



Original Research Paper

CHEMOPREVENTIVE EFFECT OF ACETOHEXAMIDE AND STIGMASTEROL AGAINST UVB- INDUCED SKIN CANCER

Ayesha Tabassum¹, Mariyam Jameela^{1*}, R.K.JAT²

1. Department of Pharmaceutical Sciences, Jawahar Lal Technological University, Hyderabad, India

2. Department of Pharmaceutical Sciences, Rajasthan University of Health Sciences, Jaipur, India

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ABSTRACT

Acetohexamide (Dymelor) is an Oral Hypoglycemic agent, which was shown to reduce the UV induced DNA damage in Xeroderma pigmentosum patients. But the studies on Acetohexamide has not been thoroughly investigated for UV B irradiation induced carcinogenesis in mice. Stigmasterol is one of the plant-derived sterols which is known to possess various antitumor and anticancer properties. The objective of this study is to comparatively evaluate the chemopreventive effect of Acetohexamide and Stigmasterol in Skin Cancer-induced by UV-B irradiation in Swiss Albino Mice. The chemopreventive role of Acetohexamide and Stigmasterol induced skin papillomagenesis was evaluated in UV-B irradiation induced mice using the parameters like tumor incidence, tumor burden, tumor weight, tumor volume, and the excised skin was subjected to histopathological studies. The results from the histopathological findings are in support of the antitumor activity of Acetohexamide and Stigmasterol. Moreover, the study reveals that the mechanism of the Chemopreventive action against UV B induced skin cancer may be due to the Antioxidant properties of the compounds.

Corresponding author :

Mariyam Jameela

Department of Pharmaceutical Sciences, Jawahar Lal Technological University, Hyderabad, India

e-mail-mariyam786@gmail.com.



1. INTRODUCTION

Skin disease is the most well-known kind of growth in the world. It is typical for the body to supplant old cells with new ones. Skin tumor frequently creates on regions of the body that get a great deal of sun introduction, for example, the face, ears, mouth, and back of the hands. Threatening melanomas can grow even in places that are not presented to the sun. The danger of creating skin malignancy is higher in individuals who have encountered long haul introduction to the sun. Skin disease is likewise more probable in individuals who utilize tanning beds¹. Others in danger incorporate those with a family history of skin malignancy, individuals with reasonable skin, individuals who have different moles or are moderately aged or more seasoned. Around 80% of these new skin tumor cases will be basal cell carcinoma (BCC), 16% will be squamous

cell carcinoma, and 4% will be melanoma. On a preventive well being note, it has been assessed that general use of sunscreen with a sun protection factor (SPF) of 15 or more prominent for the initial 18 long stretches of life would decrease the lifetime frequency of non-melanoma skin diseases by 78%. In spite of the fact that non-melanoma skin tumors (basal and squamous cell carcinomas) are the most widely recognized sorts of malignancies in people, melanoma positions as the 6th most basic disease in men and the seventh most regular in ladies. In spite of the fact that the quantity of non-melanoma skin diseases is stunning, both basal cell and squamous cell carcinomas have a superior to 95% fix rate if recognized and treated early.

Simply, sunburn and UV light can harm the skin, and this harm can prompt skin disease². There are obviously other deciding components,

including heredity and the earth. Both the aggregate sum of the sun got throughout the years, and overexposure bringing about sunburn can cause skin growth. A great many people get 80% of their lifetime presentation to the sun by 18 years old.

2. MATERIAL AND METHODS

The objective of this study is to comparatively evaluate the chemopreventive effect of Acetohexamide and Stigmasterol in Skin Cancer-induced by UV-B irradiation in Swiss Albino Mice. The UVB radiation-induced inflammatory response stimulated skin angiogenesis and carcinogenesis. Vascular endothelial growth factor (VEGF) is a proangiogenic factor which is over expressed by UVB radiation. Stability studies of the topical application performed as per International Conference on Harmonization (ICH) guidelines³. Acetohexamide (Dymelor) is an Oral Hypoglycemic agent, which was shown to reduce the UV induced DNA damage in Xeroderma pigmentosum patients but the studies on Acetohexamide has not been thoroughly investigated for UV B irradiation induced carcinogenesis in mice.

