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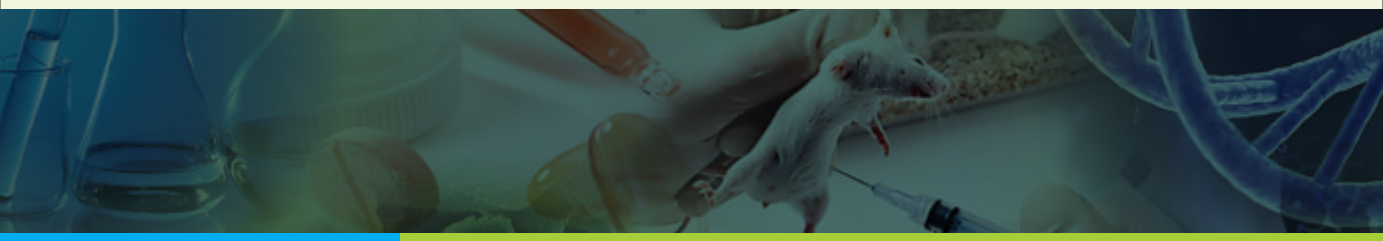

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Content

EDITORIAL MESSAGE

It gives us immense pleasure to announce the second issue of Nirma University Journal of Pharmaceutical Sciences in 2023. It is a bi-annual Journal launched exclusively to publish research and review papers and articles on the topics and issues in the area of Pharmaceutical Sciences.

Institute of Pharmacy was established in the year 2003 under Nirma University. This year marks the completion of 20 years of our Institute from its establishment. Institute of Pharmacy, Institute has been ranked 37th in India Ranking 2023 by Ministry of Human Resource Development, (MHRD), Government of India in its National Institutional Ranking Framework (NIRF). The institute received 1st rank by GSIRF 2023 with Five Star Rating.

We sincerely hope that the Nirma University Journal of Pharmaceutical Sciences (NUJPS) will achieve its mission and goals in the field of pharmaceutical sciences with unwavering dedication and perseverance.

We congratulate all the authors and co-authors whose papers are published in this issue and express our sincere thanks to them.

Members of the editorial team are determined to make the NUJPS a significant journal.

Wish you all a Happy New Year 2024 from an editorial team member of Nirma University Journal of Pharmaceutical Sciences (NUJPS).

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REVIEW ARTICLE

CHALLENGES AND NOVEL APPROACHES FOR THE TREATMENT OF ONYCHOMYCOSIS

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ABSTRACT

Onychomycosis is a fungal disease of nail and approximately half of the patients of nail disorders suffers from it. The current review focuses on human nail, disease, factors affecting drug penetration for understanding the challenges in drug delivery, as well as, current and novel approaches for the treatment of onychomycosis. Human nail composed of compressed keratinized cell act as major barrier for drug penetration for treatment of damaged nail. Variety of factors affecting drug penetration through human nail like molecular weight, partition coefficient, affinity to keratin, pKa and ionization, type of vehicle, use of penetration enhancers, etc are to be taken into consideration while design and development of topical formulations. Apart from oral treatments, mechanical approaches as nail abrasion, physical approaches like iontophoresis, etching, laser, ultrasound have also been explored for penetration enhancement. Formulation scientists are working towards development of novel nano-formulations like nanoparticles, SLN, NLC, liposomes, spanlastics, nano-emulsions, etc. Lastly, the review focuses on several attempts for drug penetration studies using human nail or bovine hoof membrane.

Keywords: Onychomycosis, Nail drug delivery, Transungual formulations

INTRODUCTION

Human Nail

The human nail **(fig.1)** is comprised of bunch of keratinized cells as approximately 25 flattened dense dead layers which are tightly bound to one another, and having average thickness of about 0.5 to 1 mm and the growth rate of 2 to 3 mm per month of nail matrix in

single direction [1]. Structurally, the human nail is hard, translucent and having the convex structure, which contains alpha keratin in the fibrous form linked with cystine disulphide bonds (about 3.2% sulfar on dry basis) to provide tensile strength [2]. Nail plate can be further subclassified as dorsal, intermediate and ventral layer with ratio of 3:5:2 thick, whereby an intermediate plate layer exhibit softness and flexibility. Human nail is considered as most resistant barrier for penetration of any external material including drug molecules; and hence it become difficult to treat when affected adversely.

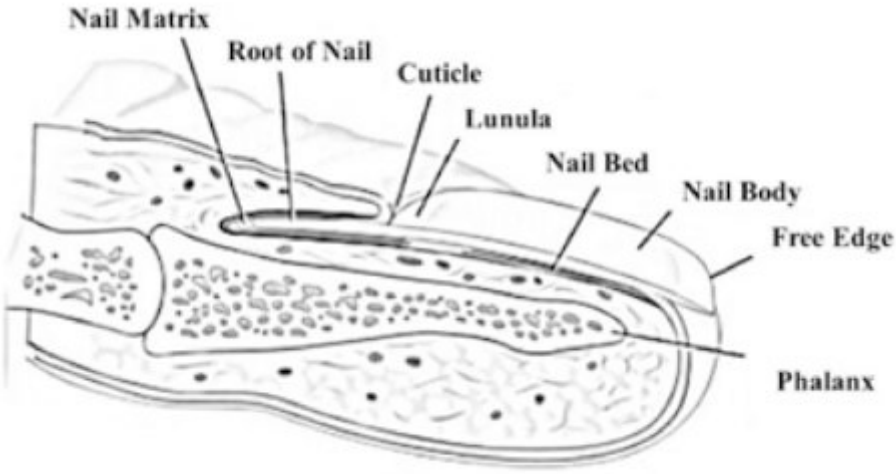

Figure 1: Anatomy of Normal Human Nail

Nail Diseases

Human nail is susceptible to wide range of disease or disorders as shown in **Table 1**, ranging from discolouration as yellow, white or red, pigmentations, brown colour bands, brittle nails, ingrowing nails, thicken nail plates, as well as infection [2]. Few of the disorder may be an adverse effect of systemic drugs as well as few reflects the diagnostic hint for other major ailment in body. Other major disease nail psoriasis is an inflammatory auto-immune disease which is observed in more than 80% of the patients having skin psoriasis [3].

Table 1 : Types of nail disorder with possible causative reason

Type of Nail Disorder	Possible causative reason
Nail Pitting	Psoriasis
Absent Part	Anonychia congenita
Cuticle invasion	Lichen pianos
Pigmentation & Ridging	Monilla
Distal Onycholysis	Tinea
Spoon Nails	Iron deficiency Anaemia
Discolored & Inverted Edges	Ectodermal dysplasia
Clubbing	Hypoxia, Malignancy or Toxins
Bitten Nails (Short)	Anxiety
Splinter Haemorrhage	Bac. Endocarditis
Yellow	Bronchiectasis, Lymphoma & Edema
Half & Half	Hepatic Necrosis
Ridging	Rheumatoid arthritis
Longitudinal Brown lines	Addisons's, Breast cancer & Melanoma
White Nails	Anemia
Red Nails	SLE, Polycythemia
Horizontal White & Pink Bands	Nephrotic Syndrome
Brittle Nails	Hypothyroidism

Onychomycosis

Onychomycosis, also known as tinea unguium, is one of the major nail infections which occurs at skin beneath the nail bed that comprises about 50% of the nail diseases, and it accounts for about one fifth of the population may develop it during life span [4]. In majority of the cases, the patient ignores it considering the cosmetic changes due to age or nature of work. If left untreated on-time, may cause discomfort as well as spread of infection to nearby tissues. Onychomycosis is originated from combining two Greek words “onyx” a nail having “mykes” a fungal infection. Onychomycosis development is observed due to microbial infections namely (i) Dermatophytes like Trichophyton rubrum as well as mentagrophytes; (ii) the Non-dermatophyte fungi like species from Acremonium, Alternaria, Aspergillus, Fusarium, Scytalidium, etc. as well as (iii) the Yeast like Candida albicans; among all, it has been observed that more than 90% were caused by Trichophyton family alone.

Onychomycosis can be further divided in to five different subtypes [5] by expert as

per the location and spread of disease. The more prevalent type of onychomycosis is Distal Lateral Subungual Onychomycosis (DLSO/DSO), causing nail bed and nail plate underside invasion. The Proximal White Subungual Onychomycosis (PWSO/PSO) is comparatively rare type and more observed in immunocompromised patients, whereby the fungal infection of proximal nail occurs through the cuticle which results as a white or yellow colour change of nail, keeping distal in ordinary condition. White superficial onychomycosis (SWO/WSO) is also a less common sub-type, shows opaque or white colour spots on the external nail plate getting amalgamate and spread over a period of time. Candida onychomycosis (CO) is observed in patients having the long-term muco-cutaneous yeast infection by *C. krusei, C. tropicalis* and *C. parapsilosis*. Lastly, most severe condition whereby the nail plate becomes thick and dystrophic is known as Total dystrophic Onychomycosis (TDO) as late-stage nail disease.

Poor hygiene conditions and/or weakened immunity are the obvious causes of nail disorder; however, the prevalence has been also observed with older age, improper cutting of nails, chronic exposure to pathogenic fungi, repeat trauma in the nail region as well as in few cases concurrent disease like diabetes; and the immunocompromised patients may observe the recurrence.

FACTORS AFFECTING NAIL PERMEATION

For the treatment of nail disorders, the drug must penetrate into the most resistant barrier of dead thick keratinized cell layer of nail plate. Traditionally, it has been observed that softening of nail through pre-treatment with aqueous solutions helps to improvise skin penetration to some extent; however, few chemical treatments have also resulted in loosening of the nail plate leading to complete detachment of nail causing traumatic condition for patient. Several drug related factors must be taken into consideration for design and development of treatment protocol.

Molecular Weight of Drug

The obvious relationship of molecular weight of drug for penetration through any membrane is inverse relation – i.e. larger molecules are difficult to penetrate; and the same in true for nail plate also. Moreover, the nail is hardest tissue of body as most resistant for penetration into or through the nail plate. Few researchers have proposed the “free volume theory of diffusion” for establishing the relationship as dependence of molecular weight on permeability to determine diffusion coefficient [6-8]. Studies revealed that hydration has increased the nail porosity, facilitated permeability of drug as reduction of resistance to diffuse by performing the comparative studies of contact with aqueous media against fully hydrated nail. Based on pharmacodynamic outcomes, it

can be correlated that drug with the molecular weight less than 350 gm per mole have exhibited desirable penetration of anti-fungal drug in onychomycosis treatment.

Partitioning of Drug Molecule

The partition coefficient (i.e. Log P) of molecule for octanol vs water has been an indicative of lipophilicity and predicts the permeation across membrane for majority of the biological membranes. However, for nail plate permeation, the studies revealed the plot of log of partition vs log of permeation resulted in zero value of slop indicating that the permeation is not dependent on lipophilicity of drug molecule. Moreover, the hydration studies have revealed that permeation is positively affected by increasing hydrophilicity of membrane, which suggest that unlike other biological membranes which are treated lipidic bilayer, the nail plate should be considered as hydrophilic hydrogel layer.

Affinity to Keratin

The keratin protein consists of major nail composition, and binding or affinity of drug to keratin is expected to significantly affect the drug performance in nail drug delivery. Binding data for drug to plasma protein are available for majority of drugs; however, very few have studied the drug binding to keratin matrix [9]. Few researchers have studied the drug binding of commonly used drug and penetration enhancers for nail treatment with either defatted keratinized powder with different drug concentration as in-vitro study, as well as, ex-vivo or in-vivo study with human nail. Study discovered that keratin binding is not only reducing drug permeability but also reduces anti-fungal activity of drug, demanding the higher concentration of drug to maintain similar activity. However, further it has been observed that keratin binding is reversible process leading to reduced activity in initial duration and providing prolonged drug action as per depletion of concentration as a function of time.

pKa & Ionization of Drug Molecule

Above factors suggested hydrophilic vehicle for nail drug delivery, leading to consideration of pka and degree of ionization of drug molecule in vehicle and its effect on site of action [10]. Uncharged molecules as well as drug at higher concentration exhibits higher permeation than charged molecules. The keratin in human nail exhibits iso-electric point between 4.0 to 5.0, i.e. it carries positive charge on nail for vehicle having pH below isoelectic point and negative charge for higher pH vehicle. The proof of concept observed by few researchers as study on miconazole, benzoic acid, pyridine, etc exhibited significant impact on diffusion and permeability by change in vehicle pH [11].

Type of Vehicle

The discussion propagates towards use of hydrophilic vehicle for nail delivery; however, it has several bottlenecks in

topical formulations as fast drain-out during routine washing or wiping and comparatively lesser adherent to nail plate. Hydrophilic solvents cause swelling of keratin fibrous to create pores and increases drug diffusion. Several studies conducted whereby comparison of use of alcohols, co-solvents, DMSO, etc which exhibited that change in solvent was not affecting the nail barrier, the drug permeation is independent of type of vehicle. It has also reported that lipophilic material and alcohol as cosolvent has reduced permeation, which may be due to deswelling of keratin fibres.

Use of Chemicals as Penetration Enhancer

The nail barrier resistance can be reduced by targeting the stability of keratin, which can be compromised by chemical attack on polar bonds, disulphides bonds, hydrogen linkages and peptide linkages in keratin structure. Urea and salicylic acid have been widely explored as nail softening agents, whereby urea act as keratolytic agent and salicylic acid is considered to dissolve the intracellular cement like glue structure [12]. However, there are several negative reports indicated absence of penetration enhancement or reduction i.e. 5-flourouracil even though softening of nail observed, which may be due to change in other factors like pH of vehicle. Sulfhydryl group containing molecules can cause disulphide cleavage like mercaptoethanol, cysteine, acetyl-cysteine, etc. Synergistic improvement in penetration have been observed by simultaneous use of urea with acetyl-cysteine.

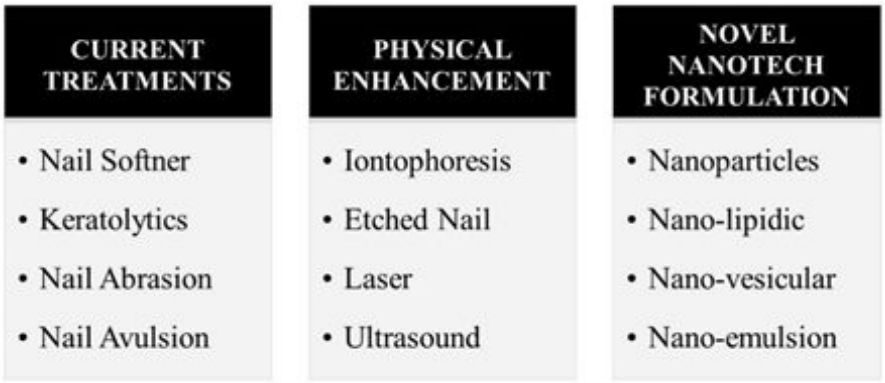

Figure 2: Treatment Approaches for Onychomycosis

CURRENT APPROACHES

Conventional Approaches

Multiple approaches for treatment of nail disorder explored includes oral delivery, physical removal of nail, use of nail-softner and penetration enhancers for topical drug delivery, etc [13,14]. Historically, the surgical removal of damaged nail for allowing fresh growth of new nail was considered as first line of treatment option; however, the panic trauma to patient needs a high level of pain threshold and currently it is considered as out-dated approach. Oral administration of drugs is still in practice by few of the prescribers, which may deliver small fraction of drug to desired site of action and may result in unwanted adverse reaction. Currently, topical administration of drug along with use nail softner &/or penetration enhancer are being practised; however, the desired outcome is yet to achieve, which indicated the need of novel approaches to increase penetration enhancement with minimal adverse effect and higher patient compliance.

Mechanical Approaches

Nail abrasion technique involves the application of rough sand paper to abrase carefully the top surface of nail plate (mainly on the edges) with appropriate intensity as per the pain threshold of patient before the application of topical formulation [15]. This technique reduces the nail thickness, removes the fungal infection mass from the surface and exposes the infected nail bed. Use of drill has also employed to make hole in nail plate for direct delivery of topical medication to lower surfaces. Total or partial nail avulsion involves surgical removal of entire nail or affected area using local anaesthesia.

