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DEVELOPMENT OF IMPLANTABLE DRUG DELIVERY SYSTEM OF EMBELIN FOR THE TREATMENT OF BREAST CANCER

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Abstract

Embelin is a traditional herbal medicine that exhibits anti-cancer effects in human breast cancer cells. However, the therapeutic effect of Embelin as Implantable Drug Delivery System is not yet determined. Embelin is having drawbacks like poor bioavailability and toxicity in systemic circulation, so we opt for Implantable Drug Delivery System. In this study, we optimised, developed and evaluated the Implantable Drug Delivery System of Embelin as pellets. The prime objective of the study is to prolong the drug release as much as possible using a combination of naturally occurring biodegradable polymer such as Guar gum, Chitosan, Xanthan gum, Locust bean gum. In this study, integration of Embelin and Chitosan was achieved via Granulation, with the aid of Guar gum as an excipient, followed by Extrusion /spheronisation. In vitro drug release study revealed the chitosan blended Embelin loaded implant possesses a longer, yet steadier, sustained drug release behaviour than the other three bio polymeric implants. It exhibits prolonged drug release of up to nine days and Korsmeyer's-peppas plot were exhibited.

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Introduction

Cancer refers to a group of disease that arises from mutated cells that have acquired the capacity to proliferate indefinitely and evade apoptosis, which eventually leads to tumour formation and subsequent invasion to surrounding tissues [1-2]. Breast cancer is the most commonly occurring cancer in women and the second most common cancer overall [3]. It refers to a group of disease in which cells in breast tissue change and divide uncontrollably, typically resulting in a lump or mass. The risk factors include, familial history, alcohol use, overweight, use of birth control pills, post-menopausal hormone use, exposure to large amount of radiation at younger age, lack of exercise, never having children or having first child after age 35, not breast feeding, weight gain, older age at menopause and younger age at first period. The risk factors can be limited by maintaining a healthy diet, adding exercise to our daily routine, reduce alcohol intake, limit menopausal hormone usage and if possible, breast feed [4-6]. Current cancer treatment options include surgical intervention, chemotherapy, and radiation therapy or a combination of these options. There are many chemotherapy drugs used for breast cancer treatment, including alkylating agents (such as cyclophosphamide), anthracyclines (such as epirubicin), antimetabolites (such as methotrexate), anti-mitotic (such as vinorelbine), and taxanes (such as paclitaxel). The agents are nonselective and can also damage healthy normal tissues, causing severe unintended and undesirable side effects, e.g., loss of appetite and nausea [7]. An important challenge in treating cancer is to find a technology for controlled targeted drug delivery and release to eradicate tumour cells without sparing normal cells [8]. The merits of Implantable Drug Delivery System include they can be manufactured cost-effectively and administered to a desired site to achieve the goal of safety, efficacy and patient acceptance, The polymeric components may have additional functions, such as structural support and improvement of biocompatibility or stability [9]. The implantation is typically done in subcutaneous or intramuscular tissue A significant advantage of biopolymeric Implantable Drug Delivery Systems is that they do not demand surgical extraction after use, as they can be degraded naturally by the body [10]. Different biopolymers such as guar gum, xanthan gum, chitosan, locust bean gum is used. The drug release from biodegradable polymeric systems is controlled either by diffusion, degradation or combination of both [11]. Pelletization is an agglomeration process that converts fine powders or granules of bulk drugs and excipients into small, free-flowing, spherical or semi-spherical units, referred to as pellets. The pellet formation and growth may occur in a number of ways: Agitation, Compaction, Extrusion /spheronization, Drug layering, Globulation.

Embelin extracted from Embelia ribes berries, commonly known as Vidanga [12]. Embelia ribes has been shown to possess astringent, carminative, stimulant, antioxidant, anti spermatogenic, antibacterial, antianxiety, anticancer, anti-inflammatory, antidiabetic, antidyslipidemic, analgesic and cardioprotective activity [13]. Embelin, the natural small molecular inhibitor of XIAP. It is an NF-kB blocker and potential suppressor of tumorigenesis, The ability of Embelin to prevent tumour growth is by inducing apoptosis and activating pro-apoptotic genes required in controlling the metastatic spread of cancer. Due to poor oral bioavailability and undesired nature of hydrophobicity and systemic toxicity when administered infusion, implant is proposed.

