RESEARCH
www.commprac.com
ISSN 1462 2815

EVALUATION AND IN VIVO ANTI-ARTHRITIC POTENTIAL OF POLYHERBAL OINTMENT

Praveen Kumar [1] and Alok Kumar Dash [2]*

[1] Institute of Pharmacy, V.B.S. Purvanchal University, Jaunpur, Uttar Pradesh, India.
[2] Assistant Professor, Institute of Pharmacy, V.B.S. Purvanchal University, Jaunpur, Uttar Pradesh, India. *Corresponding Author Email: dr.alokdash@gmail.com

DOI: 10.5281/zenodo.10991294

Abstract

Emblica officinalis, Datura stramonium, Nerium indicum, Curcuma longa, and Acorus calamus are medicinal plants that are employed in the management of chronic inflammation-associated ailments, including arthritis. A polyherbal ointment was formulated and assessed in this investigation as a potential treatment for arthritis in a rodent model. The aim of this research endeavor is to develop and assess the anti-arthritic properties of a topical herbal ointment comprising extracts of Datura stramonium, Nerium indicum, Curcuma longa, Acorus calamus, and Emblica officinalis in rodents. Physical appearance, net content, viscosity, extrudability, pH, spreadability, and primary skin irritation tests were all assessed for the Polyherbal ointment. The formulation of the topical herbal ointment was subjected to a stability study in accordance with ICH recommendations, and its anti-arthritic activity was assessed using Freund's Complete Adjuvant (FCA) induced arthritis method. In addition to assessing body weight, paw volume was measured. The formulated ointments behaved as intended, were stable, and exhibited homogeneity. The paw volume and body weight reductions observed in the animals provided evidence for the ointment formulation's anti-arthritic properties.

Keywords: Arthritis, Topical Herbal Ointment. Medicinal Plants, Body Weight.

INTRODUCTION

Rheumatoid arthritis (RA) is a multifaceted autoimmune disorder distinguished by persistent joint inflammation resulting in joint rigidity, discomfort, inflammation, and possible joint impairment. In contrast to osteoarthritis, which is caused by progressive joint degeneration, rheumatoid arthritis (RA) arises from an erroneous targeting of self-tissues by the immune system, specifically the synovium, the membrane lining that envelops the joints.

The immune response initiates a series of inflammatory processes that culminate in joint deformity and thickening of the synovium. [1,2] RA can also affect larger joints, including the knees, shoulders, and hips, in addition to the minor joints of the hands, wrists, and ankles. RA symptoms frequently manifest progressively, beginning with mild stiffness and discomfort in the joints that worsens with time. Typically symmetrical in nature, the condition manifests on both sides of the body and may induce systemic manifestations including fatigue, illness, and weight loss.

Nonetheless, the severity and course of rheumatoid arthritis (RA) can significantly differ among individuals, as some may encounter intermittent moderate symptoms, whereas others may confront a more incapacitating and progressive ailment. RA can impact various organs and systems beyond the joints, potentially resulting in complications including inflammation of the eyes, rheumatoid nodules, lung involvement, and cardiovascular disease. [3,4] Critical to the management of RA and the prevention of long-term joint injury and disability are early diagnosis and treatment.

A combination of medications, including biologic agents, disease-modifying antirheumatic drugs (DMARDs), and nonsteroidal anti-inflammatory drugs (NSAIDs), is frequently used in treatment strategies to reduce inflammation and pain, delay the progression of the disease, and preserve joint function. [5] RA patients may also experience improvements in their overall health and symptoms through the adoption of healthy lifestyle habits, including regular exercise, weight management, and stress control, in addition to medication. [6,7]

Notwithstanding the obstacles presented by rheumatoid arthritis (RA), continuous investigations and progressions in therapeutic approaches instil optimism regarding improved control and prognoses for individuals afflicted with this chronic autoimmune disorder. By receiving comprehensive care and support from healthcare professionals, individuals diagnosed with rheumatoid arthritis (RA) can enhance their quality of life through effective symptom management. Numerous integral biochemical, molecular, and cellular pathological components and pathways of RA have been elucidated through intensive research, resulting in the development, marketing, and discovery of novel therapeutics that target a number of RA's seminal components.

Rheumatoid arthritis (RA) is distinguished by elevated populations of immune cells, including lymphocytes and macrophages, within the synovial space. Additionally, there is a notable accumulation of free radicals (FR), primarily reactive oxygen species (ROS), which have the potential to cause permanent damage to articular tissue by oxidizing its biomolecules.

