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Anticancer Activity of *Panchatikta Ghrita* in 7,12-dimethylbenz(a)anthracene (DMBA) Induced Skin Carcinogenesis in Swiss Albino Mice

Nivedita Deshbhratar[1], Raman Belge[2], A.P. Somkuwar[3], Umap Swati[4]

[1]P.G. Scholar, Dept. of Rasashastra & Bhaishajya Kalpana, Shri Ayurved Mahavidyalaya, Nagpur
[2]P.G. Guide, Professor & H.O.D. Dept. of Rasashastra & Bhaishajya Kalpana, Shri Ayurved Mahavidyalaya, Nagpur
[3]Professor & H.O.D. Dept. of Pharmacology, Nagpur Veterinay College, Nagpur
[4]Assistant Professor, Dept. of Pharmacology & Toxicology, Nagpur Veterinary College, Nagpur.

Corresponding Author: Nivedita Deshbhratar

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ABSTRACT

Aim of the present study was to evaluate the anticancer effect of orally administered Panchatikta ghrita (Ghee) in 7,12-Dimethylbenz(a)anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice. Skin squamous cell carcinoma was induced at the shaved back of mice by applying DMBA (25 µg in 0.1 ml acetone/ mice) twice weekly for 7 weeks. Tumor formation (100%) was observed in 7 weeks of treatment in DMBA alone. As during radio and chemotherapy body's natural cells are also killed and low immunity develops, Panchatikta ghrita controls these adverse effects of both and helps better recovery and life styles. Oral administration of Panchatikta ghrita (Ghee) completely prevented the formation of skin tumors, hyperkeratosis, hyperplasia, epidermal erosion, dermal invasion and pearl formation. Results of the present study suggested that Panchatikta ghrita had potent anticancer and chemopreventive effect in DMBA induced skin carcinogenesis.

Keywords: Panchatikta ghrita, Chemoprevention, Hyperplasia, Epidermal erosion, Hyperkeratosis.

INTRODUCTION

Skin is the largest organ of human body. It contains three kinds of cells, squamous cells, basal cells and melanocytes. [1] Skin cancer, the most common form of human cancer goes to three distinct phases that is initiation, promotion and progression. [2]

Skin cancer develops in the epidermis. [3] Oxidative stress is the cause of skin cancer leading to DNA damage in the skin that includes DNA base damage. DNA single and double stand breaks and cross-linking between DNA and proteins. [4] Uncontrolled release of reactive oxygen species (ROS) is involved in pathogenesis of a number of human skin disorders including cutaneous neoplasia. [5] Premature ageing of skin and tumor initiation occurs if ROS are excessively generated in the skin. [6] Development of novel strategies to prevent skin cancer is a desirable goal to reduce the incidence of skin cancer.

Ayurveda a science of health and longevity; has tried many herbal and Rasayana remedies with varying degree of success, but its main significance lies in its preventive approach. [7] Over the past decade, herbal medicines have been accepted universally, and they have an impact on world health and international trade. Hence, medicinal plants play an important role in the health care system of a large number of the world's population. [8]

According to Ayurveda there is no specific mention of skin carcinoma in the

Ayurvedic text; but its signs and symptoms can be correlated with Kushtha. By the nirukti of kushtha "anything that creates abnormality in the skin or damages it is called as kushtha. [9]

Panchatikta Ghrita (PTG) is mentioned in Bhaishajya Ratnavali in Kushtha rogadhikar. It contains Nimba, Patol, Kantakari, Guduchi, Vasa and Goghrita. PTG cures eighty types of vataj vikara, fourty types of pittaja vikara and twenty types of kaphaja vikara. [10]

Various works have been done on the contents of PTG to prevent carcinogenesis in the Vivo and Vitro studies. Like chemopreventive activity of Azadirachta indica in Murine skin cancer and anticancer activity of Azadirachta indica in chemically induced skin cancer. [11] [12] [13][14]

Chemopreventive Property of Trichosanthes dioica Root Against 3-Methylcholanthrene-induced Carcinogenesis in Albino Mice. [15]

Chemopreventive potential of Tinospora cordifolia on skin Carcinogenesis in Mice. [16]

In Vivo and In vitro Anticancer activity of Adhatoda vasika. [17]

