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Anticancer Activity of Flavonoids Rich Extract of Melissa Officinalis Flower in DMBA-induced Dermal Carcinoma in Albino Rat Model

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ABSTRACT:

Plant and herbs used in the folk and traditional medicine have been accepted currently as one of the main source of chemoprevention drug discovery and development. Around 60% of currently used anticancer agents are derived in one- way or another from natural sources, including plants, marine source and micro-organisms. The main aim of research work to provide Anticancer activity of *M. officinalis* flower in animal model DMBA-induced skin carcinoma. The present research work is to find out the potential effect of flower extract containing rich amount of flavonoids. To find an alternative for present allopathic medication to minimize side effects and adverse drug reaction with cheaper cost compared to synthetic medication. Fresh 1% DMBA solution was prepared by dissolving 2.5mg of DMBA in 0.25 ml of acetone to each rat and applied topically on shaved skin of hind back region of rats at weekly intervals. A total 5 doses of DMBA solutions were used in this experiment. In two-step carcinogenesis, DMBA solution is administered to the skin once as an initiator, and croton oil is applied three times per week after two weeks as a promoter. The experiments were performed in accordance to the experimental protocol approved by the Institutional Animal Ethical Committee that confirms to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). In this study selected 5 groups of rats and each group consist of 6 animals. In present research work studied the Morphological parameters of animals body such as body weight, tumor burden,tumor weight, tumor yield, etc. To that end, the *Melissa Officinalis* for skin integrity maintenance that also exhibit potential antitumor activity might be considered promising alternatives for the therapy of skin pathologies.

Keywords: DMBA, Skin Cancer, *Melissa Officinalis*, Morphological Parameters.

1. Introduction

Cancer is a complex disease that is normally associated with a wide range of escalating effects both at the molecular and cellular levels. It therefore seems unlikely that chemoprevention follows simplistic rules and formulations. The old saying "Prevention is always better than cure" is particularly true in the case of cancer where a cure, if at all possible, is associated with high cytotoxic loads and/or invasive procedures.[1] Skin cancer is the out-of-control growth of abnormal cells in the epidermis, the outermost skin layer, caused by unrepaired DNA damage that triggers mutations. These mutations lead the skin cells to multiply rapidly and form malignant tumors. The human body is made of living cells which grow, divide into new cells, and die. Cell division is a continuous process in the human body and is a replacement of dying cells.[2]

1.1. DMBA:

7,12-dimethylbenz[a]anthracene appears as yellow to greenish-yellow crystals or a yellow solid. Odorless. Maximum fluorescence at 440 nm. Bluish-violet fluorescence in UV light.

1.1.1. Mechanism of DMBA in skin carcinogenesis:

Initiation is generally elicited by the application of a low dose of carcinogen such as 7,12 - dimethylbenz[α]anthracene (DMBA). This treatment causes an activating, oncogenic mutation of the H - ras gene in skin epithelial cells. Those cells are promoted by repeated application of a non - carcinogenic promoter such as croton oil. Tumor promoters do not directly change the DNA sequence, however, they elicit a wide range of cellular and biochemical changes related to cell growth and differentiation. Initiated cells are expanded by promoter treatment and develop into premalignant papillomas. The progression step in DMBA induced skin cancer is a spontaneous process facilitated by genetic instability; loss of p53 gene is frequent.

Some papillomas acquire the ability to invade as a result of the progression step and become squamous cell carcinomas (SCCs).[3]

1.2. Cyclophosphamide:

The majority of the antineoplastic effects of cyclophosphamide are due to the phosphoramide mustard formed from the metabolism of the drug by liver enzymes like cytochrome P-450. Hepatic enzymes first convert cyclophosphamide to hydroxycyclophosphamide and then subsequently metabolized to aldophosphamide. Aldophosphamide is cleaved to the active alkylating agent phosphoramide mustard and acrolein.

The phosphoramide metabolite forms cross-linkages within and between adjacent DNA strands at the guanine N-7 position. These modifications are permanent and eventually lead to programmed cell death.

1.3. Plant Profile: [5]

1.3.1.Common Name

Billilotan, Balm, lemon Balm.

1.3.2. Botanical Description

Kingdom: Plantae,

Division: Magnoliophyta,

Class: Magnoliopsida,

Order: Lamiales,

Famiy: Lamiaceae,

Genus: Melissa,

Species: M. officinalis

Binominal name: Melissa officinalis

Fig.1. *Melissa Officinalis* flower

1.3.3. Chemical constituents: [6]

The main components of the essential oil are 39% citronellal, 33% citral (citronellol, linalool) and 2% geranial. In oil contains three terpinene, phenol carbon-acid (rosmarinic acid), and flavonglychoside acids in low ratio. Also found caffeic acid, several flavonoids (luteolin-7- Oglucoside, isoquercitrin, apigenin-7-Oglucoside and rhamnocitrin), ferulic acid, methyl carnosoate, hydroxycinnamic acid, and 2- (3',4'-dihydroxyphenyl)-1,3-benzodioxole-5-aldehyde and some other aldehydes: betacaryophyllene, neral, and geranyl acetate.

