

Research : In Vitro Antiarthritic Activity of synthesized Silver Nano particles from Extract of Merrmia dissecta**Rucha Pancham, Urwashi Lanjewar, Swapnil Dhanvij****DEPARTMENT OF PHARMACOLOGY (PG) MAHARASHTRA INSTITUTE OF PHARMACY ARMORI ROAD BETALA, BRAMHAPURI DIS. CHANDRAPUR MAHARASTRA - 441206 INDIA.****ABSTRACT:**

Objective: The present investigation deals with the study of in vitro anti-arthritis activity by inhibition of protein denaturation method by bovine serum albumin method and egg albumin method. Merremia dissecta Linn plant is a used in parts of leaf. It is used in the treatment of gout, Antipathogenic, antimicrobial, anti diuretics, Alzheimer, and piles in Ayurvedic medicine, and traditionally used for the bone fracture.

Method: The air-dried powder of *M. dissecta* Linn (leaves parts) was extracted using a Soxhlet apparatus with methanol as solvent. And preparation of silver nanoparticles. The extracts and its silver nanoparticles were concentrated under reduced pressure. The activities were carried out using the following concentration (100, 250 and 500 µg/ml) and compared with diclofenac as standard drug. It has significant in vitro anti-arthritis in both the methods.

Result: The Methanolic extract of *M. dissecta* possessed significant anti-arthritis property in ME of Merremia dissecta extract than compared to MEMD its silver nanoparticles.

Conclusion: Activity may be due to the presence of the chemical profile such as phenolic acid, flavonoid (leuteotin), Germacrene and β-sitosterol. The results of the study have suggested in the use of *M. dissecta* Linn and its silver nanoparticles as a potent anti-arthritis in several applications.

Keywords: *M. dissecta* Linn, Silver Nanoparticles, Anti-arthritis, Bovine serum albumin, Methanol, Diclofenac.

INTRODUCTION

In the very last few decades, there is a tremendous growth in the region of herbal medicine. It is coming popularized in both developing and developed countries due to its natural origin because of its lesser side effects. Herbal remedies provide a lot of drugs for the treatment of internal diseases which are considered to be stubborn and incurable by other system of medicines [1].

Arthritis is an autoimmune disorder characterized by pain, swelling, and inflexibility [2]. Rheumatoid joint inflammation influences more or less 1% of the populace around the world. Its etiology is still obscure [3]. However, advances in understanding the pathogenesis of the disease have raised the development of new therapeutics, with improved outcomes. Rheumatoid arthritis may quickly progress into a multisystem inflammation with irreversible joint destruction and increase the risk of humanity. However there are no effective treatment for arthritis Disease. Merremia dissecta also known as almovine is an ayurvedic herb used to reduction of joint pain

and inflammation. To the potential use of *Merremia dissecta* in rheumatic disease. *Merremia dissecta* is one of the widely used plant claimed by the traditional system of medicine to have its positive effect on stiffness of joint, pain, muscle and reduction of inflammation functioning. In this report, a simple and eco-friendly chemical reaction for the green synthesis of silver nanoparticle from *Merremia dissecta* has been developed. Here we report for first time, to the best of our knowledge, the synthesis of nanoparticle of varying size using methanolic extract of leaves of *Merremia dissecta* at room temperature. Nanoparticle shows better effect than extract on Arthritis disease.

Centella asiatica (CA), commonly named gotu kola, is an Ayurvedic herb used to enhance memory and nerve function. To inves-

tigate the potential use of CA in Alzheimer's disease (AD), we examined the effects of Healing with medicinal plants is as old as mankind itself. Before the concept of history began, humans undoubtedly acquired life benefits by discovering medicinal and aromatic plants that were food and medicine.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups. people use herbal medicine because- Personal preferences for Herbal medicine, Perception of safety, Accessibility & Affordability An alternative approach to healthcare, Efficacy of treatment.

Nano drug delivery systems for herbal extracts :

Herbal drugs have a potential future for enhancing the activity and overcoming problems. Phytotherapeutics need a scientific approach to deliver the components in a sustained manner to increase patient compliance and avoid repeated administration. This can be achieved by designing NDDSs for herbal constituents. NDDSs not only reduce the repeated administration to overcome non-compliance but also help to increase the therapeutic value by reducing toxicity and increasing the bioavailability [7].