PHYSICAL APPROACHES

Iontophoresis

Delivery of active moiety by creating an electromotive force across the membrane is the key principle of iontophoresis technique, which has been widely explored for transdermal, ocular, dental, orthopaedic, etc fields with an aim of penetration enhancement compared to conventional topical delivery. Multiple variation possible as per type of membrane, active moiety as permeant, solvent vehicle and additives, which may lead to interaction between electric field and ionic charge of molecule, creation of flow pathway, pore induction, etc. Researchers have explored iontophoresis for transungual drug delivery for several molecules and reported as multi-fold improvement in penetration compared to passive diffusion [10, 16-18]. The studies have varied the pH of solvent, ionic strength, buffer concentration, current density, etc and reported their significant impact on permeation.

Pre-treatment as Etching

The damaged nail is etched by applying phosphoric acid solution or gel as surface modifying agent to create micropores on

dorsal surface as pre-treatment process before topical application of conventional formulation [19]. Researchers have supported the significant change in etched nail as increase in roughness factor compared to untreated nail using advance microscopic techniques, as well as multi-fold rise in permeation of conventional topical formulation. Prolong bioadhesion of sustained release film was observed as added advantage due to higher roughness to increase the therapeutic outcome.

Laser and Photodynamic Therapy

The laser exposure to damage nail with or without presence of carbon dioxide have been explored by researchers. Daily application of topical drug on laser treated nail have shown rapid healing of disease with mild of no pain. The targeted delivery to affected nail area by avoiding peripheral tissue damage with high efficacy and minimal discomfort are the key merit of laser treatment [20]; however, high cost, need of sophisticated equipment, and long-term photo ageing would be bottlenecks of same. In the similar approach, light source is impacted on the fungal infected area by applying photosensitizer, which in-turn generate oxygen species locally to destroy fungus growth. The technique offers high efficacy and sensitivity by avoid drug interactions; but may also cause pain, burning and erythema.

Ultrasound

Phonophoresis is a non-invasive technique which employs application of ultrasound to create temporary micropores to enhance topical drug delivery. It has been a proven technique for transcellular delivery in joints, muscles and nerves; and hence recommended for nail delivery by several researcher groups; however, no detailed documented proof of concept for ungual delivery available.

NOVEL NANOTECH-BASED FORMULATION

Nanoparticles

In past few decades, nanoparticles have been explored for many drugs having solubility &/or permeability, as it offers many advantages faster penetration due to nano-scale and solubility improvement due to higher surface area. Several techniques have been employed by scientists as top-down approaches (reducing micro-scale particles to nano-scale) as well as bottom-up approaches (precipitation, spray drying, emulsion solvent evaporation, salting-out, etc). Matrixing of drug in to polymeric nanoparticles offers advantage of sustained release. Researchers across the globe have attempted nanoparticles [21-23] of anti-fungal drugs and delivered as suspension or embedded into gel formulation. Study revealed that in-depth penetration was observed with nanoparticles for 1 week of treatment compared to conventional. Nanoparticles of pullulan exhibited lesser irritation and proven safe for prolong treatment.

Lipidic Nano-Formulations

Lipidic nano-formulations have gain a lot of attention due to various merits offered like ease of formulation, biocompatibility, biodegradation, improved stability (photo-degradation and oxidation) of drug by engulfing into lipid matrix, etc. Although, the hydrophilic formulations recommended for nail delivery, researchers have explored using lipidic nano-formulations along with other physical or chemical approaches of penetration enhancement and showcased the improved result against conventional formulations. Solid lipid nanoparticles (SLNs) and its extended version Nanostructured lipidic carriers (NLCs) have been formulated for antifungal drugs for nail delivery and co-administered with urea as penetration enhancer. Formulation scientists observed that water dissipation has been reduced because of solid lipid matrix, which indicated that the occlusivity of SLN was higher comparted to that of NLC [24].

Nanovesicular Formulations

Liposomes as unilamellar or multilamellar vesicular systems designed majorly using phospholipids and cholesterol as bilayers, which allows amphillic systems for hydrophilic drug as well as hydrophobic drug encapsulation. Ethosomes (i.e. use of ethanol in liposomes) and liposomal poloxamer gel formulation have been studied and found superior for nail penetration. Flexible nanovesicular systems also known as transferosomes and spanlastics formulations using surface active agents like tween and span have proved almost double penetration than that of marketed preparation [25,26]. In another study, the co-administration of keratolytic agents have showcased improved therapeutic compared to marketed formulation.

Nanoemulsion

The microemulsion or nanoemulsion formulated with blend of surfactant and co-surfactants or co-solvents to enclose the hydrophobic drug containing oily globules at nano-scale in hydrophilic vehicle. Improved solubility, stability and penetration enhancement of nanoemulsion have been proven superior to that of lesser steady liposomal formulations [27-29]. Additionally, improvement in antimycotic actions were also reported due to drug solubilization in nanoglobules. Formulation scientists have further embedded into gel as nanoemulgel [30] to improve retention for better therapeutic outcomes.

CHARACTERIZATION OF TRANSUNGUAL FORMULATION

The physicochemical characterization of the transungual formulations like nail lacquer, solutions, gel, cream, ointment, spray, film, emulsion, etc would be as per standard protocols. For nano-based formulations the size specific characterizations shall include particle size distribution, zeta potential, use of advanced microscopic techniques like SEM, TEM, AFM, etc. The antifungal

activity of the drug can be tested using growth inhibition studies in well-planned protocol in micro-biotech labs. Recent advancement in the field is TurChub® cells developed by MedPharm (UK) as modified franz diffusion cell containing human nail as barrier for drug delivery against reservoir compartment containing fungus grown in agar media [31]. The major area of focus for performance of formulation as nail delivery is to ensure penetration of drug, which can be characterised by in-vitro, ex-vivo or in-vivo evaluation using animal or human nail.

Nail Penetration Study

It is always desirable to characterized formulation on human nail plates for getting real analysis; however, it is difficult to manage the human nails in large quantity as comparative and repeat studies, as well as, nail clippings are generally hard, curve and small in size that are difficult to handle in experimental set-up to get high level of accuracy of method [32]. Alternatively, the scientists are using the bovine hoof [33] as biomimicking nail plate membrane to overcome above issues of human nail, but the differences swelling capacity, density of keratin layers, complexity of network, resistance to drug penetration, etc. are to be taken into consideration. Studies have proven that human nail are more resistant than bovine hoof membrane and researchers have also established the mathematical relationship for same to extrapolate the findings [8]. The attempts were also made to develop the biomimicking human nail using human hair keratin as film with adequate resistance. Majority of the characterizations employs use of modified franz diffusion cell whereby drug is expected to be travelled from formulation to pass through nail plate membrane barrier hold in diffusion cell, and drug concentration were measured in reservoir compartment at regular interval ranging from hours to days [34]. Modification to technique also includes pretreatment as hydration by soaking human nail, use of chemical or physical techniques for penetration enhancement; as well as use of cotton ball beneath the nail plate to keep it continuously hydrating.

CONCLUSION

Nail diseases are common yet not addressed much for innovations in novel formulation, novel treatments and detailed clinical investigations, due to limited drug suitable for penetration to most resistant barrier as nail plates. Detailed investigation of human nail properties, factors affecting nail penetration, drug suitability study, etc may open the newer avenue for young and budding researchers. The use of nanomaterials in the formulation may raise a hazard-related concern; however, for nail delivery, the risk is very low due to comparatively less vascularisation than that of nasal, mucosal, ocular, etc. A topical formulation for treatments of onychomycosis will have less stringent regulatory requirements compared to oral delivery. Employing novel physical,

chemical and formulation approaches may be offer promising outcomes; however, establishing standardized protocol for characterization and in-vitro in-vivo correlation are future requirements.

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REVIEW ARTICLE

LUMPY VIRUS OUTBREAKS IN INDIA

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ABSTRACT

Animal husbandry is a vital sector of the Indian economy. The emergence of new illnesses, which is a big worry for livestock owners, is currently one of the primary obstacles facing the livestock and dairy sectors. The transboundary illness lumpy skin disease (LSD), which affects cattle and water buffaloes, is significant economically. A virus known as the lumpy skin disease virus (LSDV) causes lumpy skin disease (LSD). This virus belongs to the Capripoxvirus genus of the Poxviridae family. Recently, there has been a 7.1% morbidity rate among cattle in India due to LSD. Clinical signs of this illness usually involve fever, loss of appetite, and unique nodules on the skin's mucous membranes found in the mouth, nose, udder, genital, and rectum. They also include a decrease in milk supply, abortion, infertility, and occasionally death. It may be possible to stop the spread of the illness by vaccination, stringent quarantine rules, and vector control.

Keywords: Lumpy skin disease, Viral infection, Transboundary spread, Outbreak, India.

INTRODUCTION

As India is still thriving from the impact of COVID-19, there it comes another virus "Lumpy Skin Disease" (LSD) that makes an appearance in India. Also known as Neethling virus [1].

Lumpy Skin disease which has been considered an endemic situation that is emerged in 1929 Africa has been gulping the states of Gujarat and Rajasthan [1]. This disease has spread to around 17 states in the country. Which is a devastating threat to large domestic ruminants [2].

Lumpy skin disease is caused by a virus known as Capripoxvirus (CaPV), which belongs to the Poxviridae family and is closely related to the viruses that cause goat and sheep pox [3]. The viruses that cause sheep pox (SPPV) and goat pox (GTPV) belong to the same genus as the virus responsible for lumpy skin disease [4]. This is "an emerging threat to livestock worldwide" and has an impact on domestic water buffalo (Bubalus bubalis) and dairy cattle

(Bos taurus) [3] [4]. Ticks or other insects that feed on blood, including some types of flies and mosquitoes, or other insects that bite for food, can spread it [5]. Additionally, the illness can be transferred between animals directly in some circumstances as well as through fomites (items or materials that are prone to transmit the infection, such as clothing, utensils, and furniture) [6]. Since LSDV is not zoonotic, it cannot be transmitted from animals to people. This virus has no effect on humans [6].

In India, which has the most cow population in the world (303 million), the illness has spread to 15 states in just 16 months in 2019 [6]. Odisha announced the first LSD epidemic in August 2019, and five districts were dealing with the exotic cow disease [6].

A certain viral strain was found in Maharashtra in September 2020 [7]. Gujarat has sometimes reported instances throughout the past few years as well, but in the current circumstances, the spread of the virus and the number of recorded deaths are the main causes for concern [7]. Disease may lead to problems with animal welfare and considerable output losses, which also have a huge negative impact on India's economy and animal welfare [7].

In May 2022, the first instance was observed in the Kutch hamlet of Kaiyari in Lakhpat Taluka [8]. Kutch and Jamnagar are two of the Gujarat state's hardest-hit areas; the effects are less severe in the other districts [8]. 15 out of Gujarat's 33 districts have been affected by the 2022 LSD outbreak [8]. According to official statistics (Animal Husbandry Department), over 40,222 cattle have been infected, and 1,021 animals have died in the past few weeks in the state of Gujarat. Reportedly 294,000 have been vaccinated [8].

A total of 2,111 cow fatalities have been reported as of August 8 in Rajasthan, followed by 1,679 in Gujarat, 672 in Punjab, 38 in Himachal Pradesh, 29 in Andaman & Nicobar, and 26 in Uttarakhand [9].

Jodhpur, Barmer, Jaisalmer, Jalore, Pali, Sirohi, Bikaner, Churu, Ganganagar, Hanumangarh, Ajmer, Nagaur, Bhilwara, Tonk, Jaipur, Sikar, Jhunjhunu, Alwar, Dausa, Chittorgarh, Bharatpur, Dholpur, Karauli, Banswara, Rajsamand, Pratapgarh, Dungarpur and Udaipur have all reported cases [9].

The disease has caused the greatest impact in Ganganagar (Rajasthan), resulting in 3,672 deaths. Jodhpur follows with 2,426 deaths, then Hanumangarh with 2,167, Nagaur with 2,099, Barmer with 1,973, Jalore with 1,765, and finally Bikaner with 1,704. Out of the 512,140 animals infected, more than 461,643 have received treatment [9].

Circular, hard nodes that resemble lumps on the animal's hide (skin) are a sign of infection [10]. A nodular skin lesion can range in size from 10 to 50 mm [10]. The number of lesions varies and may be minimal in situations of moderate infection

before progressively increasing in severity in seriously affected animals [10].

Nearly a week after viral infection, fever starts to develop. This first fever might reach 41 °C (106 °F) or higher and last for a week [10].

When animals with skin lesions, mucous membranes in the mouth and nasal cavities, as well as ocular discharge, excrete infectious virus, which may contaminate shared feeding and drinking water locations, virus transmission takes place [11]. Which further cause transmission of virus to another bovine, thus in this way a chain is generated which eventually leads to an epidemic [11].

Greater vulnerability exists in thin-skinned cattle than in native breeds with thicker skin [12]. While newborn calves are at a higher risk and can display the characteristic lesion within 24 to 48 hours, all animals of different ages are prone to contracting the disease [12]. According to reports, the virus may linger in desiccated (dry) crusts for up to 35 days, in necrotic skin nodules that are considered to be "dead" or "corpse" for up to 33 days, and in air-dried hides for at least 18 days [12].

It is believed that skin lesions are the main infection sites in cattle [13]. Certain arthropods, particularly blood-sucking insects such as mosquitoes and ticks, as well as the Stomoxys calcitrans fly, the Adeyes aegypti mosquito, and certain tick species like Rhipicephalus and Amblyomma spp., can transmit the LSDV virus. The disease can be spread through infected food and water, as well as bodily fluids like saliva, nasal secretions, and semen during the later stages of the illness [13].

The virus can infect females during natural mating or artificial insemination because it persists in the semen of infected bulls [10]. The corneas of both eyes may occasionally develop severe ulcerative lesions as well, which in the worst instances might result in blindness [10].

Virus can also be transmitted to calves those drinks infected bovine milk [11]. Disease symptoms include fever, lack of appetite, salivation, Symptoms such as nasal discharge, lacrimation, swollen lymph nodes, a notable decrease in milk production, and weight loss are commonly observed in cases of LSD [11]. It is common for pregnant cows and buffaloes to experience miscarriage. Unfortunately, in certain situations, diseased animals may also perish as a result [14].

The state's first lumpy care centre has been established by the Jaipur Greater Municipal Corporation at the city's Hingonia Gaushala [12].

Infected animals are recovered within three weeks when treated with anti-allergy and antibiotic medicines [15].

The best way of prevention is to keep the diseased animal in isolation [16]. No other animal should be allowed to approach the ill especially their baby calves or consume its remaining water or feed [16].

There is no proper treatment so far for LSDV, but several traditional medicines are used for the treatment and various anti-allergy, antibiotics medicines are very helpful [16].

At present, there is no known cure for lumpy skin disease. Thus, treatment mainly focuses on managing the clinical symptoms [17]. The vaccine currently being administered is the same one used for the goatpox virus [17]. According to reports, two institutes under the Indian Council of Agricultural Research have successfully created a domestic vaccine for the illness [17].

A vaccine called Lumpi-ProVacInd has been created through a collaboration between the National Equine Research Centre in Hisar, Haryana and the Indian Veterinary Research Institute in Izzatnagar, Bareilly, both institutes under the Indian Council of Agricultural Research (ICAR) [12]. Which the Union government is planning to commercialise [12].

CONCLUSION

More than 60% of India's rural population depends on the livestock industry for a living and nutritional sustenance. The livestock industry is crucial to the Indian economy. The recent epidemic of Lumpy skin disease (LSD), which requires considerable attention to reframe our vision to see livestock health and production holistically, is one of the issues this living asset is currently confronting. Instances of lumpy skin disease (LSD) are not typical virus outbreaks. The disease's recent expansion, which began in June 2022 and moved into disease-free areas, is evidence of its importance in terms of epidemiology and the economy. The illness causes death, decreased draught power, decreased milk output, infertility, abortions, culling, and losses in hide quality, according to the cattle owners. It also causes weight loss and infertility. An estimated financial loss of Rs. 35,000 to 80,000 (approximately) each dead animal recorded during the present epidemic is a significant loss for a farmer whose livelihood depends on the production of livestock. The morbidity rate is typically up to 50% and the death rate is typically around 1-5%.

