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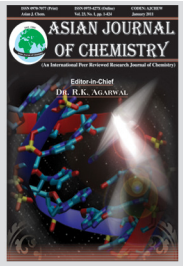

Chemotherapeutic Role of *Bis*-3-azophenyl-4-hydroxy-6-methyl-pyran-2-one Iron(II) in Chemically Induced Skin Cancer in Albino Rats

SAMINA SHAHID[1], SHAFIQ-UR-REHMAN[2,*], FAHIM A. QURESHI[2] and AHMAD ADNAN[2]

[1]Institute of Chemistry, University of the Punjab, Pakistan
[2]Department of Chemistry, Government College University, Lahore-54000, Pakistan

*Corresponding author: Tel: +92 333 4566845; E-mail: shafiqchemist@gmail.com

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The present study was designed to observe the effect of *bis*-3-azophenyl-4-hydroxy-6-methyl-pyran-2-one iron(II) in 7,12-dimethylbenz-(a)anthracene (DMBA) followed by 12-O-tetradecanoyl-phorbal-13-acetate (TPA), induced chemical cacinogens on the skin of albino rats. Eighty albino rats were divided into four groups. First and second groups (A & B) were used as control. In third group C, 7,12-dimethylbenz(a)anthracene was given 100 mg/mL as single dose while 12-O-tetradecanoyl-phorbal-13-acetate was given 10 mg/mL in acetone twice a week. Different types of tumors were observed. In group D, all the chemical carcinogens (7,12-dimethylbenz(a)anthracene and 12-O-tetradecanoyl-phorbal-13-acetate) were applied locally in the same dose, route and schedule, after the induction of skin cancer, the complex (*bis*-3-azophenyl-4-hydroxy-6-methyl-pyran-2-one iron(II)) was applied by following the same route.

Key Words: 7,12-Dimethylbenz(a)anthracene, 12-O-tetradecanoyl-phorbal-13-acetate, *Bis*-3-azophenyl-4-hydroxy-6-methyl-pyran-2-one iron(II), Skin cancer.

INTRODUCTION

Reproduction of cells is a physiological process that occurs in almost all tissues under different conditions. The steadiness between proliferation and apoptosis is tightly regulated to ensure the consistency of organs and tissues. Mutations in DNA that lead to cancer interrupt these orderly processes. Leong and Leong[1] realized tumor behaved in a body in different ways. The unrestrained and often rapid proliferation of cells can lead to either a benign tumor or a malignant tumor (cancer). Benign tumors do not extend to other parts of the body or invade other tissues and they are rarely a threat to life. Malignant tumors can invade other organs, spread to distant locations (metastasize) and become life intimidating.

Robert Bentley (1861) of King College, London, noted the antitumor properties of an extract of the common May apple (*Podophyllum peltatum*). Scientists soon had the ability to analyze such natural products to determine active ingredients. Compounds in the plant are responsible for the observed therapeutic properties.

2-Pyrones and their derivatives have been widely recognized compounds on account of the fact that they are natural products. J.N. Collie carried out ground breaking research to prepare triacetic lactone[2]. Pyrones has displayed remarkable utility as antibacterial and antifungal activities[3]. The transitional metal complexes with hetrocyclic systems containing nitrogen and sulphur atoms have been studied extensively because of their antitumor potential[4-7]. It is an established fact that most of the transition metals also posses similar properties having combined the two, we attained complexes, which have much enhanced activity[8]. The study was undertaken with the intention of studying the effects of chemical carcinogens on the skin of rats with initiation-promotion protocol and the use of previously reported complex *bis*-3-azophenyl-4-hydroxy-6-methyl-pyran-2-one iron(II) as therapeutic agent[9].

EXPERIMENTAL

Eighty albino rats were selected for experimental study. They were divided into four groups (20 each) A, B, C, D. The animals in the experiment were healthy and adult rats and were obtained from Veterinary Research Institute, Lahore, Pakistan. The rats were kept under optimal atmospheric and hygienic conditions. All the four groups kept in separate iron cages fitted with removable gauze lids that had been especially designed for keeping the cages clean. The cages were labeled with their respective identification markings.