Anti- Arthritic Activity of *Merremia dissecta*:

Merremia dissecta (L.) Urban, family convulvulaceae (CA), is known as Nagin, kaliaphumari, alamovine in Ayurvedic medicine. –*Merremia dissecta* is Native of Tropical and America whereas distributed globally in India, Sri Lanka, Myanmar, China, Pakistan, Indonesia, Australia, French.

Currently, nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed because of their efficacy in the management of pain, inflammation, and rheumatic disorders. However, their long-term therapeutic use is often associated with adverse effects such as gastrointestinal ulcers and renal insufficiency.

These reasons, the use of medicinal plants has become the new strategy of several studies to develop and introduce new drugs with greater safety and efficiency. Hence, this study seeks to

determine the anti-inflammatory, and anti-arthritis activities of leaf extract of *Merremia dissecta* a medicinal plant that have been used traditionally to treat inflammation, rheumatic diseases.

MATERIALS AND METHODS

Materials

Drugs and chemicals

All reagents procured were analytical grade.

Methodology

Collection and authentication of plant :

The fresh leaves of *Merremia dissecta* were collected from its natural habitat from Pusad, Maharashtra in India. The identity of the plant was authenticated by Dr.Sakhare sir, Department of Ayurvedic Mahavidyalaya Pusad, MUHS University Nashik. A voucher specimen was deposited at herbarium, Department of Botany Ayurvedic college Pusad, Maharashtra.

- **Preparation of crude extract :**



fig.No.01: soxhlet extraction leaves of *Merremia dissecta*

The leaves of *Merremia adissecta* was thoroughly rinsed with water and shade dried at room temperature in hygienic condition. The leaves of *Merremia dissecta* was powdered manually and extracted with methanol using soxhlet extraction technique. Extraction was performed until the cycle periods of 48-72hr.and solvent become colourless. The methanolic extract was concentrated using rotary evaporator and stored for further use.percentage yield of after extraction 14.95 W/V.

- **Preparation Of Synthesized Silver Nanoparticles:**

Synthesis of merremia dissecta silver nanoparticles:

5 ml merremia dissecta extract was taken in a beaker and paced on a magnetic stirrer with hot plate. To this 50 ml of 1Mm silver nitrate solution was added drop wise with constant stirring 120 rpm at 50-600°C. The colour change of the solution was checked periodically. The colour

change of the solution from yellow to brown colour indicates the formation of silver nanoparticles.

Separation of silver nanoparticles:

The synthesized merremia dissecta silver nanoparticles were separated by centrifugation using REMI centrifuge at 5000 rpm for 15 minutes. The supernatant liquid was discarded and the pellets were collected stored.



Fig.No.02 : Preparation of Herbal Nano particle.

EXPERIMENTAL DESIGN

The pharmacological potential of *Merremia dissecta* were screened by In-vitro antiarthritic Method .

IN-VITRO SCREENING

Antiarthritic activity

Inhibition of protein denaturation using bovine serum albumin

In this method, anti-arthritic activity of methanolic extract of the plant *Merremia dissecta* for Silver Nanoparticles was evaluated by % inhibition of denaturation. In this method bovine serum albumin (BSA) was used. The standard drug was Diclofenac sodium(100µ/ml).

0.5% Bovine Serum Albumin (BSA): Dissolved 500mg of BSA in 100 ml of water.

Method:

Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%W/V aqueous solution) and 0.05ml of MEMD solution of various concentrations.(100,250,500)

Test solution (0.5ml) consist of 0.45ml of bovine serum albumin (0.5%W/V aqueous solution) and 0.05ml of silver Nanoparticles solution .

Test control (0.5ml) consists of 0.45ml of BSA (0.5% W/V) and 0.05 ml of distilled water.

Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%w/v aqueous solution) and 0.05ml of Diclofenac sodium (100µg/ml) of various concentrations. (Vaijayanthimala P etal2018)

Procedure:

1. 0.05 ml various concentrations (100, 250,500 ug/ml) of test dugs MEMD ,its silver nanoparticles(100,250,500)and standard drug diclofenac sodium (500, 100, 250,500

ug/ml) were taken respectively and 0.45 ml (0.5% w/V BSA) mixed. The sample were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was using UV-Visible spectrometer at 255nm.

