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SUPPRESSION OF DMBA/CROTON OIL-INDUCED MOUSE SKIN CANCER PROMOTION BY FORMULATED HERBAL GEL CONSISTING OF QUERCETIN AND KAEMPFEROL

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ABSTRACT

A herbal gel formulation was made by using Quercetin and Kaempferol. Formulation efficacy study was carried out using the initiator 7, 12 Dimethylbenz (a) anthracene (DMBA) and with promoter croton oil induced skin carcinoma on albino mice. The parameters of tumour incidence, tumour burden, tumour volume, tumour weight and the morphological examinations were studied. Results revealed that the formulated herbal gel have a fine effect to suppress the tumour in albino mice with the concentration of 20 mg/kg as compared to DMBA treated and standard group.

Key words: Herbal gel, (Quercetin and Kaempferol), 7, 12 Dimethylbenz (a) anthracene (DMBA), croton oil induced skin carcinoma

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INTRODUCTION

The development of skin cancer is a major global public health threat. Ultraviolet (UV), e.g., solar ultraviolet B (UVB) and solar ultraviolet (UVA), radiation are the main causes of skin cancer. The incidences of basal cell carcinoma, squamous cell carcinoma, and melanoma continue to rise despite the advent and use of sunscreen agents with high SPF constituents. Early detection and treatment are essential in improving survival rates, yet skin cancer is a cancer that is largely preventable altogether. Current sunscreen formulations have proven inadequate for fully protecting persons from the DNA- damaging effects of UV radiation. Sunscreen usage may sometimes create a false sense of safety as individuals may over expose themselves to sunlight. Studies have demonstrated that flavones possess anti-oxidant, anti-mutagenic, anti-carcinogenic, anti-inflammatory, anti-proliferative, and anti-progression properties (1). In addition, Birt et al.,(2) used an *in vivo* mouse model to demonstrate that topical application of apigenin prior to UVB-irradiation significantly reduced, by up to 90%, the incidence of skin cancer. Other groups have demonstrated apigenin's ability to protect mice against colon cancer. Researchers have found that apigenin

induces reversible, cell-cycle arrests at G l and G2/M phase of the cell cycle. It was further discovered that apigenin mediates an inhibition on the cell cycle through multiple mechanisms including direct and indirect inhibition of the mitotic kinase p34cdc2, as well as the induction of the cell cycle inhibitor p21 WAFl in a p53-dependent manner. Loss of Gl/S and/or G2/M cell cycle checkpoint controls leads to transformation and cancer progression. Initiation and progression through the cell cycle is largely controlled by proto-oncogenes that promote cell proliferation and tumor suppressor genes that function to slow or halt cell growth. Mutations in either proto- oncogenes and/or tumor suppressor genes predispose cells to a compromised Gl/S checkpoint by shortening the length of time spent in Gl or G2/M.

Globally, the incidences of melanoma and non-melanoma skin cancer are found to be increasing than other forms of the cancers due to various risk factors like excess exposure to ultraviolet B radiation, latitude, climatic conditions, environmental pollutants, occupational carcinogens, active / passive smoking, ageing, family history etc. On the other hand, the depletion of stratospheric ozone is implicated as one of the major risk factor for skin carcinoma. In India, though no clear data is available on the prevalence/incidence of skin cancer, indirect surveys indicate that non-melanoma skin cancers (NMSCs) may be on the rise in India. Melanoma cells develop resistance to chemotherapeutics very rapidly and thus complicate the treatment. Thus the search for newer therapeutics is more important for effective treatment/management of skin cancer. Plant based drugs are being used for chemoprevention and also to suppress the malignancy of cancer. Flavonoids are one of the major secondary metabolites obtained from plant sources which exhibit broad beneficial effects in human health. For example, Quercetin is found in abundance in vegetables and fruits. Quercetin is reported to prevent the oxidation of low-density lipoproteins (LDL) by scavenging the free radicals. It also reported to impart beneficial effects in the treatment of cancer, chronic inflammation and atherosclerosis.

