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Carbopol thickened nanoemulsions of chemotherapeutics to treat DMBA-induced breast cancer

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Abstract--Breast cancer is the most frequent invasive malignancy in women globally. The goal of this study was to prepare mitoxantrone and 4-methyl umbelliferone nanoemulsions followed by hydrogel preparation viz. MTO-NE-Gel and 4-MU-NE-Gel and characterized for various parameters like visual assessment, pH, rheology, ex vivo studies, and in vivo studies in 7,12-dimethylbenz(a)anthracene-induced breast cancer in swiss albino mice. The pH, viscosity, and spreadability were found to be 5.62 ± 0.05 , $13107 \text{ cP} \pm 658$, and $5.3 \text{ g.cm/s} \pm 0.28$ for 4-MU-NE-Gel and 5.75 ± 0.03 , $13025 \text{ cP} \pm 705$, and $4.06 \text{ g.cm/s} \pm 0.45$ for MTO-NE-Gel. The percent cumulative amount of drug permeated for 4-MU suspension, MTO Solution, 4-MU-NE-Gel, and MTO-NE-Gel were found to be $51.26\% \pm 5.22$, $15.54\% \pm 3.91$, $89.093\% \pm 6.8$, and $97.242\% \pm 4.51$ respectively after 8 h which was significantly higher in hydrogel formulations. The outcome of pharmacodynamic studies reveals a significant reduction in tumor weight and tumor volume for MTO-NE-Gel and 4-MU-NE-Gel treated mice as compared to MTO-Gel and 4-MU-sus-Gel. Histopathological evaluation of mammary tumors indicates improvement in the tissue anatomical features and a significant decreasing trend in levels of tumor marker CA15-3 was also observed. Based on the promising results of this investigation, it can be concluded that prepared

hydrogels can be a potential non-invasive therapy for the management of breast cancer.

Keywords---mitoxantrone; 4-methyl umbelliferone; breast cancer; DMBA; nanoemulsion based hydrogel; hydrogel

Introduction

Breast cancer is the most common cancer in women around the world and it can be cured in 70-80 % of individuals with early-stage and non-metastatic illnesses. In 2020, around 2.3 million breast cancer cases in females were reported, and due to this breast cancer has surpassed lung cancer as the most prevalent type of cancer worldwide, with 11.7% of total cancer cases. It is the sixth greatest reason for cancer mortality globally with 685,000 fatalities. Breast cancer is the most widespread type of cancer in women, reporting for 1 in every 4 cases and 1 in every 6 deaths, and it is the most common disease in the majority of countries (159 out of 185 countries) and for mortality in 110 countries (GLOBOCAN, 2020). In 85% of cases, it begins in ductal cells of the breast whereas in 15% of cases the glandular tissue lobules are affected. In the initial stages of malignant development i.e., inside the duct or lobule, the possibilities of metastasis are less and generally, no symptoms are observed (WHO, 2021).

Over 60% of Breast Cancer patients present with negative lymph nodes at an early stage, and more than half of cases are categorized as luminal (estrogen receptor (ER) positive HER2 negative) tumors. Even after their obvious excellent prognosis, the luminal lymph nodes' negative tumors can show variable behavior and lead to poor prognosis and the enhanced need for systemic therapy, such as targeted chemotherapeutic treatment, in combination with hormonal therapy (Rakha and Pareja, 2021).

The tumor microenvironment (TME) is a multifaceted condition that includes the presence of extracellular matrix (ECM), different cellular secretory factors, stromal cells, proteins, immune cells, blood and lymphatic vessels, RNAs, and other elements. TME contributes to carcinogenesis, tumor progression, metastasis, and recurrence. Surgery, radiation, immunotherapy, chemotherapy, or combinatorial therapy are now the most frequent treatments for breast cancer metastases. Novel drug delivery systems and nanoemulsions are considered to be a newer approach to enhancing therapeutic efficacy, have received a lot of interest in recent years, and have been investigated in the context of treatment (Gu.*et al*, 2021).

In recent years, many novel drug delivery techniques have been suggested to increase the efficacy of chemotherapy and decrease side effects, such as nanotechnology, which can enable selective drug accumulation in tumor tissue through passive and active targeting approaches. Although nanoemulsions have numerous benefits for controlled delivery, some have burst release, poor bioadhesion, and permanent deformation, making them unsuitable for long-term use. Hydrogels, a further efficient drug delivery method, are made up of a significant quantity of water and a cross-linked polymer network that has remarkable biocompatibility, low cytotoxicity, and a strong drug-encapsulating

ability and have been widely used for cancer therapy in recent years. Topical treatments are intriguing, but medication composition and dose must be thoroughly investigated. In the context of a topical method that bypasses first-pass metabolism in the liver, these low plasma levels indicated reduced systemic toxicity. These factors, taken together, significantly encourage the development of localized strategies for breast cancer prevention (Lazzeroni.*et al*, 2012).

Hyaluronan is a protagonist in the initiation and advancement of tumors, notably breast cancer. Hyaluronan's contribution to tumor development is based on its metabolism, which includes the regulation of HA synthases and hyaluronidases, which determine the amount and size of hyaluronan molecules, as well as the binding of hyaluronan to its cellular receptors, primarily CD44 and RHAMM, which trigger various oncogenic signaling pathway (Karalis.*et al*, 2019). 4-MU is a derivative of coumarin. 4-MU is a well-known medication that is already in use in humans. It's known as "hymecromone," and it's utilized in a variety of nations for its choleric, and biliary spasmolytic properties. 4-MU is usually regarded as a selective HA production inhibitor. 4-MU therapy causes tumor cell growth inhibition and apoptosis, which is coherent with HA's function in the cell survival mechanism. Exogenous HA was able to repair the apoptotic impact of 4-MU on smooth muscle cells. Along with the recognized function of HA in angiogenesis, 4-MU therapy is claimed to inhibit the development of new blood vessels necessary for metastases (Nagy.*et al*, 2015).

