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Original Article

PHARMACOLOGICAL EVALUATION OF HYDROALCOHOL AND CHLOROFORM EXTRACTS OF NYCTANTHES ARBOUR-TRISTIS L. FOR ANTIOXIDANT, ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

PRABHJOT KAUR[1], PRIYANKA KASHYAP[1], NEETI KATOCH[1], SUMEET GUPTA[2], RANDHIR SINGH*

[a]Department of Pharmacology, Maharishi Markandeshwar College of pharmacy, Maharishi Markandeshwar University, Mullana, Ambala, India, [b]Department of Pharmacology, Faculty of Pharmacy, Maharishi Markandeshwar College of pharmacy, Maharishi Markandeshwar University, Mullana, Ambala, India.
Email: randhirsingh.dahiya@gmail.com

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ABSTRACT

Objective: The study was designed to evaluate the anti-oxidant, anti-inflammatory and analgesic activity of hydroalcohol (HA) and chloroform (CH) extracts of the whole plant of *Nyctanthes Arbour-tristis* L in laboratory animals.

Methods: HA and CH extracts of the whole plant were prepared by Soxhlet apparatus for 72 h and subjected to preliminary phytochemical screening and in-vitro and in-vivo anti-oxidant activity. The anti-inflammatory activity was evaluated by employing carrageenan induced paw edema in rats and analgesic activity was evaluated using acetic acid induced writhing test and tail flick method in mice.

Results: The preliminary phytochemical analysis of extracts indicated the presence of terpenoids, flavonoids, phenols, tannins, alkaloids, glycosides, saponins, fats and fixed oils, proteins and amino acids. HA and CH extracts were found to possess strong in-vitro and in-vivo antioxidant activity. Oral administration of 100, 200 and 400 mg/kg of HA and CH extracts significantly attenuated paw edema in rats. The percentage inhibition of glacial acetic acid induced writhing in HA and CH extracts was found to be 19.78%, 38.45%, 50.52% 47.22%, 63.06%, 72.55% at 100, 200 and 400 mg/kg dose respectively. Similar results were observed in the tail flick model indicating the analgesic activity.

Conclusion: The results showed that HA and CH extract of *Nyctanthes arbour-tristis* L. Produced significant antioxidant, anti-inflammatory and analgesic activity in rats and mice respectively.

Keywords: Carrageenan, Paw edema, Tail flick, Writhing.

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [1]. *Nyctanthes arbour-tristis* L. (Oleaceae) is one of the well known medicinal plants native to India, distributed in the sub-Himalayan regions and southward to Godavari. Different parts of the plant are known to possess activity against various diseases [2]. Various studies showed that the extract of Oleaceae species possesses effective antioxidant, anti-inflammatory and analgesic activity. Search for analgesic and anti-inflammatory secondary metabolites proved alkaloids, flavonoids, steroids and terpenoids as analgesic [3], whereas alkaloids, fatty acids, polyphenolics (flavonoids, lignans, phloroglucinols, quinines, phenylpropanoids, stilbenes and diarylheptanoids), steroids, terpenoids [4, 5], saponins and polysaccharides [5] behave as anti-inflammatory agents. So the present study was designed to investigate anti-oxidant, analgesic and anti-inflammatory activity of chloroform and hydroalcohol extracts of whole plant of *Nyctanthes arbour-tristis* L.

MATERIALS AND METHODS

Preparation of extract

The whole plant of *Nyctanthes arbour-tristis* L. (leaves, roots, seeds and flowers) were collected from Choudhary Devi Lal herbal garden in November and December, 2012 and authenticated by Dr. Sunita Garg, Head Raw Material Herberium and Museum Delhi, National Institute of Science Communication and Information Resources (specimen no: NISCAIR/RHMD/consult/2013/2322/102). The plant was dried in shade and ground to fine powder. Equal quantity of powder was mixed with chloroform and hydroalcohol (60:40, ethanol: water) and subjected to successive extraction with in the Soxhlet apparatus for 72 h. The extract was concentrated using the rotary evaporator under reduced pressure and stored under refrigeration.

