



International Journal of Medical Research & Health Sciences

www.ijmrhs.com Volume 2 Issue 3 July - Sep Coden: IJMRHS Copyright ©2013 ISSN: 2319-5886

Received: 7th May 2013

Revised: 5th Jun 2013

Accepted: 8th Jun 2013

Research article

ANTI INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF NYMPHAEA ALBA FLOWER IN SWISS ALBINO MICE

*Jacob Jesurun RS¹, Senthilkumaran Jagadeesh², Somasundaram Ganesan³, Venugopala Rao K⁴, Madhavi Eerike⁴

¹Department of Pharmacology, Shri Sathya Sai Medical College and Research Institute, Chennai, Tamilnadu, India

²Department of Pharmacology, Sree Balaji Medical College and Hospital, Chennai, Tamilnadu

³Department of Pharmacology, Mahatma Gandhi Medical College and Research Institute, Pondicherry.

⁴Department of Pharmacology, Chettinad Hospital and Research Institute, Chennai, Tamilnadu

*Corresponding author email: drsrsjj@gmail.com

ABSTRACT

Inflammation plays an important role in various diseases with high prevalence within populations such as rheumatoid arthritis, atherosclerosis and asthma. The aim of the present context to investigate the anti inflammatory activity of extract of Nymphaea Alba flower (NAF) in Swiss Albino mice. The anti inflammatory activity was test using acute inflammatory models like acetic acid-induced vascular permeability chronic models like; cotton-pellet induced granuloma. Oral administration of ethanolic extract of Nymphaea Alba flower at the doses of 100mg/kg and 200mg/kg of body weight in mice. Diclofenac sodium used as standard drug Exhibited dose dependent and significant anti-inflammatory activity in acute (acetic acid-induced vascular permeability, $p < 0.001$) and chronic (cotton pellet granuloma < 0.001). But at the dose of 200mg/kg of extract shown high significant than 100mg/kg. Hence, the present investigation established ethanolic extract of Nymphaea Alba flower has anti-inflammatory activity.

Keywords: Nymphaea Alba Flower, Ethanolic extract, Anti-inflammatory, Diclofenac sodium.

INTRODUCTION

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, and thermal and mechanical injuries¹. Most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces

prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema, and pain. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required². Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicine used for the

treatment of inflammation-related diseases like arthritis, asthma, and cardiovascular disease³. Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions⁴.

However, the long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes⁵⁻⁷. Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates^{8,9}. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs.

The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs having been used in the treatment of diseases and for revitalizing body system in almost all ancient civilization. Ayurveda, the Science of Life, has provided a rationale basis for treatment of various ailments. Pain, inflammation and fever are very common complications in human beings.

Nymphaea alba, also known as the European White Waterlily, White Lotus, or Nenuphar, is an aquatic flowering plant of the family Nymphaeaceae. It grows in water from 30-150 centimeters deep and likes large ponds and lakes. The leaves may be up to thirty centimeters in diameter and they take up a spread of 150 centimeters per plant. The flowers are white and they have many small stamens inside. The root of the plant was used by monks and nuns for hundreds of years as an anaphrodisiac, being crushed and mixed with wine. It is rich in tannic acid, gallic acid, alkaloids, sterols, flavonoids,

glycosides, hydrolyzable tannins and high-molecular-weight polyphenolic compounds¹⁰

MATERIALS & METHODS

Experimental Animals: Swiss albino mice (20 to 25 g) were obtained from King Institute, Chennai, India. The animals were housed in standard environmental conditions (12 h light/12 h dark; 22±2°C) for one week prior to the experiments to acclimatize to the laboratory conditions. They were allowed free access to tap water and pellet rodent diet. The animal care and experimental protocols were in accordance to the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Ethical approval was obtained from Institutional Animal Ethics Committee Chettinad hospital and research institute, chennai.

Preparation of plant extract: *Nymphaea alba* flower (NAF) powder purchased from Mother Herbs (P) Ltd Delhi. A fine dried powder (25 mesh) of sample (*Nymphaea alba* flower) was extracted using 50 ml of 70% ethanol at 75 °C for 2.5 h by reflx. The extracts were filtered through Whatman No.4 filter paper under reduced pressure, frozen and then lyophilized (Ly-8-FMULE, Snijders). All the samples were redissolved in 70% ethanol at a concentration of 5.0 mg/ml .