This, in turn, contributes to the disease's progression. Therefore, arthritis can be classified as an autoimmune disorder characterized primarily by inflammation. Inflammation is the tissue response to a physical or chemical injury, bacterial invasion, or exudation from blood vessels, resulting in pain, erythema, heat, and oedema. It is characterized by a reactive state of hyperemia and exudation.

Prolonged anti-arthritic treatment focuses on inhibiting key mediators of chronic inflammation, including lymphocytes T, interleukins (tumour necrosis factor alpha, TNF-), and enzymes (induced cyclooxygenase), in an effort to regulate these processes or reduce the degenerative effects they have on articular tissue. [8] Rheumatoid arthritis, in summary, is an intricate and multifaceted disorder that necessitates a holistic and individualized therapeutic approach.

Healthcare providers can assist individuals with rheumatoid arthritis in attaining optimal health outcomes and leading fulfilling lives by attending to the physical, emotional, and social dimensions of the disease. By means of persistent investigation, inventive thinking, and cooperative efforts, it is possible to further enhance our comprehension and management of rheumatoid arthritis, thereby ultimately bettering the quality of life for the millions of individuals impacted by this chronic autoimmune disorder. [9,10]

Anti-arthritic activity of polyherbal ointments containing Datura stramonium, Nerium indicum, Curcuma longa, Acorus calamus, and Emblica officinalis has not been documented. In light of this investigation, we have therefore disclosed this.

2. MATERIALS AND METHODS

2.1. Collection of Materials

The following plants were collected from the surroundings of the Jaunpur Campus of Veer Bahadur Singh Purvanchal University: Acorus calamus, Curcuma longa, Emblica officinalis, Datura stramonium, and Nerium indicum.

2.2. Preparation of Plant Material

An elevated temperature of 40°C was applied to a heated air grill in order to desiccate the phytoconstituents from the following plants: Emblica officinalis fruits, Curcuma longa rhizome, Nerium indicum leaves, Datura stramonium seeds and Acorus calamus rhizome, respectively.

Following processing with a Willy machine, the coarse powder derived from the desiccated plant components was transferred to a container that had been tightly sealed. Using ethanol and a soxhlets apparatus, individual components of the plant were extracted. The concentrates obtained were altered through the process of refining. The concentrated extracts were stored in desiccators for additional utilization. [11, 12]

2.3. Formulation of Ointment

Fusion was used to produce the constituent of the ointment. By dissolving at a temperature of 70 degrees Celsius, the constituents of the base were consolidated in the basin. The ingredients were refrigerated for designated time intervals after dissolving while being continuously agitated at a temperature of 70^{o}C. Triturating the base material with the active ingredients by means of a mortar and pestle constituted the ointment formulation process.

Datura stramonium seeds, Nerium indicum leaves, Curcuma longa rhizome, Acorus calamus rhizome, and Emblica officinalis fruits are the constituents utilized in the formulation of ointment base and polyherbal ointment. The ethanolic extract comprises the subsequent excipients: methyl paraben (0.2 g), propyl paraben (0.1 g), stearic acid (15 g), white wax (2 g), yellow vaseline (8 g), and propylene glycol (8 g), triethanolamine (1 g) is present in each extract.[13,14]

2.4. Evaluation of Polyherbal Ointment

2.4.1. Appearance:

A visual examination was conducted to assess the clarity, color, and appearance of the ointment prior to its further evaluation. Regarding patient compliance, the test is critical.

2.4.2. pH:

A digital pH meter was utilized to ascertain the pH of the test formulation. A constant reading was obtained by dipping the electrode into the ointment formulation after dissolving one gram of ointment in 25 ml of distilled water.

2.4.3. Determination of viscosity:

The dial type viscometer (LV) was utilized to ascertain the viscosity of the ointment. Non-Newtonian in nature, spindle no.4 is implemented in the system.

2.4.4. Spread ability:

The ideal ointment must have low spread ability values but good consistency. Spread ability of formulation was determined by an apparatus suggested by Multimer et al, which was fabricated itself in the laboratory and used for the study. It consisted of a wooden block provided with two glass slides.

The lower slide is fixed on a wooden block and the upper slide with one end tied to a glass slide and the other end tied to a weight pan. An excess of ointment (2.5gm) between two glass slides and 1000g weight was placed on slides for 5 min to compress the sample to a uniform thickness. Weight was added to the pan.

The time (seconds) required to separate the two slides, was taken as a measure of spreadability.

