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DEVELOPMENT OF A TOPICAL ANTI-INFLAMMATORY GEL FROM VITEX NEGUNDO EXTRACT AND EVALUATING IT

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ABSTRACT

Transdermal route offers several potential advantages over conventional routes. These advantages includes avoidance of first pass metabolism, predictable and extended duration of action, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in the blood levels, and most important it provides patient convenience. But one of the major problems for efficient drug delivery is low penetration rate. Gel system showed topical delivery with higher transdermal flux and higher skin deposition and became an attractive option as it has several desirable advantages. Prepared is a prescription topical retinoid sold as a cream or gel. This medication is approved for treatment of psoriasis, acne, and sun damaged skin. To confirm the presence of vesicular structure, formulations were visualized under microscope at different magnified fields, which showed presence of lipid bilayer as well as spherical structure of vesicles. Using the same microscopic method and special software "particle size analysis", size of vesicle was determined for sonicated. Vesicular size was found to be in the range of 0 – 5.483 µm. Vesicular size was reduced up to 3 folds by sonication in the evaluation. After confirmation regarding existence of vesicles and their size, drug entrapped by vesicular system was evaluated by ultra-centrifugation. Which showed effective sub dermal deposition and indicated better sub dermal action. The present study is related to the preparation of the transdermal gel for the treatment of inflammation by using the extracts of vitex negundo by the study it can be concluded that prepared gel is promising route of drug administration. Even though the TDDS faces the problem of drug permeation because of the rigid stratum corneum, it can be overcome by the use of penetration enhancers such as ethanol. The size of the Gel can be reduced by sonication

thereby improving the skin permeation properties of gel. By encapsulating gel the frequency of dosing can be reduced as gel cause the delivery of drug for almost 10hrs.

KEYWORDS: *Preparation of the transdermal gel for the treatment of inflammation by using the extracts of vitex negundo by avoiding the fluctuation in the blood levels.*

INTRODUCTION

Vitex negundo, commonly known as the Chinese chaste tree, five-leaved chaste tree or horseshoe vitex,is a large aromatic shrub quadrangular, densely whitish, tomentose branch lets. It is widely used in folk medicine, particularly in South and Southeast Asia. Vitex negundo is an erect shrub or small tree growing from 2 to 8 m (6.6 to 26.2 ft.) in height. The bark is reddish-brown. Its leaves are digitate, with five lance late leaflets, sometimes three. Each leaflet is around 4 to 10 cm (1.6 to 3.9 in) in length, with the central leaflet being the largest and possessing a stalk. The leaf edges are toothed or serrated and the bottom surface is covered in hair. The numerous flowers are borne in panicles 10 to 20 cm (3.9 to 7.9 in) in length. Each is around 6 to 7 cm (2.4 to 2.8 in) long and are white to blue in colour. The petals are of different with the middle lower lobe being the longest. Both the corolla and calyx are covered in dense hairs. The fruit is a succulent drupe, 4 mm (0.16 in) in diameter, rounded to egg-shaped.

IDENTIFICATION OF VITEX NEGUNDO

Common names of Vitex negunda in different languages include:

- Bengali : Nirgundi, Nishinda, Samalu
- English : Five-leaved chaste tree, Horseshoe vitex, Chinese chaste tree
- Hindi : Mewri, Nirgundi, Nisinda, Sambhalu, Sawbhalu
- Sanskrit : Nirgundi, Sephalika, Sindhuvara, Svetasurasa, Vrikshaha

- Tamil : Chinduvaram,Nirnochchi,Nochchi,Notchi,Vellai-nochchi
- Telugu : Sindhuvara, Vavili, Nalla-vavili,Tella-vavili.

Chemistry

The principal constituents of the leaf juice are Castilian, isoorientin, chrysophenol D, luteolin, P–hydroxybenzoic acid and D-fructose. The main constituents of the oil are sabinene, linalool, terpinen-4-ol, β-caryophyllene, α-guaiene.

Uses

Vitex negundo is the richest source of stable Vitamin C. This richness in Vitamin C makes the Nirgundi one of the best anti-inflammatory and anti-biotic (not by killing the cells…but by increasing the immunity of the body). Nirgundi is a drug of choice for problems where inflammation has occurred like- pharyngitis, tonsillitis, endometriosis and even orchitis.

