SYNOPSIS

1. TITLE

Development and Characterization of Apigenin Transfersomal System for Skin Cancer

2. INTRODUCTION

Skin cancer is one of the most common types of cancer. Skin cancer is the uncontrolled growth of abnormal cells in the skin. According to current estimate, 40-50 % of Americans, who live to age 65 have skin cancer at least once. Each year, Cancer develops when DNA, the molecule found in cells that encodes genetic information, becomes damaged and the body cannot repair the damage. These damaged cells begin to grow and divide uncontrollably. When this occurs in the skin, skin cancer develops. As the damaged cells multiply, they form a tumor. Since skin cancer generally develops in the epidermis, the outermost layers of skin, a tumor is usually clearly visible. This makes most skin cancers detectable in the early stages. More than 1.5 million skin cancers are diagnosed annually and the incidence of skin cancer is rising as well. About 80 percent of skin cancers are basal cell carcinoma, 15 percent squamous cell carcinoma, and 5 percent invasive melanoma. More than 75 percent of skin cancer deaths are due to malignant melanoma. Exposure to ultraviolet (UV) radiation is associated with a variety of harmful effects ranging from photoaging to skin cancer.UVB (290 to 320 nm) directly damages the cellular DNA leading to the formation of the 6-4 cyclobutane pyrimidine dimmers, and UVA (320 to 400 nm) indirectly damages the DNA via the production of oxygen reaction species. Apigenin a naturally occurring plant flavone (4', 5, 7,-trihydroxyflavone) abundantly present in common fruits and vegetables including parsley, onions, oranges, tea, chamomile, wheat sprouts and some seasonings. Apigenin has been shown to possess remarkable anti-inflammatory, antioxidant and anti-carcinogenic properties. The cancer chemopreventive properties of apigenin were first demonstrated by Birt *et al.* who described the antimutagenic and anti-promotion properties of apigenin through inhibition of TPA-induced ornithine decarboxylase activity in mouse skin. These initial studies with apigenin generated further interest in the development of apigenin as a chemopreventive and/or chemotherapeutic agent. Li *et al.* established a short-term *in vivo* system to evaluate topical formulations of apigenin and to determine whether apigenin is effective when delivered as a topical preparation to local skin lesions. It was observed that topical application of apigenin was capable of targeting local tissue. Another study by Li and Birt, demonstrated the *in vivo* and *in vitro* percutaneous absorption of apigenin using different

vehicles. Through these studies it was apparent that delivery of apigenin into viable epidermis appears to be a necessary property for an apigenin formulation to be effective in skin cancer prevention. Studies have shown that apigenin is effective in the prevention of UVA/B-induced skin carcinogenesis in SKH-1 mice. Topical application of apigenin has been shown to inhibit UV-mediated induction of ornithine decarboxylase activity, reduce tumor incidence and increase tumor-free survival in mice. Several other studies have provided evidence that apigenin prevents UV-induced skin tumorigenesis by inhibiting the cell cycle and cyclin-dependent kinases. Novel drug delivery system is advantageous in delivering the drugs at predetermined rate on desired site of action which minimizes the toxic effects with the increase in bioavailability of the drugs. Application of novel vesicular drug delivery systems with phytoconstituents can lead to enhanced bioavailability, increased solubility and permeability, thereby reducing the dose and hence, side effects.

3. OBJECTIVE OF RESEARCH

The objectives of the proposed research work are:

- To prepare nanocarrier vesicular system of apigenin for achieving the sustained drug release.
- To achieve enhanced therapeutic effect and increased permeability.
- To study the efficacy of the prepared formulation in animal model of skin cancer.

4. BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD

The exhaustive literature survey furnished the information about photoprotective of herbal drugs of different categories with natural flavonoids and their derivatives for enhancement of solubility, stability, bioavailability and permeability etc. Till date, very little work, related to a natural plant flavone apigenin based novel drug delivery system for skin cancer, which can give a fruitful contribution in this area, is available. We enumerate some of important contribution in the following way: -

- **Gao et al., (2013)** prepared nanocrystals of apigenin by the supercritical antisolvent process for dissolution and bioavailability enhancement. The pharmacokinetic study of apigenin nanocrystals, in comparison to coarse powder, was also performed in rats after a single oral dose.
- **Zhai et al.,(2013)** studied that apigenin-loaded micelles were fabricated by a thin-film dispersion method and possessed a sustained release property. Polymeric micelle

formulation become a potential nanocarrier for apigenin as a poorly water soluble drug.

