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CHEMOPROTECTIVE EFFECT OF MELATONIN ON DMBA/CROTON OIL INDUCED CARCINOGENESIS IN SKIN OF SWISS ALBINO MICE

SHIMAL **M**OHAMMED **A**MIN **O**MAR* and **B**USHRA **M**OHAMMED **A**MIN MOHAMMED**
*Dept. of Basic Science, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region-Iraq.
** Dept. of Biology, College of Science, University of Duhok, Kurdistan Region-Iraq.

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ABSTRACT

Melatonin, a hormone secreted by the pineal gland, is known to have anti-mutagenic, antioxidant and oncostatic functions. This beneficial action of melatonin has been explained in terms of its ability to scavenge free radicals and the activities of anti-oxidant enzymes. The present study was carried out to evaluate the anti-tumor activity of melatonin on the two stage process of skin carcinogenesis, A significant reduction in tumor incidence, tumor burden, tumor yield, and cumulative number of papilloma was observed in mice treated with 10mg/kg of melatonin as compared to the positive control group treated with DMBA plus croton oil. Furthermore, Histopathological alterations in the carcinogen-treated control animals were also observed in the form of epidermal hyperplasia, keratinized pearl formation, and acanthosis in skin and tumors, whereas these were found to be reduced significantly in melatonin group were administrated before DMBA administration. The results thus can be suggested that melatonin may function as an endogenous anti-mutagenic and oncostatic agent.

KEYWORDS: DMBA/Croton Oil, Skin Carcinogenesis, Melatonin, Antioxidants.
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INTRODUCTION

DMBA, a polycyclic aromatic hydrocarbon, has been widely used to induce cancer in animal models (Mestas and Hughes, 2004). 7,12-dimethylbenz(a)anthracene (DMBA), an environmental pollutant commonly found in cigarette smoke, is a potential carcinogen that causes DNA damage (Balmain and Brown, 1988). Further, it cause cross link between DNA and proteins, triggering genomic instability and inflammation, which induce cancer (Rastogi et al., 2007).

It had been considered that using of natural and synthetic agents is as the best pharmacological approach to prevent cancer induction and progression which may prevent genetic mutations (Tiwari *et al*., 2015).

Melatonin, is one of the natural hormones that inhibits tumor genesis in different experiments, both in vivo and in vitro. Studies regarding melatonin and cancers show that melatonin exerts its anti-cancer effect via three mechanisms: inhibition of cell proliferation, stimulation of differentiation, and apoptosis (Blask et al., 2005) and (Viswanathanet al., 2007). Melatonin (N-acetyl-5-methoxytryptamine) is a hormone widely found in animals, plants and microbes. It synthesized from tryptophan and secreted by the pineal gland and perhaps all organs (Grantet et al., 2009) and (Reiteret al., 2017).Including the gastrointestinal tract, bone marrow, eyes, lymphocytes and skin .The synthesis and secretion of melatonin are regulated by the 'master biological clock' located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Reiter, 1991).

Melatonin functions as a cell protector because it is not synthesized in a single organ and does not exert effects upon a specific target organ. It has been recognized that melatonin is a molecule with paracrine, autocrine, effects, which exerts diverse receptor-dependent and receptor-independent actions (Tan et al., 2003),(Hardeland et al., 2009) and (Luchetti et al., 2010).

Moreover, melatonin is known to possess potent immunomodulating, antiproliferative, antioxidant and endocrine-modulating properties

shimal.omar@uod.ac, bushra.mohammed@uod.ac

(Vijayalaxmi et al., 2002) and (Srinivasan et al., 2008).

Melatonin's effects as an antioxidant include: a) cleaning free radicals; b) increasing antioxidative enzymes; c) stimulating mitochondrial oxidative phosphorylation and decreasing electron leakage; and d) stimulating other antioxidant effects (Reiter et al., 2003).

