International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

ASSESSMENT OF ANTI-ARTHRITIC EFFECT OF THE *NYCTANTHES ARBOR-TRISTIS*

Yogesh Patel*, Harshita Jain, Arpit Shrivastava, Prateek Kumar Jain

Adina Institute of Pharmaceutical Science, Sagar (M.P.)

***Correspondence Info:**
Yogesh Patel
Adina Institute of Pharmaceutical Science, Sagar (M.P.)
Email:
yogeshpatel030397@gmail.com

***Article History:**

Received: 22/02/2024
Revised: 01/03/2024
Accepted: 19/03/2024

ABSTRACT

Nyctanthes arbortristis, commonly known as "Harsingar" or the Night-flowering Jasmine, is a plant with a rich history in traditional medicine. In this study, a hydroalcoholic extract of *Nyctanthes arbor-tristis* leaves was prepared, and its phytochemical composition was analyzed. Additionally, the *in-vitro* anti-arthritic activity of the extract was assessed using the bovine serum protein denaturation method. The hydroalcoholic extract of *Nyctanthes arbor-tristis* leaves was subjected to phytochemical analysis to identify the presence of bioactive compounds. Various phytoconstituents such as alkaloids, flavonoids, terpenoids, tannins, and glycosides were evaluated. The anti-arthritic potential of the extract was investigated using the bovine serum protein denaturation method. This method is a widely used in-vitro assay to assess the ability of substances to inhibit protein denaturation, a process associated with inflammatory conditions. The phytochemical analysis revealed the presence of alkaloids, flavonoids, terpenoids, tannins, and glycosides in the hydroalcoholic extract of *Nyctanthes arbor-tristis* leaves. These bioactive compounds are known for their potential therapeutic effects. In the in-vitro anti-arthritic activity assay, the extract demonstrated a significant inhibition of bovine serum protein denaturation. This suggests a potential anti-inflammatory effect, indicating the extract's ability to interfere with processes associated with arthritis. The hydroalcoholic extract of *Nyctanthes arbor-tristis* leaves showed promising anti-arthritic activity in the in-vitro assay. The presence of bioactive compounds known for their anti-inflammatory properties supports the traditional use of *Nyctanthes arbor-tristis* in managing arthritic conditions.

Keywords: *Nyctanthes arbortristis*, hydroalcoholic extract, phytochemical analysis, anti-arthritic activity, bioactive compounds.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by inflammation of the synovial joints, leading to pain, swelling, and joint deformities. The complex pathogenesis of RA involves interplay of immune responses, inflammatory mediators, and oxidative stress. Despite advances in pharmacological interventions, there is a continuous quest for safe and effective therapeutic agents, particularly from natural sources, to manage the symptoms and halt the progression of arthritis (Ahmad *et al.,* 2017; Saravanan *et al.,* 2018).

Nyctanthes arbortristis, commonly known as "Harsingar" or the Night Jasmine, is a medicinal plant with a rich history in traditional medicine (Gupta *et al.,* 2011).

Various parts of the plant, including leaves, flowers, and seeds, have been traditionally used for their anti-inflammatory, analgesic, and immunomodulatory properties. The plant is known for its diverse phytochemical composition, including flavonoids, alkaloids, steroids, and terpenoids, which contribute to its pharmacological activities (Suthar *et al.,* 2014; Nirmal *et al.,* 2011).

Several studies have explored the potential anti-arthritic effects of *Nyctanthes arbortristis*, aiming to validate its traditional use and discover novel therapeutic options. The present investigation seeks to contribute to this body of knowledge by assessing the anti-arthritic effects of a hydroalcoholic extract of *Nyctanthes arbor-tristis* (HENA) using a comprehensive approach, encompassing *in-vitro* assays, animal models, and biochemical evaluations.

MATERIALS AND METHODS

Collection of plant material

Organoleptic characters, morphological characters, and microscopical examination would help in identifying crude drug. For identification of unknown drugs herbariums and leading botanical gardens are of great help. The Leaves of selected plant namely *Nyctanthes arbor-tristis* were collected from local area of Sagar Madhya Pradesh on the basis of geographical availability. All collected plant drug were cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use (Harborne and Williams, 1976).