- **Sachan *et al.* ,(2013)** prepared drug carrier transfersomes a novel tool for vesicular drug delivery system, which can respond to an external stress by rapid and energetically inexpensive, shape transformations. Such highly deformable particles can be used to bring drugs across the biological permeability barriers, such as skin.
- **Anitha et al., (2012)** reported that Plant phenolics are one candidate for prevention of harmful effects of UV radiation on the skin. Additionally, plants contain a lot of other substances which can be useful for skin care.
- **Man et al., (2012)** studied demonstrate that topical apigenin inhibits acute inflammation and subacute dermatitis as indicated by improved epidermal permeability barrier function. Therefore, apigenin could be useful in treating acute and subacute dermatitis.
- **Lin et al., (2012)** studied that the anti-hepatoma activity of apigenin is as effective as 5-FU and its apoptotic mechanism might be mediated through the p53-dependent pathway and the induction of p21 expression.
- **Modhi *et al* , (2012)** prepared transfersomes are new dominants for transdermal drug delivery. This high deformability gives better penetration of intact vesicles. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.
- **Seema *et al* ,(2012)** vesicular systems have been realized as extensively useful carrier systems in various scientific domains. Over the years, vesicular systems have been investigated as a major drug delivery system due to their flexibility to be tailored for varied desirable purposes.
- **Dhiman *et al* ,(2012)** reported there is a great potential in development of novel drug delivery system for valuable herbal drugs as it provides efficient and economical drug delivery. Also, the trend of incorporating NDDS for herbal drugs has also been adopted at industrial scale.
- **Mimeault et al,(2012)** reported that Despite great interest in using the curcumin as chemopreventive or therapeutic agent. Thus, these future investigations should lead to more chemically stable and effective curcumin formulations that could be used as dietary substances, in safe conditions for cancer prevention.

- **Muller et al., (2011)** apigenin nanosuspensions were prepared using the combination technology. These skin formulation to overcome the poor solubility and dermal application as efficient antioxidant.
- **Kaur et al., (2011)** studied the photoprotective nature of the P. granatum extract was confirmed and development of cream incorporated with extract loaded nanotransfersomes was successfully done. The application of herbal extract loaded nanovesicular creams could be utilized for the prevention from ultraviolet radiations generated premature aging.
- **Kulkarni *et al* , (2011)** have studied the Application of novel drug delivery systems to phytoconstituents can lead to enhanced bioavailability, increased solubility and permeability, thereby reducing the dose and hence, side effects. A number of plant constituents have exhibited enhanced therapeutic effect at similar or less dose when incorporated into novel drug delivery systems as compared to conventional extracts. Hence, there is great potential in development of novel drug delivery system for valuable herbal drugs.
- **Ulrich et al ,(2011)** multiple studies have evaluated the use of RCM in melanoma and nonmelanoma skin cancer and have demonstrated high sensitivity, specificity rates and discuss other clinical applications of this emerging technique.
- **Shukla et al., (2010)** studied that apigenin has gained particular interest over the years as a beneficial and health-promoting agent because of its low intrinsic toxicity and because of its striking effects on normal versus cancer cells, compared with other structurally related flavonoids.
- **Saraf *et al* ,(2009)** this review focused on list of herbs, formulations and evaluation parameters researches have to be carried out to validate simple, economic and rapid methods to carry out efficacy studies of herbal photoprotective formulations.
- **Chuang et al., (2009)** reported that apigenin represents a promising chemotherapeutic agent, which may be used in combination with immunotherapy for the treatment of advanced stage cancers.
- **Mukerjee *et al* , (2009)** showed that Curcumin-loaded PLGA nanospheres were prepared by using a s/o/w emulsion solvent evaporation technique. PLGA nanospheres are capable of delivering curcumin over a prolonged period achieving a sustained delivery of curcumin, thus making it a potential candidate for cancer therapy.

