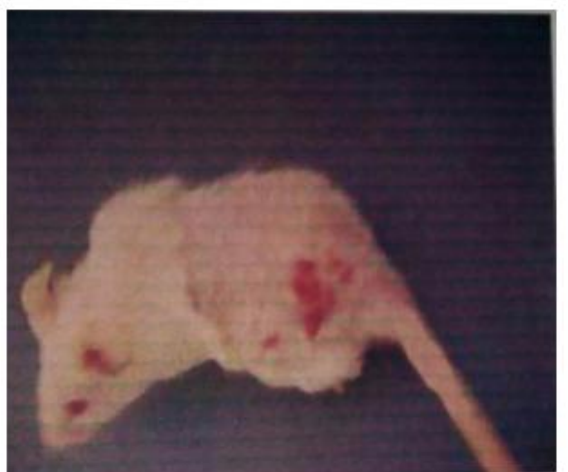

Fig.3: V.D.E (200mg/kg)

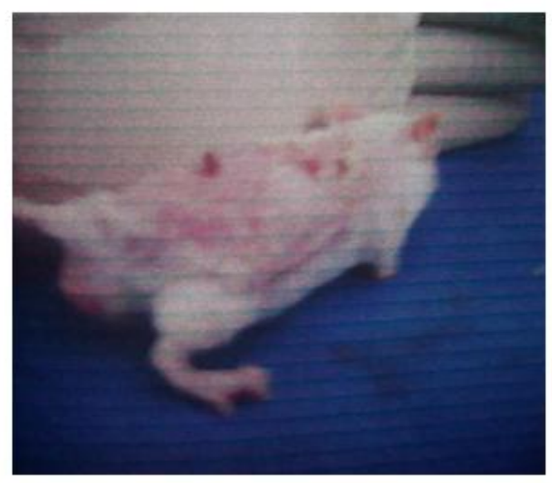

Fig.4: V.D.E (400mg/kg)

DISCUSSION

Different parameters in relation to skin papilloma in mice skin were observed. Among them, body weight of mice shows anticancer activity, because during cancer promotion weight of mice decreases due to cancer side effects on immunomodulatory system. Weight differences were present in drug treated and standard group while weight decreased in only control treated group. Tumor means at least one papilloma on mice and tumor incidence was high in vehicle only group and least was present in standard group. But in exact treated group tumor incidence was

significantly low as compared to control treated group. Mortality index was also high in control group and this was significantly less in extract treated group. High incidence of tumor yield and tumor burden shows cancerous state and according to the result, drug treated groups was having less tumor yield and tumor burden. These data are showing that extract was effective to reduce diameter of skin papilloma.

Decreasing in GSH concentration showed oxidative nature of carcinogen and overcoming this, may be reason for anti-cancer activity. 400mg/kg increased GSH level as compared to 200mg/kg but these differences were statically nonsignificant. Both the drug treated groups show anticancer activity and this may be due to increase in catalase activity but statistically, drug shows dose depended effect. Drug treated groups were significantly less effective as compared to standard but both groups are effective in increasing catalase activity.

CONCLUSION

Skin papilloma study on mice showed that tumor incidence, tumor yield, cumulative tumor incidence and tumor burden effectively reduced by *Vinca difformis* extract and 200mg/kg dose was significantly more effective as compared to 200mg/kg dose. Present study showed that extract at dose 200mg/kg reduced the LPO level and increased the GSH, catalase enzyme activity at significant level as compared to vehicle treated group. There was no significant difference present between standard and 400mg/kg extract dose possessed in respect to LPO level. LPO level is directly linked to oxidative stress. By the histopathological evaluation, concluded that epidermal and dermal layers were uniformly arranged with epidermis having normal layer of keratin in DMBA and croton oil with extract treated mice. Although there was abnormally thickened epidermis observed in skin tumor section but the extent of keratinocyte pearls was much less as compared to DMBA and croton oil treated tumors. The extent of lesion was much less in DMBA and croton oil with extract treated mice tumors as compared to DMBA and croton oil treated tumors. The results revealed that the extract possessed anticancer potential for papilloma on mice skin.

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