It was calculated using the formula:

$$S = m.l / t$$

Where,

S = spreadability

M = weight tied to upper slide

t = Time taken

l = length of slide 7 cm

Shorter time interval, to cover distance of 7 cm, indicates better spreadability.

2.4.5. Skin irritation

Ten healthy male and female volunteers were selected for skin irritation testing. 100 mg ointment was applied on an area of 2 cm^2 for 6 hours, on the interior surface of the upper arm and covered with cotton bandage. After 6 hr the sites were cleaned with acetone and readings were noted.

2.4.6. Washability

The formulation was applied on the skin and then ease extends of washing with water was checked.

2.4.7 Extrudability

A closed collapsible tube containing about 20 g of ointment was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the ointment was extruded. The amount of the extruded ointment was collected and weighed.

2.5. Pharmacological Screening

2.5.1 Animals

For the purpose of assessing anti-arthritic properties, Deshpande Laboratories Pvt. Ltd. in Bangalore, India, selected Wistar strain rats (12 weeks old, robust weight, weighting 150-200 g of either sex). The rats were housed in the animal house. Temperature (23±2^oC), humidity (50±5% RH), and light and dark cycles lasting 10-14 hours were utilized to maintain controlled conditions. [15]

The animals were provided with sterile paddy husk bedding and unrestricted access to food and water within polypropylene enclosures that were individually occupied. Committee for the Purposes of Control and Supervision on Experiments on Animals (CPSCEA) and Institutional Animal Ethical Committee Register No. CPCSEA-approved ethical standards guided the design and execution of the experiments. 1582/PO/Re/S/11/CPCSEA is the consensus.

2.5.2 Anti-arthritic activity

FCA-induced arthritis paradigm [16] in rats was utilized to evaluate the efficacy of the topical herbal ointment formulation when applied topically. Four distinct groups of six rodents were established for each of the four categories. As the normal control, Group 1 was topically applied with an emollient (ointment) base.

A suspension of deceased Mycobacterium tuberculosis bacteria (Genei, Bangalore) 0.1 mL (0.1% w/v) was administered via liquid paraffin injection into the left hind foot in the sub-plantar region of rodents to induce arthritis in groups second, third and fourth. Group 2 was designated as the arthritic control. 21 days were permitted for the development of arthritis in Groups 2, 3 and 4 that were administered FCA.

At four, eight, fourteen, and twenty-one days into the experiment, body weight and rat paw volume were determined for the control and treatment groups, respectively, using a digital Vernier calliper. Once the onset of arthritis was confirmed, the left knee joint regions of Group 3 (which served as the reference standard) and Group 4 were topically treated with diclofenac sodium ointment (Voveran ointment, purchased from a community pharmacy shop) and the herbal ointment formulation, respectively, for 22 to 42 days.

Weight assessments were conducted on the rats and treatments, as well as the volume of the rat paws, using a digital Vernier caliper (Mitetoyo digital caliper, Japan), on the 25th, 29th, 35th, and 42nd days of the treatment period. The animal's scores were visually documented at the conclusion of the 42nd day. (Examine Table II)

2.5.3. Body weight

As shown in Table III, the induction of arthritis in rodents resulted in an average increase or decrease in body weight across all groups. The arthritic control group exhibited a decrease in body weight, while the groups treated with topical polyherbal ointment formulation and diclofenac sodium ointment demonstrated an increase in body weight, in comparison to the control group of normal rodents.

3. RESULT

Table 1: Evaluation of polyherbal ointment

S.No.	Parameters	Results
1.	**Appearance:**	Smooth, homogenous, translucent
2.	**pH**	7.02
3.	**Viscosity**	3247cps
4.	**Spreadability**	14.36 g. cm/sec
5	**Skin irritation**	Non irritant
6.	**Washability**	Good
7.	**Extrudability**	Good

Figure 1: Image of Polyherbal Ointment

Rat paw volume (mm)

Table II: Evaluation of anti-arthritic activity of poly herbal ointment in FCA induced arthritic rats

Groups	Initial	After 21 days	25th day	29th day	35th day	42nd day
Normal control	4.58±0.17	5.29±0.33	5.59±0.20	5.39±0.15	5.71±0.15	5.85±0.66
Arthritic control	4.76±0.14	9.75±0.19	10.25±0.31	10.86±0.25	10.79±0.33	10.56±0.19
Diclofenac Sodium topical ointment (1% w/w)	5.23±0.18	10.67±0.23	10.23±0.25	9.76±0.19	8.57±0.44	8.56±0.09
Topical herbal ointment	5.15 ±0.29	10.45±0.17	10.57±0.29	8.96 ±0.26	9.11±0.19	8.95±0.28

