

## Evaluation of Anticancer Activity of Liposome's Vincristin-Vinblastin, Curcumin Transdermal Patch on Swiss Albino Mice.

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### Abstract

Skin cancer is still a major cause of morbidity and mortality worldwide. Skin overexposure to ultraviolet irradiations, chemicals, and several viruses has a capability to cause severe skin-related disorders including immunosuppression and skin cancer. These factors act in sequence at various steps of skin carcinogenesis via initiation, promotion, and/or progression. These days cancer chemoprevention is recognized as the most hopeful and novel approach to prevent, inhibit, or reverse the processes of carcinogenesis by intervention with natural products. Phytochemicals have antioxidant, antimutagenic, anticarcinogenic, and carcinogen detoxification capabilities thereby considered as efficient chemopreventive agents. the liposomal carrier, but increased clearance for liposome encapsulated vinblastine ( $t_{1/2} = 9.7$  h) relative to vincristine ( $t_{1/2} = 18.5$  h). Transdermal drug delivery systems are polymeric formulations which when applied to skin deliver the drug at a pre determined rate across dermis to achieve systemic effects. Anticancer drugs produces side effect in body and it can be reduces it by applying to skin in form of trans dermal patch for skin cancer. So we were evaluate liposomes of Vincristine +Vinblastine one liposome's and Curcumin other in transdermal patch anticancer activity. We were desineng anti skin cancer activity on Swiss albino mice, for this studies we used DMBA and croton oil for producing skin papilloma on mice during 3 month. We evaluate tumor size , number of tumor reduces by applying patch on Lipo-VVC TDP near tumor side. The result we observed that tumor regression size and number were reduced and compare with standard drug vincristine (IP) produced less lethal condition in mice.

**Key words:** LipoVVC TDP (Liposomal Vincristine-Vinblastine Curcumin Trance dermal patch), Liposome, Trance dermal patch, and anticancer.

### Introduction

Skin cancer is currently the most common type of human cancer worldwide. In the United States, the annual incidence rate of skin cancer is increasing each year, representing a growing public concern<sup>1</sup>. It has also been anticipated from the current records that nearly half of all Americans are susceptible to develop skin cancer at least once up to the age of 65. According to an estimate by the American Cancer Society 76,250 men and women have been diagnosed with skin cancer and 12,190 men and women could have been died (9,180 from melanoma and 3,010 from other nonepithelial skin cancers) in the United States in year 2012. Even if considerable progress has been done in developing effective

skin cancer treatment modalities, skin cancer is still a global health problem of concern.<sup>2</sup> The main types of skin cancer are the following. (i) Basal cell carcinoma, the most common easily treated form of skin cancers and accounts for 90% of all cases. Long-term exposure to sunlight is the main cause of it. (ii) Squamous cell carcinoma is the second most common type of skin cancer. It is easily treated when found early, but in a small percentage of cases this cancer has metastasis potential. Both basal cell and squamous cell carcinoma are together known as nonmelanoma type of skin cancer. (iii) Melanoma is responsible for 75% of all skin cancer-related deaths<sup>3</sup> despite the fact that it accounts for less than 5% of all skin cancer cases. Approximately 65–90% of melanomas are caused by exposure to ultraviolet (UV) light.<sup>4</sup> (iv) Kaposi's sarcoma, a more slow-growing form of skin cancer, occurs in elderly men of Italian or Jewish ancestry and is caused by a Herpes family virus. It is an aggressive AIDSrelated form affects about one third of patients with AIDS.

## Cause of Skin Carcinogenesis

Skin, a major environmental interface for the body, is unintentionally or occupationally exposed to a number of harmful stimuli such as UV irradiation, viruses, and several chemical Carcinogenic agents (Figures 1 and 2).

