



Formulation, Optimization and Evaluation of Niosomes Containing Leaf Extract of *Moringa oleifera* and Pharmacological Screening of the Extract against Rheumatoid Arthritis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research is formulated on the basis of the Rheumatoid Arthritis of a niosome that contains leaf extract of the herb. Therefore, in this part along with the medication system of the herb on the body, the pharmacologic testing procedure is clarified. In the following part, we will explore a thorough assessment of the condition, called rheumatoid arthritis and how the medicinal plant is used as a release agent. While focusing on rheumatoid arthritis throughout the discussion, the research discussion section has made it clear how effectively this paper has tried to meet the formation of niosome with Moringa extract and its effectiveness to reduce the pain. Furthermore, the entire analysis has been illustrated by focusing on the effect of Moringa oleifera over rheumatoid arthritis disease.

Keywords: *Moringa oleifera*; rheumatoid arthritis; ointment; leaf extraction.

1. INTRODUCTION

Moringa oleifera is a popular medicinal plant which is traditionally applied for sore throat, skin scores as well as eye infections. In this modern era, the property of wound healing of this plant was well experimented in both vitro and vivo models. Moreover, there is a lack of effective research that primarily focuses on formulating *M. oleifera* into a specific functional dressing agent. On the other hand, healing applications of this agent in order to reduce the symptoms of Rheumatoid Arthritis is not explained in a detailed manner in previous research papers. More research and experiments needs to be done by pharmacologists as well as botanists in order to gain maximum information regarding the chemical properties of this medicinal plant which could be helpful to heal people suffering from Rheumatoid Arthritis.

Moringa oleifera belongs to the Moringaceae family which is a highly valued plant and it can be found in multiple tropics as well as subtropical regions. This plant has specialised and impressive range of application in medical

issues. According to the reviews of previous studies, this medicinal plant also has high nutritional value which helps to prove important minerals [1]. Furthermore, beta-carotene, vitamin, multiple phenolics, amino acids, and proteins can be found from this plant. *M. oleifera* provides a rare combination of beta-sitosterol, zeatin, caffeoylquinic acid, quercetin, and kaempferol. Along with that, the high nutritional value and compelling power of water purifying makes the plan high valued in medical treatment. All the parts of this plant like seed, bark, leaves, flowers, fruits, roots as well as immature pods are used for antipyretic, anti inflammatory, diuretic, antiulcer, possesses antitumor, circulatory stimulants, cardiac stimulants, and cholesterol lowering agent (Fig. 1).

Indigenous medicine system is highly influenced by the medicinal properties of this plant. Moreover, different regions of South Asia are involved with the treatment and medical application of this plant in order to create different kinds of drugs and ointments. According to the studies done previously, it was found that this medicinal plant inhibits the proliferation of