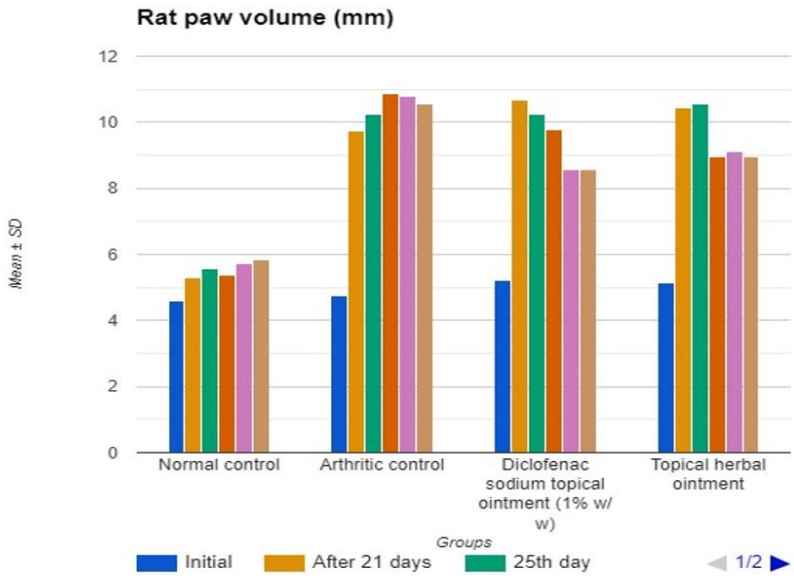
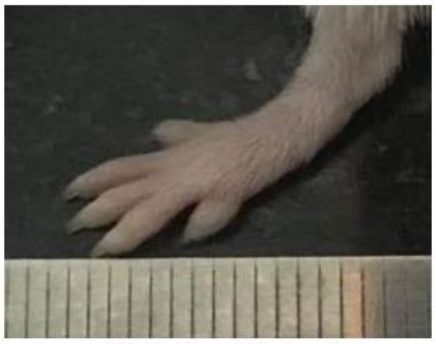
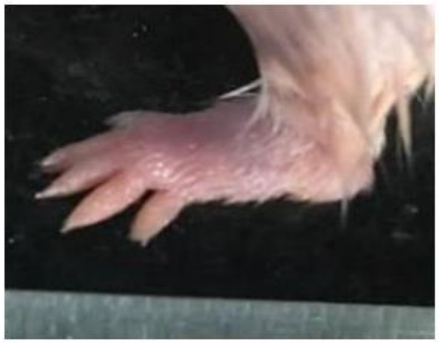


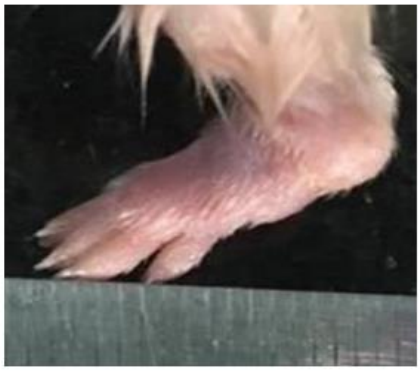
Figure II: Evaluation of anti-arthritic activity of poly herbal ointment in FCA induced arthritic rat



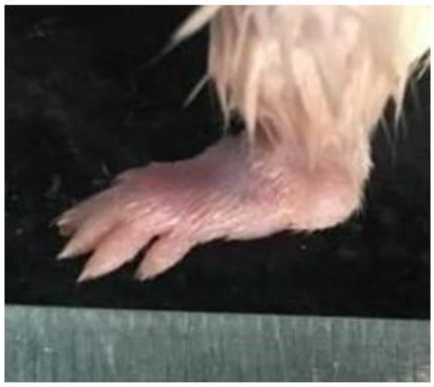
Un InducedUntreated



InducedUntreated



Test Formulation



Diclofenac Sodium

Table III: Effect of diclofenac sodium, poly herbal ointment formulation on body weight changes in FCA Induced arthritic rats