Drugs and chemicals

All the chemicals and reagents used were of analytical grade. Chloroform and ethanol were used for extraction procedure. The in-vitro antioxidant assays were performed using Gallic acid and ascorbic acid as standard solution and other reagents are Folin-Ciocalteu reagent, sodium carbonate, potassium ferricyanide, trichloroacetic acid, ascorbic acid hydrogen peroxide, 2,2-diphenyl-1-picrylhydrazyl(DPPH), sodium dodecyl sulphate, thiobarbituric acid, acetic acid. 1, 1, 3, 3-tetra methoxy propane (standard solution), DTNB [5, 5`-dithiobis (2- nitrobenzoic acid)], disodium hydrogen phosphate and glutathione was used in in-vivo antioxidant assay. Diclofenac sodium, aspirin (obtained as gift samples from Hindustan Pharmaceuticals) and Pentazocine (obtained as gift sample from Ind-swift) were used as standard drugs for anti-inflammatory and analgesic activity. Others reagents are acetic acid (Qualikems Fine Chemicals Pvt. Ltd.) and carrageenan (Sigma-Aldrich).

Phytochemical analysis

The extracts were subjected to preliminary phytochemical analysis to identify the presence of chemical constituents such as terpenoids, flavonoids, phenols, tannins, alkaloids, glycosides, saponins, fats and fixed oils, proteins and amino acids [6, 7, 8].

In-vitro antioxidant activity

Estimation of total phenolic compounds

Total soluble phenolics in the extracts were determined with Folin-Ciocalteu reagent according to the method using gallic acid as a standard phenolic compound [9]. The absorbance of the blue colour was observed at 760 nm. The concentration of total phenols was expressed as mg/g of dry extract [10]. All determinations were performed in triplicate. Total content of phenolic compounds in plant extract was determined as µg of gallic acid equivalents (GAE).

Reducing power assay

The reducing power of the extracts was determined using ascorbic acid as a standard the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated the increased reducing power [11].

Reducing power ability (%) = [(Absorbance of Control - Absorbance of test Sample) / (Absorbance of Control)] x 100

Hydrogen peroxide-scavenging activity

The Hydrogen peroxide-scavenging activity of extracts was determined by the method of Ruch et al., (1989). The extract (20-320µl/ml) was dissolved in 3.4 mL of 0.1M phosphate buffer (pH 7.4) and mixed with 600 µL of 43 mM solution of hydrogen peroxide. The absorbance value (at 230 nm) of the reaction mixture was recorded at 10 min intervals between zero and 40 min. for each concentration, separate blank sample was used for background subtraction. The percentage of hydrogen peroxide scavenging effect is calculated using following formula.

Percentage H_2O_2 scavenging effect = $[A_0 - A_1 / A_0]$ X 100

Where A_0 absorbance of blank, A_1 absorbance of standard or extract.

Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical-scavenging activity

The free radical-scavenging activity of the NA extracts was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH [13]. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in water at different concentrations (10 - 320 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical-scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

% inhibition = $(A_0 - A_t) / A_0$ x 100;

Where A_0 was the absorbance of the control (blank, without extract) and A_t was the absorbance in the presence of the extract. All the tests were performed in triplicate and the graph was plotted with the mean values.

Experimental animals

Wistar rats (170-220 g) and Swiss albino mice (22-26 g) were used in the study. The animals were obtained from the animal house of M. M. College of Pharmacy, Mullana, Ambala. All the animals were kept in plastic cages with stainless steel coverlids and rice straw was used as bedding material. The animals were facilitated with environmental conditions of photoperiod (12:12 h dark: light cycle) and temperature (25 ± 2°C). Animals were kept on commercial animal diet and water *ad libitum*. The study protocol was duly approved by Institutional Animal Ethics Committee (I. A. E. C).