The animals were allowed and facilitated to acclimatize in the animal house for one week before the experiment was started. Two chemicals were used as carcinogens, 7,12-dimethylbenz(a)anthracence (DMBA) was used as an initiator and 12-O-tetradecanoy1-phorbol-13-acetate (TPA) was used as promoter in the study. These chemicals were obtained from

TABLE-1
GROUP OF RATS ON THE BASIS OF TREATMENTS

Group (30 weeks)	Carcinogen (15 weeks)				Curative (15 weeks)	
	DMBA in acetone 100 µg/mL		TPA in acetone 10 µg/mL (after 2 weeks of DMBA)		Complex in DMF 10 µg/mL (after 15 weeks of carcinogensis)	
	Route	Schedule	Route	Schedule	Route	Schedule
A	-	-	-	-	-	-
B	-	-	-	-	-	-
C	Topical	Single dose	Topical	Twice a week till 15 weeks	-	-
D	Topical	Single dose	Topical	Twice a week till 15 weeks	Topical	Twice a week till 30 weeks

DMBA = 7,12-dimethylbenz(a)anthracene; TPA = 12-O-tetradecanoyl pharbol-3-acetate, Complex = *bis*-3-azopheny1-4-hydroxy-6-methy1-pyran-2-one iron(II)

Sigma Chemical Company. 7,12-Dimethylbenz(a)anthracence (100 µg/mL of acetone) was applied on the skin of the albino rats to test its effects as an initiator of skin cancer. This solution was prepared each time only a short time before its use and its temperature was maintained at 20-25 ºC. Insulin syringe was used for the application of solution. 12-O-tetradecanoyl-phorbal-13-acetate (10 mg/mL of acetone) was applied twice a week after 7,12-dimethylbenz(a)anthracence to observe its role as a substance that promoted skin cancer[10]. The solution of 12-O-tetradecanoyl-phorbal-13-acetate was prepared by mixing 1 mg of dry 12-O-tetradecanoyl-phorbal-13-acetate powder in 100 mL of acetone. Acetone and DMF 0.2 mL were used as vehicles for all topically applied chemicals. However, only acetone and DMF was used on the skin of the rats belonging to the groups A and B to confirm its role as placebo.

After 1 week of acclimatization of the rats, the back of each rat was shaved off hair (5 cm × 5 cm area) with electric clippers after 3 days before the first dose was administrated. 12-O-Tetradecanoyl-phorbal-13-acetate and 7,12-dimethylbenz-(a)anthracence are known carcinogens so high protective measures were taken during the application of drugs. All the animals were divided into four groups (A-D) of twenty rats each. First and second (A and B) groups were selected as control and were treated biweekly topical application of acetone and DMF administered with a insuline syringe on a biweekly basis.

Particulars of lesion recorded: Every week, loss of hair and gross morphological features such as ulcers were closely observed in each animal and if found, were then measured carefully with vernier calipers throughout the experiment. After the completion of 15 weeks, the lesions and the surrounding skin of each animal was also closely examined (by a true cut fine needle biopsy) with a microscope. to determine the extent of histopathological changes, such as papiloma malignant fibrous histiocytoma, atrophy, fibrosarcoma, chronic inflammation, squamous cell carcinoma in situ, extensive squamous cell carcinoma and osteoma at the end of the experiment. The lesions were then diagnosed according to the histopathological changes. Ether was used to anesthetize the rats. All the animals were sacrificed after giving the anesthesia in a glass jar after 30 weeks. After removing sections of the rats' dorsal skin, sections that contained or surrounded the lesion were removed for further cutting. The cancerous and surrounding tissues were washed two to three times with 10 % formalin and were then used for histopathological studies.

Histopathological studies: To prepare the tissues for histopathological examination[11-13], the following steps were taken: fixation, which preserved cells in condition similar to the ones present during life, inhibited bacterial decomposition and also preserved loss of any easily diffusible substance by appropriate coagulation and strengthening of the tissues against the decomposing effects during various stages in tissue processing), gross examination and sectioning of the tissues: processing for the section: clearing: wax impregnation: embedding; storage of blocks; trimming of blocks; section cutting; removal of paraffin wax; hydration; staining oiling; clearing and mounting. The identifications were ultimately got confirmed from an expert, Dr. Ehsan Hashmi, Pathology Department of King Edward Medical College, Lahore, Pakistan.

