ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 5, 2023

Online - 2455-3891
Print - 0974-2441

Review Article

TOPICAL FORMULATION CONSTITUTED WITH TRANSFEROSOMES FOR THE TREATMENT OF NON-MELANOMA SKIN CANCER

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Received: 08 December 2023, Revised and Accepted: 31 January 2023

ABSTRACT

Overexposure to UV-B radiation causes an evolution in the strands of DNA of skin membrane cells, resulting in non-melanoma skin cancer. With the addition of excipients and nanovesicular structures such as transferosomes that boost the permeability rate and pharmacological activity, a formulation containing curcumin, kaempferol, trans-resveratrol, and apigenin have been developed which possess strong anti-inflammatory and anti-proliferative potential. The formulation quickly penetrates the stratum corneum and acts on cancer cells, inhibiting metastasis and angiogenesis by interfering with signaling molecules in the three primary mitogen-activated protein kinase pathways: extracellular-signal-regulated kinase, c-Jun N-terminal kinases, and p38. It blocks pro-inflammatory cytokines such as lipopolysaccharide, tumor necrosis factor-alpha, IL1, IL6, COX-2, LOX, oxidative stress, and lowers the levels of matrix metalloproteinase (MMP)-3, MMP-9, and vascular endothelial growth factor. The yield value, sensory testing, spreadibility, dynamic viscosity, water content, pH, specific gravity, anti-microbial preservative concentration, microbiological limit, sterility testing, contaminants, uniformity of dosage, and assay on RAW264.7 cell line will all be used to evaluate the formulation. The O/W cream that has been produced will be significantly more successful than traditional cancer treatments, and it will have no side effects, protects the patient from recurrence of cancer and inexpensive treatment.

Keywords: Non-melanoma skin cancer, Curcumin, Kaempferol, Trans-resveratrol, Apigenin, Transferosomes.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i5.47033. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

In Caucasians, non-melanoma skin cancer (NMSC) is the most frequent kind of cancer. The incidence rate of NMSC has increased by 10% each year, and 2–3 million new cases of NMSC are detected each year around the world. NMSC rates rise as you get closer to the equator, with the highest reported rates in Australia's northern regions [1].

Photodynamic therapy and other old techniques can shrink cancer cells to a certain extent, but they come with a slew of adverse effects, including cancer recurrence and a slew of other issues such as anemia, weight loss, diarrhea, and constipation.

There are several types of skin cancer: Actinic keratosis (AK), squamous cell carcinoma (SCC), and basal cell carcinoma (BCC).

Traditional techniques and chemotherapy, such as photodynamic therapy, used to treat NMSC are very expensive, have significant side effects, and the recurrence rate of NMSC is very high after these exhausting stages. Due to their less adverse effects, cost efficiency, and simplicity, non-invasive methods are becoming more popular.

Curcumin, kaempferol, trans-resveratrol, and apigenin are all lipid-soluble substances with powerful anti-inflammatory and anti-proliferative properties, yet when taken orally, they have no effect on skin cancer, due to low oral bioavailability and maximum plasma levels are both quite low. Because these chemicals are highly unstable at pH 7.4, they needed to be given in a more efficient manner.

NMSC

AK

AK is a premalignant lesion caused by too much exposure to UV radiation which causes DNA damage.

In several cases of AK, there may be a formation of horn which resembles a real animal horn. The cutaneous horn appears as a funnel-shaped growth that extends from a red base on the skin. In most cases, AK mutates into SCC. Tumors that arise from AK are aggressive locally but generally metastasized after only after a long period [2,3].

SCC

SCC is a common tumor that typically arises on sun-exposed sites. This tumor has a higher incidence in males than females. It is mainly caused by UV-B light exposure that causes widespread DNA damage and high mutations. Patients with rare autosomal disease *xeroderma pigmentosum* are at greater risk. TP53 mutations are common which is caused by mutations in RAS and loss of function mutations in NOTCH receptors [4,5].