Groups	Initial body weight (g)	Body wt. after 21days of FCA induction	Body wt. after treatment 25th day	Body wt. after treatment29th day	Body wt. after treatmet 35 thday	Body wt. after treatment 42nd day	Weight gain (g)
Normal Control	156.5± 0.84	181.3±1.70	183.7±1.89	186.76±1.54	195.56±1.12	201.76±1.25	20.46
Arthritic control	157.5± 0.76	154.78±0.23	152.20±0.94	140.50±1.34	147.00±1.18	144.70±1.43	-10.08
Diclofenac sodium topical ointment (1% w/w)	156.00 ±1.29	153.20±1.28	154.00±1.37	166.20±1.38	158.50±1.45	160.70±1.34	7.5
Topical herbal ointment formulation	155.57 ±1.65	153.67±1.42	153.34±1.71	154.00±1.44	155.70±1.32	158.43±1.83	4.76

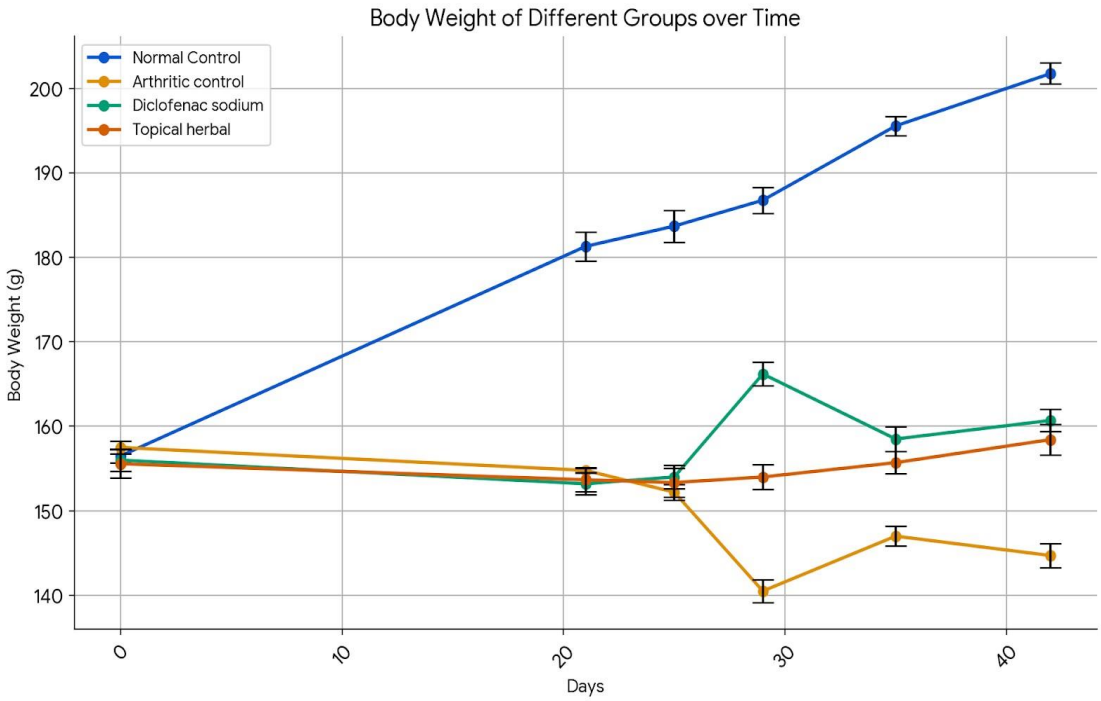


Figure III: Effect of diclofenac sodium, poly herbal ointment formulation on body weight changes in FCA Induced arthritic rats

5. CONCLUSION

Rat paw volume alterations were documented on the 25th, 29th, 35th, and 42nd days following topical application of diclofenac sodium ointment and the polyherbal ointment formulation for a period of 22 to 42 days (Table II). An increase in limb volume was indicative of the onset of arthritis in the arthritic control groups. Groups treated with diclofenac sodium ointment and topical herbal ointment exhibited a notable decrease in paw volume on the twenty-first day following FCA induction.

As demonstrated in Table II, the arthritic test scores indicated that the diclofenac sodium ointment-treated and topical herbal ointment formulation-treated groups experienced a significant reduction in the discomfort associated with FCA-induced arthritis.
As a result of a positive quality control evaluation and in vitro release characteristics that were consistent with commercially available diclofenac sodium ointment, the formulation was chosen for an anti-arthritic study.

As shown in Table III, the induction of arthritis in rodents resulted in an average increase or decrease in body weight across all groups. The arthritic control group exhibited a decrease in body weight, while the groups treated with topical polyherbal ointment formulation and diclofenac sodium ointment demonstrated an increase in body weight, in comparison to the control group of normal rodents. Studies that support the formulation's efficacy in treating patients with joint inflammatory disorders may account for the developed topical herbal ointment formulation's anti-arthritic activity.