Materials and methods

The investigation made use of a diverse range of chemicals, each contributing to specific aspects of the research. These chemicals and their respective sources are as follows: Embelia ribes berries were obtained from the local market in Kerala, while n-hexane, acetic acid, methanol, and sodium hydroxide were sourced from Nice Chemical in Cochin. Dichloromethane was provided by Chemdyes Corporation in Rajkot, Gujarat, and petroleum ether, along with potassium dihydrogen orthophosphate, came from Spectrum Reagent and Chemical, also based in Cochin. Guar gum, xanthan gum, and locust bean gum were acquired from Yarrow Chem Product in Mumbai, and Mefenamic acid was supplied by Research Lab Fine Chem Industries, also in Mumbai. Chitosan was obtained from Hi Media Laboratories, Mumbai.

Extraction and Isolation of Embelin

The berries of Embelia ribes were coarsely powdered and undergo the process of extraction i.e., 250 mg of dried powder is extracted with 500 ml of n- hexane in Soxhlet apparatus for 6 hours. The residue is obtained by removing the solvent by distillation and the obtained residue is washed with 500 ml of cold petroleum ether. The residue is dissolved in a mixture of 250 ml dichloromethane and 250 ml of methanol and kept for crystallisation for 24 hours which then undergo recrystallisation by washing the crystals with n-hexane. The golden coloured Embelin crystals were obtained and were characterised using organoleptic properties.

Characterisation of isolated Embelin

The physical appearance was determined through visual observation. For confirmation Embelin crystals dissolved in petroleum ether and dilute ammonia solution. To assess the melting point, a capillary melting point apparatus was **employed. UV-spectroscopy was carried out using a Shimadzu UV Spectrophotometer.**

For TLC profiling, 1 mg of isolated embelin was dissolved in 10 ml of dichloromethane. 10 µl test sample was applied to a pre-coated silica gel G aluminium plate. The chromatogram was developed using mobile phase composed of Ethyl acetate, Toluene, Methanol, and Formic Acid in a specific ratio. TLC plate was developed and observed under ultraviolet light, and the Rf value was determined. 1H NMR was done using sophisticated NMR spectrophotometer and frequency was 400MHZ.

For FT-IR spectroscopy, spectra were obtained using a FT-IR spectrophotometer. The potassium bromide (KBr) disk method was utilized, which involved mixing of the drug powder with spectroscopic KBr and compressing it in a vacuum press to create a disk. The Infrared Spectrum was recorded by scanning over a wave number range of 400-4000 cm. Characteristic IR spectra and peaks were observed and compared with the spectrum and peaks of the reference Embelin spectrum.

development of Embelin Implant

Table 1. Formulation composition

Formulation code	Ingredients
F1	Embelin (1%) + Guar gum (99%)
F2	Embelin (1%) + Xanthan gum (99%)
F3	Embelin (1%) + Locust bean gum (99%)
F4	Embelin (1%) + Chitosan (5%) + Guar gum (94%)

Pilot study is conducted to optimise the level and type of biodegradable polymer required to extend the drug release using Embelin. Implants were prepared with different biopolymers such as guar gum, xanthan gum, chitosan, locust bean gum etc.The Embelin mixed with biopolymer in a glass mortar. After that small quantity of water is added and mixed thoroughly, until it becomes a sticky dough mass. The dough mass was fed into the cylinder of the extruder and was extruded in the form of long rods through the nozzle. The rods were cut into 2.5 sized implants, which is then dried at 40 ^{o}C. In case of F4, drug is mixed with previously soaked chitosan. In order to make it in the form of a dough mass, it is mixed with guar gum powder and extruded.

Pivotal study for the development of Embelin Implant

Pivotal study is conducted to develop pellets of Embelin and to conduct various quality control tests for evaluation. Implants were prepared with Chitosan as per the formula given in Table:1. Embelin in combination with chitosan and guar gum were used. They undergo pelletization and then implants are developed.