LSD outbreak management techniques should be thoroughly implemented. These include raising awareness of LSD, limiting animal movement, isolating affected animals, keeping an eye on stray animals, cleaning and disinfecting the area, controlling insects, and ultimately, safely disposing of carcasses. Preventive vaccination should be carried out in mission mode in high-risk locations, such as the borders of afflicted states and districts, and infected animals should be identified and documented.

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RESEARCH ARTICLE

REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY(RP-HPLC) METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SILDENAFIL CITRATE AND DULOXETINE HYDROCHLORIDE IN COMBINE DOSAGE FORM

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ABSTRACT

Sildenafil citrate (SIL) and Duloxetine (DULO) is a combination, which is a serotonin-norepinephrine reuptake inhibitor (SNRI). The primary goal of the endeavor is to develop and evaluate a stability-indicating reverse-phase liquid chromatography (RP-HPLC) technique for determining Sildenafil citrate and Duloxetine hydrochloride in the combined dosage form. Estimation of Sildenafil citrate and Duloxetine hydrochloride in pharmaceutical dosage form was carried out using Thermoscientific C18BDS- (25 cm x 4.6 mm x 5 mm). The mobile phase used consisted of Acetonitrile: Phosphate buffer (50:50%v/v), and the pH of the mobile phase was adjusted to 4.0 using 0.5%v/v Orthophosphoric acid. The flow rate was 1.0mL/min. The UV detection was done at 290nm. The injection volume was 20µl. Forced degradation studies including hydrolytic (acidic, basic, and neutral), oxidative, and thermal, have been carried out according to ICH Q1A (R2) guidelines. Potential degradation was found in acidic, oxidative, and basic environments. The development of the method was validated according to ICH guidelines, and the findings were deemed acceptable. The devised stability-indicating technique effectively evaluated sildenafil citrate and duloxetine in the pharmaceutical tablet dosage form in the presence of a

degradation product, with test results showing satisfactory recovery.

Keywords: Sildenafil citrate, Duloxetine hydrochloride, Stability indicating, RP-LC, Forced degradation, Method validation.

INTRODUCTION

Sildenafil citrate5-{2-Ethoxy-5-[(4-methylpiperazin-1-yl)sulfonyl]phenyl}-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d]pyrimidin-7-one [1]. Duloxetine(methyl[(3S)-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl)propyl]amine)[2]. a serotonin-norepinephrinereuptake inhibitor(SNRI)[3-4], Sildenafil Citrate and Duloxetine HCl active pharmaceutical ingredient(API)is not official in any Pharmacopoeia.Several HPLC (High-performance liquid chromatography) and UV analytical methods have been reported [5-22], however, there is no method available for testing the forced degradation behavior of this drug in its combined dosage form, so testing the forced degradation behavior of this drug under various conditions such as hydrolytic (acidic, basic), oxidative, and thermal according to ICH Q1A (R2)[21] is an ongoing task. The key objectives of the research are to (1) build a way to indicate that is simple, particular, selective, sensitive, linear, accurate, robust, precise, and stable, and (2) perform a forced deterioration study in compliance with ICH guidelines. (3) To validate the established methods in compliance with ICH Q2 (aR1) guidelines.

EXPERIMENTAL

Materials and reagents

Sildenafil Citrate standard and Duloxetine HCl standard were obtained as a gift sample from Sunrise Pvt. Ltd. HPLC grade solvents Acetonitrile, Phosphate Buffer were purchased from Merck, Rankem.

Instrumentation

The HPLC was carried out on Shimadzu LC-10AT consisting of an auto-injector, sample manager and quaternary gradient pump with SPD20A PDA detector. The output signal was monitored and processed using LC-Solution software. A thermal degradation study was done using a hot air oven (Labline, India). Hydrolytic degradation was done using a digital water bath (Meta lab Scientific Industries Ltd., Mumbai, India). All pH measurements were carried out on a pH meter (Lab India, India), and weighing was done using a Mettler Toledo, Schwerzenbach, Switzerland. An ultrasonic bath sonicator (manufactured by, 5510, Branson Ultrasonic's Corporation, Danbury, CT, USA) was used for dissolving samples.

Conditions

The separation was accomplished on a Thermo scientific C_{18} BDS column (25 cm x 4.6 mm x 5 m) at pH 4 using Acetonitrile: Phosphate buffer (50:50% v/v) as the mobile phase. The mobile phase was filtered using 0.45 mm membrane filter paper & sonicated for 20 minutes.

Acetonitrile: Phosphate buffer (50:50% v/v) was used as a diluent. The flow rate was set to 1 mL/min and the detection wavelength (λ) at 290 nm. The run time was 7 minutes.

Preparation of Solutions

Preparation of standard stock solution of Sildenafil citrate

Sildenafilcitrate (25 mg) was weighed & transferred into a 25mLvolumetric flask (VF). 25mL of mobile phase was added and sonicated to dissolve. To obtain 100 µg/mL working standard solutions, a 1 mL aliquot of the stock solution was diluted to 10 mL with diluent.

Preparation of standard stock solution of Duloxetine HCl

Duloxetine HCl (25 mg) was weighed & transferred into a 25mLvolumetric flask (VF).15mL of mobile phase was added and sonicated to dissolve & make up the volume with mobile phase and mixed it. A 0.6 mL aliquot of the standard stock solution was diluted to 10 mL with diluent to make up the working standard solutions of 60 µg/mL.

Preparation of a standard stock solution of Sildenafil citrate & Duloxetine HCl

Sildenafil citrate (100 mg) and Duloxetine HCl (60 mg) were precisely weighed and transferred to a 100 mL volumetric flask, dissolved in the sufficient mobile phase, and diluted up to the mark with mobile phase to achieve a final concentration of 100 µg /mL Sildenafil citrate and 60 µg /mL Duloxetine HCl.

Preparation of the solution Sildenafil citrate & Duloxetine HCl

The sample was prepared by taking 20 tablets into mortale pestle, crushed it & the powder was taken into 100 ml of volumetric flask, diluted with 50 ml of mobile phase, placed in an ultrasonic water bath for 10 mins to achieve optimal solubility of the active components, and diluted to the mark (1000 µg /mL of SIL and 600 µg /mL of DULO). Soltion was filter with whatman filter paper no. 0.45 mm. From this, 1mL of this solution was taken into 10mL volumetric flask & the volume was made up to 10 mL with mobile phase. (SIL 100µg/mL and DULO 60µg/mL).

FORCED DEGRADATION STUDIES

Forced degradation experiments were carried out according to ICH Q1(A) R2 guidelines to determine the drug's stability under various stress conditions such as hydrolysis (acidic, basic, neutral), oxidation, & thermal.

Forced degradation sample stock preparation:

Sildenafil citrate (100 mg) and Duloxetine HCl (60 mg) were precisely weighed and transferred to a 100 mL volumetric flask, where they were dissolved in an appropriate amount of mobile phase (Acetonitrile: Phosphate buffer (50:50)) and diluted to the mark. (The solution

contains 1000 µg /mL Sildenafil citrate and 60 µg /mL Duloxetine HCl. The resulting solution was known as the standard degradation solution. Using 0.45mm membrane filter paper, the final solution was filtered.

Neutral hydrolysis

Neutral hydrolysis was performed using water. 1 mL of forced degradation sample stock preparation was taken and added into 10 mL of volumetric flask. This resulting solution was kept under waterbath at 70°C. After some time, cool the contents to room temperature before adding diluents to make up the volume. (SIL at 100 µg/mL and DULO at 60 µg/mL).

Acidic hydrolysis

Acidic hydrolysis was performed using 0.5N HCl. 1 mL of forced degradation sample stock preparation was taken and added into 10 mL of volumetric flask. Add 2 mL of 0.5N HCL solution and mix. This resulting solution was kept under waterbath at 70°C after 1 hour to cool at room temperature. Samples were neutralized with 2 mL of 0.5N NaOH & solution was diluted. (SIL 100 µg/mL and DULO 60 µg/mL).

Basic hydrolysis

Basic hydrolysis was performed using 0.5N NaOH. 1 mL of forced degradation sample stock preparation was taken and added into 10 mL of volumetric flask. Add 2 mL of 0.5N NaOH solution and mix. This resulting solution was kept under waterbath at 70°C after 1 hour to cool at room temperature. Samples were neutralized with 2 mL of 0.5N NaOH & solution was diluted. (SIL 100 µg/mL and DULO 60 µg/mL).

Peroxide degradation

Peroxide degradation was performed using 3% H2O2. Transfer 1mL offorced degradation sample stock preparation into 10mL of volumetric flask & add 2mL 3% H2O2 and put the volumetric flask in a water bath for 2 hours at 70°C, After the time period remove the flask from the waterbath and allow to cool at RT and then makeup the volume with diluent. (100µg/mL of SIL and 60µg/mL of DULO).

Thermal degradation

Thermal degradation was performed in a vacuum oven. Sildenafil citrate (100 mg) and Duloxetine HCl (60 mg) were precisely weighed, taken into petri plate & kept under vacuum oven at 105°C. and transferred to a 100 mL volumetric flask, where they were dissolved in an appropriate amount of mobile phase (Acetonitrile: Phosphate buffer (50:50)) and diluted to the mark. (The solution contains 1000 µg /mL Sildenafil citrate and 60 µg /mL Duloxetine HCl. The resulting solution was known as the standard degradation solution. Using 0.45mm membrane filter paper, the final solution was filtered.

METHOD VALIDATION

The following method was utilized & validated in accordance with ICH standards[ICH, 1996.][23]

System Suitability Parameters

Five times repeating injections (n=5) were used to establish system suitability in terms of retention time (tR), number of plates (NTP), tailing factor, and peak area using 100 µg /mL of SIL and 60µg /mL of DULO, which were produced from a stock solution of 1000 µg /mL of SIL and 600 µg /mL of DULO.

Linearity&Range (n=5)

The linearity response data was identified by examining five unique calibration curve levels ranging from 50 to 150µg /mL for Sildenafil citrate (50, 75, 100, 125, and 150µg /mL) and 30-90 µg /mL (30, 45, 60, 75, and 90µg /mL) for Duloxetine HCl. The calibration curve was plotted against peak area (AU) vs concentration (µg /mL).

Accuracy(n=3)

The recovery study was carried out using the standard addition technique, in which known amounts of standard powders of Sildenafil citrate& Duloxetine HCl were added to the pre-analyzed samples at 50%, 100%, and 150% levels.

Precision

The precision parameter was established using three variables: repeatability, intra-day precision, and inter-day precision.

Repeatability(n=6):

Method precision was established by analyzing six sample preparations under the same chromatographic condition as per method. Six replicates of sample were prepared at the test concentration and injected on the same equipment and on the same day. Relative standard deviation (RSD) or percentage coefficient of variation(%RSD) should not be more than 2%.

Intraday Precisionand Interday Precision

The intra day precision was performed by using 3 different concentrations 50, 100, and 150µg/mL for Sildenafil citrate (n=3) and 30, 60, and 90µg/mL for Duloxetine HCl (n=3) prepared from the stock solution of 100µg/mL of Sildenafil citrate and 60µg/mL of Duloxetine HCl & injected on the same day while in inter-day precision, the solution was injected on

three consecutive days.

LODand LOQ

The calibration curve approach was used to calculate the Limit of Detection (LOD) and Limit of Quantification (LOQ) based on the standard deviation of the response and the slope.

$$LOD = 3.3 \times \sigma / S$$

$$LOQ = 10 \times \sigma / S$$

Where,
σ = Standard deviation of the intercept&
S = Slope of the calibrationcurve

Estimation of Sildenafil citrate & Duloxetine HCl in tablet dosage form by the proposed method (n=5)

Twenty tablets were weighed and crushed finely. 60 mg of Duloxetine and 100 mg of Sildenafil citrate are present in each tabletThe stock solution is made by dissolving equivalent to 10 mg of tablet powder in 10 ml mobile phase, placing it in an ultrasonic water bath for 10 minutes to ensure complete dissolution of the active components, and diluting it to the desired concentration with mobile phase (100µg/ml Sildenafil citrate and 60µg/ml Duloxetine HCl). Take 1 ml of this solution and add 1 ml of SIL from a standard stock solution of 100µg/ml land make up to 10 ml to get 100µg/ml Sildenafil citrate and 60µg/ml Duloxetine HCl were produced, applied to an HPLC column, and evaluated under optimum chromatographic conditions.

RESULT AND DISCUSSION

Optimization of the method

Many runs were performed using mobile phases consisted of solvents of varying polarity and concentration levels to establish a suitable mobile phase for the efficient separation of SIL & DULO. For the method development, various mobile phase systems, as well as concentration levels, were tested. The mobile phase consisting of Acetonitrile: phosphate buffer (50:50% v/v) with pH-4 provided the highest resolution with strong well-defined peaks and Rt values of 3.66 min± 0.02 and 5.28 min± 0.02 for SIL & DULO, respectively. The overlain chromatogram of the both drug were acquired on the HPLC instrument to determine the analytical wavelength for quantification. SIL and DULO demonstrated significant absorbance at around 290nm, chosen as the analytical wavelength for analysis.

Forced degradation studies

Optimization

In hydrolysis (acidic&basic), degradation was beginning with concentration (0.5N) & high temperature(T) (70°C) while peroxide degradation was beginning with low concentration(3%). The greatest concentration of NaOH was not used in basic hydrolysis since the sample was discovered to be unstable and easily precipitated out, thus a low concentration (0.5 N) was used. In thermal, degradation was beginning with a high temperature (105°C) because SIL and DULO melting point are 189-190°C and 118-122°C respectively. In acidic, basic, peroxide & thermal conditions, sample were analysed from 1 hrs to 24 hrs. In water & thermal conditions, less than 5 degradation was observed while in acidic, basic & peroxide conditions, degradation was performed. Detailed of force degradation were described in **Table 1.**

Table 1: Summary of Forced Degradation Studies of Sildenafil Citrate and Duloxetine Hydrochloride

Stress	*SIL*			*DULO*		
	Peak Area	*Retention Time*	*%Degradation*	*Peak Area*	*Retention Time*	*%Degradation*
As such	1402.21	3.65	-	883.87	5.280	-
Water hydrolysis	1340.20	3.653	4.42	873.16	5.277	1.21
Acid	1253.59	3.653	10.59	793.29	5.277	10.24
Base	1155.72	3.653	17.57	730.07	5.277	17.37
Oxidation	1180.84	3.650	15.78	749.02	5.270	
thermal	1398.20	3.657	0.28	843.57	5.283	4.55

Neutral hydrolysis

In neutral hydrolysis with water at 70°C for 3 hours, six degradation products were obtained: DP1 (degradation product), DP2, DP3, DP4, DP5, and DP6. DP1 and DP3 were possible degradation products with 4.42% and 1.21% degradation, respectively. (detailed shown in **Fig.1**).

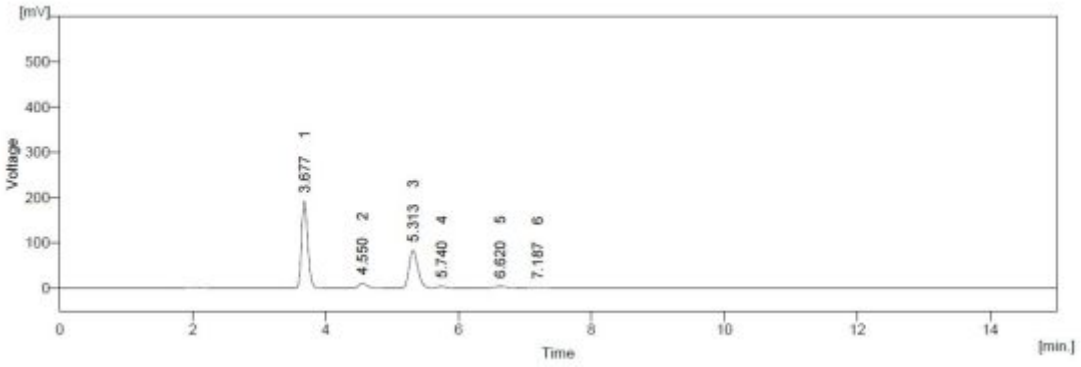

Fig .1 Chromatogram of Neural Hydrolysis of Standard mixture at 290 nm

Acidic hydrolysis

For 1 hour of acidic hydrolysis with 0.5 N HCl at 70°C, eight degradation products were obtained: DP1 (degradation product), DP2, DP3, DP4, DP5, DP6, DP7, and DP8. DP1 and

DP4 were possible degradation products, with 10.59% and 10.24% degradation, respectively. (detailed shown in **Fig.2**).