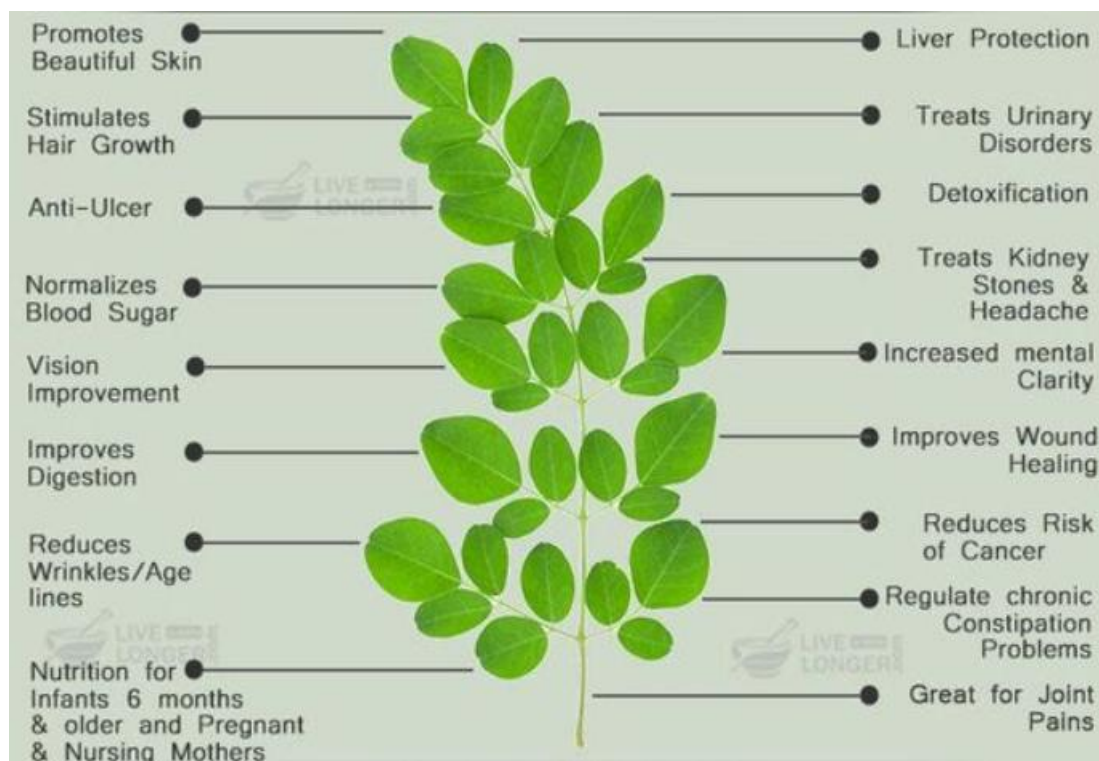


Fig. 1. Benefits of *Moringa*

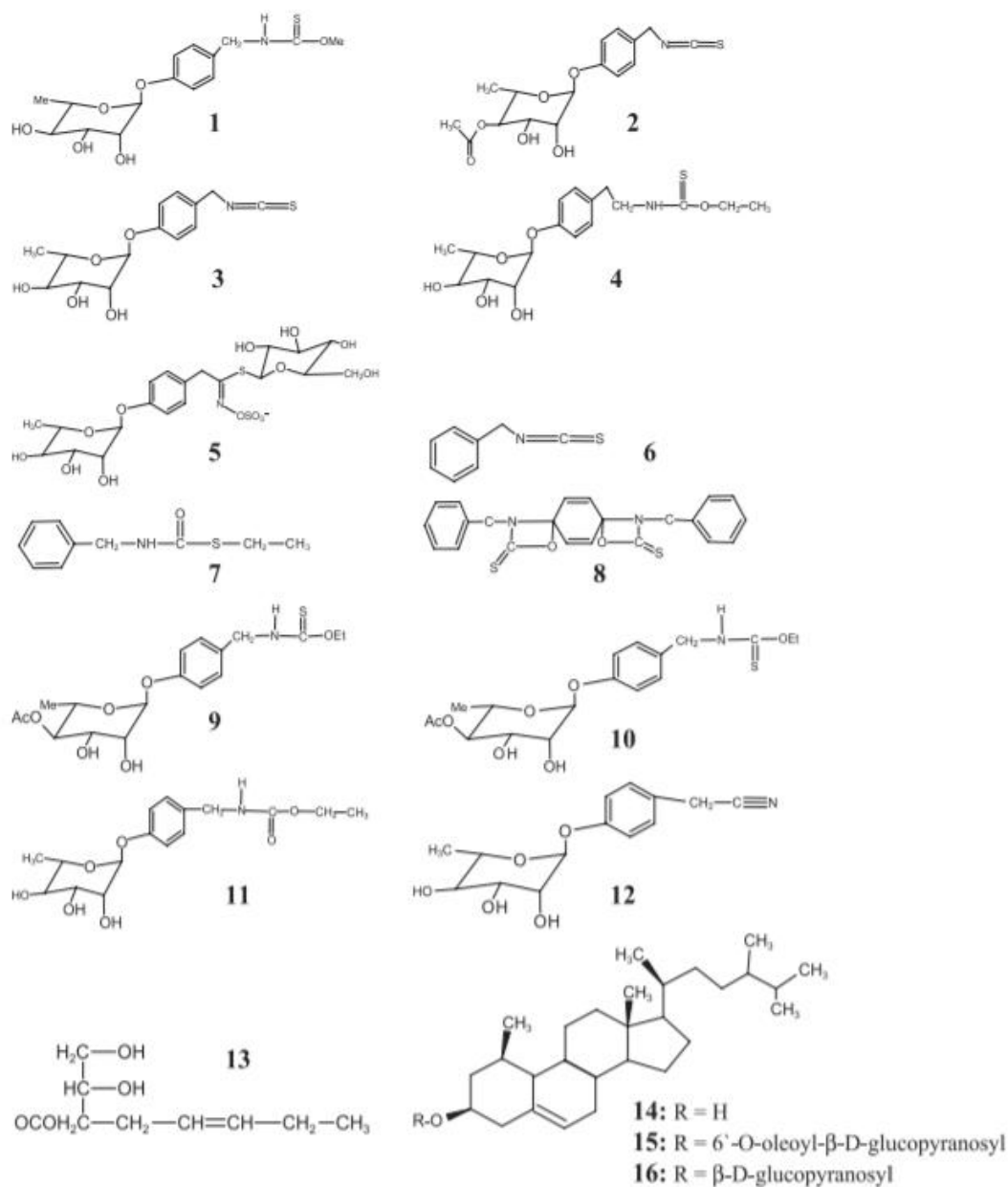


Fig. 2. Phytochemical properties of *Moringa oleifera*

multiple cell lines which consists of breast cancer MBD, lung cancer A549 and so on. However, apoptosis is identified in the cellular homeostasis by some selective removal of all the damaged cells. Apart from this, anticancer properties have also been found in this plant. It can be stated that Rheumatoid arthritis or RA refers to an autoimmune type of disease which can create damage to the body parts by following joint pains [2]. However, this joint damage commonly happens in both of the sides of the body. An

ointment is prepared through the leaf extraction of *M. oleifera* which consists of niosomes. As a result of the application of this ointment on the affected area the complications of RA can be resolved (Fig. 2).

This study is based on the formulation of a niosome containing leaf extract of the plant which is used in order to treat Rheumatoid Arthritis. Therefore, the pharmacological screening process will be explained in the section below

along with the drug mechanism of the plant on the human body [3]. A detailed evaluation of the disease named Rheumatoid Arthritis will be explained in the section below and how the medicinal plant is being used as a relief agent will be discussed in the following section of the study. In this regard, the evaluation process followed by the optimisation process of collecting niosome containing leaf extract is also included in the section below.

2. METHODOLOGY

2.1 Formulation of Niosome Containing Leaf Extract of *Moringa oleifera*

Ointments are considered as the tropical formulations which offer better compliances to the patient. Thereby, the patient got relief from the pain by applying the ointment in the affected area. According to the reviews of multiple researchers, ointment is basically a semisolid dosage which contains >50% of