Evaluation of Implant

Formed Embelin implant undergo certain evaluation such as diameter of implant, weight variation, swelling index, stability testing, dissolution studies, drug polymer interaction studies etc.

The diameter of the implant was measured accurately using Vernier callipers14. For dissolution studies, the implants were immersed in a 400 ml phosphate buffer dissolution medium at pH 7.35 within a USP Dissolution Type 1 Apparatus. This buffer solution was prepared from sodium hydroxide and potassium dihydrogen phosphate, and the apparatus was placed in a water bath at 37°C and agitated at 60 rpm. At predetermined intervals, 3 ml samples were withdrawn and replaced with an equal volume of fresh buffer. The amount of drug released from the implants was quantified through UV absorption spectroscopy at 274 nm [15]. Weight variation analysis involved the random selection of samples, each of which was individually weighed on an electronic balance.

The swelling index was evaluated by immersing the implants in a swelling solution with phosphate buffer at a specific pH. After one hour, the implant's weight was measured, and any excess solution was gently removed by tapping the surface with a dry piece of filter paper. The degree of swelling for each implant formulation at a given time was

calculated using a specific formula, where Wt. and Wo represents the sample's weight at any given time and in the dry state, respectively [16].

$$H = \frac{Wt. - W0}{W0} \times 100$$

Additionally, an assay was conducted by adding 100 ml of phosphate buffer at pH 7.4 to the sample. The mixture was sonicated for 20 minutes to dissolve both the drug and the polymer. It was then analysed spectrophotometrically at a wavelength of 290 nm. The content of Embelin was calculated by comparing the results with a standard solution. Formulation was stored at 40°C/75% RH as per ICH guidelines and various physicochemical parameters were monitored periodically for 3 months17to conduct stability testing.

Results and discussion

Extraction and isolation of Embelin

Embelin is successfully extracted and confirmed by physicochemical evaluation. The isolated embelin is found to be an orange coloured solid, having melting point 1430C. The percentage yield was found to be 1.4%w/w. The isolated compound was found to be soluble in organic solvents like chloroform, methanol etc and insoluble in water. It gives purple coloured precipitate with petroleum ether and dilute ammonia solution. The identification of compound was also done by TLC.The Rf value of isolated Embelin found to be 0.75 which is very identical to that of standard Embelin 18. The isolated embelin is characterised by various spectral analysis (UV, IR, NMR). UV spectral analysis was carried out for the identification of embelin and the absorption peak was found to be at 339.2nm. The NMR spectra obtained is also identical to that of standard Embelin. IR revealed the functional groups present in Embelin. All spectra were identical to that of standard Embelin.

Formulation of pellet using guar gum

Drug is released by the end of 48thhr as depicted in the **figure 1**. On exposure to dissolution fluids,
the polymer gets hydrated and forms a viscous gel layer that slows down further penetration of dissolution fluid towards the core of the pellet.

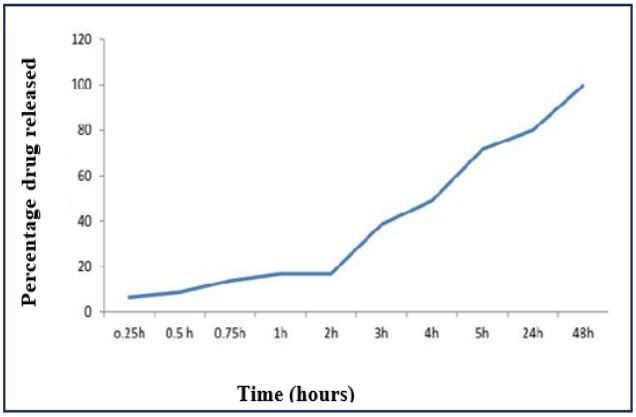

Figure1. Invitro drug release of F1(Embelin +Guar gum)

Formulation of pellet using Xanthan gum

The highest drug release of up to 92% is achieved in 24 hrs as shown in **figure 2.** The drug release progressively increases as time proceeds.