Group A and B: The animals of groups A and B were kept controlled. However, these animals received doses of acetone and dimethyl formamide the vehicles used for dilution of carcinogens and *bis*-3-azophenyl-4-hydroxy-6-methyl-2*H*-pyran-2-one iron(II) Table-1.

Group C: The animals of this group were administered with 7,12-dimethylbenz(a)anthracence at 100 mg/1 mL of acetone topically as single dose on the shaved dorsum of the albino rat skin and 12-O-tetradecanoyl-phorbal-13-acetate was given twice a week till 15 weeks to test the cancer inducing effects of 7,12-dimethylbenz(a)anthracence and 12-O-tetradecanoyl-phorbal-13-acetate (Table-1).

Group D: Animals of group D were given carcinogens (7,12-dimethylbenz(a)anthracence and 12-O-tetradecanoyl-phorbal-13-acetate) in the same schedule as for other animals (Table-1). In this group complex was given locally after 15 weeks of carcinogenesis in a dose 20 mg/0.2 mL twice a week for next 15 weeks to observe the response of locally applied given complex against chemical carcinogens (Table-1).

In this group we saw the chemotherapeutic effect of complex. After completion of 30 weeks, biopsies were taken to see the chemotherapeutic response of locally applied given complex against chemical carcinogenesis.

RESULTS AND DISCUSSION

Group A and B: Forty albino rats that were used as control groups (A and B) in which biopsy of skin showed normal cells (Fig. 1).

Group C: In group C all the chemical carcinogens (7,12-dimethylbenz(a)anthracence and 12-O-tetradecanoyl-phorbal-13-acetate) were applied locally produced 100 % lesions. Maximum number of lesions was encountered in this group (100 %). All these animals received topical application of 7,12-dimethylbenz(a)anthracence and 12-O-tetradecanoyl-phorbal-

TABLE-2
DISTRIBUTION OF LESIONS OBTAINED IN DIFFERENT GROUPS

		Groups (each 20)				
		A	B	C	D (before complex)	E (after complex)
Benign lesions	EPA	-	-	7	7	-
	Pap	-	-	3	3	-
	Dys	-	-	3	3	-
	OST	-	-	1	-	-
	Total	-	-	14	13	-
Malignant lesions	SQCCIS	-	-	2	3	2
	SQCC	-	-	2	2	2
	MFH	-	-	2	1	1
	Total	-	-	6	6	5
Total lesions (%)		0	0	20 (100)	19 (95)	5 (25)

EPH: epidermal hyperplasia; Pap: Papilloma; Dys: Dysplasia; SQCCIS: Squamous cell carcinoma *in situ*; SQCC: Squamous cell carcinoma; MFH: Malignant fiberous histiocytoma; OST: Osteoma; Complex = *Bis*-3-azopheny1-4-hydroxy-6-methy1-pyran-2-one Iron(ll)

13-acetate. In group C 14 animals developed benign lesions, which were epidermal hyperplasia (07), dysplasia (03), papilloma (03) and osteoma (01). There were (06) malignant lesions, which were squamous cell carcinoma *in situ* (02) squamous cell carcinoma (02) (Fig. 2) and malignant fibrous histiocytoma (02) (Fig. 3). Most of the rats have chronic inflammation and precancerous changes in early weeks. Hair loss was observed on specific area on third week where 7,12-dimethylbenz(a)anthracence and 12-O-tetradecanoyl-phorbal-13-acetate were applied locally and post application of 7,12-dimethylbenz(a)anthracence and 12-O-tetradecanoyl-phorbal-13-acetate slight bleeding and ulceration, which was not too deep was observed at 15 weeks. Small size out growths were also observed (papilloma) at 15 weeks in the treated area.