Mitoxantrone is a dihydroxy anthracenedione analog that has shown therapeutic effects in metastatic breast cancer, acute non-lymphoblastic leukemia, chronic myelogenous leukemia, and non-Hodgkin's lymphoma (Faulds.*et al*, 1991). It has received clinical approval and has efficacy against a broad range of antineoplastic action; it has been widely utilized in treating breast and prostate cancer. The suppression of deoxyribonucleic acid and Ribonucleic acid synthesis by intercalation into deoxyribonucleic acid and Ribonucleic acid-base pairs, as well as topoisomerase II inhibitor, an enzyme involved in deoxyribonucleic acid repair, is the possible mechanism for the anticancer activity of mitoxantrone. Myelosuppression, phlebitis, alopecia, nausea, vomiting, and diarrhea, are only a few of the typical adverse effects linked with mitoxantrone treatment (Ugwu.*et al*, 2005). Despite this, Mitoxantrone's therapeutic effectiveness is severely limited due to its high systemic toxicity and lack of specificity. Nanoemulsion may be able to alleviate these mitoxantrone adverse effects. This nanotechnology can effectively load and deliver medication, target it for site distribution, and increase drug accumulation while reducing side effects (Tao.*et al*, 2018).

Chemically produced mammary cancer in rodents has been usually employed to mimic human breast carcinogenesis throughout the years. Every mouse and rat model has its own set of benefits and drawbacks. Single doses of carcinogens like 7,12-Dimethylbenz[a]anthracene(DMBA) or nitrosomethylurea can be utilized to produce mammary tumorigenesis in vulnerable mice and rat strains (Abba.*et al*, 2016). The two main mammatrophic carcinogens, DMBA, and nitrosomethylurea can easily produce mammary cancers without the requirement for irradiation (Tsubura.*et al*, 2011).

According to the research cinnamaldehyde, has been shown to have 59 potential aims in the therapy of mammary carcinoma. Following that, gene ontology and the Kyoto Encyclopedia of Genes and Genomes enrichment study (WHO, 2021) revealed 83 physiological processes and 37 signaling pathways, including the peroxisome proliferator-activated receptor and PI3K-Akt pathway, which are closely related to tumor cell apoptosis, were linked with breast cancer (p 0.05) therapy.

In vitro cell line study showed that cinnamaldehyde inhibits cell growth, and changes in cellular structure, inhibits cell migration, invasion, and capabilities, and promotes cell death (Liu.*et al*, 2020).

The goal of this work was to incorporate MTO-NE and 4-MU-NE in a hydrogel system to enhance the viscosity for the ease of topical application. In this study the presented data to back up our theory, which asserts that MTO-NE and 4-MU-NE incorporated in the hydrogels demonstrated improved solubility, dissolution, and permeability, as well as promising absorption and bioavailability in vivo which may help in the management of breast cancer.

Method

Material

Mitoxantrone hydrochloride and 4-methyl umbelliferone were purchased from Cayman chemical, China, and Sigma Aldrich, India respectively. Dimethylbenz(a)anthracene (DMBA) and Rhodamine B were purchased from Sigma Aldrich, India, and Cinnamaldehyde oil was purchased from Loba Chemie, India. The rest of the chemicals and solvents utilized were of analytical grade.

Preparation of nanoemulsion

Preparation of 4-MU-NE and MTO-NE

Two separate nanoemulsions viz. 4-MU-NE and MTO-NE were prepared by utilizing an aqueous microtitration process with cinnamaldehyde, Tween-80, and Polyethylene Glycol 400(PEG-400) as the oil phase, surfactant, and as a co-surfactant respectively. 4-MU being oil soluble is solubilized in the oil phase and MTO being water-soluble is solubilized in water. In a 5 mL glass container containing cinnamaldehyde(as oil phase), Tween 80 and PEG-400 (S_{mix}) were added, and the mixture was mixed thoroughly using vortex mixture until the oil and surfactant phases were miscible. This combination was diluted with water to make nanoemulsions (Ali and Hussein, 2017).

Preparation of 4-MU-NE and MTO-NE based hydrogel

Carbopol 940 was used to convert 4-MU-NE and MTO-NE into a hydrogel system. This was accomplished by allowing 1.5 percent w/w of Carbopol 940 to expand for 12 hours in double-distilled water. After 12 hrs, 4-MU-NE and MTO-NE were separately added to the swelled Carbopol along with triethanolamine for effective crosslinking (Oktavia Indrati.*et al*, 2020).

Characterization of nanoemulsion loaded hydrogel

Organoleptic Evaluation

All developed nanoemulsions-based gel formulations were observed physically for the color and visual phase separation (Mulia.*et al*, 2018).

Physicochemical characterization

pH & Viscosity

Using a previously calibrated pH meter (Mettler Toledo, Langacher, Switzerland), the pH of 4MU-NE-Gel and MTO-NE-Gel was measured at room temperature (Gull.*et al*, 2020).

A Brookfield viscometer (DV II + Pro, US) of the cup and bob type was used to evaluate the viscosity. This work was carried out at room temperature using spindle #64 to produce a 60 rpm shear rate for the prepared formulations(Archana.*et al*, 2015).