References

1) Lipsky PE, Rheumatoid arthritis. In: Harrison's Principles of Internal Medicine,(Eds. Kasper DL. Fauci AS, Hauser SL, Longo DL & Jameson JL, McGraw Hill,New York: Braunwald E; 2005. p. 1968.

2) Patil KR, Patil CR, Jadhav RB, Mahajan VK, Patil PR, Gaikwad PS. Anti-arthriticactivity of Bartogenic acid isolated from fruits of Barringtonia racemosaRoxb. (Lecythidaceae). Evid Based Complement Alternat Med. 2011;(2011): 785245.

3) Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-s498.

4) Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).

5) Mandal S, Bhumika K, Kumar M, Hak J, Vishvakarma P, Sharma UK. A Novel Approach on Micro Sponges Drug Delivery System: Method of Preparations, Application, and its Future Prospective. Indian J of Pharmaceutical Education and Research. 2024;58(1):45-63.

6) Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: a Pharmaceutical Perspective. *Community Practitioner*, *21*(2), 29–37. https://doi.org/10.5281/zenodo.10642768

7) Singh, M., Aparna, T. N., Vasanthi, S., Mandal, S., Nemade, L. S., Bali, S., & Kar, N. R. (2024). Enhancement and Evaluation of Soursop (Annona Muricata L.) Leaf Extract in Nanoemulgel: a Comprehensive Study Investigating Its Optimized Formulation and Anti-Acne Potential Against Propionibacterium Acnes, Staphylococcus Aureus, and Staphylococcus Epidermidis Bacteria. *Community Practitioner*, *21*(1), 102–115. https://doi.org/10.5281/zenodo.10570746

8) Khalilullah, H., Balan, P., Jain, A. V., & Mandal, S. (n.d.). *Eupatorıum Rebaudıanum Bertonı (Stevıa): Investıgatıng Its Antı-Inflammatory Potentıal Vıa Cyclooxygenase And Lıpooxygenase Enzyme Inhıbıtıon - A Comprehensıve Molecular Dockıng And Admet*. *21*(03), 118–128. https://doi.org/10.5281/zenodo.10811642

9) Mandal, S. (n.d.). *Gentamıcın Sulphate Based Ophthalmıc Nanoemulgel : Formulatıon And Evaluatıon , Unravellıng A Paradıgm Shıft In Novel Pharmaceutıcal Delıvery Systems*. *21*(03). https://doi.org/10.5281/zenodo.10811540

10) Mandal, S., Tyagi, P., Jain, A. V., & Yadav, P. (n.d.). *Advanced Formulation and Comprehensive Pharmacological Evaluation of a Novel Topical Drug Delivery System for the Management and Therapeutic Intervention of Tinea Cruris (Jock Itch)*. *71*(03).

https://doi.org/10.5281/zenodo.10811676

11) A Dash, J Mishra, D Dash -Phytochemical Investigation and Pharmacological screening of Platycladus orientalis; International journal of pharmaceutical Science and research ,Volume-4,Issue-1,(2014)

12) AK Dash, J Mishra, DK Dash -Antidiabetic activity and modulation of antioxidant status by Ocimum canum in streptozotocin-induced diabetic rats: European Scientific Journal February 2014 edition vol.10, No.6

13) Chatterjee GK, Pal SP. Search for anti-inflammatory agents from Indianmedicinal plants: a review. Indian Drugs. 1984;21:413.

14) Ren Y, Lu L, Guo TB, Qiu J, Yang Y, Liu A, Zhang JZ. Novelimmunomodulatory properties of berbamine through selective downregulation of STAT4 and action of IFN gamma in experimental autoimmuneencephalomyelitis. J Immunol. 2008;181:1491–8.

15) Alamgeer, Chabert P, Akhtar MS, Jabeen Q, Delecolle J, Heintz D, Garo E,Hamburger M, Auger C, Lugnier C, Kim HJ, Oak MH, Schini-Kerth VB.Endothelium-independent vasorelaxant effect of a Berberis orthoboric rootextract via inhibition of phosphodiesterase's in the porcine coronary artery.Phytomedicine. 2016; 23: 793–9

16) Mizushima,y.;tsukada,w.;akimoto,T.AModification of rat adjuvant arthritis for testing anti-rheumaticdrugs.*J.Pharm.Pharmacol*.,v.24,n.10,p.781- 785,1972.