1.3.4. Traditional uses: [7]

Historically lemon balm has been said to possess sedative/tranquilizing, anti-gas, fever- reducing, antibacterial, spasmolytic, hypotensive, memory-enhancing, menstrual-inducing, and thyroid-related effects; antiviral and antioxidant activities; antifungal, antiparasitic, and antispasmolytic activities; flatulence; asthma; bronchitis; amenorrhea; cardiac failure; arrhythmias; ulcers; and wounds. Besides, it has been said that it is effective in treatment of headaches, indigestion, colic, nausea, nervousness, anemia, vertigo, syncope, malaise, insomnia, epilepsy, depression, psychosis, and hysteria. Traditionally the aerial part has been used for the treatment of cancer but there is no scientific proof. So, the present study was undertaken to investigate the effects of *Melissa Officinalis* on anticancer activity.

2. Experimental work:

2.1. Preparation of animal model:

Thirty male wistar rats of 6 to 7 weeks of age and weight about 150- 200 gm were used in the present experimental study. All the animal experiments were performed following the guidelines and prior approval of Institute Animal Ethic Committee and Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). Two rats were housed in each polypropylene cages under standard housing condition with food and water.[8]

2.2. Preparation of DMBA solution:

Fresh 1% DMBA solution was prepared by dissolving 2.5mg of DMBA in 0.25 ml of acetone to each rat.[9]

2.3. Preparation of Herbal drug doses:

200 mg/kg, 400 mg/kg dissolved in water, respectively; doses decided as per previously studied oral acute toxicity study according to OECD guidelines 423.[10]

2.4. Acute oral toxicity study:

Rat treated with 300mg/kg and 2000mg/kg doses of EOMO presented a slight reduction in the locomotors activity and not present signs of toxicants effects which could be detected by an alteration in body weight measured for 14 days. One death was registered in the group treated with 2g/kg, and zero death at the second step (OECD 423). The animals quickly recorded their normal activity and growth after a period of 24hours. All animals did not show any changes in the general appearance during the observation period. This result indicates that the LD50 was higher than 2000mg/kg.[11]

2.5. Evaluation of anticancer activity:

Fresh 1% DMBA solution was applied topically on shaved skin of hind back region of rats at weekly intervals. A total 5 doses of DMBA solutions were used in this experiment. In two-step carcinogenesis, DMBA solution is administered to the skin once as an initiator, and croton oil is applied three times per week after two weeks as a promoter. These protocols were followed for 16 weeks. [12]

2.6. Treatment Protocol:

Group I- (Negative control): Normal rats (6) belonging to this group were treated with water for 16 weeks.

Group II- (Positive control): Rats (6) belonging to this group were treated with a single topical application of 1% DMBA solution to a shaved area of skin. After two weeks, croton oil (1% w/v in acetone) was applied topically three times per week.

Group III- (Standard): Rats (6) belonging to this group were treated with oral administration of cyclophosphamide (50 mg/kg body weight) starting from the time of croton oil application till the end of experimentation. DMBA was given as the same in the group II.

Group IV- (Treatment-1) (Moderate dose): Rats (6) belonging to this group were treated with oral administration of Melissa Officinalis flower extract (200 mg/kg body weight) starting from the time of croton oil application till the end of experimentation. DMBA was given as the same in the group II.

Group V- (Treatment-2) (High dose): Rats (6) belonging to this group were treated with oral administration of Melissa Officinalis flower extract (400 mg/kg body weight) starting from the time of croton oil application till the end of experimentation. DMBA was given as the same in the group II.

2.7. Morphological Parameters: [13]

Body weight: The weight of each rat was measured weekly.

Cumulative number of tumors: The total number of tumors appeared till the termination of the experiment were estimated.

Weight: The weight of each tumor was measured at the termination of experiment.

Tumor incidence: The number of rat carrying at least one tumor was expressed as a percentage incidence

Tumor burden: The average number of tumors per tumor-bearing rat was estimated.

Tumor yield: The average number of tumors per rat was calculated.