Dose preparation

Different doses 100mg/kg, 200mg/kg and 400mg/kg of both extracts (HA and CH) were prepared by triturating with 0.5% CMC.

***In-vivo* antioxidant assay**

Estimation of lipid peroxidation

The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation was performed according to method of Ohkawa et al., (1979). 0.2 mL of supernatant of homogenate was pipetted out in a test tube, followed by addition of 0.2 mL of 8.1% sodium dodecyl sulphate, 1.5 mL of 30% acetic acid (pH 3.5), 1.5 mL of 0.8% of thiobarbituric acid and the volume was made up to 4 mL with distilled water. The test tubes were incubated for 1 h at 95^{0}C, then cooled and added 1 mL of distilled water followed by addition of 5 mL of n-butanol-pyridine mixture (15:1 v/v). The tubes were then centrifuged at 4000 g for 10 minutes. The absorbance of developed pink colour was measured spectrophotometrically at 532 nm. A standard calibration curve was prepared using 1-10 nM of 1, 1, 3, 3-tetra Methoxy propane. The TBARS values were expressed as nanomoles per mg of protein.

Estimation of reduced glutathione

The reduced glutathione (GSH) content in tissue was estimated using method of Beutler et al., (1963). The supernatant of homogenate was mixed with trichloroacetic acid (10% w/v) in 1:1 ratio. The tubes were centrifuged at 1000 g for 10 min at 40^{0}C. The supernatant obtained (0.5 mL) was mixed with 2 mL of 0.3 M disodium hydrogen phosphate.

Then 0.25 mL of 0.001 M freshly prepared DTNB [5, 5`-dithiobis (2-nitrobenzoic acid) dissolved in 1% w/v citric acid] was added and absorbance was noted spectrophotometrically at 412 nm. A standard curve was plotted using 5-50 µM of reduced form of glutathione and results were expressed as micromoles of reduced glutathione per mg of protein.

Anti-inflammatory activity

Carrageenan induced paw edema

Animals were divided into nine groups and each group consists of six animals and kept on fasting overnight. The different doses of extracts were administered to animals one hour before the administration of carrageenan. After 1 hour carrageenan was injected into the sub-planter region of the hind paw of rats and paw volume was measured at 0, 1, 2 and 3rd hr. by using Plethysmometer (Medicaid Systems, Chandigarh), [16].

Group I: Normal control.

Group II: Inflammation control group received vehicle only (0.5% Carboxymethylcellulose).

Group III: Standard group treated with Diclofenac sodium (100 mg/kg *p. o.*).

Group IV: Inflammation + (HA 100 mg/kg *p. o.*).

Group V: Inflammation + (HA 200 mg/kg *p. o.*).

Group VI: Inflammation + (HA 400 mg/kg *p. o.*).

Group VII: Inflammation + (CH 100 mg/kg *p. o.*).

Group VIII: Inflammation + (CH 200 mg/kg *p. o.*).

Group IX: Inflammation + (CH 400 mg/kg *p. o.*).

Analgesic activity

Tail flick method

Animals were divided into eight groups and each group consists of six animals. Firstly, basal reaction time to radiant heat was measured by placing the tip (last 1-2 cm) of the tail on radiant heat source and cut off period of 6 seconds was observed to prevent damage to tail. Different doses of HA and CH extracts were administered to mice and subjected to same procedure and reaction time was noted at 0, 15, 30 and 60 min [17]. The average values of reaction time after each time interval was calculated and statistically compared between treated groups and control group.

Group I: Normal control.

Group II: Standard group treated with Pentazocine sodium (30 mg/kg *p. o.*).

Group III: Radiant heat + (HA 100 mg/kg *p. o.*).

Group IV: Radiant heat + (HA 200 mg/kg *p. o.*).