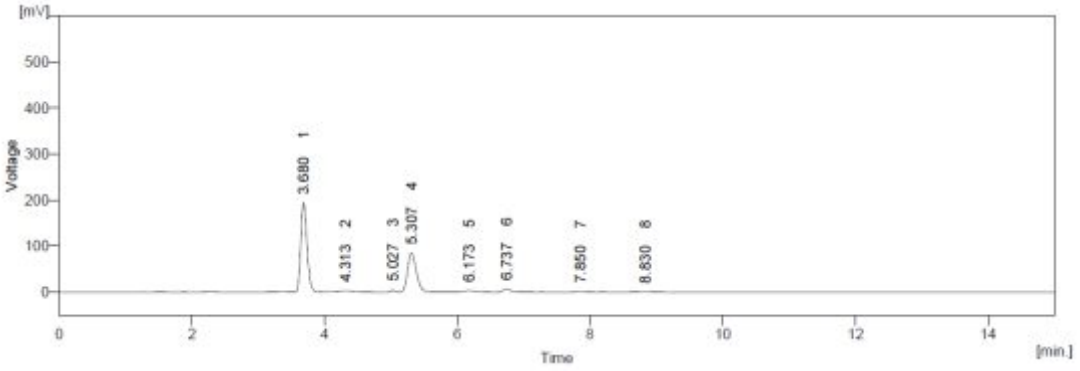

Fig .2 Chromatogram of Acid Degradation of Standard Mixture at 290 nm

Basic hydrolysis

Basic hydrolysis with 0.5 N NaOH at 70°C for 1 hour yielded eight degradation products: DP1 (degradation product), DP2, DP3, DP4, DP5, DP6, DP7, and DP8. DP1 and DP3 were possible degradation products with 17.57% and 17.37% degradation, respectively. (detailed shown in **Fig.3**).

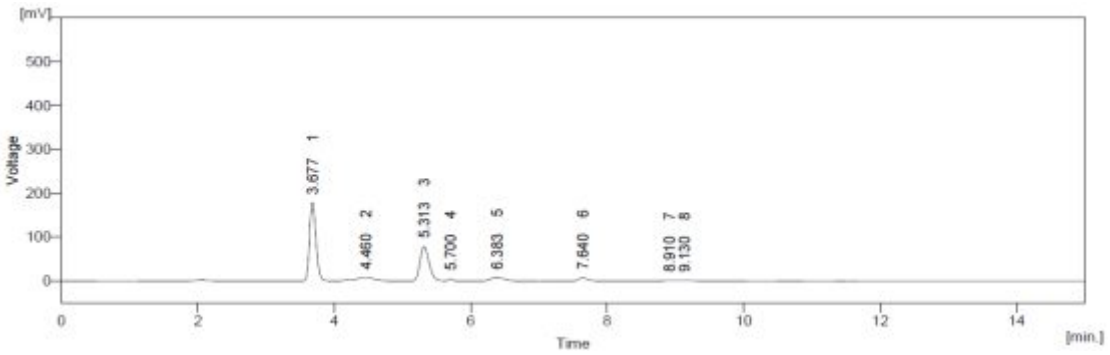

Fig .3 Chromatogram of Base Degradation of Standard Mixture at 290 nm

Peroxide degradation

Peroxide degradation using 3% H2O2 at room temperature for 2 hours yielded six degradation products: DP1 (degradation product), DP2, DP3, DP4, DP5, and DP6. DP1 and DP4 were possible degradation products with 15.78% and 15.25% degradation, respectively. (detailed shown in **Fig.4**).

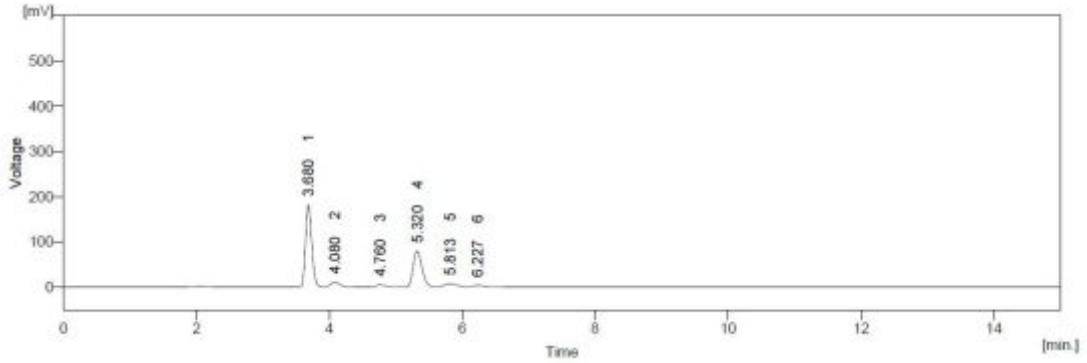

Fig.4 Chromatogram of Oxidation Degradation of Standard Mixture at 290nm

Thermal degradation

Thermal degradation at 105°C for 8 hours yielded six degradation products: DP1 (degradation product), DP2, DP3, DP4, DP5, and DP6. DP1 and DP5 were possible degradation products, with 0.28% and 4.55% degradation, respectively. (detailed shown in **Fig.5**).

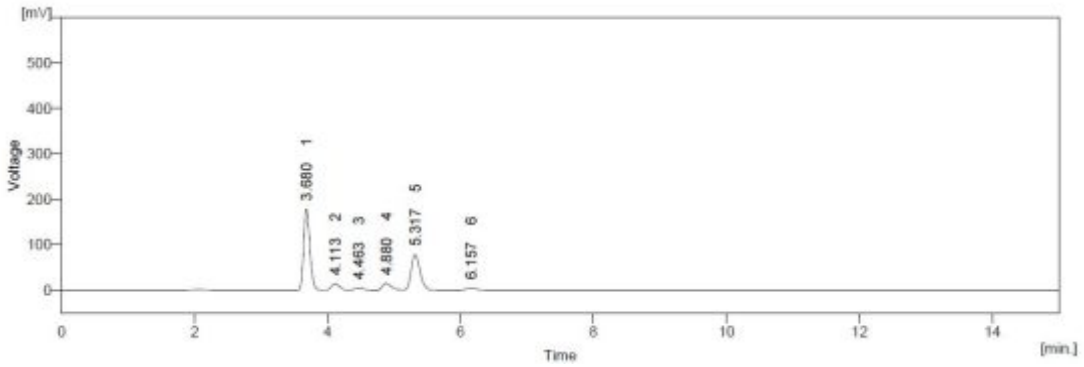

Fig.5 Chromatogram of Thermal Degradation of Standard Mixture at 290 nm

Method Validation

System Suitability Parameters

Table 2 shows the results of the system-suitability test parameters. The percentage RSD for all parameters was determined to be less than 2%. This implies that the system is suitable.

Table 2: System Suitability Parameters (SSP) for SIL & DULO (n=5)

System Suitability Parameters (SSP)	DRUGS	
	SIL±SD	DULO±SD
Retention time(min)	3.65±0.01	5.28±0.05
Tailing factor(T)	1.43±0.07	1.38±0.03
Number of theoretical plates (N)	7026±27.82	7280±35.32
Resolution (R)	7.65	

Linearity

The method was found linear in the range of 50-150µg/mL for SIL and in the range of 30-90µg/mL for DULO. **Table 3** displays the correlation coefficients, y-intercepts, and slopes of the regression lines for the 2 medicines. The linearity of the method wasproved by the value of the regression coefficient (R^2) of SIL and DULO as shown in **Fig 6 (a)** and **Fig 6 (b)** respectively.

Table 3: Linearity Data of SIL & DULO(n=5)

Parameters*	SIL	DULO
Linearity range	50-150 µg/mL	30-90 µg/mL
Linearity regression equation	y=40.187x+78.554	y=602.8x+681.35
Slope ± SD	14.107	14.844
Intercept ± SD	6.0688	2.7914
Correlation coefficient (r^2)	0.999	0.999

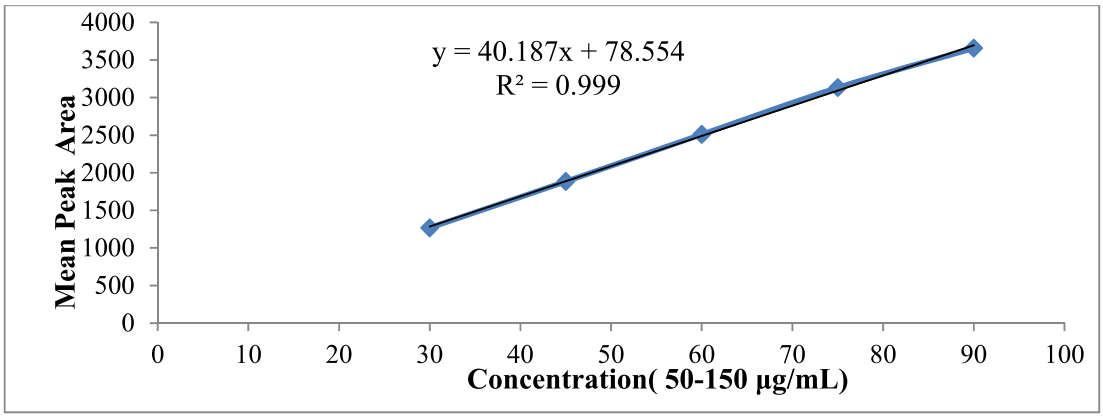

Fig. 6: (a)Calibration Curve for SIL (50-150 µg/mL)

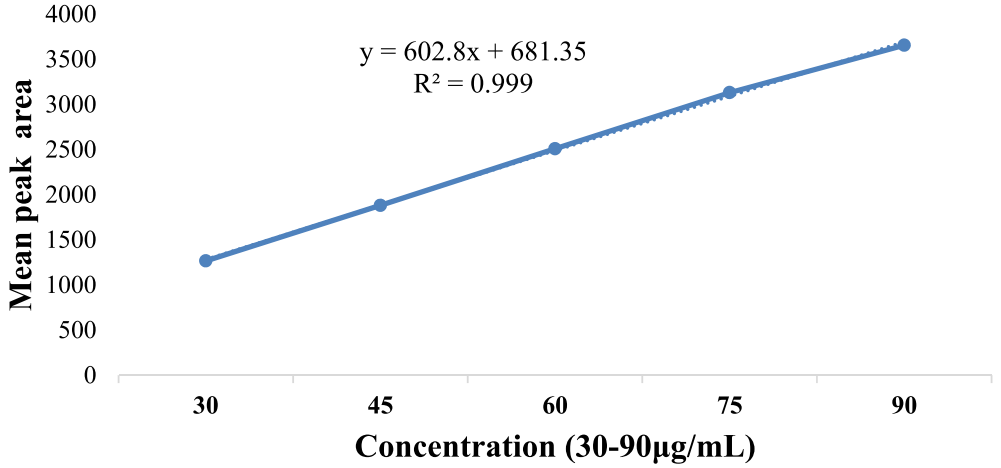

Fig. 6: (b) Calibration Curve for DULO (30-90µg/mL)

Specificity

The specificity of the proposed method was assessed by injecting about 20 µL of the SIL and DULO blank or working standard and sample solution into the HPLC system and recording chromatograms as shown in (**Fig.7 (a), (b), (c),** and **(d)**, respectively. under optimal chromatographic conditions. The chromatograms of standard SIL and DULO, as well as the sample, revealed two peaks with 100% peak area & Rtvalues of 3.66 min± 0.02 & 5.28 min± 0.02 for SIL and DULO, respectively.

Fig. 7 (a) Chromatogram of Blank

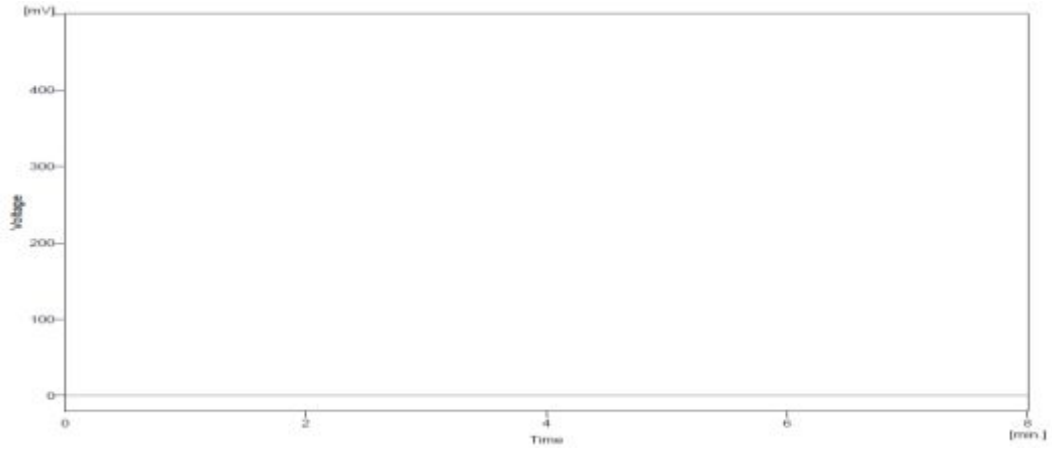

Fig. 7(b) Chromatogram of Standard SIL (100µg/mL)

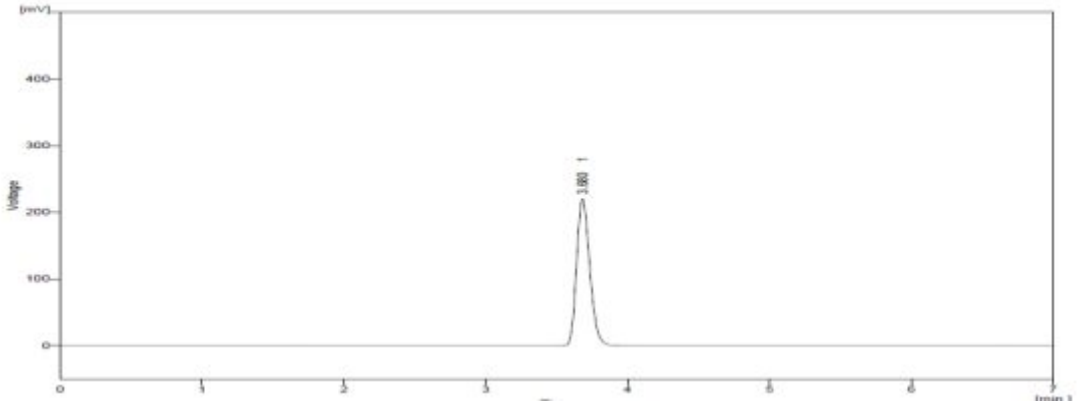

Fig. 7(c) Chromatogram of Standard DULO (60µg/mL)

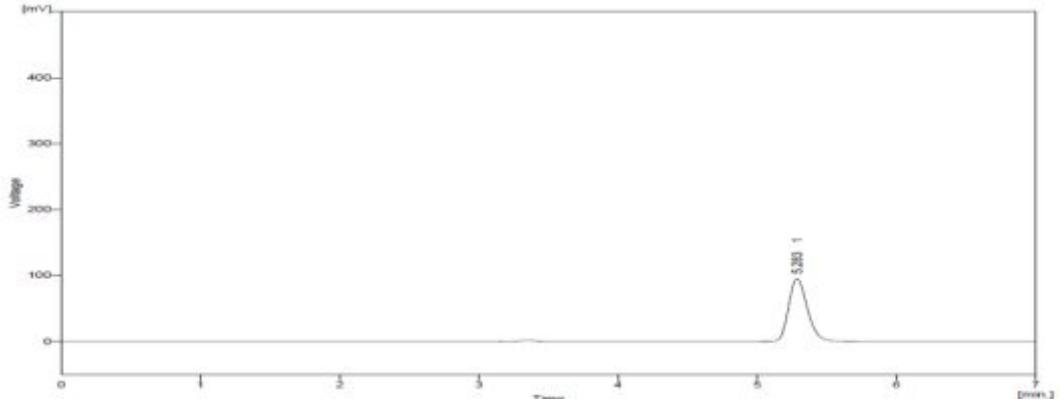

Fig. 7(d)Chromatograms of SIL (100 µg/mL) and DULO (60 µg/mL) Sample Solution

Accuracy

Tests for recovery were carried out at 50%, 100%, and 150% of the test concentration. At all three levels, the percentage recovery of SIL and DULO was considered to be satisfactory (**Table 4**). The percentage recoveries for SIL and DULO were discovered to be in the range of 100.22-100.57% and 100.35-100.72%, respectively.