1. Effect on doshas – Good for pacifying Vata and Kapha
2. Taste – Bitter, pungent, astringent
3. Energetics – Heating
4. Main Traditional Action: analgesic, spleen and liver tonic, antipyretic, anti- hyper and hypothyroidism
5. Other Traditional Uses: Leucoderma or vitiligo, consumption, inflammations, eye diseases, spleen enlargement, bronchitis, asthma, biliousness, painful teething of children arthritis, intestinal worms, fever, ulcers, skin diseases, nervous disorders and leprosy.

- Herb Powder helpful in soothing the inflammation and swelling of joints and muscles.
- Nirgundi is best analgesic, anti-inflammatory, antibacterial.
- Nirgundi is used for cleaning and healing wounds
- Nirgundi is also used as a hair tonic
- Nirgundi leaves after heated are tied over the affected part in headache, scrotal swelling, and arthritic pain.
- Decoction prepared form Nirgundi leave powder is used for tub bath in endometritis, colitis and orchitis, in these conditions it reduces the obstruction of blood and amakapha, increases perspiration, reduces edema and also relieves pain.
- Gargles with decoction of Nirgundi leaves powder are useful in pharyngitis, stomatitis, difficulty in deglutition and inflammation.
- Nirgundi is an analgesic, brain tonic and alleviates vata, therefore useful in headache, sciatica, rheumatic arthritis, and synovitis, improves memory.

- Nirgundi improves appetite and digestion. It is useful in loss of appetite, anorexia, ama-dosha, hepatitis and is anthelmintic.
- Nirgundi improves blood circulation process.
- Nirgundi improves ventilation therefore used in Kapha dominant cough, asthma, pneumonia and pleurisy.
- Nirgundi useful in dysuria and anuria. In gonorrheal infection it helps in passing urine smoothly.
- Nirgundi improves menstrual flow therefore used in dysmenorrhea and obstetric conditions.
- Nirgundi is used as a anupan or a main drug in typhoid and malaria fever.
- Nirgundi is stimulated every part of the body, therefore it acts as a rasayan in body.
- The oil is used to treat sinus conditions, wounds, ulcers, syphilis, and skin disease.
- In strengthening hair, reduce swelling and nausea, Helps curing swollen joints and its pain. Use 61.8% of the oil. In vitro and animal studies have shown that chemicals isolated from the plant have potential anti- inflammatory, antibacterial, antifungal and analgesic activities

Inflammation

(Latin, inflammation) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.

Inflammation is a protective response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair.

The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.

Too little inflammation could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the organism. In contrast, chronic inflammation may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). Inflammation is therefore normally closely regulated by the body.

Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

Inflammation is not a synonym for infection. Infection describes the interaction between the action of microbial invasion and the reaction of the body's inflammatory defensive response — the two components are considered together when discussing an infection, and the word is used to imply a microbial invasive cause for the observed inflammatory reaction. Inflammation on the other hand describes purely the body's immunovascular response, whatever the cause may be. But because of how often the two are correlated, words ending in the suffix –it is (which refers to inflammation) are sometimes informally described as referring to infection. For example, the word urethritis strictly means only "urethral inflammation", but clinical health care providers usually discuss urethritis as a urethral infection because urethral microbial invasion is the most common cause of urethritis.

Causes Physical

- Burns
- Frostbite
- Physical injury, blunt or penetrating
- Foreign bodies, including splinters, dirt and debris
- Trauma
- Ionizing radiation.

Biological

- Infection by pathogens
- Immune reactions due to hypersensitivity
- Stress.

Chemical

- Chemical irritants

- Toxins
- Alcohol.

Psychological

- Embarrassment
- Excitement.

Types

- Colitis Phlebitis
- Bursitis
- RSD/CRPS
- Rhinitis
- Tendonitis
- Tonsillitis
- Vasculitis
- Cystitis
- Dermatitis.

Cardinal signs

Infected ingrown toenail showing the characteristic redness and swelling associated with acute inflammation. Acute inflammation is a short-term process, usually appearing within a few minutes or hours and begins to cease upon the removal of the injurious stimulus. It is characterized by five cardinal signs. An acronym that may be used to remember the key symptoms is "PRISH", for pain, redness, immobility (loss of function), swelling and heat.

The traditional names for signs of inflammation come from Latin:

- Dolor (pain)
- Calor (heat)
- Rubor (redness)
- Tumor (swelling)
- Function laesa (loss of function).