- **Patel et al., (2007)** this review examined the cancer chemopreventive effects of apigenin in an organ-specificity format, evaluating its limitations and its considerable potential for development as a cancer chemopreventive agent.
- **Liu et al., (2005)** studied that apigenin may inhibit human lung cancer angiogenesis by inhibiting HIF-1 and VEGF expression, thus providing a novel explanation for the anticancer action of apigenin.
- **Birt et al., (1997)** evaluated that apigenin is effective in the prevention of ultraviolet-B light (UVB) induced skin carcinogenesis. Mouse skin carcinogenesis was induced by exposure to a total dose of 40 J/cm2 UVB over 11 weeks. Reduction in cancer incidence (52% inhibition) and an increase in tumor free survival in comparison with control mice ($P < 0.01$).

5. NOTEWORTHY CONTRIBUTION IN THE FIELD OF PROPOSED WORK

Each year, millions of people find out that they have skin cancer. Skin cancer is almost 100% curable if found early and treated right away. From the exhaustive literature review, it was observed that the poorly water soluble/bioavailable drug with bioflavonoid apigenin based polymers improves/enhances the solubility, stability and bioavailability. However, very few literatures are available and reported on the development of novel delivery system of based on these apigenin drugs. Therefore, keeping in mind this concept, the proposed research work will contribute in the formulation development and characterization of apigenin based novel drug delivery for photochemoprotective is a unique nanocarrier delivery system. The quantitative and qualitative results will provide the evidence that nanovesicular formulation development in this study will be become a potential nanocarrier for apigenin as a skin cancer. One of the most desirable goals in cancer chemoprevention is the identification of natural agents with demonstrable efficacy against defined molecular targets. And the work will contribute globally by improving health related quality of life in skin cancer patients.

6. PROPOSED METHODOLOGY OF THE RESEARCH WORK

The proposed work will be carried out according to the following plan:

1. Procurement of apigenin drug and other consumables

2. Preformulation studies
    - Identification of drug by chemical test, UV and IR Spectrometry.

- Solubility studies in aqueous and non-aqueous solvents.
- Partition coefficient studies.
- Preparation of standard curve of drug apigenin.
- Preparation of different pH media selection
- Compatibility study

3. Selection of polymers.
4. Preparation of transfersomes of apigenin.
5. Characterization of prepared formulation for
    - Entrapment efficiency
    - Drug content
    - Vesicle morphology
    - Vesicle size distribution and zeta potential
    - No. of vesicles per cubic mm
    - Degree of deformability or permeability measurement
    - Surface charge and charge density(Zetasizer)
    - In-vitro drug release
6. Optimization of various parameters-
    - Formulation parameter.
    - Processing parameter.

7. Stability studies
    - Accelerated stability studies at different temperature and humidity levels for changes in morphology and drug content.
    - Release study of aged products

8. *In vivo* studies
    - Ex vivo skin permeation studies
    - Cytotoxicity studies
    - Penetration ability study (CLSM)
    - Primary skin irritation studies
    - Skin surface biopsy studies
    - Animal models of skin cancer incidence

9. Compilation and presentation of data

7. EXPECTED OUTCOME OF THE PROPOSED WORK

The objective of proposed research work is lead to the development of novel Photoprotective Vesicular approaches for sustained Drug Delivery System of Apigenin for higher exposure on site of action to achieve the desired therapeutic potential for skin cancer. Application of novel drug delivery systems to phytoconstituents of bioflavonoid is lead to enhanced therapeutic effect, increased permeability and thereby reducing the dose and hence, side effects.