Table 4: Accuracy Data of SIL & DULO (n=3)

Drug	Spike level%	Taken	Spiked Amount	Amount Recovered	SD	% recovery
SIL	50	50	75	75.31	0.52	100.39
	100	50	100	100.57	0.66	100.57
	150	50	125	125.26	0.80	100.22
DULO	50	30	45	45.26	0.52	100.56
	100	30	60	60.56	0.66	100.72
	150	30	75	75.25	0.80	100.35

Precision

Repeatability(n=6)

The repeatability data for SIL and DULO are shown in Table 9. %RSD for SIL and DULO were found to be 0.72% and 0.89% respectively. **Table 5** displays the repeatability outcomes.

Drug	Conc.	1	2	3	4	5	6	Standard Deviation	%Relative SD
SIL	**100**	1417.46	1430.46	1406.12	1416.75	1431.46	1410.45	10.32	0.72
DULO	**60**	892.46	896.41	883.21	890.23	894.45	907.47	8.00	0.89

Intraday precision and Inter day precision(n=3)

The data for intraday precision for SIL and DULOis shown in Table10. The % RSD for SIL and DULO was found to be0.98-1.74% and 0.99-1.74% respectively. The % RSD was found to be 0.64-1.06% and 0.64-1.07% for SIL and DULO respectively. The results of repeatability are as shown in

Table 6: Intraday & Interday Precision of SIL & DULO (n=3)

Drug		SIL			DULO	
Conc. (µg/ml)	**50**	**100**	**150**	**30**	**60**	**90**
Intraday precision						
Mean peak area	706.1757	1411.23	2121.864	444.9153	889.624	1340.416
± S.D.	7.54	12.01	13.72	4.76	7.57	8.63
% R.S.D	1.06	0.85	0.64	1.07	0.88	0.64
Interday precision						
Mean peak area	704.815	1416.938	2113.992	444.0517	893.2373	1333.567
± S.D.	6.96	14.59	36.89	4.40	9.20	23.26
% R.S.D	0.98	1.03	1.74	0.99	1.03	1.74

The % RSD for repeatability, intra-day precision, and inter-day precision were all less than two, indicating that the approach was precise.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD & LOQ were found to be 0.540 & 1.639 µg/mL for SIL and 6.230 & 18.879 µg/mL for DULO respectively. Similarly, LOQ for SIL and DULO were found to be 1.639 and 18.879 respectively, indicating the sensitivity of the developed method.

ANALYSIS OF MARKETED FORMULATION

The new approach was used to examine the marketed formulations that are SIL, and DULO. The sample chromatogram exhibited just 2 peaks at Rt values of 3.66 min & 5.28 min for SIL and DULO, implying that no excipients included in the tablet formulation interfered. The SIL and DULO concentrations were determined by comparing the samples peak areas to those of the standard. (**Table 7**).

Table 7: Assay Data of SIL & DULO (n=5)

Drug name	**Amt added per 1 ml of solution**	**% Amt found**	**SD**	**RSD**
SIL	100 mg	100.54%	1.29	1.29
DULO	60 mg	100.40 %	1.14	1.14

CONCLUSION

The simple, specific, selective, linear, accurate, robust, sensitive & stability indicating method was developed. The forced degradation study was performed as per ICH Q1A (R2) guideline. In acidic conditions, satisfactory deterioration was attained(10.59% for SIL & 10.24% for DULO), DP1& DP4 werefoundas a potentdegradation product, Inbasiccondition(17.57% for SIL & 17.37% for DULO), DP1& DP3 were found as apotentdegradationproduct, Inoxidation(15.78% for SIL & 15.25% for DULO), DP1& DP4 were found as a potent degradation product,Inwater hydrolysis(4.42% for SIL &1.21% for DULO), DP1& DP3 were found as a potent degradation product,The degradation was achieved in thermal condition(4.55% for DULO), DP5 wasb found s a potent degradation product. There was no degradation found in the thermal condition for Sildenafil Citrate.

LIST OF ABBREVIATIONS

I. SIL,Sildenafil

II. DULO, Duloxetine

REFRENCES

[1] Drug profile: sildenafil citrate http://www.drugbank.ca/drugs/DBOO 03

[2] Drug profile : duloxetine hydrochloride, http://www.drugbank.ca/drugs/DB00476

[3] "Sildenafil citrate",Oct-2012 http://www.drugs.com/monograph/sildenafilcitrate.htmL

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RESEARCH ARTICLE

METHOTREXATE AND HYDROXYCHLOROQUINE COMBINATION THERAPY IN THE TREATMENT OF RHEUMATOID ARTHRITIS BASED ON CLINICAL DISEASE ACTIVITY INDEX SCORE

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ABSTRACT

In the Indian population, the incidence of rheumatoid arthritis (RA) is found to be 0.75%. Methotrexate (MTX) is a folate antagonist which is approved as a first-line drug by the U.S. Food and Drug Administration (FDA) for the treatment of RA. MTX monotherapy has its limitations. Hydroxychloroquine (HCQ) belongs to the category of disease-modifying antirheumatic drugs (DMARDs). Thus, the present work aims to study the effectiveness of MTX and HCQ combination therapy in the management of RA based on the Clinical Disease Activity Index (CDAI) Score. A Bicentric Retrospective Observational Study was conducted on RA patients managed with the combination therapy of MTX and HCQ in the tertiary care hospitals of Ahmedabad city. Demographic information, CDAI score, RA factor, comprehensive therapy plan, and treatment results (remission) were all obtained using devised data input forms. The baseline CDAI score and CDAI score after one year and two years were noted. A total of a 576 RA patients on combination therapy of MTX and HCQ were enrolled to participate in the research study, of which 84% i.e. n=484 were female and

16% (n=92) were male. Data indicated that out of a total 576 patients, 26.2% (n=151) belonged to the age group of 40-49 years and 26.04% (n=150) were identified to be of age between 50-59 years. Interpretation of the results revealed that all groups significantly improved after receiving the combination therapy. However, remission was achieved in 56 patients (9.7%) with combination therapy. Thus, treating active RA with the combination of MTX and HCQ is efficacious.

Keywords: Rheumatoid Arthritis, Methotrexate, Hydroxychloroquine, Combination Therapy, Clinical Disease Activity Index Score

INTRODUCTION

Rheumatoid arthritis (RA) is a type of autoimmune disorder that is characterized by a chronic and often progressive inflammatory illness with polyarticular symmetric joint and systemic symptoms. The incidence of RA worldwide between 1980 and 2019 was reported to be 460 per 100,000 people, with some regional and methodological differences [1]. Amongst the Indian population, the prevalence of RA is 0.75% in adult populations [2]. Studies show that women are more likely than men to acquire RA due to hormonal variations [3,4]. Males have a decreased risk of RA because testosterone and progesterone depress the immune system and estrogen boosts humoral immunity [5-8].

Methotrexate (MTX) (structure is shown in **Fig. 1**) is a folate antagonist and the first-line drug approved by the U.S. Food and Drug Administration (FDA) for the pharmacotherapeutic management of RA [9,10]. Monotherapy with MTX has its own limitations. MTX induces liver toxicity [11, 12], pulmonary toxicity [13, 14], and stomatitis [15]. Even with extensive patient monitoring, improvements in disease parameters are evident quite quickly when using MTX alone, but they tend to plateau after approximately six months without any

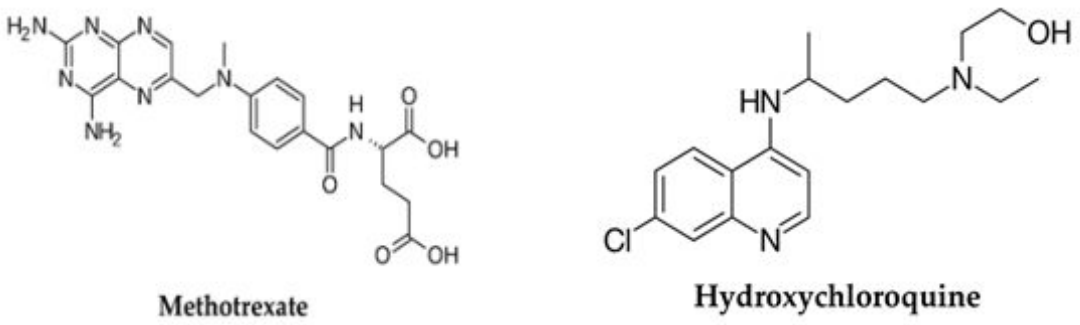

Figure 1: Structure of Methotrexate and Hydroxychloroquine

further improvement [16, 17]. Therefore, a combination of other disease-modifying antirheumatic drugs (DMARDs) with MTX will be the preferred pharmacotherapeutic approach for managing RA. The DMARDs reduce phagocytosis, T-cell activation, and pro-inflammatory cytokines such as tumour necrosis factor. They also have a marginally beneficial impact on those with active RA and systemic rheumatoid diseases, serving as immunomodulators and having marginal anti-inflammatory effects [18-21]. Hydroxychloroquine (HCQ) (structure is shown in **Fig. 1**) belongs to the category of DMARDs and is reported to be a safer drug than methotrexate when it comes to hepatic side effects [22]. Additionally, the potency, safety profile, and dosage of MTX are all reported to be improved by HCQ [23]. The extent of severity in RA is expressed as the Clinical Disease Activity Score (CDAI), which represents a simple summation of joints involved in the disease [24]. The study therefore aims at evaluating the effectiveness of the combination therapy of MTX and HCQ in the treatment of RA on the basis of CDAI.

METHODOLOGY

A bicentric retrospective observational study was carried out on RA patients receiving combination therapy of MTX and HCQ in the tertiary care hospitals of Ahmedabad city. Pediatric patients were excluded from the study. Informed consent from the enrolled patients were taken. Data entry forms were designed, and the data collected were demographic details, CDAI score, RA factor (RF), detailed treatment plan, and outcomes (remission) of the treatment. After approval from the Ethics Committee, subject data was collected over a period of January 2016- July 2021 and scrutinized according to inclusion and exclusion criteria. The data was collected in self-designed data entry forms. The CDAI score was recorded at baseline, after first year and second year of the study. The data was handled confidentially, with no access to any personnel other than the researchers. The data was analyzed statistically using SPSS 20 software with Friedman test (moderate and high severity groups) and Wilcoxon signed-rank test (low severity group).

RESULTS AND DISCUSSION

A total 576 patients RA patients on combination therapy of MTX and HCQ were enrolled to participate, out of which 84% i.e. 484, were female while 16% (n=92) were male. This can be attributed to the fact that the hormonal changes that females go through during menopause increase the risk of developing the disease. Apart from this, estrogen can enhance the humoral immunity in several systems while androgens and progesterone are considered as natural immunosuppressor and thus males are at a lower risk of developing RA [3-8].

It was found that out of total 576 patients, 26.2% (n=151) lie in the age group of 40-

49 years and 26.04% (n=150) lie in the age group of 50-59 years **(Fig. 2)**. The study shows similar results to another study conducted in Gujarat which observed that the age group of 40-59 years is mostly affected with RA [25]. The antibody test results of patients for the diagnosis of RA revealed that, 88.6% (n=514) patients tested seropositive for rheumatoid factor (RF), while 11.4% (n=64) patients tested seronegative **(Fig. 3)**. Seropositive patients have RF while seronegative patients do not have RF. However, significant local and systemic symptoms of RA persisted even in the absence of RF and therefore considered to be RA patient.

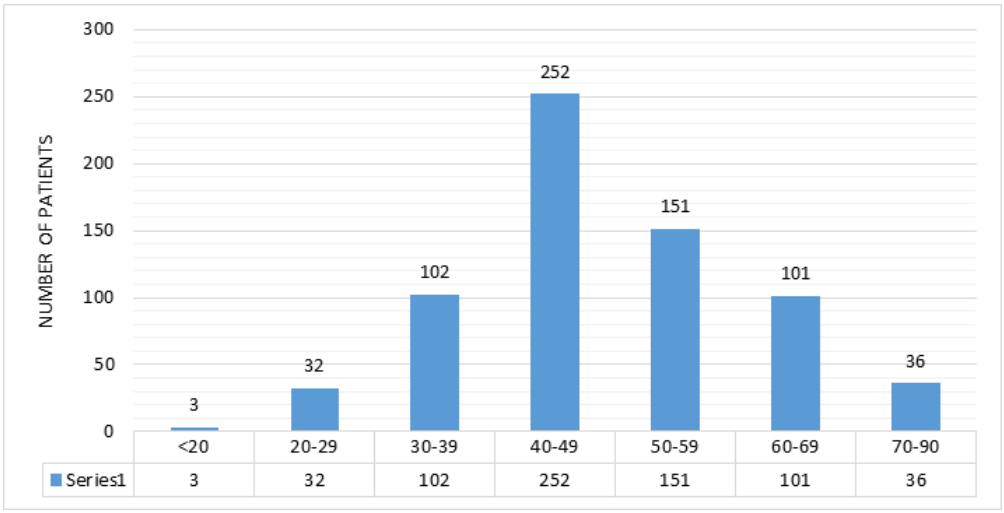

Figure 2: Age Distribution Among the Enrolled Patients (Age is Expressed in Years)

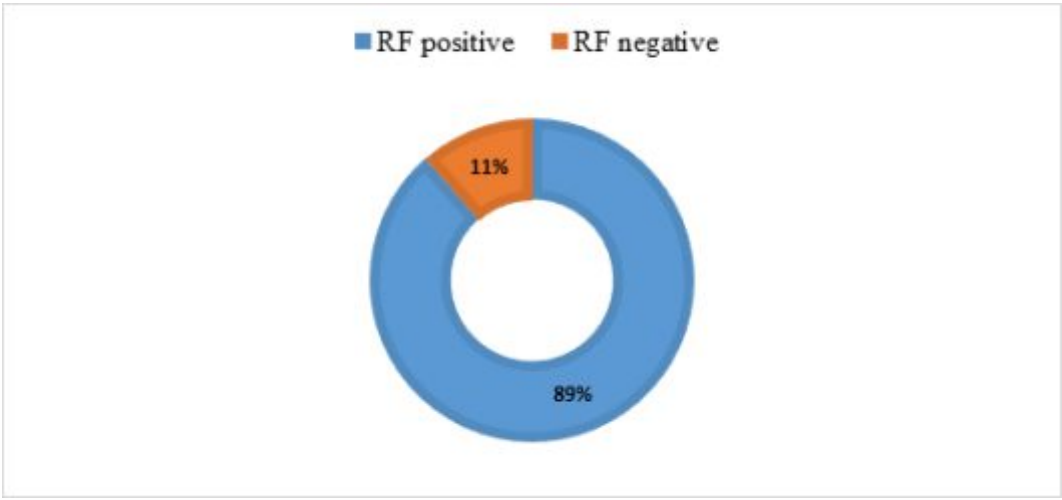

Figure 3: Distribution of Patients Based on Rheumatoid Factor (RF).

Based on CDAI score, 9.7% (n=56) of the total patients had low disease severity, 49.13% (n=283) had moderate disease severity, and 40.7% (n=235) had high disease severity. Significant improvement with the given combination therapy of MTX and HCQ in all group with low, moderate and high disease severity were observed **(Table 1)**. Remission was considered when CDAI score is less than and equal to 2.8. Remission was achieved in 56 patients (9.7%) based on a reduction in CDAI score. The patients with baseline low CDAI score often discontinue treatment and therefore remission was not mostly achieved in that case. High and moderate severity patients were followed up till two years and there was significant decrease in the CDAI score from the baseline indicating high recovery from the initial state. The mean score was 8.01 ± 3.78 and 13.69 ± 6.43 in moderate and high CDAI score category, respectively after treatment for 2 years (Table 2,3). These results are in conformity with the earlier published studies that show the effectiveness of MTX and HCQ combination in RA patients [26, 27].

