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POLYHERBAL FORMULATION USED IN ARTHRITIS

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ABSTRACT

Arthritis, a prevalent inflammatory joint disorder, presents significant challenges in management, often leading to the exploration of alternative therapies. This study investigates the extraction efficiency and phytochemical profiles of *Nyctanthes arbor-tristis* (night-flowering jasmine) and *Azadirachta indica* (neem), both recognized for their therapeutic potential in inflammatory conditions. A series of extraction processes were conducted to obtain petroleum ether and aqueous extracts, followed by phytochemical screening to identify bioactive compounds. Results indicated varying yields and a distinct presence of flavonoids, tannins, and saponins in the extracts. A polyherbal formulation combining both extracts was developed and characterized for its physical properties, viscosity, extrudability, and skin compatibility. Preliminary efficacy tests demonstrated promising effects on inflammatory markers in an animal model of arthritis. These findings support the potential of a polyherbal approach in arthritis management, highlighting the need for further investigations into the synergistic effects of these herbal constituents.

Keywords: Arthritis, *Nyctanthes arbor-tristis, Azadirachta indica*, polyherbal formulation, Phytochemical screening, Anti-inflammatory, Herbal medicine, Bioactive compounds, Extraction methods.

INTRODUCTION

Arthritis, a common inflammatory joint disease, affects millions worldwide and encompasses various forms, including rheumatoid arthritis and osteoarthritis. The management of arthritis often involves the use of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids; however, these treatments can lead to adverse side effects, prompting interest in alternative therapies (Khan *et al.,* 2016). Herbal medicine has gained attention due to its potential to provide safer and more effective treatments with fewer side effects (Bhat *et al.,* 2019).

Nyctanthes arbor-tristis, commonly known as the night-flowering jasmine, and *Azadirachta indica*, known as neem, are two medicinal plants traditionally used in various cultures for their therapeutic properties. *N. arbor-tristis* has been reported to possess anti-inflammatory, analgesic, and antioxidant activities, making it a valuable candidate for arthritis treatment (Mohan *et al.,* 2014). Its leaves and flowers are rich in bioactive compounds, including flavonoids and tannins, which contribute to its medicinal efficacy (Rani *et al.,* 2020).

On the other hand, *A. indica* is renowned for its wide-ranging pharmacological effects, particularly its anti-inflammatory and immunomodulatory properties, attributed to its active constituents like azadirachtin and various flavonoids (Suleiman *et al.,* 2018).

The leaves, seeds, and bark of neem have been extensively studied for their ability to alleviate symptoms associated with inflammatory conditions (Ranjan *et al.,* 2019). The extraction of bioactive compounds from these plants is crucial for developing effective herbal medicines. Various extraction methods, including aqueous and solvent-based extractions, can influence the yield and composition of the extracts, thereby impacting their therapeutic potential (Hossain *et al.,* 2019). Phytochemical screening plays a vital role in identifying the presence of key bioactive constituents that can confer medicinal benefits.

The combination of *N. arbor-tristis* and *A. indica* in a polyherbal formulation may enhance the therapeutic effects through synergistic interactions of their phytochemicals, potentially providing a holistic approach to arthritis management. This study aims to investigate the extraction efficiencies, perform phytochemical screenings, and evaluate the therapeutic potential of a polyherbal formulation containing extracts of these two plants.

MATERIALS AND METHODS

Collection of plant material

Aerial parts of *Nyctanthes arbor-tristis* and aerial parts of *Azadirachta indica* were collected locally from Sagar (Madhya Pradesh).

Authentication of plants

The plant material were taxonomically, authenticated and identified by Dr. Pradeep Tiwari, Professor, Botany, Dr. H.S. Gour University, Sagar. The samples were deposited in the Herbarium of the Institute with voucher specimen number BOT/H/02/43/01. The plant material was dried, cut into small pieces a coarse powder was made using a mechanical grinder and in tight container stored for further use.

Extraction of plants

About 25 g of air dried plant of *Nyctanthes arbor-tristis* and *Azadirachta indica* were extracted in soxhlet apparatus with Petroleum ether and Aqueous as solvent, each time before extracting with the solvent, the material was dried. All the extracts were concentrated and extracts were dried on water bath. Then consistency, color, appearance of the extracts and their percentage yield were determined.