**A) UV Radiation.** Epidemiological data around the world have been evocative of that the exposure of skin to UV radiation is the main ecological carcinogen in the development of both melanoma and nonmelanoma types of skin cancers. UV irradiation stimulates clonal expansion of aberrant skin cells, resulting in skin carcinogenesis via involvement of multiple cellular signaling pathways.<sup>5</sup>

**B) Chemicals.** Over the last few years occupational skin cancer cases are reported and have mainly been owing to industrial exposure of human being to chemical carcinogens such as polycyclic aromatic hydrocarbons (PAH) and arsenic. PAH from shale oil, tar, pitch, raw paraffin, creosote, asphalt, and chimney soot have been associated with risk to skin cancers. Workers from industries in which PAH are produced (coal, coke and aluminium production plants, steel and iron foundries, and exposure to diesel engine exhaust fumes) are at higher risk of skin cancer.<sup>6</sup>

**C) Viruses.** Viruses are another agent which transforms keratinocytes by activation of cancer-promoting genes. Apart from this, several viral proteins act as oncogenes which lead to the cellular proliferation. Viral skin carcinogenesis is highly concerned with immune deficient host, in such cases lower T cell reactivity and antigen presenting cells in skin assist in viral escape and tumorigenesis.<sup>7</sup>

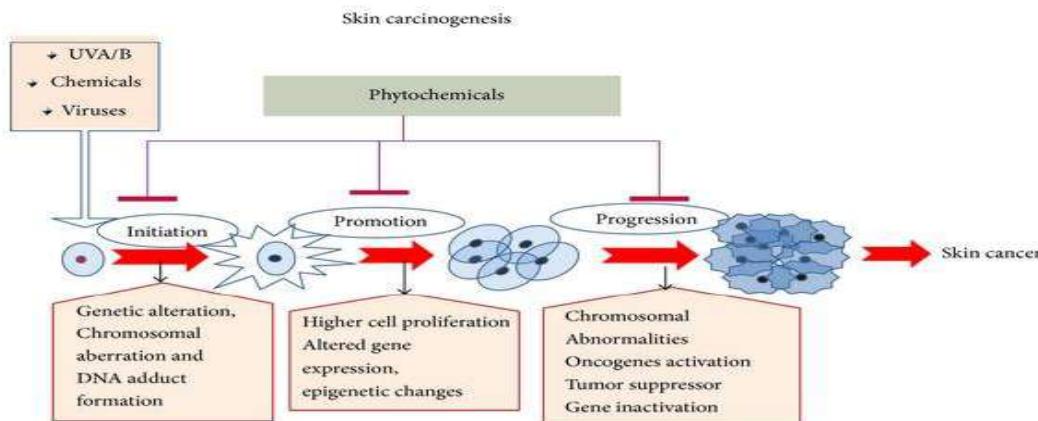


Figure 1: Skin cancer develops in series of events in multiple steps; however in most of studies, skin carcinogenesis is progressed in three key steps, that is, initiation, promotion, and progression, and many phytochemicals could prevent the abrupt changes in each of the steps to reverse the process of developing skin cancer.

## Mechanistic Pathways of Skin Carcinogenesis

*a. Mitogen-Activated Protein Kinases (MAPKs) Signaling Pathway.* MAPKs, a family of serine/threonine-specific protein kinases belonging to the CMGC (CDK/MAPK/ GSK3/CLK) kinase group, carcinogenesis and UV irradiation is reported to act as inducer of it.<sup>8</sup>

*b. Phosphatidylinositol-3-kinase (PI3K)/Akt Pathway.* PI3K is a member of lipid kinase family and generates phosphatidylinositol- 3,4,5-trisphosphate (PI(3, 4, 5)P3). PI(3, 4, 5)P3 is a second messenger essential for the translocation of Akt to the plasma membrane where it is phosphorylated and activated by phosphoinositide-dependent kinase (PDK) 1 and PDK2. Activated Akt alter the function of several substrates involved in the regulation of cell survival, cell cycle progression, and cellular growth. In recent years, it has been shown that PI3K/Akt signalling pathway components are frequently altered in cancers.<sup>9</sup>

*C. The JAK-STAT Pathway.* the JAK-STAT3 pathway has been suggested as playing a critical role in cell transformation and carcinogenesis. Intervention of this pathway will offer opportunities for the design of new chemopreventive and chemotherapeutic approaches

*D.Cyclooxygenase (Cox).* Derived prostaglandins (PGs) exhibit multiple functions in severe and chronic skin inflammation provoked by various physical (UV light, wounding) and chemical (TPA, arachidonic acid) injurious stimuli. COX has two main isoforms as COX-1 and COX-2. COX-1 is constitutively expressed in most tissues, whereas COX-2 is inducible by a variety of tumor promoting agents.<sup>10</sup>