BCC

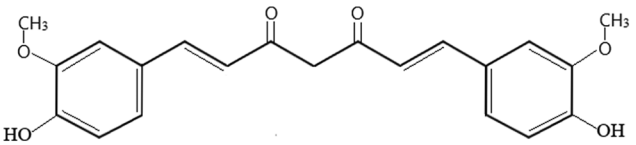
BCC is a common slow-growing cancer that rarely metastasizes. It tends to occur at sites that are exposed to sunlight and in lightly pigmented individuals. The main cause for BCC is loss of function mutations in PTCH1, a tumor suppressor gene that negatively regulates hedgehog signaling (SHH); hence, tumors exhibit constitutive SHH pathway activation (SHH signaling is an important regulator of embryonic development), and patients with gorlin syndrome are also manifest BCC. A mutation in TP53 is caused by UV-B damage also common in both familial and sporadic BCC [6,7].

People suffering from immunity-related diseases are more prone to NMSC. It estimates that up to 85–90% of NMSC is in immunocompromised individuals. Patients suffering from a rare cell-mediated immunity disorder, *Epidermodysplasia verruciformis*, are also very prone to NMSC. In transplant recipients, the risk of SCC increases by 125–250-fold and the risk of BCC increases by 10–16-fold. Patients with human immunodeficiency virus-acquired immunodeficiency syndrome or non-Hodgkin lymphoma also develop aggressive SCC [8,9].

Cause type of NMSC (Table 1)

Materials and methods

Curcumin



Curcumin (diferuloylmethane) is derived from turmeric (*Curcuma longa* Linn). Overall curcumin comprises 3–4% of whole turmeric constituents. It is responsible for the yellow color of turmeric, and it comprises curcumin I (90–94%), curcumin II (6–8%), and curcumin III (0.5–1%).

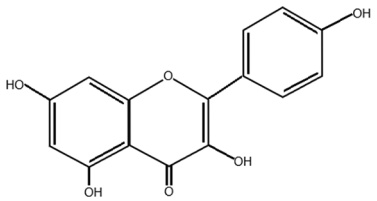
It has anti-inflammatory, antioxidant, anti-carcinogenic, hypotensive, anti-mutagenic, anticoagulant, antiprotozoal, antifertility, anti-aging, hepatoprotective, antidepressant, anti-venom, antidiabetic, antibacterial, anti-fibrotic, antifungal, antiviral, antiulcer, and hypocholesterolemic potential.

The peak plasma concentration of curcumin is only 1.8 ng/mL. It shows little or no side effects even at high doses. Curcumin is highly unstable at pH 7.4 and degrades quickly but quite stable at acidic pH [10,11].

How curcumin works

- Curcumin binds efficiently with (cyclooxygenase-2) COX-2 and then stop its conversion into PGG2 hence reduces the production of prostaglandins
- Similarly, curcumin binds with (Lipoxygenases) 5-LOX and reduces the productions of LTs [12]
- Curcumin inhibits iNOS and which stops the production of nitric oxide
- Curcumin is also involved in PPARγ activation which suppresses airway hyper responsiveness, which reduces inflammation and epithelial hyperplasia in the airway
- Inhibits the production of interleukins: IL-1, IL-2, IL-6, IL-8, and IL-12 [13]
- It also decreases inflammatory mediator (IL-4, IL-5, and IL-13) synthesis and release which reduces mucus hypersecretion and inhibits collagen deposition
- Regulates chemokine monocyte chemoattractant protein
- Curcumin is also proven to work against the activity of histamine and β-hexosaminidase releases from IgE. Hence, it is also acts as mast cell stabilizer
- Curcumin also degrades the production of IκB-α, which further stops the activation of nuclear factor kappa B (NF-κB), a pro-inflammatory transcription factor [14]
- Curcumin inhibits TNF-α, which is mediator of inflammation [15]
- It shows inhibitory action on Janus kinase STAT signaling.