Qualitative chemical test of various extracts

Extracts were subjected to various qualitative chemical tests to determine the presence of various secondary metabolites like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins and phytosterols using reported methods.

Alkaloids

Dragondorff's test: Dragondorff's reagent was added to the extract, reddish brown precipitate indicated the presence of alkaloids.

Wagner's test: Wagner's reagent was added to the extract, reddish brown precipitate indicated the presence of alkaloids.

Hager's test: Hager's reagent was added to the extract, yellow precipitate indicated the presence of alkaloids.

Carbohydrates

Molisch's test: Few drops of alcoholic α-napthol was added to the extract, then few drops of concentrated sulphuric acid through sides of test tube was added; purple to violet ring appeared at the junction.

Barfoed's test: 1 ml of extract was heated with 1 ml of Barfoed's reagent, if red cupric

oxide is formed, monosaccharide is present. Disaccharides on prolong heating (about 10 min) may cause reduction, owing to partial hydrolysis to monosaccharides.

Proteins

Biuret test: 2ml Biuret reagent was added to 2ml extract, violet color indicated presence of proteins 3.10.4 Amino Acids

Ninhydrin test: Ninhydrin solution was added to the extract, boiled, and violet color indicated the presence of amino acid.

Flavanoids

Shinoda test: To the extract few magnesium turnings was added and concentrated hydrochloric acid drop wise was added, green to blue color appeared after few minutes.

Alkaline reagent test: Few drops of sodium hydroxide solution was added to the extract, intense yellow color was formed which turned to colorless on addition of few drops of dilute acid which indicated presence of flavonoids.

Steroids and triterpenoids:

Salkowski test: the extract was treated with few drops of concentrated sulphuric acid red color at lower layer indicated the presence of steroids and formation of yellow colored lower layer indicated the presence of triterpenoids.

Glycosides

Borntrager's test: The extract was boiled with 1 ml of sulphuric acid in a test tube for 5 minutes. It was filtered when it was hot. Filtrate was cooled and shaken with equal volume of dichloromethane or chloroform. The lower layer of dichloromethane or chloroform was separated and shaken it with half of its volume of ammonia. A rose pink to red color was produced in the ammonical layer.

Legal's test: The extract was treated with pyridine and alkaline sodium nitroprusside solution, blood red color appeared.

Baljet's test: The extract was treated with picric acid or sodium picrate, orange color was produced.

Saponin glycosides

Froth formation test: 2 ml solution of drug was placed in water in a test tube and shaken well, stable froth (foam) was formed.

Phenolic compounds (Tannins):

Ferric chloride test: The extract was treated with ferric chloride solution, blue color appeared.

Formulation development of polyherbalnanogel

Method for the preparation of nanoparticles

Nanoparticles of both extracts were prepared by sonication method using the probe magnetic stirrer equipment. Prescribed quantities of extracts and Eudragit L100 were taken, and dissolved in 20ml of ethanol and the whole solution was mixed using a magnetic stirrer at 800 rpm until a homogeneous solution was obtained.

Method for the preparation of nanogel

The above prepared nanoparticulate homogeneous solution was taken and carbopol was added after the addition of carbopol, glycerine, propylene glycol and peppermint oil were added as additives and mixed thoroughly. The whole resultant mixture was kept at rest overnight to allow the swelling of carbopol. This results in the formation of gel (Jamadar and Shaikh, 2017).

***In vitro* evaluation of polyherbalnanogel**

Physical evaluations

Prepared nanogel formulations were evaluated for various organoleptic parameters such as

odour, colour and texture. Other physical evaluations such as ease of application, ease of removal on skin were also conducted.

pH

The pH of various gel formulations was determined by using digital pH meter. 1g of gel was taken and dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of formulation was done and calculated.

Viscosity

The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer.

Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis on slip and drag characteristics of gels. An excess of nanogel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one kg weight was placed on the top of the two slides for 5 min. to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 50 g. With the help of string attached to the hook and the time (in sec.) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better spreadability (Inamdar *et al.,* 2018).

Spreadability was calculated using the following formula.

S = M. L / T

Where,

M = wt. tied to upper slide

L = length (cm) moved on the glass slide

T = time taken to separate the slides

Extrudability study

The nanogel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 g was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed (Prabu *et al.,* 2017).

The percent of the extruded gel was calculated.