In the past decades, it has been shown to prevent the beneficial effects of naturally occurring, which was found to prevent carcinogenesis. Plant-derived substances had been explored for prevention and treatment of the UVB radiation-mediated skin disorders⁴. A variety of Phytosterols from the plant-derived phytochemicals have been reported to show antioxidative, anti-inflammatory, antimicrobial, and anticancer effects. Stigmasterol is one of the sterol, originally isolated from plants.

It is present in different herbs and fruits such as onion, almond, red wine, and orange. Stigmasterol shows evidence of some pharmacological properties such as antioxidant, anti-inflammatory, and anticancer effects. Moreover, It has been reported that stigmasterol has various pharmacological properties, including antiosteoarthritic, hypoglycemic, antimutagenic, antioxidant, and anti-inflammatory activity⁵.

3. EXPERIMENTAL SETUP

UV-B irradiation induced Skin carcinogenesis in Mice

Chemicals

Acetohexamide and Stigmasterol each (250 mg/kg.b.wt.p.o) dissolved in 0.5% dimethyl sulfoxide (DMSO) and made up with water to obtain the final required concentration for oral administration.

Animals: Swiss Albino mice were purchased from CPCSEA approved animal house, Sainath agencies, Bapujinagar, Musheerabad, Hyderabad-48. Mice having the age of 21 days and 30 gm body weight was used for the study. All the mice were kept at room temperature of 22 °C under 12 h light/12 h dark cycle in the animal house. Mice were fed with commercial pellet diet (animal quarantine) and water *ad libitum* freely throughout the study. All animal procedures were performed after approval from the Institution of Animal Ethical Committee (IAEC Ref No.: IAEC/AUCOPH/2018/28) and in accordance with the recommendations for the proper care and use of laboratory animals.

Experimental Groups

The mice selected randomly and divided into 4 different treatment groups with 6 mice in each group:

Group 1: Vehicle control-Distilled water

Group 2: Negative Control-UV-B irradiation

Group 3: Acetohexamide (250 mg/kg.b.wt.p.o) + UVB irradiation

Group 4: Stigmasterol (250 mg/kg.b.wt.p.o)+ UVB irradiation performed at 180 mJ/cm² thrice a week for 6 weeks. One hour prior UVB exposure, mice pretreated with administration of Acetohexamide and Stigmasterol in respective groups.

Procedure

1. Skin of 3 x 3 cm² back area of animals was shaven three days before the commencement of experiment, and only those animals in the resting phase of hair cycle were selected for the study.

2. A total of 24 selected animals were randomly divided into 4 groups (I, II, III and IV) to evaluate the chemo preventive role of Acetohexamide and Stigmasterol induced skin papillomagenesis.

3. At the end of 6th week, the animals were sacrificed through cervical decapitation and the entire liver and skin

6. Liver was perfused immediately with ice cold 0.9 % w/v sodium chloride, thereafter carefully removed, trimmed free of extraneous tissue.

8. Finally, the skin Tumours were excised out and the following parameters were studied.

- (a) Tumour incidence (% of animals that develop at least one Tumour)
- (b) Tumour burden (average number of Tumour per animal)
- (c) Tumour volume
- (d) Tumour weight
- (e) Histopathological examination

Parameters to be assessed to determine anti cancer activity

1. **Body weight:** Change in mean body weight was measured weekly.

2. **Tumour Assessment:** The UVB-irradiated skin of the mice was examined once a week for tumor appearance⁶. Tumor growth that have to be >1 mm in diameter and that persisted for at least 2 weeks considered as tumors and recorded. The dimensions of all the tumors on each mouse recorded at the termination of the experiment⁷.

Tumor volumes calculated using the Hemiellipsoid model formula:

Tumor volume = $\frac{1}{2} (4\pi/3) (l/2) (w/2) h$, where l=length, w=width, and h=height.