Melatonin antioxidant property acts in normal cells or tissues under oxidative stress, via direct scavenging reactive oxygen species (ROS), inducing antioxidant enzymes or activating nuclear factor erythroid 2-related factor 2 (Nrf2)-dependent antioxidant defense system (Tan et al., 2007). It functions as a powerful antioxidant to protect nuclear and mitochondrial DNA from damage (Galano et al., 2011) and (Galano et al., 2013).The oncostatic and tumor inhibitory effects of melatonin, in a variety of experimental models and clinical conditions have been an increasing area of interest (Maitra et al., 2019).

During the past several decades, there has been an explosion of work to identify the different actions of melatonin, especially in relation to cancer, Recent studies on melatonin activity as an anticancer agent demonstrated that melatonin prevents human cancer cell growth in

cancer cell cultures and in various animal model systems suggesting the possession of pleiotropic anti-proliferative activity towards a variety of tumors (Jung and Ahmad, 2006) and (Tian et al., 2017).

Reiter et al (2017) extensively summarized the literature showing that melatonin mitigates a number of cancer types at the initiation, progression and metastasis levels; the processes by which melatonin restrains cancer development and growth have been often described, whereas other roles appear to be merely epiphenomena of a more complex action of melatonin that should be thoroughly investigated.

Melatonin was found to acquire cell preservation activity in normal cells, working as an antioxidant. In contrast, exposure to melatonin, induces apoptotic cell death in cancer cells, limiting the growth rate and size of the tumor. It is a potent scavenger of hydroxyl radical, peroxynitrite anion, superoxide, and singlet oxygen, also possessing a potent direct chain- breaking antioxidant activity (Reiter et al., 2003). Experimentally it has been shown that melatonin plays some roles in skin physiology such as hair growth cycling, fur pigmentation, and melanoma control. Melatonin suppresses ultraviolet (UV)-induced damage to skin cells and exerts strong antioxidant effects on UV-exposed cells (Slominski et al., 2005). Melatonin is transformed to 6-hydroxymelatonin and N 1 – acetyl-N 2 –formyl-5-methoxy-kynuramine in melanocytes, keratinocytes, and fibroblasts primarily. All three types of cells in the skin express the metabolism of melatonin and its endogenous production (Kim et al., 2013). Melatonin treatment on, 7,12-dimethylbenz(a)anthracene (DMBA) has been shown to induce chromosomal mutation in bone marrow cells of swiss albino mice and because melatonin has excellent antimutagenic, antioxidative, and oncostatic properties, there is a likely possibility that melatonin would protect against the skin carcinogenicity of DMBA. (shimal and bushra, 2019).

To the best of our knowledge, there are a few animal studies on the effect of melatonin in the prevention of skin carcinogenesis. thus , In this in vivo study We aim to evaluate the modulating influence of Melatonin on two stage skin carcinogenesis in mice induced by DMBA by measuring tumor incidence, tumor diameter, accumulating number of tumor, tumor burden, tumor yield, and examination the histopathological changes in the DMBA /croton induced tumors in mice skin before and after melatonin injection .

MATERIALS AND METHODS

Chemicals:

Melatonine hormone 99% and the initiator, 7, 12-dimethylbenz (a) anthracene (DMBA) was purchased from ACROS organics -Belgium, and Croton oil (used as promoter) were procured from Alfa Aesar-USA. 100 mg of DMBA dissolved in 100 ml Acetone, and then 1ml of solution (single dose) applied on the shaved region of mouse skin. Croton oil was mixed in acetone to give a solution of 1% dilution while 10mg/kg of melatonin were applied by injection intraperitoneally after dissolving in ethanol and diluting in double distilled water, Other analytical grade chemicals were purchased from local sources.

Experimental animals

Random-breed male Swiss albino mice (Mus musculus), strain Balb/c (25-30 gm, 8-10 weeks) were obtained from University of Duhok, college of Science animal house. The animals were kept in climate controlled facility at standard laboratory conditions such as temperature 24±2°C, with a photoperiod of 12 h light/12 h dark cycle. The mice were fed with standard locally prepared diet and water ad libitum. And in order to study the effect of melatonin on two stage skin carcinogenesis ,melatonin (10mg/kg)was applied daily at 8 p.m. The animals were acclimatized for one week prior to the start of the experiment.