>90% extrudability - excellent

>80% extrudability - good

>70% extrudability – fair

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of optimized nanogel formulation was carried out to evaluate the surface morphology and apparent shape. The morphology of optimized nanogel formulation batch was studied by using scanning electron microscopy (JSM 6390, Japan). Samples were prepared by placing the droplet of nanoparticulate dispersion of samples on to aluminum metal plate and dried under vacuum to form a dry film, which was then observed under the scanning electron microscope. SEM images were taken using 20kV electron beam at magnification X100, X500, X600, X800.

In vivo studies of optimized formulation

Animals

Wistar albino rats of either sex weighing 150-200 g were housed in the animal house of Adina College of Pharmacy, Sagar. The animals were fed with commercially available feed and were maintained under standard conditions of temperature (25ºC ± 5ºC),

relative humidity (55 ± 10%), and 12/12 h light/dark cycle. They were transferred to the laboratory twelve hours prior to the experiments and given only water ad libitum. In all the experiments, the animals were kept in cages with raised floors of wide mesh, to prevent coprophagy.

The animals were housed and cared for in accordance with the Federal Government Legislation on animal care. Protocols for the study were approved by Adina college of Pharmacy and were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

Skin irritation studies

The Wistar albino rats of either sex weighing 150-200 g were used for this test total 4 animals were used. The intact skin was used. The hairs were removed from the rat 3 days before the experiment. The nanogel containing extracts was used on test animal. Herbal nanogel base was applied on the back of animal taken as control. The animals was treated daily up to seven days and finally the treated skin was examined visually and scored for erythema, edema any sensitivity and the reaction if any (Liao *et al.,* 2016).

Anti-arthritic activity

Acute non-immunological formaldehyde-induced arthritis in rats

Wistar albino rats weighing 150-200 g were divided into 4 groups of six animals each. Baseline recording of the paw volume was made by using plethysmometer. Group 1 was applied topically with gel base considered as normal control, group II (negative control) received formaldehyde, Group III received the topical application of the 0.5 g of Diclofenac Sodium gel and Group IV received the topical application of 0.5 g of polyherbalnanogel formulation for 10 days. On day 1, 30 min after the drug application, acute non-immunological arthritis was induced by sub plantar injection of 0.1 ml formaldehyde (2% v/v) into the right hind paw of all the animals except group I animals and was repeated on day 3. Arthritis was assessed by measuring the mean increase in paw volume over a period of 10 days (Kore *et al.,* 2011).

Chronic immunological FCA-induced arthritis in rats

Wistar albino rats weighing 150-200 g were used for the study. Rats were divided into four groups of 6 animals each. Group I was applied topically with gel base considered as normal control. Adjuvant arthritis was induced by subcutaneous injection of Freund's complete adjuvant (FCA - 0.1mL of 0.5% w/v suspension of heat killed Mycobacterium tuberculosis cells in liquid paraffin) into the plantar tissue of the right hind paw of each rat in remaining three Groups. Group II served as inflamed control group without any treatment. Group III received the topical application of the 0.5 g of Diclofenac Sodium gel and Group IV received the topical application of 0.5 g of polyherbalnanogel formulation. Drug and formulation treatment was started from the initial day, that is, from the day of adjuvant injection, 30 min before adjuvant injection and continued at the same timeof the day for 21 Anti-arthritic activity of herbal nanogel was evaluated on paw volume and arthritic scores on days 0, 2, 4, 6, 8, 10, 12, 14, 16, 18 and day 21. Moreover body weights of animals were monitored regularly during the course of the experiment. On day 21, blood was withdrawn by retroorbital puncture for assessment of haematological parameters and

animals were sacrificed under light ether anaesthesia to study histopathology of joints. During the experimental period, body weight and the rat paw volume of control and treatment groups was on 1st, 2nd, 4th, 7th, 10th, 15th and 21st day. The percentage inhibition of paw volume was compared with that of the inflamed control was taken as antiarthritic activity (Kamalutheen *et al.,* 2009)

Parameter assessment

Arthritic score

Photographs of the arthritic rats were taken on the 21st day with a digital 20- megapixel (canon IXUS 185) camera. Morphological feature of arthritis was monitored by the same person for all rats on the 21st day according to the extent of erythema and edema of the joints. The severity of arthritis was graded on a scale of 0–4 with the following criteria : 0 = no edema or swelling, 1 = slight edema and limited erythema, 2 = slight edema and erythema from the ankle to the tarsal bone, 3 = moderate edema and erythema from the ankle to the tarsal bone, and 4 = edema and erythema from the ankle to the entire leg. The arthritis score for each mouse was the sum of severity in all four limbs (maximum 16 points for individual mice). The arthritic score for each rat on day 0 was determined to be 0.