Process of acute inflammation

The process of acute inflammation is initiated by resident immune cells already present in the involved tissue, mainly resident macrophages, dendritic cells, histiocytes, Kupffer cells and

mast cells. These cells possess surface receptors known as pattern recognition receptors (PRRs), which recognize (i.e., bind) two subclasses of molecules: pathogen-associated molecular patterns(PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs are compounds that are associated with various pathogens, but which are distinguishable from host molecules. DAMPs are compounds that are associated with host-related injury and cell damage.

At the onset of an infection, burn, or other injuries, these cells undergo activation (one of the PRRs recognizes a PAMP or DAMP) and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow causes the redness (rubor) and increased heat (calor). Increased permeability of the blood vessels results in an exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (tumor). Some of the released mediators such asbradykinin increase the sensitivity to pain (hyperalgesia, dolor). The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly neutrophils and macrophages, outside of the blood vessels (extravasation) into the tissue. The neutrophils migrate along a chemotactic gradient created by the local cells to reach the site of injury. The loss of function (function laesa) is probably the result of a neurological reflex in response to pain.

In addition to cell-derived mediators, several a cellular biochemical cascade systems consisting of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response. These include the complement system activated by bacteria and the coagulation and fibrinolysis systems activated by necrosis, e.g. a burn or a trauma.[5]

The acute inflammatory response requires constant stimulation to be sustained. Inflammatory mediators are short- lived and are quickly degraded in the tissue. Hence, acute inflammation begins to cease once the stimulus has been removed.

Vasodilation and increased permeability

These mediators staging framework at the inflammatory tissue site in the form of a fibrin lattice - as would construction scaffolding at a construction site - for the purpose of aiding phagocytic debridement and wound repair later on. Some of the exuded tissue fluid is also funneled by lymphatic's to the regional lymph nodes, flushing bacteria along to start the recognition and attack phase of the adaptive immune system.

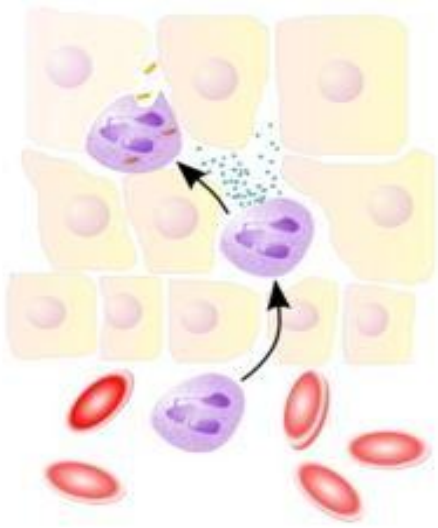
Plasma cascade systems

- The complement system, when activated, creates a cascade of chemical reactions that promotes opsonisation, chemo taxis, and agglutination, and produces the MAC.
- The kinin system generates proteins capable of sustaining vasodilation and other physical inflammatory effects.
- The coagulation system or clotting cascade, which forms a protective protein mesh over sites of injury.
- The fibrinolysis system, which acts in opposition to the coagulation system, to counterbalance clotting and generate several other inflammatory mediators.

Cellular component

The cellular component involves leukocytes, which normally reside in blood and must move into the inflamed tissue via extravasation to aid in inflammation. Some act as phagocytes, ingesting bacteria, viruses, and cellular debris. Others release enzymatic granules that damage pathogenic invaders. Leukocytes also release inflammatory mediators that develop and maintain the inflammatory response. In general, acute inflammation is mediated by granulocytes, whereas chronic inflammation is mediated by mononuclear cells such as monocytes and lymphocytes.

Leukocyte extravasation



Neutrophils migrate from blood vessels to the infected tissue via chemotaxis, where they remove pathogens through phagocytosis and degranulation.

Main article: Leukocyte extravasation

Various leukocytes are critically involved in the initiation and maintenance of inflammation. These cells must be able to get to the site of injury from their usual location in the blood, therefore mechanisms exist to recruit and direct leukocytes to the appropriate place. The process of leukocyte movement from the blood to the tissues through the blood vessels is known as extravasation, and can be divided up into a number of broad steps:

1. **Leukocyte margination and endothelial adhesion**: Activated tissue macrophages release cytokines such as IL- 1 and TNFα, which bind to their respective G protein-coupled receptors on the endothelial wall. Signal transduction induces the immediate expression of P-selectin on endothelial cell surfaces. This receptor binds weakly to carbohydrate ligands on leukocyte surfaces and causes them to "roll" along the endothelial surface as bonds are made and broken. Cytokines from injured cells induce the expression of E-selectin on endothelial cells, which functions similarly to P-selectin. Cytokines also induce the expression of integrin ligands such as ICAM-1 andVCAM-1 on endothelial cells, which further slow leukocytes down. These weakly bound leukocytes are free to detach if not activated by chemo kines produced in injured tissue. Activation increases the affinity of bound integrin receptors for ICAM-1 and VCAM-1 on the endothelial cell surface, firmly binding the leukocytes to the endothelium.
2. **Migration across the endothelium, known as transmigration, via the process of diapedesis:** Chemokine gradients stimulate the adhered leukocytes to move between endothelial cells and pass the basement membrane into the tissues.
3. **Movement of leukocytes within the tissue via chemotaxis:** Leukocytes reaching the tissue interstitium bind to extracellular matrix proteins via expressed integrin’s and CD44 to prevent their loss from the site. Chemo attractants cause the leukocytes to move along a chemotactic gradient towards the source of inflammation.

Resolution of inflammation

The inflammatory response must be actively terminated when no longer needed to prevent unnecessary "bystander" damage to tissues. Failure to do so results in chronic inflammation, and cellular destruction. Resolution of inflammation occurs by different mechanisms in different tissues. Mechanisms that serve to terminate inflammation include:

- Short half-life of inflammatory mediators in vivo.
- Production and release of transforming growth factor (TGF) beta from macrophages
- Production and release of interleukin 10 (IL-10)
- Production of anti-inflammatory lipoxins

- Down regulation of pro-inflammatory molecules, such as leukotrienes.
- Up regulation of anti-inflammatory molecules such as the interleukin 1 receptor antagonist or the soluble tumor necrosis factor receptor (TNFR)
- Apoptosis of pro-inflammatory cells
- Desensitization of receptors.
- Increased survival of cells in regions of inflammation due to their interaction with the extracellular matrix (ECM)
- Down regulation of receptor activity by high concentrations of ligands
- Cleavage of chemokines by matrix metalloproteinases (MMPs) might lead to production of anti-inflammatory factors.

LIST OF MATERIALS

Name Of Material
VITEX NEGUNDO
Carbopol
PEG4000
Methanol
Methyl paraben
Water

Fresh leaves of Vitex negundo were collected. They washed under running tap water and the surface sterilized using 70% ethanol. The peels were shade dried for 3 to 4 weeks. Then the leaves were subjected to drying for about 6-8 days.

Plant Extracts Preparation

The dried leaves were collected for extraction. Fifty grams of plant material was weighed in an motor and made into fine powder such that it should pass through the sieve no 40.Followed by transferring of this powder into 500 ml beaker .To the above beaker 375ml of ethanol and 125 ml of water is added and kept a side for 72hours (70:30).which is filtered by using vacuum filter.

The extract which contains required chemical constituents was subjected to evaporation process using Rota evaporator for the complete evaporation of solvent material. After the complete evaporation of solvent the powder material was get freed from even traces amounts of solvents using lyophilisation technique. The pure powder material was subjected to specific tests for compounds using standard tested for its anti-Inflammatory properties using various.

PHYTOCHEMICAL ANALYSIS

The ethanolic extract of vitex negundo was subject to preliminary chemical screening for the presence and absence of active physic chemical constituents by following methods.

Test for alkaloids

Treated with dilute HCL and filtered, the filtrate is treated with following alkoliodal agents Mayer's reagent.

Appearance of cream colour indicates presence of alkaloids.

Dradendroff's test

Appearance reddish ppt by addition of dragendr off reagent indicates the presence of alkaloids.

Hager's test

Appearance of yellow colour ppt after addition of hager's reagent indicates presence of alkalolids.

Quinoline alkaloid test

Addition of glacial acetic acid to the extract gives reddish brown fumes, while extract gives blue fluorescence in u.v.

Test for carbohydrates

Small quantity of alcohol extract is dissolved in distilled water by using cyclometer. which is subjected to to detect the presence of sugars.

1) MOLISH REAGENT
2) FEHLING'REAGENT
3) BENEDICT'S REAGENT

4) BARFOED'S REAGENT

Test for proteins Biuret's test

Addition of copper sulphate and NAOH solution to the extract gives violet colour indicating the presence of examined proteins.

Millions test

Treatment of extract with million's reagent gives pink colour indicating the presence of proteins.

Test for flavonoids

5ml of the extract solution will be hydrolysed with 10% v/v sulphuric acid and cool then, it will extract with diethyl ether and divided into three portions in three separate test tubes.1ml of dilute sodium carbonate, 1ml of 0.1N sodium hydroxide, 1ml of strong ammonia will be added to the first, second and third respectively.in each test tube, development of yellow colour demonstrate the presence of flavonoids.