Kaempferol, on the other hand, is one of the commonly flavonoids seen in plant based foods and herbs used in traditional medicines. Several reports demonstrate the possible association and consumption of diet rich in kaempferol and decreased risks of several diseases like cancer, cardiovascular, neurological and ageing (3-10).

MATERIALS AND METHOD

Experimental Animal

Male Albino mice Having age of 21 days and 30 gm body weight were used throughout the study. All mice were kept at room temperature of 22◦C under 12 hr light/12 hr dark cycle in the animal house. Mice were fed with standard food. Animals were fed with commercial pellet diet and water ad libitum freely throughout the study. All animal procedures were performed after approval from the IAEC (institution of animal ethical committee) and in accordance with the recommendations for the proper care and use of laboratory animals.

Experimental Design

Table-1 Experimental mice were divided into 6 groups of eight animals

Group	Treatment
Group I	Mice (N=8) of this group were given topical application of acetone (100 µl/mouse) for 12 weeks
Group II	Mice (N=8) of this group were topically applied a single dose of DMBA (100g / 100µl of acetone) over 3x3 cm² shaven area of the mice skin. Two weeks later, croton oil (1% in 100µl of acetone) was applied, as a promoter three times in a week animals of this group for 12 weeks, which served as carcinogen control group

Group III	Mice (N=8) of this group were topically applied a single dose of DMBA (100g / 100µl of acetone) over 3x3 cm² shaven area of the mice skin. Two weeks later, 5FU gel (20mg/kg) were topically applied 30 minutes before croton oil (1% in 100µl of acetone) was applied, as a promoter three times in a week animals of this group for 12 weeks
Group IV	Mice (N=8) of this group were topically applied a single dose of DMBA (100g / 100µl of acetone) over 3x3 cm² shaven area of the mice skin. Two weeks later, formulated herbal gel (20mg/kg,) were topically applied 30 minutes before croton oil (1% in 100µl of acetone) was applied, as a promoter three times in a week for 12 weeks
Group V	Mice (N=8) of this group were topically applied a single dose of DMBA (100g / 100µl of acetone) over 3x3 cm² shaven area of the mice skin. Two weeks later, formulated herbal gel (10mg/kg,) were topically applied 30 minutes before croton oil (1% in 100µl of acetone) was applied, as a promoter three times in a week for 12 weeks
Group VI	Mice (N=8) of this group were topically applied a single dose of DMBA (100g / 100µl of acetone) over 3x3 cm² shaven area of the mice skin. Two weeks later, formulated herbal gel (20mg/kg) were topically applied 30 minutes before croton oil (1% in 100µl of acetone) was applied, as a promoter three times in a week for 12 weeks

Procedure

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. A total of 40 selected animals were randomly divided into 5 groups (I, II, III, IV, V & VI) to evaluate chemo preventive role of formulated herbal gel against DMBA/ croton oil induced skin papillomagenesis. At the end of 12th week, the animals were sacrificed through cervical decapitation and the entire liver and skin was removed from the selected dorsal area for biochemical study soon after the sacrifice of animals. Small pieces of skin were taken and crushed in masticator. Homogenates were prepared in Tris KCl, 10 per cent TCA, phosphate buffer and 30 percent KOH for estimation of LPO, GSH, SOD CATALASE activity and TOTAL PROTEINS contents respectively. Liver was perfused immediately with ice cold 0.9% sodium chloride, thereafter carefully removed, trimmed free of extraneous tissue. The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris-HCl buffer, pH 7.5 for estimation of LPO, GSH, SOD CATALASE activity and TOTAL PROTEINS contents respectively. Finally, the skin tumors were excised out and the following parameters were studied (a) Tumor incidence (% of animals that develop at least one tumor)(b) Tumor burden (average number of tumor per animal) (c) Tumor volume (d) Tumor weight (e) Histopathological examination (11).