MATERIALS AND METHODS

Chemicals:- 7,12-dimethylbenz(a) anthracene (DMBA), a polycyclic aromatic hydrocarbon, is a procarcinogen and thus needs metabolic activation to become an ultimate carcinogen. The active metabolite, dihydrodiol epoxide, generated during the metabolic activation of DMBA binds and causes damage to DNA. Excessive reactive oxygen species are also generated during metabolic activation of DMBA. It is widely used as an initiator and promoter to induce skin carcinogenesis in Swiss albino mice. [18] DMBA induces skin carcinogenesis and it is commonly employed to study Chemoprevention by Panchtikta Ghrita in DMBA induced skin carcinogenesis in Swiss albino mice. The inducer, DMBA was purchased from Sigma Chemical Co. USA.

Animals:- Healthy Swiss albino mice of either sex, weighing 25-35 g were selected for the study.

HOUSING:

All the mice were kept under standard management conditions as per the norms of CPCSEA.

Adult Swiss albino mice of either sex, 7-8 weeks old and weighing 25-35 g were used for conducting this study. 6 animals were housed in one polypropylene plastic cage containing rice husk as bedding material. They were maintained under control conditions of temperature (25 +/- 2º C) and light (14 hr light / 10 hr dark) and provided standard mouse feed and water ad libitum.

FEEDING:

The animals were provided with ad libitum balanced pelleted feed and wholesome and purified drinking water throughout the experiment.

BEDDING MATERIAL:

Clean sterilized dried rice husk was used as a bedding material for the experimental animals. Bedding material was changed on every alternate day.

Experimental Animals:

Approval for the experimental protocol was obtained from Institutional Animal Ethical Committee (IAEC) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The proposed research work was conducted on Swiss Albino mice, which was procured from the CPCSEA recognized Laboratory. Swiss albino mice around 20 - 35 g of either sex were used for the present study.

Method of preparation of Panchatikta Ghrita:-

- Panchatikta Ghrita was prepared in department of Rasashastra and

Bhaishajyakalpana as per method described in Bhaishajya Ratnavali. [19]

- For the preparation of Panchatikta Ghrita, authenticated raw materials were used.
- All the drugs were collected from local market, in dried form.
- First did Ghrita Murchhana was done. [20]
- Then Panchatikta kwatha was prepared according to Bhaishajya Ratnavali. . [21]
- After that PTG was finally prepared according to Bhaishajya Ratnavali. Once Ghrita (Ghee) siddhi lakshana get observed, heating was stopped accordingly. [22]

Experimental Design -

In Vivo Study -

The mice were divided into five groups. Mice of either sex selected for the study,6 in each groups. Skin carcinogenesis was developed in Swiss albino mice according to the method of Azuine and Bhide. [23] Depilatory cream was applied to remove hair from back of each mice and the mice were left untreated for two days. Mice having no hair growth after two days were selected for the study.

A) Inclusion Criteria: Healthy Swiss albino mice, Weight between 25 -35 g were selected for the study.

B) Exclusion Criteria: Mice which were infected were excluded from the study.

INDUCTION OF SQUAMOUS CELL CARCINOMA:

Animals were divided into 5 groups of 6 each. Skin carcinogenesis was developed in Swiss albino mice according to the method of Azuine and Bhide. Depilatory cream was applied to remove hair from the back of each mice and mice were left untreated for two days. Mice having no hair growth after two days were selected for the study.

DMBA (25 µg in 0.1 mL acetone) twice weekly was applied on back of shaved mice.

Group 1 -Positive Control group- normal regular healthy diet. Mice received no other treatment.

Group A - Mice were orally administered with PTG (100 mg/kg body wt) by gastric gavage, starting 1 week before the exposure to the carcinogen.

Group A, B, C, D mice were applied with DMBA (25 µg in 0.1 ml acetone/ mice) twice a week for 7 weeks.

At the end of experimental period all animals were sacrificed by cervical dislocation.

Section of skin from carcinoma induced mice exhibited a well differentiated squamous cell carcinoma with the formation of keratin pearls and infiltration of lymphocytes into the underlying dermis. Epidermal hyperplasia, dermal invasion, hyperkeratosis, epidermal erosion was also evident in skin sections.

Dose selection:

According to Sharangdhar Samhita, the dose of PTG is 1 pala (4 tola).