Spreadability study

The spreadability experiment was performed using the equipment described by (Multimer, 1956). The apparatus consisted of a wooden block with a gauge and two glass slides with a pan fixed on a lever. In between the two glass slides, the excess formulation is sandwiched. To achieve uniform thickness, the formulation was compressed using 100 g weight by placing it on the upper glass slide for 5 min. To the pan, 100g weight was added. Spreadability was determined by the amount of time it took to separate the two slides in seconds.

The following formula was used to calculate the spreadability

$$S = (m \times l)/t$$

here S stands for spreadability, length of glass slide as l, m for weight fixed to the top slides, and t for the time taken to separate the slide from each other in seconds (Sabale and Vora, 2012).

Ex vivo permeation study

This study employed a vertical Franz diffusion cell system, with the whole assembly (Logan Instruments Corp., NJ, USA) consisting of three permeation cells. Each cell had an effective permeation area of 0.6 cm² and the donor and receiver compartments had a capacity of 1 ml and 5 ml, respectively. (Khuroo.*et al*, 2014).

From the swiss albino mice mammary, the shaved and treated skin was taken, thereafter skin was placed between the donor and receiver compartments such that the stratum corneum faces upward. The skin was stabilized for 15 minutes with 7.4 pH phosphate buffer saline (PBS) before the initiation of the permeation study. For the whole procedure, the temperature and stirring rate were set at 37 ± 1 °C and 100 rpm, respectively. Once the equilibrium has been established the former medium was replaced with a 7:3 v/v combination of phosphate buffer saline (pH 7.4) and ethanol (Ahmed.*et al*, 2019).

4-MU-NE-Gel and MTO-NE-Gel permeation profiles (Table 1) were compared to permeation profiles of 4-MU suspension (4-MU-sus-Gel) and MTO- Accurately weighed 1 g of all of these formulations were administered to the mice's skin via the donor compartment. At predetermined intervals, a 1 ml sample was drawn from the receiver compartment and replaced with an equivalent volume of fresh medium. (Ahmed.*et al*, 2019). For the MTO and 4-MU quantification, the HPLC technique that has previously been validated was utilized. Using the following equation, the cumulative quantity of drugs passed through the mice's mammary skin (Q_n) at a certain interval was calculated (Gull.*et al*, 2020).

$$Q_n = \frac{C_n \times V_0 + \sum_{i=1}^{n-1} C_i \times V_i}{S}$$

Pharmacodynamic study

Ethical compliance in animal handling

The research began after official approval from the CPCSEA and the IAEC (Approval No. CPCSEA/IAEC/AIP/2020/08/25). Animal House, Amity Institute of Pharmacy, Amity University, Sec 125, Noida, 201313, Uttar Pradesh (Registration No.1327/PO/ReBi/S/10/CPCSEA) was used for conducting the research work.

Animal model

36 Swiss albino mice (3 weeks age), (weight 23.2 ± 0.91 g) were procured. The animal facility was kept at a constant temperature of $22 \pm 2^\circ\text{C}$, with adequate relative humidity and a 12-hour light/dark cycle. A standard pellet diet and purified water ad libitum were given to the animals throughout the study. All animals were handled and tested according to the protocol. For the *in vivo* study on albino mice, the mice were divided into 6 different groups, each having six animals (Minari, 2014).

Preparation of standard gel (4-MU-sus-Gel and MTO-Gel)

4-MU (20mg/kg) suspension was prepared by suspending it in 0.1%w/v of carboxymethyl cellulose (CMC) then the suspension was incorporated into the swollen Carbopol 940 system along with a few drops of triethanolamine resulting in the formation of Standard 4-MU-Sus-Gel similarly Standard MTO-Gel was prepared by dissolving MTO (2mg/kg) in double-distilled water followed by incorporated into the swollen Carbopol 940 system along with a few drops of triethanolamine.

Treatment modalities

The vehicle control group, Group I, received solely sesame oil as a vehicle through oral administration. Mice in Group II (Disease Control group) were given 50 mg/kg DMBA mixed in sesame oil, orally one time a week for four weeks., Here day 0 is considered the start of the experiment i.e., the day when tumor volume reaches 0.5 cm in dimension, and day 18 is the last day of dosing. From group III to group VI, dosing starts after mammary tumor development by DMBA administration, for each mice day 0 is considered as the start of the experiment i.e., the day when tumor volume reaches 0.5 cm in dimension. In Group III i.e, Standard Mitoxantrone treated group, there are the age-appropriate mice, which received MTO-Gel 2.0 mg/kg on days 0,3,7, and 10 topically. In Group IV i.e MTO-NE-Gel treated group, MTO-NE-Gel 2.0mg/kg was applied topically by using a spatula at the tumor site on days 0,3,7, and 10. In Group V i.e, Standard 4-MU treated group,4-MU-Gel 20 mg/kg, was administered every day for 14 days, i.e., from day0 to day14. To the Group VI animals, i.e 4-MU-Gel treated group, 4-MU-NE-Gel 20mg/kg was applied topically by using a spatula on the tumor site for 14 days, i.e. from day 0 to day 14 of the experiment.

The animals were treated for 2 weeks. The experimental and control animals were closely monitored daily, and their weights were taken once a week. Each mouse has six pairs of mammary glands, which were examined, touched, and palpated

daily. The fundamental characteristics of mammary carcinogenesis, such as tumor weight and tumor mean volumes, were analyzed to observe whether any abnormal masses were developing early. The mice were sacrificed by cervical dislocation after completion of the study (Karimi.*et al*, 2019).