Table 1: Effectiveness of MTX+HCQ in Enrolled RA Patients Based on CDAI Score

Disease Severity	CDAI Score		
	Baseline	**After 1 year**	**After 2 years**
Low (n=56)	7.77 ± 1.79	4.91 ± 1.91*	DT
Moderate (n=283)	18.03 ± 2.85	12.68 ± 3.25*	8.01 ± 3.78*
High (n=235)	28.31 ± 6.44	20.42 ± 5.46*	13.69 ± 6.43*

CONCLUSION

Results of the present study conclude that treating active RA in all the range of disease severity with combination of MTX and HCQ is efficacious as remission is significantly increased based on CDAI score.

Values are expressed as mean±SD.

*P value <0.001 compared to baseline CDAI is considered to be statistically significant.

DT: Discontinued treatment

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REVIEW ARTICLE

THE ROLE OF HERBAL COSMETICS FOR THE MITIGATION OF THE PSORIASIS

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ABSTRACT

Psoriasis is a chronic inflammatory skin disease with a strong genetic predisposition and autoimmune pathogenicity. The potential molecular targets for psoriasis are JAK, STAT3, Interleukin 8. By inhibiting theses targets it marks in alteration in immune response and suppresses the abnormal activation of inflammatory cascade like psoriasis. The lack of possible cure and certain adverse reactions to several synthetic treatments has led to extensive research for anti-psoriatic activity in herbal based formulation. The recent synthetic treatments available for treating psoriasis include phototherapy, oral medications like methotrexate, cyclosporine, and azathioprine but due to severe side effects of phototherapy which include pain, uneven pigmentation and scarring and certain side effects of oral medications like methotrexate increased the risk of liver fibrosis, cyclosporine can lead to hypertriglyceridemia. Due to these severe side effects which can lead to discomfort in the body. Therefore, the herbal preparations which are naturally available can avoid this problem and can be used to treat psoriasis. This review aimed for the exploration of the herbal cream formulation containing pure herbs, viz. oil extracts and methanolic extracts, extract of leaves of basil, thyme tulsi, turmeric, neem, beeswax, olive oil, rose oil assessed the antipsoriatic activity of various cream formulation.

Keywords: Psoriasis, Herbal Cream, Herbal Excipients, Molecular Targets

INTRODUCTION

Psoriasis is a recurrent, chronic inflammatory skin condition [1-16]. The most prevalent type of psoriasis is plaque (psoriasis vulgaris), which is characterised by reddish patches which is

coated in silvery white scales. The red patches are usually symmetrically distributed on the body part like knees, palm of hand and chest, as well as the scalp, elbows. They can grow into enormous plaques with a core patch of normal skin, forming geometrical patterns. The nails are become brittle, irregularly laminated and thicker in many cases.

The term 'psoriasis' refers to a variety of clinical conditions, the most prevalent and well-known of which being psoriasis vulgaris, often known as plaque psoriasis [17-24]. Given the prevalence of psoriasis vulgaris in comparison to other types of psoriasis [25-30].

Psoriasis normally appears gradually, although it can also appear suddenly. psychological disturbances, Injury to the skin and acute infection are all possible precipitating events. During the summer, the lesions usually become less severe and sometimes disappear, probably due to the action of sunlight. Extensive sloughing of the outer layer of the skin, with consequent irritation, and psoriatic arthritis are severe psoriasis consequences. Individuals with psoriasis, on the other hand, are often in good health. Researchers believe that the fundamental causes of psoriasis are the result of complicated interplay between genetic and environmental factors due to the disorder's heterogeneity in progression and severity [31-46].

Epidemiology

Psoriasis affects both men and women is most common between the ages of 10 and 30, but females and those mostly those female which have family history are more likely to develop it. Its onset age is bimodal, with men's peaks at 30–39 and 60–69 years and women's peaks 10 years earlier. It is especially common in colder climates. Psoriasis affects about 2% to 3% of the population in the US. Between 0.05 and 0.3 percent of Asians, on the other hand, suffer from depression. [7, 16-17]

Pathogenesis

Skin barrier changes associated with psoriasis. The stratum corneum (SC), which is connected to the protein-rich corneocyte, which is mostly composed of ceramides, cholesterol, and free fatty acids, is where the skin's barrier function is located. [10].

Regular corneocyte regeneration maintains the skin's water content, adaptability, and structural soundness while also treating any impairment [12]. Continuous exposure to environmental factors, chemicals, and harsh detergents and soaps, can have a significant impact on the skin's organizational and useful properties, leading to negative morphological and physiological changes to the skin throughout time [11].

A major issue with the psoriatic skin barrier's dysfunction is skin dryness, which is caused by an abnormal and flawed desquamation (shedding) process in

which visible scales made of corneocytes are shed. This results in the cosmetically unattractive rough texture of dry skin and excessive transepidermal water loss (TEWL), which in turn causes discomfort and itching. Irritators, allergens, and microbes can penetrate damaged, dry, and brittle skin that is unable to effectively bind and hold water, causing irritation, inflammation, and infection. [10-14]

Molecular targets for the psoriasis

JAK Inhibitors

Topical treatment for mild-to-moderate psoriasis has been successfully explored using the JAK1 and JAK2 inhibitor roxolitinib. According to study, uxolitinib is an effective topical medication with modest systemic absorption. Less than 1% of the plasma concentrations required for systemic action in healthy volunteers were present in patients taking topical treatment, showing that ruxolitinib reduces the growth of psoriasis plaques locally rather than through systemic effects. [38]

Tofacitinib has undergone the most rigorous scientific testing and is thought to be both a topical and oral administration. Stage I and II clinical studies on tofacitinib, confirmed dose-structured development in patients with psoriasis as compared to the fake treatment bundle. [38]

Bruton's tyrosine kinase inhibitor

In intrinsically resistant cells like dendritic cells (DCs) and gamma delta T cells, Bruton's tyrosine kinase (BTK) has been shown to carry out critical signalling functions. [39]

Small molecules focused on RORγt

Suffocating Th17 cell separation by focusing on RORγ**t** with tiny atom opposing agonists might be a promising treatment for psoriasis. Furthermore, little atoms disrupting RORt are expected to be safer than worldwide immunosuppressive specialists like cyclosporine. [40]

Stat3 as a healing goal for the remedy of psoriasis

Administration of STA-21-containing treatment to psoriasis patients in nonrandomized research. Following two weeks of topical STA-21 medication, the psoriatic injuries of six out of the eight patients improved. Therefore, we think that focusing on Stat3 could result in a psoriasis treatment [41].

The interleukin-8 receptor a potential target for antipsoriatic therapy

The development of psoriasis is thought to be significantly influenced by interleukin-8. Cyclosporine, calcitriol, calcipotriol, or dithranol all had a dose-dependent effect on interleukin-eight binding to cultured human keratinocytes [42,43].

AVAILABLE TREATMENT OF PSORIASIS

There is no permanent cure for psoriasis but there are a number of therapies

available like synthetic and herbal treatment to help with the skin symptoms.

Synthetic treatment of psoriasis

Topical psoriasis therapies made of synthetic materials come in a range of shapes (such as creams and gels) and often reduce inflammation and scaling. For instance, retinoids (vitamin A derivatives) and synthetic forms of vitamin D act by slowing down the division of skin cells, whereas corticosteroids, coal tar cream, and salicylic acid reduce inflammation. Psoriasis can be treated using phototherapy, which includes exposing the skin to UV rays [1]. Emollients and moisturizers can help reduce flare-ups. The greatest lotions, creams, and ointments for dry skin are typically heavy, oily products.

Salicylic acid removes the scales that develop on psoriasis areas. It comes in a variety of forms, including liquids, foams, gels, soaps, shampoos, pads made of fabric, and patches. When used in conjunction with other skin treatments, it is extremely beneficial. Other drugs function more effectively when dead skin flakes are removed.

Coal tar might improve the appearance of your skin by slowing the formation of skin cells. It also comes in a variety of forms, such as shampoo for psoriasis of the scalp. The inferior goods are sold over-the-counter.

Mild steroids (corticosteroids) reduce inflammation and decrease skin cell proliferation to prevent accumulation. They are available in various strengths. For delicate places like your face or neck, as well as skin-fold locations like your groin or armpit, weaker formulations may be effective. A prescription from a doctor is necessary for stronger corticosteroids.[8]

Drawbacks of the synthetic treatment of psoriasis

While phototherapy offers advantages, there are some disadvantages as well, including pain, uneven pigmentation, and scarring. A higher risk of skin cancer has also been connected to long-term therapy. The most effective psoriasis drugs impair the immune system, leaving patients susceptible to a number of infections and illnesses that can be fatal. Despite this, oral medications are routinely used as a last resort to treat psoriasis. Examples of oral anti-inflammatory drugs include methotrexate, cyclosporine, and azathioprine. Oral biologics (made from human or animal proteins) are drugs that alter the immune system by destroying malfunctioning immune cells. Biotechnological treatments for psoriasis include infliximab (Remicade), etanercept (Enbrel), and guselkumab (Tremfya) [1]. The side effects of oral medication which is used to cure psoriasis is show in Table 1. [9] Retinoids, which are vitamin A-based medications, can increase your risk of liver disease.

Table 1: Conventional Systemic Therapy and Associated Risk

Drug	Associated Risk
Methotrexate	Liver Fibrosis
Acitretin	Hypertriglyceridemia
Cyclosporine	Nephrotoxicity
Phototherapy	Erythema

HERBAL TREATMENTS FOR PSORIASIS

People can manage their symptoms with the use of medicines and natural therapies. Many herbs can help with psoriasis symptoms by reducing inflammation or slowing the proliferation of skin cells without any adverse effect.

Mahonia aquifolium

This plant has antiproliferative properties, which means it can slow down skin cell proliferation. This capacity aids in the treatment of psoriasis, which causes skin cells to divide too quickly, resulting in scaly skin and plaques.

Aloe vera

Aloe vera contains chemicals that may assist to decrease inflammation and modify the immune system. Aloe vera may benefit from these ingredients since they allow it to Helps to keep the skin from drying out by soothing the skin and reducing inflammation.

Other all-natural remedies

There is currently insufficient evidence to suggest that alternative herbal therapies are effective psoriasis treatments. However, the following herbs may be useful therapy choices for folks to viz. neem, extracts of sweet whey, capsaicin, curcumin. For treating symptoms, vitamin D creams may be as effective as corticosteroid treatments. Corticosteroids, on the other hand, were more successful for scalp psoriasis. [18] There are several disadvantages of the synthetic treatment of psoriasis like phototherapy and oral medication while In herbal treatment of psoriasis ,there are less adverse effect on the skin and other body part than the synthetic that is why we can prefer more herbal formulation or medication than synthetic medication.

HERBAL EXCIPIENTS WHICH CAN BE USED FOR THE PREPARATION OF HERBAL FORMULATION MEANT FOR THE TREATMENT OF PSORIASIS

Tamarind seeds

The core of Tamarindus indica (family Fabaceae) is used to make tamarind xyloglucan. For viscosity and adhesion, this is required. Tamarind gum is a carbohydrate having a glucosyl, xylosyl, & galactosyl ratio of 3: 2: 1. The wet

granulation approach was utilised to manufacture matrix tablets from polysaccharide derived from tamarind seeds, and the effects on release of drug were investigated. Tablets with varying polymer proportions have been developed, resulting in decreased drug release and higher polymer content. The tamarind gum-based tablets are capable of passing through the bulk of the drug. [20,21].

Fenugreek seeds

Trigonella foenum graecum Linn., popularly known as fenugreek, is an annual fragrant leguminous plant which is known as fenugreek. These herbs have been utilised for their medicinal effects for a long time. Because of their bioactivity, degradability, simplicity of access, and low cost, natural polysaccharides found in the Fenugreek plant are used as culinary and pharmaceutical excipients. Flatulence, diarrhea, cholera, lack of appetite, indigestion, difficulty breathing, eye diseases, have all been treated with this. It has anti urolithic, antihypertensive, antidandruff, anti-inflammatory, anti-oxidant, and anti-inflammatory properties. The mucilage content of fenugreek seed is high. [22]

Karaya gum

The polysaccharide gum karaya has an acidic pH. This molecule is made up of glucose, rhamnosis, & galactic acid. Gum's high viscosity prevents it from being used as a binder & disintegrant for typical dosage forms. After many results study revealed that an altered gum karaya disintegrated quickly in the tablets, the capability of gum karaya was studied [26].

Guar gum

Guar gum is a type of gum that comes from the guar plant. Guar gum is comparable to locust bean gum in that it is mostly made up of a polymer of galactose & mannose with various proportions. Guar gum is used as an adhesive and dissolving agent in tablet formulations. This emulsion stabilizer is quite useful. It is not affected by the pH, water content, or solubility of the tablet matrix. It is not always bright white and discolours with time in alkaline tablets. [28]

Lubricants

Lubricants as shown in Table 5 can avoid components from sticking to capsule machine and clumping together. Lubricants prevent the material and the die wall from grinding together while producing and ejecting the tablet, which is critical for proper tablet formation and ejection. Lubricants promote product flow by reducing inter-particle friction. Lubricants are often split into two types: hydrophilic and non-hydrophilic.

Table 2 List of natural Lubricants

No.	Excipients	Source
1	Stearic acid	Animal
2	Castor oils	Castor seeds
3	Sodium chloride	Minerals
4	Paraffin oil	plant of paraffin

PRESERVATIVES

Preservatives are frequently used to increase the shelf life of many food and medicinal products. To stop microorganisms from altering and degrading during storage, especially in products with a water content, preservatives are required.

Clove essential oil

Clove oil contains the highest amounts of phenolic substances including polyphenols, eugenol acetate, gallic acid, as well as ß-pinene, limonene, linalool, and benzaldehydes, 2-Heptanone, ethylhexanoate at lesser concentrations. Clove's antibacterial and antioxidant properties, among other things, make it very desirable. As per the World Health Organization, people should ingest 25mg/kg of body weight each day. Eugenol is absorbed fast through the tongue.

Neem oil

This herb is unique in that it has therapeutic qualities throughout the entire plant. Herbal remedies are made from seeds, and leaves, flowers, bark, which are all significant elements of the plant.

CONCLUSION

This leads us to the conclusion that synthetic treatments, such as oral medicines and phototherapy, frequently have harmful side effects. Herbal creams and other formulations have shown to be quite effective at treating psoriasis with fewer side effects. It has been found that several different herbs, including turmeric extract curcumin, block the phorbol ester-induced activation of the transcription factors NF-B and AP-1 and useful for the treatment of psoriasis.

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REVIEW ARTICLE

A REVIEW ON SEMECARPUS ANACARDIUM LINN

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ABSTRACT

A powerful ethnomedicinal plant from the Anacardiaceae family known as "Bhallatak" or "Bhilawa" is *Semecarpus anacardium* Linn. and has strong therapeutic value. Several treatments use bhilawa, both conventionally and ethnobotanically. *S. anacardium* nut phytochemical examination reveals biologically active substances such bhilawanols, biflavonoids, minerals, phenolic compounds, amino acids, and vitamins, and which demonstrate varied therapeutic characteristics. In their clinical work, traditional healers and doctors employ formulations of *S. anacardium*. Numerous formulations are available on the market; some of the more popular ones are Bhallatakasav, Bhallatak Parpati, Amritbhallatak Avaleha, Suran vatak, Narsimha choorna and Sanjeevani Vati. *S. anacardium's* pharmacological effects, including its hypoglycemic, CNS stimulant, anti-cancer, antioxidant, antiatherogenic, antibacterial, anti-inflammatory, and also promoter activity of hair growth, have been proved in many research study.