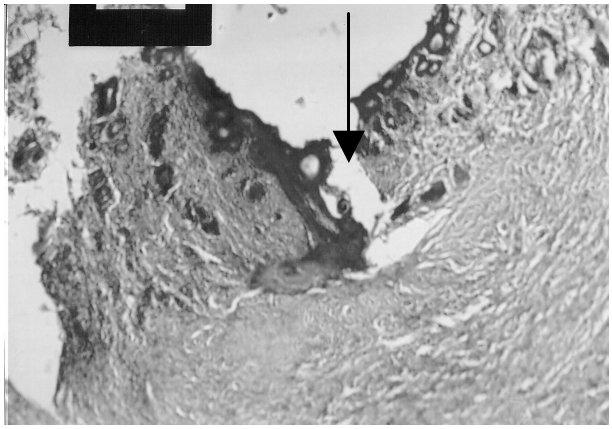

Fig. 1. Photomicrograph showing normal cells (arrow) in the skin of rat (H and E, X 40)

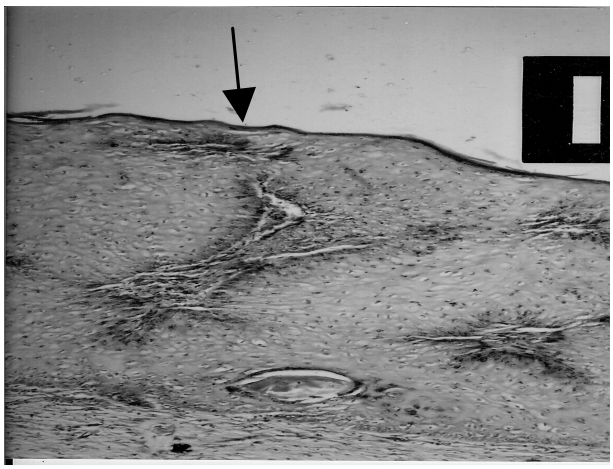

Fig. 2. Photomicrograph showing (arrow) histology of squamous cell carinoma (arrow) (H & EX 10) stage II grade-1

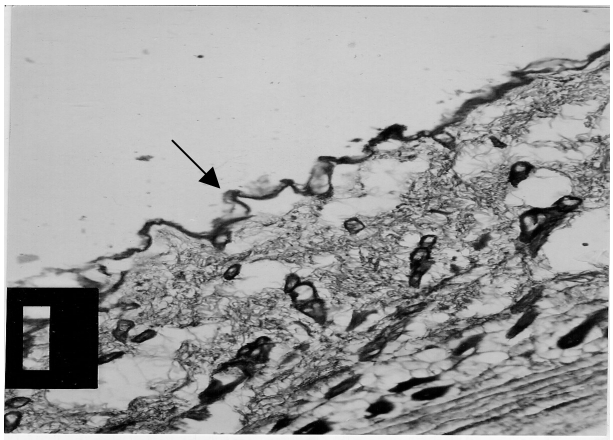

Fig. 3. Photomicrograph showing histology of malignant fibrous histiocytoma (arrow) (H and E X 40)

Group D: 13 animals developed benign lesions, which were hyperplasia (07), papiloma (03), dysplasia (03). There were (06) malignant lesions in this group which were squamous cell carcinoma *in situ* (03), squamous cell carcinoma (02) and malignant fibrous histiosytoma (01) (Fig. 2) after 15 weeks of carcinogensis (Table-2). When chemotherapy was given 13 animals were cured, which were suffering from benign lesions while squamous cell carcinoma in situ, squmous cell carcinoma and malginent fibrous hyperplasia were not cured however there were no death of any animal in this group. And no further progression of malignancy was seen in this group.

No tumor developed in the first and second group. However, albino rats belonging to the third group to whom no chemotherapy was given, all malignant tumors became worse and three animals bearing these malignant tumors died before the completion of experimental period. Present study suggested that use of iron complex decreases the risk of malignant conversion of benign tumors, because all benign tumors and pre-malignant lesions were cured with the use of *bis*-3-azophenyl-4-hydroxy-6-methyl-pyran-2-one iron(II). Present findings are consistent with Jun *et al.*[14]. They also used iron chelates as an antitumor. It is suggested that if complex is given

in early stage of tumor, the results are 100 % correct but in the case of malignant tumors, no good results were found. This study concludes the chemotherapeutic effect against malignant tumors was not satisfactory. However, it was not disappointing. There is immense scope for further research on these complexes. However, for the purpose of current study it was imperative to lay down certain parameters in order to achieve the desired results.

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