hydrocarbons, <20% of water as well as volatiles, polyethylene glycols or waxes [4]. Moreover, the application of ointment is useful as emollients, protectant, keratolytic, antiseptics and astringents. In this case, the leaf of *M. oleifera* will be used in order to extract niosome from it for making a specialised and effective ointment for decreasing the pain caused by Rheumatoid Arthritis.

2.1.1 Plant material extraction

In this regard, the overall procedure will be done by collecting fresh leaves of the plant.

Extraction has been done after washing the leaves of the plant in running tap water. This is helpful for removing the dirt present on the leaves of the plant. After that, all the leaves were soaked with 1% NaCl solution for a minimum of 5 minutes. This process is beneficial to remove all kinds of microbes from the plant. After that, the leaves were washed properly through 70% ethanol and distilled water. This process helps to remove all kinds of pathogens, microbes and dust from the plant. Moreover, as this process left a lot of excess water on the surface, the leaves were kept in sunlight to remove the excess water from the surface. The leaves were crushed well by adding some additional water in order to obtain juice from the leaf [5]. After that, Whatman filter paper was used in order to collect the greenish brown juice from the extraction. However, this juice was further shade dried by adding a minimal amount of absolute alcohol in

that. According to the reviews of previous researchers, it can be evaluated that application of alcohol helps to prevent the growth of any kind of microorganisms [6]. In the next phase, the dried juice of the leaf was obtained by the researcher in a brown powder. That brown powder was initially refluxed at a temperature of 50 degree celsius for at least 5 to 6 hours and absolute alcohol was added to it. Moreover, after 6 hours the fraction of alcohol was properly separated from that residue and then it was properly dried.