Induction of Tumor: For the induction of skin tumors, dorsal hair between the cervical and caudal portions of the animals of Group 1 to 6 were removed using a surgical clipper, two days prior to the initiation of the experiment, and one ml DMBA (100 mg / 100 ml acetone) was applied. After 14 days, the tumor initiation by DMBA was promoted with the topical application of one ml of 1% croton oil (1ml croton oil /100ml acetone), thrice a week for the next 14 weeks.

Experimental design

During the 16 weeks of experimentation, mice were observed daily and weighed at the end of the sixteenth week ,to test the Chemopreventive efficacy of melatonin against DMBA and croton oil-induced mouse skin papillomagenesis, Sixty five mature male mice in the resting phase of growth were selected from the inbred colony and divided into six different groups. The dorsal skin was carefully shaved (2cm diameter) by electric clipper to avoid injuries two days before application of the initiator. Only those tumors which persisted for two weeks or more, with a diameter greater than 1 mm, have been taken into consideration for the final evaluation of the data. Skin tumors, which regressed after one observation, were not considered for the counting. The groups were as follow:

1. DMBA+ Croton oil (+ve group); n=15 mice: animals were treated with a single dose of DMBA (100 mg DMBA /100 ml of acetone) over the shaven area of the skin, Croton oil 1% (1ml croton oil /100ml acetone) applied after 14 days of DMBA application. The treatment with croton oil was trice weekly and continued up to the sixteenth week at alternate days.

2. Pre-Treatment group; n=15mice: in this pre initiation treatment group, animals were injected intraperitoneally with melatonin (10mg/kg) at 8 p.m for 14days, then a single dose of DMBA solution applied over the shaven part of skin, two weeks later mice were injected with melatonin (10mg/kg) 3 times/week followed by applying 1% Croton oil on the shaven part of the skin until the end of the experiment.

3. Post-Treatment group; n=15: In this post-initiation treatment group, a single dose of DMBA solution applied topically to the shaven part of the mice skin, then after two weeks mice were administered with melatonin (10mg/kg) injection 3 times/week followed by applying 1% Croton oil on the shaven part continued to 16 weeks (Pandey & Agrawal, 2009).

4. Croton oil group; n =10: these animals were treated with 1 ml of Croton Oil solution (1ml croton oil/100 ml acetone) applied topically to the shaven part of the mice skin (3 times /week) over 16 weeks.

5. Acetone group; n = 5: animals of this group were treated with 1 ml of acetone on the dorsal clipped portion (3 times /week) over 16 weeks.

6. Melatonin group; n=5: animals of this group were injected intraperitoneally with melatonin 10mg/kg (3 times /week) over 16 weeks.

chemo-modulatory efficacy of melatonin on DMBA/Croton oil induced skin carcinogenesis.

For determining the in vivo chemo-modulatory efficacy of melatonin on DMBA/Croton oil induced skin carcinogenesis, the following parameters were used :

I. Morphological examination

The parameters studied after the completion (i.e. 16 weeks) of the experiments are discussed below: The number of skin tumors on each affected mouse was recorded. Skin papilloma were defined as lesions with a diameter >1 mm that were present for at least two consecutive observations (Pandey & Agrawal, 2009).

1.Tumor incidence: The number of mice carrying at least one tumor expressed as a percentage incidence.

2.Tumor yield: The average number of papilloma per mouse.

3.Tumor burden: The average number of tumors per tumor bearing mouse.

4.Cumulative number of tumors: the total number of tumors carried by all mice.

5.Tumor Diameter: The diameter of each tumor was measured using electronic caliper.

6.Measurement of body weight: The body weight of the individual mouse was recorded initially and till the end of experiment or death of the mouse. A continuing rise in body weight after the beginning of the treatment was observed in all mice groups and such weight was found to be near normal at the end of the experimental period.

II.Histological evaluation

A part Skin of sacrificed animals were removed after end of the experiment (16 week) and fixed in 10% formalin fixative for 24 h. Dehydration of the tissue was done in ascending series of alcohol, embedded in paraffin wax, and 4-µm thick sections were prepared and studied using a light microscope (Olympus VANOX-S Germany) and digital images were captured. The slides were evaluated by a qualified experienced pathologist masked to the experimental groups and treatments. A minimum of 10 fields for each slide were examined and scored.

