DESIGN AND DEVELOPMENT OF BREAST CANCER RESEARCH USING NANO SUSPENSION AND EFFECTIVE DOSING AND TRACKING DEVICE.

INSTRUMENT AND FORMULATIONS DEVELOPMENT

Cancer has become one of the ten leading causes of death in North America. It is estimated that there are nearly 2 to 2.5 million cancer cases at any given point of time in India. Over 7 to 9 lakh new cases and 3 lakh deaths occur annually in India due to cancer, whereas in Karnataka there are about 1.5 lakh prevalent cases of cancer and about 35,000 new cases are added to this every year. Based on the consolidated report of cancer registries the overall common cancer sites in South India are stomach for males and cervix for females. According to the Canadian Cancer Society, the Canadian breast cancer death rate is going down as a result of better screening and more effective treatments. This is also the case in most industrialized countries. So the major contribution to decrease of death rate of breast cancer can only the improving the existing screening scheme and make it available on lowest cost as much as possible even free.

OBJECTIVES OF THE PROJECT:

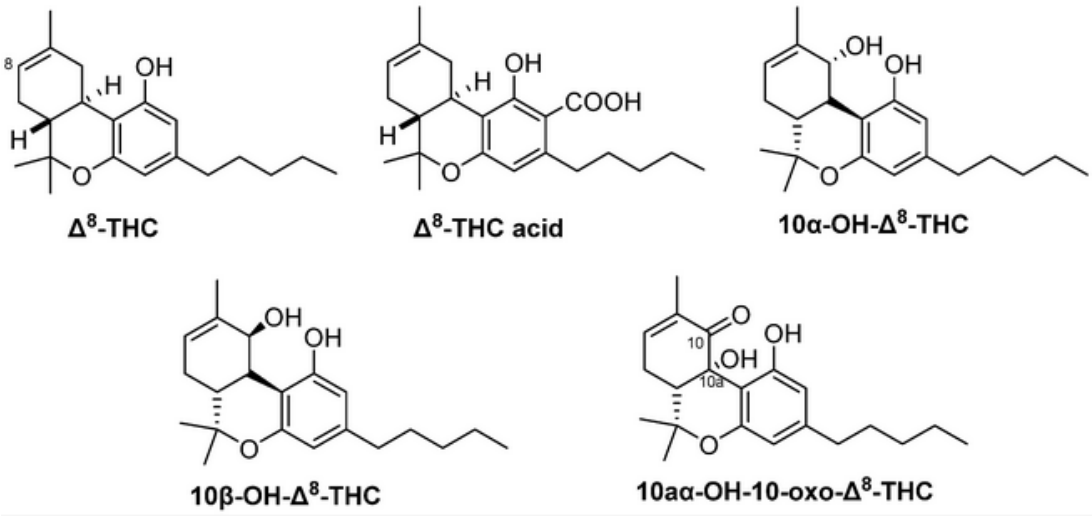
1. Develop the cost-effective medical device to deliver precise dose and collect vital information
2. Develop the effective formulation suitable for the delivery device utilizing nano technologies
3. Development of algorithms/technologies for the early detection for breast cancers

APPLICATIONS OF THE INSTRUMENTS / SYSTEM / FORMULATIONS TO BE TAKEN UP FOR DEVELOPMENT.

1. Medical device developed by IVPMED
2. AI platform integrated within the medical device
3. Nano suspension (oil in water)