Kaempferol

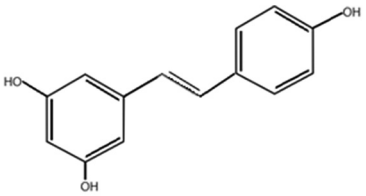


Kaempferol is found in various plant parts, such as seeds, leaves, fruits, flowers, and even vegetables. Kaempferol possess cardioprotective, neuroprotective, anti-inflammatory, anti-diabetic, antioxidant, antimicrobial, antitumor, and anti-cancer potential [16,17].

How kaempferol works against cancer

- Kaempferol triggers apoptosis in cancer cells
- It arrests the cell cycle at G2/M phase
- It downregulates the signaling pathways and phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) [18]
- It expresses epithelial-mesenchymal transition-related markers (N-cadherin, E-cadherin, Snail, and Slug), and matrix metallopeptidase 2, metastasis-related markers [19]
- It induces the activation of cysteine proteases, caspases-3, 7, 9, and Poly (ADP-ribose) polymerases which are involved in apoptosis initiation, hence preventing the accumulation of reactive oxygen species (ROS)
- Kaempferol inhibits AP-1 activation (AP-1 is a downstream molecule which is regulated by mitogen-activated protein kinase (MAPKs), it is a transcription factor involved in *COX-2* gene expression); hence, it inhibits UVB-induced COX-2 expression [20]
- It inhibits UVB-induced phosphorylation of extracellular-signal-regulated kinase (ERKs), p38, and c-Jun N-terminal kinases (JNKs).

Trans-resveratrol

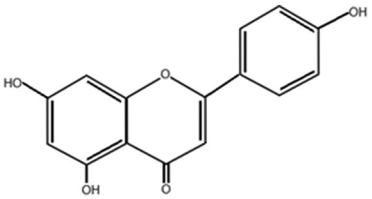


Resveratrol is a pleiotropic phytochemical which belongs to the stilbene family. Stilbenes are the secondary metabolites which are produced by the plants in response to stressful conditions, such as fungal infection or UV radiations. It possesses anti-microbial, anti-oxidant, anti-aging, anti-inflammatory, anti-estrogenic, cardioprotective, and anti-cancer properties. Furthermore, it has been reported that resveratrol can reverse multidrug resistance in cancer cells [21,22].

How trans-resveratrol works against cancer

- Decreases free radical scavenging incidence
- Resveratrol effectively hinders the development skin tumors by inducing apoptosis, which was indicated by the induction of cytochrome *C*-release, the expression of bax, p53, and APAF-1, and the inhibition of Bcl-2 [23]
- Decreases the expression of COX-2, and ornithine decarboxylase [24]
- It also decreases hyperplasia
- It inhibits activation of NF-κB through inhibiting the IκB-α kinase activation, and hence down-regulating pro-proliferation genes, such as cIAP-2, survivin, cyclin D1, Bcl-xL, Bcl-2, XIAP, Bfl-1/A1, and TNF-α receptor-associated factor 2 [25]
- It also suppresses matrix metalloproteinase (MMP)-3, MMP-9, COX-2, and vascular endothelial growth factor (VEGF)
- Resveratrol causes inhibition of signal transducers and activators of transcription 3
- It exerts an anti-oxidant effect with a reduction in H_2O_2 and lipid peroxidation in the skin
- It also decreases the levels and expressions of hepatic TNF-α, interleukin-1β (IL-1β), and IL-6 [26].

Apigenin



Apigenin is a flavonoid which is present in grapefruit, parsley, onions, oranges, chamomile, and wheat sprouts. It has a number of valuable bioactive functions including antibacterial, antiviral, anti-proliferative, anti-inflammatory, antioxidant, antiangiogenic, and anticancer activities, treatment for rheumatoid arthritis, autoimmune disorders, Parkinson's disease, and Alzheimer's disease [27,28].