Statistical analysis

The data of experiments were submitted to SPSS software (SPSS,2013), in order to illustrate the effect of studied groups (treatments) on the studied characters and parameters. Both one-way ANOVA and GLM procedures for group effect and time effect, respectively were used to analyze the data according to the following models:

$y_{ij} = \mu + G_i + e_{ij}$ *…….(Model 1)*

Where:

y_{ij}= the studied character; μ= *overall mean;* G_i= *effect of group;* e_{ij} = *experimental error.*

$y_{ijk} = \mu + G_i + \delta_{ij} + t_k + (\tau * t)_{ik} + e_{ijk}$

…….(Model 2)

Where:

t_k = the effect of time k

$(\tau * t)_{ik}$ = the effect of interaction between group i and time k

δ_{ij} = random error with mean 0 and variance σ2 δ., the variance between animals (Subjects) within groups and it is equal to the covariance between repeated measurements within animals.

The differences among means were computed using Duncan multiple range test (Duncan, 1955), at 0.05 level of significant

RESULTS

Effect of Melatonin on Morphological features:

1. Effect of Melatonin on morphological examination of tumor

The incidence of tumors starting from 5th week in DMBA/Croton oil-treated animals developed papillomas (87% incidence) at the end of 12thweeks. The pre initiation treatment group 2 at doses of 10 mg/kg significantly reduced the incidence of papilloma comparing with the positive group (Table 1). While the post initiation treatment of Melatonin group 3 animals (73%)reduced tumor incidence but not significantly

The groups received melatonin intraperitoneally, DMBA alone, acetone and croton oil topically, showed 0% tumor incidence (Table 1).

In Group2 (pre-initiation) and Group 3(Post-initiation) animals received melatonin significantly reduced ($p<0.05$) the cumulative number of tumors (total number of papilloma till the end of the experiment) 1.56 and 1.4 respectively as compared to the positive control Group1 32.53 (Table 1). Concerning The tumor diameter, the groups 2 and 3 show significant ($p<0.05$) reduction in tumor diameter as compared to DMBA/Croton oil applied mice (Table 1). In positive control, group 1 animals ,The average number of tumors per mice (Tumor yield)was 3.93 and the average number of tumors per tumor bearing mice (Tumor burden) 4.54 ,Tumor yield and tumor burden in the group 2 and 3 animals were significantly reduced 1.13, 1.18 and 0.87,2.13 respectively comparing with positive group.

None of the mice from the group 1(croton oil), group 2 ,the vehicle-treated group (acetone alone), group 3 (DMBA alone), and group4 (melatonin alone) developed tumors (Table1). Moreover Redness and inflammation were observed in mice skin of treated groups 1 ,2 ,3 and hair loss was observed in skin of all groups except group 2 and group 6.

Table (1): Effect of Melatonin on Morphological appearance of tumor (Means ± se) .

GROUPS	TREATMENTS	Tumor incidence	Cumulative Number of tumors	Diameter of tumors	Tumor Yield	Tumor Burden	TUMOR INCIDENCE %
1	POSITIVE CONTROL DMBA+C.O	0.87 ± 0.091 a	32.53 ± 5.23a	3.02 ±.0.45 a	3.93 ± 0.86 a	4.54 ±0.87 a	87 ± 0.09a
2	(BEFORE) MELATONINE + SINGLE DOSE DMBA + C.O	0.53± 0.133b	1.56 ±.42 b	1.46 ± 0.42 b	1.13 ± 0.39 b	1.18 ±0.41 b	53± 0.13b
3	(AFTER) SINGLE DOSE DMBA + C.O+melatonine	0.73 ±0.118ab	1.49 ± 0.31 b	1.49 ±0.31 b	0.87 ±0.17 b	2.13 ±0.52 b	73 ± 0.12ab
4	CROTON OIL ALONE	0.00 ±.000c	0.00 ±.00 c	0.00 ±.00 c	0.00 ± 0.00 b	0.00 ±.00 c	0.00 ±.000c
5	ACETON ALONE	0.00 ±.000c	0.00 ±.00 c	0.00 ±.00 c	0.00 ±.00 b	0.00 ±.00 c	0.00 ±.000c
6	MELATONIN ALONE	0.00 ±.000c	0.00 ±.00 c	0.00 ±.00 c	0.00 ±.00 b	0.00 ±.00 c	0.00 ±.000c

The data were expressed as mean ± SEM (n=5-15), M: Melatonin; N. Co : Negative control; DMBA: 7,12-Dimethylbenzanthracene; P. Co : positive control; The different letters in the column are significantly different at level ($p < 0.05$).