At the termination of the experiment, mice sacrificed and skin samples collected for the analysis of various biomarkers of interest⁸. The incidence and multiplicity of carcinoma will also be recorded in the same experiment.

3. Histopathological Analysis :

The experiment was terminated at the end of the 6th week of Tumour promotion and mice were sacrificed for histopathological analysis. Skin samples obtained from dissection were fixed in 10 % formalin before being processed in an automatic tissue processor by standard protocols. The processed tissues were embedded in paraffin wax, sectioned with microtome at a thickness of 4 µm and stained with Haematoxylin and Eosin (H&E) stain using routine protocol. Stained slides were observed under a light microscope and digital micrographs of the slides were taken.

4. Data analysis: All the values are represented as Mean $\pm$ SEM from 8 animals per group. Statistical differences of the mean were analyzed using one-way analysis of variance (ANOVA) followed by turkey's multiple comparison test using graph pad prism 5.0. P value < 0.05 was considered as statistically significant (*, ** indicates $p < 0.05$ and 0.01 , respectively vs. control). The results are represented as Mean + SEM from 6 animals per group.

4.RESULTS AND DISCUSSION

Table 1 Tumour Assessment - Effect of drugs on tumour burden, tumour incidence & tumour volume

Groups	Control	Negative Control	Group-III	Group- IV
Tumour burden	0.00 $\pm$ 0.00	2.00 $\pm$ 2.08b**	5.86 $\pm$ 2.79 b*	4.14 $\pm$ 3.07 b*
Tumour incidence (%)	0.00 $\pm$ 0.00	57.14 $\pm$ 53.45**	85.71 $\pm$ 37.79b	71.43 $\pm$ 48.75 b*
Tumour volume (mm ³)	0.00 $\pm$ 0.000	7.71 $\pm$ 7.27 b*	14.43 $\pm$ 6.45b*	11.29 $\pm$ 7.80 b*

Values are expressed as the mean $\pm$ S.D; Statistical significance' p' value are calculated by one way ANOVA followed by Dunnett's test.

a – Comparisons are made between the Positive control group Vs Negative control group,

b – Comparisons are made between Test1 & Test 2 Vs negative control group.

p value * represents $p < 0.05$, ** represents $p < 0.01$

Tumour Incidence:

The number of mice carrying at least one Tumour expressed as percent incidence .

There is a significant increase in the tumour incidence in the UV induced group as compared to that of the normal control groups. There is a significant decrease in the tumour incidence in the Test & Test 2 group as compared to that of the standard⁹.

Tumour Burden: The average number of Tumours per Tumour bearing mouse

There is a significant increase in the tumour burden in the UV induced group as compared to that of the normal control groups. There is a significant decrease in the tumour burden in the Test 1 & Test 2 group as compared to that of the standard.

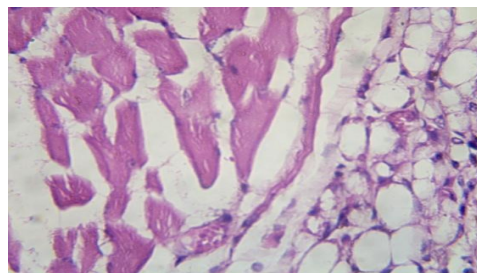
Tumour Volume Volumes for radiotherapy are vital to its successful execution. This requires the best possible characterisation of the location and extent of tumour¹⁰.

There is a significant increase in the tumour volume in the UV induced group as compared to that of the treated groups. There is a significant decrease in the tumour volume in the Test 1 & Test 2 group as compared to that of the standard.