Keywords: *S. anacardium*, Medicinal plant, Pharmacological Activity

INTRODUCTION

Plants are essential to human survival and the foundation of life on earth. The local population typically relies on neighboring forested regions to meet their needs for things like medicine, wood, timber, wild veggies, fuelwood, and a variety of other things. Many cultures have used plants and herbs for thousands of years to treat disease and promote wellness. The use of Indian herbal remedies is becoming more widely accepted worldwide. Almost all

therapeutic formulations used in Ayurveda are made from plants. In addition to their active components, minerals, alkaloids, volatile oils, vitamins, glycosides, alcohols, acids, esters, and other compounds make plants and herbs beneficial. Since ancient times, higher plants have been important in maintaining human health as sources of therapeutic chemicals. Different types of conventional medications are referred to as complementary and alternative medicine (CAM) in other parts of the world. Any treatment used in conjunction with (complementary) or in substitute of (alternative) conventional medical treatment is referred to as complementary and alternative medicine (CAM). In alternative medicine, medicinal plant formulations are frequently used, especially for illnesses that cannot be treated with current methods [1]. Natural products are the source of more than 50% of all contemporary clinical medications [2] and are crucial to the pharmaceutical industry's drug research efforts [3].

S. anacardium Linn. is the most adaptable, one of the best, and widely used plant as a household cure, which is found in the sub-Himalayan region, Orissa, Bengal, Bihar, and central parts of India. Since many years ago, it has been extensively utilized throughout India. The Greek words simeion, which means marking or trace, and carpus, which means nut, are the origin of the word *S. anacardium* means "heart-shaped marking nut" and is similar to cardium. It is referred to as Bhilaavaa, Bhallataka, and Serankottai in the Unani, Ayurvedic, and Siddha medicine, respectively.

In Ayurveda bhallatak are noted to possess madhur, ushnavirya, kashayras, ushnagunas, madhurvipakand, snigdh and tikshna qualities [4,5]. It has several karmas includes Bhootanashan (anti-devil), Kaphavatashamak (alleviates kapha & Vatadosha), Medhya (beneficial to brain), Pittasanshodhak (expels out pitta dosha), Vrishya (aphrodisiac), Chedana (excisional functions), Bhedan (incisional function), Bruhan (anabolic in effect), Vanhikar (improves digestive fire) due to that it indicated for many diseases like Arsha (Haemorrhoids), Grahani (Inflammatory bowel disease), Shwitra (Vitiligo), Udar (Ascites), Shotha (Inflammation), Kushtha (Skin disorders), Gulma (Abdominal mass), Krumi (Helminthiasis), Adhman (Flatulence), Vran (Wounds), Jwar (Fever) etc [6].

Bhallataka is classified by Maharsi Charaka as Dipaniya, an appetizer, Bhedaniya, a herb that breaks up buildup, Mutrasangrahaniya, an antidiuretic, and Kusthaghna, an antidermatosis. A popular medication for the treatment of piles of Kapha and Vata types is bhallataka. Additionally, it has the capacity to cause contact dermatitis, which might result in allergic symptoms. Because of its high potency, it is only used after purification processes. The fruit, gum, and oil have all been employed for its medicinal powers since ancient times, and this plant has been

dubbed “HAIF PHYSICIAN” in Ayurveda. The nut of this plant contains biflavonoids, phenolic compounds, vitamins, minerals, bhilawanols, and amino acids, according to chemical and phytochemical tests. Numerous extracts of nut medicines from this plant are beneficial against a wide range of illnesses, including infections, malignancies, and rheumatoid arthritis, among others. However, isolating its active ingredient and figuring out the structure-function relationship can substantially benefit in understanding the pharmacological activity of its nut.

The purpose of this review is to provide an overview of *S. anacardium's* description, phytochemistry, medicinal activity, and recently identified effects and applications.

TAXONOMICAL CLASSIFICATION

Plantae (Kingdom), Tracheobionta (Subkingdom), Spermatophyta (Super division), Magnoliophyta (Division), Magnoliopsida (Class), Rosidae (Subclass), Sapindales (Order), Anacardiaceae (Family), *Semecarpus* (Genus), *anacardium* (Species)

Synonyms

Sanskrit: Arusharah, Antahsattva, Arzohita, Ballata (Ballata, Bhallata,), Aruskara (Arukara), Bhallatakah, Visasya, Viravrksa.

English: Marsh Nut, Indian Marking Nut Tree, Oriental Cashew Nut

Hindi: Bhelwa, Bhel (Bhela), Bhilwa, Bhilv (Bhilawa)

Gujarati: Bhilamu

Telugu, Nallajeedi

Tamil: Erimugi (Erimuki)

Assamese: Bhelaguti

Marathi: Bibha, Bibba

Bengali: Bhelatuki, Bhela

Malayalam: Chera, Alakkuceru

Oriya: Bhollataki

Kannada: Bhallika, Bhallataka, Karigeri, Goddugeru

Kannada: Bhallataka, Bhallika, Goddugeru, Karigeri

Punjabi: Bhilawa

BOTANICAL DESCRIPTION

It is a deciduous tree having medium-sized that grows up to 3500 feet in height in the hotter regions of India and the outer Himalayas. In Bihar, Assam, Orissa, and Bengal, as well as in Central India and the Western Peninsula of East Archipelago, Northern Australia the plant is abundant where it is called the ‘marking nut’ by Europeans, because it was used by washermen as an as an indelible ink to mark clothes before washing.

The tree is medium-to-large in size, growing 15 to 25 meters tall, with a grey bark that flakes off in little, and irregular

flakes. Its leaves are simple, alternating, obovate-oblong, 30 to 60 cm long, 12 to 30 cm wide, and apex are rounded. The blooms are greenish white, in panicles, and develop in May and June along with new leaves. They are easily identified by their big leaves and the red blaze of resin that they exude, which turns black when exposed to air. Fruits are obliquely ovoid and 2–5 cm long. The fruit's upper portion, which has a cup-like shape when ripe, is smooth, fleshy, orange red in color, and sweet and delicious. It is composed of the thickened disc and calyx base. The lower base, which is rotatable, is made up of an oblong pericarp with corrosive resinous fluid-filled cells between its inner and outer laminae. The pericarp is thick, smooth, black, and lustrous. The juice of the fruit is white while it is young, but as it ripens, it turns brownish black. The nut weighs about 3.5g and has dimensions of roughly 1"x 0.75"x 0.33" [7]. Instead of wet environments, it is commonly found in drier ones. The fruit is 2-3 cm wide and ripens between December and March. no particular affinity for soil. It is a modest shade-bearer with an obliquely ovoid that is 2.5-3.8 cm long, compressed, and shines black when ripe. The disk, calyx base, and peduncle extremity are all orange in color. Gray in hue, the bark releases an irritating fluid when cut [8]. (Figure 1)

Fig.1. *S. anacardium* Plant, Fruit and Nuts

PHYTOCHEMISTRY

Bhilwanols, phenolic compounds, [9,10] biflavonoids, [11] sterols, and glycosides are the main constituents of *S. anacardium* Linn. [10,12] Bhilawanol is a mixture of *trans* and *cis* isomers of ursuhenol obtained from fruits, with the primary components being 1,2,hydroxy-3 (pentadecadienyl 82) benzene and 1,2,dihydroxy-3 (pentadecadienyl 82 ,112)

[13]. Other Compounds also presents like, Semecarpetin [14], Anacardoside [15], Jeediflavanone,[16,17] Nallaflavanone [18], Galluflavanone [19,20] Anacarduflavone [21] Semecarpuflavanone [22] Bhilawanol-A, mono-olefin I, diolefin II, Bhilawanol-B, amentoflavone anacardic acid, o-trimethyl biflavone A2, Tetrahydroamentoflavone, *O*-hexamethyl bichalcone A, *O*-tetramethyl biflavanone C *O*-tetramethyl biflavanone A1, *O*-heptamethyl bichalcone B1, *O*-dimethyl biflavanone B, *O*-hexamethyl bichalcone B2. [23] Figure 2 showed the chemical structure of few chemical constituents of plants.

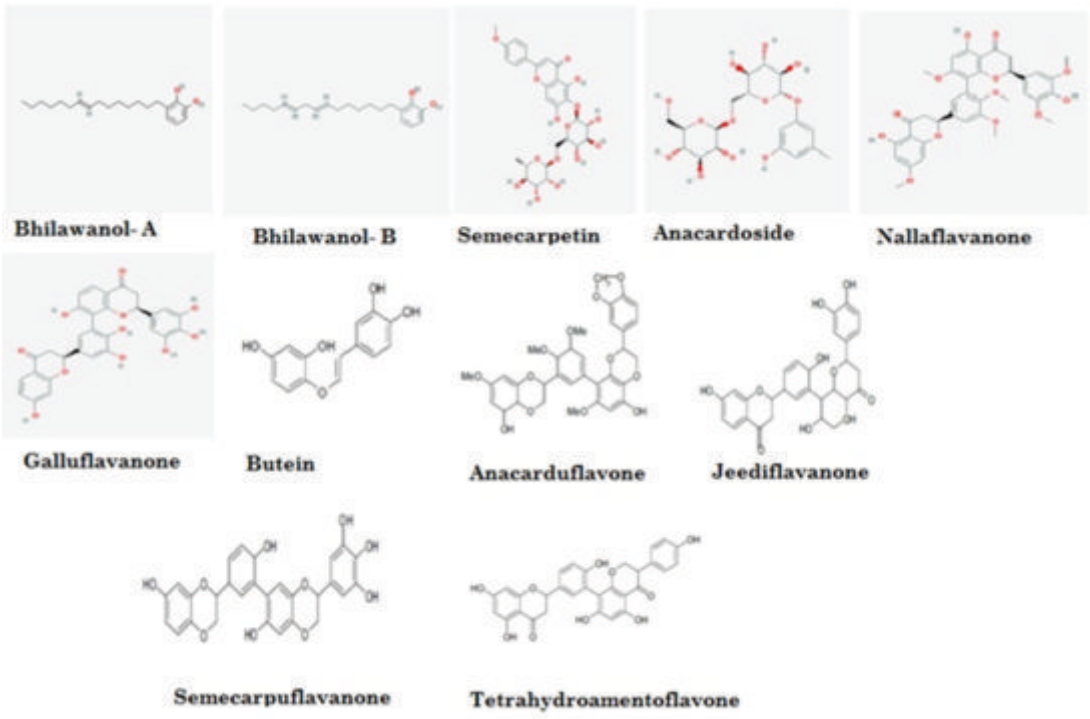

Figure 2 Phytoconstituent of *S. anacardium*

MEDICINAL PROPERTIES

S. anacardium have an astringent (kashaya), pungent (katu) and bitter (tikta) in taste (rasa) and three qualities (guna) like piercing (lkshna), unctuous (snigdha), light to digest (laghu) and veerva is hot in potency (ushna); vipaka is madhura (after digestion, undergoes sweet taste conversion); karma is vrsya, medhya, vatahara, bhedana, dipna; and are indicated in arsas, kustha, vatavyadhi, kapha vikara, krmi, gulma and grahani.

THERAPEUTIC USES

External uses- Bhallataka juice is given to the snake bite location after incisions have been made because it causes blisters and is

an antidote for snake bite. Haemorrhoids are made to dry up and fall out by burning bhallataka incense. It causes abortion when administered to the vagina. Any discomfort from an injury is relieved by applying this nut or cauterizing it with the seed.

Internal Uses

Helpful for people with hemiplegia, epilepsy, sciatic neuralgia, and nervous system impairment. Using its functions as appetizers, digestants, purgatives, and liver stimulants, the digestive system is employed to treat GIT problems. It is the greatest treatment for intestinal colic and is useful in cases of loss of appetite, digestive issues, constipation, gulma, ascites, sprue, piles, and many forms of worms. Acts as an expectorant in the respiratory system and is helpful for coughing and asthma. Within the reproductive system, it activates penile nerves and increases virility, making it an aphrodisiac. It's hot and sharp qualities stimulate the uterus. It is used to treat seminal indisposition, impotence, and dysmenorrhea; godambi is best consumed in the winter. It is eliminated via the skin. The best treatment for dermatoses, vitiligo, and vatrakta is bhallataka. Milk and shevate are helpful for joint inflammation. It functions as a tonic and rejuvenator in addition to enhancing each dhatu's agni. helpful for overall ill health.

SHODHANASANSKARA OF BHALLATAKA:

Shodhanasanskara (purification process) is the procedure by which specific chemicals are treated with processes like as rubbing, heating, and so on in order to remove their detrimental or toxic effects. Before being used for therapeutic purposes, poisonous plant medications are treated to shodhanasanskara. The shodhanasanskara method significantly lowers the toxicity of dangerous plants. One such poisonous plant that is still utilized in Indian medicine is *S. anacardium*. Bhilawanols are among the plant's hazardous chemical constituents. The detoxification/purification process known as sodana involves both purification and a decrease in the concentrations of harmful substances in fruits [24]. Following are the various methods for purification of fruits.

1. **With gomutra**: The fruits of *S. anacardium* contain tarry oil in their pericarp, which is composed of 90% anacardic acid and 10% cardol, as well as bhilwanols, semecarpol, and anacardol. Bhilwanol and anacardic acid are the culprits behind blisters, irritation, contact dermatitis, and toxicity. The fruits of *S. anacardium* are soaked in gomutra for about seven days before being rubbed with brick powder or brick gravels and ultimately washed with water in this purification process. While processing *S. anacardium* nuts, dermatitis can be prevented by using coconut oil. This process results in the decarboxylation of oil and the conversion of anacardic acid to the less poisonous anacardol. The soaking of the fruits in gomutra may cause the oil to be diminished. Fruits' irritating oil is absorbed by the brick gravel's absorbent

properties. The amount of total flavonoid and total carbohydrate content is not affected by the purification procedure, but there has been a noticeable fall in total phenolic content. *S. anacardium*'s antioxidant capacity declines, while its safety profile improves [25].

2. **With gomutra and cow milk**: Using this technique, a sharp knife is used to cut away the fruit's thalamus portion. The nuts are then exposed to fresh cow pee every day for seven days, then cow milk every day for seven days, and finally thoroughly rubbed with brick powder for three days. The nuts are cleaned every day with water prior to the addition of new cow milk or urine during the treatment with cow urine and cow milk. Such fruits are rubbed with brick gravel and left in touch with it for 3–4 days after the nuts are removed from cow milk or urine. The nuts were rinsed in hot water on the final day (18th day) to get the brick powder off. Three times this shodhana process is performed [24].

3. **With brick powder**: For shodhanasanskara, ripe bhallataka fruits that are submerged in water are chosen. Fruits that float in water are not accepted. A pottali (little cotton bag) constructed of three to four folds of cotton cloth is used to hold bhallataka fruits and ishtika churna (brick powder). Hands are used to rub this pottali with mild pressure. After the skin of the bhallataka fruit has been scraped off, brick powder is wetted with oil and then washed in hot water. bhallataka becomes shuddha (pure) through this technique. [26].

4. **With coconut water**: Bhallataka fruits are split in half, put in a swinging device called a dolayantra, and heated for a period of one to two hours. During this procedure, bhallataka turns into shuddha. Sanskara coconut oil should be used with caution during shodhana to exposed body regions such as the face, hands, legs, and other areas. [26].

5. **Frying method**: 200 g of randomly chosen fruits are placed in an iron pan, where heat is provided from underneath by the ignition of charcoal. After 4-5 minutes of heating, smoke began to emanate from the nuts. The pan holding bhallataka nuts is then covered with burning charcoal. The hot nuts caught fire right away. After two minutes, the fire is put out by spreading it with a long ladle as soon as the flaming nuts are removed from the pan to the ground. The nuts were then allowed to cool before being placed in an airtight glass container for future research. The same process was carried out three times. [27]

BHALLATAK FORMULATIONS

Fifty mahakasayas are detailed in sutra sthana of the charakasamhita. Bhallataka has described some of these mahakasayas in the deepaniya mahakasaya. [28] Kusthagna mahakasayas (a collection of plants that aid in digestion). [29, 30]

The 10 various types of Bhallataka preparations are listed in Rasayanaadhyaya [31] in the Charakasamhita are bhallatakakshir, bhallatakapalala, bhallatakakshoudra, gudabhallataka,

bhallatakataila, bhallatakasarpi, bhallatakalavana, bhallatakayusha, bhallatakatarpana, bhallatakasaktu.

