PHARMACEUTICAL RESEARCH AND INNOVATIONS

(Book Chapter)

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Editor's

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EXTRACTION TECHNIQUES TO DERIVE MOSQUITOCIDAL PHYTOCHEMICALS FROM PLANTS

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Abstract - Phytochemicals are biologically active compounds which are found in plants. They present in the various parts of the plant such as root, stem, flower, bark, fruit, peels etc. They also used as the ingredients in human nutrition and medical science. Phytochemicals classified into primary metabolites and secondary metabolites. The secondary metabolites involved in the plant defence mechanism. This property of secondary metabolites leads to the emergence of natural insecticides from plant origin. Large number of plant extracts has been reported to have mosquitocidal or repellent activity against mosquito vectors, but very few plant products have shown practical utility for mosquito control. This study reviews the extraction methods to derive the phytochemicals which has the potential to control mosquito vectors.

1 INTRODUCTION

Mosquito transmit diseases like malaria, dengue, filariasis accounted for global mortality and morbidity with increased resistance to common insecticides. Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. Who has declared mosquitoes as "Public enemy number one". Mosquitoes borne disease are prevalent in more than 100 countries across the world, infection over 700,000,000 people every year globally and 40,000,0000 of Indian population. Mosquitoes are the major vector for transmission of life threatening disease like malaria, Yellow fever, dengue fever, chikungunya fever, filiariasis, encephalitis, West Nile virus infection, etc. in almost all tropical and subtropical countries and many other parts of the world (Govindrajan *et al*., 2011; Ramar *et al.,* 2013). One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control.

One of the most effective alternative approaches under the biological control programme is to explore the floral biodiversity and enter the field of using safer insecticides of botanical origin as a simple and sustainable method of mosquito control. Further, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concerntly on both behavioral and physiological processes (Rawani *et al*, 2014).

Botanicals are basically secondary metabolites that serve as a means of defense mechanism of the plants to with stand the continuous selection pressure form herbivore predator and other environmental factors. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities (Shaalam *et al,* 2005). Insecticidal effects of plant extraction vary not only according to plant species, mosquito species, geographical varieties and parts used, but also due to extraction methodology adapted and the polarity of the solvents used during extraction. Phytochemicals have a major role in mosquito control programs. The bioactive plant ingredients can be obtained from the whole plant or from a specific part by extraction with different types of polar and non-polar solvents, such as petroleum ether, benzene, chloroform, methanol, absolute alcohol and acetone etc. A wide selection of plant from herbs, shrubs and large trees was used for extraction of mosquito toxins. Phytochemicals were extracted either from the whole body of little herbs of from various parts like fruits, leaves, stems, barks and roots

etc., of large plants or trees. In all cases where the most toxic substances were concentrated upon, found and extracted for mosquito control.

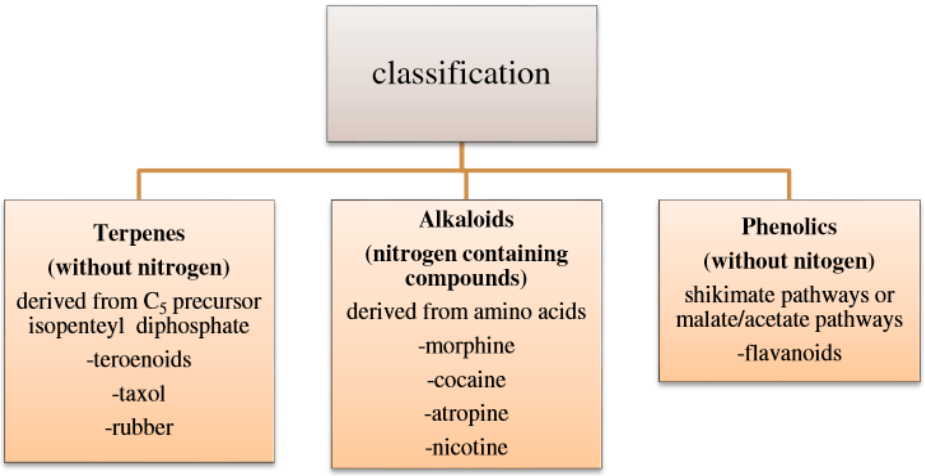
Phytochemicals derived from plant sources can act as a means to act as larvicides, insect growth regulators, repellents and oviposition attractants and can play an important role in the interruption of the transmission of mosquito- borne disease at the individual as well as at the community level (Govindarajan *et al*., 2008; Nathan *et al,* 2006). The active principles in medicinal plants are chemical compounds known as secondary plant products. Some secondary products discourage herbivores, other inhibit bacterial or fungal pathogens.

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans as medicinal ingredients and nutrients. They protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavour. Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control. Plant products can be used, either as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess. A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors, but very few plant products have shown practical utility for mosquito control. Plant products can be obtained either from the whole plant or from a specific part by extraction with different types of solvents such as aqueous, methanol, chloroform, hexane, etc., depending on the polarity of the phytochemicals (ICMR, 2003).

Primary metabolites (i.e., organic acids, amino acids, carbohydrates) can also be obtained from plant waste material and used for different purposes (Patsalou *et al,* 2017). Plants in particular produce secondary metabolites (SMs), which are not directly involved in the basic functions of growth, development, and reproduction of the organism, but are essential for long-term survival and play multiple roles, including defence against predators or attraction of pollinators (Demain *et al,* 2000). SMs are endowed with numerous biological activities, making them also extremely important for human health and well-being.

The secondary metabolites of the plants constitute a large and varied group of organic compounds that are synthesized in small quantities; they have no direct function in essential processes such as photosynthesis, respiration, solute transport, protein synthesis, nutrient assimilation, and the differentiation or formation of carbohydrates, proteins, and lipids. It is believed that secondary metabolites are related to the defense of the plant against predators and pathogens, they also act as all elopathic agents that influence growth, survival, and reproduction of other plants, attract seed pollinators and serve to face adaptation to sudden changes in temperature, humidity, light intensity and drought (Kroymann *et al,* 2011, Ramakrishna *et al,* 2011; Berini, 2018). Secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes of compounds aimed at plant growth and development, such as carbohydrates, amino acids, proteins and lipids. They can be regarded as products of biochemical "side tracks" in the plant cells and not needed for daily functioning of the plant. Plant extracts basically known as phytochemicals, active ingredients or secondary metabolites are proven to exhibit various pharmacological and biochemical actions when ingested by insect herbivores. Plant bioactivity depends on chemical compounds which may inhibit insect feeding. Toxic effects to insect pests are produced by the compounds terpenoids, steroids, phenols, flavonoids, tannins, alkaloids and cyanogenicglycosides. Primarily, phytochemicals affect the midgut epithelium and secondarily affect the gastric caeca and malpighian tubules of insects initiating anti feedant properties and eventual death of insects making their presence in plants potential for use as insecticides (David *et al,* 2000).

According to this, the secondary metabolites in plants can be divided into three large groups: terpenes, phenolic compounds, and alkaloids.



2 EXTRACTION METHODS

2.1 Conventional Methods

Bioactive compounds from plant materials can be extracted using various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents in use and the application of heat and/or mixing. To obtain bioactive compounds from plants, the existing classical techniques are: Decoction, Infusion, Soxhlet extraction, Maceration and Hydro distillation (Azmir, 2013). These extraction methods use organic solvents (such as hexane, acetone, methanol, ethanol, etc.) or water and are generally carried out under atmospheric pressure.

2.2 Objectives of Extraction Methods

All these extractions procedure share have similar objectives: Extraction of targeted bioactive phytoconstituents from the vegetal, Increase the selectivity of analytical methods, Increase of sensitivity of bioassay by increasing the concentration of targeted compounds; Convert the bioactive compounds into a more suitable form for detection and separation; Provide a strong and reproducible method that is independent of variations in the sample matrix (Smith, 2003).

2.3 Decoction

It a suitable method for the extraction of the constituents soluble in water and that cannot also been destroyed by the effect of heat (Bimakr, 2010). Decoctions are normally preferred for harder herbs like roots, barks, and seeds. It is helpful to grind or crush the whole root, bark, and seeds before preparing the decoction. During decoction, distilled water is added to the dried extract and the mixture is subjected to heating continuously for a period of time at a temperature of 100°C. Then it is allowed to cool to room temperature and filtration is performed to obtain the filtrate. That filtrate is concentrated to obtained extract. Depending on the type of plant material used, strong decoctions are prepared in two general ways. The first involves boiling the mixture longer. This is usually indicated when working with larger woody pieces of bark. Longer boiling time, up to 2 hours or more, is sometimes necessary to break down, soften, and extract the larger pieces. Alternatively, when smaller woody pieces are used yet a stronger remedy is wanted, the decoction is prepared as above (boiling 20 minutes), then it is allowed to sit/soak overnight before straining out the herb. When straining, again, make sure to press on the cut herb pieces in the strainer to get as much moisture/decoction out of the herb pieces. (Mukherjee, 2002).

2.4 Maceration

It is an old method used for medicinal preparation. It is consider as a widely and low-cost way to get natural products from plant material. The powdered solid materials is placed in a container which is stoppered and contains the solvent and is allowed to stand at room temperature for a period of 3 days with constant agitation until the soluble matter has completely dissolved. This process dissolves the plant cell wall, releasing the soluble photochemical. The mixture is then strained, the Marc (the damp solid material) is pressed, and the combined liquids are then clarified by the method of filtration or decantation after standing for some time. (Azwanida, 2015). The commonly used solvents are methanol, ethanol or a mixture of water and alcohol (Sasidharan *et al*, 2013) Maceration is an old technique as not all active phytoconstituents are extracted (De Silva, 2017). It is very simple and the cheapest because it only requires a simple container as the place for extraction, but this method requires a long time for the extraction process (Naviglio *et al*, 2019). The number of raw materials, the selection of solvents, and the correct extraction time are things that affect the effectiveness of this method.

2.5 Infusion

In this method, extraction consist in soaking the solids plants powder either cold or boiling water for a short period of time (Bimakr, 2010). The plant material is grinded into the powder, and then placed inside a clean container. The extraction solvent hot or cold is then poured on top of the material, soaked, and kept for a short period of time (Ingle, 2017). This method is suitable for extraction bioactive constituents that are readily soluble Infusions are generally prepared for immediate use, as preservatives are absent. In some cases preservatives like alcohol are used and the infusions concentrated by boiling.

2.6 Soxhlet extraction

Soxhlet extraction is a method that was suggested for extraction of lipid first by Franz Ritter Von Soxhlet, a German chemist (Grigonis *et al,* 2004). Nowadays, it is used for the extraction of valuable bioactive (solid-liquid) compounds from various natural sources. The Soxhlet extraction is a simple and convenient method for infinitely repeated cycle of extraction with a fresh solvent until complete exhaustion of the solute in the raw material (Azmir, 2013). It is a continuous solid/ liquid extraction. The solid material which is to be extracted is placed in thimble which is made of a material such that it contains solids but allows only liquids to pass through it (It acts as a filter paper).The thimble is then placed in extractor. Organic solvent is then heated in reflux due to which the vapors generated starts boiling and as the vapor rises up they are further condensed by the condenser which further fills up the thimble. This process is repeated until all the materials which is to be extracted from the solids is done.

2.7 Hydro Distillation

The name "hydro distillation" is used due to the use of water in either the liquid form or vapour form**.** Hydro distillation has long been used for the extraction of essential oils and bioactive compounds from plant materials.. Hydro distillation can be applied by either water or steam distillation, and their mixture, where the samples are packed in a closed chamber, and water is added in an adequate amount, and then the mixture boiled, or steam directly injected to the plant sample (Azmir *et al.,* 2013). In this process, the plant material is soaked in the water that is placed over a container over heat. The container material should be manufactured using copper, stainless steel or glass along with a condensing unit attached to a receiving flask. During the boiling process, the steam as well as oil vapor is captured in the condensing apparatus. Excessive water from the resultant mixture is made to dispense out through an opening in the condensing

apparatus. The final product hence obtained in the receiving flask contains only the distillate. Moreover, the excessive water is referred as 'hydrosol' that has a significant amount of plant essence can also be used in various cases.

2.8 Non-conventional Extraction Techniques

Increase in the interest of plant metabolites has encourage researchers to an increasing consideration for novels methods of extraction enabling fastening and shortening extraction times, efficient extraction, automation, and reduction of organic solvent consumption (Rafiee *et al,* 2011). Several novel extraction methods such as Ultrasound assisted extraction (UAE), Microwave assisted extraction (MAE), Supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) has taken place. These new methods are able to reduce the extraction time, decrease the volume of solvent used and improve the extraction yield (Brusotti *et al,* 2014)).

2.9 Microwave Assisted Extraction (MAE)

Microwave-Assisted Extraction is an extraction technique using electromagnetic waves (Guntero *et al,* 2017). Microwaves are often used on a laboratory scale because they are small in size and easy to operate. A microwave that works by assisting electromagnetic waves makes the extraction time faster, only a few minutes. This is because all electromagnetic waves generated are converted directly into heat (Chemat *et al,* 2014). Electromagnetic waves consist of two perpendicularly oscillatory fields namely: Electric Field and Magnetic Field, which can also be called as Microwave. These waves are used as energy vectors or information carriers. Electromagnetic waves are absorbed by the material and converted to heat energy. This is a Microwave Energy Microwaves penetrate into biomaterials and generate heat by interacting with polar molecules such as water inside the materials. Then the penetration of microwaves depth into plant matrix depends on dielectric constant, moisture content, temperature, and the frequency of the electrical field. The water contained in a plant material is responsible for the absorption of microwave energy which led to internal superheating and cell structure disruption. This action, created the diffusion of bioactive compound from the plant matrix (Takeuchi *et al*, 2009). 2450 MHz (2.45 GHz) is the most commonly used frequency for commercial microwave instruments, which has an energy output of 600-700 W.

2.10 Ultrasound-Assisted Extraction (UAE) or Sonication Extraction

UAE involves application of high-intensity, high-frequency sound waves and their interaction with materials. UAE is a potentially useful technology as it does not require complex instruments and is relatively low-cost. It can be used both on small and large scale (Dai et al, 2010). A crushed sample is placed into the ultrasonic bath after mixing it with a suitable solvent, while controlling the temperature and extraction time. (Sasidharan *et al,* 2011). UAE uses ultrasound with frequencies ranging from 20 kHz to2000 kHz. The acoustic cavitation has a mechanical effect of the ultrasound, which increases the surface contact between the sample and the solvent as well as the permeability of cell walls, maintaining a high quality of compounds. There is an alteration of Physical and chemical properties of the materials treated by ultrasound and disruption of the plant cell wall which releases the compounds and enhances bulk transport of the solvents into the plant cell. UAE uses acoustic waves in the kilohertz range that travel through the solvent producing cavitation bubbles. When the cavitation bubbles burst at the surface of the plant sample matrix, a shockwave-induced damage to plant cell wall enhances the mass transfer of phenolic compounds across cellular membranes into solution.

3 SECONDARY METABOLITES IN MOSQUITO CONTROL

The secondary metabolites act as defence mechanisms against predators. This characteristic reveals that the natural insecticides play a pivotal role in vector control, and their use represents an excellent alternative to synthetic insecticides (Marques *et al,* 2015). Many scientists identified the compounds with insecticidal activity against mosquito vectors from plants.

Compounds	**Plant**	**Activity**	**Reference**
Essential Oil Based Terpene Compounds (Diallyldisulfide, Diallyltrisulfide, Eugenol, Methyl Eugenol, Carvone, Limonene, Eucalyptol, Eudesmol, α- pinene)	*Menthapiperita*, *Ocimum sanctum*, *Eucalyptus maculata*, *Allium sativum* and *Callistemon linearis*	Larvicidal and Adulticidal Agent against *Aedesaegypti*	Rijusarma*et al,* 2019
Phytochemicals (Alkaloids, glycosides,tannin,etc)	StemBark Extracts of *Euphorbia, Balsamifera*	Mosquito Repellent Activity	Idris*et al*, 2014
Azadirachtin (limnoid group)	Neem (seeds, leaves, and other parts of the tree)	anti- feedant, ovipositional deterrence, repellency, growth disruption, sterility and larvicidal action against insect	Schmutterer, 1990
arbazole alkaloids, mahanimbine,murrayanol, and mahanine	*Murrayakoengii*	Larvicidal activity	Ramsewak, 1999
Limonene nanoemulsions (Monoterephene)	*Citrus sinensis* essential oil	Larvicidal activity	Azmy*et al,* 2019

4 CONCLUSION

In this world, large number of compounds from plant origin has shown remarkable results against the mosquitoes. This increases the option to control mosquito borne diseases. The extraction of photochemical from plant parts is a crucial step and it require proper knowledge for obtaining specific desired compounds. Extraction is not a universal one it varies depend on the plant material which is used and factors such as compound, temperature, concentration. So the method of selection should be selected very carefully and then only can extract the target compound easily.

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HORMONES AND GENES INVOLVEMENT IN THE PATHOPHYSIOLOGY OF SCHIZOPHRENIA

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Abstract - Schizophrenia is a debilitating brain disorder with a worldwide prevalence of ~1% that results in substantial morbidity and mortality. The main symptoms of schizophrenia are hallucinations, delusions, and cognitive impairments. Most cases of schizophrenia start during adolescence and early adulthood, and often have a lifelong course. The serotonin 2A receptor (HTR2A) is particularly abundant in the mammalian cortex is important in controlling cortical and is responsive to changes in serotonergic activity. Dopamine D_1 receptors are expressed at high levels on the distal dendrites of pyramidal neurons in the prefrontal cortex that are thought to be involved in working memory processes**.** GWAS have identified the α-1C subunit of the L-type voltage-gated calcium channel (*CACNA1C*) gene as a significant risk gene for schizophrenia. Dysbindin-1 gene was found to be a component of the dystrophin-associated protein complex (DPC) in skeletal muscle cells. DPC is highly expressed in the brain, in particular the cortex and the hippocampus. Many studies have shown that dysbindin-1 is one of the important potential susceptibility gene for schizophrenia. Overall purpose of this study is to find the path physiology of Schizophrenia which pave ways to control the prevalence of schizophrenia more effectively and to diagnose schizophrenia at early stage by using the estimation of biogenic amines present in the blood.

1 INTRODUCTION

Schizophrenia is a common and serious psychiatric illness [1] affecting 0.5-1% of the population in early adulthood. Despite continuing progress, current treatments continue to have significant side effects and inconsistent efficacy across patients. The disease remains incurable, with the best outcome being the control of symptoms and preservation of sufficient functionality and independence.

Schizophrenia is a disease with remarkable phenotypic heterogeneity. The symptoms are genetically divided into three categories [2]. Positive symptoms with which the general public is most familiar, include hallucinations and delusions of varying content, and are perhaps the most disruptive. Negative symptoms include lack of motivation, an hedonia, and flat affect. Cognitive symptoms such as defects in attention, concentration, working memory, and critical thinking are the most incapacitating, leading to significant disability [3]. Each patient can have a different mix of the three types of symptoms leading to an overall highly heterogeneous phenotype. Affecting symptoms may combine with the classic symptoms of schizophrenia leading to schizoaffective disorder, which some genetic researchers categorize with schizophrenia and others with bipolar disorder. Heterogeneity also manifests in the patients' response to medication, frequently resulting in multiple changes in treatment strategy during the course of the illness as patients navigate through ineffective treatments [4].

In 2010, NIMHANS, Bangalore reported that the burden of mental and behavioral disorders ranged from 9.5 to 102 per 1000 population. Another study among rural peoples of south India, in 2010 reported 37% of those who died by suicide had a mental disorder. The two most common reasons were alcohol dependence (16%) and adjustment disorders (15%). Currently mental and behavioral disorders account for about 12 percent of the global

burden of diseases. This is likely to increase 15 percent by 2020. So the present study mainly focuses on identifying the involvement of hormones and gene in schizophrenia.

2 DOPAMINE

Dopamine is synthesized from tyrosine through dopa. The first step, the conversion of tyrosine to dopa by tyrosine hydroxylase, is the rare-limiting step, and is subject to feedback regulation. The major metabolic product of dopamine catabolism in humans is homovanillic acid, and, to a lesser extent dihydroxyphentlacetic acid and 3-methoxytyramine. Concentrations of these metabolites have been examined in the brain, cerebral spinal fluid (CSF), plasma, and urine of patients with schizophrenia of increased or decreased dopamine neurotransmission.

Reduced cortical dopamine could explain hypofrontality, impaired cognition, and negative symptoms (such as an hedonia and lack to motivation). Altered sub cortical and limbic dopamine, on the other hand, could cause positive symptoms (such as hallucinations and delusions). These symptoms of schizophrenia may result from excess dopaminergic neurotransmission particularly in mesolimbic and striatal brain regions which lead to positive symptoms and finally changes into schizophrenia. There are many clinical shreds of evidence about schizophrenia that provides support for the dopamine hypothesis. In this hypothesis, the different evidence has appeared. The first evidence that in schizophrenia patient's dopamine came from amphetamine users. Amphetamine showed that too produces more dopamine and produces psychotic symptoms related to schizophrenia [5, 6].

2.1 Glutamate

Glutamate is one of the most prevalent neurotransmitters in the brain. Virtually all neurons in the brain are affected when glutamate is applied. A nonessential amino acid that does not cross the blood-brain barrier, it can be synthesized in the brain from glutamine. The dominant mode of inactivation of synaptic glutamate is via reuptake by specific, high – affinity uptake sites.

Glutamate is relevant to the neurochemistry of schizophrenia because of its role in key neural networks. Projections to and from corticol and hippocampal pyramidal neurons use glutamate as a primary neurotransmitter. These include projections to subcortical structures such as the stratum, nucleus accumbens, and ventral tegmental area: output from these areas modulated by glutamate. Thalamic projections to the cortex also employ glutamate as the major neurotransmitter. Glutamate neurotransmission is important not only for rapid synaptic transmission between these regions, but also for experience-dependent cortical plasticity and memory. This is particularly true for the voltage- sensitive NMDA receptor, a likely candidate for modulating memory traces at Hebbian synapses. Glutamate's essential role in key neural networks, memory and cortical plasticity, thus it is involved in the pathophysiology of schizophrenia.

The dopaminergic dysfunctioning may be associated with glutamatergic dysfunctioning. In this concept glutamate, dysfunctioning will lead to opening effect in the thalamocortical loop which causes to appear psychotic symptoms and well-known dopamine concentration changes. Glutamatergic receptors consist of two groups which can perform different functions and finally lead to schizophrenic symptoms appear. In these receptors, major receptors are NMDA (N-methyl, D-Aspartate) receptor which causes schizophrenia among most patients by changing dopamine level from the normal range [7, 8].

2.2 Serotonin

Serotonin is synthesized from tryptophan and is broken down into 5-hydroxyindolic acid (5-HIAA) by monoamine oxidase (MAO). Tryptophan is an essential amino acid; dietary intake of

tryptophan can affect CNS synthesis of serotonin. Serotonin synthesis is also modulated by auto receptors on nerve terminals. Synaptic serotonin is inactivated primarily by reuptake pumps on presynaptic neurons and glia; following uptke, serotonin is repackaged into vesicles or broken down to 5-HIAA. Both serotonin itself and its uptake pumps are found in blood platelets, where they play a role in clotting. In the CNS serotonin neuronal cell bodies are located in the brainstem in nine separate nuclei.