Group V: Radiant heat + (HA 400 mg/kg *p. o.*).

Group VI: Radiant heat + (CH 100 mg/kg *p. o.*).

Group VII: Radiant heat + (CH 200 mg/kg *p. o.*).

Group VIII: Radiant heat + (CH 400 mg/kg *p. o.*).

Acetic acid induced writhing test

Animals were divided into eight groups and each treatment group consists of six animals. Different doses of HA and CH extracts and aspirin were administered one hour before the administration of 0.6% acetic acid (10 ml/kg b. w., *i. p.*) in mice. The number of abdominal contractions was counted for each animal for a period of 10 minutes after 10 minutes of acetic acid injection [18].

Group I: Normal control.

Group II: Standard group treated with Aspirin (100 mg/kg *p. o.*).

Group III: Writhing + hydroalcohol extract (HA) (100 mg/kg *p. o.*).

Group IV: Writhing + hydroalcohol extract (HA) (200 mg/kg *p. o.*).

Group V: Writhing + hydroalcohol extract (HA) (400 mg/kg *p. o.*).

Group VI: Writhing + chloroform extract (CH) (100 mg/kg *p. o.*).

Group VII: Writhing + chloroform extract (CH) (200 mg/kg *p. o.*).

Group VIII: Writhing + chloroform extract (CH) (400 mg/kg *p. o.*).

Statistical analysis

The results were expressed as mean ± S. E. M. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's test. The *p*-value < 0.05 was considered to be statistically significant.

RESULTS

Phytochemical Analysis

Phytochemical screening showed presence of flavonoids, terpenoids and fixed oils and fats in chloroform extract whereas tannins, phenols, triterpenes, diterpenes, steroids and alkaloids were found in hydroalcohol extract.

***In vitro* antioxidant activity**

Estimation of Total phenolic compounds in HA and CH extracts of *Nyctanthes arbour-tristis* L.

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups and may contribute directly to antioxidative action. The amount of total phenolic compounds was found to be 20.25 mg/ml and 30.36 mg/ml Gallic acid equivalent (GAE) respectively in HA and CH extracts of *Nyctanthes arbour-tristis* L. (Figure 1).

Reducing Power of HA and CH extracts of *Nyctanthes arbour-tristis* L.

Reducing power is the measure of reductive ability of antioxidant and it was evaluated by the transformation of Fe (III) to Fe (II) in the presence of the test extracts. The concentration of ferrous ions formed can be determined by measuring the absorbance of coloured solutions at 700 nm. Higher absorbance of the given solutions indicates higher reducing power.

The reducing power of HA and CH extracts of *Nyctanthes arbour-tristis* L. was assessed as a function of their concentration. The reducing power of hydroalcohol and chloroform extracts of NA increased with increase in concentration of extract and an excellent reducing power was obtained at 320 µg/ml. EC_{50} (effective concentration at which the absorbance is 0.5) was calculated from the calibration curve and was found to be 40 µg/ml for hydroalcohol extract, 20 µg/ml for chloroform extract and 10 µg/ml for ascorbic acid (standard) (Figure 2).

Effect of HA and CH extracts of *Nyctanthes arbour-tristis* L. on hydrogen peroxide-scavenging activity

Hydrogen peroxide produces hydroxyl radicals in cells. Scavenging of these radicals by the extract is used as a test for antioxidant activity. The reduction of these radicals is seen by the decreased absorbance at 230 nm with increasing concentration of the test compound. In hydrogen peroxide scavenging assay, IC_{50} value of hydroalcohol and chloroform extracts of NA was 132 µg/ml and 93 µg/ml respectively while that of ascorbic acid was found to be 45 µg/ml (Figure 3).

Effect of HA and CH extracts of *Nyctanthes arbour-tristis* L. on DPPH free radical scavenging activity

The free radical scavenging activity of the HA extract of *Nyctanthes arbour-tristis* L. was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (2, 2-diphenyl-2-picryl hydrazyl).