Shinoda's test

The extract will be dissolve in alcohol to that one piece of magnesium followed by conc. HCL will be added in drops and heated appearance of magenta colour shows the presence of flavonoids.

Test for gums and mucilage

The extract will be treated with 25ml of absolute alcohol, and then solution will be filtered the filtrate will be for its swelling properties.

Test for glycosides

A pinch of the extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicated the presence of glycosides.

Test for saponins

Foam test 1ml of the extract was diluted to 20ml with distilled water and shaken well in test tube and noted for the formation of foam in the upper part of the test tube.

Test for fixed oils

Sodium hydroxide test mix 1ml of 1% copper sulphate and 5 drops of fixed oil then add 5 drops of 10% sodium hydroxide solution. A clear blue solution will be obtained.

Sodium sulphate test

Take 5 drops of sample in a test tube and a pinch of sodium sulphate. Pungent odour will emanate from the tube.

Constituents	**Present/Nil**
Carbohydrates	Present (isoorientin)
Proteins	Present
Flavonoids	Present (casticin)
Tannins	Present
Glycosides	Present (chrysophenol)
Fixed oils	Nil
Alkaloids	Present

Organoleptic Characters

The extracts were tested for their psycho rheological properties like color, odour and taste.

Colour	Green
Odour	Characteristic
Taste	Acrid

Ph measurement

Solution was prepared by dissolving 1gm of Extract in 30ml of distilled water (ph 7). The ph of Gel gel was determined by using digital ph meter. The measurement was done by bringing the probe of the ph meter in contact with the samples.

IN-VITRO ANTI INFLAMMATORY ACTIVITY

Anti-inflammatory activity of an extract is known by two methods

1) INVIVO METHOD
2) INVITRO METHOD

Invitro anti-inflammatory activity of drug can be determined by following methods

1. HRBC MEMBRANE STABILOZATION METHOD.
2. LIPOXYGENASE INHIBITION.
3. TRYPSIN INHIBITION.
4. NITRIC OXIDE SCAVENGING.
5. LTB4 RELEASE.

6. HISTAMINE RELEASE USINGRBL-2H3.

DRUG RELEASE STUDY FROM DIALYSIS MEMBRANE

The skin permeation of Extract from Gel formulation was studied by using an open ended diffusion cell specially designed laboratory according to the literates. The effective permeation area of the diffusion cell and receptor cell volume was 2.4 cm and 200 ml respectively. The temperature was maintained at 37 ± 0.5°C.[3]

The receptor compartment contained 200 ml of pH 6.8 phosphate buffer and was constantly stirred by magnetic stirrer at 100 rpm. The dialysis was prepared by using semi permeable membrane from egg. The membrane was tied to an open end tube. This served as the donor compartment whereas the beaker containing phosphate buffer served as the receptor compartment. Gel formulation [EF1-EF7 (20ml suspension) and for optimized Gel gel (10gm)] was applied to the dialysis membrane and the content of diffusion cell was kept under constant stirring. Then 5 ml of samples were withdrawn from receptor compartment of diffusion cell at predetermined time intervals and analysed by spectrometric method at 234 nm after suitable dilution. The receptor phase was immediately replenished with equal volume of fresh pH 6.8 buffer. Triplicate experiments were conducted for drug release studies.

In-vitro release kinetics

To analyse the in vitro release data, various kinetic models were used to describe the release kinetics. The zero order rate equation describes the systems where the drug release rate is independent of its concentration. The first order rate equation describes the release from system where release rate is concentration dependent.

Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion.

The results of in vitro release profile obtained for all the formulations were plotted in modes of data treatment as follows:

- Zero -order kinetic model– Cumulative percentage drug released versus time.
- First–order kinetic model– Log cumulative percent drug remaining versus time.
- Higuchi's model– Cumulative percent drug released versus square root of time.
- Kerseymere equation/ Peppa's model–Log cumulative percent drug released versus log

time.

Zero order kinetics

Zero order release would be predicted by the following equation:

$A_t = A_0 - K_0 t$

Where,

A_t = Drug release at time't' A_0 = Initial drug concentration

K_0 = Zero- order rate constant (hr-1)

When the data is plotted as cumulative percent drug released versus time, if the plot is linear then the data obeys Zero

– order kinetics and its slope is equal to Zero order release constant K_0.