The tumors volume was estimated using the method

$$V = \frac{w^2 \times l}{2}$$

V is tumor volume, w represented as the width of the tumor, and l represented as tumor length (Faustino-Rocha.*et al*, 2013, Padhi.*et al*, 2016)

Histopathological study

At the completion of the research, blood samples were taken through the cardiac puncture collection method under ether anesthesia, and the animals were subsequently decapitated. All mammary tumors were immediately dissected in all groups.

For histopathological investigation, the tumors were first set in 10% formalin buffer and then fixed in paraffin wax, followed by staining with hematoxylin and eosin (H&E). (Karnam.*et al*, 2017).

Assessment of tumor marker CA15-3

CA 15-3 (Cancer Antigen 15-3) is a mammary cancer tumor marker. It is one of the primary circulating prognostic markers for breast cancer, The level of the tumor marker CA15-3 in the blood was measured using a commercial kit with LIAISON® CA15-3® chemilluminescence immunoassay. The analysis was carried out according to the specifications mentioned in the kit. CA15-3 levels in the blood were analyzed in IU/ml.; (Duffy.*et al*, 2010, Karimi.*et al*, 2019)

Statistical analysis

The mean ± standard deviations were used to express all of the data using the statistical software GraphPad Prism, One-way ANOVA was performed for statistical analysis, followed by Tukey's multiple comparison test. Differences with p-values less than 0.05 were thought to be significant.

Results

Preparation of 4-MU-NE and MTO-NE based hydrogels when employing the dynamic light scattering technology, the average particle size of 4-MU-NE and MTO-NE was found to be 113.2±5.3 nm and 123.2±6.17 nm, respectively, while the polydispersity index of 4-MU-NE and MTO-NE formulations was found to be 0.254 and 0.281. The hydrogel for 4-MU-NE and MTO-NE was successfully formed with 1.5 % w/v Carbopol 940. The result of characterization is compiled in Table 1. On visual assessment, the formulations developed were found to be transparent, gel-like consistency, and no physical separation in the NE-based hydrogels was observed.

Characterization of hydrogels

pH and Viscosity

The observed pH of the 4-MU-NE-Gel and the MTO-NE-Gel, respectively, was 5.62 ± 0.05 and 5.75 ± 0.03. Because of its acidic nature, it will be physiologically compatible with the pH of the skin & will not irritate the skin.

The viscosities of 4-MU-NE-Gel and MTO-NE-Gel prepared with 1.5 %w/w of Carbopol 940 were found to be 13107 ± 658 cP and 13025 ± 705 cP respectively.

Spreadability
One of the prerequisites for a gel to satisfy ideal attributes is its spreadability. A gel formulation's treatment potential is also influenced by its spreading value. The spreadability of 4-MU-NE gel and MTO-NE gel was found to be 5.3 ± 0.28 g.cm/s and 4.06 ± 0.45 g.cm/s respectively.

Ex-vivo skin permeation studies

The penetration profiles of 4-MU and MTO nanoemulsion loaded hydrogels via dissected mice skin are illustrated in Figure 1. With increasing time, the cumulative amount of 4-MU-sus and MTO solution that pass through the unit skin area (Q_n) in the receiver medium were found to be 51.26 ± 5.22 % and 15.54 ± 3.91 % respectively. Whereas % cumulative amount of drug release for 4-MU-NE-Gel and MTO-NE-Gel were found to be 89.093 ± 6.8 % and 97.242 ± 4.51 % respectively. The release profile indicates a significant increase in drug permeation through the skin upon comparison with 4-MU-sus and MTO solution from 4-MU-NE-Gel and MTO-NE-Gel. Drug penetration from 4-MU-NE and MTO-NE gels was improved as compared with suspension and solution, respectively.

Pharmacodynamic study- Mice body weight, tumor volume, and tumor weight

As illustrated in Figure 2, the average body weights (mean \pm standard error) of animals of vehicle control (sesame oil), disease control (DMBA), standard MTO-Gel, and 4-MU-sus-Gel, MTO, and 4-MU-NE based gel can be easily investigated. The graph of vehicle control mice shows normal weight as compared to declining weight in disease control mice. Furthermore, it can be observed from the graph (Figure 2) that the weight gain of the animals in Standard MTO-Gel and standard 4-MU-sus-Gel is considerably less as compared to formulated therapies i.e., MTO-

NE-gel and 4MU-NE-Gel, respectively.

The mice in the vehicle control group did not indicate any signs or symptoms of mammary carcinoma. According to the findings of this study as shown in Figure 3, MTO-NE-Gel when compared with standard MTO-Gel significantly reduced DMBA-induced mammary cancer in Swiss albino mice with a p-value of < 0.05 whereas the 4-MU-NE-Gel exhibited a significant reduction in mammary cancer when compared with the standard 4-MU-sus-Gel treated group.

In comparison to the DMBA-treated control group, group 4, group 3, group 6, and group 5 exhibited a markedly lower tumor weight. The average tumor weight of the mice after four weeks of DMBA treatment found to be 4.418 ± 0.764 g (group 2) was significantly reduced to 2.472 ± 0.105 g (group 3) to 1.577 ± 0.156 g (group 4) and 3.89 ± 0.564 (group 5) to 2.87 ± 0.633 (group 6).