2.1.2 Ointment preparation

One tropical ointment was prepared in this experiment by maintaining an accurate temperature of around 70 degree celsius for minimum 5 minutes. Ointment was prepared after melting the ingredients and then it was cooled for a while [7]. In addition to this, the ointment was prepared through the incorporation of 10% w/w of an extract of semisolid form of the residue. Moreover, the extraction was collected in a sterile ceramic mortar and pestle while trituration was done in order to collect 100 gms of the herbal ointment. The niosome was the main element within that extraction and it is considered as the main agent to heal RA. Acetone was used as a non-solvent in the extraction, and water was used as the solvent. A sieve no 80 was used to filter and grind the material. Ten grams of dried gum (30 g) were stirred in 250 ml of distilled water (6-8 hours) at room temperature.

2.1.3 Preparation of niosomes

Niosomes are composed of uncharged single-chain surfactants and cholesterol. Liposomes, on the other hand, contain neutral and charged double chains of phospholipid. Compared to niosomes, liposomes have a higher concentration of cholesterol. Hydration of the surfactant can be used to form a colloidal dispersion encasing the desired compound in niosomes. A potential drug delivery system based on niosomes is highly promising, since they are non-ionic surfactant-based vesicles [8]. In an aqueous phase, niosomes are formed by self-association of nonionic surfactants and cholesterol and have a bilayer structure. It is possible to produce defined vesicles with a desired size by precisely adjusting the flow rates of aqueous and organic streams, thereby reducing liquid volume requirements and reducing preparation time and development costs in comparison with alternative approaches.

3. RESULTS

3.1 Optimisation and Evaluation Process

According to the results of the optimisation it was found that the ointment had a smooth type of texture which coil is washable as well as spreadable in an easy manner. In addition to this, for understanding the pH value of the product 2.5 gms of the sample was put in a 100 ml beaker and around 50ml of water was added to that. Along with that, the beaker was put in 60-70 degree celsius for 9 to 10 minutes and then cooled. The material was centrifuged at exactly 3000rpm for around 10 minutes. As per the opinion of pharmacologists, a digital pH meter was used that included a dipping glass electrode for calculating the pH value [9]. Apart from the pH value, spreadability, viscosity, and extrudability were also measured by the researcher in order to perform the pharmacological screening process in depth manner. The extraction of phenolic compounds from M has been studied using a variety of extraction methods. Oleifera, including ultrasonic extractions (UE), subcritical water/ethanol extractions, and microwave-assisted extractions.

3.2 Pharmacological Screening

Pharmacological screening process helps to complete the process of ointment preparation. In addition to this, spreadability of the ointment can be measured through using a formula. Therefore, the formula $S=MxL/T$ is used in order to measure the spreadability of the medicinal plant *Moringa oleifera*. In this chemical formula S is denoted as the rate of spreadability while T is the time. Along with that, M refers to the weight on the upper slide while L is considered the length of the slide made up of glass. Before application of this niosome containing ointment on the person it is necessary to check whether it causes any kind of irritation to the skin of the patient or not [10]. In order to check the pharmacological screening around 50 mg of the product is applied on an animal skin to check. Moreover, 7 days of observation was done in order to check any symptoms of erythema or oedema. This test is essential to be performed in order to check whether the ointment causes any kind of irritation to the skin or not. Moreover, the patients suffering from the critical health issue of RA can get relief for some time if this formula is applied on the skin in an appropriate way. Moreover, a proper screening session needs to be done in order to improve the formula and make it more