2. The control represent 100% protein denaturation .the result were compared with diclofenac .The %inhibition of protein denaturation can be calculated (Habibur Rehman et al 2015).
3. Formula, (% Inhibition =OD Control –OD Sample/OD Control)

● Inhibition of protein denaturation using egg albumin

In this model, anti arthritic activity of plant leaves of methanolic extract of merremia dissecta and its silver nanoparticles was estimated by % inhibition of denaturation by egg albumin.

Test solution:

1. The 5ml reaction mixture of test solution consists of 0.2 ml of egg albumin(from fresh hen's egg) 2.8 ml of phosphate buffered saline (PBS,Ph 6.4) and 2 ml of different concentrations of MEMD extract (100,250500 µg/ml)
2. And 0.2 ml of egg albumin, 2.8 ml of phosphate buffer saline and 2 ml of different concentrations of Nanoparticles (100,250,500 µg/ml)
3. Test control solution : 5ml of test solution consist of 0.2ml of egg albumin and 2.8ml of phosphate buffer saline and 2ml of distilled water.
4. Standard solution : 5 ml of standard solution consist of 0.2ml of eggs albumin and 2.8ml of phosphate buffer saline solution and diclofenac sodium(100µg /ml)was added. (Habibur Rahman et al 2015,vaijantimala et al 2018)
5. The pH of the solutions was adjusted to 6.4 by using 1N HCl. At next step, samples was incubated at 37°C for 20 min then heated at 70°C for 5 min. After cooling, the absorbance was measured at 660 nm using spectrophotometer. The results was compared with standard diclofenac sodium[Kosala et al 2018, Neha mohan etal2013].

The percentage inhibition of protein denaturation was calculated using the formula:

$$\left[\frac{\text{Abs of control} - \text{Abs of test sample}}{\text{Abs of Test Control}} \right] \times 100$$

Statistical analysis:

All the results were expressed as mean ± standard deviation, and all the grouped data were statistically evaluated with Graph Pad prism. Hypothesis testing methods included one-way analysis of variance followed by least significant difference test with ONE way ANOVA followed by dunnett's t-test .

RESULT

Anti-Arthritic Activity

- **Effect on protein denaturation by BSA**

Table No.01: Effect of methanolic extract of Merremia dissecta and its silver nanoparticles on heat induced protein denaturation using BSA.

All the Value are expressed as Mean ±SEM (n=3)

Sr.No	Concentration in (µg/ ml)	%Inhibition of Control groups	% Inhibition with Standard drug Diclofenac (100µg /kg)	% Inhibition with Extract MD (200mg / kg)	% Inhibition with Nanoparticles (200mg /kg)
1	100	100	71.86 ±0.70*	42.25±0.57*	44.82± 0.96*
2	250	100	95.4 1± 0.83*	72.741 ± 0.37*	71.95 ±0.70*
3	500	100	96.91±0.90*	79.98 ±0.89*	75.41±0.72*

All the Values are significant when compared to control (* p<0.05)

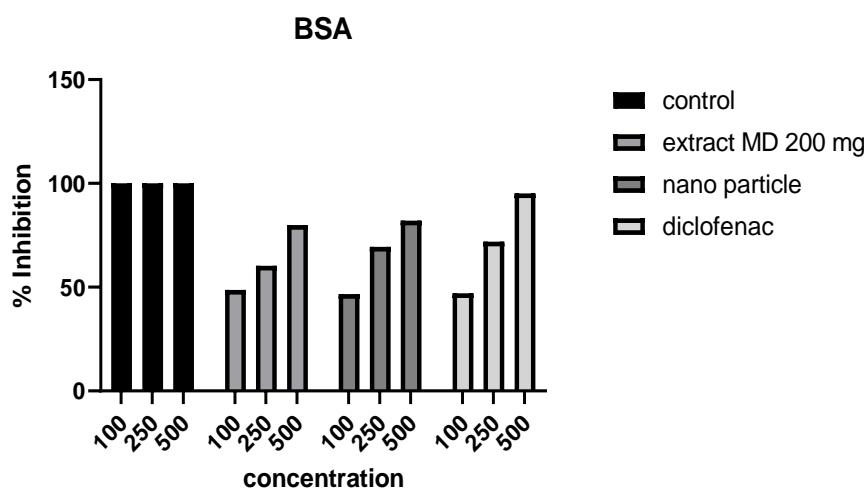


Fig. No. 1: Effect of MEMD and its Silver Nanoparticles of Protein Denaturation Process By Using BSA.