The effects of serotonin are mediated by an ever-increasing number of receptor subtypes. Currently, seven classes of serotonin receptors have been characterized: (5-hydroxytryptamine [5-HT]) – type 1 (5-HT_1) through 5-HT_7. 5-HT_2 receptors are found in the prefrontal cortex, striatum, and nucleus accumbens: 5-HT_3 receptors are found in cortical, limbic, and subcortical areas, such as the amygdala and hippocampus. Serotonin plays a critical role in synaptic mechanisms associated with learning and memory; it may also have importance neurotrophic effects during the development of adult.

2.3 GABA

GABA is the major inhibitory neurotransmitter in the brain. Virtually all neurons are inhibited by GABA, and up to 40 percent of neurons use GABA as their major neurotransmitter. Many GABA neurons are local inhibitory interneuron's, but GABA neurons in some regions (such as the striatum) are also primary efferent neurons. GABA is synthesized from glutamate via the enzyme glutamic acid decarboxylase (GAD). GABA acts at two receptor subtypes, GABAA and GABAB, the former being the more important in the CNS. A variety of drugs act at GABA receptors, including alcohol, benzodiazepines, and barbiturates. Findings implicating GABA in schizophrenia include reduced number of GAB Aergic cortical interneurons, increased GABAA receptor density in the prefrontal cortex, and reduced GABA uptake sites in the hippocampus.

2.4 Norepinephrine

Norepinephrine, another monoamine neurotransmitter, has been intensively studied in schizophrenia. Similar to dopamine and serotonin, norepinphrine neurons are located in the brainstem in a group of nuclei (including the locus ceruleus) that project to a variety of cortical and subcortical regions. Nor epinephrine acts at two receptor families, adrenergic and β-adrenergic receptors; at least seven α and three β subtypes have been cloned. Both receptor families exert their effects via changes in G-protein–mediated second messenger systems, including camp and phosphoinositol. Two neuropeptide transmitters, galanin and neuropeptide Y, are colocalized in noradrenergic neurons. Nor epinephrine and its co-transmitters are involved in a number of physiological and behavioral processes including the sleep-wake cycle, arousal, stress, and memory. Both basic and clinical studies support a role for this system in psychiatric disorders such as anorexia nervosa, bulimia nervosa, anxiety disorders, post-traumatic stress disorder, depressive disorders, substance dependence, and substance withdrawal. Many of the behavioral states mediated by the noradrenergic system are markedly altered in schizophrenia. However, more direct evidence is lacking and any changes in noradrenergic function in schizophrenia may be secondary to the agitation that frequently accompanies psychosis.

2.5 Neuropeptides

Two other interesting candidate molecules that have been studied in schizophrenia are the neuropeptides cholecystokinin and neurotensin. Both are found in a number of brain regions implicated in schizophrenia, such as the substantia nigra, nucleus accumbens, hippocampus, and various cortical regions. Both are colocalized with dopamine, GABA, glutamate, and other neurotransmitters. Several studies have reported changes in the levels of the peptides

themselves, mRNA, or receptors. For example, the following findings have had some degree of replication: reduced temporal lobe cholecystokinin peptide concentrations, reduced cholecystokin in receptor density in both temporal and frontal regions, and reduced cholecystokin in mRNA in the temporal lobe.

2.6 Dysbindin gene

Dysbindin (dystrobrevin binding protein I) was identified as a gene associated with schizophrenia through linkage to chromosome 6p [9]. The association between this locus and schizophrenia has been replicated in several subsequent studies. Dysbindin co-localizes with dystrobrevin in both muscle and brain. It is widely distributed in brain, and has been detected bothpre and postsynaptic ally, including in synaptic terminals in the hippocampus [10]. The function of dysbindin in brain is not well understood. It has been reported to influence glutamate neurotransmission [11].

Mutations indysbindin also cause Hermansky-Pudlak syndrome type 7 [12], a complex genetic disorder related to lysosomebiogenesis, which is not known to have a psychiatric phenotype. A deletion within the homologous gene inmice accounts for the phenotype known as ‘‘Sandy,’’ with albinism and bleeding disorders. While the association of dysbindin with schizophrenia has been fairly well replicated, no protein coding mutations contributing to the risk for schizophrenia have been identified. Reduced levels of expression of dysbindin message or protein have been found in schizophrenic brains [13], raising the possibility that polymorphisms in dysbindin associated with schizophrenia may modulatedysbindin expression level. In addition, knockdown of endogenous dysbindin with siRNA resulted in reduction of glutamate levels in neurons in culture, suggesting a possible synaptic consequence for reductions in dysbindin levels [14] and connecting dysbindin with the glutamate hypo function hypothesis of schizophrenia.

2.7 DAOA

The chromosome 13 locus has strong linkage regions to schizophrenia. Among other genes, this locus contains G72, now called D amino acid oxidase activator (DAOA). Several individual replication studies and ameta-analysis have supported the association of DAOA with schizophrenia, though as with other loci, the associated alleles and haplo types are not identical across studies, and some variants are located outside of the gene [15]. Functionally, DAOA activates D amino acid oxidase (DAO). DAO oxidizes D-Serine, which is a coagonist at NMDA glutamate receptors. Thus, there is some biologic plausibility for DAOA as a candidate gene, based on the glutamate hypothesis. DAOA does not have a homolog in mice, so no knockout model has been made. Further explorations of this system may be of considerable interest, especially given the potential efficacy of D-Serine in the rapeutictrials and reports of reduced D-Serine in blood and CSF in individuals with schizophrenia. VCFS includes facial dysmorphism and other features, and presumably is caused by loss of one copy of several or many genes in this region. The VCSF region includes at least 27 genes. TheTbx1gene may account for many of the physical features of VCSF [12]. It is expressed in microvasculature in brain. Inactivating mutations inTbx1have been found in one small family with VCSF or Asberger’s syndrome [12], but the relation of this gene to schizophrenia is still incompletely explored.

2.7 COMT

The gene on chromosome 22q11 that has received the most attention is catechol-O-methyl transferase (COMT). The protein product is an enzyme that participates in the clearance of dopamine from synapses, and thus couldbe involved in regulation of neurotransmission related

to schizophrenia [16].A functional polymorphism, involving the presence of either valine or methione at codon 108 (in the soluble is form of COMT, equivalent to cod on 158in the membrane-bound is form of COMT) alters enzyme activity. The methi one allele is less stable and thus has lower activity, suggesting the hypothesis that individuals with two copies of the methione allele, or with a deletion of one copy of COMT, would be expected to have higher dopamine levels in critical central synapses, perhaps especially in the prefrontal cortex.

2.8 DISC1

The translocation is between exons 8 and 9 of theDISC1gene on chromosome 1. No genes have been found at the chromosome 11 site. The translocation has not been found in many families[17], identified via a proband with schizophrenia, has a four-base deletion resulting in a frame shift and predicted C-terminal truncation of the DISC1 protein. However, the family is too small to clearly demonstrate segregation with disease, and the deletion has also been found in two presumably unaffected blood donors [18].A locus on chromosome 1 within theDISC1gene was linked to schizophrenia in a finish population [19], and the DISC1locus has emerged as apotential risk factor for both schizophrenia and affective disorder in different populations [16].

2.9 Neuregulin 1

Neuregulin 1was identified as a candidate gene via fine-mapping of a locus on chromosome 8p linked to schizophrenia [20]. A number of studies have found association with schizophrenia within the neuregulin 1region. The neuregulin 1gene is very complex, with at least 25 exonsspread over almost a mega base, with extensive alternative promoter usage and alternative splicing, resulting in multiple possible protein products. A region in the 50end of the gene appears to most consistently associate with disease.

3 CONCLUSION

We concluded from this study that Schizophrenia is a complex, chronic mental health disorder characterized by an array of symptoms, including delusions, hallucinations, disorganized speech or behavior. Study of the different genetic etiologies of schizophrenia will also improve understanding of the schizophrenia phenotype, and also understanding of affective disorder and potentially other related major psychiatric illnesses.

Schizophrenia is definitely requiring prompt treatment. Although patients can increase adaptive functioning through available pharmacological and non - pharmacological treatment options, it is hoped that future research will address gaps in treatment and potentially a cure for schizophrenia.

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MODERN ANALYTICAL TECHNIQUES

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Abstract- The spectroscopy techniques for the quantitative and qualitative estimation of drugs have been includes the various methods UV-Visible spectroscopy, Mass spectrometry, Infrared spectroscopy, Nuclear magnetic resonance, Fluorimetry, and phosphorimetry. Hyphenated techniques for the analysis of drugs follow the various techniques in combination with two of three methods i.e; LC-NMR, LC-MS, LC-IR, GC-MS, CE-MS, LC-PDAMS, LC-MS-MS, LC-NMR-MS, LCPDA-NMR-MS etc. It is very important to develop a method with minimum errors, and to overcome the faulted errors in analytical chemistry some of latest trends in analytical techniques were available which includes advancement in automated development of HPLC, RP-HPLC, LC-MS etc. These methods suggest the proper use of each technique in the better advancement of drug development process.

1 INTRODUCTION

Pharmaceuticals may develop impurities at various stages of their development, transportation and storage which makes the pharmaceutical risky to be administered thus they must be detected and quantitated. For this analytical instrumentation and methods play an important role. Analytical Technique is a branch of science that is related to the various disciplines of chemistry like physical chemistry, organic chemistry, and inorganic chemistry. It helps us to identify a substance, determine its structures, and quantitative analysis of a compound.1,2,3,4,5
Analytical techniques:

1.1 Titrimetric techniques

With the development of functional group analysis procedures titrimetric methods have been shown to be beneficial in kinetic measurements which are in turn applied to establish reaction rates.

1.2 Spectroscopic Techniques

The process of method development spectroscopic technique was the most important technique. In our pharmacopoeias this technique is based on the natural absorption of UV radiations, and other chemical reactions. Spectroscopy is totally based on the quantitative measurement, properties transmission, and wavelength function. This method has been great advantage to save time,or expenditure of labor. Also, this technique has great precision, and accuracy. In pharmaceutical analysis this method was specially applied to analyze the dosage forms in pharmaceutical industries has been increased regularly.

(a) UV-Visible Spectroscopy
(b) FTIR Spectroscopy
(c) Mass Spectroscopy (MS)
(d) Nuclear Magnetic Resonance Spectroscopy (NMR)
(e) Fluorimetry and Phosphorimetry

2 CHROMATOGRAPHIC TECHNIQUE

The separation of components of a mixture is essential in our daily life. For this purpose, different separation techniques like crystallization, filtration, evaporation, sublimation, distillation, chromatography, etc., are used based on the nature of components for separating the mixture.

(a) High Performance Thin Layer Chromatography (HPTLC)
(b) High Performance Liquid Chromatography (HPLC)
(c) Thin Layer Chromatography (TLC)
(d) Gas Chromatography
(e) Paper chromatography
(f) lon exchange chromatography

2.1 Electro Analytical Methods

Electro analytical methods are a class of techniques in analytical chemistry which study an analyte by measuring the potential (volts) and/or current (amperes) in an electrochemical cell containing the analyte. These methods can be broken down into several categories depending on which aspects of the cell are controlled and which are measured.

The three main categories are:
Potentiometry (the difference in electrode potentials is measured)
Coulometry (the cell's current is measured over time) and
Voltammetry (the cell's current is measured while actively altering the cell's potential).
Polarography: is a subclass of voltammetry that uses a dropping mercury electrode as the working electrode.
Amperometry: is the term indicating the whole of electrochemical techniques in which a current is measured as a function of an independent variable that is, typically, time or electrode potential.

2.2 Electrophoretic Technique

It is a very important technique for drug analysis in pharmaceutical fields, and the proper name of this techniques capillary electrophoresis (CE). Capillary electrophoresis technique is totally based on the electric charge ions by means of electromagnetic field. This technique is based on the automatic experimentation of chemicals. It is the main instrument used for the measurement of chemical analysis in the presence of chemical and physical equilibrium.

2.3 Kinetic Technique of Analysis

The main implementation was made regarding the principle of kinetic technique which helps the scientist to chemical instrumentation process or highly applicable in the pharmaceutical drug analysis, data analysis and method development. Kinetic methods trust the measurements of concentration changes (detected via signal changes) in a reactant (which may be the analyte itself) with time after the sample and reagents have been mixed manually or mechanically.

Multi component kinetic estimations, most often referred to as differential rate methods, are also receiving wide acceptance in the field of pharmaceutical research.

Two new approaches of multi component kinetic estimation have been proposed for dealing with overlapping spectra of components in the binary mixtures.

1. Kinetic wavelength pair method
2. H-point standard addition method

2.3 Hyphenated Techniques

For the development of method the separation technique based on the coupling separation, and online separation will acquire to develop a new method for drug analysis which is called as hyphenated techniques. The determination of drugs in biological materials is an important step

in drug discovery and drug development. To increase the potential of drug analysis the hyphenated techniques were used:

- Liquid chromatography-Nuclear magnetic resonance (LC-NMR)
- Liquid chromatography-Infrared spectrometry (LC-IR)
- Gas chromatography-Mass spectrometry (GS-MS)
- Capillary electrophoresis-Mass spectrometry (CE-MS)
- Liquid chromatography-Photodiode array-Mass spectrometry (LC-PDA-MS)
- Liquid chromatography-Mass spectrometry-Mass spectrometry (LC-MS-MS)
- Liquid chromatography-Nuclear magnetic resonance- Mass spectrometry (LC-NMR-MS)
- Liquid chromatography-Mass spectrometry (LC-MS)

2.4 Microbiology and Biological Techniques

Microorganisms have found widespread uses in the performance of bioassays for: Determining the concentration of certain compounds (e.g., amino-acids, vitamins and some antibiotics) in complex chemical mixtures or in body fluids. Diagnosing certain diseases. Testing chemicals for potential mutagenicity or carcinogenicity. Monitoring purposes involving the use of immobilized enzymes. Sterility testing of antibiotics.

Microbiological assays are used during production to determine the potency and quality control. These are used to determine the pharmacokinetics of drugs in animal and human. In antimicrobial chemotherapy to monitor, in managing, controlling the chemotherapeutic agents.

- Protein analysis & quantification
- Protein sequence analysis
- Protein structure analysis
- Protein binding analysis
- Crude protein analysis (quantification of protein).

3 PHYSICAL METHOD

Thermomechanical Analysis (TMA): is used to characterize physical properties of materials when force is applied at specified temperatures and time periods. TMA is useful for investigating properties of viscoelastic materials, such as organic polymers. These materials exhibit both viscous and elastic properties that affect their response to mechanical stresses.

4 RADIOACTIVE METHOD

4.1 Radioimmunoassay

RIA methods have been used successfully for the determination of limitless number of pharmaceutically important compounds in biological fluids. Most of these methods are now automated with separation assisted by the use of antibody bound to a solid phase matrices. shows list of the compounds that have been analyzed by RIA in biological fluids. The most important advantage of RIA in the measurement of compounds in biological fluids is the quite precision and extreme sensitivity, which cannot be achieved by other analytical techniques.

5 CONCLUSION

The spectroscopy techniques for the quantitative and qualitative estimation of drugs have been includes the various methods UV-Visible spectroscopy, Mass spectrometry, Infrared spectroscopy, Nuclear magnetic resonance, Fluorimetry, and phosphorimetry. The process of separation of drugs also depends on the chromatographic techniques includes High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC) which is an powerful separation technique, and thin layer chromatography was useful for bulk drug

screening, Gas chromatography to determines the impurities in pharmaceuticals. For the determination of drugs the electrochemical and electrophoretic technique were used. The electrochemical method helps the scientist to check the electrochemical nature of drugs by the use of voltammetry, chronocoulometry, pulse voltammetry etc. The capillary method in electrophoretic analysis of drugs was used for the quantitative estimation of drugs by applying electromagnetic field. To determine the flow system in a analytical process the flow injection analysis (FIA), and kinetic method were applies to justify the results. Hyphenated techniques for the analysis of drugs follow the various techniques in combination with two of three methods i.e; LC-NMR, LC-MS, LC-IR, GC-MS, CE-MS, LC-PDAMS, LC-MS-MS, LC-NMR-MS, LCPDA-NMR-MS etc.

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CICLOPIROX: NEW VISTAS FOR AN OLD ANTIFUNGAL AGENT

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Abstract - Irrational use of antifungal drugs along with corticosteroids has led to rampant emergence of resistant fungi; making its treatment highly complicated. Mucormycosis as post-treatment outcome of Covid-19 is the most recent noteworthy event. Ciclopirox olamine is an old, off-patent, efficacious, and safe topical antifungal of the hydroxypyridone family. Clinical trials with ciclopirox are ongoing with emphasis on its role in treatment of Taenia, Oncomycoses, and female reproductive tract cancers. Clinically, its use is well-established in the form of cream, nail lacquer, shampoos etc. with good safety profile. Erythema, irritation, redness, pain or pruritus, are documented following skin and vaginal application.

Over the years, in addition to the role of ciclopirox in killing dermatophytes, yeasts and molds, investigators have reported its beneficial effects as anti-ischemic stroke agent. It is found to alleviate brain infarction, neurological deficits and brain edema after ischemia. In addition, it is reported to be antidiabetic via modulation of ER stress and p21 activity. It may be used to treat porphyria by modulation of heme group biosynthesis. Moreover, anti-neoplastic activity against hematologic and solid tumors is noteworthy and reportedly occurs via inhibition of multiple signaling pathways like PERK, HMG-A2, DJ-1 mediated autophagy, cdc25A, mTOR, β-Catenin-c-Myc. However, contrary to its pleiotropic actions, it remains under-utilized in clinics. Hence, the aim of this review is to give a bird's eye view of the antifungal profile of the agent and provide an in-depth information of the new vistas that are emerging in latest research along with the obstacles preventing its use.

Keywords: Candida; ciclopirox olamine; dermatophyte; mycoses; pityriasis versicolor; seborrheic dermatitis; tinea; cancer.

1 INTRODUCTION

Ciclopirox is a cyclic hydroxamic acid and a hydroxypyridone antifungal drug, a unique chemical used in topical antifungal treatment regimens. Chemically, ciclopirox is an ethanolamine salt of6-cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone. It was first launched in European market after its discovery in late 1960s. Ciclopirox olaminepossess a broad spectrum of activity which includesactinomycetes, candida species, dermatophytes, molds, yeasts, other fungi, and a variety of Gram-positive and Gram-negative bacteria. Ciclopirox is available as a spray, powder, solution, cream, lotion, gel, vaginal cream, and nail lacquer. Clinically, 1% ciclopirox olaminecream and 8% ciclopirox acid nail lacquer is used.

The mechanism of action of ciclopirox involved chelation and ligand formation with intracellular trivalent metal cations. This chelation or binding restricts the availability and access of metal ions to cellular metal-dependent enzymes, which are vital for fungal cell survival. Functional genomic approaches have confirmed this recently. Amidst different metal ions iron chelation is predominant, which inhibits fungal growth. In addition, ciclopiroxis reported to inhibit secreted form of aspartyl proteinases in some Candida species, thereby hindering the adhesion of fungi to its substrates. This unique and pleiotropic mechanism of action is helpful in preservation of sensitivity and spectrum of activity. This is supported by evidence-based

medicine in the treatment of superficial mycoses orvaginal candidiasis, where ciclopirox was mainstay of the antifungal regimen.

The safety profile of ciclopirox is well documented. No systemic adverse reactions are known. Local adverse effects are limited to burning, redness, minor pain of skin. In some instances pruritis is also seen. Application on nails is associated with mild erythema. Overall it is extremely good in terms of safety. But, its use in clinics is limited probably dues to its less efficacy as compared to other potent drugs.

Furthermore, ciclopirox produced mild anti-inflammatory effects in some animal's studies, which are also evident in few clinical case-reports. The purported mechanism of anti-inflammatory effects appears to be scavenging of reactive oxygen species in addition to iron chelation. Ciclopirox was found to be the most promising compounds, inhibiting HIV and other STI-causing pathogens in a study that screened in excess of 2000 topical products in India. On further exploration, it was found that ciclopirox blocks the HIV-1 replication by inhibiting the substrate binding to enzyme deoxyhypusine hydroxylase, required for formation of unique amino acid hypusine (lysine derivative). This results in inhibition of maturation of eIF5A (Elongation factor).

Ciclopirox inhibits cell proliferation and angiogenesis, implicating its role in cancer treatment. This is supported by studies wherein ciclopirox was instrumental in treatment of malignant leukaemia, myeloma and solid tumorcell lines at pharmacologically achievable concentrations. Cciclopirox also in hibitstumor growth inhuman breast cancer xenografts, possibly involving caspase-dependent mechanism. Other than cancer, ciclopirox appears to be promising ant malarial agent as it inhibits deoxyhypusine hydroxylase. The IC50 for Plasmodium falciparum cell culture is 8.2mmol/L.

Thus, plethora of studies are implicating a potential new vista for ciclopirox, with mainstay in cancer pharmacotherapy along with some cytoprotective and anti-inflammatory effects. Owing to this, the present review aims to highlight its pharmacokinetic properties along with a glimpse of its antimicrobial profile and provide an in depth, detailed summary of current literature detailing its effects in cancer.

2 PHARMACOKINETICS

Ciclopirox is available in the form of various topical formulations hence the transdermal kinetics is discussed in detail in many reviews. Radiolabeled study also documented and confirmed that after topical administration, the drug is rapidly glucuronidated and the drug in plasma is >90 % protein bound. However, in recent times after its repurposing for cancer, oral pharmacokinetics became need of the hour. Hence, an oral suspension was formulated in 2012 and its pharmacokinetics was studies in subjects (Clinical trial ID: NCT00990587).A dose range of 5-80 mg/m2 was covered. Once daily dosing up to five days along with multiple daily dosing (Upto 4 times a days) were investigated. The absorption of ciclopirox olamine oral formulation was rapid and complete with Cmax being achieved in 0.5-4 h duration. The half-life was found to be 5.2 ± 4.3 h after single dose administration. The half-life was reportedly reduced to 2.7 ± 0.5 h after five days of administration. The drug was exclusively glucuronidated and the metabolite Cmax and AUC were 10-fold higher as compared to plain drug. Thus, rapid metabolism along with dose limiting toxicity at 80 mg/m2 were highlights. This stemmed the discovery of prodrug of ciclopirox, fosciclopirox (phosphoryl-oxymethyl ester) for parenteral administration. The pharmacokinetics after s.c. administration was studied in animals by Weir et al., 2019 and further evaluated in clinical trials (NCT03348514).

3 PHARMACODYNAMICS

Ciclopirox is an antifungal agent with antibacterial and other pleotropicactions. Ciclopirox acts by chelating intracellular trivalentcations, namely Fe3+ and Al3+. The chelation of these cations results in inhibition of essential fungal enzymes by limiting co-factor availability. It produces defects in mitochondrial electron transport processes, disrupting the energetics. It also modifies membranous organelles and causes disorganization of internal structures. Ciclopirox may also exert its effect by disrupting DNA repair, cell divisionsignals, and structures (mitotic spindles) as well as some elements of intracellular transport. Thus, it appears that ciclopirox does not act via a single mechanism but has pleotropic actions in microorganism owing to its chelation property primarily.