Chemistry and Pharmacology of Delta-8-Tetrahydrocannabinol

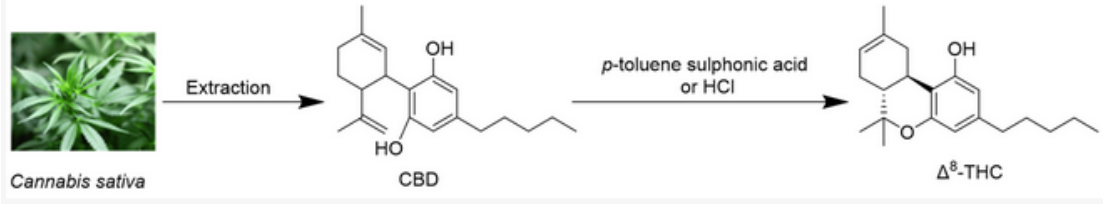
In 1966, another psychoactive cannabinoid, Delta-8-tetrahydrocannabinol (Δ^8-THC) was isolated from marijuana grown in Maryland. Δ^8-THC is gaining increased popularity due to its better stability and easier synthetic manufacturing procedures compared to Δ^9-THC. The passing of the U.S. Farm Bill in 2018 led to an increase in the sale of Δ^8-THC in the United States. Δ^8-THC is the positional isomer of Δ^9-THC and represents one of the minor groups of cannabinoids with the same chemical skeleton of tetrahydro cannabinoids. In 1985, the U.S. Food and Drug Administration (FDA) approved an oral drug product containing synthetic Δ^9-THC (Marinol® capsules) to manage nausea and vomiting resulting from chemotherapy. Seven years later, it was approved by FDA as an appetite stimulant in HIV/AIDS patients. The legal situation of Δ^8-THC in the USA varies from one state to another.



Chemical structure Δ^8-THC and its derivatives isolated from cannabis.

Synthesis of Δ^8-THC

Since the concentration of the naturally occurring Δ^8-THC in the cannabis plant is exceedingly low, its extraction holds little economic viability due to the substantial associated expenses. Consequently, almost all the Δ^8-THC on the market today is synthetically produced, predominantly from the chemical conversion of cannabidiol (CBD). CBD can be readily transformed into Δ^8-THC through acid-catalyzed cyclization reaction.



The synthetic pathway of Δ^8-THC.

Pharmacology of Δ^8-THC

The endocannabinoid system comprises the endocannabinoids, cannabinoid receptors, and enzymes responsible for the synthesis and degradation of endocannabinoids. Both cannabinoid binding receptors, CB1 and CB2, are G protein-coupled receptors that are part of the endocannabinoid system interacting and responding to cannabinoids and endocannabinoids. CB1 receptors are highly concentrated in specific brain regions and are less abundant in a more widespread manner, primarily influencing the psychoactive effects of cannabinoids. CB1 receptors affect functions like mood and appetite. Their activation is associated with the psychoactive effects of cannabis. CB2 receptors have a more limited distribution, being located in various immune cells and a small number of neurons. CB2 receptors mainly modulate

inflammation and immune cell activity without causing psychoactive effects. Both CB1 and CB2 receptors predominantly couple to inhibitory G proteins, sharing pharmacological influences with other GPCRs. Consequently, the cellular response to specific cannabinoid receptor ligands is intricately shaped by factors such as partial agonism, functional selectivity, and inverse agonism. Interestingly, Δ^8-THC exhibits the ability to competitively attach to the orthosteric sites of both CB1 and CB2 receptors, with Ki values falling within the nanomolar (nM) range. Notably, the Δ^8-THC intriguing effects are not restricted to a specific species, as evidenced by consistent impacts observed across human, rat, and mouse receptors. he antiemetic effects of 5 mg/kg and 10 mg/kg Δ^8-THC could be counteracted by a CB1 antagonist, but not by a CB2 antagonist. It is important to highlight that Δ^8-THC exhibited superior effectiveness in preventing vomiting compared to Δ^9-THC, despite its lower affinity for CB1 and CB2 receptors. Δ^8-THC competitive binding to CB1 and CB2 receptors, demonstrated with nanomolar affinity across species, showcases a nuanced molecular engagement.

