

FORMULATION & EVALUATION OF ANTI INFLAMMATORY CREAM BY USING NSAID's DRUG WITH HERBAL EXTRACT OF WITHANIA SOMNIFERA FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

***M. Lakshmi Prasanna, Dr. K. Vidyadhar, JSD. Pavan Kumar, P. Mani Kumar, D.L.Anusha, Phalguna Rao and N. Satya Pandu**

VJS College of Pharmacy, D.B.V.Raju Township, Diwancheruvu, Rajahmundry India.

Affiliated to Andhra University, Vizag.

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***Corresponding Author**

M. Lakshmi Prasanna

VJS College of Pharmacy,
D.B.V.Raju Township,
Diwancheruvu,
Rajahmundry India.
Affiliated to Andhra
University, Vizag.

1. INTRODUCTION

Diclofenac sodium (DS) is a nonsteroidal anti-inflammatory drug^[71] (NSAIDs) widely used clinically to reduce inflammation and pain in conditions such as rheumatoid arthritis, menstrual pain, dysmenorrhea, fever, osteoarthritis or acute injury. It has a short half-life in plasma (2 hrs) and only 50% of the drug reaches the circulation. Oral dose of diclofenac potassium causes an increased risk of serious gastrointestinal adverse events including bleeding, ulceration and perforation of the stomach or the intestines which could be fatal. Transdermal delivery of the drug can improve its bioactivity with reduction of the side effects and enhance the therapeutic efficacy. DS^[73] has a potent anti-inflammatory effect, but it does not penetrate well through skin and cannot reach the effective concentration at the

site of action after transdermal application. For this reason, we wanted to suggest new, alternative dosage forms for transdermal application of DS.

1.1 SKIN BARRIER AND TRANS DERMAL DRUG DELIVERY^[74]

The skin provides the largest interface between the human body and the external environment. Therefore, one of its most important functions is to regulate what enters the body via the skin, as well as what exits. In general, the skin is designed to let very little enter, since other tissues, such as the permeable epithelia of the gastrointestinal tract and lung, provide the primary means of regulated entry into the body. Likewise, the skin must prevent

excessive loss of water and other bodily constituents. The skin's remarkable barrier properties are due in large part to the stratum corneum, which represents the thin outer layer of the epidermis. In contrast to other tissues in the body, the stratum corneum consists of corneocytes (composed primarily of aggregated keratin filaments encased in a cornified envelope) that are surrounded by an extracellular milieu of lipids organized as multiple lamellar bilayers. These structured lipids prevent excessive loss of water from the body and likewise block entry of most topically applied drugs, other than those that are lipid-soluble and of low molecular weight. This poses a significant challenge to administering medications via the skin either for local cutaneous effects or as systemic therapy following their entry into superficial dermal capillaries.

1.2 STRUCTURE AND ORIGIN OF THE SKIN BARRIER

Stratum Corneum Structure and Organization

The stratum corneum is a composite material made of proteins and lipids structurally organized as “bricks and mortar”. Instead of being uniformly dispersed, the highly hydrophobic lipids in normal stratum corneum are sequestered within the extracellular spaces, where this lipid-enriched matrix is organized into lamellar membranes that surround the corneocytes. Hence, rather than stratum corneum thickness, variations in number of lamellar membranes (= lipid weight %), membrane structure, and/or lipid composition provide the structural and biochemical basis for site-related variations.

FEATURES OF THE STRATUM CORNEUM

- Primary barrier to drug absorption into skin
- Two-compartment organization: “bricks and mortar”
- Microheterogeneity within extracellular spaces: “There's more to the mortar than lipid”
- Persistent metabolic activity: dynamic changes in cytosol, cornified envelope, and interstices from inner to outer stratum corneum.
- Homeostatic links to the nucleated cell layers: barrier function regulates epidermal DNA and lipid synthesis.
- Pathophysiologic links to deeper skin layers: barrier abrogation and/or epidermal injury initiates epidermal hyperplasia and inflammation
- Stratum corneum as a biosensor: changes in external humidity alone regulate Proteolysis of filaggrin, epidermal DNA/lipid synthesis and initiation of inflammation.

Factors Affecting How Stratum Corneum Lipids Mediate Barrier Function

- Extracellular localization: only intercellular lipids play a role.
- Amount of lipid (lipid weight %).
- Elongated, tortuous pathway: increases diffusion length.
- Organization into lamellar membrane structures.
- Hydrophobic composition: absence of polar lipids and presence of very-longchain saturated fatty acids.
- Correct molar ratio: approximately 1:1:1 of three key lipids: ceramides, cholesterol and free fatty acids.
- Unique molecular structures (e.g. acylceramides).

1.3 Skin Structure And Function

The skin is the largest organ in the body, with a surface area of approximately 1.8 m² and a total weight estimated, for a typical adult of 70 kg, to be 4 kg. In normothermic conditions, the cutaneous circulation comprises 5–10% of the total cardiac output. For 70-kg human males, the skin blood flow is approximately 4.64 cm³/s or 16 700 cm³/h (Kasting & Robinson, 1993). Thus, the ratio of the total capillary flow to the corresponding skin surface area is approximately 0.93 cm/h. The skin is a heterogeneous organ, containing a number of layers as well as appendages, such as sweat glands, hair follicles, composition of the stratum corneum vary according to body region. Until the beginning of the 20th century, the skin was thought to be completely inert and impermeable to chemicals that might otherwise enter the body. While the skin does act as a barrier, it is not a complete barrier. Many chemicals do penetrate the skin, either intentionally or unintentionally, and cutaneous metabolism does occur. Because of its large surface area, the skin may be a major route of entry into the body in some exposure situations.

Functions of the skin**Barrier function**

The skin provides a sturdy, flexible, and self-repairing barrier to the exterior environment, protecting the internal body organs and fluids from external influences. It prevents loss of endogenous water and nutrients (humans are approximately 70% water) and protects against many unwanted toxic substances and pathogenic microorganisms. The skin also responds to mechanical forces (elasticity and cushioning).

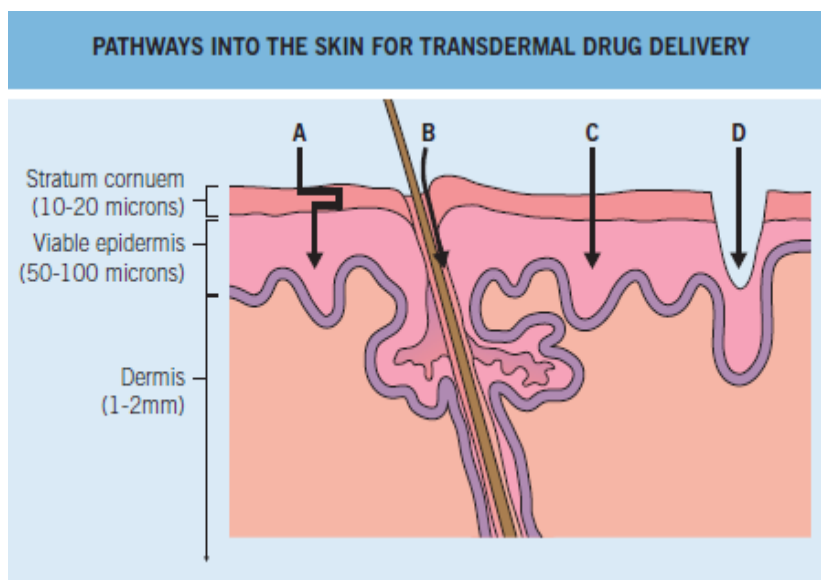


Fig no: 1. A schematic diagram of the structure of the skin

Skin structure

Based on structure and embryonic origin, the cellular layers of the skin are divided into two distinct regions. The outer region, the epidermis, develops from the embryonic ectoderm and covers the connective tissue; the dermis is derived from the mesoderm.

Epidermis

The epidermis comprises about 5% of full-thickness skin and is divided into five or six layers, based on cellular characteristics (see Fig. 1). The majority of cells in the epidermis are called keratinocytes, which are formed by differentiation from one layer of mitotic basal cells. The number of distinguishable layers is dependent upon the anatomical site.

Dermis

The dermis provides the nutritional support for the avascular epidermis. The dermis is a 0.2- to 0.3-cm-thick tissue that comprises a fibrous protein matrix, mainly collagen, elastin, and reticulum, embedded in an amorphous colloidal ground substance. The physical behaviour of the dermis, including elasticity, is determined by the fibre bundles and ground substance. The dermis is the locus of blood vessels, sensory nerves (pressure, temperature and pain) and lymphatics. It contains the inner segments of the sweat glands and pilosebaceous units. The dermis provides flexibility with strength, serves as a barrier to infection, and functions as a water storage organ.

Skin appendages

The skin appendages originate in the subpapillary dermis and consist of eccrine sweat glands, apocrine sweat glands, sebaceous glands, and hair follicles, with their associated erector muscles.

Appendages are found in most anatomical sites, although the number of each varies significantly by site. An average human skin surface contains 40–70 hair follicles and 200–250 sweat ducts per square centimetre. Sebaceous glands are most numerous and largest on the face, especially the forehead, in the ear, on the midline of the back, and on anogenital surfaces.

Transport through the skin

Percutaneous absorption includes permeation through the epidermis and uptake by the capillary network at the dermal–epidermal junction. Percutaneous absorption occurs mainly transepidermally (across the stratum corneum intracellularly and intercellularly); for many chemicals, transport through appendages is Permeation of a chemical through the stratum corneum is basically a diffusion process in which active transport plays no role. The layer with the highest resistance to diffusion is the rate-limiting membrane. For many compounds, the lipophilic stratum corneum is the primary or rate-limiting barrier. However, diffusion through the hydrophilic epidermis and dermis can be rate limiting for very lipophilic materials and/or when the stratum corneum is damaged or affected by disease.

Theoretical aspects of diffusion

Diffusion of compounds across a membrane is described by Fick's first law.

$$J = -D \frac{\partial C}{\partial x}$$

which states that the flux (rate of transfer per unit area) of a compound (J , mass/cm² per second) at a given time and position is proportional to the differential concentration change δC over a differential distance δx (i.e. the concentration gradient $\delta C / \delta x$). negative sign indicates that the net flux is in the direction of decreasing thermodynamic activity, which can often be represented by the concentration. Fick's second law describing concentration within a membrane

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}$$

Is derived by combining a differential mass balance in a membrane with Fick's first law and, when considering the skin, assuming that the compound does not bind, the compound is not metabolized, and its diffusion coefficient does not vary with position or composition.

PHYSICOCHEMICAL FACTORS AFFECTING SKIN PERMEATION

Physical state

Thermodynamically, pure powders and saturated liquid solutions of the same compounds have the same driving force for dermal absorption. However, availability to the skin surface may cause the absorption rates to be different. Absorption from dry particulates can occur even without surface moisture. However, dermal absorption of chemicals in solutions may be more rapid than absorption from particulates. Although data are limited, particle size would be expected to have an effect, with slower absorption rates from larger particles.

Molecular size/Molecular weight

Molecular size is an important factor in membrane permeation. Theoretically, molecular volume should be a better predictor of flux and the permeability coefficient K_p . However, molecular weight is often used instead, because it is more readily available and unambiguous. The equations for estimating K_p have been derived from databases containing data on primarily hydrocarbons. For most hydrocarbons, the ratio of molecular weight to molecular volume is nearly constant; thus, K_p estimates based on molecular weight are as good as those based on molecular volume.

Maximum flux

The maximum flux of a solute through the skin defines the highest exposure risk for a chemical. Maximum flux, either measured or estimated, is a better gauge for dermal absorption than a given physicochemical property of a solute. Hence, neither water solubility nor the octanol/water partition coefficient alone is a reliable indicator of the likelihood of significant dermal absorption.

Ionization

Ionized species do not penetrate the skin very well. The stratum corneum permeability coefficients for non-ionized compounds are frequently 1–2 orders of magnitude larger than permeability coefficients for ionized forms of the same compound. However, ionization effects are less evident in maximum flux estimations. The exact relationship between the non-ionized and ionized forms would be expected to depend upon the compound and the

lipophilicity of the non-ionized chemical, in particular, but also on the vehicle and salt form of the chemical used, as ion pairing may also facilitate ionized drug.

Binding properties

Permeation through the stratum corneum can be slower than expected for some compounds due to binding. Examples include certain metal ions (particularly Ag⁺, Cd²⁺, Be²⁺, and Hg²⁺), acrylates, quaternary ammonium ions, heterocyclic ammonium ions, and sulfonium salts. Other potential agents that may have slower than expected permeation possibly due to binding include diethanolamine, quinines, alkylsulphides, acidchlorides, halotriazines, and dinitro-or trinitrobenzenes. Plants have been the major source of drugs for the treatment of various disorders in Indian medicine and other ancient systems in the world, and for a long time many disorders has been treated orally with herbal medicines or their extracts .Because plant products are frequently considered to be less toxic and more free from side effects than synthetic ones. Furthermore, after the recommendations made by the WHO on, investigations on chemical constituents frommedicinal plants have become more important and the search for more effective and safer. World ethnobotanical information about medicinal plants reports that almost thousandsof plants could be used to control variousdisorders. Many herbs and plants have been described as possessing different pharmacological activity when taken orally. Some of these plants have also been pharmacologically tested and shown to be of some value in treatment of various disorders.

1.4 METABOLISM IN THE SKIN

The skin is a metabolically active organ and contains enzymes that are able to catalyse not only endogenous chemicals such as hormones, steroids, and inflammatory mediators but also xenobiotics, including drugs, pesticides, and industrial and environmental chemicals. Although the metabolism of xenobiotic compounds in the skin is intended to detoxify potentially reactive chemicals by converting lipophilic compounds into polar, water-soluble compounds that are readily excreted into the bile and urine, in some cases a compound may be activated, leading to enhanced local and/or systemic toxicity. Cutaneous activation and detoxification can be a critical determinant of systemic exposure in humansfollowing dermal absorption. If transport through the viable epidermis is rate limiting and themetabolite is less hydrophobic than the parent compound, then percutaneous absorption of the metabolized compound could be faster than that of the parent compound.

The drug-metabolizing systems of the skin

The skin contains enzymes that catalyse Phase 1 (e.g. oxidation, reduction, and hydrolysis) and Phase 2 (conjugation) reactions the specific activities of cutaneous xenobiotic-metabolizing enzymes in the skin, measured in subcellular fractions, are lower than those of their counterparts. However, although the basal activities may be relatively low, if the surface area of the skin exposed to the contaminant is very large, the metabolism in the skin is likely to make a contribution to the overall metabolism of the compound following dermal exposure. However, while the skin is a large organ, the only place that metabolism will be important for most substances will be the actual area that is exposed to the chemical. The extent to which topically applied chemicals will be metabolized depends on the chemical and the enzymes involved. Some chemical groups, such as esters, primary amines, alcohols, and acids, are particularly susceptible to metabolism in the skin.

Methodology for evaluating skin metabolism in invitro systems

The extent of cutaneous metabolism of a chemical applied to the skin is difficult to differentiate in vivo from systemic metabolism, mainly in the liver, using blood and excreta samples. In vitro studies isolate the skin from the metabolic activity in the rest of the body. The use of viable skin is essential.

Effects of skin metabolism

Cutaneous metabolism may result in: activation of inert compounds to toxicologically active species. e.g., polycyclic aromatic hydrocarbons (PAHs), such as benzopyrene and 3-methylcholanthrene.

Importance of metabolism for percutaneous absorption

That skin can metabolize compounds before they enter the bloodstream is important for risk assessment purposes and for drug delivery. However, there are conflicting views concerning the influence of local skin metabolism on percutaneous absorption.

1.5 IN VITRO TESTS FOR DERMAL ABSORPTION

In vitro methods are designed to measure the penetration of chemicals into and subsequent permeation across the skin into a fluid reservoir and can utilize non-viable skin to measure penetration and permeation only or fresh, metabolically active skin to simultaneously measure permeation and skin metabolism. Permeation across the non-living outer layer of skin, the stratum corneum, is often the rate-limiting step for percutaneous absorption.

Principles of the standard in vitro tests using skin samples

The test substance, which may be radiolabelled, is applied to the surface of a skin sample separating the two chambers of a diffusion cell. Most common methods for the evaluation of in vitro skin penetration and permeation use diffusion cells, which range in complexity from a simple two-compartment “static” cell to multi-jacketed flow-through cells.

Test chambers

Diffusion cells are of the upright/vertical or side-by-side type, with receptor chamber volumes of about 0.5–10 ml and surface areas of exposed membranes of about 0.2–2 cm². Both receptor chamber volume and the exposed surface area should be accurately measured and recorded for individual diffusion cells. Vertical cells are useful for studying absorption from semisolid formulations spread on the membrane surface and are optimal for simulating in vivo performance.

Skin preparations**Choice of skin**

The choice of skin depends on the purpose of the test and the availability of skin samples. For risk assessment purposes, human skin is preferred. As stated above, the epidermis is the major barrier for the compound, because once the permeant has transferred across the epidermis, it has access to the cutaneous circulation. In the in vivo situation, the dermis normally has little effect on the dermal absorption of compounds whose rate of absorption is limited by the stratum corneum.

Application of test substance**Test substance**

For practical purposes, the test substance ideally should be radiolabelled (preferably with carbon-14 at a metabolically stable position). However, radiolabelled scintillation counting does not distinguish between metabolites, and further analysis may be necessary. It is essential to determine radiochemical purity (pre-and post-permeation), and the possibility of tritium exchange, where appropriate, should be examined. If radiolabelling is not possible, suitable validated assay procedures must be established for the respective chemicals and metabolites.

Evaluation of the results

The terminal procedures of an in vitro dermal absorption study are slightly different following infinite and finite dosing experiments. After finite dosing, the mean maximum amount of dermally absorbed material is determined, which requires complete recovery of the test substance (90–110% or 85–115%). The quantity washed from the skin, the quantity associated with the skin (and in the different skin layers, if analysed), and the amount present in the receptor fluid should be determined.