First order kinetics

First order release could be predicted by the following equation:

$$Log\ C = \frac{\log C_O - Kt}{2.303}$$

Where

C = Amount of drug remained at time't' C_0 = Initial amount of drug.

K = First - order rate constant (hr^{-1})

When the data is plotted as log cumulative percent drug remaining versus time if it yields a straight line it indicates that the release follows first order kinetics. The constant 'K1' can be obtained by multiplying 2.303 with the slope value.

Higuchi's model

Drug release from the matrix devices by diffusion has been described by following Higuchi's classical diffusion equation,

$$Q = (D[2A - C_s]C_s\, t)^{\frac{1}{2}}$$

Where,

Q = Amount of drug release at time't'

D = Diffusion coefficient of drug in the matrix

A = Total amount of drug in unit volume of matrix Cs = Solubility of drug in polymeric matrix

t = Time (hrs. at which Q amount of drug is released).

Above equation can be simplified if we assume that 'D', 'Cs' and 'A' are constant. Then the equation becomes:

$$Q = (Kt)^{\frac{1}{2}}$$

When the data is spited according to the above equation i.e. cumulative drug release versus square root of time if it yields a straight line then it indicates that the drug was released by diffusion mechanism. The slope is equal to 'K' (Higuchi's 1963).

Korsmeyer equation / Peppa's model

To study the mechanism of drug release from the liposomal solution, the release data was also fitted to the well- known exponential equation (Korsmeyer equation/ Peppa's law equation), which is often used to describe the drug release behaviour from polymeric systems.

$$\frac{M_t}{M_\alpha} = Ktn$$

Where

Mt / Mα = Fraction of drug released at time't'

K = Constant incorporating the structural and geometrical characteristics of drug / polymer system. n = Diffusion exponent related to the mechanism of the release.

Above equation can be simplified as follows by applying log on both sides,

$$Log\frac{Mt}{M\alpha} = Log\ K + n\ Log\ t$$

STABILITY STUDIES

Stability study was carried out for Extract Gel preparation at two different temperature i.e. refrigeration temperature (4 ± 2° C) and at room temperature (27 ± 2°C) for 8 weeks (as per ICH guidelines). The formulation was subjected to stability study and stored in borosilicate container to avoid any sort of interaction between the Gel preparation and glass of container, which may affect the observations.

IN-VITRO DRUG PERMEATION STUDIES

In-vitro skin permeation study or in-vitro diffusion study has been extensively studied, developed and used as an indirect measurement of drug solubility, especially in preliminary assessment of formulation factors and manufacturing methods that are likely to influence

bioavailability.

The objectives in the development of in-vitro diffusion tests are to show the release rate and extent of drug from the dosage form. The in-vitro drug permeation study of Extract of Curcuma longa and aloe Vera from gel formulation was studied using Franz diffusion cell and the method described in methodology chapter.

The release data was obtained for all the gel formulations. Spectrometric results were obtained and given consideration to sampling loss, to calculate actual cumulative drug diffused was calculated since the volume of receptor cell was only 20 ml. The obtained diffused amount of drug was extrapolated to diffusion by unit surface area of semi permeable membrane. These cumulative values were plotted as a function of time and steady state transdermal flux was calculated from the slop of linear portion. Stability of drug and stability of vesicles are the major determinant for the stability of formulation. Studies were carried to evaluate total drug content at room temperature (27±2° C) and at refrigeration temperature (4±2° C). Samples were collected for every 2 weeks and absorbance was seen at 255nm in U.V spec.

RESULTS AND DISCUSSION

ANALYTICAL STUDY

Scanning of drug

Extract was scanned in methanol between 200 nm and 400 nm using ultraviolet spectrophotometer. Extract of Curcuma longa and aloe Vera was identified by its light absorption pattern which follows the absorption of light in the range 220 to 360 nm and a maximum absorbance at about 234 nm. A broad shoulder at about 255 nm was observed which confirm the presence of Extract of vitex negundo. Extract of Vitex negundo gave highest peak at 255 nm and the same was selected for further evaluations.