How apigenin works against cancer

- Apigenin initiates apoptosis by modulating Bcl-2, Bax, STAT-3, and Akt proteins expression [29]
- It promotes anti-inflammatory pathways including p38/MAPK and PI3K/Akt [30]
- It prevents the Iκ-B degradation and nuclear translocation of the NF-κB and also reduces COX-2 activity [31]
- It inhibits metastasis and angiogenesis by interacting with the signaling molecules in the three major MAPK pathways: ERK, JNK, and p38 in human cell culture models [32]

- It is known for suppressing cluster of differentiation 40, TNF-α, and IL-6 production through inhibition of interferon gamma-induced phosphorylation of signal transducers and activators of transcription 1 in murine microglia [33].

Transferosomes

Transferosomes are nanovesicular carrier systems that are specially designed to have at least one inner aqueous compartment that is enclosed by a phosphatidylcholine, together with an edge activator [34]. These nanovesicular carrier systems can easily pass through the pores of stratum corneum and it is able to load a large amount of API in it (Fig. 1).

EXTRACTION OF COMPONENTS

Of resveratrol from extraction *Pediomelum cuspidatum*

Extraction process will be done by reflux extraction method with the help of 95% ethanol. First, the dried roots of *P. cuspidatum* are cut down with the help of FZ102 plant disintegrator, and then, ethanol is added in the proportion of 1:6. The mixture will be left for 12 h, and then, soaked powder will be extracted out at 80°C. The extract will be pulverized into powder form of size <9 mm and filtered with help of ultrasonic cleaners. Further, the aqueous phase will be hydrolyzed with the help of HCl (pH=1), and then, liquid extraction will be done with the help of methyl tert-butyl ether. To remove impurities, eluting will be done at basic pH (8–9). Then, at the final procedure, the organic phase is dried under reduce pressure to obtain the product [35].

Table 1: Cause of different types of NMSC

UV-B	BCC
HPV	BCC, SCC
Iatrogenic immunosuppression	BCC, SCC
HIV-AIDS	BCC, SCC
Solar UV radiation	BCC, SCC
Arsenic	BCC, SCC
Occupational factors	BCC, SCC
Smoking	SCC

BCC: Basal cell carcinoma, SCC: Squamous cell carcinoma, HIV-AIDS: Human immunodeficiency virus-acquired immunodeficiency syndrome

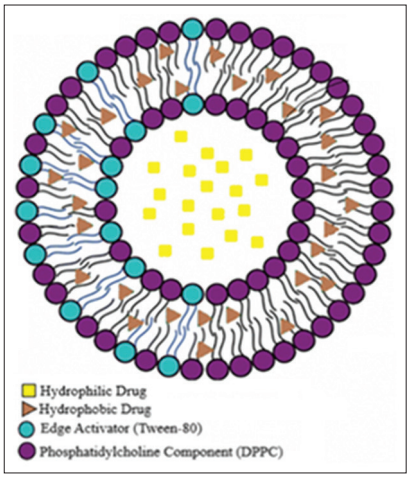


Fig. 1: Transferosomes

Extraction of kaempferol and Apigenin from *Strobilanthes crispus*

Extraction of kaempferol is done using supercritical CO_2 method on the leaves of *S.crispus*. Ethanol (99.5%) will be used as a cosolvent here. The optimal conditions for extraction are pressure (20 MPa), temperature (50°C), and dynamic extraction time (60 min). The flow of CO_2 and ethanol is maintained at 10 g and 1 g/min. Some rigid material can be used to preserve an adequate CO_2 flow rate and it also maintains the desired permissiveness of particle during extraction. Eight flavonoids were obtained which are (+)-catechin, (−)-epicatechin, rutin, myricetin, luteolin, apigenin, naringenin, and kaempferol. After this process silica gel, chromatography will be used to separate out kaempferol and apigenin from others on the basis of polarity [36].