1.6 IN VIVO TESTS FOR DERMAL ABSORPTION.^[67,68]

This chapter is an overview of the methodology for in vivo tests for dermal absorption. Although some examples are given, this is only a small part of the literature available on this subject. For a compilation of available studies, the reader is referred to databases such as that of EDETOX. The in vivo methods allow the determination of the extent of cutaneous uptake as well as systemic absorption of the test substance. The main advantage in performing an in vivo study rather than an in vitro study is that the in vivo study uses a physiologically and metabolically intact system. In vivo dermal penetration studies are carried out in laboratory animals, usually rodents, and in human volunteers.

Laboratory animal studies

The rat is the most commonly used species for animal in vivo studies, having the advantage that information from other toxicity and toxicokinetic studies is mostly obtained from this species. The male rat is the species and sex required for dermal absorption studies according to the USEPA, however, other animal species can be used when they have been shown to have skin absorption more similar to that of humans than the rat.

Principles of the standard in vivo tests

The test chemical is applied to a designated area of skin in an appropriate format (e.g. in solvent or formulation for a defined period). Body fluids, tissue, or excreta are collected at predefined intervals, and the quantity of chemical and/or metabolite in the samples is measured by a suitable analytical procedure). The collection of expired air should be considered when there is information that the chemical and/or its metabolite are excreted by this route. Preparation of the application site at least 24 h before treatment, the application site should be prepared. In the rat, for example, the hair on the shoulders and the back is removed with animal hair clippers; shaving of the application site should be avoided,

Application of the test substance to the skin The test substance preparation, which ideally is radiolabelled in a metabolically stable position, is applied to 5–10% of the surface of the skin (for rats with body weight 200–250 g) This specification of the volume is based on an unconstrained application (i.e. to prevent the solution from running over a larger area) if one were trying to simulate a skin splash.

Evaluation of the results

The quantity of non-pharmaceutical chemical or its metabolites must be determined in (OECD, 2004c).

- dislodgeable dose from the skin surface (washing water).
- Skin from the treated and non-exposed site (stratum corneum, epidermis, and dermis);
- dislodgeable dose and desquamated skin (from protective dressing post-exposure);
- Urine, faeces, and cage washing;
- Expired air, if applicable (>5% volatile metabolites of applied dose);
- Blood and remaining carcass; and.
- Solvent washing of contaminated material and application system. An adequate mean recovery is in the range of 100 • } 10%, although this recovery is higher than is often observed. Recoveries outside the given range should be justified.

Factors affecting dermal absorption in vivo

Species, strain, and sex

The skin of rats, guinea-pigs, and rabbits is more permeable than that of humans, whereas the skin permeability of pigs and monkeys is more similar to that of humans. Other possible animal models include the athymic (nude) rat skin flap model, hairless rats, hairless mice, and fuzzy rats. In a comparative study involving several species, radiolabelled haloprogin, N-acetylcysteine, cortisone, testosterone, caffeine, and butter yellow dissolved in acetone were applied to the skin of rats, rabbits, minipigs, and humans (only haloprogin and N-acetylcysteine). The dose was 4 µg/cm² skin surface applied using a non-occlusive foam pad.

Age

In a comparative study with human volunteers demonstrated that age can affect dermal absorption. Permeation of hydrocortisone, benzoic acid, acetylsalicylic acid, and caffeine was significantly lower in aged subjects, whereas the absorption of testosterone and estradiol was similar in young and aged subjects.

Anatomical site

The percutaneous absorption of acetylsalicylic acid, benzoic acid, caffeine, and benzoic acid sodium salt (radiolabelled) was measured in male Caucasians on four body sites (arm, abdomen, postauricular, forehead), using the tape-stripping method. Skin penetration, as indicated by the amount of chemical in the stratum corneum, was ranked as follows: arm abdomen postauricular forehead.

Temperature and humidity conditions

Increased percutaneous absorption rates were seen for 2-butoxyethanol vapours with raised temperature and humidity conditions. The mean "baseline" (25°C, 40% relative humidity, shorts and T-shirts) percentage of dermal absorption was 11% (range 9–14%) of the "whole-body" burden. For each subsequent exposure, a single parameter was changed: humidity (60%, 65%), temperature (20°C, 30°C), or clothing (minimal or overalls). At 30°C, the percentage of dermal absorption was significantly increased, with a mean of 14% (range 12–15%).

1.7 RHEUMATOID ARTHRITIS.^[71]

RA is a chronic, progressive, inflammatory autoimmune disease associated with articular, extra-articular and systemic effects. It has been reported that RA affects 0.5-1% of the adult population of developed regions.^[43,56] Although some patients have mild self-limited disease, many experience joint destruction, severe physical disability and multiple co-morbidities.^[61] Mortality rates are more than twice as high in patients with RA as in the general population^[61,62], and this gap appears to be widening.^[54] T cells, B cells and the orchestrated interaction of pro-inflammatory cytokines play key roles in the pathophysiology of RA.^[61,62] The cytokines most directly implicated in this process are TNF- α and IL-6; IL-1 and IL-17 may also play important, albeit arguably less so, roles in the disease process. The goal of this review is to summarize the complex pathobiology of RA as currently understood, highlighting the effects of major immune modulators at both articular and systemic levels. In addition, we briefly discuss how the increased understanding of the pathobiology of RA has led to the development of biologic agents that target specific immune mediators and has resulted in new and effective treatments for RA.

1.8 OVERVIEW OF RA PATHOBIOLOGY

Although the exact cause of RA remains unknown^[56], recent findings suggest a genetic basis for disease development. More than 80% of patients carry the epitope of the HLA-DRB1 cluster^[57], and patients expressing two HLA-DRB1 alleles are at elevated risk for nodular disease, major organ involvement and surgery related to joint destruction. Single-nucleotide polymorphism genotyping across the MHC has identified. Environmental factors, such as smoking and infection, may also influence the development, rate of progression and severity of RA. Various immune modulators (cytokines and effect or cells) and signalling pathways are involved in the pathophysiology of RA.^[59] The complex interaction of immune modulators is responsible for the joint damage that begins at the synovial membrane and covers most IA structures. Synovitis is caused by the influx or local activation, or both, of mononuclear cells (including T cells, B cells, plasma cells, dendritic cells, macrophages and mast cells) and by angiogenesis.^[61] The synovial lining then becomes hyperplastic, and the synovial membrane expands and forms villi.^[61] The osteoclast-rich portion of the synovial membrane, or pannus, destroys bone, whereas enzymes secreted by neutrophils, synoviocytes and chondrocytes degrade cartilage. In addition to joint symptoms, many patients experience extra-articular or systemic manifestations, or both. According to a US pharmacy claims data analysis with a mean follow-up of 3.9 years, 47.5% of 16752 patients with RA experienced at least one extra-articular or systemic manifestation. Extra-articular manifestations include rheumatoid nodules, vasculitis, pericarditis, keratoconjunctivitis sicca, uveitis and rheumatoid lung. Systemic manifestations include acute-phase protein production, anaemia, cardiovascular disease (CVD), osteoporosis, fatigue and depression.

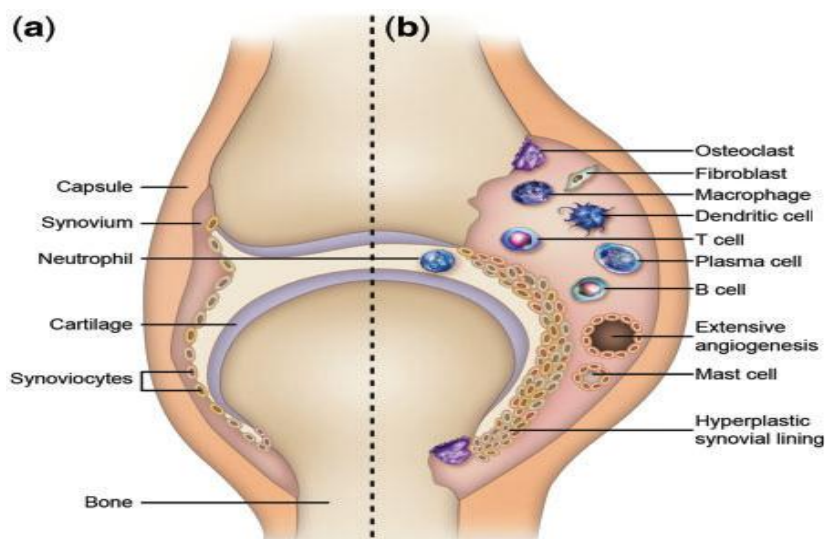


Fig no 2: Schematic view of a normal joint (a) and a joint affected by RA (b).

1.9 Effector Cells Involved In the Pathobiology of Ra

The earliest event in RA pathogenesis is activation of the innate immune response, which includes the activation of dendritic cells by exogenous material and autologous antigens.^[12,13] Antigen-presenting cells, including dendritic cells, macrophages and activated B cells present arthritis-associated antigens to T cells. Concurrently, CD4⁺ T cells that secrete IL-2 and IFN- γ infiltrate the synovial membrane. As noted previously, most patients with RA carry the epitope of the HLA-DRB1*04 cluster. These alleles share a homologous amino acid sequence on the HLA-DR b-chain that confers binding of specific peptides and affects antigen presentation to TCRs. Disease-associated HLA-DR alleles may present arthritis-related peptides, leading to the stimulation and expansion of autoantigen-specific T cells in the joints and lymph nodes. B cells contribute to RA pathogenesis not only through antigen presentation, but also through the production of antibodies, autoantibodies and cytokines. RF and anti-CCP autoantibodies are common in patients with RA. B lymphocytes express cell surface proteins, including immunoglobulin and differentiation antigens such as CD20 and CD22. Autoantibodies can form larger immune complexes that can further stimulate the production of pro-inflammatory cytokines, including TNF- α , through complement and Fc-receptor activation. T- and B-cell activation result in increased production of cytokines and chemokine's, leading to a feedback loop for additional T-cell, macrophage and B-cell interactions. In addition to antigen presentation, macrophages are involved in osteoclast genesis and are a major source of cytokines, including TNF- α , IL-1 and IL-6. Within the synovial membrane there is a great increase in activated fibroblast-like synoviocytes, which also produce inflammatory cytokines, PGs and MMPs. Synoviocytes contribute to the destruction of cartilage and bone by secreting MMPs into the SF and by direct invasion into these tissues.

1.10 Cytokines And The Impact On Effector Cells

It is well established that pro-inflammatory cytokines (e.g. IL-6 and TNF- α) are involved in the pathogenesis of RA. TNF- α and IL-6 play dominant roles in the pathobiology of RA; however, IL-1, VEGF and perhaps IL-17 also have a significant impact on the disease process. Details on the roles of these cytokines are shown in through complex signal pathways. These cytokines activate genes associated with inflammatory responses, including additional cytokines and MMPs involved in tissue degradation,^[12] this is discussed in subsequent sections. An IL-17-secreting subset of CD4⁺ cells that has a critical role in synovitis has recently been implicated in the pathogenesis of many inflammatory and auto immune

diseases, including RA. The presence of TH17 cells in the SF and peripheral blood of patients with RA suggests the involvement of this potent proinflammatory cytokine in RA pathology. An *in vivo* study has shown that CIA was markedly suppressed in IL-17-deficient mice. Additionally, the ubiquitous expression of IL-17 receptor (IL-17R) on fibroblasts, endothelial cells, epithelial cells and neutrophils indicates that this cytokine has the potential to influence a number of pathways and effector cells involved in RA.

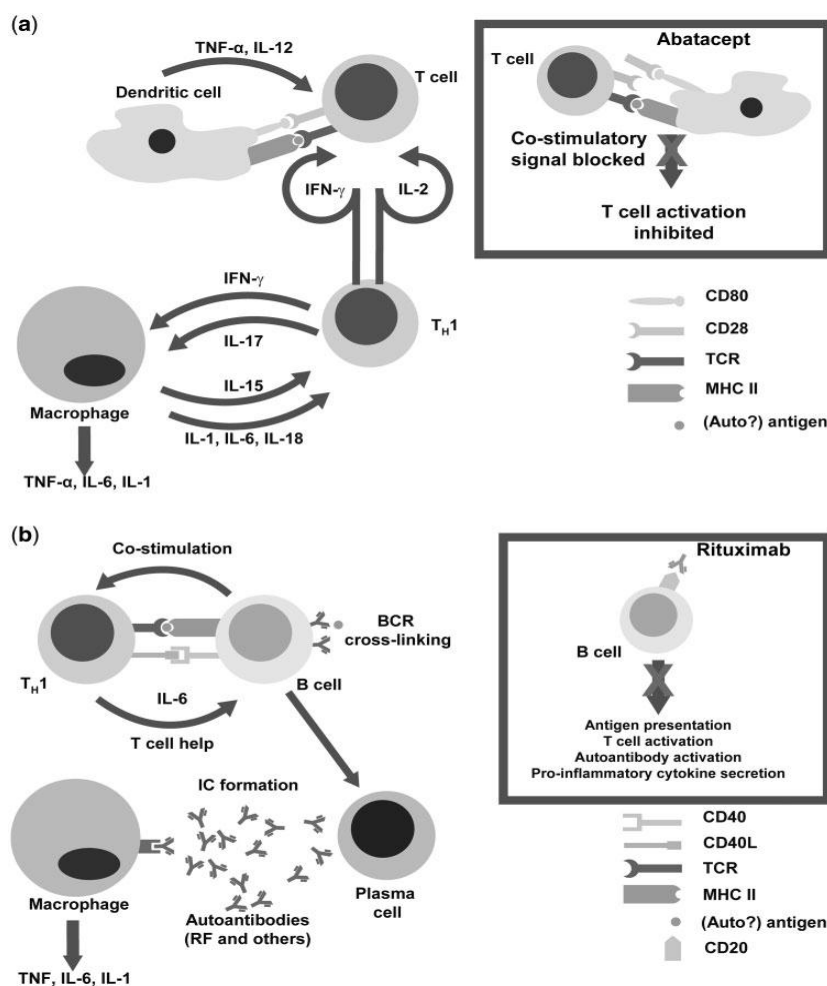


Fig no: 3 Consequences of the activation of effector cells by cytokines

Role of cytokines in RA joint effects inflammation

TNF- α , IL-6 and IL-1 are key mediators of cell migration and inflammation in RA.^[13] IL-6, in particular, acts directly on neutrophils through membrane-bound IL-6R, which in turn contributes to inflammation and joint destruction by secreting proteolytic enzymes and reactive oxygen intermediates.^[8] Furthermore, an *in vitro* study with fibroblasts from patients with RA demonstrates the role of IL-6 in promoting neutrophil recruitment by activated fibroblasts. Although untreated fibroblasts were able to recruit neutrophils, recruitment was

inhibited in the presence of anti-IL-6 antibody.^[2] The authors concluded that while IL-6 can directly recruit neutrophils, recruitment may also occur indirectly through fibroblasts.

Bone and cartilage destruction

Osteoclasts are multinucleated cells formed by the fusion of mononuclear progenitors of the monocyte/macrophage family. The primary mediators of bone destruction, these cells populate the synovial membranes of patients with RA and are polarized on bone.^[2] Macrophage-driven osteoclastogenesis requires the presence of macrophage colony-stimulating factor (MCSF) and results from the interaction of the RANK and the RANK ligand (RANKL). RANKL expression is regulated by pro-inflammatory cytokines such as TNF- α , IL-1, IL-6 and IL-17. MCSF, IL-6 and IL-11 can also support human osteoclast formation from peripheral blood mononuclear cells by a RANKL-independent mechanism. The principal cause of bone erosion is the pannus, which is found at the interface with cartilage and bone. Angiogenesis is a key process in the formation and maintenance of pannus because invasion of cartilage and bone requires increased blood supply. In patients with RA, many pro-angiogenic factors are expressed in the synovium, but VEGF, a potent cytokine, plays the central role in new blood vessel development.

Role of cytokines in systemic

The acute-phase response (APR) is the change in the concentration of certain plasma proteins, such as CRP, hepcidin, serum amyloid A, haptoglobin and fibrinogen, following protein synthesis alterations within hepatocytes. IL-6 has the greatest effect on acute-phase protein levels, although IL-1, TNF- α , TGF- β 1 and IFN- γ are also contributory. Elevated levels of CRP, a major acute-phase protein, can be detected within 4 h of injury, with peak values usually occurring within 24-72 h. Although an APR generally lasts for only a few days.

EFFECTS OF RA

Acute-Phase Protein Production

Some components may persist indefinitely. Increased levels of CRP may exacerbate disease-related tissue damage and contribute to the development of further complications, such as CVD. A prospective observational study that evaluated patients within 1 year of their RA diagnosis and then 3 years later found that an elevated baseline CRP level was a significant predictive factor for radiographic damage at the latter evaluation. The relationship between CRP elevation and CVD is discussed later in this review.

Anaemia

After CVD, the most common systemic manifestation of RA is anaemia, which occurs more frequently during the early stage of the disease. In patients with early RA, IL-6 levels are significantly higher in patients with anaemia than in persons without anaemia. Additionally, haemoglobin levels are inversely correlated with IL-6 levels. IL-6 is required for the induction of hepcidin during inflammation and rapidly induces hypoferric anaemia in humans. Hepcidin, a peptide produced by hepatocytes, is thought to be the principal iron-regulatory hormone and the key mediator of anaemia in patients with chronic disease. Plasma hepcidin inhibits iron release from macrophages in the spleen and iron uptake in the duodenum. In vivo data in wild-type mice have shown that after a turpentine-induced inflammatory response, liver hepcidin expression is increased and serum iron is decreased. Conversely, in IL-6 knockout mice, hepcidin levels are reduced, whereas iron levels are slightly increased in response to turpentine treatment. In humans, serum hepcidin levels have been shown to be highest in patients with RA and anaemia, whereas the lowest levels are reported in healthy adults.