7. REFERENCES:

- Adnan, O. Abu-Yousif, Kimberly A. S., Spiro G., et al. Enhancement of UVB-Induced Apoptosis by Apigenin in Human Keratinocytes and Organotypic Keratinocyte Cultures. *Cancer Res* 2008; 68:3057-3065.
- Anitha,T., Medicinal plants used in skin protection. *Asian J Pharm Clin Res,*2012; 5(3):35-38.
- Androutsopoulos, V.P., Papakyriakou, A., Vourloumis, D., Dietary flavonoids in cancer therapy and prevention: Substrates and inhibitors of cytochrome P450 CYP1 enzymes. *Pharmacology & Therapeutics* 2010; 126: 9–20.
- Birt, D.F., Mitchel, D., Gold,B., et al. Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. Anticancer Res. 1997; 17(1A):85-91.
- Caltagirone , S.,Rossi, C., Poggi, A., et al. Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. Int.J. Cancer 2000; 87(4):595-600.
- *Dhiman, A., Nanda, A. and Ahmad, S., Novel herbal drug delivery system (NDDS): the need of hour . IPCBEE,* 2012; 49: 71-75.
- *Dayan N., Touitou E., Carrier For Skin Delivery of Trihexyphenidyl HCl: Ethosomes v/s Liposomes , Biomaterials,* 2002; 21: 1879-1885.
- Gao, y., Qian, S., Jianjun Zhang,J., Huang,Y., Liu,D., Preparation of apigenin nanocrystals using supercritical antisolvent process for dissolution and bioavailability enhancement. *European Journal of Pharmaceutical Sciences* 2013; 48: 740–747.
- Gradolatto, A., Basly, J.P., Berges, R., et al. Pharmacokinetics and metabolism of apigenin in female and male rats after a single oral administration. *Drug metabolism and disposition 2005;* 33:49–54, 2005.

- *Gavali, S.M., Pacharane, S.S., Jadhav, K.R.and Kadam, V.J. Cinical transfersome: a new technique for transdermal drug delivery. IJRPC, 2011, 1(3) 735-740.*
- *Goyal , A., Kumar , S., Nagpal, M., et al. Potential of novel drug delivery systems for herbal drugs. Ind J Pharm Edu Res, 2011; 45(3): 225- 235.*
- Gupta, S., and Mukhtar, H., Chemoprevention of skin cancer: Current status and future prospects. *Cancer and Metastasis* 2002*;* 21: 363–380.
- *Heather, A.E., Benson: Transdermal Drug Delivery: Penetration Enhancement Techniques. Current Drug Delivery, 2005; 2: 23-33.*
- *Honeywell-Nguyen, P.L. et al. Skin penetration and mechanisms of acti*on in the delivery of the D2-agonist rotigotine from surfactant-based elastic vesicle formulations. Pharm. Res., 2003; 20: 1619–1625.
- Henry W. Lim, H.W., William, D. James, M.D., Darrell, S., Adverse effects of ultraviolet radiation from the use of indoor tanning equipment: Time to ban the tan. *J am acad dermatol* 2011, 1-10.
- Huong, S.P., Bun, H., Fourneron, J.D., et al. Use of various models for in vitro percutaneous absorption studies of ultraviolet filters. *Skin Research and Technology* 2009; 15: 253–261.
- Horinaka, M., Yoshida, T., Shiraishi, T., et al. The dietary flavonoid apigenin sensitizes malignant tumor cells to tumor necrosis factor–related apoptosis-inducing ligand. *Mol Cancer Ther 2006*; 5:945-951.
- Jain S., Jain P. Transferosomes: A novel vesicular carrier for enhanced transdermal delivery: development, characterization and performance evaluation, *Drug Dev. Ind. Pharm*. 2003, 29: 1013-1026.
- Jain S., Mishra D., Kuksal A., Tiwary A. K., Jain N. K. Vesicular approach for drug delivery into or across the skin: current status and future prospects, www.Pharmainfo.net.
- Kulkarni, G.T., Herbal drug delivery system: An emerging area in herbal drug research. *Journal of Chronotherapy and Drug Delivery*, 2011, 2, 211-219.
- Kumar, A., Pathak, K. and Bali, V. Ultra- adaptable nanovasicular system : a carrier for Systemic delivery of therapeutic agents. *Drug Discovery Today* 2012, vol.17, 1233-1241.
- *Kaur, C.*D., *and Saraf, S., Herbal Photoprotective Formulations and their Evaluation. The Open Natural Products Journal, 2009; 2: 71-76.*