2.9. Euthanasia:

Cervical dislocation: Firm pressure is applied at the base of the skull, along with a sharp pinching and twisting of the thumb and forefinger. At the same time, the tail is pulled backward. This severs the spinal cord at the base of the brain or within the cervical spine area (the upper third of the neck).[14]

2.9.1. Methods of carcass disposal after euthanasia:

Lime inhibits pathogens by controlling the environment required for bacterial growth. Calcium hydroxide (hydrated lime) is an alkaline compound that can create pH levels as high as 12.4. At pH levels greater than 12, cell membranes of pathogens are destroyed. The high pH also provides a vector attraction barrier (i.e., prevents flies and other insects from infecting treated biological waste).[15]

3. Result and Discussion:

3.1. Physical parameters of extracted powder:

Sr. no.	Parameters	*Melissa Officinalis* flower extract
1.	Appearance	Brown fine powder
2.	Odour	Characteristic
3.	Taste	Characteristic
4.	Solubility	Soluble in water and alcohol

Table 1. Evaluation of physical parameters of Melissa Officinalis flower extract

3.2. Preliminary phytochemical screening:

Sr. no.	Chemical tests	Results
1.	Flavonoids	+ve
2.	Alkaloids	+ve
3.	Essential oil	+ve
4.	Carbohydrate	+ve
5.	Protein and amino acid	-ve
6.	Total Phenolic compounds	+ve
7.	Nitrogen Compounds	+ve
8.	Saponin	-ve
9.	Glycosides	+ve
10.	Triterpenes	+ve

Table: 2. Evaluation of phytochemical present in Melissa Officinalis flower extract

Qualitative phytochemical analysis revealed the presence of secondary metabolites like alkaloids, flavonoids, cardiac glycosides and triterpenes, etc. However, the MO extract was rich in flavonoids.

3.3. Progression and development of cancer:

After being exposed to 1% DMBA carcinogen for 10 weeks, the rats developed skin tumours. The first signs of DMBA administration were erythematous response, necrosis, and ulceration, which were followed by hyperplasia and tumour growth (2 weeks). The gross features of the tumours included multicentric soft sessile or pedunculated growth, which in later stages grew into large cauliflower growth in rats which continued to grow and showed necrosis and ulceration. In the present study, tumour development was observed in the rats which might be due to the optimum number of doses with higher concentration and weekly application. The large tumour formation was observed by 10th weeks after the 1st application of DMBA with application of croton oil.

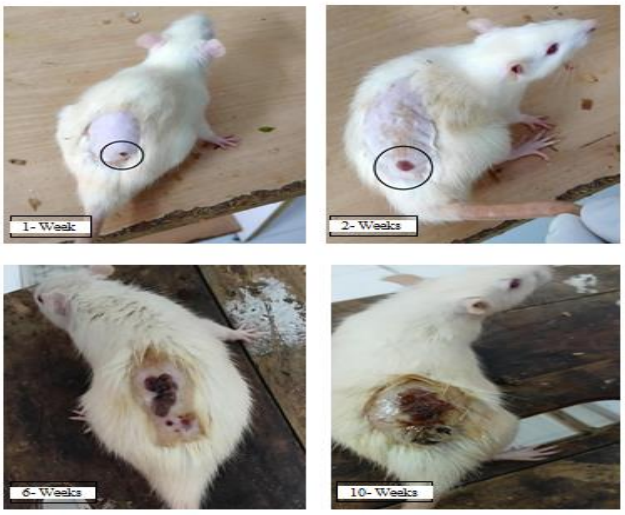

Fig.2. Development stages of skin tumor following application of DMBA Carcinogen

3.4. Evaluation of morphological parameters:

3.4.1. Antitumorigenic potential of MOE causes change in Body weight:

Negative Control	Positive Control	Standard	Treatment-1	Treatment-2
168	201	170	173	173
172	198	176	177	170
176	199	180	183	171
167	210	177	180	172
173	199	183	190	175
170	180	172	170	171
Average				
171	197.8333	176.3333	178.8333	172
1.36626	3.995136	1.977653	2.937308	0.730297

Table: 3. Final body weight of the rat

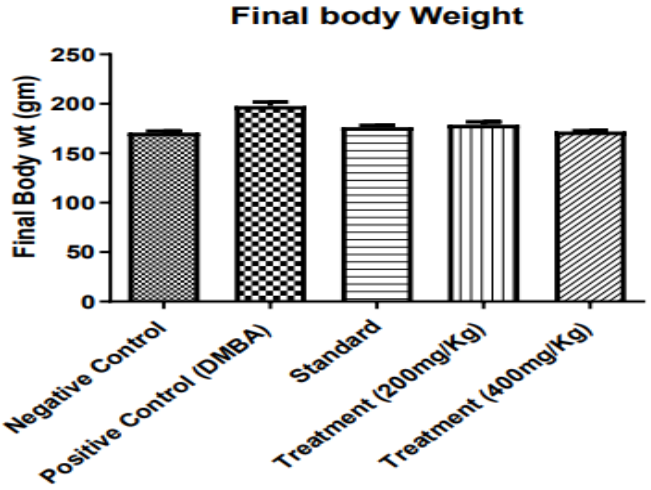

Fig.3. Rat body weight variations among each group

The body weight was found to be gradually increased during experimentation in Group II as compared to the group I, but it was found to decrease in the carcinogen-treated Group III and both Groups IV and V and following SDs significantly different * P< 0.05, ** P< 0.01, *** P< 0.001.