1. Effect of Melatonin on changes in body weight of mice

A gradual increase in body weight was noted in all animals of the different groups after the beginning of treatment and such weight was found to show significant changes in body weight through the experimental protocol (table 2). No apparent toxicity was observed in terms of change in body weights.

Table (2): Effect of Melatonin on changes in body weight of mice

GROUPS	Weight before treatment SE	Weight after treatment SE
POSITIVE CONTROL	29.13 ± 1.86	36.00 ± 0.38
BEFORE MELATONINE + DMBA + C.O	31.17 ± 0.59	36.80 ± 1.13
AFTER DMBA + C.O + MELATONINE	31.21 ± 0.62	33.15 ± 2.49
CROTON OIL ALONE	31.10 ± 0.54	37.65 ± 0.79
ACETON ALONE	31.17 ± 0.47	37.53 ± 0.50
MELATONIN ALONE	31.82 ± 0.58	37.73 ± 0.51
Sig. level (P)	NS	NS

Effect of melatonin on histopathology of skin

Generally, the dermal layer is composed of loose connective tissue and dense connective tissue known as the papillary and reticular layers, respectively. We observed no changes in the structure of the skin in melatonin treated group which show normal skin layers that is, epidermis, dermis, and basal layer (Figure1, A-a). promoter treatment; Croton oil (alone) group showed excessive thickening of epidermis layer, hyperkeratosis, initial formation of papilloma originated from epidermis, and initial formation of pearls cells. epidermal irregular acanthosis, mild mixed inflammation in dermis and in defined granuloma formation (Figure1, B-b). Acetone (alone) group: showed thin layer of epidermis, dermal edema and thin layer of epiderm, no other changes seen. (Figure1 C-c). Carcinogen and promoter treatment show evidence of papilloma, features of early papilloma focal mixed inflammation with necrosis and dilated vascular channals , abnormal thickening of epidermis, and deposition of keratinocytes pearls, (Figure2, D-d).

Administration of the Melatonin during the pre-initiation stage demonstrated an evidence of papilloma , focal surface ulceration plus mixed inflammation in dermis , a lower degree of epidermal hyperplasia, sever inflammation with hemorrhage in dermal and hypodermal layers (Figure2, E-e). The slides in group 3(post-treat.) animals exhibited initial formation of pearls cells originated from epidermis with slight formation of papilloma, excessive thickening of epidermis layer shows evidence of papilloma , dermal inflammation, focal surface epithelia ulceration (Figure2, F-f).