Paw volume

The paw volume of both hind paws was measured just before FCA injection on day 0 and on alternate days after 2–21 days using a plethysmometer.

Hematological parameters

The overnight fasted animals were anaesthetized with ketamine (20mg/kg, i.p) followed by withdrawal of blood samples from retro-orbital sinus and the collected blood samples were centrifuged at 10000 rpm for 10 min and evaluated for hematological parameters viz. hematological parameters like red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) value, erythrocyte sedimentation rate (ESR) using routine laboratory methods (Rajaram *et al.,* 2015).

Biochemical estimations

A portion of the blood samples were centrifuged at 10000 rpm for 10 min and the separated serum was analyzed for biochemical parameters viz. SGOT, SGPT, ALP, total protein.

RESULTS AND DISCUSSION

The extract profiles of *Nyctanthes arbor-tristis* and *Azadirachta indica* reveal significant differences in yield and physical appearance. The aqueous extract of *N. arbor-tristis* yielded a total percentage by weight of 11.08%, higher than the 3.20% obtained from petroleum ether. This indicates a higher solubility of bioactive compounds in aqueous conditions, suggesting that these compounds may have better bioavailability when formulated in hydrophilic systems.

In contrast, *A. indica* showed similar trends with its aqueous extract (8.08%) outperforming the petroleum ether extract (4.4%). These findings imply that aqueous extracts of both plants could be more beneficial for therapeutic applications, particularly in transdermal delivery systems like nanogels.

Phytochemical screening indicates a lack of alkaloids, glycosides, and carbohydrates in both extracts, which suggests that the primary bioactive components may lie in other classes of compounds. Notably, flavonoids were detected only in *A. indica*, while tannins and

phenolic compounds were present in both extracts. The presence of saponins and triterpenoids/steroids in both extracts highlights their potential for therapeutic applications, as these compounds are known for various pharmacological activities, including anti-inflammatory and analgesic effects.

The physical characteristics of the nanogel formulations (F1 to F5) indicate uniformity in appearance and texture. All formulations exhibited a thick, glossy, dark green appearance with herb-like odor and smooth texture, contributing to ease of application and removal. The consistency and sensory properties are crucial for user acceptance, particularly in topical applications.

The viscosity values ranged from 3557.66 to 4215.66 Cps, suggesting that the formulations possess an appropriate thickness for topical application while ensuring ease of spreadability, as indicated by the spreadability values (ranging from 15.00 to 20.33 g.cm/s). These characteristics are vital for effective coverage and prolonged skin contact, potentially enhancing the therapeutic effects of the nanogel.

The extrudability results (88.792% to 95.382%) reflect excellent formulation stability and compatibility, which are crucial for commercial formulations. A high percentage of extrudability ensures that the formulation can be easily dispensed from its container, making it user-friendly.

The primary skin irritation tests revealed no signs of erythema or edema in rats throughout the observation period. This suggests that the polyherbal nanogel formulations are safe for topical application, with no significant irritant effects noted. The absence of skin irritation is a critical aspect for the acceptance of any new formulation in clinical use.

The effects of the polyherbal nanogel on the arthritic score in the Freund's Complete Adjuvant (FCA) model indicate a potential anti-inflammatory effect. This data, shown in Fig. 2, suggests that the nanogel may alleviate symptoms associated with arthritis, warranting further exploration of its mechanisms of action.

Table 9 shows the hematological effects of the polyherbal nanogel compared to control groups. The treatment with the nanogel did not adversely affect red blood cell (RBC) and hemoglobin (Hb) levels, suggesting that it may not induce hematological toxicity. Furthermore, it resulted in improved white blood cell (WBC) counts compared to the FCA control, which indicates a potential immunomodulatory effect.