Sr. no.	Groups	Cumulative number of tumors	Weight of tumors	Tumor incidence	Tumor burden	Tumor yield
1.	Group I (Negative control)	-	-	-	-	-
2.	Group II (Positive control)	4.0 ±0.89	5.71 ±2.39	100 %	5.7 ± 0.46	5.7 ± 0.46
3.	Group III (Standard)	1.4 ±0.81	1.71 ±1.34	20.66 %	3.67 ± 1.15*	1.77 ± 0.25***
4.	Group IV (Treatment -1)	2.0 ± 1.05	2.10 ±1.11	31.02 %	4.99 ± 0.71*	3.97 ± 0.47***
5.	Group V (Treatment -2)	1.5 ± 0.95	1.72 ±1.25	21.89 %	4.60 ± 0.99*	3.03 ± 0.40***

Table.4. Chemopreventive potential of MOE on chemical-induced skin carcinogenesis in rat

The cumulative number of tumors in the carcinogen-treated control group was noted as 4.0 ± 0.89, which were significantly reduced to 1.4 ±0.81 in the Standard group, 2.0 ± 1.05 in the Treatment-1 group, and 1.5 ± 0.95 in the Treatment-2 group after MOE administration. The tumor yield exhibited a significant decline, i.e., 5.7 ± 0.46 in carcinogen treated, 1.77 ± 0.25, 3.97 ± 0.47*** and 3.03 ± 0.40, in the MOE-treated Groups IV–V, respectively, when compared with Group II. The tumor burden was noted as 5.7 ± 0.46 in the carcinogen-treated control group, and it was also significantly decreased to, 3.67 ± 1.15 in standard and 4.99 ± 0.71, 4.60 ± 0.99 after the administration of MOE in the experimental groups. Animals of group II exhibited 100% tumor incidence after the treatment with DMBA/croton oil alone, while the animals of Groups I did not show any tumor appearance. After treatment of MOE, tumor incidence decreases to 20.66% in group II and 31.02%, 21.89% in group IV-V as compared with group II. Data are presented as mean ± SD, Statistical comparison: control versus experimental *p < 0.05, **p < 0.01, ***p < 0.001 MOE; SD.

4. Discussion:

Currently utilised methods for treating cancer include chemotherapy and radiation, but they also have a number of drawbacks and side effects. In order to lessen the burden of cancer worldwide, the quest for innovative preventative medicines is gathering speed. Due to the inclusion of antioxidative and anti-inflammatory compounds, natural dietary supplements have been shown to have several chemopreventive characteristics.

Bioactive substances found in *Melissa Officinalis* flower include a number of flavonoids such quercitin, apigenin and other flavonglychoside, alkaloids, etc. Scientific evidence suggests that the presence of flavonoids and antioxidant properties is associated with the anticarcinogenic potential of MOE. The rat chemical-induced multistage skin carcinogenesis model is a particularly useful model to examine the genetic and biochemical changes. In the present experiment, the topical application of DMBA was used to initiate carcinogenesis because skin absorption was reported to be the fastest route of entry for these polycyclic aminohydrocarbons. Croton oil contains 12-O-tetradecanoylphorbol-13-acetate, which is used for skin tumor promotion by the production of reactive oxygen species and hydroperoxides in keratinocytes.

In the current investigation, rats given DMBA/croton oil alone had 100% tumour incidence, high tumour yield, tumour burden, and comulative number of tumor due to their carcinogenic propensity in the absence of therapy. The cumulative number of tumours was significantly reduced after MOE administration, and the morphological parameter was similarly reduced. Severe destruction of the skin histology was observed in Group II after the carcinogen treatment alone with croton oil. Squamous Cell Carcinoma (SCC) growth was characterised by epidermal hyperplasia and dermal invasion due to hyperproliferation. The carcinogenic impact of DMBA/croton oil was reflected by the production of sticky pearls, tumour nests, and acanthosis, which was observed to be reduced following administration of MOE at various group of treatment.

5. Conclusion and Future scope:

From a future perspective, the phytochemical composition and pharmacological effects attributed to Melissa officinalis represent an opportunity to create new controlled release systems with the potential for targeted delivery. The controlled release systems developed so far represent a future perspective for the development of new systems. These systems may contain other materials as a delivery system, or those made so far may be improved. With many substances, essential oils, and natural plant extracts available, many materials can be functionalized in order to develop controlled release systems. Materials such as silica, polysaccharides, polymers, and lipids can be used as encapsulation carriers. Functionalized controlled release systems can be enhanced with various substances so that they can be used, depending on the desired field.

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