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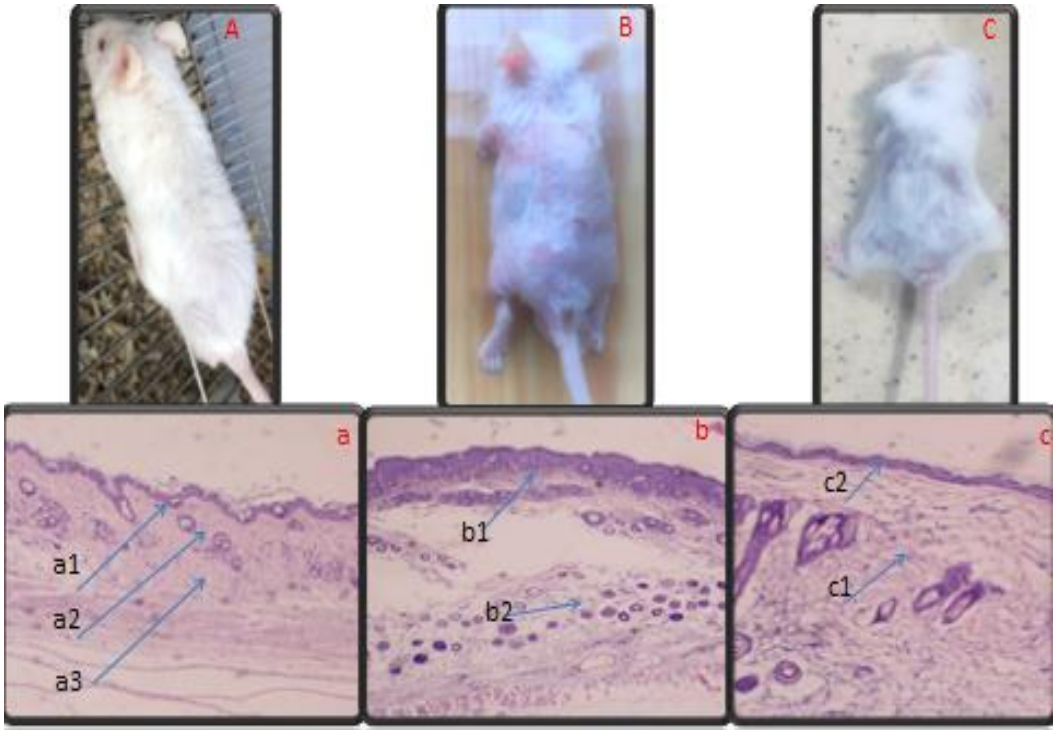

(A). Negative control , shows normal skin layers, (a1) epidermis (a2)dermis (a3) hypodermis ,no changes seen. (B). Croton oil (alone) variable changes , including epidermal irregular acanthosis (b1) , mild mixed inflammation in dermis and in defined granuloma formation(b2). (C). Acetone (alone) ,show only dermal edema (c1) and thin layer of epiderm