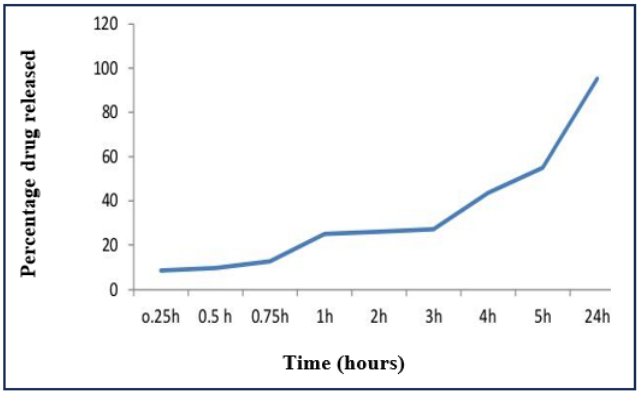

Figure 2: Invitro drug released from F2(Embelin+ Xanthan gum)

Formulation of pellet using Locust bean gum

It is found out that Locust bean gum has got synergistic effect with xanthan gum when used in matrix tablets. Locust bean gum has property to increase the solubility of some lipophilic drugs. The in vitro and in vivo studies revealed that locust bean gum and chitosan was capable of protecting the drug from being degraded in stomach or small intestine19 as shown in **figure 3.**

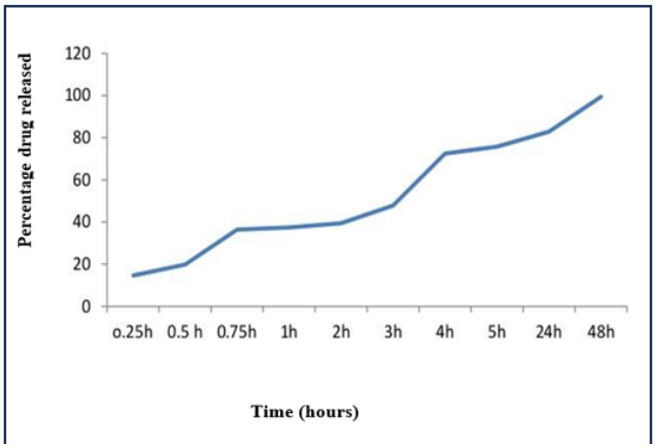

Figure 3. Invitro drug released from F3(Embelin +Locust bean gum)

Formulation of pellets of embelin using Chitosan (F4)

The release pattern of Embelin from implants demonstrated a three-phase release profile: a fast release of up to 46.2% during initial 24 hours, followed by a drug release of up to 74.5% in the following 5 days, thereafter sustained the drug release for 9 days. Thus, the drug release was maintained for a period of 9 days and found to berelease 96.9% at 216thhr. The drug release sharply increases as time progress as shown in figure 4. Due to the hydrophilic nature of natural polymer Chitosan is expected to absorb water and sustain the drug release.

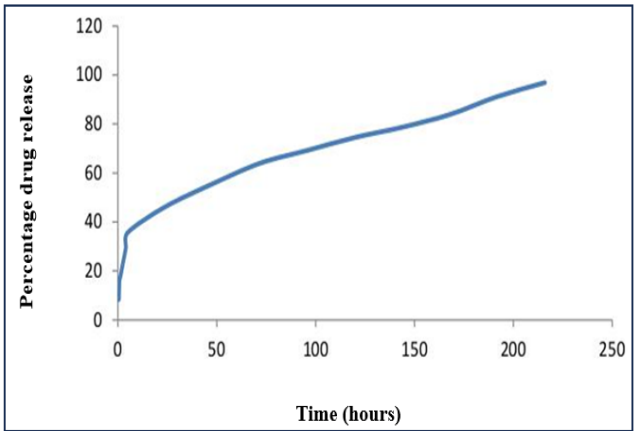

Figure 4. Invitro drug released from F4 (Embelin +Chitosan +Guar gum)