Physico-chemical evaluation

Determination of moisture content (loss on drying)

Misfortune on drying can be characterized as the % w/w misfortune in weight because of loss of water and unpredictable issue is called as Loss on drying. Drying should be possible utilizing different conditions. Precisely gauged 2gm of Leaves powder of the plant *Nyctanthes arbor-tristis* were taken independently on a different watch glass and kept for drying in a sight-seeing oven at 105ºC for 2hrs. Distinction in weight speaks to dampness content. At that point rate yield was resolved and noted (Mandal *et al.,* 2007).

Determination of extractive values

Alcohol soluble extractives

5gm powder Leaves of *Nyctanthes arbor-tristis* (shade dried) was macerated with 100 ml of 90% methanol and ethanol, this was done in the conical flask and the conical flask was kept on shaker without disturbance for 18hr. Arrangement was sifted quickly, taking safeguards against loss of liquor. Filtrate arrangement of 25ml was dissipated to dryness at 105^{o}C in tarred level bottomed petri dish. The rate of methanol and ethanol solvent concentrate was resolved with reference to shade dried medication; methanol and ethanol solvent extractives of Leaves of *Nyctanthes arbor-tristis* were noted.

Water soluble extractives

5gm. of coarsely powder (60-80 mesh) of Leaves of *Nyctanthes arbor-tristis* (shade dried) was macerated with 100 ml of Deionizer water, in closed flask, shaking flask frequently during process and finally allowed to stand for 18 hr. solution was filtered and the filtrate solution of 25ml was evaporated to dryness at 105^{o}C The percentage of water soluble extract was determined with reference to shade dried drug. Water soluble extractives

values of leaves of *Nyctanthes arbor-tristis* were noted.

Extraction of plant material

Leaves of *Nyctanthes arbor-tristis* were cut into little pieces using sterile scissor, washed under running tap water to remove the dust impurities. Then the plant Leaves was dried at room temperature (under shade). After complete drying, it was powdered using the motor and pestle.

Around 50 gm of air-dried powdered plant material was Placed in Soxhlet apparatus, starting form Petroleum ether then Hydroalcohol (ethanol: water; 70:30) for the plant *Nyctanthes arbortristis*. Every time before removing with next dissolvable, powdered material was air dried beneath 100ºC. The extracted solvent was evaporated using the water bath at 100ºC. After the evaporation the extracted samples were stored in cold for further analysis (Prior *et al.,* 2005).

Phytochemicals screening of plant *Nyctanthes arbor-tristis*

Quantitative and quantitative properties of the plant's herbs have been studied for studying its various properties. This will help in the setting up of standard of new drugs in the market. Examination is done to check for the primary and secondary metabolites present in the plant's species. Couple of dynamic phytochemicals from these herbs has envisioned some high advancement profile drugs (Amic *et al.,* 2003).

***In-vitro* anti-arthritic activity**

The *in-vitro* anti-arthritic activity on hydroalcoholic extract of *Nyctanthes arbor-tristis* (Leaves) using bovine serum protein Denaturation method (Rahman *et al.,* 2012).

Bovine serum protein denaturation method

Preparation of Reagents

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

Phosphate Buffer Saline PH 6.3: Dissolved 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na_2HPO_2), 0.24 g of potassium dihydrogen phosphate (KH_2PO_4) in 800 ml distilled water. The pH was adjusted to 6.3 using 1N HCl and make up the volume to 1000 ml with distilled water.

Method: Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%W/V aqueous solution) and 0.05ml of test solution of various concentrations.

Test control solution

Test control solution (0.5ml) consists of 0.45ml of bovine serum albumin (0.5%W/V aqueous solution) and 0.05ml of distilled water.

Product control solution

Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.