Analgesic and Hypothermic Activities

In a short-term study, dose-dependent pain relief and body temperature decrease were observed in rats after Δ^8-THC administration. These effects were noticeable within the range of doses of Δ^8-THC and accompanied by complete tolerance. In the vaporized form, employing the tail withdrawal assay in male rats, a mixture containing three parts CBD to one-part Δ^8-THC produced an immediate analgesic effect when compared to vapor from the control vehicle. Upon oral ingestion, Δ^8-THC exhibited an analgesic effect that was dose-dependent when measured by the hot-plate method. Interestingly, this analgesic effect was found to be comparable in potency to Δ^9-THC, but both were notably less potent than morphine.

Anticancer Activities

Several scientific in vitro and in vivo studies have explored the potential anti-cancer effects of Δ^8-THC. These investigations aim to understand how this compound may impact different kinds of cancer cells and their proliferation. The findings consistently reveal its ability to impede critical processes such as DNA synthesis, cancer cell growth, and cellular respiration across a spectrum of cell types, including murine leukemia cells, human cancer cells, and lymphoma cells. Notably, Δ^8-THC demonstrates promise in inhibiting multidrug resistance in certain cancer cells and induces a range of cellular responses, from apoptosis and autophagy to modulation of

molecular markers associated with cell cycle progression. Furthermore, in vivo experiments on mice carrying lung adenocarcinoma suggest that Δ^8-THC may not only retard tumor growth but also enhance the life span of treated animals. These cumulative results support the potential therapeutic relevance of Δ^8-THC in combating cancer through diverse mechanisms, warranting further exploration and clinical investigation.

Antidepressant Activities

The antidepressant effects of Δ^8-THC were evaluated, using the automated mouse-forced swim and tail suspension tests. Interestingly, doses of 1.25, 2.5, and 5 mg/kg administered intraperitoneally demonstrated a U-shaped dose-response in terms of their antidepressant action.

Toxicity of Δ^8-THC

Following the receipt of 104 reports detailing adverse events in individuals who had consumed Δ^8-THC products between December 1, 2020, and February 28, 2022, the Food and Drug Administration (FDA) released a report underscoring the potential hazards linked with Δ^8-THC products and emphasized the necessity for regulatory oversight and public awareness. The FDA report highlights five major significant concerns regarding Δ^8-THC products. Firstly, these products have not undergone FDA assessment or approval for safe usage, potentially posing risks to public health. There are worries about inconsistent formulations, incorrect labeling, and varying Δ^8-THC levels. Some products may be misleadingly labeled as “hemp products”, potentially leading consumers to underestimate their psychoactive effects. Secondly, the report expresses apprehension about products claiming therapeutic benefits without FDA approval. This could endanger consumers, as the safety and efficacy of such products have not been confirmed. The data presented indicates possible adverse events, particularly among pediatric patients, emphasizing the need for caution. Thirdly, Δ^8-THC shares similar psychoactive effects with Δ^9-THC, indicating a comparable level of impairment. Fourthly, concerns are raised about the manufacturing process, which may involve potentially harmful chemicals and lead to contaminants in the final product. Finally, manufacturers’ packaging Δ^8-THC products in ways appealing to children is a notable concern, as this could lead to unintentional exposure.