Sushrut and Vagbhatt have suggested using roughly 1,000 bhallatak seeds for the course of one therapeutic course of Vardhman prayog. Bhallatak is currently employed as a primary or minor ingredient in several formulations. Bhallatakasav, sanjeevani vati, amritbhallatak avaleha, narsimha choorna, suran vatak, bhallatak parpati, and other formulations are regularly used. Bhallatak is subjected to shodhana (purification and detoxification) before being used for medical purposes. [32] (Table 1)

Table 1. Bhallatak containing Formulations used in Markets

Formulation	Product Nature	Dose	Indication
Amrut bhallatakavaleha	Electuary	1-2 tsf, 2 times a day	Vitalizer, General tonic
Narsimha choorna	Powder	1-2 gm, 2 times a day	Restrorative
Suran vatak	Pills (500 mg each pill)	2 pills, 2 times a day	Anorectal and Piles diseases
Sanjeevani vati	Pills (250 mg each pill)	2 pills, 3 times a day	Diarrhea, Dysentery
Bhallatakasava	Wine	2-4 tsf, 2 times a day	Asthma, Neuralgia
Bhallatak parpati	Powder	250 mg, 3 times a day	Rheumatic diseases

Precaution required during consuming bhallataka containing formulation

Bhallataka must be consumed with a substantial amount of milk, rice, and ghee. After taking the formulation, avoid walking in sunrays, excessive sexual intercourse, salt, meat eating, exercise, and oil massage. Formulations of Bhallataka are contraindicated in pitta illnesses, pregnant women and children, patients with a history of hemorrhage, diarrhea, and nephritis. [26]

Manifestation of toxicity of bhallataka

Contact of Bhallataka fruits or blossoms with the body is one of the causes of Agantuja shotha according to Charaka samhita. If Bhallataka juice (even in trace amounts) comes into contact with the body, it causes severe burning feeling and ulcer. When it comes into touch with the face, it generates an intense burning sensation due to the presence of inflammation and ulcer. Some people are allergic to bhallataka, which manifests as itching all over the body, black and bloody

urine, red patches, fever, diarrhea, blisters. Oligouria, murky urine, and irritation at the anus and penis may also be discovered. There have been some reports of bhallataka having a negative influence on pile treatment.

Treatment of bhallataka toxicity

Sesamum paste rubbed with milk of buffalo and mixed with Ghee is applied locally or locally rubbed *Glycyrrhiza glabra* and sesamum paste, or rubbed shalapatra (*Desmodiun gangetictum*). In shotha caused by bhallataka, paste of sesamum with milk of goat and butter or black clay is employed. For quick relief from bhallataka shotha, some local applications are as follows - Mixture of *Cedrus deodara*, *Brasica juncea*, *Cyperus rotundus* and butter or mixture of *Amaranthus spinosa* juice and butter or mixture of butter, sesamum, sugar and milk Or *Azadirachta indica*, sesamum, sesamum oil are boiled together and made concentrated to apply locally. When signs of toxicity are seen, bhallataka medicine is stopped, and coconut white albumen, *Tamarindus indica* leaf juice, sesame seeds, or coconut is given to consume. Ghee, coconut oil, and lead lotion are administered externally. *Terminalia bellirica*, a specialized antidote for the toxicity of Bhallataka, is used. For sudden reactions and systemic effects, *Terminalia bellirica* fruit rind and bark decoction or powder preparations work well. It is also possible to utilize medications that reduce pitta, such as milk, clarified butter, and other medications with a low potency. Due to inappropriate handling of equipment and disposal of media used in the shodhana technique, there were five occurrences of contact dermatitis that developed during various stages of shodhana sanskara of bhallataka fruits. The affected individuals were instructed to apply crushed *Azadirachta indica* leaves externally to the affected areas and internally to take sarivadyasava 30 ml three times daily after meals and triphala churna 5 gm twice daily before meals. [32]

PHARMACOLOGICAL ACTIVITY

Analgesic activity: Ilanchezhian Rangasamya used the tail flicking method to test the analgesic activity of three distinct *S. anacardium* extracts, including petroleum ether, methanol and chloroform extracts. As a standard reference, they have employed acetyl salicylic acid (aspirin). At 50 mg/kg, the methanol extract demonstrated a substantial analgesic effect. They discovered that the extract of methanol was more powerful than the extracts of chloroform and petroleum ether [33].

Hypoglycemic effect

Arul investigated how dried, ripe *S. anacardium* nuts in ethanolic extract affected blood sugar levels. Both regular and streptozotocin-induced hyperglycemias in rats were studied for the impact. Levels of blood glucose were decreased by 100 mg/kg dose of the ethanolic extract of *S. anacardium*, but no antihyperglycemic effect was visible. [34].

Hypolipidemic effect

S. anacardium, *Emblica officinalis*, and honey are all included in the modified Siddha preparation known as Kalpamrutha (KA), which was created by Krishnamurthy after extensive research on the differences in lipids, lipid-metabolizing enzymes, and lipoproteins in malignant animals. The impact of *S. anacardium* and kalpamrutha on elevated levels of free cholesterol, phospholipids, total cholesterol, free fatty acids and triglycerides, and decreased levels of plasma cholesterol, the liver, and the kidney was also investigated. Levels were found to be normal in cancer-suffering animals. [35]

Hepatoprotective effect

S. anacardium's antioxidant and protective effects against lead acetate-induced toxicity was the subject of Abirami's study of the plant. He examined the plant's phytochemicals, including alkaloids, flavanoids, tannins, resins, proteins, and carbohydrates, and which are probably substance for its hepatoprotective effectiveness. [36]

Anthelmintic activity

Pal has investigated the anthelmintic effects of several *S. anacardium* nut extracts on adult Indian earthworms. They discovered that *S. anacardium* extracts in petroleum ether and chloroform exhibit greater anthelmintic action than those in ethanol and water solution [37].

Anti-cancer activity

Mathivadhani investigated the inhibitory effects of Nut extract of *S. anacardium* on the T47D- Cancer cell line of human breast. At the molecular level, it was accompanied by an increase in Bax and a decrease in Bcl, as well as caspase, PARP, cytochrome c, and internucleosomal DNA fragmentation [38]. Leukemic induced mice when treated with *S. anacardium* nut milk extract shown restored energy metabolism, according to Sugapriya. Treatment for *S. anacardium* was contrasted with imatinib mesylate, when Leukemic animals were given *S. anacardium* nut extract, the results indicated that the leukemic cells had been cleared from internal organs and bone marrow [39].

Arulkumaran looked into the effectiveness of the Kalpaamruthaa preparation, which contains honey, dried *Emblica officinalis* fruit powder, and *S. anacardium* nut milk extract, in preventing peroxidative damage and aberrant levels of antioxidant. Preparation comprising Kalpaamrutha and *S. anacardium* has demonstrated anticancer action in breast cancer induced by dimethyl benzanthracene. [40]

Prabhu investigated the anti-mutagenic properties of *S. anacardium*. They chose mice for this investigation that had received two doses (250 and 500 mg/kg, i.p.) of *S. anacardium*, which demonstrated showed a significant inhibition of induced aberrations at the 12 h pretreatment period.

The results on the reduction of induced chromosome aberrations clearly show that SA serves as an antioxidant because of the presence of flavonoids which scavenge free radicals. The action of SA oil extract has definite beneficial role against mitomycin-C induced mutagenicity and its administration may be protective and therapeutic. [41]. Krishnarajua discovered that the brine shrimp lethality test was used to check the cytotoxicity of aqueous preparations of medicinal herbs. With an LD50 of 29.5 µg *Semecarpus anacardiaceae*, one of the 120 species studied, demonstrated notable cytotoxicity [42].

Joseph investigated the anticancer properties of an Ayurvedic *S. anacardium* nut preparation. He had given one group the ayurvedic remedy that contained *S. anacardium* and another group its nut milk extracted. He discovered that both liver enzymes and the HCC - Hepatocellular Carcinoma marker had increased after 154 days of testing, associated with neoplastic alterations in the liver and had decreased in the *S. anacardium* milk extract-treated group. The Ayurvedic medicine and the effects of doxorubicin were positively correlated. This study showed that *S. anacardium* milk extract is effective in the treatment of hepatocellular cancer [43].

Protective effect on CNS

Farooq studied the effects of Nut extract of *S. anacardium* on the Central Nervous System (CNS), focusing on its nootropic and locomotor properties. Vinutha discovered that the cholinergic cells losses, particularly in the forebrain basal, are associated with a decrease in the neurotransmitter acetylcholine (ACh). The *S. anacardium* inhibits acetylcholinesterase, hence lengthening the half-life of acetylcholine. *S. anacardium* can help with cognitive decline and memory improvement [44].

Anti-inflammatory activity

Sushma used a carrageenan-induced rat paw edema model to examine the anti-inflammatory effects of an ethanolic extract of Nuts of *S. anacardium* in albino rats. Ethanolic extract of Nuts shown dose-dependent anti-inflammatory effects [45]. *S. anacardium* considerably reduced the cotton pellet granuloma and carrageenan-induced paw edema, according to Ramprasath's research [46]. *S. anacardium* has been shown to have anti-inflammatory properties for both non-immunological and immunological causes, according to Satayavati and Bajpai [47]. In hepatocellular carcinoma, Premlatha has reported that Nut extracts of *S. anacardium* gives immune-modulatory potency, tumor regulating activity, also give anti-oxidative and membrane-stabilizing activity, its restore the glucose level and regulate the mineral and found a potent effect against the hepatocarcinogen aflatoxin B1 [48]. Salvem noted that Tetrahydroamentoflavone (THA), a biflavonoid, was the main active ingredient that was isolated from the Ethyl acetate extract of *S. anacardium*. THA inhibited

prostaglandin synthesis by cycloxygenase (COX-1) *in vitro* and showed dose dependent anti-inflammatory activity in carrageenan-induced paw edema experiment [49]. Bhitre synthesized Petroleum ether, Ethanolic, Methanolic, Ethyl acetate, and Chloroform extracts of Nuts of *S. anacardium* and investigated their anti-inflammatory effect in albino rats using the carrageenan-induced paw oedema approach which showed all extract had anti-inflammatory activity which was comparable to aspirin [50].

Singh studies the in vitro anti-inflammatory activity of ethanolic extract of Nuts of *S. anacardium* using synovial fluid mononuclear cells and peripheral blood of healthy individuals and Rheumatoid Arthritis (RA) patients. Extract of *S. anacardium* shows inhibition of the LPS-induced production of pro inflammatory cytokines IL-12p40 and IL-1β but had no effect on IL-6 and TNF-α [51].

Mythilypriya showed that extract of *S. anacardium* have anti-inflammatory activity using an Adjuvant-Induced Arthritic rat model [52].

Antioxidant activity

Shanmugam discovered that albino rats receiving Kalpaamruthaa had normal lipid peroxide levels as well as antioxidant defenses in *S. anacardium* [53]. Veena assessed the antioxidant status of control and experimental animals' blood as well as key organs (liver, kidney, and breast tissue). When a medicine (*S. anacardium* and kalpaamrutha) was supplied to cancer patients, it was discovered that it decreased lipid peroxidation and improved antioxidant activity [54].

Sahoo studied the antioxidant activity of an Hexane, Methanol, Chloroform and ethyl acetate extract of *S. anacardium* stem bark and the ethyl acetate extract had the best antioxidant activity. The ethyl acetate extract of the stem bark of *S. anacardium* generated a bright yellow solid crystal, which was recognized as butein. This chemical was found to have antioxidant activity [55].

Antimicrobial activity

Sharma investigated the antifungal efficacy of *S. anacardium* at 400 mg/ml concentration against (*Aspergillus fumigatus* and *Candida albicans*). Both fungi were reported to have growth inhibition, cell size reduction, and decreased sporulation [56].

Sharma discovered that nut oil of *S. anacardium* has high antibacterial activity against numerous Gram positive and Gram negative bacteria [57]. Mohanta synthesized organic solvent and aqueous extracts of the *S. anacardium* and tested them for antibacterial and phytochemical characteristics using the disc diffusion method. At 100 mg/ml, Aqueous and Petroleum Ether extract inhibited *S. flexneri* and *S. aureus* and respectively, while chloroform extract inhibited *V. cholera*, *P. aeruginosa*, and *B.*

licheniformis, and ethanolic extract inhibit the *P. aeruginosa* and *S.aureus* [58].

According to Nair, the alcoholic extract of dry Nuts of *S. anacardium* exhibited bactericidal activity *in vitro* against three gram negative strains (*P. vulgaris E. coli, and S. typhi*) and two gram positive strains (*C. diphtheria* and *S. aureus*). According to studies, Alcohol extracts of the plant's leaves and green fruit have anti-bacterial capabilities [59].

Anti-spermatogenic effect

S. anacardium extract consumption had an anti-spermatogenic effect in male albino rats as indicated by a decrease in spermatogenic cell and spermatozoa counts. According to Sharma's research, changes in the androgen metabolism may be to blame for the cauda epidydamis' decreased sperm density. Meiotic and post-meiotic germ cells were extremely sensitive to levels of androgen, and changes in androgen levels in the testes may have an impact on how spermatocytes develop into spermatids [60].

According to Narayan, *S. anacardium*'s aerial part's aqueous extract had spermicidal properties. *S. anacardium* fruit extract administered orally to albino rats causes spermatogenic arrest. Sperm motility and density were found to have significantly decreased. The number of primary spermatocytes, secondary spermatocytes, and spermatids were likewise significantly decreased as a result of the fruit extract feeding. These findings unequivocally demonstrate *S. anacardium*'s anti-spermatogenic action [61].

Antiatherogenic effect

According to Mary, the primary factor contributing to the development of atherosclerosis is an imbalance between pro-oxidants and antioxidants. *S. anacardium* exhibits antioxidant properties. At low concentrations, it has the ability to scavenge superoxide and hydroxyl radicals [62].

Hypolipidemic and hypocholesterolemic activity

In rats fed an atherogenic diet, Tripathi has found that Nut extract oil of *S. anacardium* at a dose of 1 mg/100 g dramatically decreased cholesterol levels in serum and elevated HDL levels [63].

Memory enhancing effect

S. anacardium enhances cholinergic activity to enhance memory [64]. *S. anacardium* nut extract in methanol exhibits nootropic action. Fruit shodhana may be caused by cholinesterase activity being inhibited and have lower nootropic activity [65].

Cardioprotective effect

Asdaq examined that hydroalcoholic extract of Nuts of *S. anacardium* in isoproterenol induced myocardial injury in rat. In comparison to isoproterenol control, mice treated with low and high dosages of

Nut extract had decreased blood CK-MB activities and increased CK-MB activities in heart tissue. Both low and high dosages of nut extract considerably lowered the LDH activity in the serum, however neither dose had any effect on the LDH activity in the heart tissue when compared to isoproterenol as a control. Therefore, it can be said that *S. anacardium* has the ability to lessen the cardiac damage that isoproterenol causes in rats [66].

Aphrodisiac activity

Male mice were used in Gupta's evaluation of the effects of *S. anacardium* chloroform extract. The conventional medication Penegra (Sildenafil citrate) was compared to mounting behavior and mating performance.Male mice's mounting behavior and mating prowess were both found to be greatly improved by *S. anacardium* extract. The *S. anacardium* extracts improved the sex behavior of male mice [67].

Anti-tuberculosis activity

Singh conducted a study to isolate, characterize, and assess the bioactive components of *S. anacardium* nuts that were extracted using GC-MS. *S. anacardium* nuts were solvent extracted using Methanol, petroleum ether, ethyl acetate, and water. The bioactivity of each extract was examined in relation to the potential pathogen *M. tuberculosis*. *In vitro* bioassay studies on nut extract revealed anti-tuberculosis efficacy [68].

CONCLUSION

One of the most significant medicinal plants that can be utilized as an alternative medicine is *S. anacardium*. In their clinical work, traditional healers and doctors use *S. anacardium* (Bhallatak). According to numerous studies, the extract from *S. anacardium* nuts has a variety of phytochemicals that can combat a number of ailments. Shodhana method can reduce *S. anacardium*'s toxicity. The nut extracts exhibit a range of properties, including those that are antiatherogenic, antioxidant, anti-inflammatory, antibacterial, CNS stimulant, anti-reproductive, anticarcinogenic, hypoglycemic, and hair growth promoter. Studying the plant's traditional applications, such as its capacity to heal wounds, requires more work.

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