3.1 Spectrum

Ciclopirox is one of those unique chemicals that exhibits activity against a plethora of microbes including fungi. The broad spectrum of activity is summarized in table 1. Its broad antifungal spectrum includes Candida, Trichophytons, Microsporum, Cryptococcus, Saccharomyces, Aspergillus and Fusarium species. The antimicrobial spectrum encompasses some gram-negative species viz., Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae, and some Gram-positive species viz., Staphylococcus aureus, b-haemolytic Streptococcus group A, Micrococcus luteus, Micrococcus sedentarius, Corynebacterium minutissimum, Brevibacterium and Corynebacterium. Recent reports have also documented the antiviral properties of ciclopirox on Herpes Simplex Virus-Type I and II.

4 ANTI-CANCER RESEARCH AND CICLOPIROX

Ciclopirox olamine anticancer properties are revealed as a result of drug repositioning studies. Ciclopirox been studied for its antitumor properties and its efficacy is demonstrated in human rhabdomyosarcoma, breast carcinoma, colon adenocarcinoma, bladdercarcinoma, esophageal carcinoma and hematologic malignancies (See table 2 for details). However, the molecular mechanism of these effectsis not established. Some of the studies have found accelerated degradation of Cdc25A protein to be responsible for inhibition of cell proliferation (Shen et al., 2017). Another study spotted down regulation of the WNT/β-catenin signaling pathway in esophageal tumor cell inhibition (Al-Dali et al., 2018; Ryan et al., 2018). In addition, study in pancreatic cancer model have identified reduced Bcl-xL and survivin levels along with activation of a panel of caspases, especially caspase-3 (Mihailidou et al., 2018). Studies in colorectal cancers document activation of PERK-dependent endoplasmic reticulum stress by ciclopirox as a cause for cancer cell death (Qi et al., 2020).Huang et al., have reported the inhibition of HMGA2-a non-histone chromatin protein. HMGA2 possess pleotropic actions in cell cycle regulation, apoptosis, DNA repair, and epithelial–mesenchymal transition (Huang et al., 2019). Furthermore, it is also shown to reduce HPV E6/E7 on cogene expression and thereby remains effective in human papilloma virus induced cancers (Braun et al., 2020). One of the studies have implicated down regulation of intracellular ferritin and inhibition β-catenin-c-Myc signaling pathway in a study using T-all cell lines (Wu et al., 2016). The drug's intracellular iron chelator property is also thought to be important in hematologic malignancies (Minden et al., 2014).

In conclusion, the search for novel agents via repositioning studies has yielded an agent, which exhibits pleiotropic actions on multiple intracellular proteins that play an important part in the cell survival and cell differentiation and multiplication pathways. Thus, it appears to be a lucrative agent being effective via numerous pathways reducing the tendency to develop resistance.

4.1 Anti-inflammatory research and Ciclopirox

Numerous recent studies have reported that ciclopirox has the potential to be repurposed as a cytoprotective and anti-inflammatory agent. A recent study reported ciclopirox to be effective in Sytox Green uptake assay (Regdon et al., 2021). Ischemic stroke resulting from acute cerebral thrombosis has severe clinical consequences. A recent work suggests that ciclopirox could be a promising compound to reduce multiple ischemic injuries by providing neuroprotection after single dose administration in a rat model (Feng et al., 2020). It is also reported to be effective in moderate-to-severe scalp seborrheic dermatitis (Veraldi et al., 2019).

4.2 Future Prospects

The global market of ciclopirox olamine is continuously growing owing to its steadily increasing use in Ringworm, Athlete's foot, Tinea, Candida and gynecological diseases. The recent reports documenting its anticancer, anti-inflammatory and anti-malarial activity has spurred an increase in the number of clinical trials actively engaging seeking new regimens involving ciclopirox. In fact, oral formulation in terms of its prodrug is also being sought for its successful use by parenteral therapy. So, there is a need for researchers to work in the domain of NDDS especially nano-particles based NDDS to ensure availability or feasibility of oral route for the use of Ciclopirox olamine. If established it could prove a milestone in establishing this drug for its purported use in cancer and other related conditions and pave way for many more vistas in future.

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NANOCARRIERS FOR TREATMENT AND MANAGEMENT OF ANTIFUNGAL ACTIVITY

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Abstract - A fungus infection is the most common global skin health issue. They are usually treated with topical or systemic antifungal therapy. Topical therapy is usually preferred due to its targeted therapy and less side effects. Due to their unique structural and functional characteristics, advanced topical carriers overcome many biopharmaceutical challenges associated with conventional drug delivery systems like poor retention and low bioavailability. Evidence from literature suggests topical antifungal nanocarriers with anti-fungal agents provide superior results with minimal side effects. Different types of nanocarriers are widely used for topical antifungal medication, including Solid-Lipid nanoparticles, Microemulsions, Liposomes, Niosomes, Microsponge, Nanogel, Nanoemulsion, Micelles etc.In this article we summarize recent advances in topical carriers that are employed to promote the therapeutic effectiveness of anti-fungal drugs.

Keywords: Nanocarriers, Antifungal, Nano particles, Nanomaterials, Infectious disease, Pharmaceutical science.

1 INTRODUCTION

Fungal infection is a leading skin disease around the world. The declared prevalence of fungal infection is approximately 40 million people in developing and underdeveloped countries. Fungal infection may be superficial and invasive. Superficial infections are more frequent and affect 25-30% of the population, while invasive fungal infections are less prevalent but have excessive morbidity and motility rates(1-5).Fungi usually attack the surface of the skin during the initial stage, then invade the deeper layer by desquamation. Candida species are among the fungi which are the most superficial dermal infections(6).In India, approximately 7 percent of all infected patients die each year. About 15-20 % of patients suffer from chronic or recurrent fungal infections(7).

The five broad categories of antifungals are polyenes, azoles, allylamines, echinocandins and antimetabolites. Polyenes have a double bond conjugated alternatively within the framework of their annular structure of macrolides. The anti-fungal family includes nystatin, amphotericin B and pimaricin. Amphotericin B forms an ergosterol complex and modulates cell membrane permeability, resulting in cell content leakage and therefore cell death. Azoles consist of five organic cycles separated through two (imidazole) or three (triazole) nitrogen molecules. The mechanism of action of azoles is the 14α demethylation of lanosterol, a major step in ergosterol biosynthesis. The most highly regarded members of the allylamine family are morpholine and terbinafine. Allylamines inhibit the biosynthetic pathway of ergosterol, which is a component of the fungal cell membrane through the transformation of squalene into squalene epoxide via squalene epoxidase. Examples of echinocandins are Caspofungin, micafungin and anidulafungin(8).

Nanotechnology is a powerful tool in the field of medicine to fight a plethora of illnesses, such as cancer and Cardiovascular diseases. Thus, it is not surprising that minuscule structures (<1000 nm) have been extensively used as a deliveryvehicles of a variety of therapeutic substances, ranging from small. molecular drugs, genes and biopharmaceutical drugs (e.g., proteins, peptides) and diagnostic imaging agents. Nanocarriers are colloidal drugcarrier

systems whose submicron particle size is usually less than 500 nm(9).Nowadays, nanoparticles are very popular for their wide range of applications in various biological, pharmaceutical and medical fields(10). Improvements in pharmacokinetics and biodistribution, reduced toxicity, improvements in solubility and stability, controlled release, and administration of site – specific therapeutic agents are just a few. some of the characteristics nanocarriers can integrate into the drug delivery systems. Structurally, they are only more than 100 nm in size. A wide variety of drugs, like small hydrophobic and hydrophilic drugs, vaccines and biological molecules can be controlled with these nanoparticles(11). Nano-structured systems have been shown to be beneficial as excellent carrier of antifungal drugs and can be effective in targeting skin layers. Nano-technologies began to be used in the 1990s. Several kinds of nanocarriers have been developed and studied for their effectiveness in the treatment of different diseases. These nanocarriers could contribute to the administration of drugs and active halves at the target site atoptimum therapeutic levels (12). Nanoparticles are encouraging antifungal carriers. In this respect, the effectiveness of approaches based on nanoparticles as vectors of drugs or to combat microbes is exemplary. Topical formulations based on solid-lipid nanoparticles, liposomes, nanoemulsions and so on have been reported to have the greater capacity to overcome the skin permeation barrier (13-15). This review starts with a brief overview of the characteristics of solid lipid nanoparticles and discusses the relevance of performing systematic studies.(16) The major applications, as well as the benefits that this type of nano carrier offers in certain antifungal activity are discussed. Next, Pathophysiology of fungal disease are described. Safety and toxicity issues are also addressed. Our work offers a unique perspective, addressing the biopharmaceutical aspects of these nanovesicles using descriptive statistics from leading-edge research on solid lipid nanoparticles.

2 PATHOPHYSIOLOGY OF FUNGAL DISEASES

The fungi that cause the greatest number of illnesses are Aspergillus, Candida, Coccidioides, Penicillium and Cryptococcus. Fungal infections are brought onwhile an invading fungal species colonises a specific body region, and when the body's natural immune mechanism is outdated. A fungus reproduces and frees micropores locally, which are then propagated into its extensive environment. Primary skin and lung infections are caused by inhalation of these micropores or come into contact with the surface of the body(17-19). Fungal infections include mostly dermatophytosis, athlete's foot, candidiasis, itchiness of jock or tinea cruris, and ringworm or tinea corporis(20,21).

2.1 Dermatophytosis

It is derived from dermatophytes, keratinophilic fungi which can invade keratinized tissues (for example, nails, hair and skin).They invade, infect, and persist in the stratumcorneum and sometimes penetrate beneath the surface of the epidermis and itsappendages. Depending on microscopic features, dermatophytes are categorised as Epidermophyton, Microsporum and Trichophyton species(22-24).

2.2 Athlete's Foot

It is also known as Tinea pedisis, a common fungal infection which affects the foot region of the body. In general, they are sports zones because the fungus spreads completely in a warm and moist environment – for example Sports equipment, footwear, socks, change room and moist cloths(24).

2.3 Candidiasis

In females, vaginal yeast infections are due to Candida albicans. Enlargement of the candida disrupts the normal stability of yeast and bacteria in the vaginal organ. Basically, Candida is the reason behind fungal infections in the mouth and vaginal region. The vaginal aphthosis contains feelings of tanginess, discomfort and a warm sensation. In general, oral aphthosis is a pale spot on the tongue observed in pediatrics(25).

2.4 Jock Itch

Itchy jocks (Tinea cruris), a fungal disease of the skin, survive wet and hot temperatures. Hence, it grows out of control over the inner thighs, buttocks and groin. Jock itching propagates through direct interaction with an infected patient or fungal zone(26).Indocile infections are regarded as easy to deal with, but tinea cruris is very difficult.In jock itches, the groin region is infected, and high temperature, greed and foul odour are the most common symptoms. In infectious patients, the most commonly observed symptoms are red spots and swelling(27)

2.5 Ringworm

Ringworm (Tinea corporis), a skin disease that causes itching of the athlete's foot and jock (27).Dermatophytes are primarily responsible for this fungal infection. Those ringworm spots are red, spherical and irritating rashes (28).

3 NANOCARRIER IN FUNGAL INFECTIONS

Nanocarrier have unique optical, electrical, mechanical or chemical characteristics that have advantages compared to the conventional dosage form(29). Nanotechnology is concerned with the creation of devices and materials. A wide range of nanocarriers used as drug delivery systems for treating fungal diseases include phospholipid vesicles (such as liposomes, transferosomes, ethosomes, transethosomes etc); non-phospholipid vesicles (spanlastics and niosomes); nanostructured lipid carriers (NLCs); solid lipid nanoparticles (SLNs);nano-emulsion; polymeric nanoparticles (like chitosan-based nanoparticles); metal oxide nanoparticles; silver nanoparticles (AgNPs); carbon nanotubes (CNTs); and dendrimers(30,31).An overview of nanocarriers used against different dermatophyte is presented in Fig no. 1. Antifungal antibiotics and used to treat various infections are given.

4 APPLICATION OF NANO CARRIERS SYSTEM FOR FUNGAL INFECTION

4.1 Liposomes–

- Due to the liposome formulation of econazole hyperkeratosis, focal thickening of the stratum corneum, dyskeratosis, and parakeratosis are totally eliminated.
- 5-fluorocytosine, and amphotericin-B together with the known antileishmanial drug pentamidine.
- Ciclopirox Olamine mucoadhesive liposomes Miconazole nitrate (MCZ-N) liposomes (32).

Transferosomes –

- Terbinafine for onychomycosis.

Ethosomes –

- Econazole nitrate [ECZ-N] ethosomes used to treat Ringworm.
- Fluconazole Ethosomes used to treat variety of fungal and yeast infections(33).

Transethosomes –

- Transethosomes of Econazole Nitrate used to treat athlete's foot, jock itch, and ringworm.

Niosomes –

- Ketoconazole Niosome used to treat athlete's foot, Jock itch, ringworm, and other candidiasis.

Spanlastics –

- Luliconazole Spanlastics to Augment the Antifungal Activity against Candida albicans.

Solid lipid nanoparticles –

- Sustained release of Ketoconazole in SLN/ Dextran hydrogel used to treat athlete's foot, Jock itch, ringworm, and other candidiasis(34).

Nanolipid carrier –

- Cyclothiazide Ketoconazole in NLC hydrogelused to treat athlete's foot, Jock itch, ringworm, and other candidiasis.

Nanotubes –

- Amphotericin B conjugated to carbon nanotubes used for serious fungal infections and leishmaniasis.

Chitosan nanoparticles –

- Antifungal activity of natural compound chitosan and its nanoparticles forms against Candida albicans, Fusarium solani and Aspergillus niger. Chitosan nanoparticles (35).

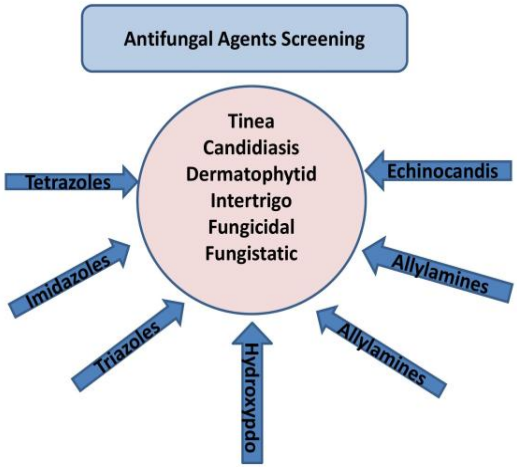

Fig. 1 Antifungal Antibiotics and used to treat various infections.(40)

5 TOXICITY OF NANOCARRIERS

Nanoparticles are increasingly being used in different areas. NPs were investigated for cellular toxicity, immunotoxicity and genotoxicity. Although the number of NPs types and applications continues to increase, there are few studies to characterize their post-exposure effects and treat their potential toxicity(36). Compared to conventional substances in a microscale, NP (diameter less than 100 nm) occurs on a huge reactive surface capable of interacting with biological systems and causing toxic effects. Most antifungals have low solubility in water, which tends to indicate lower absorption and bioavailability. It can be enhanced by forming complexes with

nanocarriers. Due to their nanoscale, they have greater interactions with biological surfaces, with both positive and negative effects (37). Nanoparticle toxicity may be related to their size, as suggested by studies of ultrafine particular matter in the respiratory tract(38). The toxicological effects of ultrafine particles are determined based on their size and propensity to agglomerate. These are also known as pass biological barriers, such as the skin, vascular endothelium and BBB; consequently, affecting the absorption, distribution and excretion of these particles(39-42).

6 CONCLUSION

Fungal infections are increasingly threatening human health. Inappropriate and irrational use of antifungal chemotherapy has resulted in the development of multidrug resistant fungal pathogens, undesirable toxicity and poor therapeutic efficacy. According to the current literature, new and alternative drug delivery systems currently focusing on a variety of research activities. The trapping of antifungal in nanoformulation allows better therapeutic activity and, in many cases, a prolonged effect by reactive release functions to stimuli. Through the proper ligand marking to the formulation, the target specific delivery of antifungal drug leading to a lower dose and a lower side effect is possible. According to some reports, nanoformulations may have broad-spectrum antifungal activity, and their mechanism of actions are different from conventional antifungals, so they may be able to overcome antibiotic resistance. A number of nanoformulations based on magnetic nanoparticles, dendrimers, liposomes and polymers are considered future antifungals. Long-term use of antifungal drugs are linked to potential side effects, patient non-compliance and reduced bioavailability at the target site that limits its clinical potential. To solve this problems, safe and effective novel drug delivery systems, which will decrease the dose with higher the concentration of drug in the target organ which have low systemic concentration, is highly desirable.

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IN VITRO MUTAGENICITY OF GMELINA ARBOREA ROXB (GAMBHARI) EXTRACT BY AMES ASSAY

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Abstract - Gmelina arborea Roxb (family Verbenaceae) commonly known as 'Gambhari' tree, the various parts of the plants are widely used in diarrhoea, anti-pyretic, thirst, anemia, leprosy, ulcers, consumption, strangury, vaginal discharges. We tested the genotoxic potential of G. arborea in Gram-negative bacteria Salmonella typhimurium TA98 (MTCC 1252) and Salmonella typhimurium TA100 (MTCC 1252) were used for the Ames assay using number of revertants as the toxicological endpoints. Aqueous extract of Gmelina arborea roxb (AEGA) was tested at the various concentrations 5, 10, 15 and 20 mg. The number of revertants significantly increased in strains TA98 and TA100 with and without S9 activation. The AEGA, when assessed with the strain TA98, displayed mutagenicity index (MI) between 1.2 to 2.2, without metabolic activation and between 1.2 to 2.1 with metabolic activation. With the strain TA100 the MI was between 1.1 to 1.5 without metabolic activation and between 1.04 to 1.1 with metabolic activation. The mutagenic indices were raised in strains TA98 and TA100 by metabolic activation. In this study, we investigated the effect of G. arborea on Gram-negative bacteria Salmonella typhimurium using number of revertants to assess the genotoxicity of the herb.
Keywords: Ames assay, Revertants, Genotoxicity.

1 INTRODUCTION

Gmelina arborea Roxb (Family Verbenaceae), an important commercial timber species has been used in Ayurveda Since ancient times. A large proportion of medicines used by the locals in India are still derived from plants or their extracts and little is known about the safety and efficacy of such alternative treatments [1-3]. In vivo and in vitro studies have shown that some natural constituents of plants parts (fruits, leaves, roots) play a modulating role on xenobiotic effects [4]. While some herbs might be pharmacologically or clinically effective, they are not necessarily free of toxicity and side effects. Therefore, investigation into the traditionally used medicinal plants is valuable as a source for potential chemotherapeutic drugs and as a safety measure for the continued use of medicinal plants [5]

The Ames test is a biological assay to assess the mutagenic potential of chemical compounds by affecting the structure of DNA. As cancer is often linked to DNA damage, the test also serves as a quick assay to estimate the carcinogenic potential of a compound. Since, the standard tests for carcinogenicity done on rodents take years to complete and are expensive to do. The procedure is described in 1970s by Bruce Ames and his group at the University of California, Berkeley[6].

The use of the Ames test is based on the assumption that any substance that is mutagenic (for the bacteria used in his test) may also turn out to be a carcinogen; that is, to cause cancer. Although, in fact, some substances that cause cancer in laboratory animals (dioxin, for example) do not give a positive Ames test (and vice-versa). The test is still considered an important part of assessing the safety of new chemicals due to the ease and low cost of the test [7-8].

The bacterium used in the test is a strain of Salmonella typhimurium that caries a defective (mutant) gene making it unable to synthesize the amino acid histidine (His) from the ingredients in its culture medium, so that they require histidine for growth. However, some types of mutations (including this one) can be reversed, a back mutation, with the gene regaining its function. These revertants are able to grow on a medium lacking histidine. The tester strains are

specially constructed to have both frameshift and point mutations in the genes required to synthesize histidine, which allows for the detection of mutagens acting via different mechanisms. The tester strains also carry mutations in the genes responsible for lipo polysaccharide synthesis, making the cell wall of the bacteria more permeable, and in the excision repair system to make the test more sensitive. The bacteria are spread on an agar plate with a small amount of histidine. This small amount of histidine in the growth medium allows the bacteria to grow for an initial time and have the opportunity to mutate. When the histidine is depleted only bacteria that have mutated to gain the ability to produce its own histidine will survive. The plate is incubated for 48 hours. The mutagenicity of a substance is proportional to the number of colonies observed[7-8].

2 MATERIALS AND METHOD

Plant material: Plant material used in this study was Gmelina arborea roxb. The leaves of Gmelina arborea roxb were shade dried and reduced to coarse powder in a mechanical grinder. The powdered material obtained was then subjected to extraction using Distill water in a Soxhlet extractor. The extract obtained was evaporated at 100°C to get a semisolid mass. The extract prepared at the Department of Pharmacology, Acharya and B.M. Reddy College of Pharmacy, Bangalore, India. The stock solution of plant extract (AEGA) 100 mg mLG1 was Prepared with sterile water and filtered through syringe filter (0.45 µL). Plates were exposed to various concentrations 5, 10, 15 and 20 mg of AEGA.

Microorganisms: Clinical strains of two human pathogenic bacteria of Gram-negative bacteria Salmonella typhimurium TA98 (MTCC 1252) and Salmonella typhimurium TA100 (MTCC 1252) were used for the Ames assay. All the microorganisms were obtained from Institute of microbial technology (IMTECH), Chandigarh and maintained in the Department of Pharmaceutical Microbiology & biotechnology, Acharya & B.M. Reddy College of pharmacy, Bangalore.

Chemicals and reagents: Alcohol (Gauri Industries limited, Mandya), Agar (Himedia), Agarose (GibcoBRL, USA)., Anesthetic ether (Karnataka Fine Chemicals, Bangalore), Benzyl penicillin (Sigma), D-Biotin (Himedia), Bovine Serum Albumin (BSA) (Himedia), Citric acid monohydrate (SRL, Bangalore), Dipotassium hydrogen ortho phosphate (Karnataka Fine Chemicals, Bangalore)., Disodium hydrogen ortho phosphate Dihydrate (Karnataka Fine Chemicals, Bangalore), Glucose (SRL, Bangalore), L-Glutamine (Sigma), L-Histidine (Sigma), Magnesium chloride hexahydrate (Karnataka Fine Chemicals, Bangalore), Magnesium sulfate Heptahydrate (Karnataka Fine Chemicals, Bangalore), Nutrient Agar (Himedia), Potassium dihydrogen phosphate (Karnataka Fine Chemicals, Bangalore), Di sodium hydrogen phosphate (Karnataka Fine Chemicals, Bangalore), Sodium ammonium hydrogen phosphate (Karnataka Fine Chemicals, Bangalore) and Di potessium hydrogen phosphate (Karnataka Fine Chemicals, Bangalore). All chemicals used in this study were of analytical grade purity and all test solutions were freshly prepared before each experiment.