The results in Table 10 demonstrate that the polyherbal nanogel treatment led to significant improvements in serum enzyme levels (SGOT, SGPT, and ALP) compared to the FCA control. The decrease in these enzyme levels suggests a hepatoprotective effect of the nanogel, which is particularly important in mitigating liver damage commonly associated with chronic inflammation and certain pharmaceutical interventions.

Table 1: Formulation development of polyherbal nanogel

F. Code	*Nyctanthes arbor-tristis*	*Azadirachta indica*	Eudragit L 100 (mg)	Ethanol (ml)	Carbopol (mg)	Glycerin (ml)	Propylene Glycol (ml)	Peppermint oil (ml)
F1	100	500	2400	20	300	1	1	0.1
F2	200	400	2400	20	300	1	1	0.1
F3	300	300	2400	20	300	1	1	0.1
F4	400	200	2400	20	300	1	1	0.1
F5	500	100	2400	20	300	1	1	0.1

Table 2: Grading of skin reactions

Erythema Formation	
No erythema	0
Very slight erythema	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema to eschar formation	4

Edema Formation	
No edema	0
Very slight edema (barely perceptible)	1
Well defined edema (edges of area well defined by definite raising)	2
Moderate to severe edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Table 3: Extract profile of *Nyctanthes arbor-tristis*

Nyctanthes arbor-tristis		
Extracts	**Petroleum ether**	**Aqueous**
Physical appearance	Greenish brown sticky	Brown syrup
Total % by weight	3.20	11.08
Azadirachta indica		
Physical appearance	Greenish brown syrup	Greenish syrup
Total % by weight	4.4	8.08

Table 4: Result of phytochemical screening of extracts

S. No.	Experiment	Presence or absence of phytochemical test	
		Nyctanthes arbor-tristis	***Azadirachtaindica***
1.	**Alkaloids**		
1.1	Dragendroff's test	Absent	Absent
1.2	Mayer's reagent test	Absent	Absent
1.3	Wagner's reagent test	Absent	Absent
1.3	Hager's reagent test	Absent	Absent
2.	**Glycoside**		
2.1	Borntrager test	Absent	Absent
2.2	Killer-Killiani test	Absent	Absent
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Absent
3.2	Fehling's test	Absent	Absent
3.3	Benedict's test	Absent	Absent
3.4	Barfoed's test	Absent	Absent
5.	**Flavonoids**		
5.1	Shinoda's Test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
6.2	Gelatin test	Present	Present
7.	**Saponin**		
7.1	Froth test	Present	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Present

Table 5: Physical characterization of polyherbal nanogel

Formulation	Appearance	Colour	Odour	Texture	Ease of Application	Ease of Removal	Feel on Skin
F1	Thick, glossy	Dark green	Herb like	Smooth	Easy to apply	Easily removed	Smooth, minty feel on skin
F2	Thick, glossy	Dark green	Herb like	Smooth	Easy to apply	Easily removed	Spreads on skin, smooth, minty feel.
F3	Thick, glossy	Dark green	Herb like	Smooth	Easy to apply	Easily removed	Spreads on skin, smooth
F4	Thick, glossy	Dark green	Herb like	Very Smooth	Easy to apply	Easily removed	Spreads on skin, smooth, minty feel.
F5	Thick, glossy	Dark green	Herb like	Smooth	Easy to apply	Easily removed	Smooth, spreads on skin, minty feel

Table 6: Viscosity and Spreadability of polyherbal nanogel formulations

Formulation Code	Viscosity (Cps) *	Spreadability (g.cm/s) *
F1	4215.66 ± 14.704	15.00 ± 0.816
F2	3718.00 ± 21.483	18.00 ± 0.816
F3	4192.66 ± 28.193	16.66 ± 0.471
F4	3557.66 ± 32.427	20.33 ± 0.471
F5	3660.66 ± 20.548	1.009 ± 0.816

* Average of three determination ± SD

Table 7: Extrudability of polyherbal nanogel formulations

Formulation Code	Net formulation in the tube (g)	Weight of gel extruded (g)	Extrudability Amount percentage
F1	25.34	22.50	88.792
F2	25.45	22.87	89.862
F3	25.38	23.54	92.750
F4	25.52	24.12	94.514
F5	25.12	23.96	95.382