CVD

The incidence of CVD events in patients with RA is more than three times that in the general population and this increase is not entirely explained by traditional risk factors. RA is associated with a spectrum of pro-atherogenic changes linked to systemic inflammation. Release of TNF- α , IL-6 and IL-1 from synovial tissue alters the function of distant tissues, including adipose tissue, skeletal muscle, liver and the vascular endothelium.^[3] These changes result in insulin resistance, dyslipidaemia, increased global oxidative activity and endothelial dysfunction. RA-related dyslipidaemia is characterized by low total and high-density lipoprotein (HDL) cholesterol, elevated triglyceride and lipoprotein (a) levels and an increase of small, dense low-density lipoprotein (LDL) species.^[3] Although the reduction in inflammation in patients with severe RA following treatment with a biologic agent may result in increased levels of total, HDL and LDL cholesterol (and perhaps triglycerides), inflammation reduction decreases CVD risk. Contrary to our understanding of the link between hyperlipidaemia and CVD, the increases in total cholesterol, LDL and triglyceride levels that may follow treatment for severe inflammation should be considered a consequence of inflammation reduction, not a CVD risk factor.

Osteoporosis

Osteoporosis is a common systemic manifestation of RA. The increased prevalence observed in this patient population consequently results in an elevated risk of bone fracture.^[6] In vivo data support a major role for IL-6 in RA-related osteoporosis.^[12] IL-6 transgenic mice, which have high circulating levels of IL-6, have osteopaenia, a condition involving accelerated bone resorption caused by increased osteoclastogenesis and reduced bone formation caused by decreased osteoblast activity.^[13] However, IL-6-deficient mice with oestrogen deficiency after ovary ectomy do not experience an increase in the number of osteoclast precursors or bone loss.

Fatigue and Depression

Persistent fatigue and high rates of depression are commonly reported in patients with RA. Corticotropin-releasing hormone, a key regulator of the hypothalamic_pituitary_adrenal (HPA) axis and the overall stress system, is associated with fatigue, dysthymia, irritability and depression. Case_control studies have demonstrated that the HPA axis is dysregulated to varying degrees in patients with RA. HPA axis dysregulation has been reported to be caused in part by the release of various cytokines, including TNF- α , IL-1 and IL-6. Thus the fatigue and depression frequently observed in persons with RA are primarily mediated by the up-regulation of cytokines known to be associated with its pathology.

2. REVIEW OF LITERATURE

Mohammed Salman Khaleel et al., (2014)^[1]

Physical characteristics of the fabricated tablet like hardness, weight variation, friability and drug content were found to be within the acceptable limits. The diclofenac sodium matrix tablet were prepared by wet granulation method, with a solvent such as Isopropyl alcohol, lactose was used as diluents, Magnesium Stearate was used as lubricant. In-vitro release of drug was performed in Simulated Gastric Fluid (SGF) pH-1.2 for two hours and Simulated Intestinal Fluid (SIF) pH-7.4 for subsequent 10 hours by USP-I dissolution apparatus, in 900 ml at $37.5 \pm 0.5^\circ\text{C}$ (stirring speed was 70 rpm). SCMC and Sodium alginate exhibited greater drug content than Metolose SR. A better sustained drug release (98.53) was obtained with the matrix tablet (F-12) made-up of the SCMC and Sodium Alginate than with Metolose SR and Sodium Alginate. It is cleared through the dissolution profile of Diclofenac sodium from matrix tablets prepared using different polymers were indicated an increase in the polymer ratio retarded the drug release to a greater extent.

Prasuna Sundari Pingali et al., (2014)^[2]

Objective of the present study was to formulate and evaluate capsules of Ashwagandha phytosomes. There are many herbal extracts having excellent in-vitro activity but less in-vivo activity because of their macromolecular size and poor lipid solubility, which result in poor absorption and bioavailability problems. Many of these problems can be overcome by formulating novel drug delivery systems. Phytosomes provide better absorption and bioavailability than the conventional herbal extracts. This project aims in improving the drug release characteristics of Ashwagandha by formulating Ashwagandha phytosome capsules. Ashwagandha Phytosomes were produced by a process in which standardized plant extract was bound to phospholipids, producing a lipid compatible molecular complex. Ashwagandha phytosome complexes were characterized by particle size, zeta potential, scanning electron microscopy (SEM), Fourier transform infrared spectroscopy and in vitro drug release. The results showed that the average particle size and zeta potential of optimized Ashwagandha phytosomes formulation were 98.4nm and -28.7 mV. In vitro drug release studies revealed that the cumulative % drug release of capsules of Ashwagandha phytosomes was found to be 76.8%.

Casilda M Balmaceda et al., (2014)^[3]

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a standard treatment for osteoarthritis (OA), but the use of oral NSAIDs has been linked to an elevated risk for cardiovascular and gastrointestinal adverse events and renal toxicity. Topical NSAIDs are thought to afford efficacy that is comparable to oral formulations while reducing widespread systemic drug exposure, which may provide a benefit in terms of safety and tolerability. As result, European treatment guidelines have, for many years, recommended the use of topical NSAIDs as a safe and effective treatment option for OA. Following the recent approval of several topical NSAID formulations by the US Food and Drug Administration, US treatment guidelines are increasingly recommending the use of topical NSAIDs as an alternative therapy and, in some cases, as a first-line option for OA. NSAIDs.

Apurva gupta et al., (2014)^[4]

Withania somnifera (Linn.) Dunal (Solanaceae) has long been used as an herb in Ayurvedic and indigenous medicine and has received intense attention in recent years for its chemopreventive properties. Objective: The present study focuses on the effect of *W. somnifera* root powder on the behavioural and radiological changes in collagen-induced

arthritic rats. Materials and methods: The rats were randomly divided into five groups: normal control, arthritic control, arthritic rats treated with *W. Somnifera* root powder and arthritic rats treated with methotrexate (at dose level 0.3 mg kg^{-1}). The treatment with *W. somnifera* (daily) and methotrexate (weekly) was initiated from the 20th day post collagen immunization and continued up until the 45th day. Arthritis was assessed macroscopically by measuring paw thickness, ankle size and body weight. Arthritic pain was assessed by toe-spread and total print length of the affected paw. Functional recovery due to the oral treatment of *W. somnifera* and methotrexate was assessed by sciatic functional index and rota rod activity. Results: Administration of *W. somnifera* root powder (600 mg kg^{-1}) to the arthritic rats significantly decreased the severity of arthritis by effectively suppressing the symptoms of arthritis and improving the functional recovery of motor activity and radiological score.

Muhammad Razi Ullah Khan et al., (2014)^[5]

Non-steroidal Anti-inflammatory drugs have their origin as the derivatives of plants, which were observed to have their therapeutic effects in different disease states. They have the advantage of local action without developing central adverse effects and cognitive impairments. Side effects have been well described, although partly neglected. Topical delivery of NSAID has its therapeutic applications in management of pain and inflammation in Rheumatoid Arthritis patients. Rheumatoid Arthritis is a chronic systemic inflammatory disorder that may affect many tissues and organs but principally attacks the synovial joints. It can be disabling and painful condition, which can lead to substantial loss of functioning and mobility if not adequately treated.

Muhammad Razi Ullah Khan et al., (2014)^[6]

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drug groups. These drugs are used dermal or systemically in treatment of various rheumatic diseases, including Rheumatoid arthritis (RA), as well as for Osteoarthritis, low back pain and some joint diseases. Diclofenac sodium is a well-tolerated NSAID because of its limited numbers of adverse effects and topical formulation has excellent permeation and absorption into the skin and is frequently prescribed for the long term treatment of Rheumatoid arthritis, Osteoarthritis and ankylosing spondylitis. The present investigation was to develop novel cream formulation containing Diclofenac sodium in combination with most effective and

potent natural anti-inflammatory agent curcuma longa, which is reported to possess strong anti-inflammatory effects in Rheumatoid arthritis and Osteoarthritis.

Yallappamaharaj R. Hundekar et al.,(2014)^[7]

The purpose of this research was to prepare and evaluate a cubosomes of diclofenac sodium, a non-steroidal anti-inflammatory drug (NSAID) by using different ratios of mono glyceride and polymer by top-down technique accompanied by homogenization. Nine formulations of diclofenac sodium cubosomes were prepared by using GMO as lipid emulsifier and Poloxamer 407 (P-407) as polymer in varying concentration and P-407 was also used as permeation enhancer and stabilizer. A number of NSAIDs have been developed for the treatment of generalized muscle and joint pain. Unfortunately these medicaments have major side effects caused by systemic administration.

Sigimol Joseph et al.,(2014)^[8]

The aim of present work is to develop Diclofenac sodium loaded biocompatible microspheres to reduce the dosing frequency, Gastro intestinal side effects and hence to improve patient compliance. The microspheres were prepared by emulsion-thermal cross-linking process at two different drugs: polymer ratios Diclofenac sodium (DS), is a potent drug in the NSAID group having non-steroidal, anti-inflammatory properties and is widely used in the treatment of rheumatoid arthritis. Diclofenac sodium with its low oral bioavailability and short plasma half-life is an ideal candidate for formulation as a sustained release drug delivery system. The prepared microspheres were evaluated for particle size, drug entrapment efficiency (EE) and in vitro drug release study.

Enkelejda goci et al., (2014)^[9]

Objective: The present research has been undertaken with the aim to develop a topical gel of diclofenac sodium (DS) 1%, evaluation of its physico-chemical characteristics and in vitro drug release through pig skin using vertical diffusion cell. Methods: In the presented work was prepared a hydrophilic diclofenac sodium gel of hydroxyethylcellulose (HEC). Skin permeability of the preparation was evaluated in vitro using abdominal hairless pig skin, into water medium at 37°C and determined using spectrophotometer UV at 276nm.

Sonam Vats et al.,(2014)^[10]

These modern days there is an upsurge in topical formulations such that it can be prepared by varying physico-chemical properties and providing better localized action. The patient

adherence to topical formulations is significant in relation to chronic skin diseases, like fungal infections, acne, psoriasis. Emulgel is one of the recent technology in NDDS used topically having characteristics of dual control release i.e. emulsion as well as gel. Despite of many advantages of gels there is a major limitation of delivering the hydrophobic drug. Henceforth emulgel has been emerged as an auspicious topical drug delivery system for hydrophobic drugs and proves a boon for dermal care and cosmetology.

Anil K. Gupta et al., (2014)^[11]

Chrono-pharmaceutics includes pharmaceutical application of “Chronobiology” in drug delivery. Chronobiology is the study of biological rhythms and their responses to other metabolic functions of body. Diseases such as bronchial asthma, hyper-cholesterolemia, ulcer, diabetes, arthritis, myocardial infarction, angina and hypertension show symptomatic changes due to circadian rhythmicity. The chronobiology, chronopharmacology and chronotherapeutics of pain have been extensively reviewed.

Soni Manish et al., (2014)^[12]

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. The present work deals with the development and evaluation of the poly herbal cream containing hydro-alcoholic extract of neem leaves (*Azadirachta indica*), turmeric (*Curcuma longa*) and aloe (*Aloe vera*). Although various topical herbal formulations for acne are available in the market, we propose to make use of hydro-alcoholic extract of neem (*Azadirachta indica*) leaves, turmeric (*Curcuma longa*) and aloe (*Aloe vera*). The plants have been reported in the literature having good anti- microbial, anti-oxidant and anti-inflammatory activity. The present study was to prepare and evaluate the polyherbal cosmetic cream comprising extracts of natural products such as aloe, turmeric and neem. Different types of formulations oil in water (O/W) herbal creams namely F1 to F4 were formulated by incorporating different concentrations of stearic acid and cetyl alcohol.

Tahsildar Apeksha Ganesh et al.,(2013)^[13]

The rationale of this review on pharmaceutical gel is topical drug administration through hydrogel drug delivery system anywhere in the body by the route of ophthalmic, rectal, vaginal and skin. Hydrogels constitute a group of material which are used in numerous biomedical disciplines and are still developing for new promising applications. Hydrogels are three

dimensional cross-linked polymernetworks that can respond to the fluctuations of the environmental stimuli. These biomaterials can incorporate large quantum of biological fluids and swell. When swelled, they are soft & rubbery and resemble the living tissue, exhibiting excellent biocompatibility.

L. P. Hingmire et al., (2013)^[14]

In the present investigation, an attempt has been made to increase therapeutic efficacy, reduce frequency of administration, and improve patient compliance, by developing sustained release matrix tablets of diclofenac sodium using natural polymers such as Xanthan gum, locust bean gum, sodium alginate. Sustained release matrix tablets of diclofenac sodium, were developed by using different drug: polymer ratios, such as 1:0.5; 1:1 and 1:1.5. Xanthan gum, locust bean gum, sodium alginate, combination of locust and xanthan, locust and sodium alginate were used as matrix former, the tablets were compressed by direct compression method using 8 mm flat faced punches. Compressed tablets were evaluated for uniformity of weight, content of active ingredient, friability, hardness, thickness, swelling index & in vitro dissolution using paddle method, and swelling index.

MD. Tabasum et al., (2013)^[15]

The aim of this work was preparation and evaluation of diclofenac sodium controlled release matrix tablets using various proportions of natural polymer *Abelmoschus esculentus* mucilage powder (i.e., Drug:Polymer ratio-1:0.25, 1:0.5, 1:1, 1:1.5, 1:2) as release controlling factor by Wet Granulation method. The tablets were evaluated for various parameters like friability, weight variation, hardness, drug time, content uniformity. In vitro drug release characteristics of dosage form was evaluated in 6.8 pH phosphate buffer. All the formulations followed zero order kinetics along with diffusion mechanisms. From In vitro release data, formulation F4 containing Drug:Polymer (1:1.5) showed maximum drug release.

Preeti Sagar Nayak et al., (2013)^[16]

An experiment was carried out to adjudge the effect of different drying techniques of post-harvest on colour and saponin content of Safed Musli (*Chlorophytum borivillianum*), a medicinal plant belonging to the family Liliaceae, has been traditionally used as adaptogenic drug. The present study was done on drying of fresh Safed Musli root in different condition like: shade, sun, solar and cabinet dryer. The moisture of about 62% is removed in 45 h when dried in shade and the temperature varies from 15 to 18°C, whereas in sun dried, the time

taken was about 16.45 h and the temperature range is 25 to 30°C. Further less time that is, 5 h is required to dry the sample in solar cabinet dryer for 54 to 60% moisture loss and the temperature inside the cabinet was observed to be 39°C.

Prakash Pawan et al., (2013)^[17]

In the present investigation an attempt has been made to study the formulation and evaluation of matrix tablets of diclofenac sodium using natural mucilage of *Hibiscus rosa-sinensis* and *Abelmoschus esculentus* as a release retardant. The mucilage was extracted from the leaves of *Hibiscus rosa-sinensis* and *Abelmoschus esculentus* using acetone. The mucilage was characterized for physiochemical and powder characteristics. The matrix tablets were formulated using different drug polymer ratio (1:0.25, 1:0.5, 1:0.75, 1:1) of *Hibiscus* mucilage and *Abelmoschus esculentus* mucilage. The developed formulations of tablets were evaluated for pre-compression and post-compression parameters.

K.Suria prabha et al., (2013)^[18]

Diclofenac Sodium, a non-steroidal anti-inflammatory agent is frequently prescribed for the long term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The drug undergoes substantial first pass effect and only 50% of drug is available systemically. Further, the drug is known to induce ulceration and bleeding of the intestinal wall. To avoid the adverse effect, alternate routes of administration have been tried by investigators. Diclofenac Sodium, a non-steroidal anti-inflammatory agent is frequently prescribed for the long term treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The drug undergoes substantial first pass effect and only 50% of drug is available systemically sodium within 50 minutes which was also found to be economic when compared to other marketed products.

Tikshdeep Chauhan et al., (2013)^[19]

Wide choice of vehicles ranging from solids to semisolids form has been used for skin care and topical treatment of dermatological disease. The oral use of Diclofenac sodium is not much recommended as it has many side effects, thus this gel formulation is made for better patient compliance and to reduce the dose of drug and to avoid the side effects like liver damage and kidney damage. The different pre-formulation studies i.e. Ultraviolet, Infrared, and Organoleptic study confirms its purity.

Dheeraj T Baviskar¹ et al., (2013)^[20]

Purpose: To develop diclofenac sodium gel using high molecular weight hydroxyl propyl methylcellulose (HPMC) and Carbopol 934P for topical and systemic delivery. **Methods:** Diclofenac sodium gel was prepared with HPMC K100M and Carbopol 934P as gelling agents. The formulations were examined for pH, spreadability, consistency, viscosity, homogeneity, drug content and stability. In vitro drug release was evaluated using Franz diffusion cell. Carrageenan induced rat paw oedema model was used for the evaluation of the anti-inflammatory activity of the gels. A commercial diclofenac sodium gel product was used as the reference drug. **Results:** Formulations containing glycerin as permeation enhancer gave drug release patterns comparable to that of the reference product. The drug content of F2, F5 and F9 was 99.81, 99.75 and 99.96 %, respectively.

Sujith S Naire^t al., (2012)^[21]

In this study creams were formulated based on the anti-oxidant potential of herbal extracts and its evaluation. Selected plant parts are dried and extracted using 70% alcohol by maceration. The extract was tested for antioxidant activity by superoxide scavenging activity. Quality evaluation of the product was assessed by using different evaluation methods. No change of the physical properties was observed; the pH was in a proper range (approximately pH6). The marker Curcumin was present in the extract, formulation and the peak was comparable with standard Curcumin obtained by HPLC. The formulations showed good spreadability, no evidence of phase separation and good consistency during this study period.