Morphological Assessment of Formulated Herbal Gel on DMBA and Croton Oil induced Skin Carcinoma on Albino Mice

During the period of 16 weeks of experimentation, mice of all groups were weighed carefully, examined once a week, skin papillomas and these were recorded. The following parameters were taken into consideration: Body weight, latency period of tumor formation, percentage of tumor incidence, tumor burden and tumor volume was observed and measured at weekly interval. Only tumors that persisted more than one week with diameter greater than 1 mm were taken into consideration for data analysis. Latency period of tumor formation was determined when the first tumor appeared. Percentage of tumor incidence was calculated by dividing the number of tumor-bearing mice with the total number of mice in a particular group and multiplied with 100%. Tumor burden was obtained by dividing the total number of tumors with the number of tumor-bearing mice in a

group. Tumor volume was measured by multiplying Π/6 to the length, width and height of tumor (11).

RESULTS AND DISCUSSION

In vivo evaluation of Quercetin and Kaempferol in formulated gel on the initiator 7, 12 Dimethylbenz (a) anthracene (DMBA) and promoter croton oil induced skin carcinoma on albino mice.

During the period of 16 weeks of experimentation, mice of all groups were weighed carefully, examined once a week and skin papillomas were recorded.

There is a significant increase in the tumour incidence in the DMBA induced group as compared to that of the normal control groups. There is a significant decrease in the tumour incidence in the Test 1(Quercetin + Kaempferol gel) and Test 2 (Quercetin + Kaempferol gel) group as compared to that of the standard 5FU gel (Fig-1).

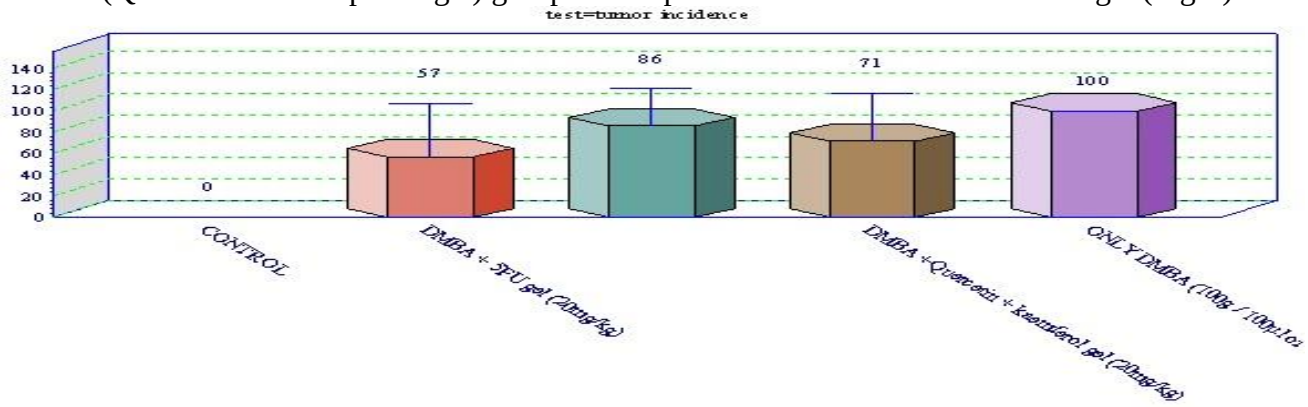

Figure-1 Tumor incidence (a – Comparisons are made between the Positive control group Vs Normal control group, b – Comparisons are made between Methothrexate (0.1mg/kg) i.p, Low Dose (5mg/kg) p.o & High Dose (20mg/kg) p.o with positive control group, p value * represents p<0.05, ** represents p<0.01, ***represents p<0.0001)

There is a significant increase in the tumour burden in the DMBA induced group as compared to that of the normal control groups. There is a significant decrease in the tumour burden in the Test 1(Quercetin + Kaempferol gel) and Test 2 (Quercetin + Kaempferol gel) group as compared to that of the standard 5FU gel (Fig-2).