Fig. (1): effect of treatments on histopathology of skin

shimal.omar@uod.ac, bushra.mohammed@uod.ac

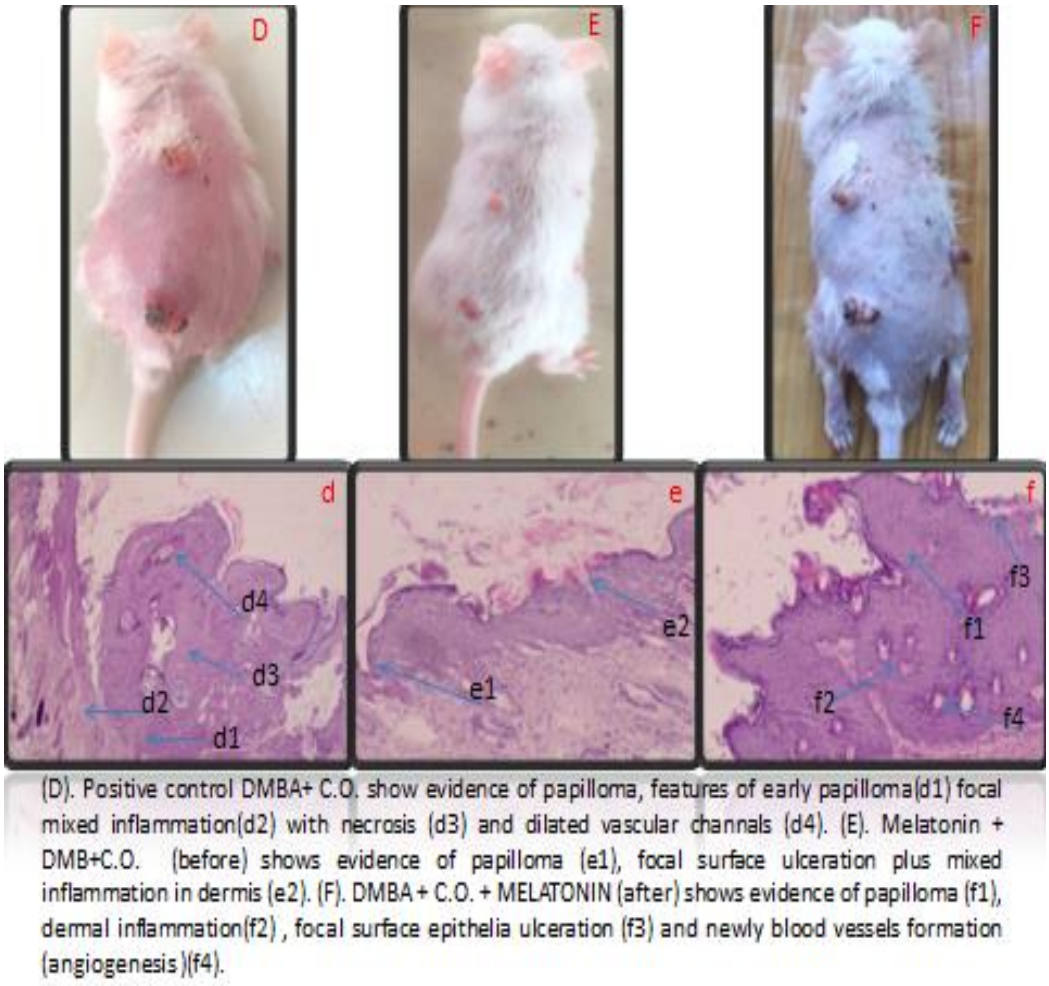

Fig. (2): effect of treatments on histopathology of skin.

DISCUSSION

The findings of present study reveal that Melatonin inhibit DMBA/croton oil induced two stage skin carcinogenesis due to its anticarcinogenic effect. The mouse skin mode is one of the most widely model for chemical carcinogenesis studies, Epidermal neoplasia can be induced in this model by two different protocols, i.e. complete carcinogenesis and two stage carcinogenesis (Wu and Pandolfi, 2001).

and such research results is likely to be directly relevant to understanding the development of many human epithelial cancer (Coghlan et al., 2000).

Application of 7,12 dimethylbenz(a) anthracene (DMBA) / croton oil induced two stage skin carcinogenesis through three sequential steps of tumor initiation, promotion, and progression, also helpful for study of genetic and biochemical alterations caused by them (Ward et al., 1986).

In the present investigation DMBA /croton oil (Group 1)used as topical application because it was reported that fastest way of absorption of DMBA carcinogen was found in skin tissue and the Repeated topical application of the tumor promoter Croton oil on mouse skin involves both oxidative burst as well as inflammation , DMBA is metabolized to DMBA-trans-3,4-diol, an intermediate inductive of DNA mutations through alteration of DNA base pairs, initiation of chronic inflammation and excessive generation of Reactive Oxygen Species ROS plays an important role in the process of mutagenesis and carcinogenesis particularly in tumor promotion. They can induce oxidation of almost all biomolecules such as lipids, DNA, RNA and proteins, leading to DNA strand break by modulating different biochemical pathways

and gene expression ,thus playing a key role in cancer development by transforming the normal cells to malignant pathways (Walentinsson and Levan, 2001),(Russo et al., 1982) and (Daniel and Joyce, 1983).

In addition, 7,12-Dimethylbenz(a)anthracene (DMBA), a genotoxic agent, may reacts with DNA directly, inducing p53-dependent cytotoxicity resulting in cell death by apoptosis or giving rise to cancer (Katz et al., 2016).

The toxic metabolites of DMBA are capable to cause mutations at codon (61) of the Ha-ras 1 gene appears to be a critical step in formation of mouse skin tumor in both complete and the two-stage tumorigenesis model with DMBA (Bizub et al., 1986).

In present experiment carcinogen treated animals exhibited 87% tumor incidence and highest cumulative number of tumors. However, the pre treatment with Melatonin Group 2 (Table1) show that Skin papilloma in pre initiation treatment was more efficient in decreasing; tumor incidence; suggesting that the inhibition in the metabolism of DMBA to its active form within the promotion phase of carcinogenesis. 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.