FTIR Spectrum of Embelin pellets

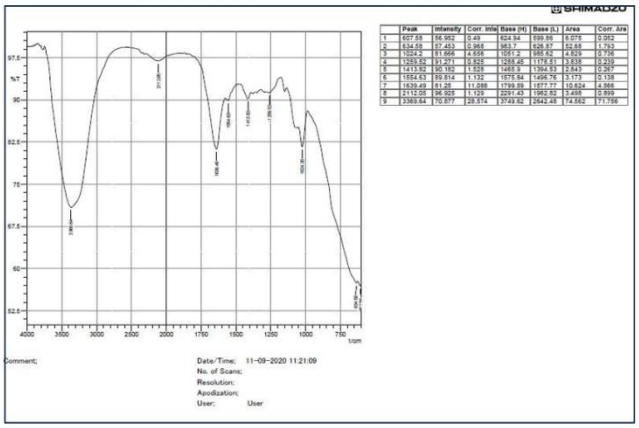

Figure 5. Spectrum of Embelin pellets

FT -IR spectrum shown in Figure 5. Revealed that there is no chemical interaction between embelin and biopolymers. The method of preparation does not affect the stability of Embelin. The formulated implant is then evaluated for various parameters and their values are within the pharmacopeial limit. The drug content in all the batches of formulation was found to be between 89% and 99.4%. Implants mean diameters were almost uniform in all the batches of implant formulations and were found to be in the range of 1.01 mm to 1.02 mm. The weights of all the implant formulations (n=6) were found to be in the range of 5.95±0.05 to 6.02±0. 03mg.Percentage of drug content was found to be between 89.0±0.02 and 99.4±0.02%. The swelling index was found in the range of 2.39 to 3.19.

Drug release rate kinetics

Table 2. Release rate kinetics data of the implant formulation

Mathematical model	Value of R2
Higuchi model	0.93
Zero order model	0.97
Korsmeyer-Peppas model	0.98
First order model	0.96

Table 2: illustrates the drug release kinetic data obtained from the release profile of implants. Korsmeyer-Peppas model gave a good fit for the drug release profile of all implant with greater regression coefficients in comparison to other models as shown in figure 6.

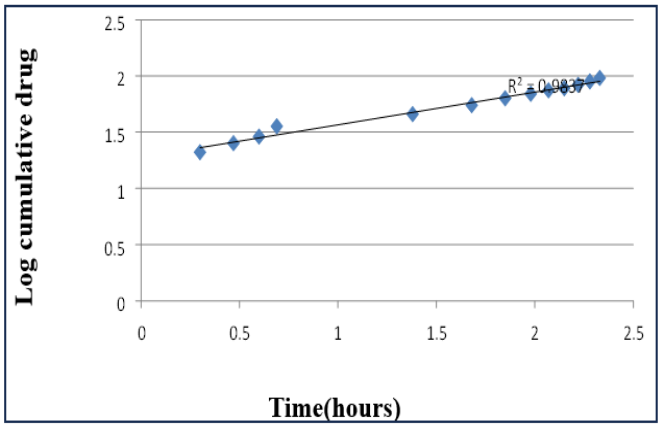

Figure 6. Korsmeyer-Peppas model

Conclusion

Cancer refers to a group of disease in which the cells have acquired the capacity to proliferate indefinitely and evade apoptosis. In this research work, we made an attempt to develop anImplantable Drug Delivery System of Embelin which can be used for the treatment of breast cancer. Embelin is a traditional herbal medicine that exhibits anti-cancer effects in human breast cancer cells. The objectives of the study are to extract Embelin from Embelia ribes berries and to formulate and evaluate Implantable Drug Delivery System of Embelin in the form of pellets. The Embelin is extracted from Embelia ribes berries. Pilot study using four naturally occurring biodegradable polymers was conducted to optimise the level of biopolymers required for the extent of drug release. The results shown that the combination of Chitosan and Guar gum with Embelin is able to prolong the drug release for 9 days. The pivotal study isdone to develop pellets of Embelin and to conduct evaluation processes. The implants were evaluated for drug content, length, diameter, weight variation, swelling index, stability testing and dissolution characteristics. Therefore, the Implantable Drug Delivery System of embelin was formulated, evaluated and studied.

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Conflict of interest

Authors are declared that no conflict of interest.

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Ethical Consideration and Inform Consent

Not applicable

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