Tolerance

It is worth noting that tolerance to the biological effects of Δ^8-THC has been a recurring observation in several scientific publications. These studies have consistently pointed out that over time, the response to Δ^8-THC can undergo significant alterations. This phenomenon has been extensively documented, shedding light on the complex nature of how the body interacts with this compound. Mice became completely tolerant to the hypothermic effects and partially tolerant to extended phenobarbital-induced sleeping times and catalepsy within 38 days of daily intravenous Δ^8-THC administration. Moreover, Δ^8-THC -tolerant mice were cross-tolerant to the body temperature-lowering effects of chlorpromazine, but not to morphine or pentobarbital. When considering morphine interactions, a study did find that morphine-tolerant mice treated with Δ^8-THC induced heightened catalepsy rather than cross-tolerance. Furthermore, the development of cross-tolerance to the prolongation of pentobarbital-induced sleep by Δ^8-tetrahydrocannabinol and 11-hydroxy-Δ^8-tetrahydrocannabinol was reported in mice. In a study exploring the reducing power of Δ^8-THC against seizure manifestations, chronic administration of intraperitoneal 15 mg/kg Δ^8-THC rapidly led to tolerance in preventing seizure activity. Only two intraperitoneal injections were required to induce tolerance. At elevated levels of Δ^8-THC, the ability to counteract epileptic activity seems to diminish in Senegalese baboons following neural discharge at the targeted stimulation site. In a short-term study regarding the cardiovascular system, a relationship between the administered dose (2 mg/kg) of Δ^8-THC and a decrease in heart rate was evident. Notably, a complete tolerance to the heart rate reduction occurred in just 13 days. The immunomodulatory effects of 60 mg/kg of Δ^8-THC against direct hemolytic plaque-forming cells in the mice spleen showed a state of reduced responsiveness (hypo responsiveness) developed when a pretreatment regimen was employed.

Clinical Trials on Δ^8-THC

Resources discussing the clinical trials on Δ^8-THC are very limited; however, most of its potential benefits are dependent on marketing claims. The first clinical study was conducted in 1973 on six participants for the comparison between Δ^8-THC and Δ^9-THC following orally and intravenously administered doses. The study participants were orally administered drug-containing chocolate cookies containing 20 mg and 40 mg of Δ^8-THC and 20 mg Δ^9-THC. The trials were conducted over three weeks intervals. Both Δ^8-THC and Δ^9-THC clinical effects were evaluated by a narrative log covering every 30 min for up to 5 h with the records of pulse rate,

blood pressure, and conjunctival color. The study results showed that all three treatments produced similar somatic, psychic, and perceptual effects. The lower Δ^8-THC dose (20mg) produced the least clinical effects with slower onset and shorter duration of action. For intravenous administration, both Δ^8-THC and Δ^9-THC solutions in 95% Ethanol (1 mg per 0.20 mL) were injected into a normal saline solution after the start of dripping. In a single experiment, three volunteers received Δ^8-THC, while four subjects received Δ^9-THC. Δ^8-THC was administered to the three subjects in a total of six separate doses. The participants experienced the same qualitative symptoms of Δ^9-THC. Moreover, the intensity and duration of clinical effects produced by the treatment with Δ^8-THC were dose-dependent. Generally, the study showed that Δ^8-THC produced slightly weaker effects than Δ^9-THC. The authors concluded that both Δ^8-THC and Δ^9-THC produce the same clinical effects when administered intravenously, however, Δ^8-THC exhibited approximately 2/3 the potency of Δ^9-THC, when administered orally.

Active Pharmaceutical Ingredients (API) in nanoform

A proven approach to improving the dissolution properties of poorly water-soluble active component(s) molecules is to reduce particle size of the solid drug, as increased surface area of the dissolving drug particles correlates with increased dissolution rates. Reduction of particle size to nanoparticle range produces dramatic increases in surface area and thus the greatest opportunity for dissolution rate enhancement via this mechanism. Therefore, nanoparticle active component(s) delivery can provide faster dissolution, improved bioavailability and ultimately enhanced clinical efficacy.

Nanoparticles are solid, colloidal particles consisting of macromolecular substances that vary in size from 5 - 1000 nano meters. The drug of interest is dissolved, entrapped, adsorbed, attached or encapsulated into the nanoparticle matrix. The nanoparticle matrix can be comprised of biodegradable materials such as polymers or proteins. Depending on the method of preparation, nanoparticles can be obtained with different properties and release characteristics for the encapsulated therapeutic agents (Sahoo SK and Labhasetwar V, Nanotech approaches to drug delivery and imaging, DDT 8:1112-1120, 2003).