Acute Toxicity Studies : The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose.¹¹

Acetic acid-induced vascular permeability: The Animals were divided in four groups each group having six animals. Group 1: Control (Distilled Water vehicle 1% (w/v) 10ml/1kg, (p o), Group 2: Treated with NAF 100 mg/ kg (p o), Group 3: Treated with extract of 200mg/ kg (p o), Group 4 : Standard drug diclofenac (10

mg/kg) received by intra peritoneal. After 1h of administration of extract and diclofenac sodium, mice were injected with 0.25 ml of 0.6 % (v/v) solution of acetic acid intraperitoneally (ip). Immediately, 10 ml/kg of 10 % (w/v) Evans blue was injected intravenously via tail vein. After 30 min of Evan's blue injection, the animals were anaesthetized with ether anesthesia and sacrificed. The abdomen was cut open and exposed viscera. The animals were held by a flap of abdominal wall over a Petri dish. The peritoneal fluid (exudates) collected, filtered and made up the volume to 10 ml using normal saline solution and centrifuged at 3000 rpm for 15 min. The absorbance (A) of the supernatant was measured at 590 nm using spectrophotometer ¹².

Cotton pellet-induced granuloma: The mice were divided into four groups, each group consisting of six animals. After shaving of the fur, the animals were anaesthetized (80 mg/kg ketamine i.p) and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized cotton pellet (100 ± 1 mg) was inserted in the groin area. All the animals received diclofenac sodium (10 mg/kg i.p) as standard, vehicle (distil

water), and extract 100 and 200 mg of extract orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion¹⁰. On the 8th day, animals were anesthetized the cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37 C for 24 h and dried at 60 C to constant weight. The difference between the initial weight and the final weight of the cotton pellet gives the amount of granulation tissue formed. The percentage inhibition of granulation tissue formation was measured by the following method,

$$\% \text{ Inhibition} = (X - Y)/X * 100,$$

Where X = mean increase in cotton pellet weight of rats in the control group = mean increase in cotton pellet weight of mice in the drug treated group¹³.

Statistical analysis: The mean ± SEM values were calculated for each group. Significant difference between groups was determined using analysis of variance (ANOVA) followed by Dunnett's t test. P<0.05 was considered as significant.

RESULTS

Table. 1: Acetic acid-induced vascular permeability

Group	Treatment	Dose	OD at 590 nm	Inhibition (%)
I	Control (Distilled water)	10 ml/kg	0.1655±0.00205	-
II	NAF	100 (mg/kg)	0.13275±0.0023*	20.35
III	NAF	200 (mg/kg)	0.11075±0.0016*	33.54
IV	Diclofenac Sodium	10 (mg/kg)	0.09425±0.0014*	43.13

*p<0.05 as compared to vehicle treated group, NAF *Nymphaea alba* flower powder

Table. 2: Cotton pellet-induced granuloma

Group	Treatment	Dose	Granuloma dry wt	Inhibition (%)
I	Control(Distilled water)	10 (ml/kg)	67.6675±0.6013	-
II	NAF	100 (mg/kg)	60.8275±0.613*	10.1
III	NAF	200 (mg/kg)	39.3075±0.541*	41.5
IV	Diclofenac Sodium	10 (mg/kg)	28.455±0.6213*	57.3

*p<0.05 as compared to vehicle treated group. NAF *Nymphaea alba* flower powder

DISCUSSION

Acetic acid-induced vascular permeability:

Effect of ethanolic extract of *Nymphaea Alba* flower (100 and 200 mg/kg), Diclofenac.sod. (10 mg/kg) and control vehicle on acetic acid-induced increased vascular permeability in rat was studied. Results of the activity showed that extract at dose (100 and 200 mg/kg) significantly inhibited (Table no.1) the vascular permeability (20.35%, 33.54 and 43.13% respectively) when compared with vehicle control group (Table-1) Acetic acid induced vascular permeability indicates acute phase of inflammation where there is increased vascular permeability and migration of leukocytes in to the inflamed area occurs¹⁴. Decreased concentration of dye with respected to absorbance indicates reduction in permeability

Cotton pellet-induced granuloma : In cotton pellet induced granuloma formation study, regarded as an animal model for sub acute inflammation, there was a statistically significant ($p < 0.05$) reduction in granuloma formation at all doses in comparison to the control group. (Table no 2) The extract exhibited 10.1% and 41.5 % inhibition of granuloma formation at the doses 100 and 200mg/kg b.w respectively, whereas diclofenac sodium showed 57.30% when compared to control. The cotton pellet-induced granuloma is widely used to assess the transudative and proliferative components of chronic inflammation¹⁵. The weight of the wet cotton pellets correlates with transude material and the weight of dry pellet correlates with the amount of granulomatous tissue. It is well known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. This effect may be due to the cellular migration to injured sites and accumulation of collagen, an important mucopolysaccharide¹⁶. Decreasing granuloma tissue, prevention of occurring of the collagen fiber and suppression of mucopolysaccharids are

indicators of the ant proliferative effect by NSAIDs.

Nymphaea Alba all the parts of the plant have medicinal uses in traditional system of medicine. It is used as an aphrodisiac, anodyne, antiscrophulatic, astringent, cardiotoxic, demulcent, sedative and antiinflammatory. Further, it also produces calming and sedative effects upon the nervous system, and is useful in the treatment of insomnia, anxiety and similar disorders¹⁷⁻²⁰.