Extraction of curcumin from *C. longa*

Soxhlet extraction method will be done for extraction of curcumin. The rhizomes of *C. longa* are dried in oven at 105°C for 3 h and then triturated using mortar and screened through a sieve size of 80 to obtain a particle size of 0.18 mm. Sample of *C. longa* powder will be weighed and embedded in a thimble and transferred in the Soxhlet apparatus which then filled with acetone as the extraction solvent. The extraction experiment will be carried out at 60°C for 8 h. After, completion of the extraction, the acetone will be separated out from using rotary evaporator in vacuum at 35°C [37].

PREPARATION OF CREAM

Procedure

- Oil phase contains: Shea butter, polo wax, glycerol stearate, cetearyl alcohol, and PEG-100 are dispersed into olive oil
- Aqueous phase contains: Distilled water in mixed with vegetable glycerine
- In a round-bottom flask, the phospholipids dipalmitoyl phosphatidylcholine and edge activator (tween-80) are dissolved in an appropriate (v/v) ratio of chloroform and methanol. All APIs curcumin, kaempferol, trans-resveratrol, and apigenin are added at this stage. A rotary vacuum evaporator is used to evaporate the organic solvent above the lipid transition temperature under reduced pressure to generate a thin layer. To eliminate the last residues of the solvent, keep it under vacuum. The thin film is then hydrated using a buffer solution with a pH of 7.4 by rotating it for a specific amount of time at a specific temperature. To obtain tiny vesicles, the resultant vesicles are inflated at room temperature and sonicated in a bath or

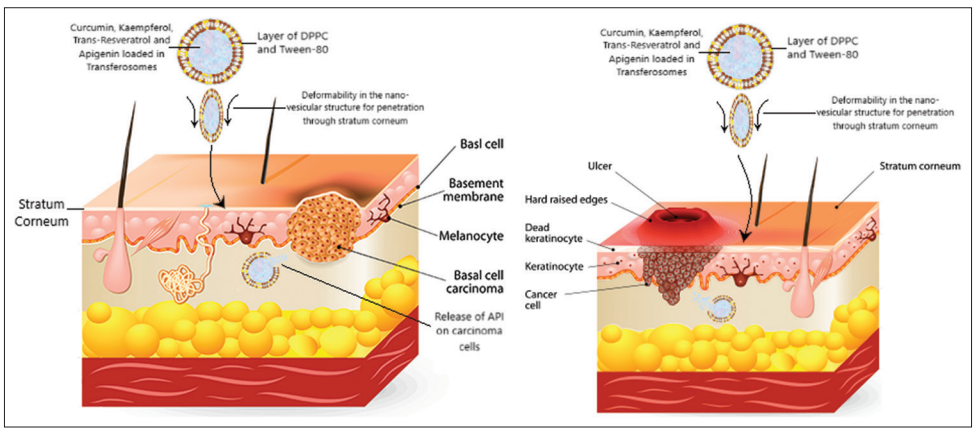


Fig. 2: Diagrammatic representation of delivery of API loaded in transferosomes at the cancer sites

probe sonicator. Extrusion across a sandwich of 100 nm to 200 nm polycarbonate membranes homogenizes the sonicated vesicles.

- The oil phase will be prepared by melting the waxes, cetearyl alcohol, glycerol stearate, and PEG-100 (emulsifiers) at 110°C in olive oil on the hot water bath for about 20–25 min. After preparation of the oil phase, the formulation is kept aside to reduce its temperature
- The aqueous phase will be prepared by dissolving the vegetable glycerine in distilled water, after the addition the weight of the aqueous phase will be measured. Then, the water phase is warmed to 75–80 °C and stirred properly until it is dissolved properly. Weight is measured again if needed pre-heated distilled water is added to make up the volume
- After preparation of both aqueous and oil phases, the aqueous phase is slowly added to the oil phase and emulsified with the help of a silverson emulsifier at 3400–3500 rpm at 75°C and 165 bar pressure. Furthermore, the transferosomes prepared at the initial stage will be added to mixture of water and oil phase and then again mixed with silverson emulsifier at same conditions
- After emulsification, the formulation is left to cool down. When the temperature of the formulation is dropped to 40°C then, add preservative (euxyl k712), pH stabilizer (glycol distearate), and synthetic vitamin E in the formulation with moderate agitation and continuous stirring
- In the last, the mixture will be stirred again for 15 min until the formulation became uniform a semisolid drug-loaded cream.