Typically, nanosuspensions of active pharmaceutical compounds are prepared by application of extremely high shear conditions to a mixture of the compound, an aqueous dispersion medium, and milling media (herein, high-shear milling). In most high-shear milling applications the milling media is a small polyester prill driven by the shear forces to which it is subjected, and acts like a microscopic ball mill, impacting the pharmaceutical compound present in the mixture (generally, the active pharmaceutical compound (APC) is present in the slurry initially as a powder, in the form of a macroscopic particulate. Typical equipment employed in preparing such nanosuspensions is a high-shear mixer in which a stainless-steel impeller rotates at multiple thousand RPM in a chamber holding a mixture of the active pharmaceutical compound, aqueous dispersion medium, and milling media.

The advantages of using nanoparticles for oral drug delivery, especially for dosing poorly soluble drug molecules, are well known and have been documented for over 40 years.

Nanosuspensions is the potent approach to improve the solubility and bioavailability of poorly aqueous soluble drug entities. Nanosuspensions may be administer through a variety of routes involving oral, transdermal, ocular, parenteral, pulmonary, etc. with solving the different issues.

Size distribution and stability study

The mean particle size and the width of particle-size distribution are important characterization parameters which govern the saturation solubility, dissolution velocity, physical stability and even biological performance of oil-in-water emulsion. The polydispersity index (PDI) is a measure of the heterogeneity of a sample based on size. Polydispersity can occur due to size distribution in a sample or agglomeration or aggregation of the sample during isolation or analysis.

Cannabinoid	Concentration (mg/ml)	Average Diameter (nm)	Polydispersity Index
Δ^8-THC	10.0	40.0	0.18
	50.0	62.0	0.19
	125.0	96.7	0.29

The oil-in-water emulsions in a nanoform were subjected to a stability test at 40°C, at room temperature (22°C) and under the condition of storage under cooling (4°C).

For the determination of the stability of the nano emulsions, each was stored in a transparent container in a thermostat at 40°C, at room temperature (22°C) and under cooling (4°C), and the stability was determined by the average size of the droplets for each formulation (Tables 9, 10 and 11). The average size measurements were made to the formulations in closed vials, stored undisturbed, without exposure of light and recorded for a period of more than 2 years.

Stability test at 4°C (RH 56%; CBD – 5mg/ml; Melatonin – 5 mg/ml; number of measurements – 5; PDI < 0.15)

Concentration of Δ^8 THC (mg/ml)	Initial Size (nm)	Size after 3 months (nm)	Size after 12 months (nm)	Size after 25 months (nm)
10	8.57± 0.21	10.31± 0.28	10.71± 0.25	11.08± 0.27
50	22.0± 0.11	22.64± 0.17	25.7± 0.22	27.8± 0.25
125	86.1± 0.31	88.7± 0.33	92.5± 0.30	98.9± 0.33

Stability test at 25°C (RH 56%; CBD – 5mg/ml; Melatonin – 5 mg/ml; number of measurements – 5; PDI < 0.15)

Concentration of Δ_8 THC (mg/ml)	Initial Size (nm)	Size after 3 months (nm)	Size after 12 months (nm)	Size after 25 months (nm)
10	8.57± 0.21	11.01± 0.18	11.07± 0.15	12.17± 0.17
50	22.0± 0.11	24.71± 0.10	25.12± 0.20	28.01± 0.18
125	86.1± 0.31	89.25± 0.23	90.51± 0.18	99.02± 0.23

Stability test at 40°C (RH 56%; CBD – 5mg/ml; Melatonin – 5 mg/ml; number of measurements – 5; PDI < 0.15)

Concentration of Δ_8 THC (mg/ml)	Initial Size (nm)	Size after 3 months (nm)	Size after 12 months (nm)	Size after 25 months (nm)
10	8.57± 0.21	11.17± 0.18	11.71± 0.20	12.08± 0.31
50	22.0± 0.11	22.24± 0.20	25.17± 0.28	27.92± 0.20
125	86.1± 0.31	87.75± 0.25	90.68± 0.42	99.1± 0.53

Nebulizers as the delivery method

Initially, nebulizers have been used to deliver aerosolized medications in the treatment of patients with pulmonary diseases. Nebulizers are the aerosol device of choice when patients cannot coordinate inhalation and actuation needed for the use of the pressurized metered-dose inhalers (pMDIs) or are not able to provide the necessary inspiratory flow required by the dry powder inhaler (DPI) for effective aerosol drug delivery. Nebulizers are divided into three categories: jet nebulizers, ultrasonic nebulizers, and mesh nebulizers.