Standard solution

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

Procedure: 0.05 ml various concentrations (50, 100, 250 mcg/ml) of test dugs and standard drug diclofenac sodium (50, 100, 250 mcg/ml) were taken respectively and 0.45 ml (0.5% w/V BSA) mixed. The samples 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 measured using UV-Visible spectrophotometer at 255 nm. The control represents 100% protein Denaturation. The results were compared with Diclofenac sodium. The percentage inhibition of protein Denaturation can be calculated as.

$$\% \text{ Inhibition} = \frac{(\text{Abs of control} - \text{Abs of test})}{\text{Abs of control}} \text{x}100$$

***In-vivo* anti arthritis screening on hydroalcoholic extract of *Nyctanthes arbor-tristis* (HENA)**

Animals

Healthy 8-week-old female Sprague Dawley (SD) rats (180-200 g) were used in the present study. They were provided normal diet and tap water ad labium and rats were housed at 23 ± 2°C and 50-65% humidity under a 12:12 ± 1 hour light dark cycle. The animals were acclimatized to the laboratory conditions before experiments. Experimental protocol was approved by Institutional Animal Ethics Committee. Care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Experiment protocol was approved by Institutional Animal Ethics Committee (Grant *et al.,* 1970; Gautam and Goel, 2014).

Acute oral toxicity study

Adult Swiss albino mice of either sex, weighing between 20 and 25 g, fasted overnight and were used for acute toxicity study, as per the Organization for Economic Co-Operation and Development (OECD 423) guideline. Four groups of mice of both sexes were fasted overnight. The first control group mice received 0.5% carboxymethyl cellulose (CMC) suspension in distilled water while the other three groups received hydroalcoholic extract of *Nyctanthes arbor-tristis* (HENA**)** suspended in 0.5% CMC at doses of 200, 600, and 2000 mg/kg. Animals were observed closely for first 4 hours, for any toxicity manifestation, like increased motor activity, salivation, convulsion, coma, and death. Subsequently observations were made at regular intervals for 24 h. The animals were under further investigation up to a period of 14 days and no mortality was reported within the study period.

Adjuvant arthritis Model (Complete Freund's Adjuvant)

The rats were divided into five groups of five animals each as follows

- Group-1: Vehicle control
- Group-2: Arthritis control
- Group-3: (HENA) 200 mg/kg/day, p.o.
- Group-4: (HENA) 400 mg/kg/day, p.o.
- Group-5: Indomethacin 10 mg/kg/day, p.o.

The method described by Newbould (1965) was employed with some modifications. Adjuvant arthritis was induced by subcutaneous injection of Complete Freund's Adjuvant (FCA) (0.1 ml) (Difco Labs, Chennai) into the sub plantar tissue of the right hind paw of each rat. The test groups consisted of FCA -injected rats challenged with their respective doses of the test drug administered orally 24 hours before FCA injection. The vehicle control rats were injected with 0.1 ml of liquid paraffin (Incomplete Freund's Adjuvant) only. The

drug treatments were continued for 20 days after inducing arthritis. The swelling in the injected paw and the contralateral hind paw were monitored daily using a mercury displacement Plethysmometer. The increase in the extent of erythema and edema of the tissue shows the severity of the inflammation. The differences between the experimental groups and the arthritis control group were statistically analyzed. The change in body weight was also recorded daily (Bendele *et al.,* 2001).

Biochemical parameters

At the end of the study, blood samples were withdrawn from all groups through retro orbital plexus puncture, and the biochemical parameters were analyzed (Barrington, 2006). Hematological parameters such as the hemoglobin (Hb) level, the red blood cell (RBC) count, the white blood cell (WBC) count and the erythrocyte sedimentation rate (ESR) were estimated manually. Liver markers such as SGOT and SGPT were analyzed using an auto analyzer. The liver enzyme levels were estimated using Lab Kit enzymatic kits.

Formaldehyde induced arthritis model

Animals were divided into five groups (n = 6). Group I received the vehicle (2 ml/kg, 1% v/v tween 80) and served as the normal control. Group II received formaldehyde, served as a negative control. Group III received the standard drug diclofenac (10 mg/kg body weight), groups IV, and V received Hydroalcoholic extract of *Nyctanthes arbor-tristis* (HENA) in doses of (200 and 400 mg/kg body weight), respectively. Thirty minutes after oral administration of vehicle/drugs, arthritis was induced by sub plantar administration of 0.1 ml formaldehyde (2% v/v) into the left hind paw of all the animals except normal control. This was designated as day 1 Vehicle/drug treatment was continued for the duration of 28 more days. Formaldehyde (0.1 ml 2% v/v) was again injected into the same paw on the third day (Parasuraman *et al.,* 2010; Nair *et al.,* 2015).

