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Short communication

Anti - Inflammatory Activity of Flowers of *Nymphaea alba* by *HRBC* Membrane Stabilization Method

O.Sai Koushik, V.Himaja, P.Srinivasa Babu, Ramadoss Karthikeyan*

Department of Pharmacognosy, Vignan Pharmacy College, Vadlamudi 522213. AP, India
*Corresponding author Email:- rkcognosy@gmail.com

To evaluate the anti inflammatory activity from the flowers of *Nymphaea alba*. Human red blood cell membrane stabilization (HRBC) method has been used as a method in estimating the anti inflammatory property of *Nymphaea alba* flowers. The in vitro method showed significant anti inflammatory property of different concentrations tested. The hydroalcoholic extract at a concentration of 500µg/ml showed significant activity when compared with the standard control ibuprofen.

Key words: *Nymphaea alba* flowers, Hydro alcoholic extract, HRBC membrane stabilization method

Inflammation is a reaction of living tissues towards injury and it comprises systemic and local responses. *Nymphaea alba* is a member of the family Nymphaeaceae. Traditionally Nymphaea alba commonly called as "European White Water lily, is an aquatic flowering plant of the family Nymphaeaceae, The family Nymphaeaceae consists of several important medicinal plants with wide range of pharmacological, biological activities and interesting phytochemical constituents (Madhu-sudhanan et al., 2011; Bose et al., 2012). Hypo tonicity induced HRBC membrane lysis was taken as a measure in estimating the anti inflammatory property of various extracts of *Nymphaea alba* (Karthikeyan et al., 2013). Thus human blood cell membrane stabilization (HRBC method) has been used as a method in estimating the anti inflammatory property. The family Nymphaeaceae consists of several important medicinal plants with wide range of pharmacological, biological activities and interesting phytochemical constituents. The main action of anti inflammatory agents is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid to prostaglandins. Since human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of Hypotonicity induced HRBC membrane lysis was taken as a measure in estimating the anti inflammatory property of various extracts of *Nymphaea alba*. Thus human blood cell membrane stabilization (HRBC method) has been used as a method in estimating the anti inflammatory property. The present study aimed to authenticate the traditional anti inflammatory activity of this species by in vitro anti inflammatory screening.

Materials and methods

Preparation of extracts

The plant material was collected from the plant *Nymphaea alba*. Which are collected during the month of December at Sekuru, Guntur (Dist) of Andhra Pradesh. Then it

was authenticated by Dr SM. Khasim, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna nagar, Guntur. The shade dried flowers were powdered and extracted with soxhlet apparatus using hydroalcoholic (yield 5.8%). The samples were prepared by suspending the residues in hot water and used for anti - inflammatory study.

Chemicals and instruments

All chemicals used in the study were of analytical grade. Dextrose, Sodium citrate, Citric acid, Sodium chloride, Sodium hydroxide and Dihydrogen phosphate was purchased from SD fine chemicals, Mumbai. Reference standard Ibuprofen was obtained from Cipla Ltd, Bangalore. Systronics 220 (Double beam) spectrophotometer was used for the estimation of anti inflammatory activity.

Preliminary Phytochemical Screening

Preliminary Phytochemical screening was performed by using standard protocol (Kokate et al., 2005).

In vitro anti – inflammatory activity

The anti inflammatory activity of flower extract of *Nymphaea alba* was determined by HRBC membrane stabilization method. Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of (2% dextrose, 0.8% sodium citrate, 0.05% citric acid & 0.42% sodium chloride in water). The blood was centrifuged at 300 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) & 10% v/v suspension was made with isosaline.

The assay mixture contained the drug (concentration as mentioned in Table 1). 1 ml phosphate buffer (0.15M, pH7.4), 2ml of hyposaline (0.36%) % 0.5 ml of HRBC suspension. Diclofenac was used as the reference drug. Instead of hyposaline, 2ml of distilled water was used as control. All the assay mixtures were collected at 37^0c for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated using colorimeter at 560 nm. The percentage heamolysis was calculated by assuming the heamolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the following formula (Saleem et al., 2011; Goodman et al., 2006).

$$\% \text{ protection} = 100 - \frac{\text{O.D of drug treated sample}}{\text{O.D of control}} \times 100$$

Results and discussion

Preliminary phytochemical screening

In the present study the preliminary phytochemical screening was studied in broad sense to explore its chemical nature. The results were tabulated in Table 1.