Dose fixation:

The dose is calculated by extrapolating the human dose to animal based on the body surface area ratio by referring to the table of Paget and Barnes.

Conversion formula: Human dose x 0.0026 (conversion factor for mice)

Test drug PTG = Human dose x 0.0026

= 48000 x 0.0026 =124.8mg/ 20g body wt of mice.

Standard drug = 5 % 5-FU (Fluorouracil) applied locally on affected site.

The experiment was performed using 5 groups:

Sr .No.	Group Name	Sample Size	Intervention/ Drug	Dose mice	Route	Duration treatment
1	Control group	6	Normal healthy diet	Normal feeding	Oral	7 week
2	Test gr(A)	6	PTG before induction	124.8mg/20gm body wt	Oral	One Week
3	Test gr (B)	6	PTG after induction	124.8mg/20gm body wt	Oral	2 week
4	Test gr (C)	6	5 -FU	Local as required	Local	2 week
5	Test gr (D)	6	PTG + 5-FU	Oral + Local	Oral + Local	2 week

Experimental parameters studied - Histopathology

We did Histopathology of each group's sacrificed skin of mice and after that revealed as SCC.

OBSERVATION AND RESULT

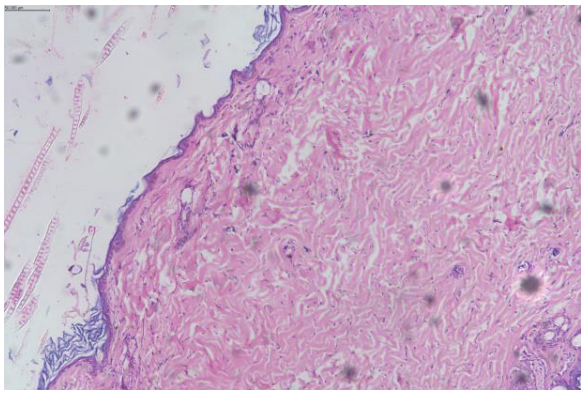

Fig 1- normal mice skin

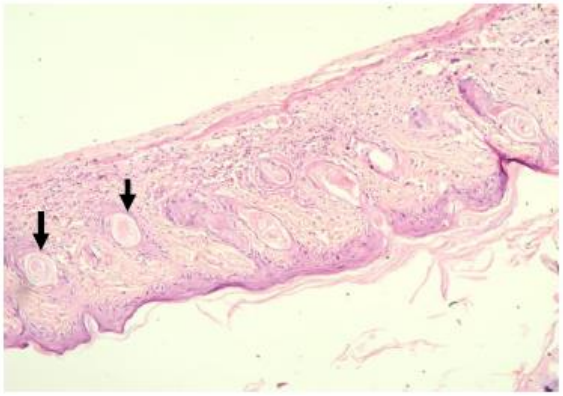

Fig2- Induction of SCC

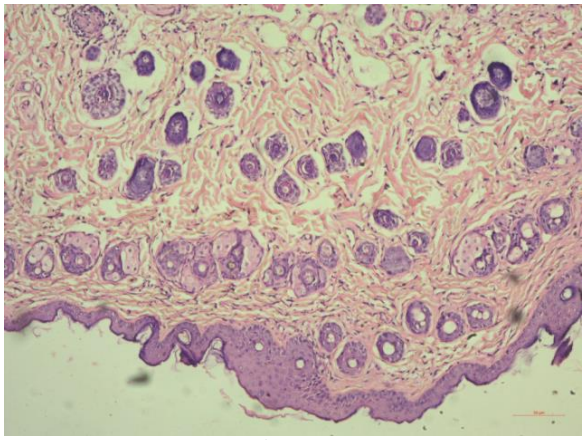

Fig4-Group B PTG after induction

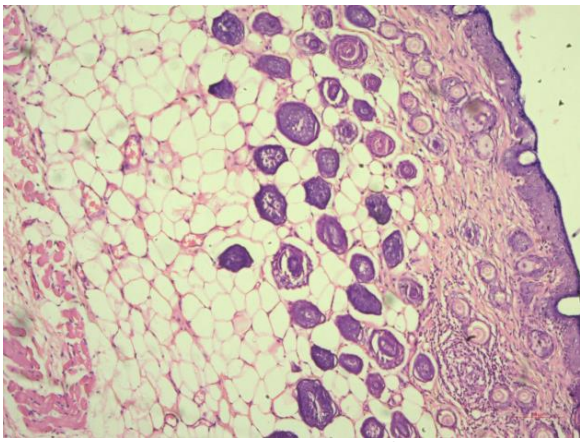

Fig3-Group A PTG before induction

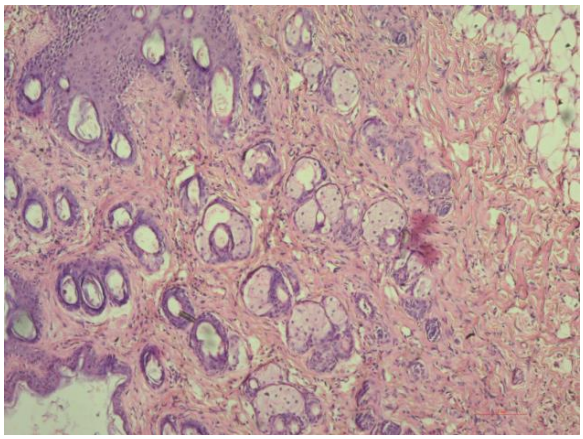

Fig5-Group C5-FU(local)

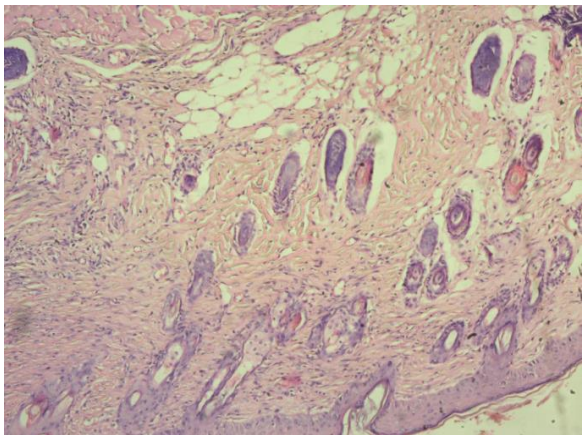

Fig6-Group D PTG (oral)+5-FU(local)

Fig 1: Section of skin showing normal histoarchitecture (H&E x100)
Section of skins from control group revealed normal histoarchitecture having epidermis layer, dermis layer, hair follicle and sebaceous glands (Fig. I)
Fig 2: Skin from tumor after induction SCC showing keratin pearls (arrows) and infiltration of lymphocytes into the underlying dermis (H&E x100)
Fig 3 : Skin from group A showing mild to moderate hyperplasia and lack of pearl formation (H&E x100)