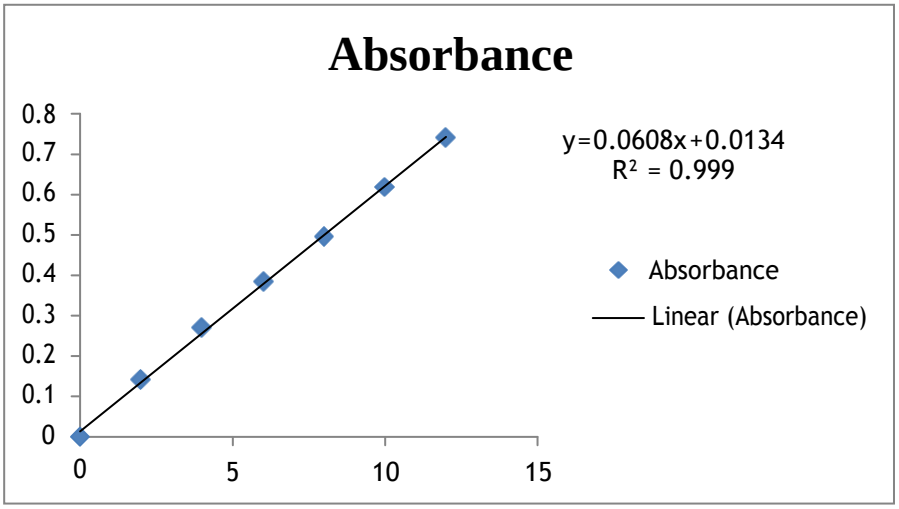
Calibration curve in water (make up with ph 6.8 phosphate buffer)

Standard solutions of different concentrations were prepared and their absorbance was measured at 255 nm. Calibration curve was plotted against drug concentrations versus absorbance as given.

Determination of λmax of Extract of vitex negundo in methanol-- λmax = 255 nm

Concentration (µg /ml)	Absorbance
0	0
2	0.14
4	0.269
6	0.383
8	0.496
10	0.618
12	0.740

Standard graph of Extract of vitex negundo



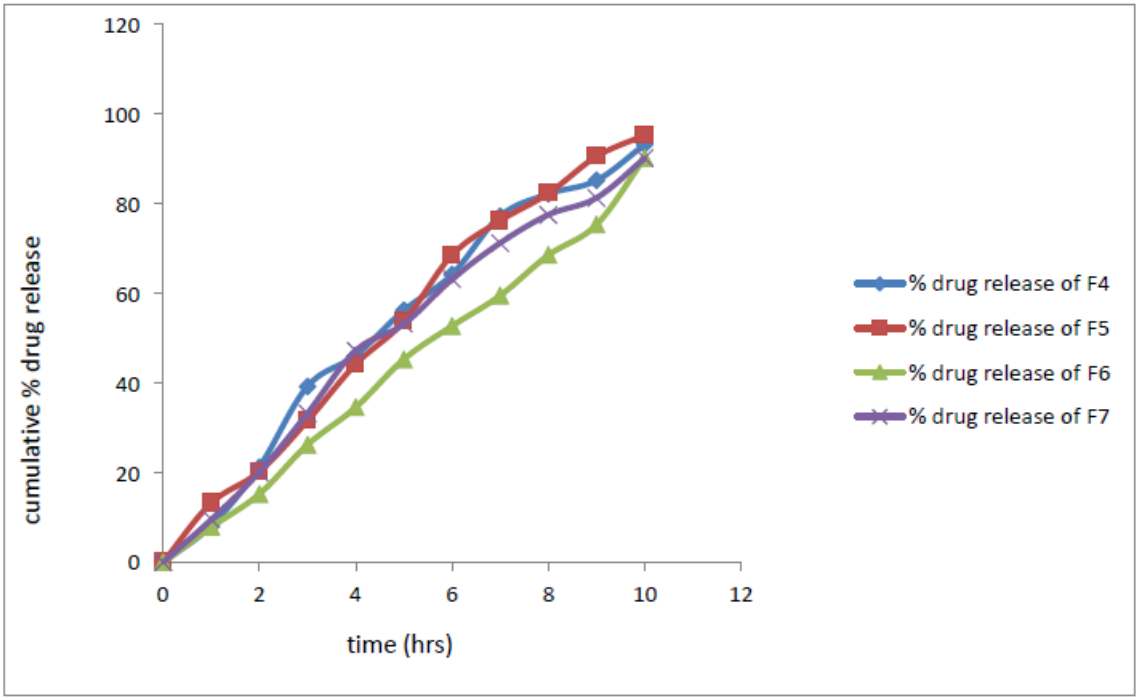
PREPARATION OF DOSAGE FORM FROM EXTRACT OF VITEX

NEGUDO VITEX Drug release Profile

	Extract	carbapol	Peg4000	ethanol	Distilled water	Methyl paraben
F1	200mg	300mg	75mg	10ml	5ml	10mg
F2	200mg	300mg	100mg	10ml	5ml	10mg
F3	200mg	300mg	125mg	10ml	5ml	10mg
F4	200mg	300mg	150mg	10ml	5ml	10mg
F5	200mg	300mg	175mg	10ml	5ml	10mg
F6	200mg	300mg	200mg	10ml	5ml	10mg