Reagents and media

0.5 mM histidin/0.5 mM biotin solution for the top agar in mutagenicity test, Vogel-Bonner medium E (50 x strength stock) for minimal agar base, 40% glucose (autoclaved sterile), Minimal glucose plates for mutagenicity test, Top Agar for mutagenicity test, 0.2M Sodium phosphate buffer, Fresh 80 mM NADP (Triphosphopyridine nucleotide, sodium salt, hydrate), Metabolically activated rat liver S-9 fraction.

The Salmonella Mutagenicity Assay: [9-11].

The mutagenicity assay with S. typhimurium was performed as described by Maron and Ames. The test was based on the plate incorporation method, using S.typhimurium test strains TA98 and TA100 with and without an exogenous metabolic system: S9 fraction in S9 mixture.

2.1 Procedure

Prepared serial concentrations 5, 10, 15 and 20 mg/ml of AEGA, and solutions for negative controls. Dissolved a top agar aliquot (100 ml) in microwave oven. Added 1/10 volume (10 ml) of 0.5 mM histidine/0.5 mM biotin solution to the molten top agar. Mixed thoroughly by swirling. Dispensed 2 ml of molten top agar to each of culture tubes held at 45°C in a heating block. Caped the tubes. Prepared S-9 mix. Added 0.1 ml of a fresh overnight culture of the test strain, and 0.5 ml of PBS (PBS can be added with 0.5mM bio/his or optionally 0.5 ml of cold S-9 mix) to each agar tube. Both control with and controls without S9 mix are necessary. Vortexed the mix for 3 sec at low speed. Poured the mix on a minimal glucose agar plate. Quickly tilted and rotated to spread the top agar evenly on the plate. Allowed several minutes for hardening. Added different concentration of AEGA (500 µl) in each test control plate. Incubated the invert plates at 37°C in a dark vented incubator. After 48 h incubation, checked the background lawn of the plates and scored the revertant colonies using the automatic colony counter.

3 RESULTS:

Effect of AEGA on different strains of Salmonella typhimurium (Ames test).

S.No.	AEGA Mg/Plate	Revertants/Plate in *Salmonella typhimurium* strains			
		TA98		TA100	
		-S9	+S9	-S9	+S9
1.	Control	20±1.15	25±0.66	82±2.08	110±3.46
2.	5	25±0.57ns(1.2)	35±1.15ns(1.4)	95±2.08ns(1.1)	115±1.15ns(1.04)
3.	10	30±2.30**(1.5)	40±2.30**(1.6)	102±0.88**(1.2)	126±1.52**(1.1)
4.	15	38±1.73**(1.9)	47±4.04**(1.8)	110±3.46**(1.3)	130±3.60**(1.18)
5.	20	43±1.00**(2.1)	55±2.64**(2.2)	120±6.55**(1.5)	142±3.60**(1.2)

Mutagenic activity expressed as the mean±SEM (MI) of the number of revertants/plate in bacterial strains TA98 and TA100 exposed to the AEGA, at various doses, with (+S9) or without (-S9) metabolic activation, in triplicate cultures. Asterisk symbol indicates**=$p<0.01$= very significant, ns = $p>0.05$= Not significant.

Table shows the number of revertants/plate, the mean±SEM and the mutagenicity index (MI) after the treatments with the AEGA, in the two different strains of Salmonella typhimurium, with or without metabolic activation. The AEGA was mutagenic to the strains TA98 and TA100 with and without S9 activation.

The number of revertants significantly increased in strains TA98 and TA100 with and without S9 activation. The AEGA, when assessed with the strain TA98, displayed mutagenicity index (MI) between 1.2 to 2.2, without metabolic activation and between 1.2 to 2.1 with metabolic activation (Table 6). With the strain TA100 the MI was between 1.1 to 1.5 without metabolic activation and between 1.04 to 1.1 with metabolic activation. The mutagenic indices were raised in strains TA98 and TA100 by metabolic activation.

4 DISCUSSION

The Ames test is a biological assay to assess the mutagenic potential of chemical compounds by affecting the structure of DNA. As cancer is often linked to DNA damage, the test also serves as a quick assay to estimate the carcinogenic potential of a compound.

AEGA, significantly increased the revertants/plate and MI in the two different strains of Salmonella typhimurium, with or without metabolic activation (Table 6). The AEGA was mutagenic to the strains TA98 and TA100 with and without S9 activation. The mutagenic indices were raised in strains TA98 and TA100 by metabolic activation.

The existence of compounds in the extract may cause base substitution (in TA100) and frame shift (in TA98 strains) mutations[11].

Tannins are described in the literature as antimutagenic, antioxidant and antitumoral compounds. In the presence of certain metal ions, tannins are pro-oxidant, cytotoxic and genotoxic. The genotoxic activity of tannins in cells of mammals caused single-stranded DNA breaks, detected by the comet assay [11].

Flavonoids are recognized for their antioxidant activity which is related to other beneficial effects: antibacterial, anti-inflammatory, antiallergic, antiviral, anticarcinogenic and hepatoprotective action. The antioxidant activity of the Flavonoids is directly related to the chemical structure, mainly to the number and position of phenolic OH-groups in the structure. However, several studies credit the flavonoid with pro-oxidative and mutagenic activities and these activities are also related to the structure of the molecules[11].

Phytochemical analysis of the AEGA identified the presence of tannins and Flavonoids which might caused the detected genetic damage and involved in the observed mutageniticity.

Acknowledgement

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PROTEOLYSIS TARGETING CHIMERA- AN EMANATING PROTEIN DEGRADATION TECHNOLOGY

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Abstract - Proteolysis-targeting chimera (PROTAC) has been emerging technology for targeted protein degradation. PROTAC is a heterobifunctional molecule comprised of ligand- mostly a small molecule inhibitor of the targeted protein linked to another ligand of E3 ligase via flexible linkers. It promotes the degradation of Protein of Interest (POI) by forming a Ternary complex with E3 ligase. E3 ubiquitin ligase induces the ubiquitination of POI which is further degraded by endogenous 26s proteasomes known as a ubiquitin-proteasome system (UPS) which helps in the degradation of misfolded/unused proteins. PROTAC regulates the protein function via degrading protein instead of inhibiting them, developing sensitivity to drug-resistant targets and the possibility of affecting non-enzymatic functions. PRTOACs have been extensively studied all over the world and have been shown to outperform not only in cancer but also in immunological disorders, viral infections, and neurological diseases. Here review discusses different POI recruiting different E3 ligases connected with help of different units of the linker and also highlights what all PROTAC molecules have been established by different pharmaceuticals as well as biotechnological industries.

1 INTRODUCTION

PROTAC (Proteolysis Targeting Chimera) is a heterobifunctional molecule comprising of ligand mostly a small molecule inhibitor of a targeted protein linked to another ligand of E3 ligase via different flexible linkers. PROTAC is becoming an appealing technology for regulating the protein of interest by causing degradation.1 Many excellent reviews have been published in different literature some of the mares are summarized here Sun et al described PROTAC as a chemical knockdown approach presenting novel and divergent biology comparable to other gene-editing tools.1 Goa et al PROTAC is an emerging technique with a novel therapeutic strategy in drug development attracting various academic institutions and pharmaceutical companies. 2Li et al signify PROTAC technique mimicking pharmacological protein inhibition and complementing nucleic acid-based gene lockdown technology for targeted protein reduction.3 Ishida et al specify PROTAC working catalytically and sub-stoichiometrically which specifies that it gets recycled over again and again and shows fractional occupancy at POI leads to higher degradation than conventional inhibition.4Pei et aldescribed heterobifunctional PROTAC as capable of targeting an undruggable target of about 85% human protein indicating greater possibilities in the therapeutic field. 5The mechanistic action of PROTAC(Figure no.1) is to use the ubiquitin proteasomes system to ubiquitinate the targeted protein. Here E3 ternary complex is formed between Target, PROTAC, and E3 ligase, where E2 ubiquitin-conjugating enzymes translocate the ubiquitin molecule to the lysine residues on the target protein surface. After the polyubiquitination, recognition of lysine leads to the signal generation via proteasome which facilitates the degradation of the targeted protein.5One of the major advantages of PROTAC is that it induces complete protein degradation rather than inhibition so that's why chances of resistance have been lowered and PROTAC molecules are believed to be therapeutically more efficacious than the conventional inhibitor.6 PROTAC role is different from conventional action of a drug as first of all PROTAC molecule does not need to bind to the active site of the target rather it achieves degradation of targeted protein by binding to the non-active site of targeted protein via moderate binding force.7

1.1 Historical Aspects of PROTAC

The concept of PROTAC molecule was introduced in 2001 by Craig M. Crews and Raymond J. Deshaises and reported the first peptide PROTAC molecule.8 In 2004 VHL E3 ligase-based Poypeptide based PROTAC molecule was established and then Craig and Crew synthesized small molecule Protac in 2008 recruiting Androgen receptor with MDM2 E3 ligase. In 2013 Craigs laid the foundation of the first pharmaceutical company focusing on PROTAC named ARVINAS. Then in 2015, PROTAC based on VHL and CRBN with nanomolar degradation was discovered. Later after some years in 2019, Arvinas after doing extensive research in the field of protac, it leads to the discovery of the first AR degradant ARV-110 which is used for the treatment of prostate cancer.

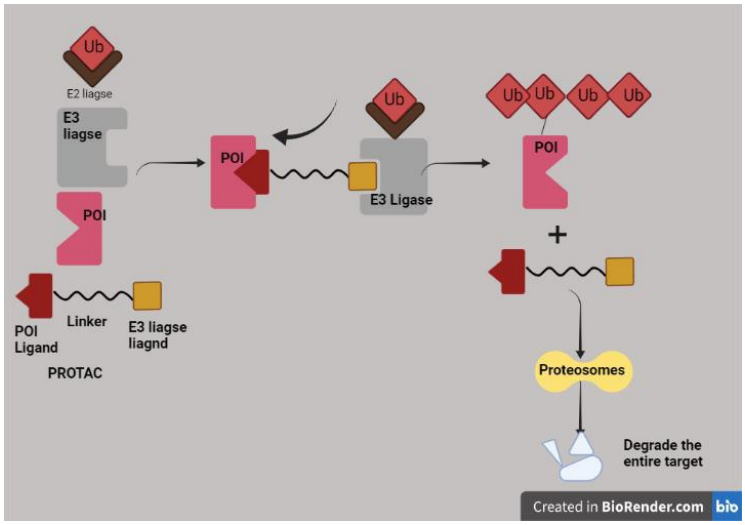

Figure 1: Mechanistic action of PROTAC molecule

ARV-110 was the first protac molecule to enter into the Phase 1 clinical trials setting a benchmark in the field of research. Later in the same year in October, Arvinas declared the initial results of the phase I clinical trials of ARV-110 and ARV-471 and reported that Oral PROTAC is safe and tolerated in cancer patients.3 Arvinas in February 2022 announced that ARV-110 has completed Phase 1 and interim Phase 2 ARDENT data and these data continue to produce anti-tumor activity and clinical benefits in a patient with metastatic castration-resistant prostate cancer.

2 OPPORTUNITIES EXEMPLIFIED BY PROTAC

Being a novel approach PROTAC technology has gained importance over time in both industry and academia. Extensive research is being carried out in both pharmaceutical and biotechnological industries. To date, there are about 30-35 proteins that have been targeted by the molecule having a major impact on proteins for cancer therapy. The different targeted proteins are nuclear receptors which include Estrogen receptor(ER), Androgen Receptor(AR) and RAR, Protein kinases, anaplastic lymphoma kinase. Crews used Enzalutamide recruiting VHL E3 ligase with suitable linkers against Androgen receptor named as ARCC-4.6 and used for both prostate cancer as well as breast cancer cell lines. The next target which has been successfully targeted is which is a bromodomain and extra terminal domain (BET) family member and were named ARV-825 recruiting pomalidomide, an E3 ligase cereblon binding moiety with BRD4 inhibitor OTX015.9 Simultaneously for protac molecule development, different E3 ligase was utilized as the Human genome comprises about >600 E3 ubiquitin ligase playing an important role in normal human cellular physiology and disease state making it an attractive target but E3

ubiquitin ligase lack deep and active binding site.4 Inhibiting E3 ubiquitin ligase via small molecules will target protein-protein interaction, making it challenging for drug design, some of the molecules have been designed against these ligases such as MDM2, VHL, and IAPs but poses some of the limitations. So E3 ubiquitin ligases are not meant for inhibiting the target rather they must be hijacked by themselves and promote target recruitment. 10Many E3ligases have been identified (Figure:2) but Von Hippel Lindau(VHL) and cereblon(CRBN)11 have gained importance due to the discovery of high-affinity PROTAC .12 To date CRBN successfully utilized targeting more than 30 different proteins which are involved in various cancer, a neurodegenerative disease associated Tau protein, immune disorder and also hepatitis C virus protein NS3C. Derivatives of pomalidomide, 4-hydroxythalidomide, lenalidomide, and alkyl-connected thalidomide were employed majorly by CRBN targeting PROTAC. Similarly, VHL13 has also been extensively used and targeted by PROTAC molecules and utilized for targeting or degrading more than 20 different proteins successfully.13-14 Currently, the PROTAC molecule has been successfully employed in the degradation of targeted protein of various diseases like cancer, neurodegenerative disease, viral infection, an immune disorder. Some of the few cases have been outlined including PROTAC molecule from AstraZeneca targeting B-cell lymphoma 6 from BCL6.P300/CBP associated factor and general control nondepressible 5(PCAF/GCN5)from GlaxoSmith Kline. PROTAC targeting Bruton tyrosine kinase from the Pfizer and another protein targeted by protac is given by GSK which is Interleukin-1 receptor-associated kinase 4 (IRAK-4).2

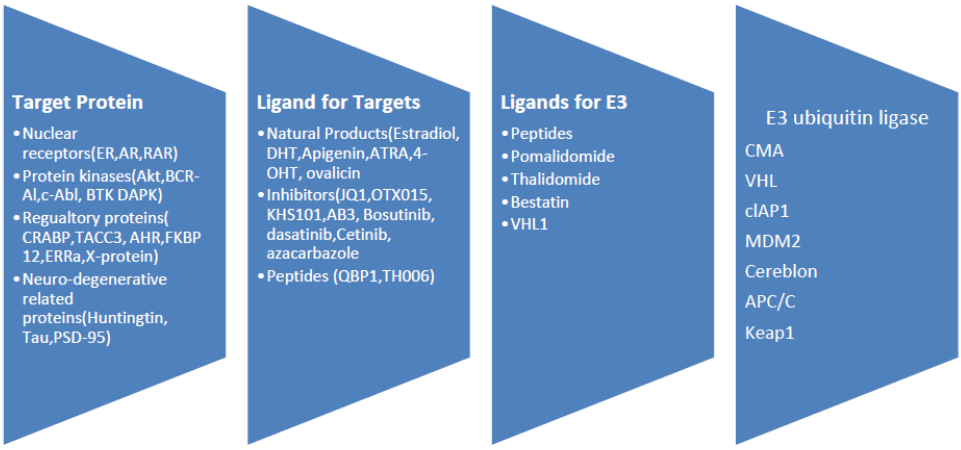

Figure 2 List of all target proteins, ligands for targets, ligands for E3 and E3 ubiquitin ligase

3 CONCLUDING REMARKS

PROTAC being a novel and powerful strategy has gained attention from both academia and industry for developing different types of different therapeutic agents relying on their unique property of protein degradation rather than inhibiting them. PROTAC acts catalytically and scope has been expanded for treating a variety of diseases and they exhibited better selectivity in comparison to conventional inhibitors. Therefore, PROTAC has been widely explored with 42 targets in publication and has outperformed in cancer as well as in immune disorders, viral infections, and neurodegenerative diseases. PROTAC showcases a very prominent and powerful approach for jumping the hurdles of drug discovery and development. On the different side, much more efforts have been needed to gain deep insights into the safety and efficacy and different target binders and E3 ligases need to be explored more.

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FORMULATION AND EVALUATION OF MICROEMULSION LOADED GEL OF ANTIFUNGAL DRUG

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Abstract - Microemulsions as drug delivery system are widely used due to their capacity to solubilize poorly water-soluble drugs efficiently. Terbinafine is mainstay of oral antifungal armamentarium and it is also used in topical formulations. Currently, formulations containing the free base and salt form are available. However, due to its poor water solubility and physicochemical properties its uptake by the skin is limited and hence new formulation approaches are continuously being done. The present investigation is an attempt to design microemulsion loaded gel of terbinafine hydrochloride. The prepared microemulsion was evaluated for globule size, zeta potential and pH. The gel was evaluated by visual characterization, drug content, in vitro drug release, and stability. The emulsion was formulated using an Smix obtained from phase diagram study. F1-F9 different formulations were prepared with varying percentage of oil, water and surfactants. Out of this F 6 formulation had 92.23 ± 0.3% drug loading and a pH of 6.2 ± 0.2. The zeta potential of this formulation was -35.21. Hence, this was used for gel preparation using Carbopol 934. Three formulations were tested out of which one with pH 6.5 ± 0.05, spreadability 21.7 ± 0.05, viscosity 6752 ± 27 and drug content 99.28 ± 0.02 was considered as the best formulation. The stability study revealed negligible changes in the drug content of selected formulation. The results confirm that microemulgel formulation of terbinafine can be used as a good alternative for topical use in antifungal armamentarium.

Keywords: Antifungal, Microemulgel, Surfactant, Carbopol 934

1. INTRODUCTION

The skin is an important component of the human body that provide protection from the outside world. Skin, on the contrary, is more vulnerable to microbial infection. Fungal infections are the most prevalent forms of skin infections and occur mostly in moist and humid climates.

A topical medication delivery system provides treatment of such a topical infection. This delivery system provides a number of advantages over other route of administration such as it avoids first pass metabolism, has local effect, GI degradation and discomfort, effective drug concentration releases at the target site. Also this therapy can be withdrawn when not needed.

However, the effectiveness of drugs is negatively affected by the stratum corneum (protective barrier of skin). To get control of the narrow penetration of drugs through the protective barrier, researchers have come about many strategies that includes niosomes, liposomes, microemulsion, solid-lipid nanoparticles, ethosomes, microspheres, etc.

Microemulsions are one of the promising sub-micron carriers for topical drug delivery. They are transparent, thermodynamically stable, dispersions of oil in water (o/w) or water in oil (w/o) and stabilized by surfactant or co-surfactant.

Microemulsions offer several advantages like high drug-loading and good skin penetration (by reducing the diffusional barrier of the stratum corneum) with acceptable biocompatibility (due to the presence of physiological lipids/oils). Furthermore, microemulsions do not require any expensive sophisticated instruments for preparation and therefore, the cost of preparation as well as time required for its preparation is less.

Recently, researchers successfully utilized microemulsion based gels for transdermal delivery of several drugs. Hence, this research aimed for the development and evaluation of terbinafine hydrochloride microemulsion loaded gel for improved transdermal delivery.

3 MATERIAL AND METHODS

3.1 Materials

Terbinafine hydrochloride was obtained as a gift sample. Carbomer, triethanolamine, and benzoic acid were obtained from S.D. Fine Chem. Ltd., Mumbai. Methanol and ethanol were obtained from LobaChemie Pvt. Ltd. Mumbai. PEG-400 and tween were purchased from Hi Media, Mumbai. Glycerine was purchased from Garima Glycerin, Indore. Castor oil was obtained from Morpheme Remedies.

4 METHOD

4.1 Solubility study of Terbinafine hydrochloride

The various component of microemulsion like castor oil, span 40, tween 20, tween 80, sunflower oil, oleic acid, PEG 400, ethanol werechosen for the solubility study of terbinafine hydrochloride. Solubility studies were examined by dissolving an excess amount of drug in each of the solvent and kept it in shaker for 3 days. The amount of terbinafine hydrochloride solubilized was analysed by UV-visible spectrophotometer.

4.2 Construction of pseudo-ternary phase diagram

Pseudo ternary phase diagram were constructed by mixing oil and specific smixratio (surfactant: co-surfactant) accurately in various ratios. The different conc. of oil and mixture of surfactant and co-surfactant were taken and ternary mixtures were formed in this ratio and quantity of water forming transparent solution was plotted in the pseudo-ternary phase diagram.

Formulation code	A (oil)	B (Smix)	C (water)
F1	0.55	5	4.44
F2	1.23	5	3.73
F3	2.16	5	2.87
F4	2.94	4.44	2.58
F5	3.84	3.84	2.32
F6	4.64	3.15	2.19
F7	5.71	2.41	1.80
F8	7.26	1.82	0.912
F9	8.56	0.953	0.478

Table 1: Formulation of microemulsion

4.3 Preparation of microemulsion

After the development of phase diagram, nine different formulations has been selected by keeping the total quantity of the formulation constant as 100% and varying all components of the system. Each formulation has been loaded with Terbinafine hydrochloride of 10mg/ml. All nine formulations have been evaluated for different parameters such as pH, In-vitro release, solubility and stability study.

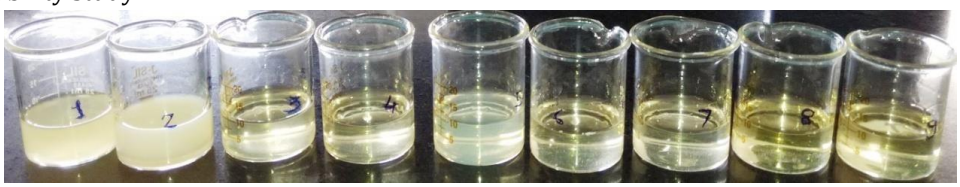

Figure 1: Formulation of Microemulsion Gel

5 EVALUATION OF FORMULATION

5.1 pH determination

The pH of each formulation was determined by using digital pH meter. 1 gm of microemulsion was taken and dissolved in distilled water and make up the volume upto 100 ml. kept it for 2 Hours prepared 1% microemulsion solution then check pH. Test carried out in triplicate reading then mean calculated. Same procedure is followed for each formulation F1-F9.

5.2 Centrifugation

The microemulsion system was centrifuged at 5000 rpm for 10 minutes to determine that the system shows signs of creaming or phase separation. The system was observed visually for appearance.

5.3 Determination of % Drug Content in microemulsion

The Micro emulsion was centrifuged at 1000 rpm for 15 min, 0.2 ml of supernatant was taken and diluted with 0.1 N HCl. Absorbance was measured at 282nm by UV Spectrophotometer. Concentration of TH was determined using standard curve equation and % drug content was calculated.

5.4 Zeta Potential and Vesicle size Measurement of Optimized Batch F6

Zeta Potential of samples was measured by Zeta sizer. Samples were placed in zeta cells and results were recorded.

5.5 Formulation development of microemulsion loaded gel

Preparation of Carbopol gel base: Carbopol 934 was weighed 0.5, 1.0 and 1.5g and dispersed in water with mild stirring. Then allowed it to swell for 24 hours to obtain 0.5%, 1.0% and 1.5% gel base. Adjust the pH by adding drop wise triethanolamine. Later added 1-2 ml of glycerin for gel consistency.

Formulation	Carbopol (in gm)
GF1	0.5
GF2	1.0
GF3	1.5

Table 2: Composition of different gel base

Preparation of microemulsion gels: Equivalent to 1% of microemulsion was incorporated into gel base by slow mechanical stirring at 25 rpm for 10 minutes. The optimized formulation was incorporated into three different gel concentration 0.5, 1 and 1.5% w/w.