Fig 4 : Skin from group B showing epidermal hyperplasia (double headed arrow) with evidence of infiltrative tumor (arrow) and infiltration of lymphocytes (H&E x100)
Fig 5: Skin from group C showing mild hyperplasia and reduced keratin pearl formation (H&E x100)
Fig 6 : Skin from group D showing mild to moderate hyperplasia, areas of necrosis and reduced keratin pearl formation with evidence of fibrosarcoma (H&E x100)

DISCUSSION AND CONCLUSION

- Histopathology of mice skin in SCC induced mice shows hyperplasia, hyperkeratosis, keratin pearl formation, epidermal thickening and epidermal erosion.
- In this present study control group (Fig.1) histoarchitecture shows normal and healthy skin tissue.
- Hyperplasia was observed in all test groups in different degrees.
- Test group A and D showed mild to moderate hyperplasia. Test group C showed mild hyperplasia while Test group B showed moderate to severe hyperplasia. Based on this we can say that PTG helps in preventing Hyperplasia.
- Keratin pearls were absent in Test group A and reduced in rest test groups with comparatively more reduction in test group B. Thus it can be stated that PTG prevented keratin pearl formation.
- Limited hyperkeratosis was observed in test group A, where as moderate hyperkeratosis was observed in rest groups. Thus PTG helped to limit hyperkeratosis.
- Infiltrative tumors were seen in test group B and fibrosarcoma was seen in test group D. Thus the tumor appearance which is repeated in carcinoma was observed in these groups but not in test group A where PTG was given as a preventive treatment. So it can be said that PTG plays an important role in restricting development of skin cancer.

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Conflict of Interest: None

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Ethical Approval: Approved

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