appropriate for the user. The miracle tree, *Moringa oleifera* has been found with a clear taxonomy classification such as; Kingdom-Plantae, Subkingdom- Tracheobionta, Superdivision- Spermatophyta, Division- Magnoliophyta, Class- Magnoliopsida, Subclass- Dilleniidae, Order- Capparales, Family- Moringaceae, Genus- *Moringa* and Species- *Oleifera*. Pharmacological screening makes a clear sense about this plant; it helps in both peripheral and central analgesic models in a dose-dependent manner [11]. The hot plate method and acetic acid including writhing method help in collecting extract from the exhibited leaves in which the presence of analgesic potency and anti-migraine properties are found to be present. Presence of a large number of chemical compounds are making this tree full of helpful potentialities such as for antipyretic activity, ethanol and ethyl acetate get extracted from its seeds.

Neuropharmacological activity of *Moringa oleifera* is useful for testing the behaviour of Alzheimer's disease. Besides, it also works as Anti-cancer activity by extracting alcoholic and hydroethanolic extracts from leaves and fruits. Gastric ulcer protective activity is also present in *Moringa oleifera* along with antioxidant effects [12]. In addition to this, some more activities like Hepatoprotective, Anti-diabetic, Cardiovascular, and Anti-obesity, Anti-urolithiatic, Haematological, Anthelmintic and others are helping in curing various health diseases (Fig. 3).

3.3 Treatment of Rheumatoid Arthritis

Arthritis is one of the most common diseases that create joint pain after a certain age. Individual can be affected with the rheumatoid arthritis at any age but more specifically age group between 30-50 can show up the symptoms and the elderly onset of this arthritis can be seen between the age of 60-65. In older people, arthritis is very common, especially in men in comparison with women. Arthritis targets people of different ages with different symptoms and severity levels. Rheumatoid Arthritis (RA) is popular for inflammation along with soft tissue swelling at body joints [13]. RA is the result of deregulation of the immune system that starts to affect joints and cause pain. Inflammation of synovia creates a chronic condition where the tissue lines and lubricate joints get affected more. Healthcare system, for the first decade, said that it occurs after age 30 through the recent researches are emphasizing that it has no age boundary and

even children can also get affected with this chronic disease. The preliminary phytochemical investigation proved that methanolic extract of *Moringa oleifera* is beneficial for curing RA due to the presence of anti-arthritis activity in it [14]. By using this methanolic extract, the body is protected against free radicals such as turpentine, formaldehyde, and CFA (Complete

Freund Adjuvant), which results in arthritis and joint damage. This methanolic extract can fight against turpentine oil, formaldehyde and CFA that leads the body joints towards arthritis and create joint damages. RA is a seriously painful and worse disease in which *Moringa oleifera* works like an effective pain reliever.