6 EVALUATION OF MICROEMULSION GELS

6.1 Determination of pH

Weighed 20 gm of gel formulation were transferred in 10 ml of beaker and measured it by using the digital pH meter. pH of the topical gel formulation should be between 6–7 to treat the skin infections.

6.2 Spreadability

A modified apparatus was used for determining spreadability of microemulsion gel. The spreadability was measured on the basis of slip and drag characteristics of the gel. The measurement of spreadability of each formulation was in triplicate and the average values are taken.

6.3 Measurement of viscosity

Gels viscosity was determined by using a Brook Field viscometer. A T-Bar spindle in combination with a helipad stand was used to measure the viscosity and have accurate readings. The T-bar spindle (T95) was used for determining the viscosity of the gels.The torque reading was always > 10%. Five readings taken over a period of 60 sec. were averaged to find the viscosity.

6.4 Drug Content

Equivalent to 10mg (Terbinafine hydrochloride) of the prepared gel was mixed with 100 ml of ethyl alcohol. Filtered the stock solution, and then aliquots of different concentrations were prepared by suitable dilutions, and the absorbance was measured at 282 nm. Drug content was calculated by linear regression analysis of the calibration curve.

6.5 In-vitro diffusion study

An in-vitro drug release study was performed using Franz diffusion cell. Firstly Dialysis membrane (Hi Media, Molecular weight 5000 Daltons) was placed between receptor and donor compartments. Microemulsion gel equivalent to 5mg of drug was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (24 ml). The diffusion cells were maintained at 37±0.5°C with stirring at 50 rpm throughout the experiment. At different time interval, 5 ml of aliquots were withdrawn from receiver compartment through side tube and analyzed for drug content by UV Visible spectrophotometer.

7 RESULTS AND DISCUSSION

Based on solubility studies, it was concluded that the solubility in the oils, surfactants and co surfactants like Span 40, Castor Oil, Oleic acid, PEG 400, Ethanol was found to be soluble and Tween 20, Tween 80 and Sunflower Oil was found to be Slightly soluble for the microemlusion preparation of Terbinafine hydrochloride. Different physicochemical properties of the selected oils were studied and were found to be favorable for oral microemlusion drug delivery system. The selected oils come under the GRAS (Generally regarded as safe) category and frequently used for the many food products.

S. No.	Component	Solubility
1	Span 40	Slightly Soluble
2	Tween 20	Slightly soluble
3	Tween 80	Soluble
4	Castor Oil	Soluble
5	Sunflower Oil	Slightly soluble
6	Oleic acid	Soluble
7	PEG 400	Soluble
8	Ethanol	Soluble

Table 3: Solubility of Terbinafine hydrochloride in different oil, surfactants and co surfactants

The phase diagramwere constructed containing different S_{mix} ratio. These phase diagram provides microemulsion region with desired concentration range. Microemulsion point were chosen for further analysis.

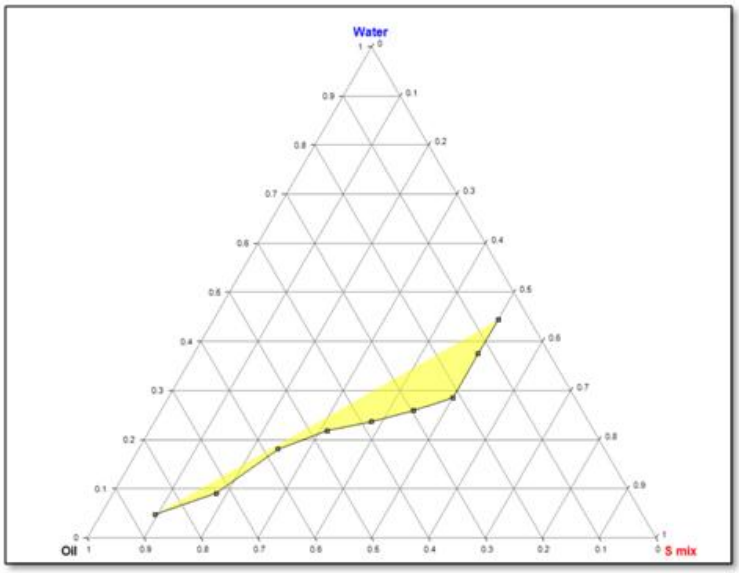

Figure 2: Construction of Phase Diagram

pH determination

S. No.	Formulation code	pH*
1	F1	6.72±0.03
2	F2	6.51±0.04
3	F3	6.79±0.02
4	F4	7.01±0.03
5	F5	6.84±0.04
6	F6	6.23±0.02
7	F7	6.98±0.03
8	F8	6.83±0.04
9	F9	6.34±0.02

***Average (Mean) of three determination**

Table 4: Results of pH of Terbinafine hydrochloride loaded microemulsion

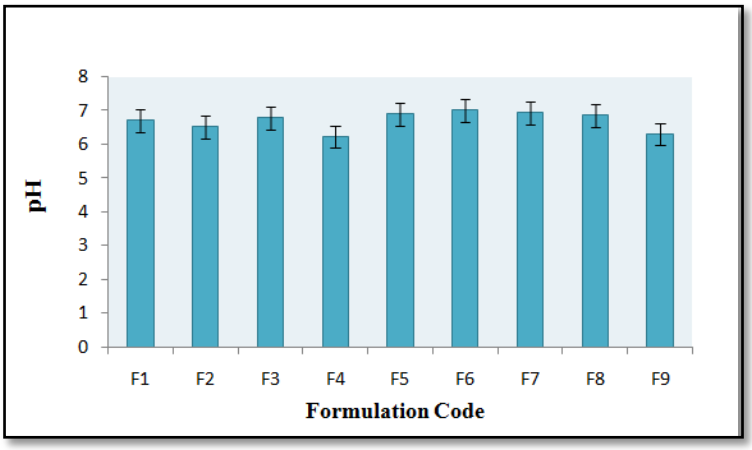

Figure 3: Graph of pH of Terbinafine hydrochloride loaded microemulsion

Centrifugation

S. No.	Formulation Code	Appearance
1	F1	Opaque
2	F2	Opaque
3	F3	Translucent
4	F4	Transparent
5	F5	Translucent
6	F6	Transparent
7	F7	Transparent
8	F8	Transparent
9	F9	Transparent

Table 5: Results of Centrifugation

7.1 Determination of % Drug Content in microemulsion

Drug content is most important in microemlusion formulation and the data found are satisfactory. It was found to be 78.85±0.35 to 92.23±0.32% which shows the good capacity of formulation to hold the drug.

S. No.	Formulation Code	% Drug Content in microemulsion*
1	F1	82.33±0.28
2	F2	78.86±0.34
3	F3	86.63±0.30
4	F4	82.27±0.42
5	F5	78.89±0.26
6	F6	92.23±0.32#
7	F7	81.22±0.12
8	F8	85.10±0.19
9	F9	78.82±0.38

***Average of three determination, #Best result among all prepared formulations.**

Table 6: Results of % Drug Content in microemulsion

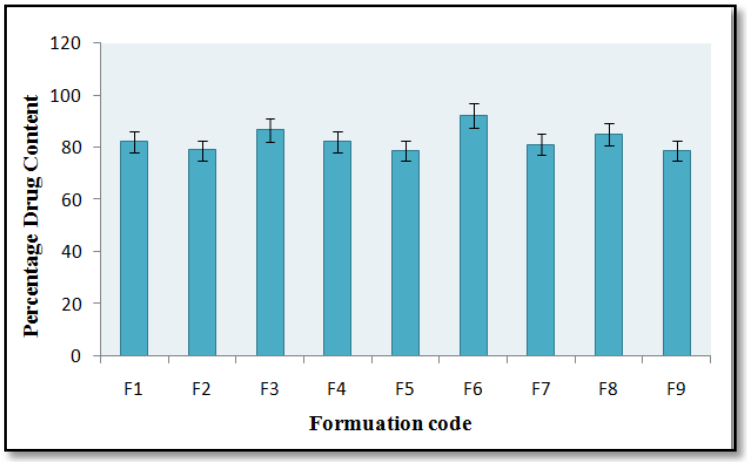

Figure 4: Graph of % Drug Content in microemulsion

7.2 Zeta Potential and Vesicle size Measurement of Optimized Batch F6

The Vesicle size analysis of the optimized formulation F6 was done using particle size analyzer (Malvern particle size analyzer). The mean droplet size was found to be 103.25nm. The particle size distribution of optimized formulation F6. Zeta potential of the optimized formulation F6 was determined using Zeta size analyzer. Zeta potential of optimized formulation was found to be -35.21mV. The zeta potential of the optimized formulation (GF3).

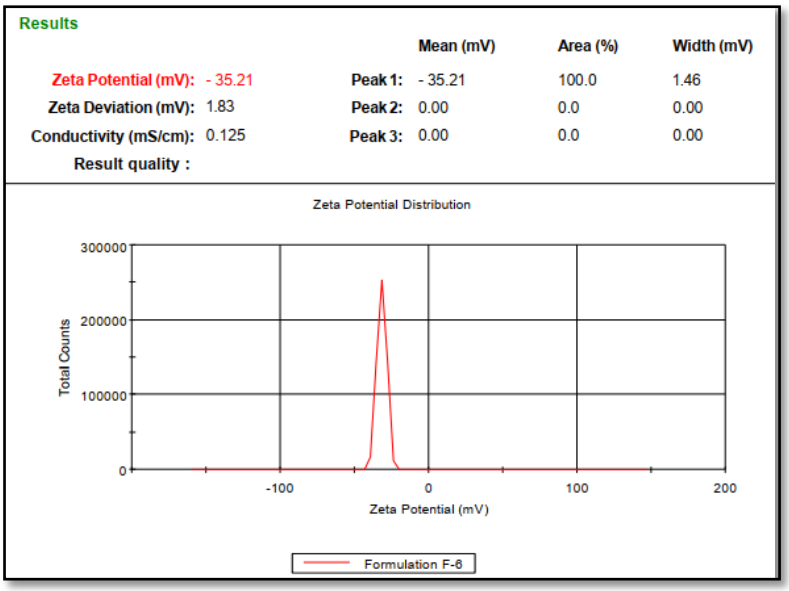

Figure 5: Result of Zeta Potential of Optimized formulation F6 (-35.21mV)

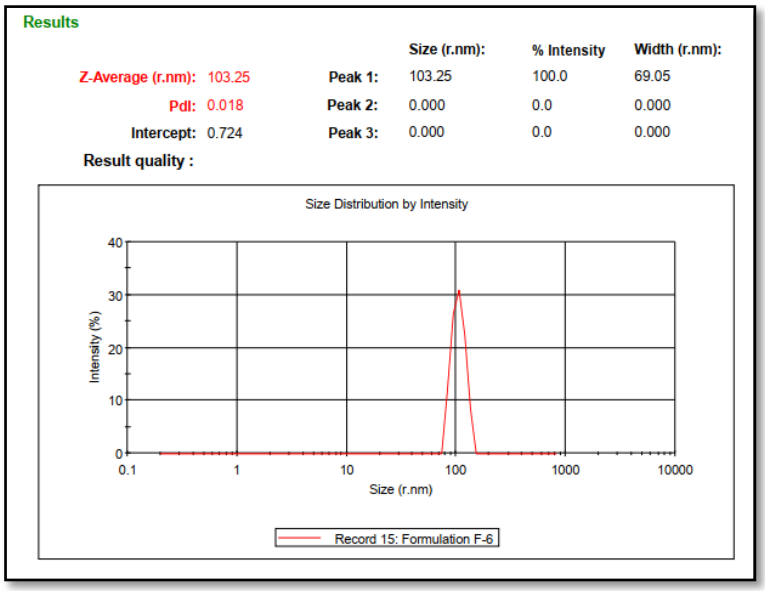

Figure 6: Result of droplet size of Optimized formulation F6 (103.25)

7.3 Results for evaluation of Microemulsion Gels

Three Different carbopol gel base prepared for optimization (0.5%, 1.0% and 1.5%) and evaluated for pH, Spreadibility, Viscosity measurements and in vitro drug release studies.

In topical drug delivery system pH plays an important role, the result of formulations shows that all the formulations are suitable for skin delivery. The pH values of the prepared gels were within acceptable limits of 6.3±0.1- 6.8±0.2.

A modified apparatus was used for determining spreadability. The spreadability was measured on the basis of slip and drug characteristics of the gels and was in the range of 20.12±0.42– 23.25±0.45gms.cm./sec. The gels should have optimum spreadability because very high and very low spreadability values indicate that the application of the gel to the site is difficult.

The results show that the viscosity of the gels increased with an increase in polymer concentration. The increase in viscosity with the polymer concentration may be due to increase in bonds between the polymer molecules which lead to formation of a hard and dense compact mass.

In vitro drug release study of Optimized formulation was carried out using modified franz Diffusion cell. The optimized formulation GF3 showed the maximum 99.65% drug release in 8 hrs. The results of stability studies of F3 formulation reveled negligible changes at 40°C ± 2°C and 75%±5% RH the prepared gel was found stable at elevated temperature and humidity.

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
GF1	98.83± 0.022	6.4±0.023	20.74±0.072	6233±34
GF2	98.55 ± 0.023	6.2±0.042	21.06±0.045	6585±28
GF3	99.28 ±0.024	6.5±0.054	21.70±0.056	6752±27

Table 6: Result of Drug Content, pH, Spreadability, Viscosity

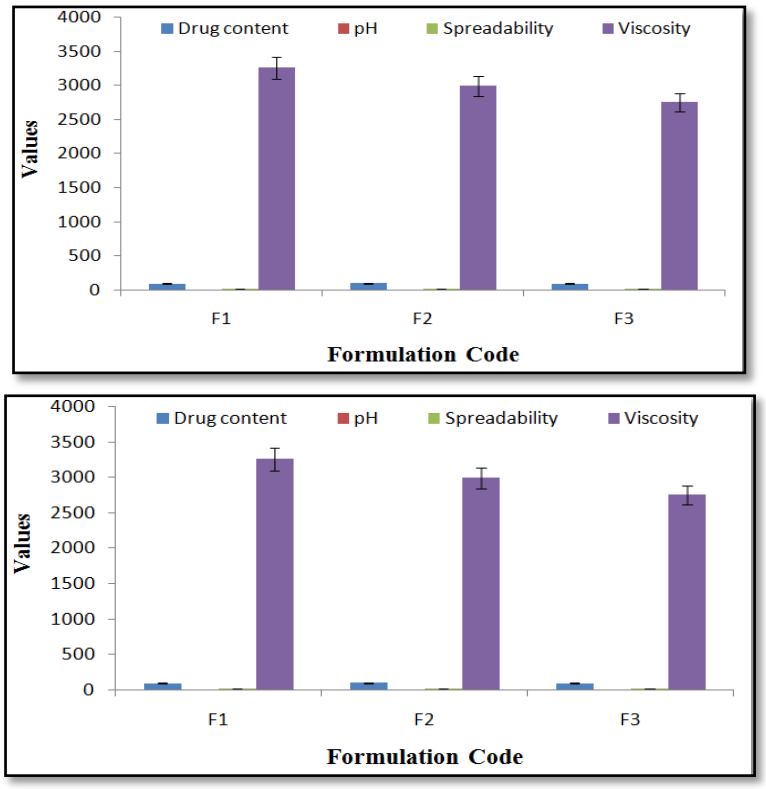

Figure 7: Graph of microemulsion gel formulations

8 CONCLUSION

The main goal of this study was to create a new drug delivery method with improved permeability, system shows less side effects, ease of administration, and prolong released of drug, Easy formation, Very small droplet size tends Very fast and very effective penetration to the skin.

The results of this study shows A microemulgel formulation was optimized, and when it was characterized for physical appearance and drug release, it appeared to be superior to commercially available Terbinafine ointment. The developed microemulsion increased solubilization capacity, a poorly water soluble drug, according to in vitro release results. Thus,

our study confirmed that the microemulgel formulation can be used for topical formulation of terbinafine hydrochloride.

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A REVIEW ON: INTRODUCTION TO OBESITY

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1. INTRODUCTION

Obesity is an abnormal growth of adipose tissue due to an enlargement of fat cell size i.e. hypertrophic obesity or increase in number of fat cells i.e. hyperplastic obesity or may be the combination of both conditions. Obesity is considered as a disease that has become world epidemic condition. As per the data of World Health Organization for year 2016, 1.9 billion adults of age 18 years and above (39%) were listed as overweight with body mass index (BMI) 25-29.9 kg/m2 and 650 million (13%) were listed as obese with BMI >30 kg/m2. More than 340 million children of age 5 to 19 years are overweight or obese. In the United States near about 100 million adults of age 18 years and above (37%) and 12.7 million children of age between 5 to 18 years (17%) are obese. Every state of United States has a greater than 20% prevalence rate of obesity with 22 states exceeding 30%. In India, prevalence of overweight and obesity is increasing way more faster than the world average. The prevalence of overweight in women increased from 8.4% to 15.5% between 1998 and 2015 and in men increased from 2.2% to 5.1% over the same period of time.

Obesity is a complex and chronic medical condition involving abnormal and excessive accumulation of body fat that cause major negative impact on human health. Obesity is not only concerned with the increasing amount of body fat but it may also increase the risk of other diseases including heart diseases, high blood pressure, diabetes, certain cancers, etc. Worldwide, pervasiveness of obesity is growing aggressively with double rates for adults and childhood obesity (children between age 6-11 years) and triple rates of adolescent obesity (age 12-19 years). A body mass index (BMI) over 25 is considered as overweight and BMI over 30 is considered as obese. It has become a matter of public health concern with serious and subtle impact on mortality, morbidity and healthcare. Obesity is generally characterized with false perceptions regarding its cause that obesity is caused mostly due to inappropriate dietary choices and lack of physical inactivity. However, as per several researches and studies it is a complicated chronic medical condition that may be caused due to several factors including interplay of multiple genes, metabolic factors, behavioral factors and environmental factors.

In a study done by expert panel from the Obesity Society, it was concluded that obesity is a complex medical condition with several occasional contributors which includes several factors that are largely beyond the control of an individual. Obesity leads to ill health, reduced quality of life, functional impairment, deliberate diseases, greater mortality, etc. Successful treatment to treat obesity may be sometimes difficult to attain but when done, produces many benefits by improving other medical issues raised due to obesity. Due to all those findings obesity was then accepted as a diseased condition. Recognizing obesity as serious health issue based on several studies, American Medical Association, in June 2013 also acknowledged for obesity as a disease.

2. EPIDEMIOLOGY OF OBESITY

Obesity is a complex multifactorial disease. The worldwide prevalence of overweight and obesity has doubled since 1980 to an extent that nearly a third of the world's population is now classified as overweight or obese. Obesity rates have increased in all ages and both sexes irrespective of geographical locality, ethnicity or socioeconomic status, although the prevalence of obesity is generally greater in older persons and women. This trend was similar across regions and countries, although absolute prevalence rates of overweight and obesity varied widely. For some

developed countries, the prevalence rates of obesity seem to have levelled off during the past few years. Body mass index (BMI) is typically used to define overweight and obesity in epidemiological studies. However, BMI has low sensitivity and there is a large inter-individual variability in the percent body fat for any given BMI value, partly attributed to age, sex, and ethnicity. For instance, Asians have greater percent body fat than Caucasians for the same BMI. Greater cardiometabolic risk has also been associated with the localization of excess fat in the visceral adipose tissue and ectopic depots (such as muscle and liver), as well as in cases of increased fat to lean mass ratio (e.g. metabolically-obese normal-weight). These data suggest that obesity may be far more common and requires more urgent attention than what large epidemiological studies suggest. Simply relying on BMI to assess its prevalence could hinder future interventions aimed at obesity prevention and control.

3 PATHOPHYSIOLOGY OF OBESITY

The changes in adipocyte activity can be considered genetic or environmental. Adipose tissue has the primary functions. Two are well recognized and defined i.e. to serve as the site for storage of energy rich fatty acids and glycerol in response to neural, endocrine and local signals for metabolism at distant sites. Third important role of adipose tissue is its function as an endocrine organ, releasing a variety of factors that regulate metabolism. On a global burden of chronic disease and disability. Currently, more than one billion adults worldwide are overweight and at least 300 million of them are clinically obese.

The rapidly rising prevalence of obesity, worldwide, has prompted re-evaluations of the definitions and diagnostic criteria, and of the extent of the burden it contributes to health care services. The relative contributions of genetics and environment to etiology of obesity have been evaluated in many studies. Although it varies from study to study, ~30% to 40% of the variance in BMI can be attributed to genetics and 60% to 70% to environment. The interaction between genetics and environment is also important. In a given population, some people are genetically predisposed to develop obesity, but that genotype may be expressed only under certain adverse environmental conditions, such as high fat diets and sedentary lifestyles. Three metabolism factors have been reported to be predictive of weight gain: a low adjusted sedentary energy expenditure, a high respiratory quotient (RQ; carbohydrate to fat oxidation ratio), and a low level of spontaneous physical activity.

Obesity is multifactorial disease that arises as a result of interaction among numerous behavioral, environmental and genetic factors and associated with the dysregulation of energy homeostasis normally maintained by the hypothalamic neuroendocrine/ neurotransmitter network. Signaling factors like leptin and various neuropeptides are important components of this complex network. Leptin is synthesized and secreted primarily from adipocytes and act centrally in the hypothalamus by binding to the leptin receptor. Circulating level of leptin are highly correlated with the level of body fat. Insulin and glucocorticoids can stimulate production of leptin by adipocytes. Low plasma concentrations of leptin and insulin (eg: during fasting and weight loss) increase food intake and decrease energy expenditure by stimulating neuropeptide Y (NPY) synthesis and perhaps by inhibiting sympathetic activity and other catabolic pathways. High leptin and insulin concentrations (eg: during feeding and weight gain) decrease food intake and increase energy expenditure through release of melanocortin and corticotropin-releasing hormone (CRH). Stimulation of the leptin receptor can lead to changes in the expression of a variety of neuropeptides. Neuropeptides that are involved in energy homeostasis can be orexigenic (appetite stimulating) and anorectic.

Table 1 Orexigenic and Anorectic peptides involved in energy homeostasis

S. No.	Orexigenic Peptides	Anorectic Peptides
1.	Neuropeptide Y (NPY)	Corticotrophin-Releasing hormone
2.	Agouti-related peptide (AgRP)	Melanocyte stimulating hormone
3.	Galanin	Cholecystokinin
4.	Orexin A and B	Glucagon like peptide 1
5.	β-endorphin	Calcitonin gene-related peptide
6.	Norepinephrine	Bombesin
7.	Growth hormone-Releasing hormone	
8.	Melanin concentrating hormone	

The involvement of most of these neuropeptides in maintaining energy homeostasis was deduced from transgenic animal studies and spontaneous mutations. β3-adrenoreceptor also has an important role in the regulation of lipid metabolism and obesity. However, the physiological function of β3-adrenoreceptor in humans has not yet been established.