Histopathology of skin

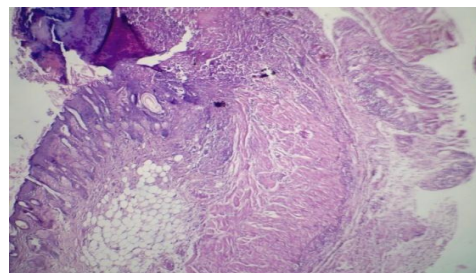
Group –I : Normal

Microscopic appearance: Section shows skin with normal epidermis. Dermis shows fibro collagenous stroma and normal appendages, sub serosa and muscular layer shows no significant pathology



Histopathology of normal skin

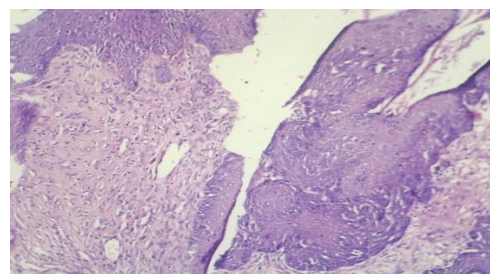
Group –II : Negative control (UV Induced)



Histopathology of negative control (UV induced) skin

Microscopic appearance: Section shows skin with epidermis showing dysplastic changes at one focus with an infiltrating neoplasm arising from it, cells are round polygonal having moderate eosinophilic cytoplasm and round vesicular nuclei with some showing nucleoli. Surrounding stroma shows scattered lymphoplasmacytic infiltrates.

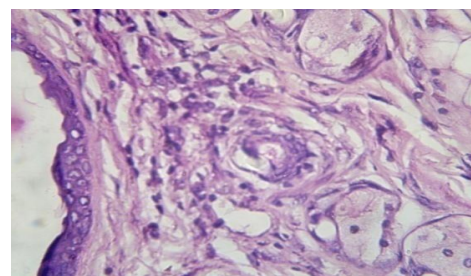
Group–III:Test-



Histopathology of Group –III

Microscopic appearance:Section shows skin with epidermis showing normal morphology. Subcutaneous tissue shows dense lymphoplasmacytic infiltrates. Surrounding stroma shows fibrocollagenous tissue.

Group –IV : Test 2



Histopathology of Group –IV

Microscopic appearance: Section shows skin with epidermis shows an infiltrating neoplasm arising from basal cell layer composed of cells arranged in glands, cells are round polygonal having moderate

eosinophilic cytoplasm and round vesicular nuclei with some showing nucleoli. Surrounding stroma shows scattered lympho plasmacytic infiltrates.

5. CONCLUSION

Plant items are normal bioactive aggravates that ensure against pressure and pathogenic assault. It has been accounted for that long haul utilization of certain therapeutic plants overpowers carcinogenesis in a few human and creature organs. We utilized a sub threshold dose of UV light as a cancer-causing agent taken to prompt skin tumors in test mice¹¹.

With respect to the commencement and promotion stages, mice thinks about demonstrate that the advancement step sets aside greater opportunity to happen and is reversible at first, so counteractive action of disease by restraint of tumor advancement is required to be an imaginative methodology¹². In the present investigation, stigmasterol could altogether repress development UV-incited papilloma as far as both frequency of tumors and the mean number of papillomas¹³.

Cell reinforcements have chemo preventive properties. Cancer prevention agents are thought to be the principal line of safeguard against oxidative pressure¹⁴, which proposes their helpfulness in lessening the danger of oxidative harm amid carcinogenesis.

Presentation to UV initiated LPO and ROS in the influenced territory of skin, prompting carcinogenesis. ROS cause permeabilization of the

external mitochondrial layer, which discharges solvent proteins from the internal film space into the cytosol, and advance caspase activation¹⁵.

Histopathological perception demonstrated that UV caused serious skin harm. The histopathology contemplate demonstrated that acetoheamide and stigmasterol repaired the deteriorated epidermal and dermal layers of skin in the gathering to mice¹⁶⁻¹⁷. Be that as it may, noteworthy histological changes were seen in the hypodermis, where there was degeneration of fat tissue. In this manner, the free hydroxyl assemble on the sweet-smelling ring is in charge of the cancer prevention agent properties. The Comet examine demonstrated that UV initiated critical DNA harm in lymphocytes¹⁸⁻¹⁹.

The present outcomes demonstrate the anticancer impact of Acetoheamide and stigmasterol at the higher focuses tried. These outcomes recommend that the anticancer action of Acetoheamide and stigmasterol might be because of its cancer prevention agent²⁰⁻²².

6.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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