The percentage of scavenging effect on the DPPH radical increases with increase in the concentrations of extract from 10 – 320 µg/ml Both HA and CH extracts decolorized DPPH due to its hydrogen donating ability. The percentage of inhibition in hydroalcohol extract varies from 34.91% (at 10 µg/ml) to 70.13% (at 320 µg/ml) and in chloroform extract, it varies from 39.01% (at 10 µg/ml) to 70.24% (at 320 µg/ml) while ascorbic acid shows 39.51% – 84.28% inhibition. In DPPH free radical scavenging assay, IC_{50} value of hydroalcohol and chloroform extract of NA was found 27 µg/ml and 27.5 µg/ml respectively whereas IC_{50} value of ascorbic acid was found to be 18 µg/ml (Figure 4).

***In-vivo* antioxidant assay**

Effect of HA and CH extracts of *Nyctanthes arbour-tristis* L. on lipid peroxidation

The formation of lipid peroxidation products was evaluated as TBARS. 2-thiobarbituric acid reactive substances (TBARS) are naturally present in biological specimens and include lipid hydroperoxides and aldehydes which increase in concentration as a response to oxidative stress.

In the present study decreased lipid peroxidation in NA treated groups indicates attenuation in oxidative stress which can be attributed to free radical scavenging activity of phytoconstituents present in *Nyctanthes arbour-tristis* L. The percentage inhibition in HA group was found to be 48.58% (100 mg/kg), 74.35% (200 mg/kg) and 79.26% (400 mg/kg) when compared to arthritis control group whereas, in CH group, the percentage inhibition of lipid peroxidation was found to be 79.26% (100 mg/kg), 84.17% (200 mg/kg) and 87.22% (400 mg/kg) (Figure 5).

Effect of HA and CH extracts of *Nyctanthes arbour-tristis* L. on reduced glutathione

Reduced glutathione is a cellular antioxidant that plays an important role in controlling oxidative stress due to H_2O_2 by getting itself oxidized to glutathione (GSSG). Increase in reduced glutathione level reflects decreased oxidative stress. The level of reduced glutathione was found to be increased in HA and CH extracts treated groups as compared to arthritis control group which indicate decrease in oxidative stress.

The percentage increase in reduced glutathione level in hydroalcohol group was found to be 52.09 (100 mg/kg), 66.72 (200 mg/kg), 76.93 (400 mg/kg) whereas, in chloroform extract group percentage increase was found to be 74.64 (100 mg/kg), 83.96 (200 mg/kg), 88.60 (400 mg/kg) (Figure 6).

Anti-inflammatory activity

Effect of HA and CH extracts of *Nyctanthes arbour-tristis* L. on paw edema

The CH extract did not produce significant attenuation in edema at 100 mg/kg whereas 200 and 400 mg/kg produced significant attenuation in 1st hr. 100, 200 and 400 mg/kg produced significant attenuation at 2nd and 3rd hr. as compared to inflammation control group and percentage attenuation was found to be 37.96%, 29.62% and 37.96% respectively. Treatment with HA extract at 100, 200 and 400 mg/kg dose produced significant attenuation in anti-inflammatory activity at as compared to inflammation control group and percentage inhibition of was found to be was found to be 50%, 49.07% and 55.55% respectively. (Figure 7, 8)

Analgesic activity

Effect of HA and CH extracts of *Nyctanthes arbour-tristis* L. on tail flick method

In tail flick model, HA and CH extracts produced significant analgesic activity as compared to control group (Figure 9, 10). The percentage inhibition with HA and CH extracts at 100, 200 and 400 mg/kg was found to be 14.56%, 36.55%, 56.02% and 24.78%, 53.36% and 83.33% respectively as compared to control group. Treatment with Pentazocine 30 mg/kg produced significant analgesic activity and the percentage inhibition of tail flick response was 88.37%.