Role of Neuropeptide Y (NPY) in Obesity:

Neuropeptide Y is most widely distributed neuropeptide in the brain as well as in the peripheral nervous system, playing an important role in the weight regulation. NPY is a potent stimulant of appetite. Six receptors of NPY have been identified; Y1, Y2,Y3,Y4,Y5 and Y6. NPY antagonists (Y1, Y5) are being evaluated as new therapeutic targets for the treatment of obesity. Bioassays have also been performed for neuropeptide Y receptors characterization.

Role of Leptin in Obesity:

Ob gene product has been identified and named as leptin. It is expressed in adipose tissue and enters the brain where it functions to reduce food intake, serum glucose and insulin levels and increases metabolic rate, ultimately leading to a reduction in body weight. Leptin mediates its effects through a specific receptor OB-R (lepr). Five different isoforms of leptin receptors have been identified. In OB/OB mice, obesity is due to mutation in OB (lep) gene associated with deficiency of leptin of leptin whereas in db/db mice and fa/fa rats, mutations in the leptin receptor (lepr) lead to obesity. Human homologue of mouse obese gene has been cloned and it is 84% homologous to the mouse protein (leptin). Leptin and leptin mRNA levels are correlated with the percentage of body fat. Generally, obese rodents and humans show increased levels of these in blood.

4. FACTORS INFLUENCING THE DEVELOPMENT OF OBESITY

Obesity is not a single disorder, but a heterogeneous group of conditions with multiple causes. Body weight is determined by an interaction between genetic, environmental and psychological factors acting through the physiological mediators of energy intake and expenditure. Hence, we can say that obesity is caused by a number of factors including genes, environment, emotional status/health, stress level, amount of sleep, several medical problems or even the medical treatment going on.

Even with the same diet and same amount of physical activity different people will have different amounts of body fat and the distribution of fat stored in the body may will also vary. Environment plays a key role in shaping an individual's habits and lifestyle. Environment can directly influence one's health. Today's society has developed a more sedentary lifestyle. Walking has been replaced by driving cars, physical activity has been replaced by technology and nutrition has been overcome by convenience foods. These changes mean that it is easy to adopt unhealthy behaviour including poor food choices and low levels of physical activity. The net effect is that we are consuming more calories and using less and the body stores these excess

calories as fat. Science shows that genetics play an important role in obesity. Genes can lead to certain disorders, which result in obesity. However, not all individuals who are predisposed to obesity become obese. Research is currently underway to determine which genes contribute the most to obesity.

5 SYMPTOMS OF OBESITY

Common symptoms for adults:

- Excess body fat accumulation (particularly around the waist)
- Sweating (more than usual)
- Shortness of breath
- Trouble sleeping
- Snoring
- Skin problems (from moisture accumulating in the folds of skin)
- Inability to perform simple physical tasks (that one could easily perform before weight gain)
- Fatigue (from mild to extreme)
- Pain (commonly in the back and joints)
- Psychological impact (negative self-esteem, depression, shame, social isolation)

Common symptoms for children and adolescent:

- Eating disorders
- Fatty tissue deposits (may be noticeable in the breast area)
- The appearance of stretch marks on the hips and back
- Acanthosis nigricans (dark velvety skin around the neck and other areas)
- Shortness of breath with physical activity
- Sleep apnea
- Constipation
- GI reflux
- Poor self-esteem
- Early puberty in girls/delayed puberty in boys
- Orthopedic problems (such as flat feet or dislocated hips)

Morbid obesity symptoms:

- 100 lbs over optimal body weight with BMI of 40 or above
- Difficulty in breathing
- Difficulty in walking
- Struggle with everyday activities

Rare symptoms:

- Pro-opiomelanocortin (POMC) deficiency obesity: Key symptoms include hyperphagia (extreme hunger) starting during infancy, early-onset obesity, and hormonal problems (such as adrenal insufficiency).
- Leptin receptor (LEPR) deficiency obesity: Key symptoms include hyperphagia, severe early-onset obesity, and hypogonadotropic hypogonadism (a condition in which the male testes or the female ovaries produce little or no sex hormones, due to a problem with the pituitary gland or hypothalamus).
- Bardet-Biedl syndrome (BBS): Key symptoms include early-onset obesity, hyperphagia, vision impairment, polydactyly (having an extra finger or toe), and kidney impairment.

6. TREATMENT

Current therapies for obesity are based on diet and exercise, a selected number of drugs for a fraction of patients, and stomach (bariatric) surgery for extremely obese individuals. Although surgery and implantable devices cloud offer a solution for significantly obese patients, traditional drug based approaches are necessary to address the needs of a broader market. Although older drugs, such as generic amphetamine like agents, are reasonably effective in the short term they carry the risks of addiction and serious cardiovascular complications.

Appetite control can be achieved through the use of agonists for appetite suppressing pathways or antagonists for appetite stimulating pathways. Much anticipation has surrounded rimonabant, a first-in-class selective cannabinoid type 1 (CB1) receptor antagonist for obesity or metabolic syndrome and smoking cessation. Over stimulation of the CB1 receptor is associated with excessive food intake, fat accumulation and nicotine dependence. In clinical studies, rimonabant produced moderate weight loss (~5% body mass). However, use of rimonabant was associated with a high dropout rate (40-50%) partly due to psychiatric side effects, such as depression, anxiety and aggression.

Activation of certain serotonin (5-HT) receptors is also known to inhibit appetite, a 5-HT agonist, fenfluramine and dexfenfluramine was a highly effective weight-loss drug, but was withdrawn from the market in the 1990s due to severe cardiac toxicity. Only two weight loss medications have been approved by the FDA for long term use, but they have failed to gain a large market share. Sibutramine, a serotonin and noradrenaline reuptake inhibitor, controls appetite by producing a feeling of satiety but because its prominent side effect is hypertension, it is not widely prescribed and is recently withdrawn from the sale globally due to increased incidence of serious, non-fatal, cardiovascular events. Orlistat reduces weight by limiting caloric intake through inhibition of the lipase-mediated breakdown of fat in the gastrointestinal tract. Unfortunately, approximately 20% of patients develop unacceptable side effects, limiting its broader use.

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FORMULATION DEVELOPMENT & EVALUATION OF FLOATING DELIVERY SYSTEM CONTAINING CINNARIZINE

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Abstract - The main objective of the present study is to formulate and characterize floating cinnarizine loaded microsphere. To increase the residence time in the stomach thus result the drug release in sustained manner and might be improving bioavailability of floating microsphere. Floating microsphere of cinnarizine was successfully prepared by solvent evaporation method and evaluated for various parameters such as, % yield, % drug entrapment, floating behavior (% buoyancy) & floating lag time. Formulated floating microsphere was found release the drug in sustained behavior continuously for a prolonged period of time for 12 hrs. Thus, the formulated cinnarizine floating microspheres can exhibit to be potential applicant for safe and effective delivery of drug in sustained manner.

Keywords: Floating delivery, Floating microsphere, Gastro retentive drug delivery system, Sustained release, Cinnarizine loaded microsphere.

1 INTRODUCTION

There are lot of development have been seen in oral controlled drug delivery system in the last few decades, this system has been limited benefits in case of drugs with a poor absorption window all over the GIT (Gastro Intestinal Tract). To modify the GIT time is one of the main challenges in the development of oral controlled drug delivery system. Gastric emptying of dosage form is significantly variable process and capacity to prolong and control the emptying time is valuable asset for dosage forms, which stay in the stomach for an extended period of time than conventional dosage forms. Certain difficulties are faced in designing controlled released systems for better absorption and enhanced the bioavailability (Audumber et.al. 2015).

Various approaches for Gastro-retentive Drug Delivery System (GDDS) have been developed to improve period of retainment of oral dosage form in the stomach. Such as floating system, swelling system and expanding system, bio adhesive system, high density system and other delayed gastric emptying devices (figure 1). Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 micro meter. The microspheres are usually unrestricted flowing powders comprise proteins or synthetic polymers, which are biodegradable in nature. The potential uses of microspheres in the pharmaceutical have been observed since the 1960's and have a number of applications (Rajkumar et.al., 2012).

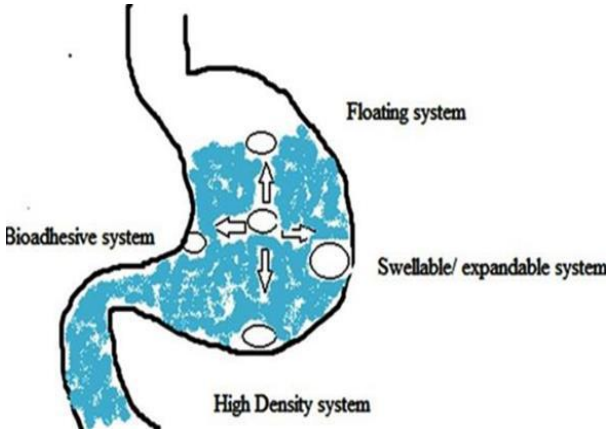


Figure 1: Approaches for GRDDS (Sharma and Khan, 2014).

When microspheres come in contact with gastric liquid, the gel formers, polysaccharides, and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. The air stuck by the swollen polymer lowers the density and confers buoyancy to the microspheres. Still, a minimal gastric content is required to permit proper achievement of buoyancy (Jagtap et.al., 2012).

2 MATERIALS AND METHODS

Cinnarizine (drug) was procured as a generous gift sample obtained from Geno Pharmaceutical Pvt. Ltd., Goa. Eudragit RLPO and Eudragit RSPO were obtained from Evonik Industries. HPMC obtained from LobaChem. Pvt Ltd Mumbai. Ethanol, Di-chloromethane and Methanol were obtained from Qualigens Fine Chemicals, Mumbai. Concentrated HCl was procured from S. D. Fine Chem. Ltd., Mumbai.

2.1 Formulation Development

Dose calculation for sustain release formulation

Required Dose = Conventional Dose (1+0.693 x £/t1/2)

£= Duration of dose

t1/2= Half-life of drug

3 DEVELOPMENT OF FLOATING MICROSPHERE OF CINNARIZINE

After performing various preformulation studies and drug polymer compatibility studies (Figure 2a & b), Floating microspheres containing cinnarizine was formulated by solvent-evaporation method (Table 1). These polymer HPMC and Eudragit RLPO are used in different proportion. The ratio of drug and polymer were dissolved in 1:2 combination of volatile solvent system of ethanol and dichloromethane. This solution was discharged gradually in slim stream into the aqueous solution of 1% polyvinyl alcohol (PVA). The mixture was nonstop agitated for 3 h at a speed of 600 rpm at 26±2°C. The floating microspheres separated by filtration, while the non-floating microspheres were disposed. The microspheres were dehydrated overnight at 41±3°C and kept in desiccant (Gunjal and Gaikwad, 2013, Kawashima et.al., 1992).

Table 1: Formulations of Prepared Floating Microspheres

S. No.	Formulation Code	Cinnarizine (mg)	HPMC (mg)	Eudragit RLPO (mg)	Eudragit RSPO (mg)
1.	F1	15	50	50	-
2.	F2	15	50	75	-
3.	F3	15	50	100	-
4.	F4	15	50	-	50
5.	F5	15	50	-	75
6.	F6	15	50	-	100

4 EVALUATION OF FLOATING MICROSPHERES

4.1 Percentage yield

The formulated floating microspheres were accumulated with a size range start from 1µm to 1000µm and balanced all the various formulations. The balanced bulk was divided by the entire quantity of all non-volatile substance which were used for the formulation of the microspheres (Joseph, 2011).

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of drug and polymer}} x\ 100$$

4.2 Drug entrapment

The several formulations of the Floating microspheres were exposed for drug content. 10 mg of Floating microspheres from all bunches were correctly weighed and crushed. The powder of microspheres was placed in 10 ml 0.1 N HCl and centrifuge at 1000 rpm. This supernatant solution is than clarified through whatmann filter paper No. 44. After separation, from this solution 0.1 ml was taken out and diluted up to 10 ml with 0.1 N HCl. The percentage drug entrapment was calculated by calibration curve technique (Sushma and Sriram, 2013).

4.3 Floating Behavior

Accurately weighted 10 mg of the floating microspheres was added in 0.1 N HCl (100 mL). The mixture was stirred at 100 rpm with the help of magnetic agitator. After 10 h, the layer of buoyant microsphere was pipetted and isolated by separation. Particles in particulate layer were separated by filtration. Particles of both types were dried in desiccators till a constant weight was found. Both the portions of microspheres were measured and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles (Sharma et.al., 2015).

$$\text{Percent buoyancy} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} x\ 100$$

4.4 Measurement of mean particle size

The measurement of mean particle size of the microspheres was obtained by photon correlation spectrometry on a submicron unit size studied on a dispersing point of 90°. Take a small amount (0.5mg) of the floating microspheres were placed in 5 ml of distilled water is used for the estimation **(Jain. et.al., 2005).**

4.5 Determination of Zeta Potential

The zeta potential studies of the floating microspheres were estimated by zeta sizer (Malvern Apparatus) via decisive the cataphoretic movement in a micro cataphoresis movement compartment. Analyzed all the samples were purified water at 25°C used same procedure repeated in three time.

4.6 Shape and surface characterization of microspheres by Scanning Electron Microscopy (SEM)

From the formulated batches of microspheres, preparations (F3) which displayed a suitable balance between the percentage releases were observed for surface morphology and shape using scanning electron microscope. Sample was secure on carbon tape and suitable gold sputtering was applied in a high vacuum evaporator. The acceleration voltage was established at 10KV during scanning.

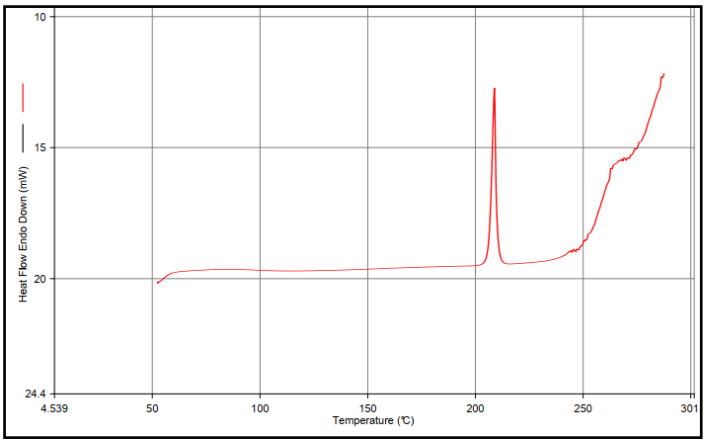
***4.7 In-vitro* release studies**

Dissolution is the procedure in which a material forms a solution. Dissolution examining measures the limit and capacity of solution formation from a dosage form, such as tablet, capsule, ointment, etc. The dissolution of a drug is significant for its bioavailability and therapeutic effectiveness. Dissolution and drug release are terms used inter changeably (Higuchi 1963, Korsmeyeret.al. 1983, Peppas et. al.1989)

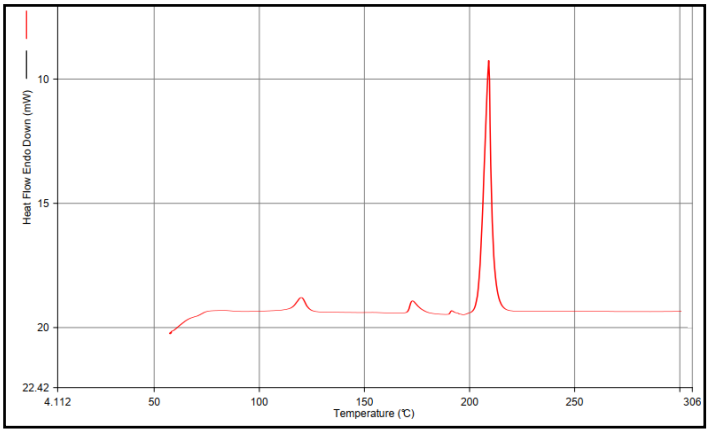
5 RESULTS AND DISCUSSION

5.1 Drug–excipient compatibility study:

Differential scanning calorimetry (DSC) is a technique that can provide information for both qualitative as well as quantitative and also the physiochemical status of drug in the microsphere. The result showed no interference among cinnarizine and excipient (Eudragit RLPO+ RSPO) (fig 2)



(a) Pure Cinnarizine



(b) Cinnarizine +Eudragit RLPO+RSPO

Figure 2: DSC Thermogram of (a) Cinnarizine (b) Cinnarizine + Eudragit RLPO+RSPO

5.2 Percentage Yield

Percentage yield of different formulations of floating microspheres are shown in table 2 and figure 3. The result of highest percentage yield was observed in optimized batch of formulation F-3 86.56±0.15% as compare to other batch of formulations.

Table 2: Percentage Yield for Different Formulations

S.No.	Formulation Code	Percentage Yield
1.	F1	78.89±0.45
2.	F2	82.23±0.32
3.	F3	86.56±0.15
4.	F4	75.57±0.26
5.	F5	79.98±0.32
6.	F6	80.23±0.45

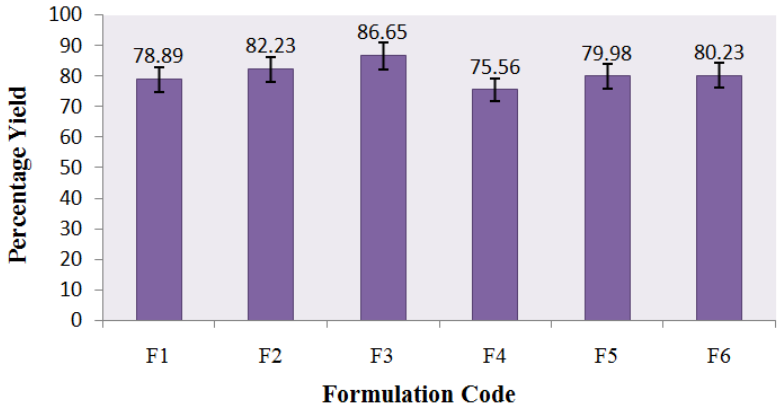


Figure 3: Percentage Yield for Different Formulations

5.3 Drug entrapment

Percent drug entrapment of floating microsphere in different formulations expressed in table 3. (Fig 4) The maximum drug entrapment was found in formulation F-3 is 79.96±0.63%.

Table 3: Percent Drug entrapment for Different Formulations

S.No.	Formulation Code	Percentage Yield
1.	F1	71.25±0.43
2.	F2	72.28±0.46
3.	F3	79.96±0.63
4.	F4	67.86±0.42
5.	F5	72.31±0.34
6.	F6	74.47±0.55

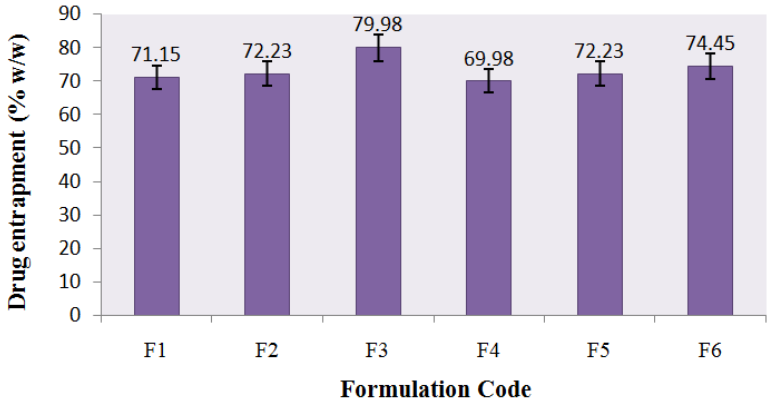


Figure 4: Percent Drug Entrapment for Different Formulations

5.4 Percentage buoyancy and floating lag period for floating microsphere

Percentage buoyancy and floating lag time studies of various formulation batches are shown in table 4, fig 5. The maximum percentage buoyancy was found to be 86.67±0.32 % and minimum floating lag period was found to be 26±1sec. The maximum percentage buoyancy and minimum floating lag period was observed in F-3 formulations.

Table 4: Percent buoyancy and floating lag period for Different Formulations

S. No.	Formulation Code	Floating Lag period (Sec)	Percentage Buoyancy
1	F1	36±3	65.57±0.45
2	F2	38±4	73.34±0.25
3	F3	26±6	86.67±0.32
4	F4	33±3	71.13±0.14
5	F5	42±5	78.86±0.56
6	F6	44±1	69.46±0.47

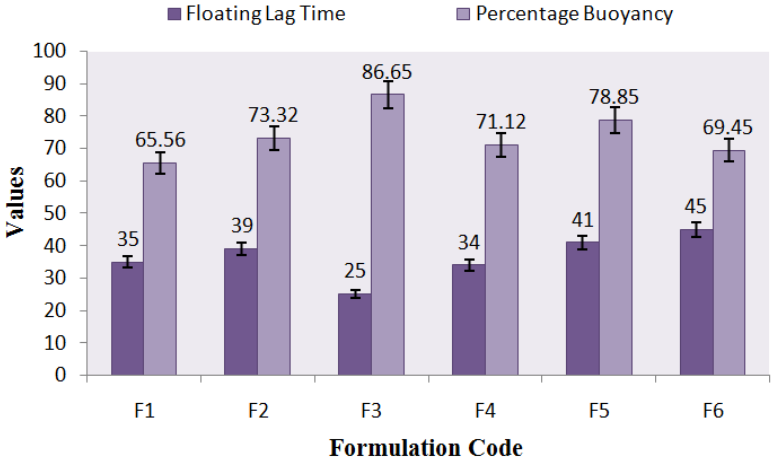


Figure 5: Floating Lag Period and Percentage Buoyancy for Various Floating Microsphere Formulations

5.5 Particle size Analysis

Determination of particle size play important role in floating microsphere and release of drug from microsphere. Particle size data of optimized microsphere formulation F3 is shown in fig 6

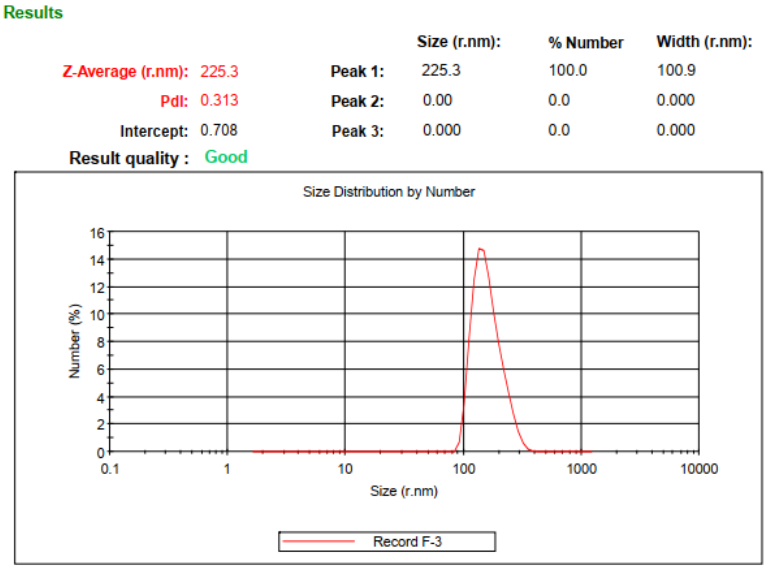


Figure 6: Particle Size Data of Optimized Microsphere Formulation F-3

5.6 Zeta Potential

The zeta potential studies of the floating microspheres were estimated by zeta sizer (Malvern Apparatus) via decisive the cataphoretic movement in a micro cataphoresis movement compartment (fig 7).