Pallab Dasgupta and Amartya De et al., (2012)^[22]

In the few decades, there has been exponential growth in the field of herbal medicines. Most of the traditional system of medicine is effective but they lack standardization. So there is a need to develop a standardization technique. Standardization of herbal formulation is essential in order to assess the quality, purity, safety and efficacy of the drug. Ashwagandha is a reputed drug mentioned in the scientific books of Ayurveda for the treatment of stress, hypertension, sleeping disorders and also as rejuvenative. The present research study deals with the comparative standardization of two marketed ashwagandha churna formulation from Dabur and Dhaka Oushodhalay.

Arun Rasheed et al., (2012)^[23]

Herbal medicines are not a simple task since many factors influence the biological efficacy and reproducible therapeutic effect. Standardized herbal products of consistent quality and containing well-defined constituents are required for reliable clinical trials and to provide consistent beneficial therapeutic effects. Pharmacological properties of an herbal formulation depend on phytochemical constituents present therein. Development of authentic analytical methods which can reliably profile the phytochemical composition, including quantitative analyses of market/bioactive compounds and other major constituents, is a major challenge to scientists and discrimination if herbs from their adulterants are reported. Nanotechnology based herbal drugs possess improved solubility and enhanced bioavailability.

Agatha Betsy et al., (2012)^[24]

Ashwagandha (*Withania somnifera*) is widely used in Ayurvedic medicine, and it is one of the ingredients in many formulations to increase energy, improve overall health and longevity, and prevent disease. The main objective of the study was to analyze the efficacy of Ashwagandha root powder with water and with milk in treatment of hypertension. The experiment was conducted on 51 stress-oriented hypertensive subjects in the age group of 40 to 70 years, selected by purposive sampling. Subjects were divided into group I and group II. Supplementation of 2gm of Ashwagandha root powder was given to group I and group II with milk and water respectively in morning. Blood pressure was also recorded over a period of three months. Overall decrease in systolic blood pressure was found though it was non-significant.

Manisha Singh et al., (2012)^[25]

The present research has been undertaken with the aim to formulate and evaluate the herbal gel containing *Ipomoea fistulosa* stem extract. The gel formulation was designed using ethanol extract of *Ipomoea fistulosa* stem and evaluated using physiological measurements. The gel was formulated using accurately weighted amount of drug extract along with other additives, poured into the fixed amount of hydrated Carbopol-934 dispersion with constant stirring. The herbal gel formulations prepared were subjected to preliminary evaluation such as pH, Spreadability, Extrudability, Drug content uniformity, Viscosity and In vitro diffusion study. The pH of all the formulations was checked and found to be compatible with the normal pH range of the skin and so chances of skin irritation are least.

Rane Rajashree et al., (2012)^[26]

Three herbal formulations namely, Amla, Ashwagandha and Shatavari Capsules, were examined for general test parameters, at different production stages, and a few basic nutritive test parameters. The general test parameters and assays were found to be satisfactory and the nutritive values were found to be quite significant. This proves that the three formulations can be used as herbal medicines, and the significant nutritive values prove that they can be used as dietary supplements.

Richa Kushwaha et al., (2011)^[27]

Ayurveda is a Sanskrit term for “knowledge of longevity.” It is the earliest health care system of India beginning over 5,000 years ago. More than 1,200 species of plants, nearly 100 minerals and over 100 animal products comprise the Ayurvedic Pharmacopoeia. Asava and Arishta are unique dosage forms discovered by Ayurveda having indefinite shelf life and it was said that the “older the better it is.” Arishtas are self-generated herbal fermentations of traditional Ayurvedic system.

Goyal.S et al., (2011)^[28]

Herbal medicine has become an item of global importance both medicinal and economical. Although usage of these herbal medicines has increased, their quality, safety and efficiency are serious concerns in industrialized and developing countries. Plants play a vital role in curing various ailments of the man and herbal remedies are getting increasing patient compliance as they are devoid of typical side effects of allopathic medicines. The allopathic system of medicine includes two conventional lines of treatment for rheumatoid arthritis, which come along with certain side effects. Hence, turning to safe, effective and time-tested ayurvedic herbal drug formulation would be a preferable option. So there is a need to investigate such drugs and their effective formulation for the better patient acceptance.

Sahu Alakh N et al., (2011)^[29]

Formulation of herbal cosmeceutical in the form of a face cream has been done. Curcuminoids from *Curcuma domestica* Val. (turmeric) have been incorporated in the formulation. Pharmacognostical standardization of turmeric has been done as per Indian Herbal Pharmacopoeia [IHP]-2002 to ensure the genuinity of the crude turmeric rhizomes. It includes taxonomical authentication, morphological characterization, powdered drug microscopy, identification tests of turmeric powder and quantitative standards - that are

foreign organic matter (0.43%), alcoholic soluble extractive (7.36%), watersoluble extractive (20.32%), total ash (8.46%), acid insoluble ash (0.76%) and loss on drying (12.52%). All the quantitative standard values are in compliance with IHP-2002. Turmeric rhizome powder has been extracted with methanol and curcumin content in the methanolic extract has been quantified spectrophotometrically.

G. Singh et al., (2010)^[30]

Withania somnifera (Ashwagandha) is a plant used in medicine from the time of Ayurveda, the ancient system of Indian medicine. The dried roots of the plant are used in the treatment of nervous and sexual disorders. From chemistry point of view, the drug contains group of biologically active constituents known as withanolides. The chemical structures of withanolides have been studied and they are widely distributed in family Solanaceae. Withaferin-A is a therapeutically active withanolide reported to be present in leaves. In animal studies, withaferin-A has shown significant anticancer activity.

Soni Hardik K et al., (2010)^[31]

The most important challenges faced by herbal formulations arise because of their lack of complete evaluation. Evaluation is necessary to ensure quality and purity of the herbal product. For evaluation of capsule containing single herb various parameters were tested. These parameters for raw material include powder characteristic study, organoleptic, physicochemical, phytochemical parameters etc., and assay of active constituent. Parameters for finished product (capsule) include uniformity of weight, pH, moisture content, disintegration time and dissolution study. HPTLC study, heavy metal analysis, microbial analysis and Nutritional value were carried out as a part of evaluation.

Mahaboob Khan Rasool et al., (2009)^[32]

Withania somnifera, popularly known as Ashwagandha is widely considered to be an integral part of Ayurvedic and Indigenous medical systems for over centuries for the treatment of various ailments. Withanolides (steroid lactone), are the major active constituents present in the roots and leaves of *Withania somnifera*. In the present study, withaferin A (active component of *Withania somnifera*), a steroid lactone was examined for its analgesic, antipyretic and ulcerogenic properties employing different experimental models in mice. For comparison purpose, non-steroidal anti-inflammatory drug indomethacin was used as standard.

U. D. shivhare et al., (2009)^[33]

High molecular weights water soluble homopolymer of acrylamide are reported to possess very high viscosity in low concentration, transparency, film forming properties and are useful in formation of gel. The diclofenac sodium gels were prepared by using different concentration of polyacrylamide for topical drug delivery with an objective to increase transparency and spreadability. These preparations were further compared with marketed diclofenac sodium gel. Spreadability and consistency of polyacrylamide gel containing diclofenac sodium (F9) were 6.5g.cm/sec and 5mm as compared to 5.5g.cm/sec and 10mm respectively of marketed gel, indicating good spreadability and consistency of the prepared gel (F9). The transparency of prepared batch F9 was good as compared to the marketed gel.

Sujith S Nair¹ et al., (2009)^[34]

In this study, creams were formulated based on the antioxidant potential of herbal extracts and evaluated. Different types of herbal creams were formulated from the ethanolic extracts of Glycyrrhiza glabra (root and stolons), Phyllanthus emblica (fruit), Lycopersicon esculentum (fruit), Curcuma longa (Rhizomes), Aloe vera (leaf) and Citrus aurantium (outer peel) namely F1, F2, F3, F4. All the formulations showed good spreadability, good consistency and no evidence of phase separation.

Naresh Ahuja et al., (2008)^[35]

A major problem being faced in ocular therapeutic is the attainment of an optimal concentration at the site of action. The bioavailability of diclofenac sodium in the form of eye drop is very low and when the drug is administered in the form of ophthalmic suspension it lead to irritation due to particle size. So in the present study diclofenac sodium gels were developed with the aim of promoting the prolong release of drug using natural polymer. Diclofenac sodium gels were sterilized and assessed for various parameters like clarity, viscosity, pH, extrudability and sterility. In *–vitro* drug release determined using dialysis membrane in phosphate buffer pH 7.4. Ocular irritation studies were performed on albino rabbits.

Steven Stanos et al., (2007)^[36]

The recent recognition of the magnitude of cardiovascular risk of both nonselective non-steroidal anti-inflammatory drugs and COX-2 selective inhibitors, in addition to the persistent concerns about the use of opioids, has brought increased attention to non-systemic, topical

analgesics. These agents have a favourable safety profile and there is increasing evidence indicating their efficacy for a variety of pain disorders.

KS Anand et al., (2002)^[37]

Use of non-steroidal anti-inflammatory drugs for the treatment of painful joint conditions like osteoarthritis, rheumatoid arthritis, arthritis of systemic lupus erythematosus, psoriasis, and other seronegative spondyloarthropathies is ubiquitous. They are the most commonly employed first line drugs for all these conditions and many others – like musculoskeletal trauma, minor aches and pains, and dysmenorrhoea. Several newer applications like prophylaxis of stroke with aspirin is now common place. Use of these drugs for the prophylaxis of conditions like Alzheimer's disease and colorectal cancer is being evaluated.

P.Sivannarayana et al., (2002)^[38]

The aim of this work is to design and evaluate diclofenac sustained release matrix tablets using *Abelmoschus esculentus* mucilage (okra gum). Diclofenac is a non-steroidal anti-inflammatory drug used in the long-term treatment of Rheumatoid arthritis. The biological half life of diclofenac is about 1-2 hr; therefore it requires multiple dosing to maintain therapeutic drug blood level. The most frequent side effects of diclofenac on long term administration are gastrointestinal disturbances, peptic ulceration. Hence an attempt was made to formulate a sustained release formulation with increased patient compliance and decreased signs of adverse effects.

R A Moore et al., (1998)^[39]

Objective: To review the effectiveness and safety of topical non-steroidal anti-inflammatory drugs in acute and chronic pain conditions. Design: Quantitative systematic review of randomised controlled trials. Data sources: 86 trials involving 10 160 patients. Main outcome measures: Measures of treatment success approximating at least 50% reduction in pain, local and systemic adverse effects. Analysis at 1 week for acute and 2 weeks for chronic conditions with relative benefit and number needed to treat.

Ernest choyra et al., (1998)^[40]

It is a progressive inflammatory autoimmune disease with articular and systemic effects. Its exact cause is unknown, but genetic and environmental factors are contributory. T cells, B cells and the orchestrated interaction of pro-inflammatory cytokines play key roles in the pathophysiology of RA. Differentiation of native T cells into Th 17 (TH17) cells results in

the production of IL-17, a potent cytokine that promotes synovitis. B cells further the pathogenic process through antigen presentation and autoantibody and cytokine production. Joint damage begins at the synovial membrane, where the influx and/or local activation of mononuclear cells and the formation of new blood vessels cause synovitis. Pannus, the osteoclast-rich portion of the synovial membrane, destroys bone, whereas enzymes secreted by synoviocytes and chondrocytes degrade cartilage. Antigen-activated CD4⁺ T cells amplify the immune response by stimulating other mononuclear cells, synovial fibroblasts, chondrocytes and osteoclasts. The release of cytokines, especially TNF- α , IL-6 and IL-1, causes synovial inflammation. In addition to their articular effects, pro-inflammatory cytokines promote the development of systemic effects, including production of acute-phase proteins (such as CRP), anaemia of chronic disease, cardiovascular disease and osteoporosis and affect the hypothalamic_pituitary_adrenal axis, resulting in fatigue and depression.

3. OBJECTIVE AND PLAN OF WORK

3.1 OBJECTIVES

The main objective of formulate & evaluate Anti Inflammatory cream is no direct contact of active drug with stomach wall. This can be a reason to remove the chances of gastric mucosal damage to a reasonable level that is caused by the use of solid dosage forms of NSAIDs. The cream formulation contains Diclofenac Sodium along with extract of withania somnifera is performs a s anti-inflammatory effect by avoiding gastric irritation & also first pass metabolism. The most convenient route of drug delivery system is topical dermal drug delivery system.

3.2 PLAN OF WORK

1. To prepare Anti inflammatory cream consisting different ratio of diclofenac sodium withwithania somnifera extract.
 2. To study the physicochemical properties of diclofenac sodium, withania somnifera and other ingredients used in this Anti inflammatory cream.
 3. To develop different formulations of diclofenac sodium with withania somnifera by using fusion method.
 4. Evaluation of diclofenac sodium with withania somnifera cream.
1. p^H.
 2. Viscosity.

3. Homogeneity.
4. Permeability release.
5. Consistency.
6. Spreadability.
7. Skin irritation.

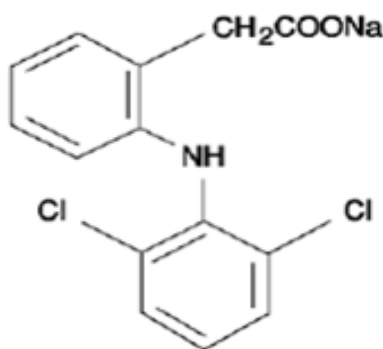
4. DRUG PROFILE

4.1 DICLOFENAC SODIUM^[53]

chemical name: 2-(2, 6-dichloranilino) phenyl acetic acid

synonym: Diclofenac acid

structural formula^[70]



Molecular weight: 296.14864

Molecular formula: C₁₄H₁₀Cl₂NNaO₂

Description: Faintly yellowish white slightly hygroscopic crystalline powder.

Odour: Virtually odorless.

Solubility: Freely soluble in methanol, soluble in ethanol, sparingly soluble in water and practically insoluble in chloroform and in dilute acid.

Storage: Store at room temperature and keep away from light and moisture.

Melting point: 284°C

PKa: 4.15

Category: Analgesic, antipyretic & anti inflammatory effects

PHARMACOLOGY^[73]**Mechanism of action**

The exact mechanism of action is not entirely known, but the primary mechanism responsible for its anti-inflammatory, antipyretic, and analgesic action is thought to be inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also appears to exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis. Diclofenac may also be a unique member of the NSAIDs. Some evidence indicates it inhibits the lipoxygenase pathways, thus reducing formation of the leukotrienes (also pro-inflammatory autacoids). It also may inhibit phospholipase A2 as part of its mechanism of action. These additional actions may explain its high potency - it is the most potent NSAID on a broad basis.

Pharmacokinetics.^[73]**Absorption**

Diclofenac is 100% absorbed after oral administration compared to IV administration as measured by urine recovery. However, due to first-pass metabolism, only about 50% of the absorbed dose is systemically available. Food has no significant effect on the extent of diclofenac absorption. However, there is usually a delay in the onset of absorption of 1 to 4.5 hours and a reduction in peak plasma levels of < 20%.

Distribution

The apparent volume of distribution (V/F) of diclofenac sodium is 1.4 L/kg. Diclofenac is more than 99% bound to human serum proteins, primarily to albumin. Serum protein binding is constant over the concentration range (0.15-105 µg/mL) achieved with recommended doses. Diclofenac sodium diffuses into and out of the synovial fluid. Diffusion into the joint occurs when plasma levels are higher than those in the synovial fluid, after which the process reverses and synovial fluid levels are higher than plasma levels. It is not known whether diffusion into the joint plays a role in the effectiveness of diclofenac sodium.

Metabolism.^[73]

Five diclofenac sodium metabolites have been identified in human plasma and urine. The metabolites include 4'-hydroxy-, 5-hydroxy-, 3'-hydroxy-, 4',5-dihydroxy- and 3'-hydroxy-4'-methoxy-diclofenac. The major diclofenac sodium metabolite, 4'-hydroxy-diclofenac sodium, has very weak pharmacologic activity. The formation of 4'-hydroxy- diclofenac is primarily mediated by CYP2C9. Both diclofenac sodium and its oxidative metabolites undergo

glucuronidation or sulfation followed by biliary excretion. Acyloglucuronidation mediated by UGT2B7 and oxidation mediated by CYP2C8 may also play a role in diclofenac metabolism. CYP3A4 is responsible for the formation of minor metabolites, 5-hydroxy- and 3'-hydroxy-diclofenac. In patients with renal dysfunction, peak concentrations of metabolites 4'-hydroxy- and 5-hydroxy-diclofenac were approximately 50% and 4% of the parent compound after single oral dosing compared to 27% and 1% in normal healthy subjects.

Excretion

Diclofenac sodium is eliminated through metabolism and subsequent urinary and biliary excretion of the glucuronide and the sulfate conjugates of the metabolites. Little or no free unchanged diclofenac sodium is excreted in the urine. Approximately 65% of the dose is excreted in the urine and approximately 35% in the bile as conjugates of unchanged diclofenac plus metabolites. Because renal elimination is not a significant pathway of elimination for unchanged diclofenac sodium, dosing adjustment in patients with mild to moderate renal dysfunction is not necessary. The terminal half-life of unchanged diclofenac is approximately 2 hours.

Drug interactions

- Warfarin: The antiplatelet effects of oral diclofenac sodium may increase the bleed risk associated with warfarin. Consider alternate therapy or monitor for signs and symptoms of bleeding during concomitant therapy.
- Rifampicin: increases the metabolism of diclofenac sodium
- Alendronate: Increased risk of gastric toxicity.
- Cyclosporine: monitor for nephrotoxicity.

Contraindications

Hypersensitivity against diclofenac sodium

- History of allergic reactions (bronchospasm, shock, rhinitis, urticaria) following the use of aspirin or another NSAID
- Third-trimester pregnancy

Uses

Diclofenac sodium is used to relieve pain, swelling (inflammation), and joint stiffness caused by arthritis. This medication may also be used to treat other painful conditions (such as dental pain, muscle aches, pain after surgery or after having a baby).