- Loan, P., Honeywell-Nguyen, Bouwstra, J.A., Vesicles as a tool for transdermal and dermal delivery. Drug discovery today, 2005, 2, 67-74.
- Li,B., Robinson, D.H., Birt, D.F., Evaluation of properties of apigenin and [G-3H]apigenin and analytic method development. J Pharm Sci. 1997; 86(6):721-5.
- Lin, C.C, Chiang, L.C., Lean, T.N., Anti-proliferative effect of apigenin and its apoptotic induction in human Hep G2 cells. Cancer Letters 2006; 237: 207–214.
- Maurya, S.D. et al., Enhanced transdermal delivery of Indinavir sulphate via transfersomes. *Int. J. Comp. Pharm*. 2012, 1, 1–7.
- Man, M.Q., Hupe, M., Sun, R., Topical Apigenin Alleviates Cutaneous Inflammation in Murine Models. Evidence-Based Complementary and Alternative Medicine 2012; 1-7.
- Mimeault, M. and Batra, S.K., Potential applications of curcumin and its novel synthetic analogs and nanotechnology-based formulations in cancer prevention and therapy. *Chinese Medicine,* 2011, 6(31), 1-19.
- Muller, R.H., Shaal,L.A., Shegokar,R., Production and characterization of antioxidant apigenin nanocrystals as a novel UV skin protective formulation. *Int. J. of Pharmaceutics* 2011 ; 420: 133– 140.
- Peppelenbosch , M.P., Ferreira, C.V., Aoyama, H., Cytotoxicity of apigenin on leukemia cell lines:implications for prevention and therapy. *Cell Death and Disease* 2010; 1-12.
- Prajapati, S.T., Patel, C.G. and Patel, C.N., Transfersomes: A Vesicular Carrier System for Transdermal Drug Delivery. *Asian Journal of Biochemical and Pharmaceutical Research,* 2011, vol. 1, 507-524.
- Patel, D., Shukla, S., and Gupta, S., Apigenin and cancer chemoprevention: Progress, potential and promise. Int. J. Oncology 2007; 30: 233-245.
- Shukla, S., and Gupta, S., Molecular targets for apigenin-induced cell cycle arrest and apoptosis in prostate cancer cell xenograft. *Mol Cancer Ther* 2006; 5:843-852.
- Svobodova, A., Walterova, D., Vostalova, J., Ultraviolet light induced alteration to the skin. *Biomed Pap Med* 2006; 150(1):25–38.
- Seema M. Jadhav, Pournima M., Karpe, M. and Kadam, V., novel vesicular system: an overview. *Journal of Applied Pharmaceutical Science,* 2012, 2(1), 193-202.
- Schatzlein, A and Cevc G, Skin penetration by phospholipids vesicles Transfersomes as visualized by means of the Confocal Scanning Laser Microscopy in

characterization, metabolism, and novel biological applications. Champaign. AOCS Press, 1995, 191-209.

- Sheo, D.M. et al., Transfersomes- A novel vesicular carrier for enhanced transdermal delivery of Stavudine: Development, characterization and performance evaluation. *J. Sci. Speculations Res.*2010, 1, 30–36.

- Svobodova, A., Psotova, J., Walterova, D., Natural phenolics in the prevention of uv-induced skin damage. J. biomed. 2003; 147(2):137–145.
- Zhaia, G., Zhaia, Y., Guoa, S., et al. Preparation and in vitro evaluation of apigenin-loaded polymeric micelles. Colloids and Surfaces A: Physicochem. Eng. Aspects 2013; 429: 24– 30.

8. PUBLICATION OF THE CANDIDATE

1. Jain,S.K., Jangdey, M.S., "Lectin Conjugated Gastro-retentive Multiparticulate Delivery System of Clarithromycin for the Effective Treatment of Helicobacter Pylori.'' *Int.J. Mol. Pharmaceutics*, 2009; 6(1) : 295-304. (Impact factor 5.5)
2. Sarwa, K.K., Mazumdar, B., Rudrapal, M., Jangdey, M.S., Verma, V.K., Capsaicinoids Content of Some Indigenous Capsicum Varieties of Assam, India. *Journal of Natural Sciences Research*, 2013; 3(4): 112-116.
3. Saraf, S., Gupta, A., Kaur, C.D., Jangdey, M.S., Saraf, S., Dermatological consequences of photosensitization with an approach to treat them naturally. *Pakistan j. Medical Science*, 2013

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