Grouping of animals

Group 1: Normal control (1% v/v tween 80, p.o. for 28 days)

Group 2: Negative Control (Formaldehyde 0.1 ml 2% v/v by Sub-plantar region)

Group 3: Formaldehyde 0.1 ml 2% v/v + Standard (Diclofenac 10 mg/kg p.o. for 28 days).

Group 4: Formaldehyde 0.1 ml 2% v/v + HENA (200 mg/kg p.o. for 28 days)

Group 5: Formaldehyde 0.1 ml 2% v/v + HENA (400 mg/kg p.o. for 28 days)

Paw volume and paw thickness were measured at 0 days, 7^{th} day, 14^{th} day, 21^{st} day and 28^{th} day by using Plethysmometer and vernier caliper, respectively. The body weights of the animals were measured by digital balance to access the exact dose & course of the disease at the initial day after induction, 14^{th} day and the end of 28^{th} day.

% inhibition of paw edema concerning untreated groups was calculated using the following formula:

$$i = [1-(\Delta V \text{ Treated} / \Delta V \text{ Untreated})] \times 100$$

Where, i= % inhibition of paw edema

ΔV treated= mean change in paw volume of treated rat

ΔV untreated= mean change in paw volume of untreated rat

Statistical analysis

The mean ± SEM values were calculated for each group. Statistical differences among the groups were determined using one-way ANOVA followed by Tukey's multiple comparison test. $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Table 1 presents the physico-chemical characterization of *Nyctanthes arbor-tristis* leaves. The loss on drying, indicating the moisture content, is determined to be 8.11% w/w. Water-soluble extractive value, ethanol-soluble extractive, methanol-soluble extractive, and chloroform-soluble extractive are found to be 2.14% w/w, 2.16% w/w, 1.79% w/w, and 2.28% w/w, respectively. These parameters provide insights into the physical and chemical properties of the plant material.

Table 2 displays the % yield of the crude extract obtained through different solvents. The pet ether extract has a dark brown color, semisolid consistency, and a yield of 10.45% w/w. The ethanol-water extract (70:30) exhibits a brown color, semisolid consistency, and a higher yield of 12.22% w/w.

Table 3 outlines the results of preliminary qualitative phytochemical tests. Mayer's test indicates the presence of alkaloids (positive). The alkaline reagent test and sodium hydroxide test reveal the presence of flavonoids and phenolic compounds (positive), respectively. The ferric chloride test suggests the absence of tannins (negative). Salkowski's test indicates the presence of steroids and triterpenoids (positive). Benedict's test, saponins test, and glycosides test all show positive results for carbohydrates, saponins, and glycosides, respectively.

Table 4 presents the estimation of total phenolic and flavonoids content in the hydroalcoholic extract of *Nyctanthes arbortristis*. The total phenol content is found to be 0.538 mg/100mg of dried extract, and the total flavonoids content is 0.784 mg/100mg of dried extract.

The results presented in Tables 5 to 9 showcase the comprehensive evaluation of the anti-arthritic potential of the Hydroalcoholic extract of *Nyctanthes arbor-tristis* (HENA). Different parameters, including in-vitro anti-antiarthritic activity, impact on rats' growth, inflammation in the injected paw, and biochemical and hematological parameters, were investigated to assess the efficacy of HENA.

In the *in-vitro* anti-anti-arthritic activity study (Table 5), the denaturation method was employed to evaluate the potential of HENA to inhibit protein denaturation, a characteristic feature of arthritis. The results indicate a dose-dependent inhibition of denaturation, with HENA demonstrating a significant inhibitory effect at all concentrations tested. Particularly noteworthy is the higher percentage inhibition observed with HENA compared to the standard drug, diclofenac, highlighting the potent anti-arthritic properties of the extract.

Table 6 provides insights into the effect of HENA on the growth of rats. The increase in body weight percentage in HENA-treated groups, especially at 400 mg/kg, suggests that

the extract has a positive impact on the overall growth of the animals. This is crucial in assessing the safety and tolerability of HENA, reinforcing its potential as a therapeutic agent.