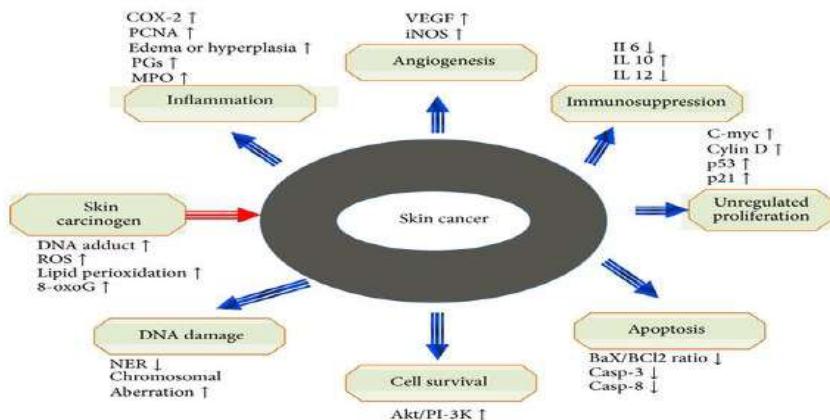


Figure 2: Molecular pathways are altered due to modulation of signaling pathway in skin cancer. Skin carcinogens lead to higher level of DNA adducts formation, ROS, lipid peroxidation, and 8-oxoG. Afterwards, major events like inflammation, angiogenesis, immunosuppression, higher proliferation, lesser apoptosis, enhanced DNA damage, and cell survival take place. Upregulated and downregulated molecules are depicted by arrows represented in upper and lower direction, respectively.

### Product Lipo VVC TDP:

The preparation of Vincristine and Vinblastine liposome and Curcumin were formulated<sup>11</sup>. After that formulation of liposome's(2mg-1mg-2mg/g) transdermal patch vincristine-vinblastine and Curcumin were formulated and evaluated also for better result.<sup>11</sup>

#### A) Papilloma model<sup>13</sup>

##### (Anticarcinogenity activity of LTP induced by DMBA and Croton oil)

Four groups of Swiss albino mice (n=6 per group) were used in the study. The animals were dorsally hair remove by hair removal cream. Group 1 animals were given, a normal diet, and tap water ad libitum daily. Group 2 animals received a single dose of DMBA (100 µg/100 µL of acetone) over the shaved area of dorsal skin after which 1% croton oil was applied to the skin three times a week for 16 weeks. After the single dose of DMBA, the group 3 animals were start treated with liposomal transdermal patch applied every three times a week for 16 weeks, and group 4 inject Cisplatin (1mg/ kg) I.P three times a week for 16 weeks, with application of 1% croton oil onto the skin 1 hour after exposure to patch. Two weeks after application of DMBA, the mice were monitored weekly for 16 weeks for the presence and size of skin tumors, body weight, and the average latency period. After 16 weeks, the mice were euthanized, and the dorsal skin was removed for histopathology and blood was taken for biochemical analysis.

Chemopreventive effect of Lipo Trancedermal VVC against skin carcinogenesis induced by DMBA and croton oil in mice

Treatment	Body weight (g)		Papillomas (n)	Tumor size (mm)	Average latency period
	Initial	Final			
Group I (Normal)	38.12±4.23	37.56±5.43	***	****	****
Group II (Papilloma)	26.26±2.16	22.75±11.24	10.16±5.03	2.06±0.37	10.10±5.17
Group III (LipoVVC) F1	28.97±1.65	25.14±12.34	3.83±0.53	1.00±0.37	11.70±4.23
Group IV (Vincristin IP)	29.57±1.55	26.15±11.97	1.50±1.07	0.93±0.24	13.10±0.76

Table no VII.3 Anticarcinogenics Activity of Liposomal VVL transdermal patch

Value are expressed as mean ± SED of 10 animals in each groups ; df=7, 14 Cumulative no tumors size ; \*\*p<0.05(n=10) compare to group II .the data compared by one way ANOVA Newman-keuls test