The effect of methanolic extract of Merremia dissecta and its silver nano particles, diclofenac in concentration (100,250,500ml) wERE found to be significant (* p<0.05) when compared to control group .

● Effect On Protein Denaturation Process By Using Egg Albumin

Table no.02: Effect of methanolic extract of MD and its Silver Nanoparticles of heat induced protein denaturation using egg albumin.

Sr.No	Concentrations	%Inhibition on with control group	%Inhibition withstand standard drug Diclofenac (100µg/mg)	%Inhibition with extract MD	%Inhibition of Silver nanoparticles
1	100	100	47.08±0.67*	48.67±0.43*	46.67±0.44*
2	250	100	71.95±0.70*	60.37±0.75*	69.48±0.69*
3	500	100	95.23±0.32*	79.98±0.89*	82.07±0.93*

All the Value are expressed as Mean ±SEM (n=3).

All the Values are significant when compared to control (*p<0.05)

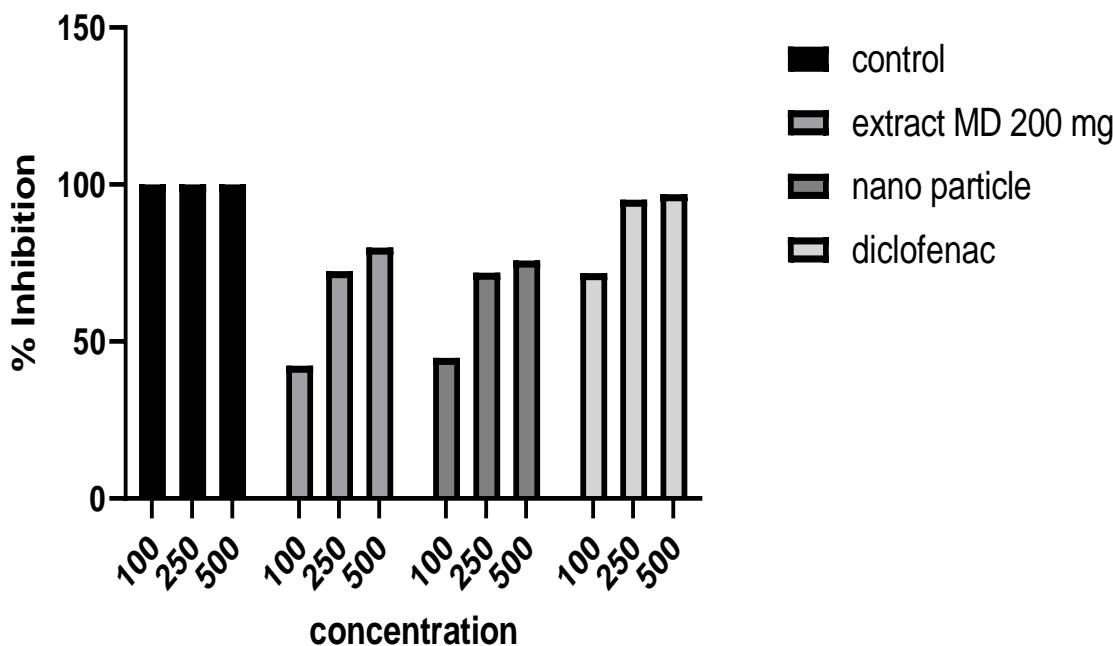


Fig.No.2.Histogram of effect on protein denaturation process by using egg albumin.