Adverse effects

Upset stomach, nausea, heart burn, diarrhea, constipation, gas, head ache, Drowsiness, and dizziness may occur. swelling of the hands or feet (edema), sudden unexplained weight gain, hearing changes (such as ringing in the ears), mental/mood changes, difficult/painful swallowing, unusual tiredness.

Precautions

Diclofenac sodium, have been reported to cause cardiovascular events, such as heart attack or stroke, both of which can result in loss of life. People with cardiovascular disease or who have risk factors appear to be at greater risk.

4.2 WITHANIA SOMNIFERA

Scientific name: *Withania somnifera* Dunal^[52]

Family: Solanaceae

Common names: Asandh, Asagandh, Ashwagandha.

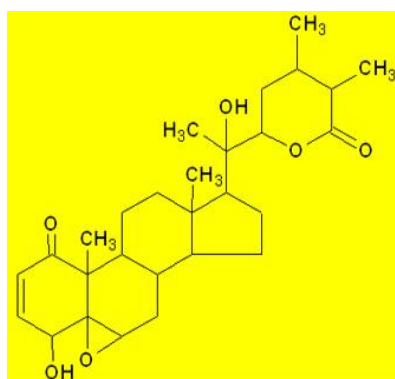
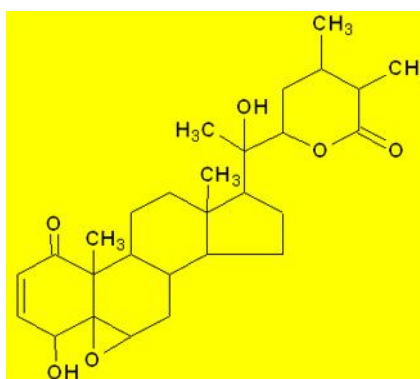


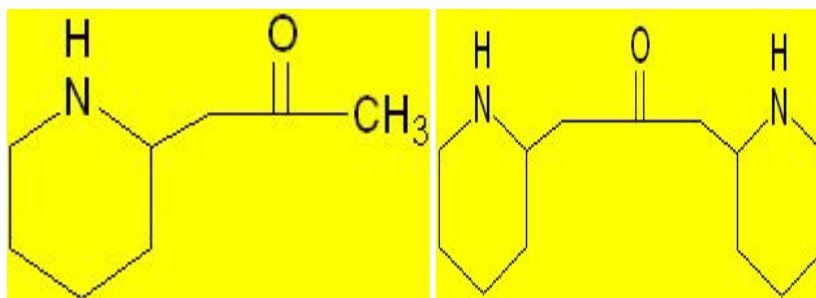
WITHANIA SOMNIFERA POWDER

Chemical Composition^[52]

Laboratory analysis has revealed over 35 chemical constituents contained in the roots of *Withania somnifera*. The biologically active chemical constituents are alkaloids (isopellertierine, anferine), steroidal lactones (withanolides, withaferins), saponins containing

an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (sitonidoside XI and X). *Withania somnifera* is also rich in iron. The roots of *Withania somnifera* consist primarily of compounds known as withanolides, which are believed to account for its extraordinary medicinal properties. Withanolides are steroidal and bear a resemblance, both in their action and appearance, to the active constituents of Asian ginseng (*Panax ginseng*) known as ginsenosides. Ashwagandha's withanolides have been researched in a variety of animal studies examining their effect on numerous conditions, including immune function and even cancer. Chemical analysis of Ashwagandha show its main constituents to be alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropene, choline, cuscohygrine, isopelletierine, anaferine and anahydrine. Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII have been isolated from root. The leaves contain steroidal lactones, which are commonly called withanolides. The withanolides have C28 steroidal nucleus with C9 side chain, with a six membered lactone ring. Twelve alkaloids, 35 withanolides, and several sitoindosides from *Withania somnifera* have been isolated and studied. A sitoindoside is a withanolide containing a glucose molecule at carbon 27. Much of Ashwagandha's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D. Further chemical analysis has shown the presence of the following Anaferine (Alkaloid), Anahygrine (Alkaloid), Beta-Sisterol, Chlorogenic acid (in leaf only), Cysteine (in fruit), Cuscohygrine (Alkaloid), Iron, Pseudotropine (Alkaloid), Scopoletin, Somniferinine (Alkaloid), Somniferene (Alkaloid), Tropanol (Alkaloid), Withanine (Alkaloid), Withananine (Alkaloid) and Withanolides A-Y (Steroidal lactones).

**Withanolide D****Withaferin**

**AnaferinDI-isopelletierine****Table no: 1 Known chemical Constituents**

Alkaloids	Withanine, Withanine, Somniferine, Tropeltigloate, Somniferine, Somnine, Nicotine, Visamine, Withasomine
Salts	Cuscohygrine, Anahygrine, Tropine, Pseudotropine, Anaferine
Steroidal Lactones	Withaferin-A, Withanone, WS-1, Withanolide E C ₂₈ H ₃₈ O ₇ , Withanolide F C ₂₈ H ₃₈ O ₆ , Withanolide G C ₂₈ H ₃₆ O ₄ , Withanolide H C ₂₈ H ₃₆ O ₅ , Withanolide I C ₂₈ H ₃₆ O ₅ , Withanolide J C ₂₈ H ₃₆ O ₅ , Withanolide K C ₂₈ H ₃₆ O ₅ , Withanolide L C ₂₈ H ₃₆ O ₅ , Withanolide M C ₂₈ H ₃₆ O ₆
Nitrogen containing compounds	Withanol C ₂₅ H ₃₄ O ₅ , Somnisol C ₃₂ H ₄₆ O, Somnitol C ₃₃ H ₄₆ O ₇
Steroids	Cholesterol, -sitosterol, Stigmasterol, Diosgenin, Stigmastadien, Sitoinsides VII, Sitoinsides VIII, Sitoinsides IX, Sitoinsides X Flavonoids: Kaempferol, Quercetin.

Medicinal properties and uses

Leaves and roots of this plant are abortifacient, aphrodisiac, diuretic, nervine tonic, alterative, narcotic, sedative, astringent, growth promoter and anthelmintic. It has anti-arthritis, anti-bacterial, anti-dote for scorpion sting, anti-stress, anti-tumour and anti-cancer activities. It is used in toning of uterus, consumption, dropsy, leucoderma, impotence, rheumatism, debility from old age, ulcer, sexual and genital weakness, assumption, rheumatic swelling, loss of memory, loss of muscular energy, spermatorrhoea, syphilis, sterility of women, blood discharge, leucorrhoea, anemia with emaciation, nervous exhaustion, multiple sclerosis, neoplasia, cancer and fatigue. Fruits and seeds are diuretic and used in coagulation of milk.

Pharmacological Activity

Centuries of Ayurvedic medical experience using *Withania somnifera* have revealed it to have pharmacological value as an adaptogen, antibiotic, abortifacient, aphrodisiac, astringent,

antiinflammatory, deobstruent, diuretic, narcotic, sedative, and tonic. Ashwagandha has been found to: Provide potent antioxidant protection. Stimulate the activation of immune system cells, such as lymphocytes and phagocytes. Counteract the effects of stress and generally promote wellness.

Anti-inflammatory activity

Research has explored the capacity of Ashwagandha to ease the symptoms of arthritis and other inflammatory conditions. These studies have proven that the herb acts as an effective anti-inflammatory agent. Its naturally occurring steroidal content is much higher than that of hydrocortisone, a commonly-prescribed anti-inflammatory. The effectiveness of Ashwagandha in a variety of rheumatologic conditions may be due in part to its anti-inflammatory properties. Rats given powdered root of *Withania somnifera* orally one hour before being given injections of an inflammatory agent over a three day period showed that Ashwagandha produced anti-inflammatory responses comparable to that of hydrocortisone sodium succinate.

Anti-stress

A study conducted by the Institute of Basic Medical Sciences at Calcutta University examined the effects of Ashwagandha on chronic stress in rodents. For a period of 21 days, the animals received a mild electric shock to their feet. The resulting stress on the animals produced hyperglycemia, glucose intolerance, increase in plasma corticosterone levels, gastric ulcerations, male sexual dysfunction, cognitive deficits, immunosuppression and mental depression. Researchers using *Withania somnifera* discovered the animals given the herb an hour before the foot shock experienced a significantly reduced level of stress.

Anti-oxidant activity

Researchers from Banaras Hindu University in Varanasi, India, have discovered that some of the chemicals found in *Withania somnifera* are powerful antioxidants. Studies conducted on rats brains showed the herb produced an increase in the levels of three natural antioxidants superoxide dismutase, catalase and glutathione peroxidase. These findings are consistent with the therapeutic use of *Withania somnifera* as an Ayurvedic rasayana. The antioxidant effect of active principles of *Withania somnifera* root may explain the reported anti-stress, cognition-facilitating, anti-inflammatory and anti-aging effects produced by them in experimental animals, and in clinical situations.

Anti-carcinogenic activity

Ashwagandha is reported to have anti-carcinogenic effects. Research on animal cell cultures has shown that the herb decreases the levels of the nuclear factor kappaB, suppresses the intercellular tumor necrosis factor, and potentiates apoptotic signalling in cancerous cell lines. One of the most exciting of the possible uses of Ashwagandha is its capacity to fight cancers by reducing tumor size. To investigate its use in treating various forms of cancer, the antitumor effects of *Withania somnifera* have been studied by researchers. In one study, the herb was evaluated for its anti-tumor effect in urethane-induced lung tumors in adult male mice. Following administration of Ashwagandha over a period of seven months, the histological appearance of the lungs of animals which received the herb was similar to those observed in the lungs of control animals.

Anti-aging activity

Ashwagandha was tested for its anti-aging properties in a double-blind clinical trial. A group of 101 healthy males, 50-59 years old were given the herb at a dosage of 3 grams daily for one year. The subjects experienced significant improvement in haemoglobin, red blood cell count, hair melanin, and seated stature. Serum cholesterol decreased and nail calcium was preserved. Seventy percent of the research subjects reported improvement in sexual performance.

Cardio protective activity

Ashwagandha has been evaluated in clinical studies with human subjects for its diuretic, hypoglycemic, and hypocholesterolemic effects. Six type 2 diabetes mellitus subjects and six mildly hypercholesterolemic subjects were treated with a powder extract of the herb for 30 days. A decrease in blood glucose comparable to that which would be caused by administration of a hypoglycemic drug was observed. Significant increases in urine sodium, urine volume, and decreases in serum cholesterol, triglycerides, and low-density lipoproteins were also seen.

Hypothyroid activity

Animal studies have shown that Ashwagandha may have an effect on thyroid activity. An aqueous extract of dried *Withania* root was given to mice daily for 20 days. Significant increases in serum T4 were observed, indicating the plant has a stimulating effect at the glandular level. *Withania somnifera* may also stimulate thyroid activity indirectly, via its

effect on cellular antioxidant systems. These results indicate ashwaganda may be a useful botanical in treating hypothyroidism

Immunomodulatory activity

A series of animal studies have demonstrated Ashwagandha to have profound effects on healthy production of white blood cells, which means it is an effective immune regulator and chemoprotective agent. In a study using mice, administration of powdered root extract from Ashwagandha was found to enhance total white blood cell count. In addition, this extract inhibited delayed-type hypersensitivity reactions and enhanced phagocytic activity of macrophages when compared to a control group.

Other Therapeutic Benefits

Further studies have also shown ashwagandha to be effective in the treatment of osteoarthritis, inflammation, stroke and tardive dyskinesia. Ashwagandha has been shown to be a potential antimicrobial agent, with antifungal activity, and moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas Aeruginosa* bacteria strains.

5. EXCIPIENTS PROFILE

5.1 STEARIC ACID^[41]

1. Non-proprietary Names.

BP: Stearic Acid

JP: Stearic Acid

PhEur: Stearic Acid

USP-NF: Stearic Acid

2. Synonyms

Acidum stearicum, cetylacetic acid, Crodacid, Cristal G, Cristal S, Dervacid, Edenor Emersol, 1-heptadecanecarboxylic acid, Hystrene, Industrene, Kortacid 1895, Pearl Steric Pristerene, stereophanic acid Tegostearic

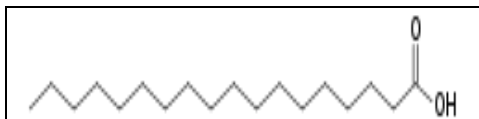
3. Chemical Name: Octadecanoic acid

4. Empirical Formula and Molecular Weight

$C_{18}H_{36}O_2$ 284.47 (for pure material) The USP32–NF27 describes stearic acid as a mixture of stearic acid ($C_{18}H_{36}O_2$) and palmitic acid ($C_{16}H_{32}O_2$). In the USP32–NF27, the content of

stearic acid is not less than 40.0% and the sum of the two acids is not less than 90.0%. The USP32–NF27 also contains a monograph for purified stearic acid. The PhEur 6.5 contains a single monograph for stearic acid but defines stearic acid 50, stearic acid 70, and stearic acid 95 as containing specific amounts of stearic acid ($C_{18}H_{36}O_2$)

5. Structural Formula



6. Applications in Pharmaceutical Formulation or Technology

Stearic acid is widely used in oral and topical pharmaceutical formulations. It is mainly used in oral formulations as a tablet and capsule lubricant; see Table I, although it may also be used as a binder or in combination with shellac as a tablet coating. It has also been suggested that stearic acid may be used in enteric tablet coatings and as a sustained-release drug carrier. In topical formulations, stearic acid is used as an emulsifying and solubilizing agent. When partially neutralized with alkalis or triethanolamine, stearic acid is used in the preparation of creams.

7. Description

Colour: Faintly yellow-colored, somewhat glossy,

State: Crystalline solid or a white or yellowish white powder.

Odour: Slight odor (with an odor threshold of 20 ppm) and taste suggesting tallow.

Molar mass: $284.48 \text{ g} \cdot \text{mol}^{-1}$

8. Pharmacopeia Specifications

Acid value: 195–212

Boiling point: 383.8°C

Density (bulk): 0.537 g/cm^3

Density (tapped): 0.571 g/cm^3

Density (true): 0.980 g/cm^3

Flash point: 113.8°C (closed cup)

Melting point: $69\text{--}70.8^{\circ}\text{C}$

Partition coefficient: Log (oil: water) = 8.2

Refractive index: 1.43 at 80.8°C

Saponification value: 200–220

Solubility

Freely soluble in benzene, carbon tetrachloride, chloroform, and ether; soluble in ethanol (95%), hexane, and propylene glycol; practically insoluble in water.^[8]

Specific surface area: 0.51–0.53m²/g

8. Uses

In general, applications of stearic acid exploit its bifunctional character, with a polar head group that can be attached to metal cations and a nonpolar chain that confers solubility in organic solvents. The combination leads to uses as a surfactant and softening agent. Stearic acid undergoes the typical reactions of saturated carboxylic acids, a notable one being reduction to stearylalcohol, and esterification with a range of alcohols. This is used in a large range of manufactures, from simple to complex electronic devices.

Soaps, cosmetics, detergents

Stearic acid is mainly used in the production of detergents, soaps, and cosmetics such as shampoos and shaving cream products. Soaps are not made directly from stearic acid, but indirectly by saponification of triglycerides consisting of stearic acid esters. Esters of stearic acid with ethylene glycol, glycol stearate, and glycol distearate are used to produce a pearly effect in shampoos, soaps, and other cosmetic products. They are added to the product in molten form and allowed to crystallize under controlled conditions. Detergents are obtained from amides and quaternary alkyl ammonium derivatives of stearic acid. Fatty acids are classic components of candle-making. Stearic acid is used along with simple sugar or corn syrup as a hardener in candies. Stearic acid is used to produce dietary supplements. In fireworks, stearic acid is often used to coat metal powders such as aluminium and iron.

5.2 BEES WAX**Beeswax^[54]**

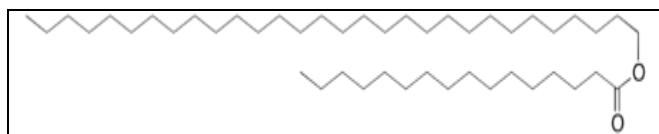
(Cera alba) is a natural wax produced by honey bees of the genus *Apis*. The wax is formed into "scales" by eight wax-producing glands in the abdominal segments 4 through 7 of worker bees, who discard it in or at the hive. The hive workers collect and use it for comb structural stability, to form cells for honey-storage and larval and pupal comfort and protection within the bee hive. Chemically, beeswax consists mainly of esters of fatty acids and various long-chain alcohols. Beeswax has applications in human food and flavoring, for example as a glazing agent. It is edible, in the sense of having similar negligible toxicity to

plant waxes, and is approved for food use in the European Union under the E number E901. However, the wax monoesters in beeswax are poorly hydrolysed in the guts of humans and other mammals, so have insignificant nutritional value. Some birds, such as honey guides, can digest beeswax.

Production

The wax is formed by worker bees, which secrete it from eight wax-producing mirror glands on the inner sides of the sternites (the ventral shield or plate of each segment of the body) on abdominal segments 4 to 7. The sizes of these wax glands depend on the age of the worker, and after many daily flights, these glands begin to gradually atrophy. When beekeepers extract the honey, they cut off the wax caps from each honeycomb cell with an uncapping knife or machine. Its color varies from nearly white to brownish, but most often a shade of yellow, depending on purity and the type of flowers gathered by the bees. Wax from the brood comb of the honey bee hive tends to be darker than wax from the honeycomb. Impurities accumulate more quickly in the brood comb. Due to the impurities, the wax must be rendered before further use. The leftovers are called slumgum. The wax may be clarified further by heating in water. As with petroleum waxes, it may be softened by dilution with mineral oil or vegetable oil to make it more workable at room temperature.