TESTING OF CREAM ON DIFFERENT PARAMETERS

Calculation of yield values

A spread meter is used to measure the flattening at a temperature of 25°C with a glass plate. Spread diameter is measured at different intervals of 5, 10, 30, 60, 120, and 180°s. The yield value is calculated after 120 s with the following formula:

$F=47{,}040 \times G \times V/\pi^2 \times D^5$

Where;

F: Yield value (dynes/cm^2)
G: Glass plate weight (g)
V: Sample size (cm^3)
D: diameter (mm) when sample spreading stopped

Sensory testing

Sensory testing determines the irritability of the cream. Sensory testing was done using single blinding three samples prepared A B C.

A. Curcumin, trans-resveratrol, kaempferol, and apigenin are not present
B. Curcumin, trans-resveratrol, kaempferol, and apigenin are present in half conc
C. Curcumin, trans-resveratrol, kaempferol, and apigenin were present as suggested.

- All the samples weighted 1 gm each. Every tester washed their hands under running tap water and air dry for 5 min
- All the testers were allowed to smell the aroma of three samples and will be allowed to rate them in a category 1–5 (where 1-poor, 2-somewhat poor, 3-somewhat good, 4-good, and 5-excellent) on the assessment paper
- Testers are given that 0.1 g dollops of cream were gently rubbed on the area of infection with their index finger in circular motion ten times
- The cream was left on the body for 5 min, and then, the rating will be done by testers in the same assessment paper
- Afterward, testers used tap water to rinse off the area where the cream has been applied
- The same procedure was repeated for all the samples A B C
- Testing is improved by asking testers to rate on appearance and texture.

Spreadability test

The cream base should spread easily without much force and not produce greater friction in the rubbing process. The spreadability test is calculated using a spreadability apparatus which is made of a wooden board with a scale and two glass slides having two pans on both sides mounted on a pulley.

A sample is placed in between the two glass slides and 100 g weight is placed on the glass slide for 5 min to compress the sample to produce a uniform thickness. A weight of 250 g is added to the pan. The time required to separate the two slides is taken as a measurement of spread ability [38].

$$S=\frac{m\times 1}{t}$$

Where,
m – Weight tied on upper slide
l – Length of glass slide
t – Time in s

pH

Determination of pH is needed for irritability check. A dollop of cream is taken (about 10 g) and diluted to 50% with distilled water to get an accurate pH reading. Dip the pH meter in the diluted cream swirl it for 1 min and note the reading.

Measurement of dynamic viscosity

The dynamic viscosity of cream influences the efficiency of the process and also the satisfaction of the customer. Dynamic viscosity will be measured using a type-E rotational viscometer. The viscosity is determined through the measurement of the torque. The dynamic viscosity of 1 mL of cream is measured with a 1 34 × R24 cone rotor at 1 rpm for 600 s at 25°C for 180 s. The reading is noted at different torque values till 100%.

Anti-microbial preservative concentration

A known concentration of Staphylococcus aureus, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus* brasiliensis will be taken. Strains of microorganisms will be suspended in five tubes one strain in each, along with cream samples which are inoculated for 28 days at room temperature and determined for every 6 h, 24 h, 48 h, 7 days, 14 days, and 28 days. The log reduction or percent reduction is calculated and compared to the acceptance criteria required by the method for acceptable preservation [39].

Measurement of water content

Water content was measured using a Karl–Fisher moisture content meter. 0.01 g of sample was measured, dissolved in absolute methanol by heating them in an ultrasonic bath at room temperature. Water concentration is measured by KF titration method.