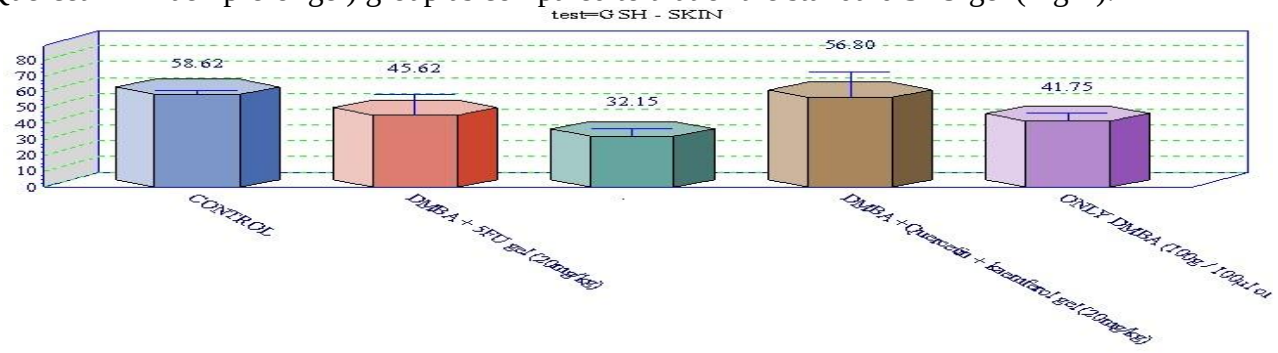

Fig-2 tumor burden (a – Comparisons are made between the Positive control group Vs Normal control group, b – Comparisons are made between Methothrexate (0.1mg/kg) i.p, Low Dose (5mg/kg) p.o & High Dose (10mg/kg) p.o with positive control group, p value * represents p<0.05, ** represents p<0.01, ***represents p<0.0001)

There is a significant increase in the tumour volume in the DMBA induced group as compared to that of the treated groups. There is a significant decrease in the tumour volume in the Test 1(Quercetin + Kaempferol gel) and Test 2 (Quercetin + Kaempferol gel) group as compared to that of the standard 5FU gel (Fig-3).

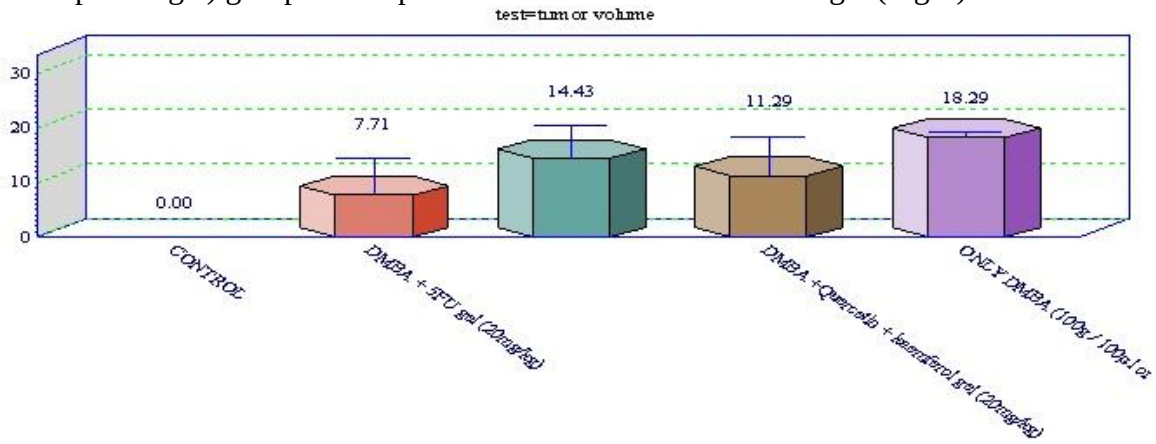

Figure-3 Tumor volume (a – Comparisons are made between the Positive control group Vs Normal control group, b – Comparisons are made between Methothrexate (0.1mg/kg) i.p, Low Dose (5mg/kg) p.o & High Dose (10mg/kg) p.o with positive control group, p value * represents p<0.05, ** represents p<0.01, ***represents p<0.0001)

Morphological Assessment of Formulated Herbal Gel on DMBA and Croton Oil induced Skin Carcinoma on Albino Mice.

Morphological Assessment of Formulated Herbal Gel on DMBA and Croton Oil induced Skin Carcinoma on Albino Mice are shown in the figure 4-12. Treated groups shows good morphological changes when compared to untreated groups.