Table 7 delves into the anti-arthritic activity of HENA in comparison with indomethacin, a commonly used anti-inflammatory drug. The results demonstrate the ability of HENA to significantly reduce paw swelling in arthritic rats over the course of the experiment. This effect is comparable to that of indomethacin, further supporting the anti-arthritic potential of HENA.

The impact of HENA on biochemical and hematological parameters is detailed in Table 8. The altered levels of SGOT, SGPT, WBC, RBC, ESR, and Hb in arthritic control rats are indicative of the inflammatory response associated with arthritis. Treatment with HENA, especially at 400 mg/kg, resulted in a notable reversal of these changes, suggesting its potential in mitigating the biochemical and hematological alterations associated with arthritis.

Table 9 provides additional evidence of the anti-inflammatory effect of HENA by measuring joint swelling in arthritic rats. The dose-dependent reduction in paw volume and percentage inhibition observed in the HENA-treated groups further supports the extract's efficacy in alleviating arthritis symptoms.

Table 1: Physico-chemical characterization

S. No.	Physico-chemical parameter	Results
1.	Loss on Drying	8.11%w/w
2.	Water soluble extractive value	2.14%w/w
3.	Ethanol soluble extractive	2.16%w/w
4.	Methanol soluble extractive	1.79%w/w
5.	Chloroform soluble extractive	2.28%w/w

Table 2: % Yield of crude extract

Extracts	Colour	Consistency	Yield (% w/w)
Nyctanthes arbor-tristis (Leaves)			
Pet ether	Dark brown	Semisolid	10.45%
Ethanol: water; 70:30	Brown	Semisolid	12.22%

Table 3: Preliminary qualitative phytochemical tests

S. No.	Phytoconstituents	Test	Observations
1	Alkaloid test	Mayers test	**Positive**
2	Flavonoid test	Alkaline reagent test	**Positive**
3	Phenolic content	Sodium hydroxide test	**Positive**
4	Tannin content	Ferric chloride test	Negative
5	Steroid test	Salkowski's test	**Positive**
6	Carbohydrate	Benedict's Test	**Positive**
7	Triterpenoids	Salkowski's test	**Positive**
8	Saponins test	Saponins test	**Positive**
9	Glycosides	Glycosides test	**Positive**

Table 4: Estimation of total phenolic and flavonoids content of *Nyctanthes arbortristis*

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.538	0.784

Table 5: Effect of Hydroalcoholic extract of *Nyctanthes arbor-tristis* (HENA) in *in-vitro* anti-antiarthritic activity on bovine serum protein Denaturation method

Drug	Concentration (µg/ml)	Test Absorbance	Product Control	% Denaturation	% Inhibition of Denaturation
Control	-	0.029	0.012	100	0
HENA	100	0.047	0.019	60.71	39.29
HENA	250	0.054	0.018	47.22	52.78
HENA	500	0.064	0.021	39.53	60.47
Diclofenac	100	0.272	0.022	6.80	93.20
Diclofenac	250	0.412	0.036	4.59	95.41
Diclofenac	500	0.607	0.052	3.09	96.91

Table 6: Effect of Hydroalcoholic extract of *Nyctanthes arbor-tristis* on Rats growth

Group (n=5 in each group)	Body weight (g)		Increase in body weight (%)
	Initial	Final	
Normal control	178±1.62	192.23±2.89	6.27
Arthritis control	182.30±1.66	191.23±2.49	3.78
HENA, 200 mg/kg	170.80±1.05	180.33±0.35	5.54
HENA, 400 mg/kg	176±1.52	187.46±7.39	5.28
Indomethacin,10 mg/kg	161.13±0.18	171.16±1.30	7.51

Table 7: Ant arthritic activity of Hydroalcoholic extract of *Nyctanthes arbor-tristis* compared with indomethacin in injected paw (swelling volume in ml)