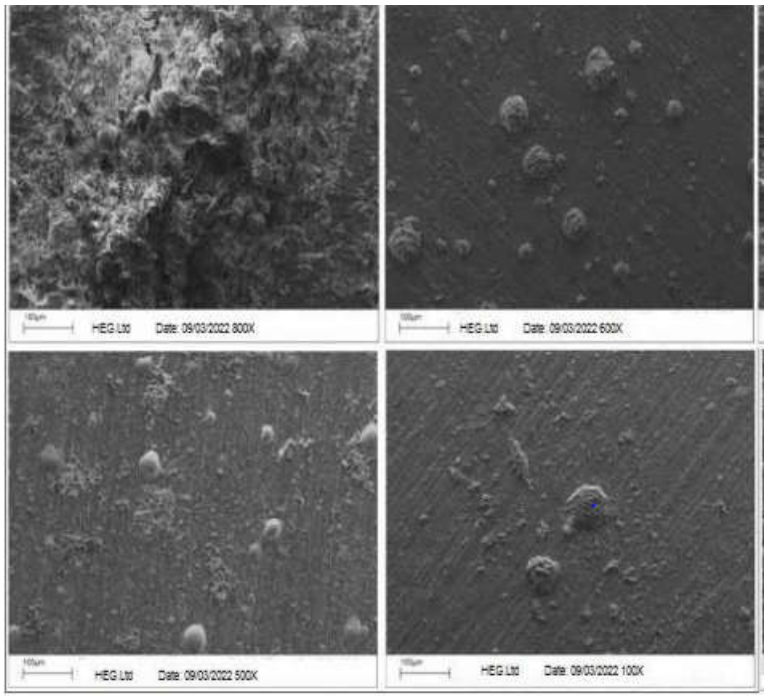

Figure 1: SEM image of formulation at different magnification

Table 8: Primary skin irritation test for polyherbal nanogel formulation

	Control	**Rat No. 1**	**Rat No. 2**	**Rat No. 3**
1h				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0
24 h				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0
48 h				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0
72 h				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0
7 days				
Erythema Score	0	0	0	0
Edema Score	0	0	0	0

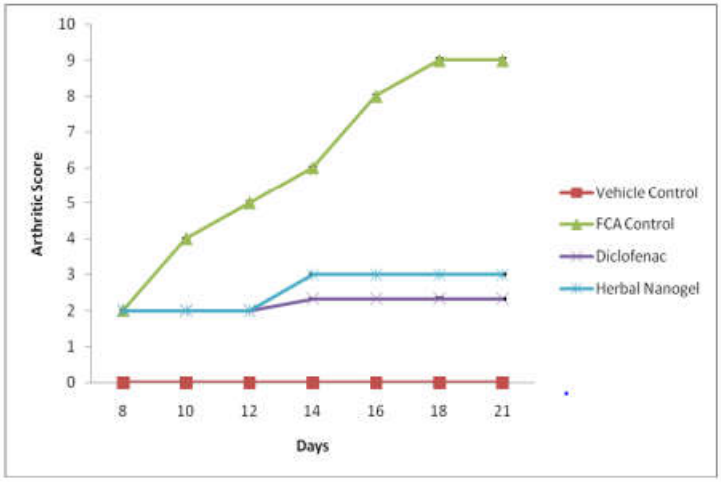

Figure 2: Effects of polyherbalnanogel on arthritic score in FCA model

Table 9: Effect of polyherbalnanogel on haematological profile

Treatment Dose (mg/kg)	RBC (×106 /µL)	WBC (×103 /µL)	Hb (g/dL)	ESR (mm)
Vehicle control	8.69 ± 0.17	11.65 ± 0.14	13.54 ± 0.21	3.63 ± 0.12
FCA control	7.83 ± 0.12	15.09 ± 0.16	11.82 ± 0.19	9.78 ± 0.16
Diclofenac	8.42 ± 0.16	12.86 ± 0.36 1	13.73 ± 0.12**	4.04 ± 0.13**
PolyherbalNanogel	8.31 ± 0.09	12.92 ± 0.65	12.78 ± 0.41	4.98 ± 0.19**

Values are calculated as the mean ± SEM, n =6 in each group

Table 10: Effect of polyherbalnanogel on various serum parameter

Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Protein(gm/dl)
FCA control	101.25 ± 12.06	72.28 ± 1.23	271.42 ± 19.18	3.32 ± 0.19
Diclofenac	50.98 ± 6.21**	47.32± 2.32**	142.76±16.46***	8.03 ± 0.34**
PolyherbalNanogel	68.21 ± 4.65*	51.42 ± 4.43*	174.54 ± 11.21**	7.16 ± .18*

Values are expressed as mean ± SEM (n=6).