Specific gravity

A pycnometer is a special glass bottle used to measure specific gravity. First, it is weighed empty and again when filled with water, the exact volume of the pycnometer is known. Specific gravity is calculated by the following formula

Specific gravity = weight of material (g)/weight of an equal volume of water (g)

Where water is used as a standard for specific gravity of solids and liquids.

Microbial limit

10 g of prepared sample cream will be dispersed in 100 mL sterile tryptic soy broth in presence of 0.25% Tween 80 and Shaken well for 15 min at room temperature.

Microbial contamination is determined by spreading a thin layer of 0.5 mL diluted prepared sample aseptically on nutrient and soybean-casein digest agar medium plates and incubate for 24–48 h at 37°C isolates which were purified and identified. A $\leq 1 \times 10^3$ CFU per gram or mL of the product is considered to be an acceptable value for topical applications [40].

Sterility testing

Prepare by diluting to about 1 ratio 10 in a sterile diluent such as peptone (1 g/L). Transfer the diluted product to a medium; incubate the inoculated media for not <14 days. Observe the cultures several times during the incubation period. Shake cultures gently each day. The incubated culture was isolated and identified.

Assay testing for anti-inflammatory response

In this assay testing, RAW264.7 cell line is prepared and tested for the presence of different types of lipopolysaccharide induced inflammatory cells. The murine macrophage RAW264.7 cell line will be maintained in dulbecco's modified eagle medium supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin and then determination of COX-2, iNOS, TNF-α, IL-1β, and IL-6 Genes by quantitative real-time PCR [41].

Release of drugs from transferosomes on squamous cell carcinoma and basal cell carcinoma

RESULTS

A formulation is prepared to treat NMSC by loading curcumin, kaempferol, trans-resveratrol, and apigenin in transferosomes then mixing them with excipients such as polo-wax, glycerol stearate, PEG-100, glycerine, shea butter preservatives, and pH stabilizers. The cream is prepared by preparing oil phase, water phase, and transferosome individually and then mixing it with the help of emulsifiers. After preparation, the cream will be tested on several factors such as yield value, sensory testing, spread ability test, dynamic viscosity, water content, pH, specific gravity, anti-microbial preservative conc., microbial limits, sterility testing, assay testing on A431 cell line to check its bio-availability, stability of the formulation, irritability on skin, content uniformity of cream, and efficient working of cream.

CONCLUSION

The formulation is a non-greasy o/w semisolid formulation applied topically which comprises curcumin, kaempferol, trans-resveratrol, and apigenin which are potent anti-inflammatory and anti-proliferative agents. Various excipients such as polowax, PEG-100, glycerol stearate, glycerine, shea butter preservatives, and pH stabilizers are used to increase the efficiency, spreadability, stability, and bioavailability of the formulation by several folds. The formulation efficiently penetrates through the skin membrane and stratum corneum and efficiently reaches cancer cells where it inhibits pro-inflammation cytokine IL-1β, iNOS, NF-κB, and suppresses COX-2; oxidative stress induced by ROS and free radicals, and improves antioxidant activity when it penetrates the stratum corneum layer. In human cell culture models, the formulation also suppresses $I\text{-}_{\kappa}B$ degradation and $NF\text{-}_{K}B$ nuclear translocation, lowers the levels of MMP-3, MMP-9, and VEGF, and inhibits metastasis and angiogenesis through interacting with signaling molecules in the three primary MAPK pathways: ERK, JNK, and p38. The composition will also provide protection against future UV-B radiation issues. Furthermore, components such as EGCG and theaflavin can be used as a substitute due to similar activity (Fig. 2) [42-54].

ACKNOWLEDGMENT

The author is thankful to the Department of Pharmacy, HIMT College, Greater Noida for providing kind guidance and excellent opportunity as well as necessary facilities for the research.

CONFLICTS OF INTEREST

The author confirms that the content of the article has no conflicts of interest.

FUNDING

This research paper received no external funding.

DATA AVAILABILITY

The original data that support the findings of this study are included in the article.

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