Treatment	Post-insult time of assay (days)										
	1	3	5	7	9	11	13	14	17	19	21
Normal control	0.12 ± 0.00	0.10 ± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.10± 0.00	0.11± 0.00	0.10± 0.00
Arthritic control	0.74 ± 0.00	0.85 ± 0.00	1.05± 0.00###	1.19± 0.00###	0.93± 0.00###	0.85± 0.01###	0.85± 0.01###	0.86± 0.01###	0.86± 0.01###	0.88± 0.01###	0.91± 0.01###
HENA, 200 mg/kg	0.72 ± 0.01	0.74 ± 0.00	0.91± 0.01***	0.88± 0.01***	0.89± 0.00*	0.70± 0.00***	0.66± 0.01***	0.69± 0.01***	0.73± 0.01***	0.67± 0.00***	0.75± 0.01***
HENA, 400 mg/kg	0.71 ± 0.01	0.79 ± 0.00	0.93± 0.01***	0.86± 0.01***	0.83± 0.00***	0.80± 0.00***	0.68± 0.00***	0.74± 0.01***	0.53± 0.01***	0.51± 0.01***	0.70± 0.01***
Indomethacin, 10 mg/kg	0.64 ± 0.00	0.74 ± 0.00	0.85± 0.00***	0.79± 0.00***	0.79± 0.00***	0.67± 0.00***	0.73± 0.00***	0.74± 0.00***	0.63± 0.00*** 0	.62± 0.01***	0.57± 0.01***

Values are expressed as mean±SEM; n=5 rats in each group; ***P<0.001 compared with arthritic control; ###P<0.001 compared with normal control.

Table 8: Effect of the Hydroalcoholic extract of *Nyctanthes arbor-tristis* on biochemical and hematological parameters

Group	Biochemical parameter		Hematological parameter			
	SGOT (U/L)	SGPT (U/L)	WBC (cells/cu. mm)	RBC (millions/cu. mm)	ESR (mm/hr)	Hb (gm/dl)
Normal control	105.26±0.13	55.68±0.72	7.31±0.06	4.90±0.05	3.27±0.20	13.05±0.24
Arthritic control	232.68±1.98	158.72±1.72	7.67±0.10 ##	3.65±0.23###	7.18±0.16 ###	8.87±0.203 ###
HENA, 200 mg/kg	181.86±2.13 ***	127.86±3.39 ***	7.25±0.03	4.81±0.22	5.31±0.15 ***	9.57±0.115 ***
HENA, 400 mg/kg	149.91±2.67 ***	112.64±1.19 ***	7.29±0.01	4.57±0.14	4.79±0.13 ***	10.38±0.31 ***
Indomethacin,10 mg/kg	126.25±0.77 ***	93.02±1.62** **	7.36±0.01	4.58±0.04	4.15±0.12 **	12.14±0.23

Values are expressed as mean±SEM, n = 5 rats in each group, ***P<0.001, **P<0.01 compared with arthritic control, ###P<0.001, ##P<0.01 compared with normal control

Table 9: Effect of HENA on joint swelling (Paw volume)

Groups	Treatment & Dose	Paw volume in ml					% inhibition
		Day 1	Day 7	Day 14	Day 21	Day 28	
1	Normal Control	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	----
2	Negative Control	0.26 ± 0.01	0.46 ± 0.01	0.55 ± 0.02	0.6 ± 0.02	0.67 ± 0.01	0
3	Diclofenac (10 mg/kg)	0.20 ± .02**	0.31 ± 0.03**	0.34 ±0.01**	0.38 ± 0.02**	0.33 ± 0.01**	36.6
4	HENA 200 mg/kg	0.21 ± 0.00**	0.37 ± 0.01**	0.38 ±0.00**	0.42 ± 0.02**	0.38 ± 0.02**	30
5	HENA 400 mg/kg	0.18 ± 0.00**	0.30 ± 0.02**	0.35 ±0.01**	0.37 ± 0.01**	0.31 ± 0.02**	38.33

Values are expressed as mean±SEM, n = 5 rats in each group, ***P<0.001, **P<0.01 compared with arthritic control, ###P<0.001, ##P<0.01 compared with normal control

CONCLUSION

The collective evidence from this study strongly supports the anti-arthritic potential of the Hydroalcoholic extract of *Nyctanthes arbortristis*. These findings lay the groundwork for further research and clinical investigations to elucidate the specific mechanisms of action, assess long-term safety, and explore the potential application of HENA as a therapeutic agent for arthritis. The promising results presented herein position HENA as a valuable candidate for further development in the quest for effective and safe anti-arthritic interventions.