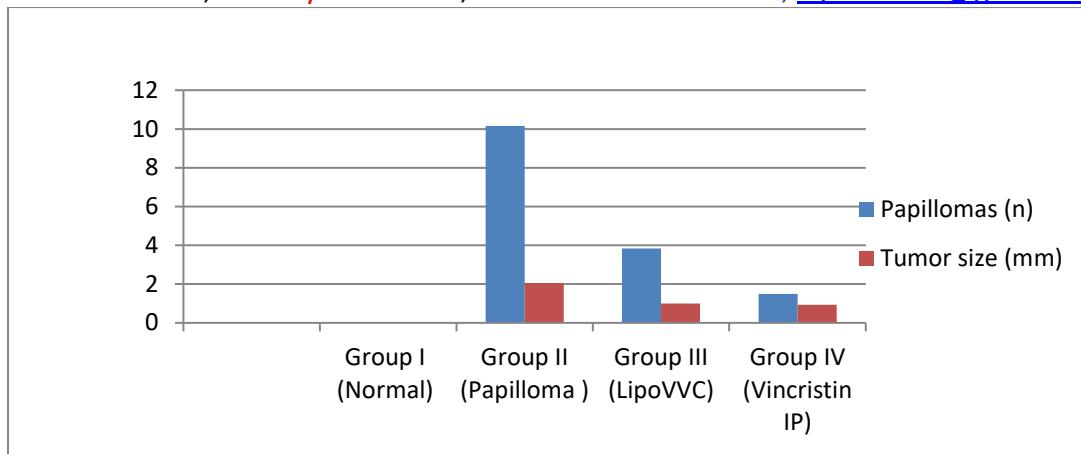
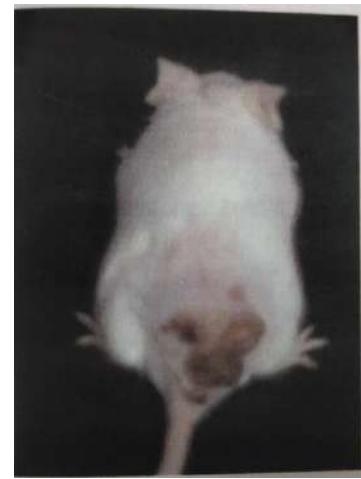


Fig: Bar diagram of papilloma no and tumor size in mm



Group I Normal



Group II DMBA + Croton Oil



Group III Lipo Transdermal Patch VVC +DMBA+ Croton oil F1    Group IV DMBA +Croton oil + Vncristin ip (2 mg /kg)

#### Examination of Oxidative stress biomarkers

On the final day of the experiment, all the animals were euthanized by cervical dislocation. The dorsal skin was removed immediately and washed in ice-cold saline (0.9% NaCl), followed by removal of extraneous material. The skin was then weighed and blotted dry. A 10% tissue homogenate of skin was prepared in 0.15 M Tris-KCl (pH 7.4), and then centrifuged at 12,000 rpm for 15 minutes. For biochemical estimation, supernatant, were used on the same day. Protein content was measured by the Bradford method<sup>14</sup> using bovine serum albumin as the standard

### Lipid peroxidation (LPO)

Lipid peroxidation (LPO) was estimated by measuring the formation of malondialdehyde.<sup>15</sup> A mixture of 0.1 mL tissue lysate and 1.9 mL of 0.1 M sodium phosphate buffer (pH 7.4) was incubated at 37°C for 1 hour. After precipitation with 5% trichloroacetic acid, the incubation mixture was centrifuged (2,300× g for 15 minutes at room temperature) and the supernatant was collected. Next, 1.0 mL of 1% thiobarbituric acid was added to the supernatant and placed in boiling water for 15 minutes. After cooling to room temperature, the absorbance of the mixture was taken at 532 nm and expressed in nmol malondialdehyde per hour/mg protein using a molar extinction coefficient of 1.56×105/M/cm.

### The glutathione (GSH) level

The glutathione (GSH) level was quantified using Ellman's reagent.<sup>16</sup> The assay mixture contained phosphate buffer, 5,5'-dithiobis-(2-nitrobenzoic acid), and tissue lysate. The reaction was monitored at 412 nm and the amount of GSH was expressed in terms of nmol of GSH/mg protein.