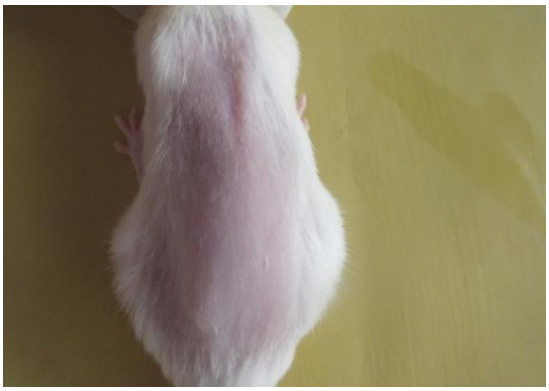

Figure- 4 Normal Skin DMBA and (Before Topical Application Of DMBA and Croton Oil)

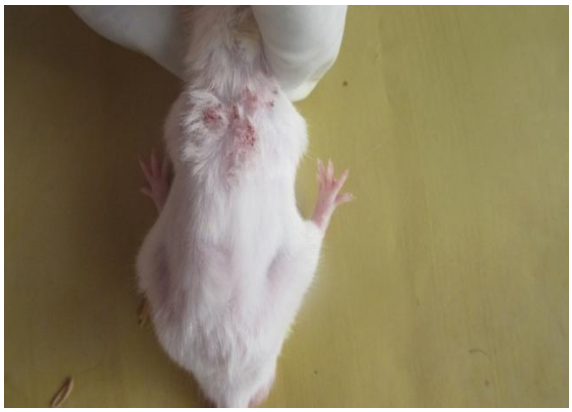

Figure-5 Tumor latency of papillomas on only croton oil treated animal

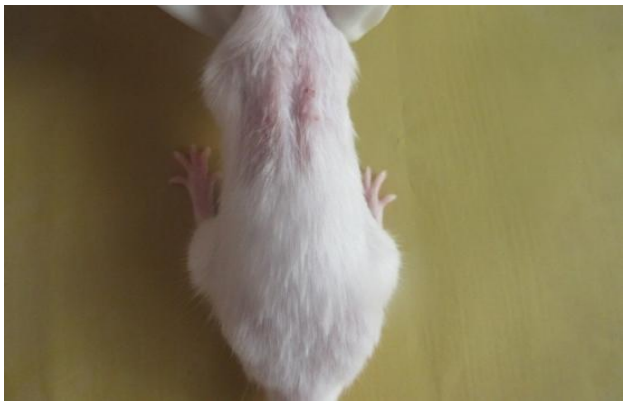

Figure-6 Tumor Latency of Papillomas on DMBA + Croton Oil and 5FU Treated Animal

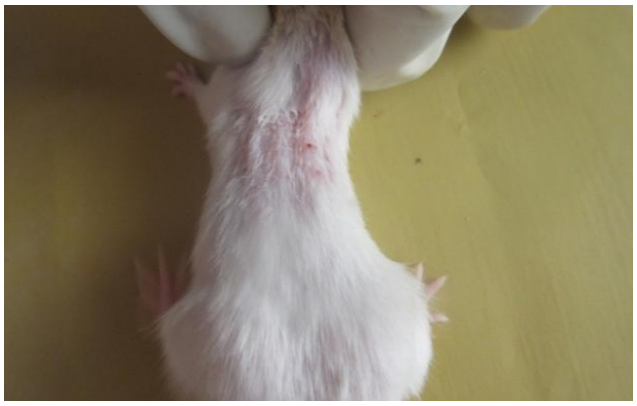

Figure-7 Tumor Latency of Papillomas on DMBA + Croton Oil and Quercetin + Kaempferol Treated animal