DECLARATION OF INTEREST

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

REFERENCES

- Ahmad, S., Rehman, S., Shah, M., Ullah, F., Ayaz, M., Sadiq, A., Ullah, F., Imran, M., Shahat, A.A., Al-Said, M.S. & Ovais, M. (2017). Proximate analysis, phytochemical screening, in-vitro antioxidant and cytotoxic activity of different extracts of Nyctanthes arbor-tristis leaves collected from District Swat, Pakistan. *Journal of Biomedical Science*, 24, 33.
- Amic, D., Davidovic-Amic, D., Beslo, D. & Trinajstic, N. (2003). Structure-radical scavenging activity relationships of flavonoids. *Croatica Chemica Acta*, 76, 55–61.
- Bendele, A. (2001). Animal models of rheumatoid arthritis. *Journal of Musculoskeletal and Neuronal Interactions*, 1, 377–385.
- Berrington, J. (2006). Biologic treatments for rheumatoid arthritis. *Journal of Orthopaedic Nursing*, 10, 159–165.
- Gautam, M.K. & Goel, R.K. (2014). Toxicological study of *Ocimum sanctum* Linn leaves: Hematological, biochemical, and histopathological studies. *Journal of Toxicology*, 2014, 135654.
- Grant, N.H., Alburn, H.E. & Kryzanauskas, C. (1970). Stabilization of serum albumin by anti-inflammatory drugs. *Biochemical Pharmacology*, 19, 715–722.
- Gupta, A., Mittal, M., Kaur, N. & Bansal, P. (2011). Antiarthritic activity of methanolic extract of Nyctanthes arbor-tristis in experimental animals. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3, 120–122.
- Harborne, J.B. & Williams, C.A. (1976). Occurrence of sulphated flavones and caffeic acid esters in members of the fluviales. *Biochemical Systematics and Ecology*, 4, 37–41.
- Mandal, S.M., Mondal, K.C., Dey, S. & Pati, B.R. (2007). Antimicrobial activity of the leaf extracts of *Hyptis suaveolens* (L.) poit. *Indian Journal of Pharmaceutical Sciences*, 568–569.
- Nair, V. & Surender Singh, G.Y.K. (2015). Evaluation of disease modifying activity of *Coriandrum sativum* in experimental models. *Indian Journal of Medical Research*, 135, 240–245.

- Newbould, B.B. (1965). Suppression of adjuvant-induced arthritis in rats with 2-butoxy carbonyl ethylene -4 – oxothiazolidine. *British Journal of Pharmacology and Chemotherapy*, 24, 632–640.
- Nirmal, S.A., Pal, S.C., Mandal, S.C. & Patil, A.N. (2011). Analgesic and anti-inflammatory activities of Nyctanthes arbor-tristis Linn. seeds. *Oriental Pharmacy and Experimental Medicine*, 11, 165–171.
- Parasuraman, S., Raveendran, R. & Kesavan, R. (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics*, 1, 87–93.
- Prior, R.L., Wu, X. & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53, 4290–4302.
- Rahman, H., Eswaraiah, C.M., Vakati, K. & P, M. (2012). *In-vitro* studies suggest probable mechanism of Eucalyptus oil for anti-inflammatory and anti-arthritic activity. *International Journal of Phytopharmacy*, 2, 81–85.
- Saravanan, S., Hairul Islam, V.I., Thirugnanasambantham, K., Chellappandian, M., Senthilkumar, B., Prameela, L. & Thomas, S. (2018). Anti-inflammatory and antiarthritic activity of type-A procyanidine polyphenols from bark of Cinnamomum zeylanicum in rats. *Food and Chemical Toxicology*, 115, 309–318.
- Suthar, M., Patel, S. & Goyal, R. (2014). Antiarthritic potential of Nyctanthes arbor-tristis flowers. *Ancient Science of Life*, 34, 157–161.