Effect of HA and CH extracts of *Nyctanthes arbour-tristis* L. on acetic acid induced writhing test

Oral administration of HA and CH extracts 30 minutes before stimulus significantly reduced the acetic acid induced writhing (Table 1). In acetic acid induced writhing test, the percentage inhibition of writhing in CH extract was 47.22%, 63.06% and 72.55% at 100, 200 and 400 mg/kg dose respectively. In HA extract, percentage inhibition of writhing was 19.78, 38.45 and 50.52% at 100, 200 and 400 mg/kg dose. Aspirin at a dose of 100 mg/kg produced significant decrease in writhing response (72.55%).

Table 1: Effect of HA and CH extracts of *Nyctanthes arbour-tristis* L. on acetic acid induced writhing test in mice.

Groups	Analgesic effect (%age inhibition) (Mean ± SEM)
Control	15.166 ± 0.929
Aspirin 100mg/kg	4.166 ± 0.437 (72.55%)
HA 100	12.166 ± 1.069 (19.78%)
HA 200	9.333 ± 0.305 (38.45%)
HA 400	7.5 ± 0.699 (50.52%)
CH 100	8 ± 0.529 (47.22%)
CH 200	5.666 ± 0.722 (63.06%)
CH 400	4.166 ± 0.437 (72.55%)

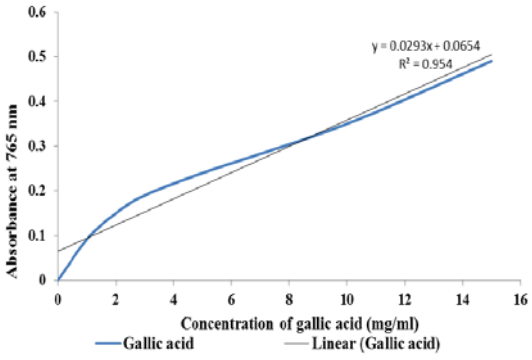

Fig. 1: Estimation of total phenol content in hydroalcohol and chloroform extracts of *Nyctanthes arbour-tristis* L.

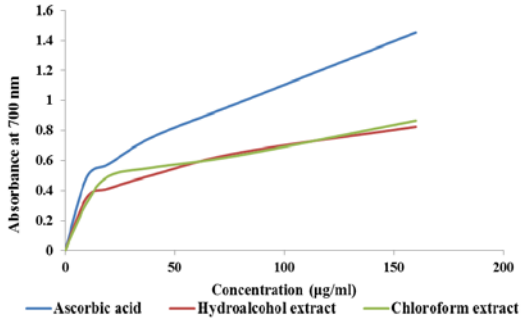

Fig. 2: Reducing power assay of hydroalcohol and chloroform extracts of *Nyctanthes arbour-tristis* L.

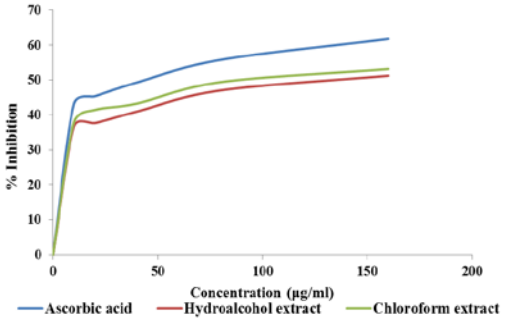

Fig. 3: Curve showing % scavenging of hydrogen peroxide in hydroalcohol and chloroform extracts of *Nyctanthes arbour-tristis* L.