Figure-8 Tumor Latency of Papillomas On DMBA + Croton Oil Treated Animal

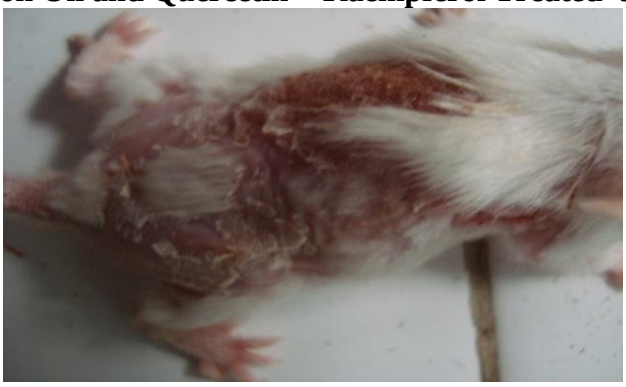

Figure-9 Only DMBA and Croton Oil Treated Animal (After 12th Week)

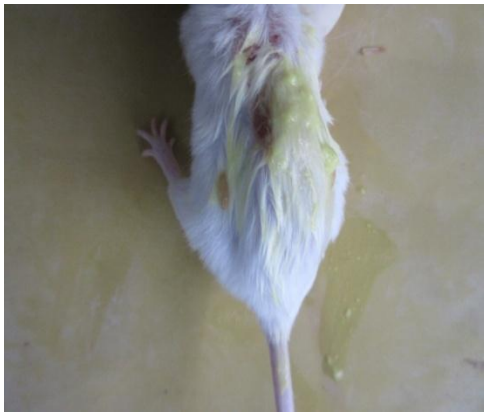

Figure-10 DMBA + Croton Oil and 5FU Treated Animal (After 12th Week)

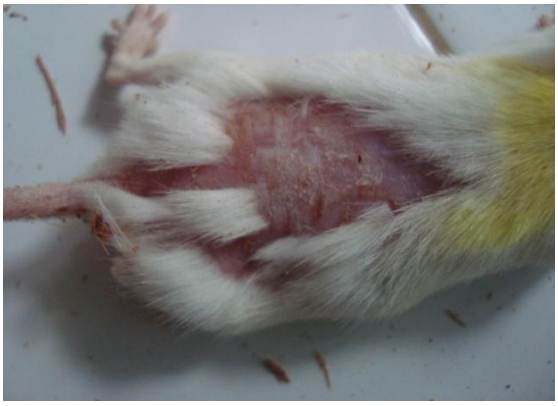

Figure-11 DMBA + Croton Oil and Quercetin + Kaempferol Treated Animal (After 12th Week)

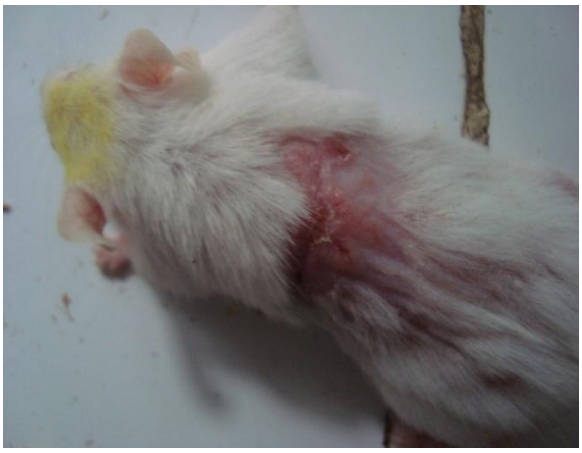

Figure-12 Tumor Latency of Papillomas on DMBA + Croton Oil and Herbal Gel Treated Animal

CONCLUSION

The formulated gel was subjected to efficacy and toxicity studies. The efficacy study was carried out using the initiator 7, 12 Dimethylbenz (a) anthracene (DMBA) and with promoter croton oil induced skin carcinoma on albino mice. The formulated herbal gel suppressed the tumour in albino mice with the concentrations of 10mg/kg & 20mg/kg when compared to DMBA treated group and standard group (5-FU) with low toxicity profile. The parameters of tumour incidence, tumour burden, tumour volume, tumour weight and the morphological examinations were compared between the group-II (DMBA (100gm/100µl of acetone) only, group-III (DMBA + 5FU gel 20 mg/kg) and group-IV (DMBA + Quercetin + kaempferol gel (1:1 ratio) 20mg/ kg). The study revealed that the formulated herbal gel have a fine effect to suppress the tumour in albino mice with the concentration of 20 mg/kg as compared to DMBA treated and standard group.

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