### Measurement of superoxide dismutase (SOD)

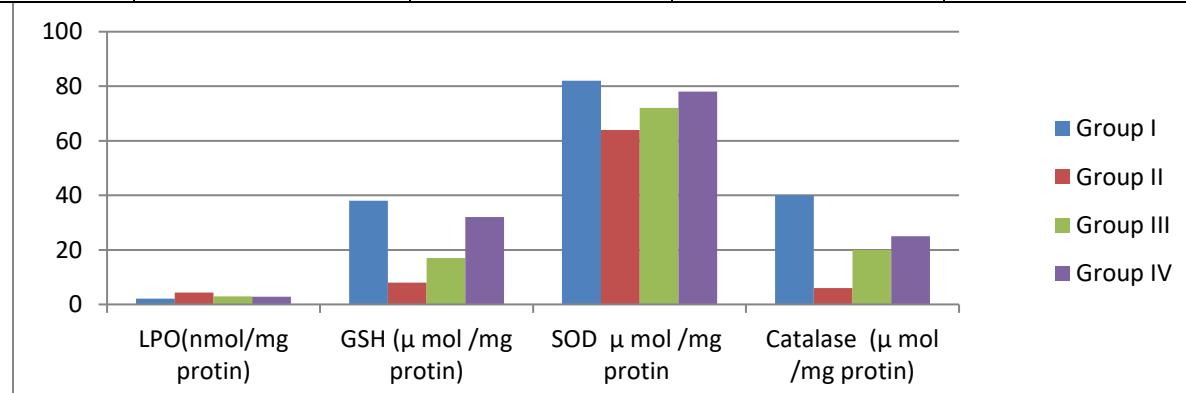
The activity of superoxide dismutase (SOD) was estimated using the method described by Kakkar et al.<sup>17</sup> The assay mixture contained sodium pyrophosphate buffer, nitroblue tetrazolium, phenazine methosulfate, reduced nicotinamide adenine dinucleotide, and tissue lysate. One unit of SOD enzyme activity is defined as the amount of enzyme required to inhibit production of chromogen (560 nm) by 50% in 1 minute under assay conditions, and is expressed as specific activity in units/min/mg protein.

### Measurement of catalase activity

Catalase activity was measured by following its ability to split hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) within 1 minute of incubation time. The reaction was then stopped by adding dichromate/acetic acid reagent, and the remaining H<sub>2</sub>O<sub>2</sub> was determined by measuring at 570 nm the chromic acetate formed by reduction of dichromate/acetic acid in the presence of H<sub>2</sub>O<sub>2</sub>, as described earlier.<sup>18</sup> Catalase activity was expressed as μmole H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein.

**Table** Induction of LPO, GSH, SOD, and catalase levels in Swiss albino mice skin

Treated	LPO (nmol/mg protein)	GSH (μ mol /mg protein)	SOD μ mol /mg protein	Catalase (μ mol /mg protein)
Group I	2.1 ±0.15	38±4.30	82±5.43	40±4.32
Group II	4.3 ±0.15	08 ±1.23	64±4.6	06±0.5
Group III	3.0 ±0.32	17±2.25	72±5.25	20±3.5
Group IV	2.8 ±0.21	32±3.5	78±4.20	25±4.2



Induction of LPO, GSH, SOD, and catalase levels in Swiss albino mice skin.

## Discussion

Plant products are natural bioactive compounds that protect against stress and pathogenic attack. It has been reported that long-term use of certain medicinal plants overwhelms carcinogenesis in several human and animal organs. Thus, it is important to identify natural plant products that could suppress or reverse the process of cancer. In the present study, we observed several beneficial effects of Lipo VVC TDP in induced skin cancer. We used a subthreshold dose of DMBA as a carcinogen followed by regular treatment with croton oil as a promoter to induce skin tumors in experimental mice. With regard to the initiation and promotion stages, animal studies show that the promotion step takes more time to occur and is reversible initially, so prevention of cancer by inhibition of tumor promotion is expected to be an inventive approach. In the current study, Lipo VVC TDP could significantly inhibit formation DMBA-induced papilloma in terms of both incidence of tumors and the mean number of papillomas. In our study, administration of Lipo VVC significantly reduced the level of LPO in mice treated with DMBA and croton oil, and as a consequence, decreased the incidence of skin tumors. We found that GSH activity was decreased in the control group (treated with DMBA + croton oil) but was increased in LIPO VCC treated mice, indicating its antioxidant activity.

Oxidative stress was observed in the control groups because LPO was high and GSH, SOD, and catalase levels were low. The beneficial effect of Lipo VCC is probably due to its ability to stimulate antioxidant enzymes in cells. The increase in enzyme activity down the production of ROS and LPO in the skin, decreased the incidence of papilloma in the areas of treated skin.

## Conclusion

The present investigation suggested that the matrix type transdermal patches of Vincristine, Vinblastine and Curcumin could be explored for the management of skin cancer. Majorly because of this studies we observed that anticancer drug can given direct site of cancer cell or near to skin it can improved tumor inhibition rate and also cover side effect without producing any lethal situation or life treating side effect

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