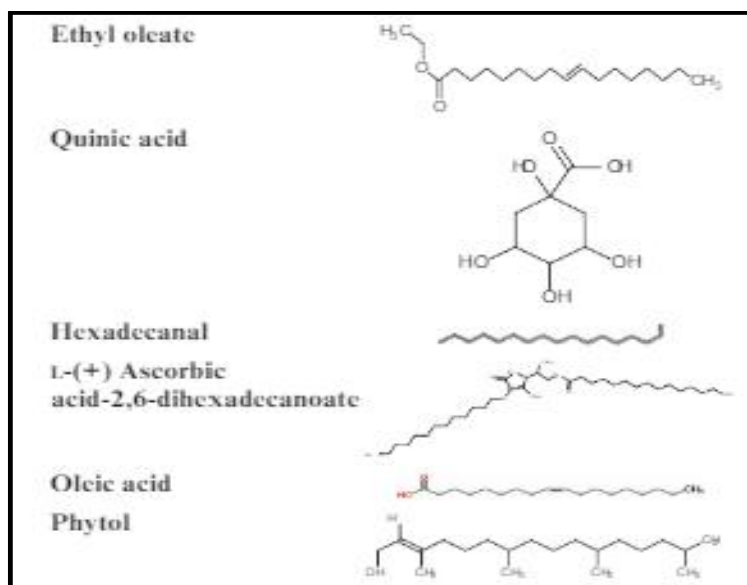


Fig. 3. Compound and chemical structure of *Moringa oleifera*

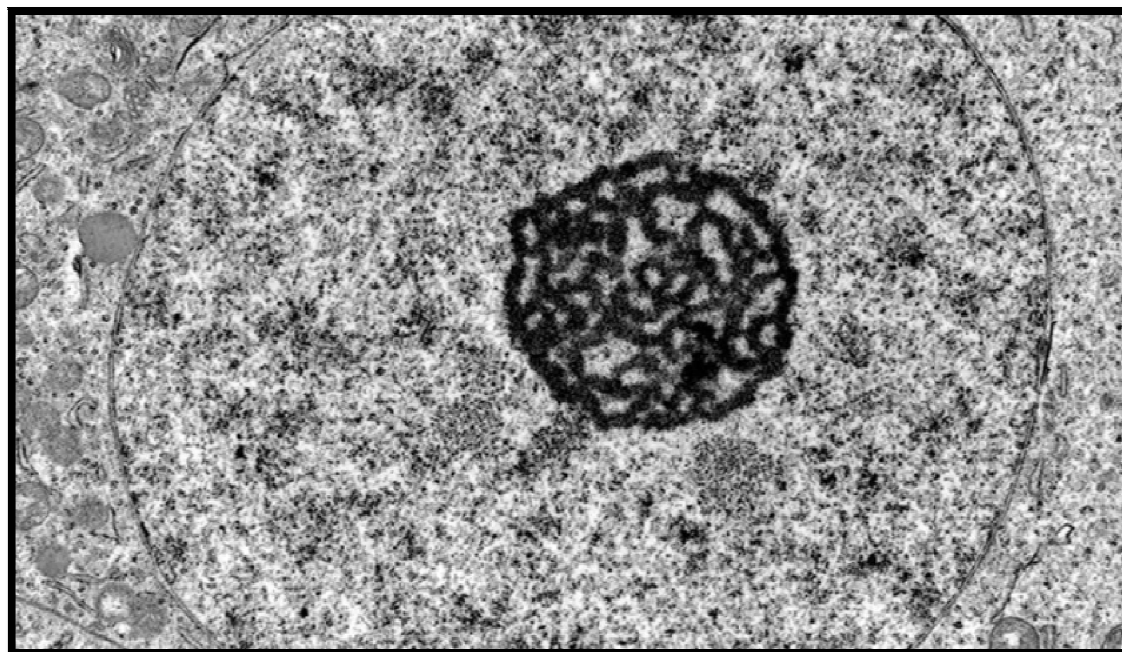


Fig. 4. TEM preparation

Moringa in various forms gets used by the professionals to reduce the pain caused by RA and hence, reduces the joints pain in a natural way. Bone health can be improved with the help of Vitamin K, Calcium and Magnesium, which all are present in *Moringa oleifera* that has brought an innovation in the healthcare industry. Furthermore, Moringa extracts are found beneficial for curing the joint pain by healing the joint ailments. The natural anti-inflammatory properties of Moringa is helping in treating inflammation, swelling and the joint pains [15]. Dose must be measured by the healthcare professionals for curing the RA in all age people as excess of any can create negative consequences. Moringa ointments are easily available on the market that can help in soothing the nerves of joints and somehow rescue the swelling that enables the patients to do exercises and cure it from the inner way. Besides, Moringa supplements are full of Vitamin A,C, E and K, protein, potassium, and calcium that bring back the strength of joints and the tissue lines start to regain its power though at a certain level [16]. Around ½ to 1 teaspoon Moringa powder can be consumed per day as per the severity of the RA and prescribed by the professionals.

4. DISCUSSION

Arthritis, especially RA is the oldest disease that is harming the bone health of old age people and now the children and young generation as well. It has been seen that *Moringa oleifera* is being used as a medicinal plant for most of the diseases that affect the human population. This study, while targeting only the RA to be cured by the plant, has been seen that the niosome containing the extract of *Moringa oleifera* is being used as an alternative medicine [17]. Seeking for alternative medicines has become much popular in the healthcare industry as not every individual has the same power to reach for medicines in a similar manner due to the side-effects of compounds. Researchers have noted that the body joints can easily absorb the anti-arthritis effect of methanolic extract of *Moringa oleifera* stem bark. Formaldehyde and turpentine oil are the most essential compounds that can reduce the pain of joints and strengthen the bones.