	Cumulative % drug release						
Time	F1	F2	F3	F4	F5	F6	F7
0	0	0	0	0	0	0	0
1	4.02	4.78	14.25	8.22	13.21	7.85	9.54
2	9.54	12.47	20.11	21.25	20.25	15.25	20.14
3	19.88	22.35	27.45	39.25	31.58	26.24	33.25
4	24.58	33.65	41.22	46.21	44.21	34.58	47.21
5	34.55	46.51	49.58	56.21	53.75	45.31	53.21
6	46.58	55.12	54.55	64.22	68.55	52.74	63.14
7	50.14	66.32	63.22	77.25	76.21	59.54	71.25

	8	60.21	72.67	70.25	82.21	82.31	68.54	77.54	
	9	72.55	80.45	80.22	85.21	90.55	75.34	81.32	
	10	78.54	86.54	85.64	93.21	95.32	90.21	90.15	



NEGUDO VITEX Cumulative % drug release Profile.

Gel formulations composed of PEG4000, Extract of Vitex negundo and ethanol were prepared using the method detailed in last chapter titled materials and methods and also according to the literature with little modification in it. Gel suspension was slight yellowish in colour and hazy in appearance after sonication. Different characteristics of extract were further evaluated and results were reported under characterization. Since the physical characterization is meant for physical integrity of the dosage form, the results were pooled at one place. Discussion on the results, described for gel formulation under the same heading.

CONCLUSION

The results of this work suggest that the compound extracted from virtex nigundo leaves have anti-inflammatory properties and this effect is increased by increasing the quantity of this compound UPTO 300mg further increase in dose results in decrease of anti-inflammatory effect., which can be used as an alternative for novel medicine. The crude fruits and leaves were collected from local gardens and which are then subjected for drying, extracted using soxhlet apparatus. The extracts were subjected to test the anti-inflammatory activity using extraction method. From the assay it was confirmed that the compounds are having anti-

inflammatory activity, IC 50 values were also determined. Therefore, pharmacological test is necessary to isolate and characterize their active compounds. Moreover, these plants extract should be investigated in vivo to better understand their safety, efficacy and properties.

SUMMARY

Transdermal route offers several potential advantages over conventional routes. These advantages includes avoidance of first pass metabolism, predictable and extended duration of action, minimizing undesirable side effects, utility of short half- life drugs, improving physiological and pharmacological response, avoiding the fluctuation in the blood levels, and most important it provides patient convenience. But one of the major problems for efficient drug delivery is low penetration rate.

Gel system showed topical delivery with higher transdermal flux and higher skin deposition and became an attractive option as it has several desirable advantages. Prepared is a prescription topical retinoid sold as a cream or gel. This medication is approved for treatment of psoriasis, acne, and sun damaged skin

To confirm the presence of vesicular structure, formulations were visualized under microscope at different magnified fields, which showed presence of lipid bilayer as well as spherical structure of vesicles. Using the same microscopic method and special software "particle size analysis", size of vesicle was determined for sonicated. Vesicular size was found to be in the range of 0 – 5.483 µm. Vesicular size was reduced up to 3 folds by sonication.

After confirmation regarding existence of vesicles and their size, drug entrapped by vesicular system was evaluated by ultra-centrifugation. Sonicated particles containing 30% w/w ethanol showed higher value i.e. 79.62%. In-vitro release was carried out using dialysis membrane. The values of drug release were F_1 78.54%, F_2 80.45%, F_3 85.64, F_4 84.21%, F_5 89.32%,. The order of drug release was found to be first order for all the formulations. Percentage drug accumulation into skin was also found to be maximum by the gel containing PEG400 350. Which showed effective sub dermal deposition and indicated better sub dermal action.

CONCLUSION

From the present study it can be concluded that prepared gel is promising route of drug administration. Even though the TDDS faces the problem of drug permeation because of the rigid stratum corneum, it can be overcome by the use of penetration enhancers such as ethanol. The size of the Gel can be reduced by sonication thereby improving the skin permeation properties of gel. By encapsulating gel the frequency of dosing can be reduced as gel cause the delivery of drug for almost 1.

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