Chemicalstructure



Physical characteristics

Triacontanyl palmitate, a wax ester, is a major component of beeswax. Beeswax is a tough wax formed from a mixture of several compounds.

DESCRIPTION

chemical formula: $C_{15}H_{31}COOC_{30}H_{61}$.

Its main components are palmitate, palmitoleate, and oleateesters of long-chain (30–32 carbons) aliphaticcohols, with the ratio of triacontanyl palmitate $CH_3(CH_2)_{29}O-CO-(CH_2)_{14}CH_3$ to cerotic acid $CH_3(CH_2)_{24}COOH$, the two principal components, being 6:1. Beeswax can be classified generally into European and Oriental types. The saponification value is lower (3–5) for European beeswax, and higher (8–9) for Oriental types.

Melting point: low of 62 °C to 64 °C (144 °F to 147 °F). If beeswax is heated above 85 °C (185 °F) discoloration occurs.

Flash point: Beeswax is 204.4 °C (400 °F).

Density: 15 °C is 958 kg/m³ to 970 kg/m³.

Specific gravity: At 15 °C [59 °F] is from 0.958 to 0.975, that of melted wax at 98 °C to 99 °C [208.4 °F to 210.2 °F] compared with water at 15.5 °C [59.9°F] is 0.822. It softens when held in the hand, and melts at 62 °C to 66 °C.

Uses

Beeswax has many and varied uses. Primarily, it is used by the bees in making their honeycombs. Apart from this use by bees, the use of beeswax has become widespread and varied. Purified and bleached beeswax is used in the production of food, cosmetics, and pharmaceuticals. The three main types of beeswax products are yellow, white, and beeswax absolute. Yellow beeswax is the crude product obtained from the honeycomb, white beeswax is bleached yellow beeswax, and beeswax absolute is yellow beeswax treated with alcohol. In food preparation, it is used as a coating for cheese; by sealing out the air, protection is given against spoilage (mold growth). Beeswax may also be used as a food additive E901, in small quantities acting as a glazing agent, which serves to prevent water loss, or used to provide surface protection for some fruits. Soft gelatin capsules and tablet coatings may also use E901. Beeswax is also a common ingredient of natural chewing gum. Use of beeswax in skin care and cosmetics has been increasing.

5.3 STEARYL ALCOHOL

1. Non-proprietary Names

BP: Stearyl Alcohol

JP: Stearyl Alcohol

PhEur: Stearyl Alcohol

USP-NF: Stearyl Alcohol

2. Synonyms^[47]

Alcohol stearyllicus; Cachalot; Crodacol S95; Hyfatol 18-95; Hyfatol 18-98; Lanette 18; Lipocol S; Lipocol S-DEO; Nacol 18- 98; Nacol 18-98P; n-octadecanol; octadecyl alcohol; Rita SA; Speziol C18 Pharma; Stearol; Stenol; Tego Alkanol 18; Vegarol 1898.

3. Chemical Name: 1-Octadecanol**4. Empirical Formula and Molecular Weight**

$C_{18}H_{38}O$ 270.48 (for pure material) The PhEur 6.0 describes stearyl alcohol as a mixture of solid alcohols containing not less than 95% of 1-octadecanol, $C_{18}H_{38}O$. The USP32–NF27 states that stearyl alcohol contains not less than 90% of 1-octadecanol, the remainder consisting chiefly of related alcohols.

5. Structural Formula: $C_{18}H_{38}O$ **6. Functional Category:** Stiffening agent.**7. Applications in Pharmaceutical Formulation or Technology**

Stearyl alcohol is used in cosmetics and topical pharmaceutical creams and ointments as a stiffening agent. By increasing the viscosity of an emulsion, stearyl alcohol increases its stability. Stearyl alcohol also has some emollient and weak emulsifying properties, and is used to increase the water-holding capacity of ointments, e.g. petrolatum. In addition, stearyl alcohol has been used in controlled-release, suppositories, and microspheres. It has also been investigated for use as a transdermal penetration enhancer.

8. Description

Stearyl alcohol occurs as hard, white, waxy pieces, flakes, or granules with a slight characteristic odour and bland taste.

9. Pharmacopoeial Specifications

Melting range: 56–628⁰C 57–608⁰C 55–608⁰C

Acid value: 41.0 41.0 42.0

Iodine value: 42.0 42.0 42.0

Hydroxyl value: 200–220 197–217 195–220

Saponification value: 42.0

Ester value: 43.0

Residue on ignition: 40.05%

Assay (of $C_{18}H_{38}O$): 595% 590.0%

10. Typical Properties

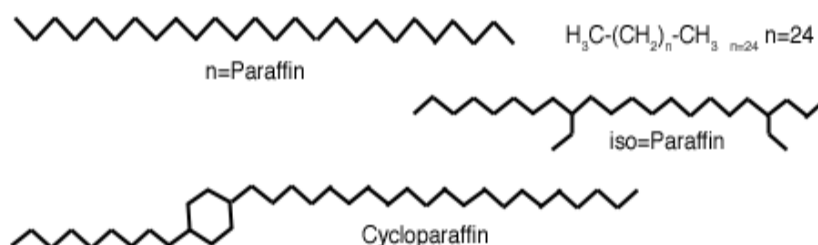
Auto ignition temperature: 4508⁰C

Boiling point:	210.58°C at 2 kPa (15 mmHg)
Density (true):	0.884–0.906 g/cm ³ (10)
Flash point:	1918 ⁰ C (open cup)
Freezing point:	55–578 ⁰ C
Melting point:	59.4–59.88 ⁰ C for the pure material.
Refractive index n_D:	60 = 1.4388 at 608 ⁰ C
Solidification point:	56–598 ⁰ C for Nacol 18-98; 55–588 ⁰ C s
Solubility:	Soluble in chloroform, ethanol (95%), ether, hexane, propylene glycol, benzene, acetone, and vegetable oils; practically insoluble in water.
Vapour pressure:	133.3 Pa (1 mmHg) at 150.38 ⁰ C
Viscosity:	9.82 mPa at 648 ⁰ C are possibly due to impurities contained in stearyl

5.4 LIQUID PARAFFIN

Medicinal liquid paraffin, also known as paraffinum liquidum, is a very highly refined mineral oil used in cosmetics and for medical purposes. This is a UK definition (British Pharmacopoeia) and the term may have different uses in other countries. The cosmetic or medicinal liquid paraffin should not be confused with the paraffin (or kerosene) used as a fuel. The term paraffinum perliquidum is sometimes used to denote light liquid paraffin. Conversely, the term paraffinum subliquidum is sometimes used to denote a thicker (more viscous/oily) mineral oil.

Chemical structure



Usage and side effects

Liquid paraffin is considered to have a limited usefulness as an occasional laxative, but is unsuitable for regular use as it can seep from the anus and cause irritation; it can interfere with the absorption of fat-soluble vitamins; it can be absorbed into the intestinal wall and may cause foreign-body granulomatous reactions; and if it enters the lungs it can cause lipid

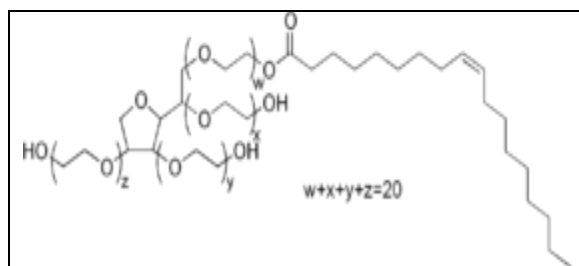
pneumonia. Liquid paraffin is also used in combination with magnesium as an osmotic laxative, sold under the trade name Mil-Par (among others)

5.5 POLYSORBATE 80

Molecular formula: Polyoxyethylene (20) sorbitan monooleate

Other names: Alkest TW, 80, Tween 80, Scattics, Canarcel.

Chemical structure:



PROPERTIES

Chemical formula	C ₆₄ H ₁₂₄ O ₂₆
Molar mass	1310 g/mol
Appearance	Amber colored viscous liquid
Density	1.06–1.09 g/mL, oily liquid
Boiling point	> 100°C
Solubility in water	Very soluble
Solubility in other solvents	ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene

Polysorbate 80 is a non-ionic surfactant and emulsifier often used in foods and cosmetics. This synthetic compound is a viscous, water-soluble yellow liquid.

Chemistry

Polysorbate 80 is derived from polyethoxylated sorbitan and oleic acid. The hydrophilic groups in this compound are polyether's also known as polyoxyethylene groups, which are polymers of ethylene oxide. In the nomenclature of polysorbates, the numeric designation following polysorbate refers to the lipophilic group, in this case the oleic acid (see polysorbate for more detail).

The full chemical names for polysorbate 80 are

- Polyoxyethylene (20) sorbitan monooleate
- (x)-sorbitan mono-9-octadecenoate poly(oxy-1,2-ethanediyl)

The critical micelle concentration of polysorbate 80 in pure water is reported as 0.012 mM.

Uses

Polysorbate 80 is used as an emulsifier in foods. For example in ice cream, polysorbate is added up to 0.5% (v/v) concentration to make the ice cream smoother and easier to handle, as well as increasing its resistance to melting. Adding this substance prevents milk proteins from completely coating the fat droplets. This allows them to join together in chains and nets, which hold air in the mixture, and provide a firmer texture that holds its shape as the ice cream melts.

Medical use

Polysorbate 80 is an excipient that is used to stabilize aqueous formulations of medications for parenteral administration, and used as an emulsifier in the manufacture of the popular antiarrhythmic amiodarone. It is also used as an excipient in some European and Canadian influenza vaccines. Influenza vaccines contain 25 µg of polysorbate 80 per dose.^[7] It is also used in the culture of *Mycobacterium tuberculosis* in Middlebrook 7H9 broth. It is also used as an emulsifier in the estrogen-regulating drug Estrasorb.

5.6SORBITOL^[49]

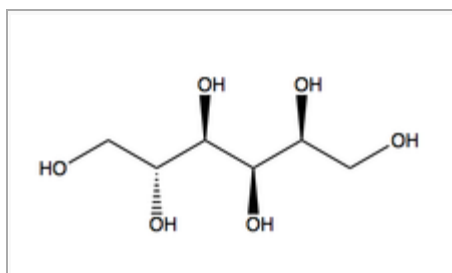
1. Non-proprietary Names: BP: Sorbitol JP: D-Sorbitol PhEur: Sorbitol USP-NF: Sorbitol
2. Synonyms: C PharmSorbidex; E420, 1, 2, 3, 4, 5, 6-hexanehexo Liponic 70-NC, Liponic 76-NC Meritol , Neosorb, Sorbitab, Sorbite, Dsorbitol, Sorbitol Instant sorbitolum, Sorbogem.

3. Chemical Name: D-Glucitol [50-70-4]

4. Empirical Formula: C₆H₁₄O₆

5. Molecular weight: 182.17

6. Chemical structure



7. Functional Category

Humectant; plasticizer; stabilizing agent; sweetening agent; tablet and capsule diluent.

8. Applications in Pharmaceutical Formulation or Technology

Sorbitol is widely used as an excipient in pharmaceutical formulations. It is also used extensively in cosmetics and food products. Sorbitol is used as a diluent in tablet formulations prepared by either wet granulation or direct compression. It is particularly useful in chewable tablets owing to its pleasant, sweet taste and cooling sensation. In capsule formulations it is used as a plasticizer for gelatin. Sorbitol has been used as a plasticizer in film. In liquid preparations sorbitol is used as a vehicle in sugar-free formulations and as a stabilizer for drug. Vitamin and antacid suspensions. Furthermore, sorbitol is used as an excipient in liquid parenteral biologic formulations to provide effective protein stabilization in the liquid state. It has also been shown to be a suitable carrier to enhance the *in vitro* dissolution rate of Indomethacin. In syrups it is effective in preventing crystallization around the cap of bottles. Sorbitol is additionally used in injectable and topical preparations, and therapeutically as an osmotic laxative. Sorbitol may also be used analytically as a marker for assessing liver blood

9. Uses of sorbitol

Use Concentration (%), Humectant 3–15, IM injections 10–25, Moisture control agent in tablets 3–10, Oral solutions 20–35, Oral suspensions 70, Plasticizer for gelatin and cellulose 5–20, Prevention of ‘cap locking’ in syrups and elixirs 15–30, Substitute for glycerin and propylene glycol 25–90, Tablet binder and filler 25–90, Toothpastes 20–60, Topical emulsions 2–18.

10. Description

Sorbitol is D-glucitol. It is a hexahydric alcohol related to mannose and is isomeric with mannitol.

Colour:	colour less
Odour:	odorless
State:	Crystalline, hygroscopic powder
Density (bulk):	0.448 g/cm ³ ; 0.6–0.7 g/cm ³ for Sorbitab SD 250; 0.5–0.6 g/cm ³ for sorbitabSD500
Density (tapped):	0.400 g/cm ³ ; 0.7 g/cm ³ for Sorbitab SD 250; 0.6 g/cm ³ for Sorbitab SD 500;
Density (true):	1.507 g/cm ³
Flowability	

Flow characteristics vary depending upon the particle size and grade of sorbitol used. Fine powder grades tend to be poorly flowing, while granular have good properties. Heat of solution -110.9 J/g (-26.5 cal/g).

Melting point: Anhydrous form: $110-1128^\circ\text{C}$

5.7 POTASSIUM HYDROXIDE^[42]

1. Non-proprietary Name

BP: Potassium Hydroxide

JP: Potassium Hydroxide

PhEur: Potassium Hydroxide

USP-NF: Potassium Hydroxide

2. Synonyms

Caustic potash; E525; kalii hydroxidum; kalium hydroxydatum; Potash lye; potassium hydrate.

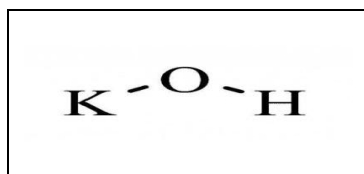
3. Chemical Name

Potassium hydroxide [1310-58-3]

4. Empirical Formula and Molecular Weight

KOH 56.11 g/mol

5. Structural Formula



6. Functional Category: Alkalizing agent.

7. Applications in Pharmaceutical Formulation or Technology

Potassium hydroxide is widely used in pharmaceutical formulations to adjust the pH of solutions. It can also be used to react with weak acids to form salts. Therapeutically, potassium hydroxide is used in various dermatological applications.

8. Descriptions

Potassium hydroxide occurs as a white or nearly white fused mass. It is available in small pellets, flakes, sticks and other shapes or forms. It is hard and brittle and shows a crystalline fracture. Potassium hydroxide is hygroscopic and deliquescent; on exposure to air, it rapidly absorbs carbon dioxide and water with the formation of potassium carbonate.

9. Pharmacopoeial Specifications

Typical Properties

Acidity/alkalinity pH = 13.5 (0.1M aqueous solution)

Melting point: 360°C; 380°C when anhydrous Solubility.

10. Stability and Storage Conditions

Potassium hydroxide should be stored in an airtight, non-metallic container in a cool, dry place.

11. Incompatibilities

Potassium hydroxide is a strong base and is incompatible with any compound that readily undergoes hydrolysis or oxidation. It should not be stored in glass or aluminium containers, and will react with acids, esters, and ethers, especially in aqueous solution.

12. Method of Manufacture

Potassium hydroxide is made by the electrolysis of potassium chloride. Commercial grades may contain chlorides as well as other impurities.

13. Safety

Potassium hydroxide is widely used in the pharmaceutical and food industries and is generally regarded as a nontoxic material at low concentrations. At high concentrations it is a corrosive irritant to the skin, eyes, and mucous membranes.

6. MATERIALS AND EQUIPMENT USED

6.1 Materials used

Table no 2: List of Materials used for the project

Ingredients	Purpose
Diclofenac sodium	Active pharmaceutical ingredient
Withania somnifera	Active pharmaceutical ingredient
Liquid Paraffin	Emollient; lubricant
Stearic Acid	Emulsifying agent; solubilizing agent;

White Bees Wax	Stabilizing agent; stiffening agent.
Stearyl Alcohol	Stiffening agent
Tween-80	Dispersing agent, solubilizing agent; wetting agent
Methyl Parabens	Antimicrobial preservative.
Sorbitol Solution	Humectant; plasticizer
Potassium Hydroxide	Alkalizing agent
Purified water	Emollient; lubricant

6.2 INSTRUMENTS USED

Table no 3: List of instruments used for the project

S.NO	INSTRUMENT USED	COMPANY NAME
1.	Physical balance	Kshitij innovation
2.	Electronic balance	SF400C
3.	UV Spectrophotometer	Systonic 2202
4.	Dissolution apparatus	LMDV-60
5.	P ^H meter	Hanna
6.	Magnetic stirrer	Kshitij innovation
7	Brookfield viscometer	Labman LMDV-60

7. METHODOLOGY

7.1 PREFORMULATION STUDIES

Prior to the development of dosage forms, the under mentioned studies were performed to ascertain the physical and chemical properties of drug molecule and other derived properties of the drug powder covering the physiological characterization of the solid and solution properties of compounds which was useful in formulating the drug into a suitable delivery system.

7.2 ANALYTICAL PREFORMULATION

a) Organoleptic Evaluation of Diclofenac. Organoleptic characters of drug were observed and recorded.

b) Identification of Drug sample-Infrared spectrum.

The infrared spectrum of Diclofenac was recorded by using FT-IR spectroscopy (shimadzu, FTR-1800 S).

c) Assay of Drug sample.

The % purity data of Diclofenac was provided by supplier of drug.

Drug-excipients interactions

Assessment of possible incompatibilities between an active drug substance and different excipients plays an important part of the formulation stage during the development of solid dosage form. Fourier Transformer Infra-Red Spectrum (FTIR).