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-25.3	Peak 1:	-25.3	100.0	2.11
Zeta Deviation (mV):	2.11	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	0.289	Peak 3:	0.00	0.0	0.00
Result quality :					

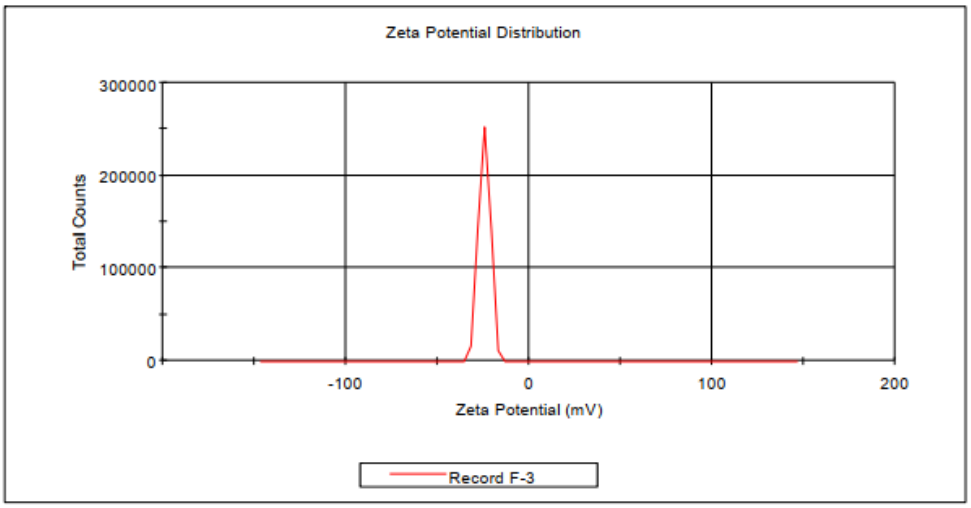


Figure 7: Zeta Potential Data of Floating Microsphere F3.

5.7 Scanning Electron Microscopy (SEM)

Shape and surface characterization of floating microspheres optimized batch of formulation F-3 was shown in fig 8

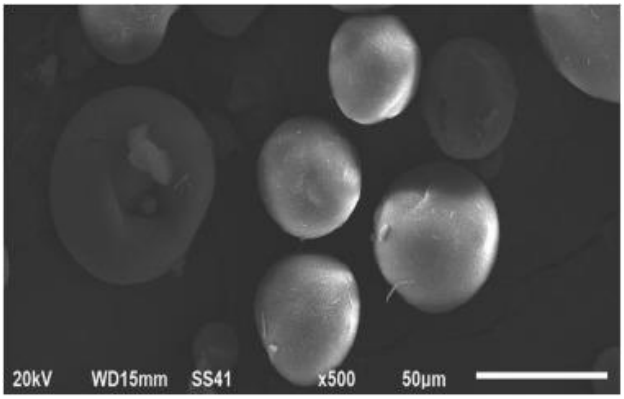


Figure 8: Graph of Scanning Electron Microscopy (SEM) of Optimized Formulation F3.

5.8 In-Vitro Drug Release of Cinnarizine Floating Microspheres

In vitro release study of cinnarizine (Drug) was performed in different batches F1-F6 using USP dissolution apparatus 2 (paddle type) in pH 1.2 for 2 hrs in the initial 2 hr, cinnarizine was not released more than 15% in formulation F-3, there after the release was increased for the next 10

hrs. The drug release pattern from microsphere of cinnarizine was in a sustained manner. And % drug release of formulation F-3 was found to be 99.45 %. (Table 5-7, fig 9-13)

Table 5: In-vitro Drug Release Studies of Formulations F1-F6

TIME (hrs)	% Drug Release						
	F1(n=3)	F2(n=3)	F3(n=3)	F4(n=3)	F5(n=3)	F6(n=3)	Marketed Formulation (CINAZIN 15 mg)
0.5	36.65±0.23	32.25±0.32	25.65±0.10	22.32±0.28	20.36±0.24	18.85±0.16	45.56±0.19
1	45.56±0.14	41.32±0.38	33.32±0.19	26.65±0.18	23.32±0.26	20.32±0.33	78.85±0.28
2	52.32±0.25	45.56±0.37	39.98±0.27	33.32±0.32	31.14±0.17	29.98±0.38	99.12±0.37
4	63.32±0.12	59.98±0.27	45.56±0.21	41.23±0.35	36.65±0.46	33.23±0.39	-
6	73.32±0.65	65.56±0.18	53.32±0.38	48.85±0.36	43.32±0.35	41.12±0.24	-
8	88.56±0.67	85.56±0.29	65.56±0.22	56.65±0.30	51.14±0.39	49.98±0.29	-
10	99.45±0.35	98.89±0.20	82.23±0.28	73.32±0.27	65.58±0.29	63.32±0.37	-
12	99.65±0.43	99.78±0.32	99.45±0.34	89.98±0.19	76.65±0.33	75.52±0.24	-

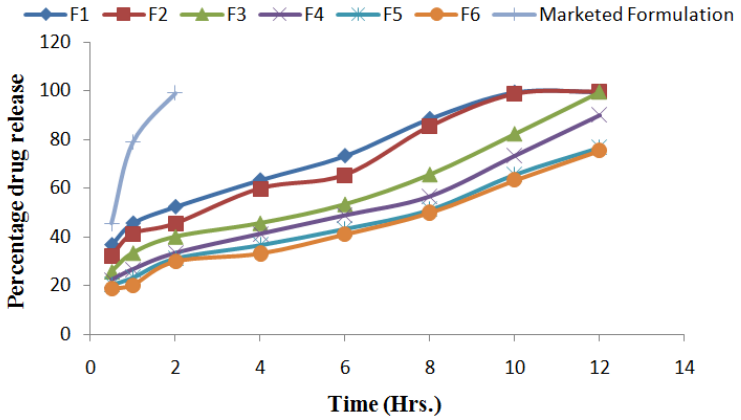


Figure 9: Graph of Release Studies of Formulations F1-F6 and Marketed Formulation

Table 6: Release Kinetics Studies of Optimized Formulation of Floating Microsphere F3

Time (h)	Square Root of Time (h)1/2	Log Time	Cumulative% Drug Release	Log Cumulative % Drug Released	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	25.65	1.347	77.79	1.891
1	1	0	33.32	1.522	66.75	1.824
2	1.414	0.301	39.98	1.699	50.02	1.699
4	2	0.602	45.56	1.780	39.75	1.599
6	2.449	0.778	53.32	1.845	30.02	1.477
8	2.828	0.903	65.56	1.915	17.75	1.249
10	3.162	1	82.23	1.971	6.55	0.816
12	3.464	1.079	99.45	2.000	0.11	-0.959

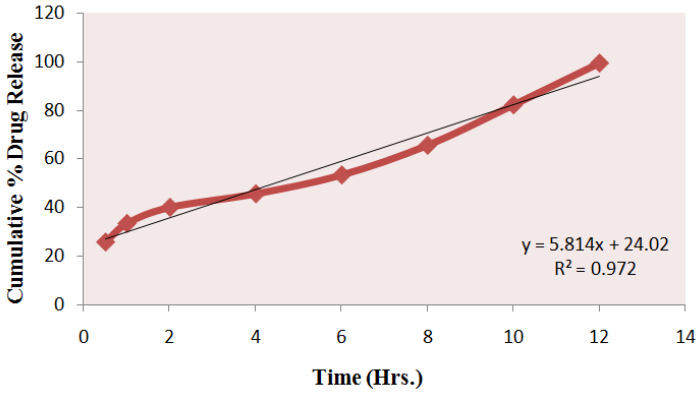


Figure 10: Zero Order Release Kinetics Graph of Optimized Formulation

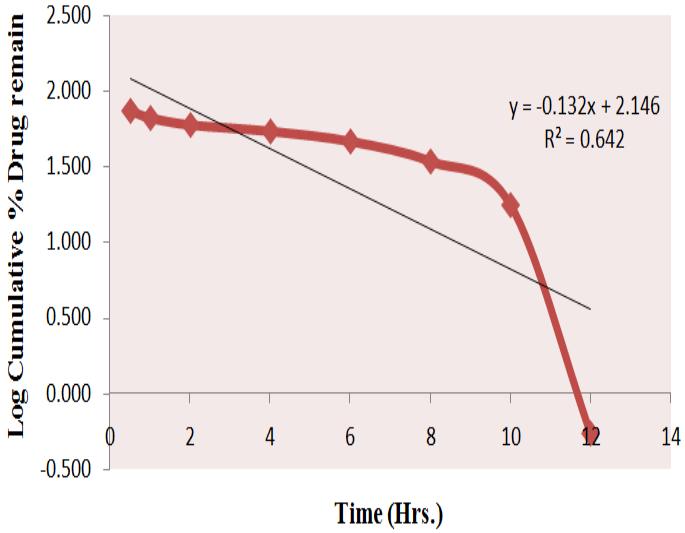


Figure 10: First Order Release Kinetics Studies Graph of Optimized Formulation

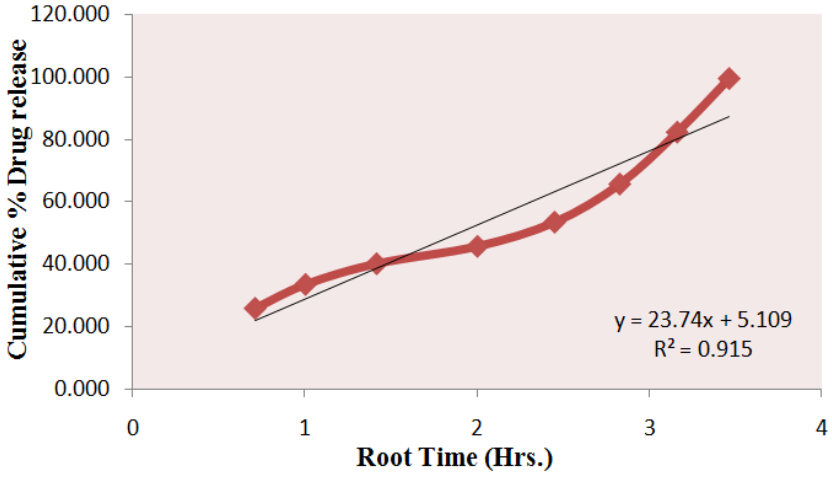


Figure 11: Higuchi Release Kinetics Studies Graph of Optimized Formulation

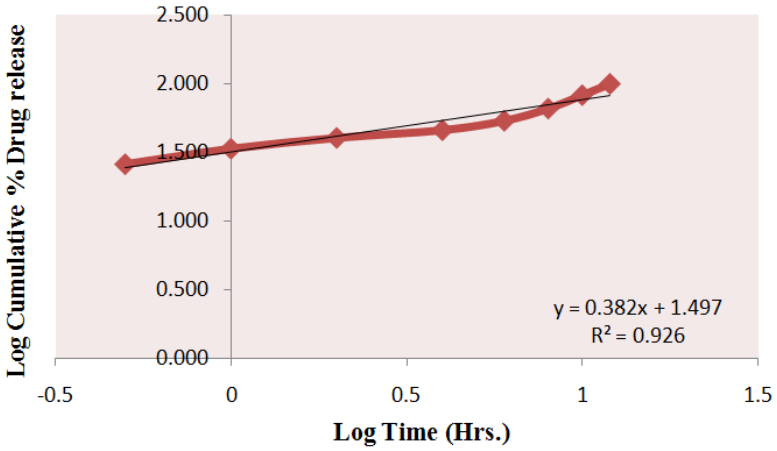


Figure 12: Graph of KorsmeyerPeppas Release Kinetics Studies of Optimized Formulation

5.9 Relative Study of Reversion constant for pick of Optimized Formulation F3

The study of *In vitro* drug release details obtained in the optimized formulation was submitted as good and this are suitable for studies performed by lined reversion investigation which giving various release kinetics such as zero order, first order kinetic equation, in order to find out the fashion of drug release follow. Then this reversion constant reading was distinguished and it was obtained a 'r2' value of microsphere as it is highest in zero order was shown 0.972. Accordingly, this represent release of drug from the formulations was found to follow zero order for floating microsphere.(Table 7)

Table 7: Relative Study of Reversion constant for pick of Optimized Formulation F3

Release Kinetics	Zero Order	First Order	Higuchi	Korsmeyer Peppas
R2	0.972	0.642	0.915	0.926

6 CONCLUSION

Floating microsphere of cinnarizine was successfully prepared by solvent evaporation method. The minimal result was showed in floating microsphere formulation F-3 such as, % yield, percentage of drug entrapment, floating behavior (% buoyancy) & floating lag time. In formulated floating microsphere was found release the drug in sustained behavior through continuous way prolonged period of time for 12 hrs. Thus, the formulated cinnarizine floating microspheres can exhibit to be potential applicant for safe and effectively delivery of drug in sustained manner.

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DESIGN OF NANOPARTICULATE SYSTEM FOR THE TOPICAL DELIVERY OF CICLOPIROX OLAMINE

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1 INTRODUCTION

Fungal infections are a universal problem and are routinely associated with high morbidity and mortality rates in immune-compromised individuals. An estimated 1.7 billion individuals suffer from fungal infections worldwide (Mota Fernandes et al., 2021). Fungal infections that are pathologically relevant can be categorized into two main types; Superficial fungal infections that affect the skin, mucous membranes, and keratinous tissues, causing ailments such as thrush, oropharyngeal candidiasis, and dermatophyte infections; and invasive fungal infections that are more life-threatening and affect sterile areas of the body such as the bloodstream, organs (lungs, liver, and kidneys), and the central nervous system(Mota Fernandes et al., 2021) Fungal infections can affect immune-competent and immune-compromised individuals equally. Critically ill COVID-19 patients in intensive care units (ICU) or on mechanical ventilation are more prone to bacterial or fungal nosocomial infections, which has caused marked increase in the cases of invasive fungal infections like mucormycosis, candidiasis, and aspergillosis. It is pivotal to understand Candida species like C.albicans, C.glabrata, C.tropicalis, and C.krusei are normal commensals inhabiting mucosal surfaces like skin, respiratory, urinary, or digestive tracts in humans. The patients who are immune compromised or on long-term pharmacotherapy have a tendency to develop mucosal candidiasis. Oropharyngeal candidiasis (OPC) caused predominantly by colonization of C. albicans can be a cause of morbidity in these patients.Mortality rate attributed to invasive candidiasis is 19–40%, which can increase to around 70% for ICU patients. Mucormycosis is a rare fungal disease with rhinoorbital-cerebral involvement being the most common type caused by inhalation of spores into paranasal sinus of susceptible individuals. Fatality rate of this fungal infection is 46% occurring due to vascular thrombosis, angioinvasion, and tissue necrosis (Jain and Taneja, 2021). Aspergillosis is another fungal disease invading the sinuses of immune compromised patients, caused by species Aspergillus fumigatus. The multitudinous increase of these fungal infections in COVID-19 patients in the past 1 year has been a cause of concern. The hosts at substantial risk of developing these fungal infections could be diabetics, immunocompromised individuals, patients on corticosteroids, and those with hematologic insufficiencies(Jain and Taneja, 2021).Globally, invasive fungal infections of aspergillosis account for 300,000 cases peryear, candidiasis accounts for 750,000 cases, and cryptococcosis (in AIDS patients)account for 223,000 cases Mortality rates are estimated to be 30% to 90%, 10% to75%, and 20% to 70% for aspergillosis, candidiasis, and cryptococcosis. (Mota Fernandes et al., 2021) At present, five structural classes of antifungal drugs are being used to treat infections. These are polyenes, azoles, ally amines, pyrimidines, and echinocandins. Although agents in these classes are effectively used as treatments today, there are some drawbacks to their use. Overuse, long treatment courses, and environmental exposure of azoles, polyenes, and echinocandins in the past decade have resulted in drug resistance. There is a high prevalence of Candida resistance to azoles and echinocandins According to the 2019 Antibiotic Resistance Threats in the United States report generated by the CDC, there were 34,800 cases of infection and 1,700 deaths caused by drugresistant Candida species. Azole resistance is likely attributable to the drug being fungistatic in nature, creating a selection pressure leading to resistance, while resistance to echinocandins is relatively recent and has emerged due to the overuse of the drug in the past decade. Aspergillus andCryptococcus are also

reported to display azole resistance.With the large numbers of fungal infections, mortality rates associated with invasive fungal infections, and shortcomings of currently used antifungal agents, there is an ever-increasing need to discover new drugs with an improved range of properties(Mota Fernandes et al., 2021). Ciclopirox olamine (CPO) is a hydroxypyridone derivative that differs in structure and mechanism of action from the other known antifungal agents. This topical antifungal agent has been in use for over three decades and received its US-FDA approval in June 2004(Sonthalia et al., 2019). The molecule exists in its free acid form known as ciclopirox and in its salt form as ciclopirox olamine (CPO). CPO 1% is equivalent to 0.77% ciclopirox. Ciclopirox remains the active compound, with no additional antifungal contribution by the olamine group. It is a broad-spectrum antifungal medication with additional antibacterial and anti-inflammatory properties (Sonthalia et al., 2019). Hydroxypyridone, CPO being the prototype, are the sole class of topical antifungal agents that have a completely different mechanism of action than other topical antifungals (azoles and allylamines). It acts through the chelation of polyvalent metalcations, such as ferric (Fe3+) and aluminum (Al3+), thereby causing inhibition of metal-dependent enzymes (cytochromes, catalase, and peroxidase) leading to disruption of cellular activities such as mitochondrial electron transport processes, energy production, and nutrient intake across cell membrane(Sonthalia et al., 2019).Ciclopirox olamine (CPO), is a BCS class II drug having low solubility and high permeability.It has a biological half-life of 1.7 h and bioavailability of < 5% with prolonged use. The aqueous solubility of the drug is low14.41mg/ml(Keshavshetti and Shirsand, 2019)

1.1 Material and Method:

Drugciclopiroxolamine, medium chain mono and diglycerides, capmul PG-8(monocaprylate), benzyl liquor, castoroil, oleic corrosive, isopropylepalmitate,isopropylmyristate,oliveoil,and sesame seed oil tween 80 tyloxapol,poloxamer,407,cremophorEL, And poloxamer188 transcutol P, polyethylene glycol400,glycerin,and ethanol.

1.2 Screening of Component:

The solubilty of ciclopirox olamine was determined in different oilviz. MCM(medium chain mono and diglycerides), capmul PG-8(monocaprylate), benzyl liquor, castor oil, oleic corrosive, isopropyle palmitate, isopropyl myristate, olive oil, and sesame seed oil by adding the excess of drug to 3 ml of different componrnts. The vials were tightly stoppered and were continuously stirred at 25±1C in a shaking water shower for 72hrs to achieve equilibrium. The equlibritated sample were removed from the shaker and centrifugate at 10,000rpm for 15 minutes .the supernatant was taken and filtered through a 0.45-µ membrane filter. The concentration of ciclopirox was determine in each oil, surfactant, cosurfactant, and combination of oil by HPLC.

1.3 Construction of Pseudo Ternary Phase Diagram:

On the basis of the solubility studies of drug, capmul PG8 was selected as the oil phase. cremophor EL, were selected as surfactant and transcutol was selected as cosurfactant respectively as per their emulsification capability for the system. Distilled water was used as an aqueous phase for the construction of phase diagrams. For the determination of existence zone of nanoemulsion, pseudoternary phase diagrams were constructed using aqueous titration method. To construct pseudo-ternary phase diagrams, the oil phase was mixed with surfactant: cosurfactant phase (cremophor and trancutolPrespectively) and the ratios of Smix (surfactant and co-surfactant mixture) used for titration are 1:0, 1:1, 1:2, 1:3, 1:4, 2:1, 3:1 and 4:1. The mixture was titrated with distilled water until it turned turbid. The volume of water used was then recorded. Water titration was continued until a clear, isotropic and thermodynamically

stable dispersion with low viscosity was obtained. plotting water phase, oil phase and surfactant: co-surfactant phase used in the experiment.

1.4 Optimization of Formulations:

Trial runs were planned by design expert 12 [state ease.inc.] software following full factorial technique.32 full factorial plan was applied for looking at three (factors) at two levels with at least 17 runs .the detail were setup by blending proper measure of surfactant and cosurfactant and afterward oil part added, blend the definition until totally scattering happens at room temperature.At that point limited quantity of medication was added and the last combination was blended by vortex until a straightforward arrangement was gotten.Details were readiedand characterized.

1.5 Preparation of Nanoemulsion

Evaluation of Nanoemulsion formulation

- **Vesical size and zeta potential**

The most common method of vesical size analysis is dynamic light scattering,which can also be integrated with the Z-potential analyzer to gain information ofsurface charge. Nanoemulsion droplets can also be measured by using the disccentrifuge analyzer which can determine particle sizes ranging between 5 nm and75 μm

1.6 Viscosityand Electric Conductivity

Viscosity and conductivity are parameters which provide information at the macroscopic level. Viscosity measurements can reveal the aspect of the existentmicelles, while conductivity measurements indicate which of the phases is continuous as well as the mechanism of phase inversion.

1.7 Formulation of Nanoemulsion Gelandits Evaluation

The optimizednanoemulsion Sf13 was selected to be formulated into gel byusing 0.5, 1.0 and 1.5g Carbopol 934, was gauged andscattered in water with gentle blending and permitted to expand for 24 hours to acquire0.5%, 1.0% and 1.5% gel. 2 ml of glycerin was added to make gel consistency then identical to 1% (Ciclopirox olamine) of nanoemulsion was fused into gel base by sluggishmechanical blending at 25 rpm for 10 minutes. The advanced definition was joined intothree diverse gel focus 0.5, 1 and 2% w/w

Formulation	Carbopol (%)
F1	0.5
F2	1.0
F3	2.0

Composition of different gel base

1.8 Spreadability

An adjusted device was utilized for deciding spreadability. The spreadability was estimated based on degree at which qualities of the gels is discovered to be spread. The alteredmechanical assembly was created two glass slides, the lower one was fixed to a woodenplate and the upper one which was connected by a snare to an equilibrium. The spreadabilitywas dictated by utilizing the equation:

$$S=ml/t,$$

Where S, is spread capacity, m is weight in the dish attached to upper slide and t is the time taken to travel a particular distance and l is the distance voyaged. For the functional reason the mass,

length was kept steady and 't' was resolved. The estimation of spreadability ofevery plan was discovered triple and the normal qualities are addressed.

1.9 Measurement of Viscosity

The viscosity of gels was dictated by utilizing a Brook Field viscometer DV-II model. A tbaraxlein mix with a helipath stand was utilized to quantify the thickness and havelegitimatereadings.The T-bar shaft (T95) was utilized for examining the thickness of the gels. The variables which influence the thickness are as per the following temperature, pressing factor and testSize and so forth which influence the consistency were kept up during the interaction. TheHelipathT-bar shaft was gone here and there giving consistency at number of focuses alongtheway. The force perusing was consistently more prominent than 10%. Five readings Assumed control throughout a time span of 60 sec. To get normal thickness.