The result of this study agree with an animal study conducted on 200 mice divided into 4 groups, benzo(a)pyrene solution was applied onto a skin site for 26 weeks. Melatonin, Metformin, or both were used in the animals in a parallel way. This promoted a significant reduction in the number and size of skin tumors (Man'cheva et al., 2011).

In another study done on the related mechanisms of cancer, melatonin inhibited the proliferation of malignant cells in breast cancer and hepatoma. Also, melatonin was reported to be an oncostatic agent via its augmentation on natural killer (NK) cells (Srinivasan et al., 2008).

Melatonin has a lipophilic structure and penetrates cell membranes easily, interacting with both intra and extracellular structures(Venegas et al., 2012).

It diffuses and concentrates in the mitochondria and cell nucleus MLT induces apoptosis of cancer cell and intrinsic pathway of apoptosis begins with cytochrome c release from mitochondria due to oxidative stress. This release stimulates intrinsic mitochondrial apoptotic pathway via activating caspases, increasing pro-apoptotic Bax levels and decreasing anti-apoptotic Bcl-2 levels in various cancer types **(**Hanikoglu et al., 2015**)**.

However, Melatonin plays a major role in restraining oxidative stress-induced apoptosis by inhibiting caspases and mitomycin C (MMC), a cancer drug that triggers caspase 8 and 9 in both intrinsic and extrinsic apoptotic pathways (Gatti et al., 2017).

Histological examinations:

The skin is the heaviest single organ of the body, accounting for about 16% of the total body weight. It is composed of the epidermis, an epithelial layer of ectodermal origin, the dermis, a layer of connective tissue of mesodermal origin. The junction of dermis and epidermis is irregular, and projections of the dermis called papillae inter digitate with invagination of the epidermis known as epidermal ridges (Junqueirza and Carneiro, 2005).

The epidermis is a stratified squamous epithelium composed mainly of keratocytes, and basal keratocytes, which are attached to the basement membrane, are undifferentiated and have proliferation potential before entering the differentiation program, they withdraw from the cell cycle and migrate toward the surface of the epidermis, leading to the formation of the outer most layer of the epidermis composed of an nucleated dead squamous (Cazanova et al., 2003).

Histological evaluation of skin sections of the animals treated with DMBA/croton oil revealed that DMBA induced epithelial thickness of epidermis that forming papillomas, the areas of epithelium layer shows in finger like projection in some sections. Congestion of blood vessels, hyperkeratosis with elongation of the rete ridges, papillomatosis and foci of vacuolated cells in acanthotic stratum malphigia (acanthosis) infiltration of mononucleated inflammatory cells also observed.

This results are in agreement with (Majed et al., 2015) and (Gopalakrishnan et al., 2019). that DMBA induced epidermal hyperplasia, dermal inflammation and keratinocyte apoptosis.

The histopathology study showed that melatonin repaired the degenerated epidermal and dermal layers of skin in the group 2 animals. However, significant histological changes were

observed in the hypodermis, where there was degeneration of adipose tissue. Thus, the free hydroxyl group on the aromatic ring is responsible for the antioxidant properties.

Skin sections of animals treated with the acetone solvent of DMBA group 5 figure1 (Cc) was showed slight hyperplasia on the epidermis surface and sever changes in the fatty bodies and hyperkeratosis, it is clear the acetone is an organic solvent most lipid are dissolves in there. For this, most fatty body changes in this group and other groups that used acetone as a vehicle of DMBA may due to acetone effects.

The general expected increase in body weight for all the experimental groups (table 2), the total body weight showed similar weight gain in experimental and control mice, total daily caloric intake, growing in age might be the cause of weight gain. DMBA topically dose administration are thought to be at lower doses that cannot affect body weight, The protection from body weight loss in treated groups, may be due melatonin immunomodulatory Melatonin that has been shown to be involved in the regulation of both cellular and humoral immunity (Srinivasan et al., 2008). That would makes healthy all the body systems and in turn increased the body weight of mice.

Our study results conclude that melatonin effectively protects against DMBA induced skin carcinogenesis ,Therefore, melatonin could be a promising candidate to prevent skin carcinogenesis. However, further studies are needed to understand the role of melatonin in the various molecular mechanisms, pertaining to antitumor activity.

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