Before and after administration with both test drugs formulations, the volume of tumors revealed that it reduced tumor growth, and MTO-NE-Gel treated mice had smaller tumors than 4-MU-NE-Gel treated animals.

When compared to the positive control group, the weekly tumor development rate in 4-MU-NE-Gel and MTO-NE-Gel treated mice was slower. The recorded data of the final tumor volume showed that the standard MTO-Gel group, test MTO-NE-Gel groups, standard 4MU-Gel, and test 4-MU-NE-Gel were 3.173 ± 0.166 cm³,

1.957±0.109 cm³, 4.78±0.421 cm³, and 3.527±0.795 cm³, respectively, which were significantly smaller than the DMBA treated group (7.23±1.24 cm³).

Histopathological study

After hematoxylin and eosin staining, the excised specimen was sectioned at 5 µm and microscopy was performed. The untreated group's sections revealed large solid tumors arranged in sheets, having large nuclei. Tumors treated with standard MTO-Gel exhibited no substantial necrosis or degenerative changes, according to histopathology examination of the collected tumors. On the other hand, tumors treated with MTO-NE-Gel exhibited regions of degeneration in the form of apoptosis. 4-MU-NE-Gel treated mice tumor tissues, demonstrated a wider region of tumor necrosis defined by cell shrinkage, fragmentation, and chromatin absence. Figure 4(a-e) shows microscopic pictures of representative tumor samples removed from mice and sectioned across the center from each treatment group.

Assessment of tumor marker CA15-3

CA15-3 is a murine monoclonal antibody (molecular weight: 300–450 kDa) generated by normal breast cells. The tumor cells produce and shed more CA 15-3 in malignant breast cancers patients. Its concentration in blood when it enters the bloodstream is found helpful as a prime serum marker to track the tumor progression (Ghosh.*et al*, 2013).

In this study, serum CA15-3 levels in group III (standard MTO-Gel), group IV (MTO-NE-gel treated), group V (Standard 4-MU-sus-Gel), and group VI (4-MU-NE-Gel treated) were substantially lower than group II i.e., the DMBA treated disease control group. As illustrated in figure 5, in group IV and group VI, a significant reduction in the level of CA15-3 was detected when compared with group III and group V respectively. It may be attributed to enhanced permeation of drug-loaded NE-based gel formulations and more drug availability at the tumor site.

Discussion

4-MU and MTO-based nanoemulsions were successfully prepared by using the ratio of oil (Cinnamaldehyde) and S_{mix} as 1:9, keeping the ratio of surfactant (tween 80) and co-surfactant (PEG 400) constant to 4:1. The outcome of the developed nanoemulsions characterization concerning droplet size and PDI was found to be acceptable as it is lying in the nano range indicating the optimum combination of oil and S_{mix}.

Furthermore, two formulations i.e., 4-MU-NE and MTO-NE-based hydrogels were successfully prepared by dispersing the nanoemulsions in 1.5%w/v Carbopol 940. On physical examination, developed nanoemulsion-based hydrogels were transparent and have gel-like consistency displaying no physical separation.

Nanoemulsion-based hydrogels showed a pH range of 5.6 to 5.7 as that of normal skin pH range i.e., 5.5 to 7.5, proving the non-irritant nature of the formulations. The viscosity of the formulation affects the spreadability of the skin. Developed formulations displayed optimum viscosity and spreadability as values mentioned in Table 1, revealing uniform application on the skin along with ensured patient

compliance. Moreover, the values of spreadability signify that formulation can be easily applied with high slip and drag.

The penetration profiles of 4-MU and MTO-loaded formulations through isolated mice skin up to 8 h displayed a better permeation profile as shown in figure 1. This might be because of the high concentration of drugs in nanoemulsion globules, which creates a concentration gradient and permeation of drugs across the skin (Anzar.*et al*, 2018). Moreover, the nanoemulsion considerably boosts the rate of penetration due to its nanosize (200 nm), as nanosized droplets may easily pass the drug across the epidermal barrier and into the stratum corneum (Parveen and Sahoo, 2011). Furthermore, the solubilization capacity of the drug as well as its permeation across the skin is increased by S_{mix} because of the disruption of the interfacial barrier and partitioning, enhances because of the nanosize. The presence of water in hydrogel leads to hydration of the skin and promotes stratum corneum cells to swell which expands drug channels and enhances overall penetration. (Naz and Ahmad, 2015).

The outcome of the average body weights (mean \pm standard error) of animals of vehicle control (sesame oil), disease control (DMBA), standard MTO-Gel (Group 3) and 4-MU-sus-Gel (Group 5), MTO-NE-Gel (Group 4), and 4-MU-NE based gel (Group 6) displayed normal weight as compared to declining weight in disease control mice. Additionally, the weight gain of the animals in Standard MTO-Gel and standard 4-MU-sus-Gel is considerably less as compared to formulated therapies i.e., MTO-NE-gel and 4MU-NE-Gel, respectively indicating regaining of their normal body homeostasis. However, the bodyweight result was in alignment with (Karimi.*et al*, 2019) work and there were no significant differences observed between the body weights of the groups.

Owing to the better penetration profiles of the developed formulations, tumor weight and tumor volume of NE-based gel-treated mice were significantly lower when compared with their free drug solution represented in Figure 3. The optimum concentration of oil and S_{mix} contributed to the effective formulation development which facilitates the permeation of nanoemulsion globules in the DMBA induced mammary cancer. Moreover, the selection of cinnamaldehyde as an oil phase synergizes the cytotoxic potential of the developed formulations. The histopathology studies of DMBA-induced mammary tumors of female mice displayed different patterns for different formulations. As shown in Figure 4, an improvement in the histopathological evaluation of mammary tumors has been observed which may be ascribed to the better permeation profile of the developed formulations as compared to their free drug solutions. Moreover, the values of tumor marker CA15-3 represented in figure 5 indicate a downward trend with developed NE-based hydrogel formulations due to enhanced diffusion of drug across the mammary tumor(Karimi.*et al*, 2019).