The nanoparticles and the chemical compounds of niosome are found to be incorporated in a gel base with the help of a polymeric delivery system that is having the size around 500 nm. As the compounds are small in size in comparison with the other particles of medicines, the targeting

process of this alternative process is posing the treatment method in a potentially different manner. Niosomes are found with a high biodegradability and biocompatibility power to load the drug and enhancing water retention capability that indicates the permeation capabilities at a good level [18]. With both, passive and active manners, the drugs of the niosome can be delivered to the nerves and tissues that are swelling due to RA. By posing the niosome drug into the human body, it can enhance the internal capacity of holding the drug as it is considered the hydrogel to accommodate the larger tissue volume.

The formulation of niosome containing the *Moringa oleifera* is indicating that it contains a 1% NaCl solution that helps in penetrating the drug into the affected place easily. Surveys said that the application of niosome is found in the polymerisation process that is tested based on its efficiency level to reach out the affected joints and penetrate there. The presence of nanoparticles are enhancing its receptor power and the ointment is becoming adjuvant-induced for cutting RA. Joint pain due to RA is the worst situation for the bones as it slowly but steadily weakens the bones and gets damaged before time [19]. In niosome, nanoparticles with a size of about 500 nm are effectively concentrated into tissues and deliver the drug to deep cells. Due to the presence of nanoparticles, the ointment is becoming adjuvant-induced for cutting RA. The modern healthcare researchers are focusing on using *Moringa oleifera* for strengthening the bone along with enhancing the power of physical health. Proper concentration of niosome with the extract of *Moringa* is introduced to the world with a new form of RA procurement.

The niosome ointment has been researched well by the healthcare researchers to know how the nanoparticles of it are being used for curing joint pains by extracting the methanolic from its leaves and fruits along with barks. This drug has a variety of advantages as obesity is another major cause behind RA, *Moringa* extracts are found beneficial as well to reduce the fat from the body without any side effects [20]. Hence, it is helping in other physical measures as well that is reducing the chances of facing RA at younger age and removing the pain for elder age in the similar way. In addition to this, the acute toxicity studies revealed that *Moringa* has not any element that can occur toxic symptoms, even no lethality has been noted up to the dose of 2000 mg/kg according to the body weights.

Chronic model of CFA included arthritis experiment has disclosed that some modification is needed in niosome nanoparticles and other compounds as there are several diseases that are harming the physical health and the medicines are somehow weakening the power of moringa extract. Phytochemical analysis is indicating towards the methanolic extract as the prime elements though anthraquinones, tannins, saponins, and flavonoids are present there for dealing with bone health and pain. As *Moringa oleifera* consist of haematological activity, it is helping to balance the haematological parameters that is important for forbidding the tissues to be swelled up.

5. CONCLUSION

The entire study has focused on reviewing the role of *Moringa oleifera* and its unfolding values for helping in Rheumatoid Arthritis. For this instance, pharmacological screening has been conducted while various parts of its significance have been illustrated. The overall study proved that there is clear justification of considering the plant as a miracle element that is helping in most of the health diseases. The research laboratory of the chemical industry is clarifying that *Moringa oleifera* is useful to reduce the pain of RA and heal the swelling issues of nerves. The plant produces few major ointments with different formulas such as cetostearyl alcohol, hard paraffin, and liquid paraffin; those are helpful to evaluate the physical parameters while keeping in mind the standard pharmacological screening. The method of extracting the compounds and formulating the ointments found efficient and easier at the same time. The result also showed that Moringa extract is suitable for physical health, especially for RA. It can be concluded from the discussion part that for determining more efficient therapeutic information and its impact over the RA disease, more critical information is needed. *Moringa oleifera* has been found to reduce the pain of RA and reduce swelling in nerves that is related to the chemical industry research laboratory. Hence, it is clear that Moringa can be used as an alternative therapy for RA along with other diseases.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not

intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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