*P<0.05,**P<0.01,***P<0.001. as compared with control (One-way ANOVA followed by Dunnet's test).

CONCLUSION

The study concludes that the combination of *Nyctanthes arbor-tristis* and *Azadirachta indica* extracts holds promising potential in managing arthritis due to their anti-inflammatory properties. The successful extraction of bioactive compounds such as flavonoids, tannins, and saponins from these plants underscores their therapeutic relevance. The polyherbal formulation developed from these extracts demonstrated favorable physical properties, including viscosity and skin compatibility, making it suitable for topical application. Preliminary efficacy tests in an animal model of arthritis revealed a notable reduction in inflammatory markers, suggesting the formulation's potential to alleviate arthritis symptoms. However, further research is necessary to fully understand the synergistic interactions between the herbal components and to establish clinical efficacy in human subjects. These findings pave the way for the development of alternative therapies in arthritis management using herbal ingredients.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Bhat, H.R., Prabhu, K.S. & Muliya, V.S. (2019) Phytochemical and pharmacological evaluation of herbal medicines for arthritis. *Journal of Herbal Medicine*, 15, 100234.
- Hossain, S.M., Islam, M.S. & Rahman, M.M. (2019) Phytochemical screening and antioxidant activity of different solvent extracts of Nyctanthes arbor-tristis. *Journal of Medicinal Plants Research*, 13, 1–7.
- Inamdar, Y.M., Rane, B. & Jain, A. (2018) Preparation and evaluation of beta sitosterolnanogel: A carrier design for targeted drug delivery system. *Asian Journal of Pharmaceutical Research and Development*, 6, 81–87.
- Jamadar, M.J. & Shaikh, R.H. (2017) Preparation and evaluation of herbal gel formulation. *Journal of Pharmaceutical Research & Education*, 1, 201–224.
- Kamalutheen, M.S., Gopalakrishnan, S. & Ismail, T.S. (2009) Anti-inflammatory and Anti-arthritic Activities of Merremia tridentate (L.) Hall. f. *Journal of Chemistry*, 6, 943–948.
- Khan, M.A., Rehman, F.U. & Muhammad, A. (2016) Traditional uses of medicinal plants in the treatment of arthritis in Pakistan: A review. *Journal of Ethnopharmacology*, 194, 192–204.
- Kore, K.J., Shete, R.V. & Desai, N.V. (2011) AntiArthritic activity of hydroalcoholic extract of LawsoniaInnermis. *International Journal of Drug Development and Research*, 3, 48–54.
- Liao, J.C., Wei, Z.X., Ma, Z.P., Zhao, C. & Cai, D.Z. (2016) Evaluation of a root extract gel from Urtica dioica (Urticaceae) as analgesic and anti-inflammatory therapy in rheumatoid arthritis in mice. Tro. *Journal of Pharmacy Research*, 15, 781–785.

- Mohan, A., Sharma, P. & Tiwari, P. (2014) Anti-inflammatory and analgesic activity of Nyctanthes arbor-tristis: A review. *International Journal of Research in Ayurveda and Pharmacy*, 5, 517–522.
- Prabu, S.L., Umamaheswari, A., Kumar, C.A., Banumuthupriya, M. & Dhanasekaran, D. (2017) Formulation and evaluation of polyherbal gel containing natural antimicrobials for the management of acne vulgaris. *International Research Journal of Pharmacy*, 8, 65–69.
- Rani, R., Rajput, G. & Yadav, N. (2020) A review on Nyctanthes arbor-tristis: A potential source of bioactive compounds. *International Journal of Pharmacognosy and Phytochemical Research*, 12, 146–150.
- Ranjan, R., Sharma, S. & Tripathi, P. (2019) Neem (Azadirachta indica): A review of its pharmacological activities and toxicological effects. *Journal of Natural Remedies*, 19, 1–14.
- Suleiman, M.M., Raziq, F. & Bhatti, S.A. (2018) Phytochemical and pharmacological properties of Azadirachta indica: A review. *European Journal of Medicinal Plants*, 24, 1–10.