Conclusion

The current research focuses on the developmental phases of a carbopol thickened nanoemulsion of chemotherapeutics viz. MTO and 4-MU along with cinnamaldehyde proceeded by characterization, permeation profile study, and *in vivo* studies on DMBA induced tumorigenesis in Swiss albino mice. The results of

ex vivo permeation studies of 4-MU-NE-Gel and MTO-NE-Gel were found to be significantly high as compared to their standard 4-MU suspension and MTO solution. A significant reduction in tumor weight and tumor volume for MTO-NE-Gel and 4-MU-NE-Gel treated mice was achieved as compared to MTO-Gel and 4-MU-Sus-Gel. A remarkable improvement in the tissue histology was noticed in the histopathological evaluation of mammary tumors accompanied by a significant decreasing trend in levels of tumor marker CA15-3. Based on the results, it can be concluded that 4-MU-NE-Gel and MTO-NE-Gel formulations can be a prospective non-invasive therapy for the management of breast carcinoma and limited adverse effects of chemotherapy.

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Tabel & Figures

Table 1: Compiled characterization of 4-MU-NE-Gel and MTO-NE-Gel.

S.No.	Formulations	Organoleptic Evaluation	pH	Viscosity (cP)	Spreadability (g.cm/s)
1	4-MU-NE-Gel	Transparent	5.62 ± 0.05	13107 ± 658	5.3 ± 0.28
2	MTO-NE-Gel	Transparent	5.75 ± 0.03	13025 ± 705	4.06 ± 0.45

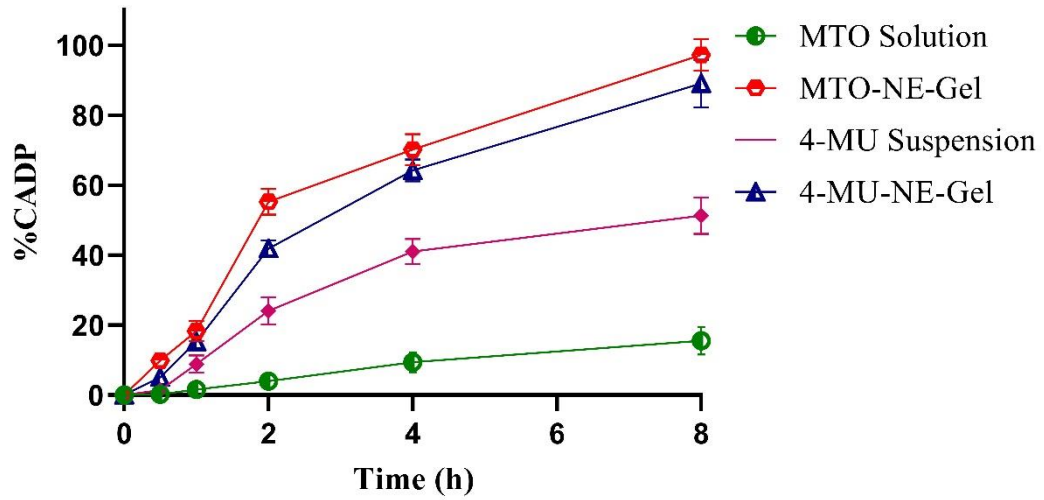


Figure 1: % CADP study of MTO solution, 4-MU suspension, MTO-NE-Gel, and 4-MU-NE-Gel through mice skin(n=3)

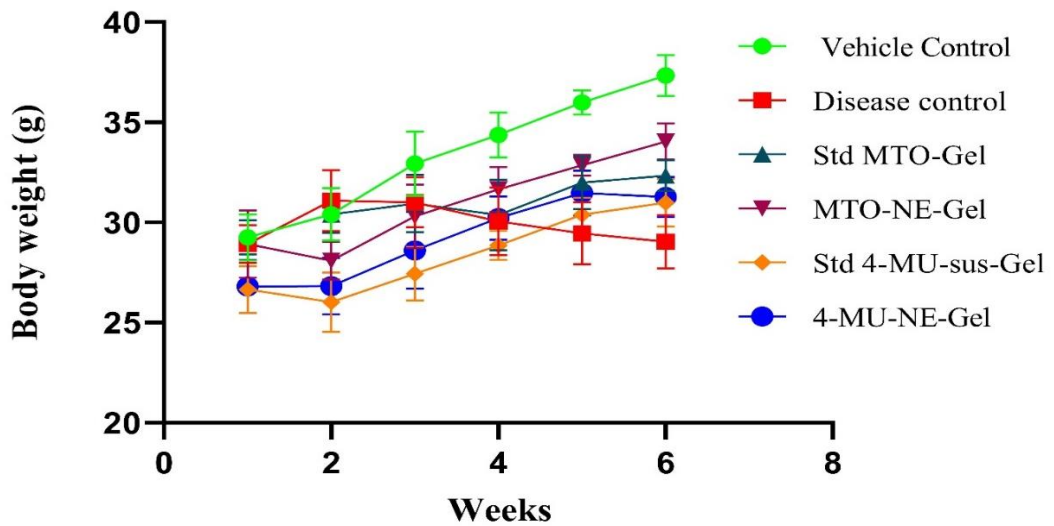


Figure 2: Effects of Standard MTO-Gel & 4-MU-sus-Gel and nanoemulsion loaded hydrogels MTO-NE-Gel & 4-MU-NE-Gel treatment on body weight rise in mice after DMBA-induced mammary cancer. The mean \pm SD for each statistic is shown. Each of the groups had six animals. At no point during the experiment, there was a significant difference in body weight between the animal groups.