FTIR STUDIES

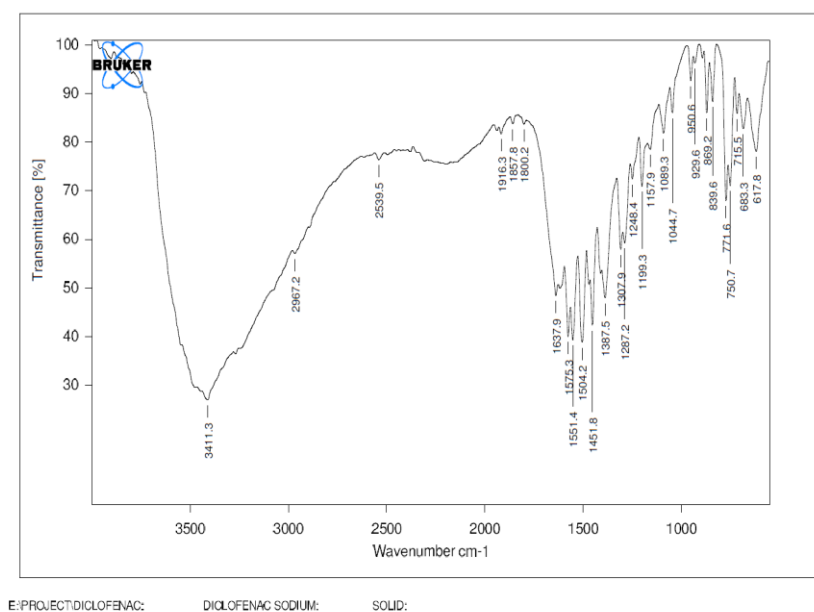


Fig no 4: FTIR Studies of Diclofenac Sodium

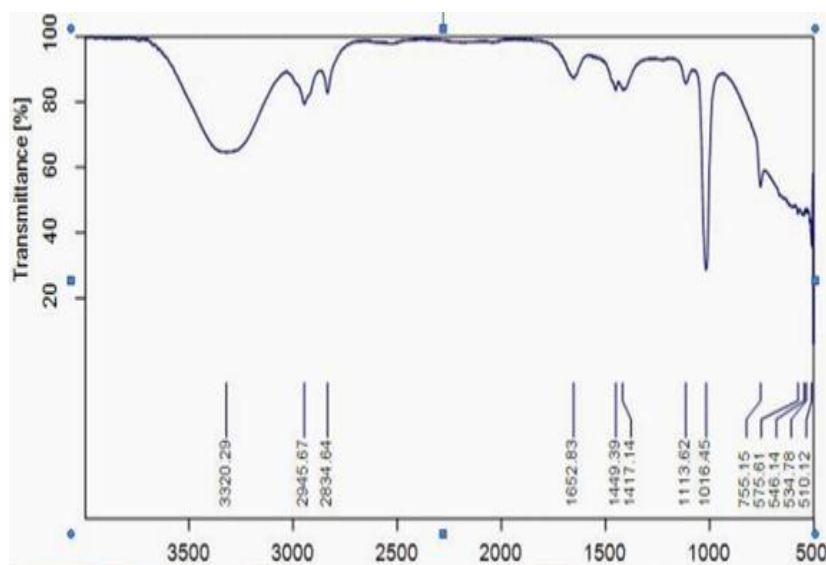


Fig no 5: FTIR Studies of Withania somnifera

Table no 4: Wave no of different functional groups present in diclofenac sodium

Peaks for groups	N-H	C-H Aromatic bending	C-H Strech	C-N Strech aromatic	C-O	C-Cl Strech	C=C Aromatic stretch
Wave no.(cm ⁻¹)	3411.3	771.6-750.7	2967.2	1387.5	1199.3	839.6-869.2	1637.9

Table no 5: Wave no of different functional groups present in Withania somnifera

Peaks for groups	O-H Strech	C-H strech Aromatic	C=O Strech	C-C Strech aromatic	C-O Strech
Wave no.(cm ⁻¹)	3439.6	2926.8	1620.5	1416.0	1321.2

7.3 PREPARATION OF 6.8 P^H BUFFER

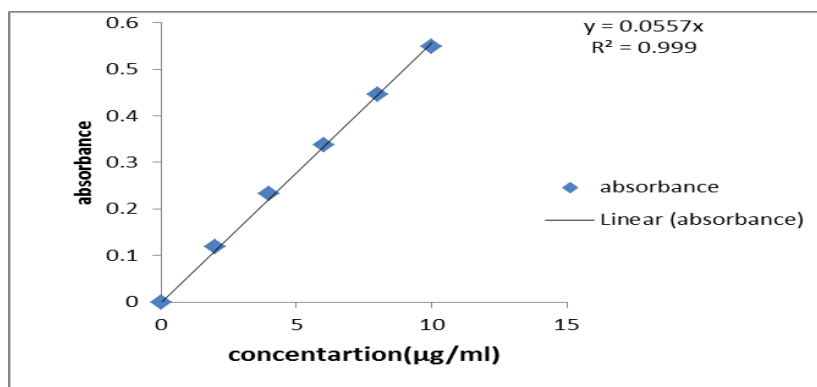
Add 6.8gm KH₂PO₄ and about 0.94gm of NaOH in 1000ml water. It directly shows pH 6.8. Adjust the pH of solution by using dilute Orthophosphoric acid or NaOH. 6.8 gm KH₂PO₄ + 0.94gm of NaOH.

7.4 STANDARD PLOT OF DICLOFENAC

The standard plot of Diclofenac in 6.8 buffer was prepared by dissolving 100mg of drug in 100ml of 6.8 buffer and it makes that the concentration of 100µg/ml. From this stock solution take 1ml and diluted to 100ml with 6.8 buffer and it makes the concentration 10µg/ml from this solution prepare different concentrations viz., 2,4,6,8,10µg/ml. The solutions were analyzed for absorbance in UV region at λ_{max} 277nm.

Table no 6: Standard Plot Of Diclofenac Sodium.

S.NO	Concentration(µg/ml)	Absorbance
1	2	0.118
2	4	0.232
3	6	0.337
4	8	0.446
5	10	0.549

**Fig 6: Standard plot of diclofenac sodium**

Preparation of Withania somnifera Extract

The roots of Withania somnifera (Aswagandha) were cleaned, washed with purified water, sliced and dried in the sun for one week. Dried roots were cut in small pieces and powered by electronic mill. 200 gm of sample was taken into a thimble and placed in an extraction apparatus. The apparatus was setup with various solvents ranging from non-polar to polar. 1 liter of solvent was added and extracted according to their boiling point for seven hours. The solvents used were chloroform (B.P. = 61°C), ethyl acetate (B.P. = 77°C), methanol (B.P. = 65°C) and acetone (B.P. = 56.53°C). After completion of extraction the dark brown extract was then cooled, concentrated by an evaporating process to get a crude dried extract.

Table no 7: formulation of diclofenac sodium cream prepared by ingredients:

Ingredients	F1	F2	F3	F4
Diclofenac sodium	1g	1g	1g	1g
Withania somnifera	-	1g	2g	3g
Liquid Paraffin	5.0ml	5.0ml	5.0ml	5.0ml
Stearic Acid	0.30g	0.30g	0.30g	0.30g
Bees Wax	5.0g	5.0g	5.0g	5.0g
Stearyl Alcohol	10.0g	10.0g	10.0g	10.0g
Tween-80	8.0ml	8.0ml	8.0ml	8.0ml
Methyl Parabens	0.12g	0.12g	0.12g	0.12g
Sorbitol Solution	6.0 ml	6.0 ml	6.0 ml	6.0 ml
Potassium Hydroxide	1.50g	1.50g	1.50g	1.50g
Purified water	63.08ml	62.08ml	61.08ml	60.08ml
Total weight in gms	100g	100g	100g	100g

7.5 Preparation Of Diclofenac Sodium Cream

In this experiment diclofenac sodium cream was prepared by fusion method. Three formulations of Diclofenac sodium cream were prepared by using different ratio of diclofenac sodium & withania somnifera extract by the following method. Required quantity of Diclofenac sodium with withania somnifera extract was formulated by the fusion method. The aqueous and oil phases were taken into beakers and heated to 75°C over a water bath. The oil phase was comprised of Diclofenac Sodium, Liquid Paraffin, Bees Wax, Stearyl Alcohol, Tween 80 and Stearic Acid while the aqueous phase was composed of Extract of withania somnifera, Methyl parabens, Sorbitol Solution and Potassium Hydroxide. Drop wise addition of the aqueous phase to the oil phase was done with constant stirring. Diclofenac cream containing the withania somnifera extract was formulated.

7.6 Evaluation of Diclofenac Sodium Cream

The prepared cream were evaluated for appearance, pH, drug content, spreadability, extrudability, permeability studies and stability studies. All the gels were visually evaluated for presence of fibres and particles.

pH

The pH values of prepared gels were checked by using a digital pH meter.

Drug content

1g each formulation containing approximately 20 mg of drug was taken in a 50 ml volumetric flask and diluted with water and shaken to dissolve the drug in water. The solution was filtered through Whatman filter paper. 1 ml of the filtrate was pipette out and diluted to 10 ml with water. The content of the drug was estimated spectrophotometrically by using standard curve plotted at 277 nm.

Spreadability

Two sets of glass slides of standard dimensions were taken. The herbal cream formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slide. 100g weight was placed upon the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of cream adhering to the slides were scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20g weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated by three times and the mean time taken for calculation.

Spreadability was calculated by using the following formula.

$$s = \frac{m \times l}{t}$$

S – Spreadability,

m – Weight tied to the upper slide (20g)

l - Length of the glass (7.5 cm)

t - Time taken in seconds

Extrudability

A closed collapsible tube containing above 20g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the cream extrudes until the pressure was dissipated.

Viscosity.^[69]

Viscosity of the gels was determined using Brookfield viscometer. Spindle type, model LVDV-E at 10 rpm. 100g of the gel was taken in a beaker and the spindle was dipped in it for about 5 minutes and then the reading was taken.

Skin irritation test

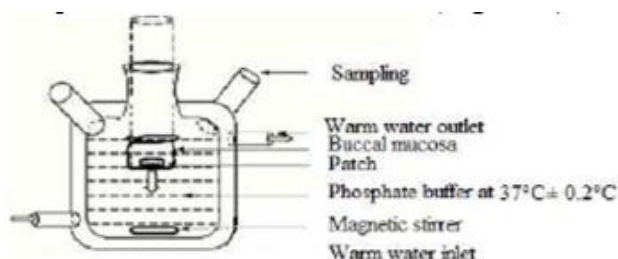
Test for irritation was performed on human volunteers. For each gel, five volunteers were selected and 1.0g of formulated gel was applied on an area of 2 square inch to the back of hand.

The volunteers were observed for lesions or irritation..

Homogeneity

All developed gels were tested for homogeneity by visual inspection after the creams have been set in the container. They were tested for their appearance and presence of any aggregates.

In vitro release testing methods



The Franz diffusion cell was used to determine the amount of the drug diffused from different formulations. Franz diffusion cells with a receiver compartment volume of 10 mL and effective diffusion area of 2.84 cm² were used to evaluate drug delivery characteristics from the eight selected compositions. A dialysis membrane (0.65 μm) was used. The receptor phase (ethanol 50 %, w/w) was continuously stirred and kept at a temperature of 32 ± 0.5°C.

during the experiments. One gram of gel formulation was placed in the donor compartment. At appropriate time, 1 mL of the sample was withdrawn from the receiver compartment and the same amount of fresh solution was added to keep the volume constant. Each experiment was run in four independent cells. The samples were analyzed spectrophotometrically at a wavelength of 276 nm and the concentration of diclofenac sodium in each sample was determined from a standard curve.

PREPARATION OF EGG MEMBRANE

In vitro release

In vitro release of the drug can be performed by diffusion flask method. Here egg membrane is used as a biological membrane.

Preparation of egg membrane

EGG MEMBRANE

Egg membrane is prepared by a small hole was made on egg and separate the egg yolk. Egg membrane was separated out by placing the egg shell in conc.Hcl till the membrane was separated from shell. Then the separated egg membrane was continuously washed with purified water to make it free from Conc.Hcl and finally cleaned or washed in alcohol then experiment is carried out. Glass tube with two ends open were taken.



At one end of the test tube egg membrane was tied and fitted to a burette stand such that surface of the membrane touches the buffer taken in a beaker which was placed on a magnetic stirrer before placing in buffer. Three ointments were taken into the three different glass tubes and spread on tied membranes and the temperature was maintained at 37°C with the help of magnetic stirrer, maintain continuous stirring. 5ml of solution from the beaker was withdrawn for every 15 minutes up to 2 hours to maintain the sink conditions, that 5ml of

sample was replaced with the fresh 5ml acetate buffer. Note the absorbance of collected sample by using UV-Visible Spectrophotometer at 276nm.

Permeability studies

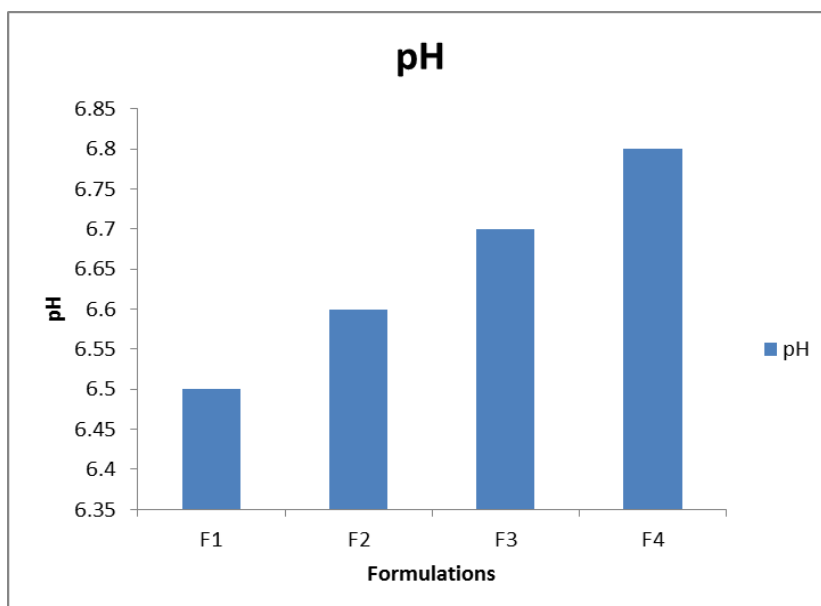


The invitro diffusion studies of the cream were performed using egg membrane. The membrane was soaked in phosphate buffer pH 6.8 for 6-8 hr & was clamped carefully to one end of the hollow glass tube. Phosphate buffer of pH 6.8 was used for in vitro release as a receptor medium. The cream sample was applied on the membrane and then fixed in between donor and receptor compartment of glass tube. The receptor compartment contained phosphate buffer (100ml) of pH 6.8. The temperature of diffusion medium was thermostatically controlled at $37^{\circ} \pm 1^{\circ}$ by surrounding water in jacket and the medium was stirred by magnetic stirrer at 500rpm. The samples were withdrawn at predetermined intervals and were replaced by equal volume of fresh fluid. The samples withdrawn were spectrophotometrically estimated at 277nm using phosphate buffer as blank.

1.p^H

Table no 8: Study of pH of different formulations

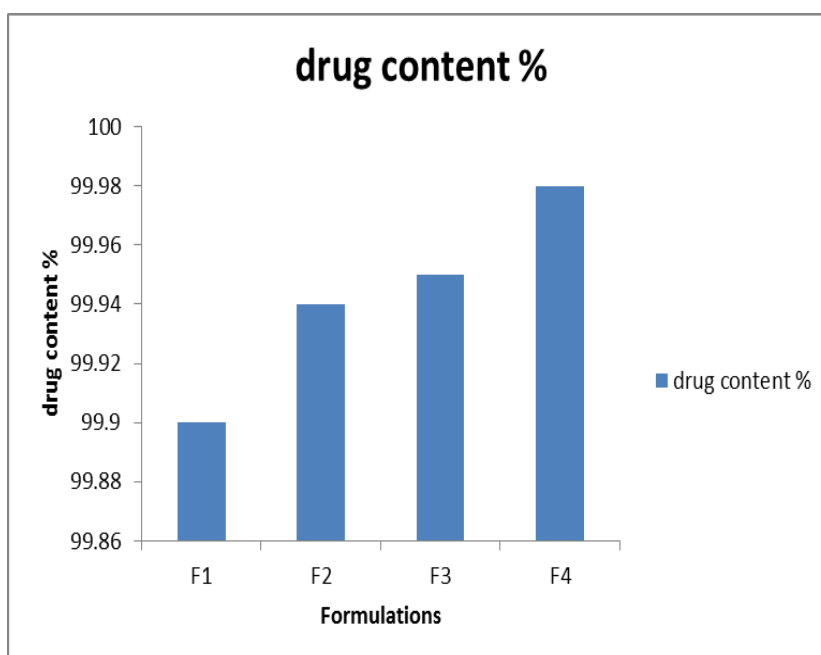
S.NO	pH \pm SD
F1	6.5 ± 0.016
F2	6.6 ± 0.153
F3	6.7 ± 0.136
F4	6.8 ± 0.134

**Fig no 7: Study of pH of different formulations**

2. Drug content

Table no 9: Calculating drug content in different formulations

Formulations	Drug content % \pm SD
F1	99.90 \pm 1.10
F2	99.94 \pm 7.40
F3	99.95 \pm 4.10
F4	99.98 \pm 1.80

**Fig no 8: drug content in different formulations**

3. Viscosity

Table no 10: Measurement of Viscosity at 6rpm & 12 rpm

Formulations	Viscosity (mPas)	
	6 rpm	12 rpm
F1	15000±0.12	10000±0.23
F2	12000±0.34	9500±0.45
F3	18000±0.11	16000±0.35
F4	20000±0.21	18000±0.31

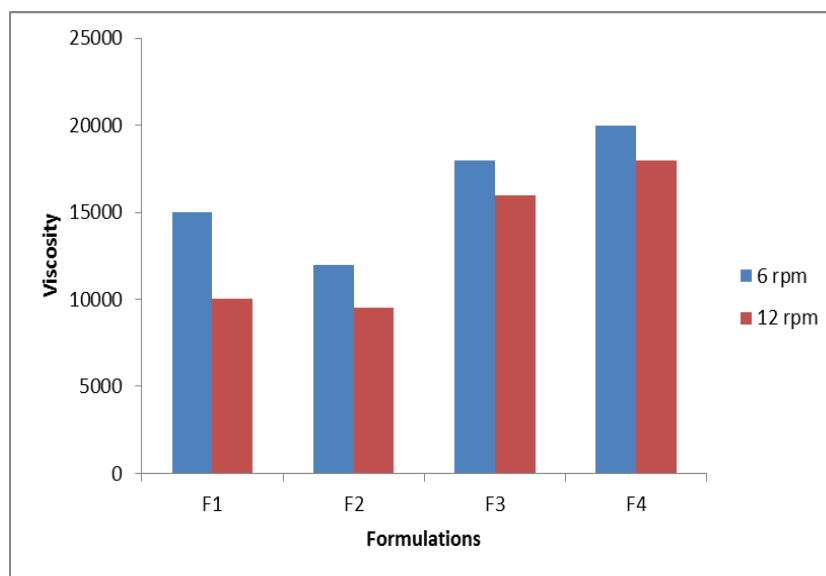


Fig no 9: Measurement of Viscosity at 6rpm & 12 rpm

Formulation Homogeneity

Table no 11: study of Homogeneity of different formulations.