While jet nebulizers are commonly used for the treatment of patients with pulmonary diseases, they are bulky and require a power source. Due to aerosolized droplets and solvent vapor that saturates the outgoing air, jet nebulizers cool the drug solution in the nebulizer and increase solute concentration in the residual volume. Jet nebulizers are effective in delivering formulations that cannot be delivered with pressurized metered-dose inhalers (pMDIs) and dry powder inhalers (DPIs). On the other hand, jet nebulizers can be difficult to use because of their need for compressed gas and additional tubing.

Ultrasonic nebulizers are more efficient and compact than jet nebulizers, they cannot be used to deliver proteins or suspensions. With the development of mesh nebulizers that use lower-frequency waves, heating issues that denature proteins during aerosol therapy are eliminated. Ultrasonic nebulizers incorporate a piezoelectric crystal vibrating at high frequencies (1-3 MHz) in order to produce aerosol. They are divided into two categories:

1. Large-volume ultrasonic nebulizers and
2. Small-volume ultrasonic nebulizers.

Whereas large-volume ultrasonic nebulizers are most commonly used to deliver hypertonic saline for sputum induction, small-volume ultrasonic nebulizers are used for delivery of inhaled medications. Ultrasonic nebulizers have many limitations compared to jet nebulizers. For instance, they have large residual volumes, an inability to aerosolize viscous solutions, and degradation of heat-sensitive materials.

Recent improvements in nebulizer technologies have led to the development of mesh nebulizers using micropump technology for aerosol production. They force liquid medications through multiple apertures in a mesh or aperture plate in order to generate aerosol. As small and portable nebulizers that are powered by either battery or electricity, they have silent operation, short treatment times, increased output efficiency, and minimal residual volume [189-192]. Advantages of mesh nebulizers include consistent and improved aerosol generation efficiency, a predominantly fine-particle fraction reaching into the peripheral lung, low residual volume, and the ability to nebulize in low drug volumes.

Aerosol therapy via nebulizers is a well-established method. New types of nebulizers have yielded a number of improvements, such as compact design, portability, shorter treatment duration, and quiet operation, that are expected to improve patient adherence to therapy.

Conventional inhaler devices have a low efficacy in targeting small airways. Smart nebulizers can be used to increase deposition to small airways by adjusting the flow and depth of each inhalation based on patients “individual inspiratory capacity”.

Smart nebulizers employ adaptive aerosol delivery (AAD®) technology, which analyses the patient’s breathing pattern in order to determine the timing of aerosol drug delivery during inhalation. They analyse pressure changes of the airflow during the first 3 breaths to determine the correct starting point for drug delivery. Then, the device continues to monitor the preceding 3 breaths throughout the treatment and adapts to the patient’s breathing pattern. This adaptation reduces not only losses of aerosol during expiration but also the variation in drug delivery during inhalation therapy while improving patient adherence to treatment [193 - 195]. The smart nebulizer system may have a user interface that can communicate information to the patient/user, including without limitation treatment progression, inhalation flow rate and breathing rate, preferably with low latency. The interface may be incorporated into the nebulizer, such as the housing, or information from the nebulizer may be communicated to a standalone device, such as a peripheral device, including for example a smartphone or tablet, for viewing. Communication of the information is not limited to visual information, such as graphics or text, but may also include audible and haptic information, communication methodologies and components.

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