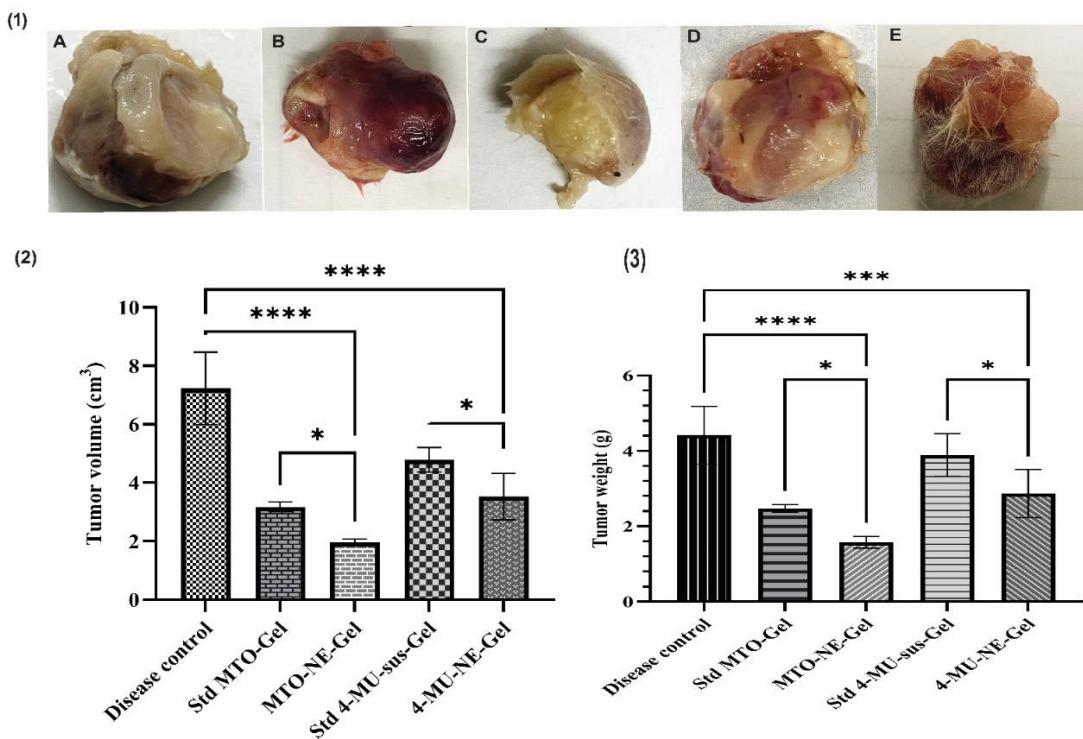


Figure 3: Effect of standard drug Gel and NE loaded Gel on DMBA induced Tumorigenesis in swiss albino mice. Figure 3(1)a-e: Images of tumor of group 2 - group 6. Figure 3(2): Tumor volume (cm³) in Swiss albino mice with mammary cancer (DMBA model) treated by Standard MTO-Gel, standard 4-MU-sus-Gel, MTO-NE-GEL, and 4-MU-NE-Gel. Figure 3(3): Effects of Standard MTO-Gel, Standard 4-MU-sus-Gel, MTO-NE-GEL, and 4-MU-NE-Gel treatments on DMBA induced mammary carcinoma on the weight of the tumor, n = 6. Data represented by one-way ANOVA test using Tukey test done for n = 6 mice per group shows mean \pm sd. Error bar indicates SEM, (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001).