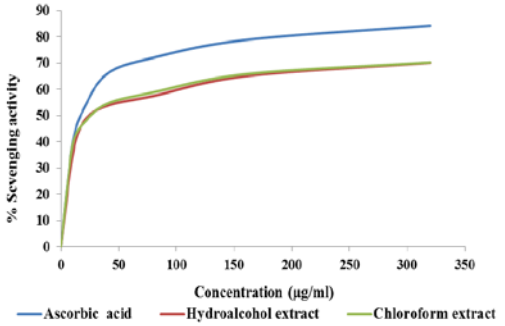

Fig. 4: Calibration curve showing % scavenging of DPPH by hydroalcohol and chloroform extracts of *Nyctanthes arbour-tristis* L.

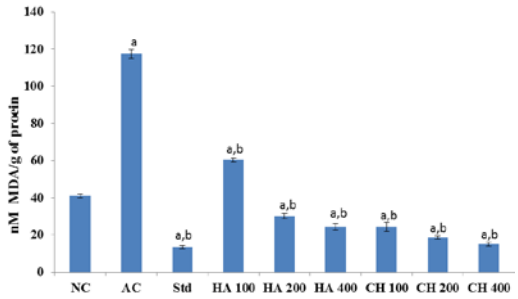

Fig. 5: Antioxidant potential of *Nyctanthes arbour-tristis* L. in TBARS assay compared to MDA in blood serum. Values are expressed as mean ± SEM a: vs control; b: vs Inflammation control.

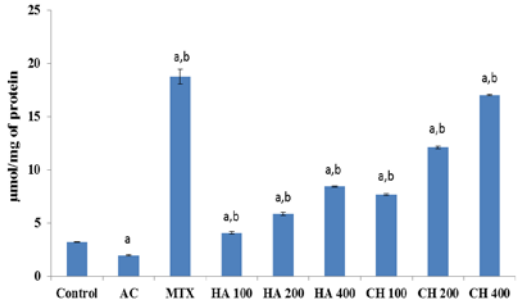

Fig. 6: Effect of hydroalcohol and chloroform extracts of *Nyctanthes arbour-tristis* L. on reduced glutathione. Values are expressed as mean ± SEM a: vs control; b: vs Inflammation control.

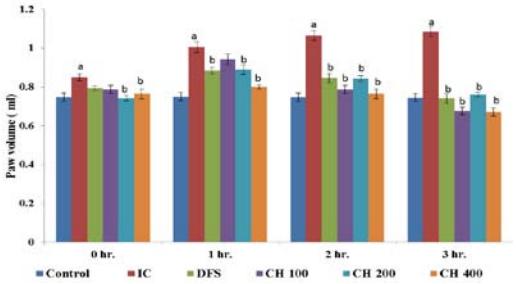

Fig. 7: Effect of chloroform extract of *Nyctanthes arbour-tristis* L. on carrageenan induced paw edema in rats. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant. a: vs control; b: vs Inflammation control.

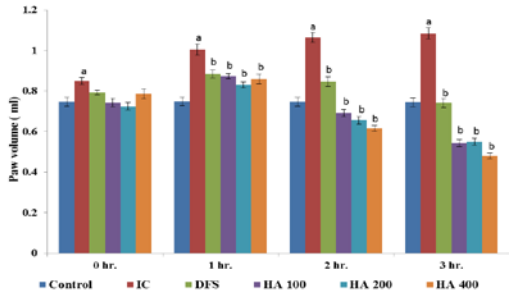

Fig. 8: Effect of hydroalcohol extract of *Nyctanthes arbour-tristis* L. on carrageenan induced paw edema in rats. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant. a: vs control; b: vs Inflammation control.

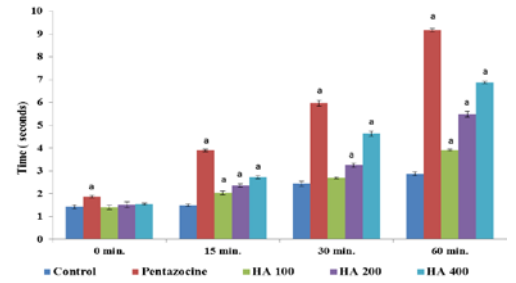

Fig. 9: Effect of hydroalcohol extract of *Nyctanthes arbour-tristis* L. in Tail flick method. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant. a: vs control, b: vs HA 100 mg/kg.