S.NO	Formulations	Homogeneity
1	F1	Good
2	F2	Good
3	F3	Good
4	F4	Good

4. Skin irritation test

Table no 12: skin irritation study results of different formulations.

S.NO	Formulations	Skin irritation
1	F1	Nil
2	F2	Nil
3	F3	Nil
4	F4	Nil

5. In vitro drug diffusion study**Table no 13: Drug permeability release from formulations F1.**

S.no	Time (hrs)	Absorbance	Amountof drug released (mg)	Cumulative % Drug released	% Drug unreleased	Log % Drug unreleased
1	0	0	0	0	100	2
2	0.5	0.157	0.560	5.60	94.40	1.974
3	1.0	0.314	1.071	10.70	89.30	1.950
4	2.0	0.471	1.903	19.03	80.97	1.908
5	4.0	0.628	2.840	28.41	71.59	1.854
6	6.0	0.785	3.560	35.60	64.40	1.808
7	8.0	0.942	4.712	47.10	52.90	1.723
9	10.0	1.177	5.823	58.30	41.70	1.620
10	12.0	1.413	6.534	65.12	34.88	1.542
11	24.0	2.826	7.632	76.32	23.68	1.374

Table no 14: Drug permeability release from formulation F2

S.no	Time (hrs)	Absorbance	Amount of drug released (mg)	Cumulative % Drug released	% Drug unreleased	Log % Drug unreleased
1	0	0	0	0	100	2
2	0.5	0.161	0.778	7.78	92.22	1.964
3	1.0	0.322	1.571	15.71	84.29	1.925
4	2.0	0.483	2.357	23.57	76.43	1.833
5	4.0	0.644	3.498	34.98	65.02	1.813
6	6.0	0.805	4.303	43.03	56.97	1.755
7	8.0	0.966	5.117	51.17	48.83	1.688
8	10.0	1.207	6.682	66.82	33.18	1.520
9	12.0	1.449	7.445	74.45	25.55	1.407
10	24.0	2.898	8.419	84.19	15.81	1.198

Table no 15: Drug permeability release from formulation F3:

S.no	Time (hrs)	Absorbance	Amountof drug released (mg)	Cumulative % Drug released	% Drug unreleased	Log % Drug unreleased
1	0	0	0	0	100	2
2	0.5	0.1662	1.012	10.12	89.88	1.953
3	1.0	0.2243	1.864	18.64	81.36	1.910
4	2.0	0.367	2.542	25.42	74.58	1.872
5	4.0	0.712	3.732	37.32	62.68	1.797
6	6.0	0.893	4.521	45.21	54.79	1.738
7	8.0	1.068	5.365	53.65	46.35	1.666
9	10.0	1.335	6.892	68.92	31.08	1.492
10	12.0	1.602	7.854	78.54	21.46	1.331
11	24.0	3.204	8.835	88.35	11.65	1.066

Table no 16: Drug permeability release from formulation F4.

S.no	Time (hrs)	Absorbance	Amount of drug released (mg)	Cumulative % Drug released	% Drug unreleased	Log % Drug unreleased
1	0	0	0	0	100	2
2	0.5	0.1994	1.376	13.76	86.24	1.935
3	1.0	0.3223	2.043	20.43	79.57	1.900
4	2.0	0.510	3.143	31.43	68.57	1.836
5	4.0	0.739	4.232	42.32	57.68	1.761
6	6.0	0.983	5.267	52.67	47.33	1.675
7	8.0	1.101	6.724	67.24	32.76	1.515
8	10.0	1.376	7.834	78.34	21.66	1.335
9	12.0	1.651	8.532	85.32	14.68	1.166
10	24.0	3.303	9.167	91.67	8.33	0.920

INVITRO DRUG DIFFUSION RELEASE STUDY

Table no 17: In vitro drug diffusion release alone Diclofenac sodium (F1)

Time (hrs)	% DRUG RELEASE
0	0±0.00
0.5	5.6±0.21
1.0	10.7±0.23
2.0	19.03±0.11
4.0	28.41±0.12
6.0	35.6±0.51
8.0	47.1±0.20
10.0	58.3±0.23
12.0	65.12±0.12
24.0	76.32±0.14

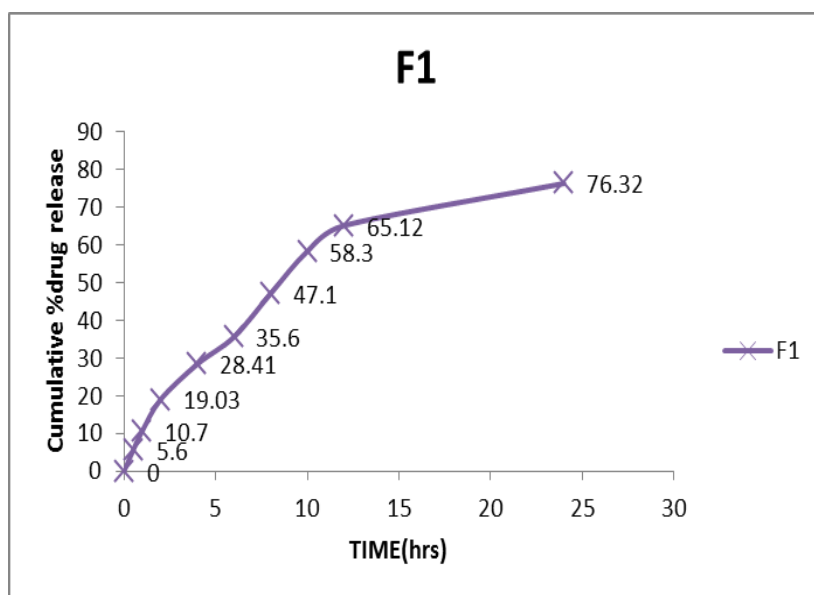


Fig no 10: In vitro drug diffusion profile (F1)

Table no 18: In vitro drug diffusion release of 1:1 Diclofenac sodium + Withania somnifera extract (F2).

TIME (min)	% DRUG RELEASE
0	0±0.00
0.5	7.78±0.11
1.0	15.71±0.98
2.0	23.57±0.12
4.0	34.98±0.12
6.0	43.03±0.34
8.0	51.17±0.12
10.0	66.82±0.14
12.0	74.45±0.24
24.0	84.19 ±0.12

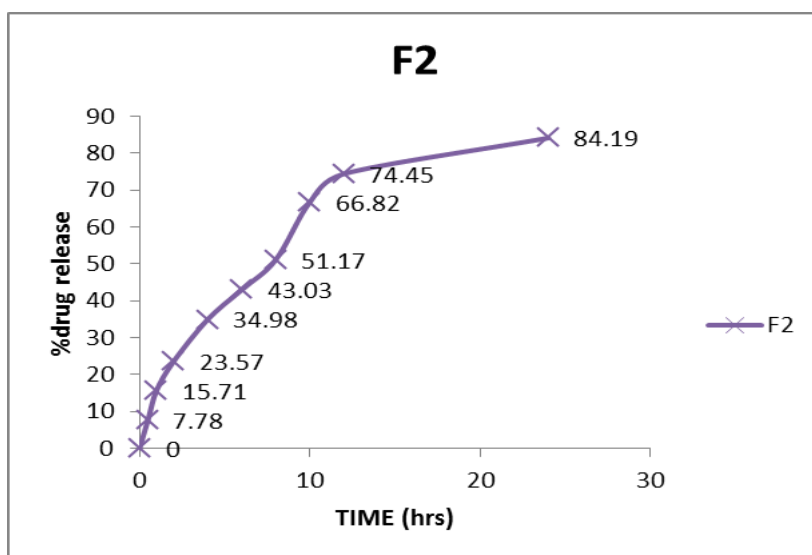


Fig no 11: In vitro drug diffusion profile (F2)

Table no 17: In vitro drug diffusion release of 1:2 Diclofenac sodium + Withania somnifera extract (F3)

TIME (min)	% DRUG RELEASE
0	0±0.00
0.5	10.12±0.12
1.0	18.64±0.34
2.0	25.42±0.12
4.0	37.32±0.13
6.0	45.21±0.19
8.0	53.65±0.13
10.0	68.92±0.42
12.0	78.54±0.25
24.0	88.35±0.32

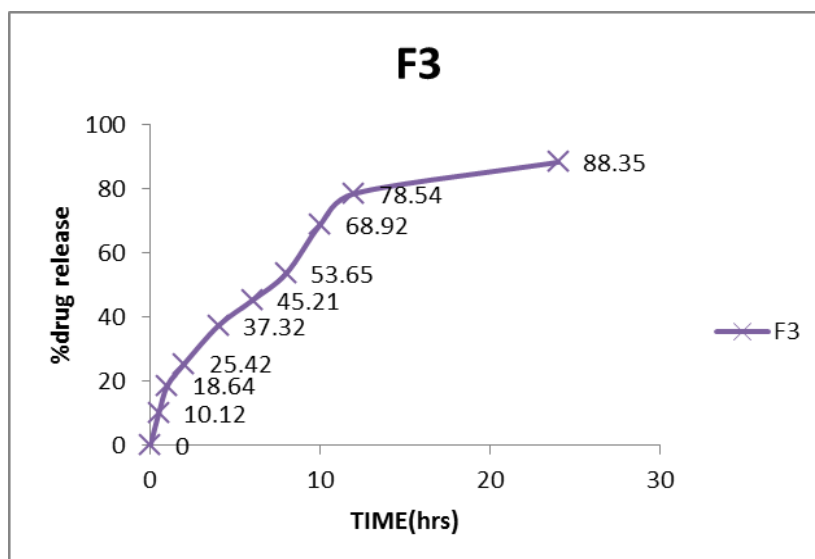


Fig no 12: In vitro drug diffusion profile (F3)

Table no 20: In vitro drug diffusion release of 1:3 Diclofenac sodium + Withania somnifera extract (F4).

TIME (hrs)	CUMULATIVE% DRUG RELEASE
0	0±0.00
0.5	13.76±0.19
1.0	20.43±0.14
2.0	31.43±0.12
4.0	42.32±0.13
6.0	52.67±0.12
8.0	67.24±0.14
10.0	78.34±0.16
12.0	85.32±0.16
24.0	91.67±0.12

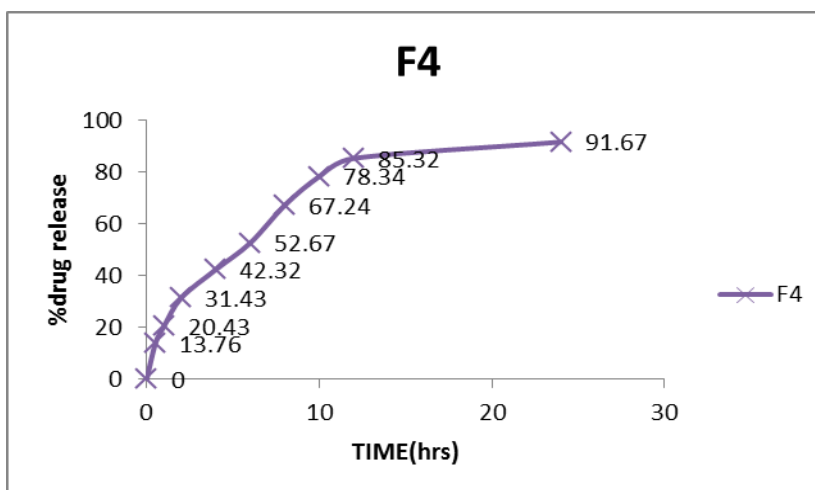


Fig no 13: In vitro drug diffusion profile (F4)

Table no 21: comparison of dissolution profile of F1, F2,F3&F4.

TIME (min)	CUMULATIVE% DRUG RELEASE	CUMULATIVE% DRUG RELEASE	CUMULATIVE% DRUG RELEASE	CUMULATIVE% DRUG RELEASE
0	0±0.00	0±0.00	0±0.00	0±0.00
0.5	5.6±0.21	7.78±0.11	10.12±0.12	13.76±0.19
1.0	10.7±0.23	15.71±0.98	18.64±0.34	20.43±0.14
2.0	19.03±0.11	23.57±0.12	25.42±0.12	31.43±0.12
4.0	28.41±0.12	34.98±0.12	37.32±0.13	42.32±0.13
6.0	35.6±0.51	43.03±0.34	45.21±0.19	52.67±0.12
8.0	47.1±0.20	51.17±0.12	53.65±0.13	67.24±0.14
10.0	58.3±0.23	66.82±0.14	68.92±0.42	78.34±0.16
12.0	65.12±0.12	74.45±0.24	78.54±0.25	85.32±0.16
24.0	76.32±0.14	84.19±0.12	88.35±0.32	91.67±0.12

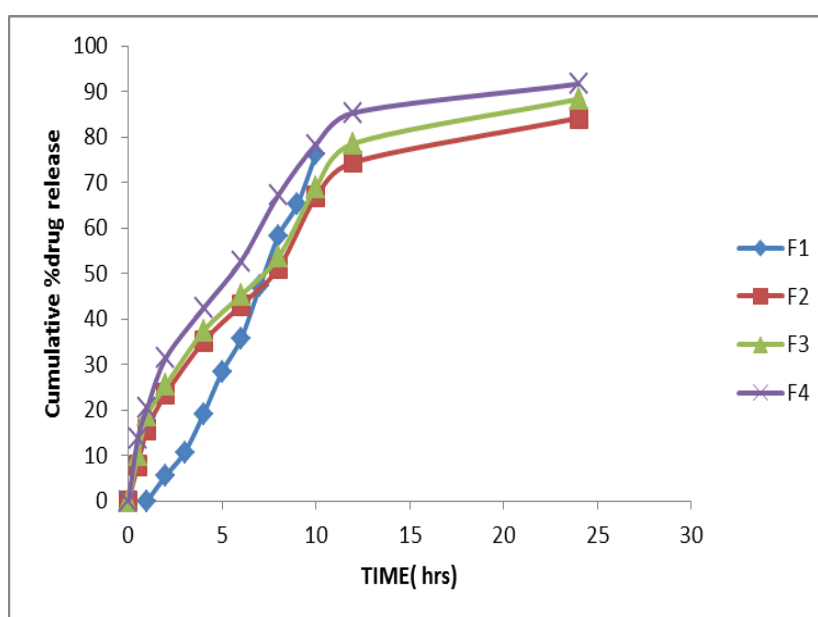


Fig no 14: In vitro Diffusion profile of F1, F2, F3 & F4

Over all parameters

Table no 22: Parameters

Parameters	F1	F2	F3	F4
pH	6.5 ± 0.016	6.6 ± 0.153	6.7 ± 0.136	6.8 ± 0.134
Viscosity 6 rpm	15000±0.12	12000±0.34	18000±0.11	20000±0.21
Viscosity 12 rpm	10000±0.23	9500±0.45	16000±0.35	18000±0.31
Drug content	99.90± 1.10	99.94 ± 7.40	99.95 ± 4.10	99.98 ± 1.80
Homogeneity	Good	Good	Good	Good
Skin irritation	Nil	Nil	Nil	Nil
In vitro drug release up to 24hrs	76.32±0.14	84.19 ±0.12	88.35±0.32	91.67±0.12

8. CONCLUSION

The present cream formulation was developed by taking into consideration that in cream formulations there is present no direct contact of active drug with stomach wall. This can be a reason to remove the chances of gastric mucosal damage to a reasonable level that is caused by the use of solid dosage forms of NSAIDs. The cream formulation contains Diclofenac Sodium along with extract of *Withania somnifera* may help prevent Rheumatoid arthritis and Osteoporosis. Diclofenac Sodium is an NSAID that is very effective to mimic the pain and inflammation in arthritis patients and *Withania somnifera* performs a synergistic anti-inflammatory effect.

From the data we have found that the prepared topical Diclofenac cream formulation with herbal extract releases of drug over a period of 24 hours. prepared cream with diclofenac alone & in combination with different ratios of *withania somnifera* shows similar dissolution profile. The dissolution of cream with diclofenac alone shows 76.32% after 1hrs whereas creams with combination of diclofenac & *withania somnifera* F2 shows 84.19% F3 shows 88.35% F4 shows 91.67%.

From the In –vitro drug diffusion study we have concluded that the cream formulation prepared, controls the release of drug for longer period of time which will be helpful to avoid the more fluctuation and also reduces the cost of therapy.

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