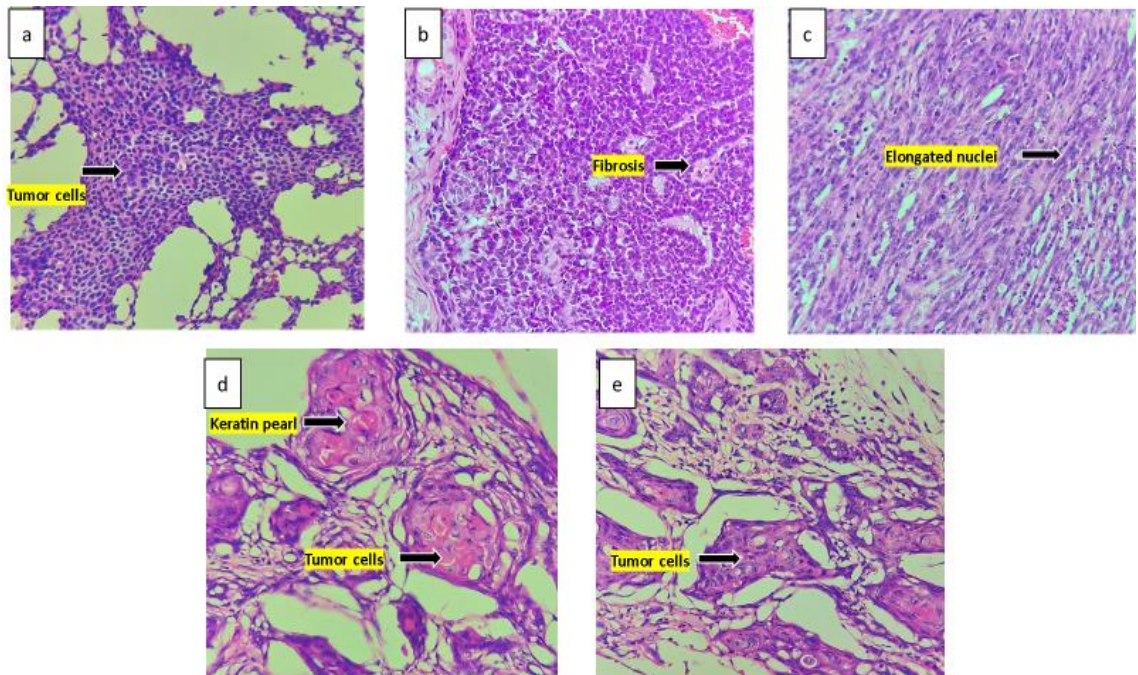


Figure 4: (a) to (e) shows the Histopathological profile of DMBA-induced mammary tumors of female swiss albino mice after 4 weeks of DMBA administration stained with hematoxylin and eosin (a)DMBA- treated tumor tissue shows the diffuse sheet of invasive carcinoma and high level of tumor-infiltrating lymphocytes (b)Figure b shows fibrosis and mild tumor infiltration in mice treated with standard MTO gel (2 mg/kg body weight). (c)Figure c presents tumor cells treated with MTO-NE-gel (2 mg/kg body weight) with a low level of tumor infiltration. The tumor cells have eosinophilic cytoplasm, indistinct cell membrane, elongated nuclei with coarse chromatin, moderate nuclear pleomorphism, and conspicuous to prominent nucleoli (d)figure standard 4MU-sus-gel treated tumor shows tumor cells arranged in nests and having eosinophilic cytoplasm, high nuclear-to-cytoplasmic ratio, with coarse chromatin and conspicuous nucleolus. Focal areas show keratin pearl formation. (e)however, the 4MU-NE-gel treatment noted a decrease in the number of inflammatory cells.

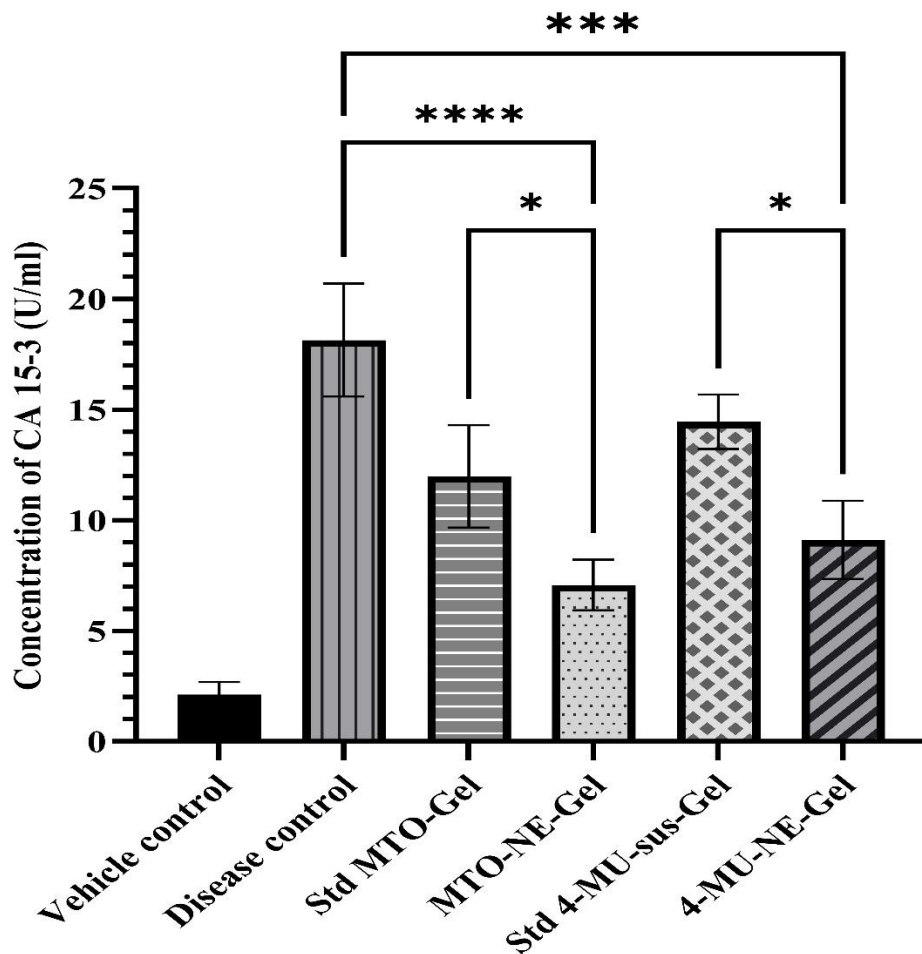


Figure 5: Representation of CA 15-3 tumor marker level in all mice groups. Data expressed as mean \pm SD. Error bar indicates SEM ($p < 0.05$).

Abbreviation

Mitoxantrone-MTO, 4-Methyl Umbelliferone- 4-MU, Hydrogel- Gel, Hyaluronic acid -HA, 7,12-Dimethylbenz[a]anthracene- DMBA, Polyethylene Glycol 400 - PEG-400