CONCLUSION

The present study reveals that ethanolic extract of *Nymphaea Alba* flower has anti-inflammatory activity in acetic acid-induced vascular permeability and cotton pellet granuloma model. In both model they exhibited anti-inflammatory effect in a dose Dependent manner which can be comparable with that of Diclofenac.sod

ACKNOWLEDGEMENT

Authors are very much thankful to Sree Balaji Medical College and Hospital Chennai and Mahatma Gandhi Medical College and Research Institute, Pondicherry for providing the facilities necessary to carry out the research work and Mother Herbs (P) Ltd Delhi for providing herbal powders.

REFERENCES

1. Oyedapo OA, Adewunmi CO, Iwalewa EO, Makanju VO. Analgesic, antioxidant and anti-inflammatory related activities of 21-hydroxy-2,41-dimethoxychalcone and 4-hydroxychalcone in mice. *Journal of Biological Sciences*, 2008;8(1):131–36
2. Anilkumar M. Ethnomedicinal plants as anti-inflammatory and analgesic agents in *Ethnomedicine: A Source of Complementary Therapeutics*, Research Signpost, India, 2010;267-91
3. Conforti F, Sosa S, Marrelli M. The protective ability of Mediterranean dietary

- plants against the oxidative damage: the role of radical oxygen species in inflammation and the polyphenol, flavonoid and sterol contents. *Food Chemistry*, 2009;112(3):587–94
4. IMS Health, IMS National Sales Perspectives TM, 2005
 5. Robert A. Antisecretory, antiulcer, cytoprotective and diarrheogenic properties of prostaglandins. *Advances in Prostaglandin and Thromboxane Research*.1976;2:507–20
 6. Peskar BM. On the synthesis of prostaglandins by human gastric mucosa and its modification by drugs. *Biochimica et Biophysica Acta*.1977;487(2):307–14, 1977
 7. Tapiero H, Nguyen Ba G, Couvreur P, Tew KD. Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies,” *Biomedicine and Pharmacotherapy*.2002;56(5): 215–22.
 8. Dharmasiri MG, Jayakody JRAC, Galhena G, Liyanage SSP, Ratnasooriya WD. Anti-inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*,” *Journal of Ethnopharmacology*. 2003;87(2):199–206
 9. Kumara N. Identification of strategies to improve research on medicinal plants used in Sri Lanka,” in *Proceedings of the WHO Symposium*, pp. 12–14, University of Ruhuna, Galle, Sri Lanka, 2001
 10. Eliana R, Ricardo T, Jose C, Galduroz F, Giuseppina N. Plants with possible anxiolytic and/or hypnotic effects indicated by three brazilian cultures - indians, afro-brazilians, and river- dwellers. *Studies in Natural Products Chemistry*. Vol. 35. Brazil: Elsevier; 2008. p. 549-95
 11. Handa SS, Sharma A. Hepatoprotective activity of andrographolide from *Andrographis paniculata* against carbontetrachloride. *Indian J Med Res*. 1990; 92:276-83
 12. Whittle BA. The use of changes in capillary permeability in determination of glutamic oxaloacetate and glutamic pyruvic transaminase. *Am J Clin Pathol*,1957;28: 96.
 13. Winter CA, Hisley EA, Nuss GW. Carageenan induced edema in hind paw of rats as an assay of anti-inflammatory drugs. *Proc Soc Exp Biol Med*. 1962;111:544–47
 14. Ojewole AOJ. Evaluation of anti-inflammatory property of *Sclerocarya birrea* (A.Rich) Hochst (Family: Anacardiaceae) stem bark extracts in rats. *J Ethnopharmacol*. 2003;85:217-220.
 15. Winter CA, Porter CC. Effect of alterations in the side chain upon anti-inflammatory and liver glycogen activities of hydrocortisone esters. *J. Am. Pharm. Assoc. Sci. Educ*.1957;46: 515-19
 16. Adnaik RS, Pai PT, Sapakal VD, Naikwade NS, Magdum CS. Anxiolytic activity of *Vitex Negundo* Linn. In experimental models of anxiety in mice. *Int J Green Pharm* 2009;3:243-47
 17. Robin D. *Nymphaea odorata*: White pond lily. *Medical Herbalism. Materia Medica pharm* 2001;11:231-33
 18. Vergeera LH, Vander VG. Phenolic content of daylight-exposed and shaded floating leaves of water lilies (*Nymphaeaceae*) in relation to infection by fungi. *Oecologia* 1997;112:481-84
 19. James AD. *Duke's hand book of medicinal plants of the bible*. USA: Taylor and Francis group.2008.p.302-5
 20. Naghma K, Sarwat S. Anticarcinogenic effect of *Nymphaea alba* against oxidative damage and hyperproliferative response and renal carcinogenesis in Wistar rats. *Mol Cell Biochem*.2005;271:1-11