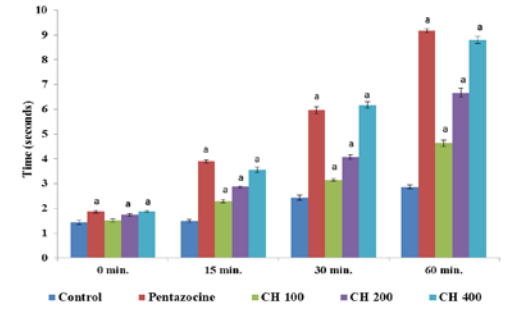

Fig. 10: Effect of Chloroform extract of *Nyctanthes arbour-tristis* L. on Tail flick method. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant. a: vs control, b: CH 100 mg/kg.

DISCUSSION

Carrageenan induced rat paw edema is a suitable model to study inflammation and inflammatory pain [19], which act by inhibiting the mediators of acute inflammation [20]. Carrageenan-induced edema is a biphasic response in which the involvement of the cyclo-oxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established [21]. The early phase of the inflammation is due to the release of histamine, serotonin and similar substances; and the later phase is associated with the activation of kinin-like substances, i. e., prostaglandins, proteases and lysosome [22, 23]. The role of PGE_2 in carrageenan induced edema and pain associated with inflammatory reaction is well documented. According to results, different doses of hydroalcohol and chloroform extracts of *Nyctanthes arbour-tristis* L. significantly attenuate oedema in both phases, but maximum percentage of inhibition was produced in later phase. This shows that *Nyctanthes arbour-tristis* L. is more effective against prostaglandins. Antioxidants are documented in several publications to mitigate the inflammatory processes and some of the antioxidant activity of plants have been ascribed to the phenolic compounds present in the plant particularly flavonoids [24, 25, 26, 27]. Flavanoids regulate cellular activities of inflammation related cells: mast cells, macrophages, neutrophils and lymphocytes. Ha et al. (2006) showed that triterpenes can inhibit synthesis of several cytokines, prostaglandin E2 and nitric oxide in LPS-stimulated macrophages.

Biochemical mediators released during disease liberate prostaglandins that promote hyperalgesia. Different doses of the HA and CH extracts of *Nyctanthes arbour-tristis* L. produced significant analgesic activity. The latency in reaction time continued upto 60 mins after oral administration of HA and CH extracts and effect of 400 mg/kg dose of chloroform and hydroalcohol extracts was comparable to that of aspirin. The peripheral analgesic effect of the test drug exhibited significant inhibition in acetic acid induced writhing test. The significant analgesic effect was attributed to the presence of flavonoid compounds which inhibited the synthesis, release or receptor responses in prostaglandin mediated effects. The presence of alkaloids, phenolic compounds and tannins has been associated with analgesic activity of *Nyctanthes arbour-tristis* L. [29, 30].

Flavonoids have been shown to possess various biological properties related to antioxidant, analgesic and anti-inflammatory mechanisms by targeting reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception [31, 32, 33].

CONCLUSION

The HA and CH extracts of *Nyctanthes arbour-tristis* L. significantly attenuated the in-vitro and in-vivo oxidative stress. The extracts also produced significant anti-inflammatory activity as well as analgesic activity.

So it can be concluded that **HA and CH extracts of** *Nyctanthes arbour-tristis* L possess anti-oxidant, analgesic and anti-inflammatory activity.

ABBREVIATIONS

NC – Normal control, AC – Arthritis control, Std - Standard, HA – Hydroalcohol extract, MTX – Methotrexate, IC – Inflammation control, DFS – Diclofenac sodium, CH – Chloroform extract

CONFLICT OF INTERESTS

Declared None

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