Table 1. Preliminary phytochemical screening

S.No	Phyto constituents in hydroalcoholic extract	Presence /absence
1	Protein	++
2	Carbohydrate	++
3	Reducing sugar	+++
4	Glycosides	+++
5	Phenol	+++
6	Tannin	+++
7	Flavones	+++
8	Saponin	++
9	Steroid	+
10	Alkaloid	++
11	Anthraquinone	-
12	Quinone	-

In vitro anti inflammatory activity

Nymphaea alba hydro alcoholic extracts at different concentrations (200,500 µg/ml) showed significant stabilization towards HRBC membrane. The percentage protection of aqueous extract at concentration 500µg/ml was higher than that of concentrations. However the percentage protection was found to be increased at higher concentrations.

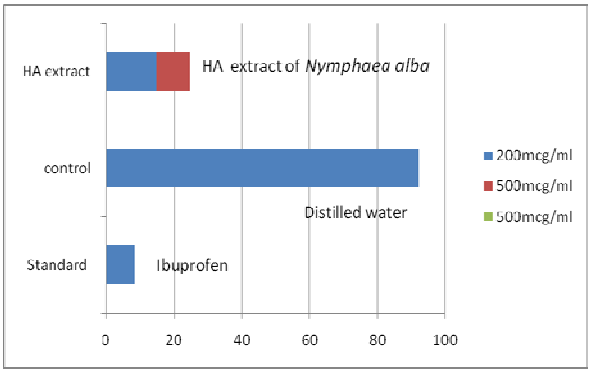

Figure 1. Bar diagram represents the protection of HRBC membrane from haemolysis

Discussion

In the present study the preliminary phytochemical screening was studied in broad sense to explore its chemical nature, it reveals the presence of considerable amount of alkaloid, steroid, phenolic substances, tannins and vitamin C (Ascorbic acid), moderately saponins and carbohydrates, trace amount of glycoside and resins were explored from the phytochemical screening. Anti inflammatory activity of its various extracts were performed to explore its bioefficiency. The study was took HRBC membrane stabilization method for screening of activity. The results revealed the hydroalcoholic extract of *Nymphaea alba* showed significant activity (membrane protection). The anti inflammatory activity and formation of collagen as well as prevention of release of inflammatory mediators is due to steroid and tannins content present in medicinal plants which has proved in earlier work (Thippeswamy et al., 2011). Hence, in this work the hydro alcoholic extract of *Nymphaea alba* has rich of steroid and tannins. Here by the activity may be attributed with the presence of steroids and tannins. The in vitro method showed significant anti inflammatory property of different concentrations tested. Further this work is suggesting to isolate the responsible phyto molecules and to correlate the mechanism of action.

Conclusion

The hydroalcoholic extract at a concentration of 500µg/ml showed significant activity when compared with the standard control ibuprofen.

References:

Anindya B , Moumita S , Sarbani DR. 2012 .In vivo evaluation of anti-diarrheal activity of the rhizome of *Nymphaea alba* (Nymphaeaceae). Orient Pharm Exp Med. 12:129–134.

Goodman and Gilman's Pharmacological Basis of therapeutics, Bruton LL (Ed).11th Ed McGrew Hill; USA. 1102-1104.

Karthikeyan R , Nagaharika Y, Kalyani V, Rasheed SK. 2013. Anti-inflammatory activity of leaves of *Jatropha gossypifolia* L. by hrbc membrane stabilization method. Journal of Acute Disease. 156-158.

Kokate CK.2005. Practical Pharmacognosy . 4th Ed. Vallabh prakashan. Delhi .107-111.

Madhusudhanan N, Lakshmi T, Gowtham kumar S, Ramakrishanan, Venu gopala rao konda, Anitha roy , Geetha R.V . 2011. Invitro antioxidant and free radical scavenging activity of aqueous and ethanolic flower extract of *Nymphaea alba*. Int. J. Drug Dev. Res. 3 (3): 252-258.

Saleem TK, Azeem AK, Dilip C, Sankar C, Prasanth NV, Duraisami R. 2011.Anti-inflammatory activity of the leaf extracts of *Gendarussa vulgaris* Nees. Asian Pac. J Trop. Biomed. 147-149.

Thippeswamy BS, Mishra B, Veerapur VP, Gupta G. 2011. Anxiolytic activity of *Nymphaea alba* Linn. in mice as experimental models of anxiety. Indian J. Pharmacol. 43:50-5.