1.10 Drug Content

Comparable to 10mg (Ciclopirox olamine) of the readied gel was blended in with 100 ml.of methanol. Aliquot of 10µg/ml was set up by appropriate weakening in the wake of siftingthe stock arrangement and the absorbance was estimated at 284nm. The estimation ofmedication content was finished by direct relapse examination of the alignment bend.

1.11 In-vitrodiffusion study

An in-vitro drug discharge study was performed utilizing changed Franz dissemination cell. Dialysis layer (Hi Media, Molecular weight 5000 Daltons) was put among receptor and giver compartments. Nanoemulsion gel identical to 5mg of medication was put in the givercompartment and the filling of receptor compartment was finished with phosphate support,pH 7.4 (24 ml). The dissemination cells were kept up at 37±0.5°C with blending at 50 rpmall through the investigation. At various time stretch, 5 ml of test arrangement were removedfrom collector compartment through side cylinder and investigate for drug content by UVVisible spectrophotometer.

2 ANTIFUNGAL ACTIVITY OF NANOEMULSION GEL

2.1 Media Preparation (Broth and Agar Media)

Agar - 2.0 gms.
Potato infusion - 20 gms.
Dextrose - 2.0 gms.
Distilled water - to make 100 ml.
pH – 7

The well diffusion technique was utilized to decide the antifungal action of the nanoemulsion gel arranged from Ciclopirox olamine utilizing standard procedure18. There were3 fixation utilized which are 10, 20 and 30 µg/ml for nano emulsion gel in antibiogramconsiders. Its fundamental component is the putting of wells with the anti-toxins on thesurfaces of agar following immunization with the organic entity tried. Undiluted over nightstock societies ought to never be utilized as an inoculums. The plates were hatched at 28oCfor 48 hr. and afterward inspected for clear zones of restraint around the wells impregnatedwith specific centralization of medication.

2.2 Stability Studies

Ciclopirox olamine loaded nanoemulsion was prepared and stored for 2 months first at cold condition (2ºC–8ºC), room temperature and at elevated temperature (50º ±2ºC) andevaluated by visual inspection.

3 RESULT AND DISCUSSION

3.1 Component selection

After performing solubility study in different oils (Fig. 1), it was found that Ciclopirox olamine exhibited maximum solubility in thecapmulPG8 (74.00±4.04).

3.2 Pseudo-ternary phase diagram study

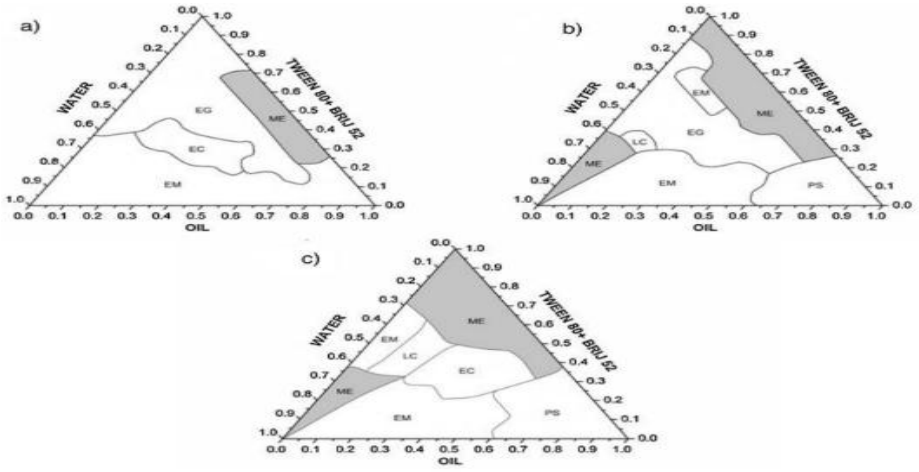
This work hasbeen carried out with several surfactant mixtures. Howevercapmul PG8 solublization in oil in water nano-emulsion for binary mixture of camphor and transcutolwas found to be maximum. The surfactant mixture that provided higher oil solubilization was camphor EL.

3.3 Optimization of Formulation Using 32 Factorial Designs

Full factorial design softwere model was applied to evaluate the effect of concentration oil ,water, and smix on size and zetz potential of the nanoemulsion an excessive data generated for all seventeen formulation suggested by the model(table)The polynomial equation generated by this experimental design (ANOVA for quadratic model) was found to be useful for futher calculations-

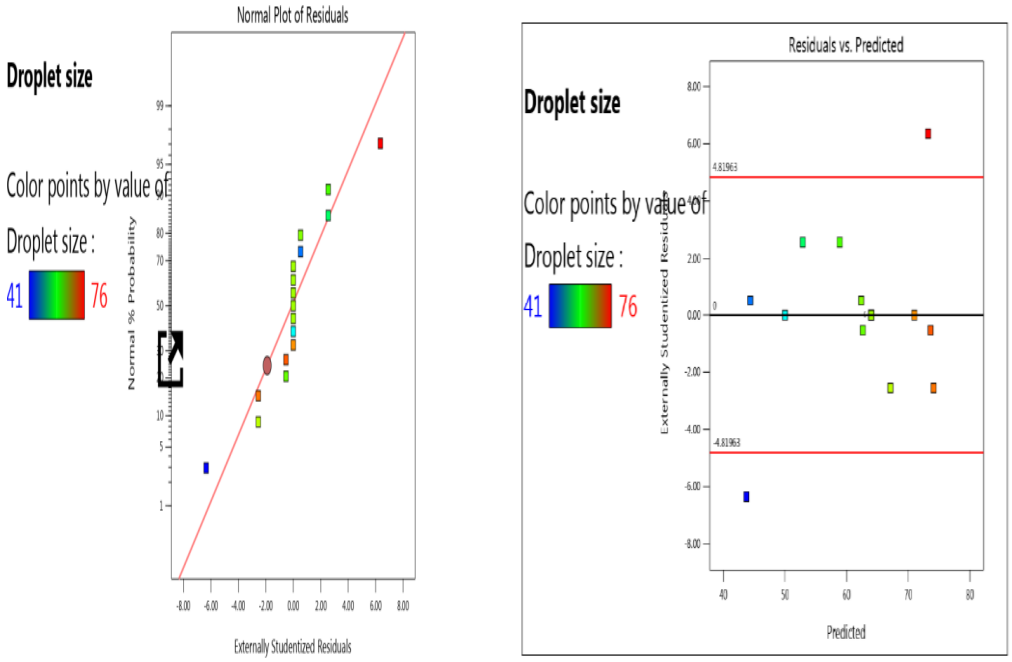
F code	Std	Run	Oil %W/V	S mix	Water
F1	6	1	30	25	40
F2	10	2	25	30	40
F3	9	3	25	20	40
F4	7	4	20	25	60
F5	16	5	25	25	50
F6	13	6	25	25	50
F7	17	7	25	25	50
F8	5	8	20	25	40
F9	14	9	25	25	50
F10	11	10	25	20	60
F11	3	11	20	30	50
F12	2	12	30	20	50
F13	4	13	30	30	50
F14	15	14	25	25	50
F15	1	15	20	20	50
F16	8	16	30	25	60
F17	12	17	25	30	60

3.4 Optimization of Formulation using Factorial 3^2 Designs

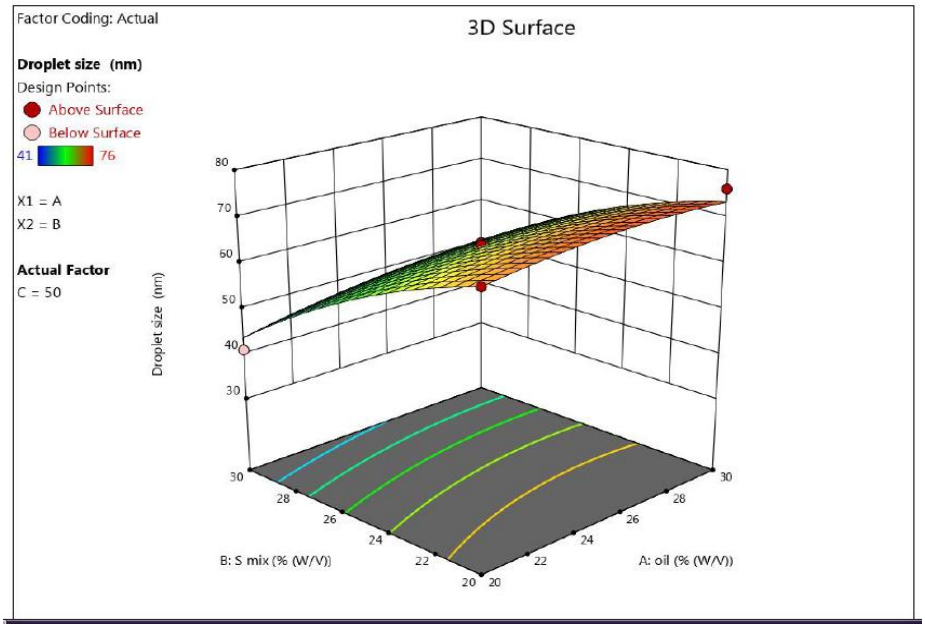


3.5 Puesdo Ternary Phase Diagram

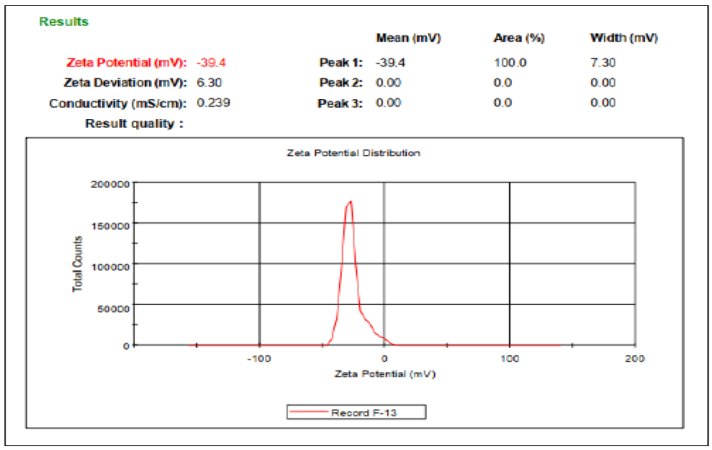
NE are portrayed by a scope of actual properties that are significant determinants of their design, drug delivery and steadiness. Pseudo ternary phase charts are frequently built to demonstrate the limits of the various stages as an element of the arrangement of the watery, oil and surfactant/cosurfactant segments.



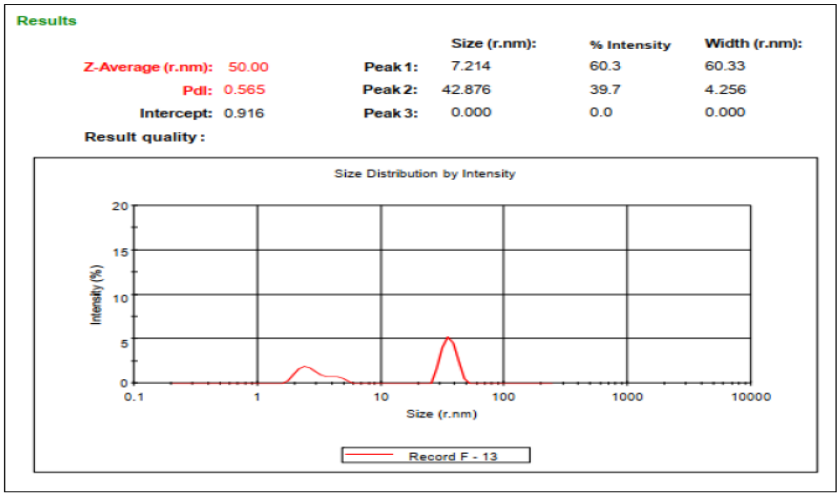
3.6 Plot Between Predictable vs. Original Droplet Size



3D Surface Diagram



3.7 Result of Zeta Potential of Optimized Formulation F13



3.8 Result of Vesicle size of Optimized Batch F13

Evaluation of nanoemulsion gel :

Code	Drug content (%)	pH	Spreadability (Gm.cm/sec.)	Viscosity (cps)
F1	92.23± 0.25	6.9± 0.2	12.2± 0.3	4200± 20
F2	98.85± 0.36	6.8± 0.1	11.1± 0.4	3560 ±35
F3	94.65± 0.21	6.5± 0.1	10.3± 0.5	3210 ±15

3.9 Results of *In-vitro* drug release data of optimized formulation F2

Time (h)	Square Root of Time (h) 1/2	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Remaining	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	0.301	18.85±0.20	1.275±0.035	81.15±0.62	1.275
1	1	0	23.35±1.02	1.368±0.055	76.65±0.55	1.368
2	1.414	0.301	39.98±0.32	1.602±0.022	60.02±0.45	1.602
4	2	0.602	56.65±0.45	1.753±0.065	43.35±0.43	1.753
6	2.449	0.778	82.23±1.20	1.915±0.044	17.77±0.26	1.915
8	2.828	0.903	98.85±0.42	1.995±0.040	1.15±0.43	1.995

3.10 Antifungal Activity of *Stand Ard Drug* and Nanoemulsion Gel Against *Candida Albicans*

S. No.	Name of drug\formulation	Zone of inhibition		
		30 µg/ml	20 µg/ml	10 µg/ml
1	Fluconazole	18±0.47	14±0.74	11±0.5
2	Nanoemulsion gel	16±0.96	13±0.86	10±0.47

3.11 Results of Stability Studies

The results of stability studies reveled negligible changes was found in 2ºC–8ºC and the prepared gel was not found stable in room temperature and elevated temperature

4 CONCLUSION

The current study has therefore successfully formulated a thermodynamically stable antifungal nanoemulsion gelcontaining ciclopirox olamine which can be retained for prolonged period of time. Such formulation achieves better local concentration of the drug with low systemic absorption of potentially toxic anti-fungal drug. Hence, the formulation has proved to be a promising approach for treatment of fungal infectio.

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IN WHAT WAY E-LEARNING IS RENOVATING THE TEACHING SEGMENT?

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1 INTRODUCTION

E-learning refers to any type of learning or teaching that is aided by technology. Whether networked or not, information and communication technologies serve as distinct means for carrying out the learning process. Even as technology advancements in terms of devices and curriculum continue, the phrase will very certainly be applied to both out-of-classroom and in-classroom electronic educational experiences. The use of a computer and a network to communicate skills and knowledge is known as e-learning. Web-based learning, computer-based learning, virtual classroom chances, and digital collaboration are all examples of e-learning applications and methodologies. The Internet, intranet/extranet, audio or video cassette, satellite television, and CDROM are all used to provide content. It can be self-paced or instructor-led and incorporates text, image, animation, streaming video, and audio, among other things. CBT (Computer-Based Training), IBT (Internet-Based Training), and WBT (Web-Based Training) are abbreviations for e-learning (Web-Based Training). This terminology, as well as versions such as e-learning, E-learning, and e-Learning, are still in use today. The terminologies will be used throughout this essay to demonstrate their applicability in the context of eLearning terminology. Electronic learning(e-learning) education methods have been made possible thanks to the Internet.

The ability of e-learning to allow students to study without regard to time or location, as well as the ability of some businesses to reduce internal training costs, has contributed to its growing popularity. Students' impressions of the use of e-learning at institutions like TATIUC (TATI University College) have become a top priority for their educational demands because of this expanding popularity. Terengganu Advanced Technical Institute (TATI) is a completely owned subsidiary of the Malaysian state of Terengganu. Terengganu Advanced Technical Institute (TATI) is a completely owned subsidiary of the Malaysian state of Terengganu. When an e- learningsystemisusedtomakethemeasilyaccessible,well-designed, learner-centered, affordable, and efficient, flexible, and withasupportivelearningenvironment,itbecomesrelevant.Rosenberg (2000) defines e-learning as the use of internet technology to give a variety of options for improving knowledge and performance It’s linked, and it focuses on the broadest concept of learning conceivable. It is made available to end users through standard Internet protocols. E-learning, according toVickyO'Leary's(2005) research, can helpafirmororganization by allowing learners to access materials such as document databases and assignments. TATIUC has taken a stride forward in upgrading its e-learning capabilities so that it can deliver better information and education to its students.

Students, teachers, professionals, and learners can access a variety of courses through the Indian government's free e-Learning portals. Audio, video, presentations, PDFs, tests, and more kinds of contentare available. A comprehensive list of freee-Learning sites has been compiled by the Indian government. A few free e-learning portals have been created by the Indian government:

1. Swayam: The objective of SWAYAM is to provide a learning platform for everyone, especially the most disadvantaged. It houses practically all the subjects taught in classrooms from 9th grade to post-secondary education. SWAYAM's official website, swayam.gov.in, contains additional information.
2. Diksha: This initiative is being led by the National Council of Educational Research and Training, which is part of India's Ministry of Education. Learners and teachers across the country can access DIKSHA via diksha.gov.in. In India, it currently covers NCERT, CBSE, and SCERT courses.
3. e-Shodh Sindhu: It will continue to give its member universities with current and archival access to over10,000 peer-reviewed papers as well as a variety of bibliographic, reference, and factual databases. At ess.inflibnet.ac.in, you may finde-Shodh Sindhu.
4. e-PG Path shala: It's a project led by the University of Georgia that's part of the Ministry of Human Resources and Development's National Mission on Education through Information and Communication Technology. epgp.inflibnet.ac.in offers interactive e- content for 70 social science, arts, fine arts and humanities, natural science, and mathematics courses.
5. Swayam Prabha: It offers 34 DTH channels with high-quality educational programming available24hours a day, seven days a week. NPTEL,IITs,UGC,CEC,IGNOU,NCERT, and NIOS supply course materials. Swayamprabha.gov.in is the website.
6. NPTEL: IIT Bombay, IIT Delhi, IIT Kanpur, IIT Kharagpur, IIT Madras, IIT Guwahati, and IIT Roorkee collaborated with the Indian Institute of Science, Bangalore, to launch the National Programme on Technology Enhanced Learning in 2003. NPTEL's website, nptel.ac.in, provides free open online engineering and core science courses

E-learning appears to be on the approach of supplanting traditional education. E-learning is frequently characterized in terms of technology. E-learning, according to Welsh et al., is "the use of computer network technology, primarily directly or through the Internet, to provide people with information and instruction." E-learning is described as the use of internet technology to present learners with a choice of possibilities, according to Rosenberg (2001). According to Rosenberg, e-learning is defined as the use of internet technology to provide learners with a variety of options (2001). While there are many different definitions of e-learning, they all have the same essential components.

Students' impressions of e-learning in university education may be influenced by a variety of circumstances. Junior students in secondary schools may have used e-learning. Senior students, on the other hand, may have had their first interaction with computers during their university studies. Regardless of age, men are thought to be more computer savvy than women. Women are less computer literate than men, and they have more computer phobia. Men's technology usagedecisions are impact edmore by their assessment. Women, on the other hand, are more affected by perceived ease of use. Different aspects of computeruse are prioritized by menand women. AS a result, young malestudentsmay adapt- learning more quickly than older female students. Two hypotheses were evaluated in determining the students' attitudes of e-learning at TATIUC: technology usage and awareness of e-learning implementation.

E-learning seems to be on the verge of becoming the new learning paradigm. Besides, E-learning is often defined in terms of technology. For example, Welsh et al. (2003, p. 246) define e-learning as the ‘‘use of computer network technology, primarily over or through the Internet, to deliver information and instruction to individuals.’’ Rosenberg (2001) shares a similar definition referring to e-learning as using internet technologies to deliver various solutions to learners. Holmes and Gardner (2006) simply state that e-learning provides us with access to resources that promote learning on anyplace and anytime basis. While the definitions of e-learning may vary, they all focus on a set of basic concepts which include learning, technology, and access.

Students' perceptions of e-learning in university education may be influenced by specific individual variables. Junior students may have experienced using e-learning in secondary schools. On the other hand, senior students may for the first time have met computers for educational purposes at university. Irrespective of age, men are supposed to be more used to computers than women. Women typically display lower computer aptitude and higher levels of computer anxiety. Research has indicated that men's technology usage decisions are more strongly influenced by perceptions of usefulness. In contrast, women are more influenced by perceptions of ease of use. Men and women focus on different aspects of using computers (Venkatesh & Morris, 2000).

Hence, it could be hypothesized that young male students are more prone to adapt to e-learning than not so young female students. In identifying the student's perception towards e-learning at TATIUC there were three hypotheses which has been putting under consideration that are technology usage and the awareness of e-learning implementation. By enabling proper knowledge flow within companies, e-learning systems provide solutions that communicate knowledge and information, support learning, and boost performance. A corporation must adopt technology solutions, methods, and resources, as well as manage them adequately, to employ e-learning effectively. A wide range of enterprises have implemented e-learning platforms such as Canvas, Blackboard, and Moodle. Students, employees, managers, instructors, institutions, and other stakeholders benefit from such systems since they promote and enhance learning processes while also facilitating knowledge transfer. Instructors and management can construct appropriate training and knowledge exchange by using features such as developing modules to organize mini course content and learning materials, or communication channels such as chat, forums, and video sharing. In recent years, the use of diverse e-learning capabilities to increase organizational and workplace learning has become a commodity. On-the-job training or knowledge growth are examples of this type of learning.

Many studies have shown that effective use of e-learning could help increase student motivation engagement, and attendance. It also increases student class participation, and improved behavior and performance on core subjects. One of the crucial factors for students' success in e-learning process is self-motivation. The Integration of information and communication technologies with the learning process depends on the participants' personal motivation. To enable students to maximize the ICT potential in their learning process, students need to be supported with their digital enhanced learning. However, many studies have shown that non-IT students need to increase the level of their technological and communication skills to be able to benefit significantly from the opportunities offered by e-learning. The lack of confidence and experience in using technology might be extra obstacle for other students. In e-learning process, students work independently, and some students might find it difficult to understand their contents, due to the lack face-to-face contact with instructors and other fellow students. All these factors indicate that these students will not be able to participate effectively and succeed in the e-learning process. Consequently, to appropriately progress and successfully use all e-learning tools to effectively access online information, some students need the necessary hardware and some specific skills. Certainly, E-Learning would increase the motivation and engagement of students for learning and help them to become self-directed independent learners. On the one hand, teachers need to develop and restructure their courses in a way that suits online requirements. It is very clear that such activities require more time and increase the workload. On the other hand, instructors and faculty members must honor, possess, and master all technical achievements and new advancements offered by E-Learning. To maintain the quality of the courses offered via E-learning, faculty members and instructors.

2 CONCLUSION

Severalstudieshave reported some important findings about online education, confuted others and presented a range of predictions about the future of online technology for educational purposes. Instructors need to understand their student motivations when teaching online classes. However, it can be difficult to assess student motivations for online learning due to the lack of personal contact between the students and instructor. One way to avoid this is to have the students complete an online assessment form on motivation. From the information obtained, a teacher can identify several strategies to engage the students and keep them motivated. Most importantly, it should be noted that more technology does not necessarily lead to better learning outcomes. Instructors who taught the participants of the study should be interviewed to get feedback to evaluate e-learning from a teacher's perspective.

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