The effect of methanolic extract of Merremia dissecta (200mg/kg), silver nanoparticles (200mg/kg) and standard diclofenac (100mg/kg) in concentration 100,250,500µg/ml were found to be significant (*p<0.05) when compared to control

DISCUSSION

The plants had been used for the prevention and cure of various ailments such as Rheumatic Arthritis and other inflammatory diseases. Natural sources such as plants have been considered as the safest and valuable treatment for the disease. From the ethno botanical knowledge, we included the plants that are used in Indian traditional systems such as herbalism, folklore and shamanism. (Chowdhary et al 2015). In vitro, anti-arthritic activity of Methanolic extract of *Merremia dissecta* and its synthesized silver nanoparticles was evaluated by inhibition of Protein denaturation process.

The ability of the synthesized silver nanoparticles to inhibit albumin denaturation has been investigated for potential anti-arthritic action. It is well documented that protein denaturation is involved in arthritic reactions and development of tissue damage during inflammation. Results of our study reveal that synthesized silver nanoparticles of methanolic extract were effective in inhibiting thermally induced albumin denaturation at all tested concentrations, indicating their capability of controlling protein denaturation involved in the arthritic process.

Protein denaturation involves the distraction of secondary ,tertiary and quaternary structure of the molecules and finally leads to cell death, it occurs mainly due to stress, high temperature, high level of acidity and high level of salt (Shilpa k etal 2018), When proteins are ruined by physical, chemical and biological agents, protein configuration get disrupted due to alteration of electrostatic, hydrogen, hydrophobic and disulphide bonds, which results into denaturation, loss of biological function and associated diseases may arise (Grant et al 1970;19) In case of Certain Arthritis ,denaturation of protein is one of the causative factor which may leads to production of auto antigens (Iain B,Dipiro 2011;manuel Rubin et al). NSAIDs are highly efficient for the management of inflammatory diseases due to their potent anti-protein denaturation activity .

Thus, the Capacity of a substance to inhibit the denaturation of protein suggesting potential for anti-inflammatory activity .On heating of Bovine Serum Albumin and eggs albumin denaturation occurs and antigens are expressed which are associated with type -3 hypersensitivity reaction and diseases such as rheumatoid arthritis.

The inhibition of protein denaturation, albumin denaturation, was studied to establish the mechanism of anti-arthritic activity of nanoparticals of *M. dissecta*. Therefore, in vitro studies on the extract of *Merremia dissecta* and its silver nanoparticles demonstrate the significant anti-arthritic activity. Hence, this mangrove plant can be used as a potent natural anti-arthritic agent. The Results of this study shows significant ($*p < 0.05$) inhibition of protein denaturation in standard drug treated(diclofenac), MD extract treated (200mg /kg) was significance ($*p < 0.05$) and Silver nanoparticles treated (200 mg/kg) groups was significance ($*p < 0.05$) compared to control group. The findings of the study exhibited a concentration dependent inhibition of protein denaturation by *Merremia dissecta* throughtout the various concentration range of 100, 250, 500 ug /ml. Silver nanoparticles throughtout at concentration 100,250,500 $\mu\text{g}/\text{ml}$ and standard drug diclofenac sodium at concentration 100,250,500 ug/ml. Silver Nanoparticles of *Merremia dissecta* showed highly significant inhibition of protein denaturation effect as compared to methanolic extract *Merremia dissecta*.

CONCLUSION

The plant extracts and herbal nanoparticles formulations would be served as an alternate therapy for the treatment of arthritis with lesser side effects.

The concept of nano medicine has risen to be the future of medicine. Advantages of using silver nanoparticles to treat arthritic and inflammation have many benefits such as low drug dose efficacy. Silver nitrate nanoparticle - mediated *Merremia dissecta* have been obtained and characterized. Synthetic optimization shows increases in plasmon resonance bands with the solute and extract concentrations as well as pH. The generated particles are found to be crystalline spherical in shape and biofunctionalized with organic molecules.

The synthesized silver nanoparticles exhibited significant inhibition of albumin denaturation, indicating a strong anti-inflammatory potential, hence, could be considered as a potential source of the anti-arthritic and anti-inflammatory drug.

Methanolic extract of *Merremia dissecta* and its nanoparticles showed the reduction of inflammation and arthritic pain. It can be concluded that MD and its nanoparticles shows significant in- vitro anti-arthritic activity.

Thus the present study concluded that Methanolic extract of *Merremia dissecta* and its silver nanoparticles possesses the anti-arthritic property.

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