

SCREENING OF ANTIPIRETTIC ACTIVITY OF AERIAL PARTS OF *NELUMBO NUCIFERA* GAERTN. IN YEAST INDUCED PYREXIA.**Deepak Gupta, Rajani G. P. *, Sowjanya K, Sahithi B***Department of Pharmacology, KLE University's College of Pharmacy,
Bangalore-560 010, Karnataka, India.****Corresponding Author:** Mobile: +919448856162, E-mail: bmruvce@yahoo.co.in**Summary**

The present study was aimed at evaluating the ethanolic and aqueous extracts of aerial parts of *Nelumbo nucifera* Gaertn. for antipyretic activity. The preliminary phytochemical screening of ethanolic and aqueous extracts of *Nelumbo nucifera* revealed the presence of flavonoids, saponins, alkaloids, aminoacids and proteins, carbohydrates, steroids and tannins. Antipyretic activity of ethanolic and aqueous extracts (200 and 400 mg/kg b.w.) of *Nelumbo nucifera* Gaertn. was evaluated by yeast induced pyrexia and basal rectal temperature was measured using telethermometer. Paracetamol (150 mg/kg) was used as a standard drug. Administration of both ethanolic and aqueous extracts of *Nelumbo nucifera* produced significant ($p < 0.001$) reduction of body temperature during yeast induced pyrexia model, which indicates that *Nelumbo nucifera* possess antipyretic activity. Antipyretic activity was found to be dose dependent. Significant antipyretic activities produced by ethanolic & aqueous extracts of *Nelumbo nucifera* may be a consequence of synergistic effect of the constituents present in the extracts namely, β sitosterol and flavonoids (kaempferol, quercetin etc). The results suggest that aerial parts of *Nelumbo nucifera* ethanolic and aqueous extracts (NNE and NNA) possess significant anti-pyretic activity.

Key Words: *Nelumbo nucifera* Gaertn.; yeast; pyrexia;**Introduction**

Fever is a complex physiologic response triggered by infections or aseptic stimuli. Elevation in body temperature occurs when the concentration of prostaglandin E₂ (PGE₂) increases within parts of the brain. Such an elevation contributes to a considerable alteration in the firing rate of neurons that control the thermoregulation process in the hypothalamus. It is now evident that most of the antipyretic drugs exert their action by inhibiting the enzymatic activity of cyclooxygenase and consequently reducing the levels of PGE₂ within the hypothalamic region. Recently, other mechanisms of action for antipyretic drugs have been presumed. These mechanisms have shed the light on the ability of antipyretic agents to reduce pro-inflammatory mediators, enhance anti-inflammatory signals at sites of injury or boost antipyretic messages within the brain. [1]

Nelumbo nucifera Gaertn. (Nymphaeaceae) also known as Indian lotus, sacred lotus, lotus. It is the national flower of India. It is a large aquatic herb with stout, creeping rhizome found throughout India. Almost all parts of the lotus plant are eaten as vegetable and also used in the indigenous system of medicine. [2]

The various parts of the plant *Nelumbo nucifera* Gaertn. are widely used in traditional / folklore medicine for the treatment of fever, cough, vomiting, blood complain, leprosy, biliousness, beneficial to the eye, diarrhoea, bleeding piles, inflammation, diuretic, cholera, as a cardio tonic. [3,4] It is also widely used in Ayurveda. Its root, seeds and rhizomes are extensively studied and reported to have many pharmacological actions. *Nelumbo nucifera* is reported to possess anti-bacterial [5], antidiarrhoeal [6], anti-psychotic [7], anti-diabetic [8], hepatoprotective [9], antipyretic (seeds) [10] and anti-oxidant [11] activities.

Materials and methods

Preparation of the Extract: The fresh aerial part of *Nelumbo nucifera* Gaertn. was collected, procured and authenticated from Regional Research Institute (Ay.) (Central Council of Research in Ayurveda and Siddha, Dept of AYUSH, Ministry of Health and Family Welfare, Govt. of India, New Delhi), Government Central Pharmacy, Annexe, Ashoka Pillar, Jayanagar, Bangalore 560011.

The authenticated leaves were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of the plant (540g) was successively Soxhlet extracted with petroleum ether, chloroform and 90% ethanol. The aqueous extract was prepared using the same marc by the processes of maceration.

Animals: Albino rats (Wistar) of either sex weighing 150-200g were selected for antipyretic activity. They were procured from Venkateshwara Enterprises, Bangalore.

Preliminary phytochemical screening: Qualitative chemical tests were conducted for ethanolic and aqueous extracts of aerial parts of *Nelumbo nucifera* to identify the various phytoconstituents. [12]

Acute toxicity studies : Acute oral toxicity study of aqueous and ethanolic extracts of *Nelumbo nucifera* Gaertn. was carried out according to OECD guidelines 425. For this study female rats (150-200g) were used. The dose of 2000mg/kg (herbal extract) was administered to the animals. The animals were observed continuously for 4h and later upto 24h for mortality and any other behavioral changes. [13]

Yeast induced pyrexia: Male albino Wistar rats were randomly divided into six groups of six animals each. Group I served as control, groups II to VI were treated groups, the animals were administered, ethanolic & aqueous extracts of *Nelumbo nucifera* Gaertn., p.o. (200 and 400mg/kg b.w.) & paracetamol 150 mg/kg b.w. respectively. Basal rectal temperature was measured using telethermometer. Twenty ml per kg of 20% suspension of dried yeast in 2% gum acacia in normal saline (sc) was administered to the rats of various groups. After 18h of yeast injection, the rectal temperature of rats was recorded. Immediately the extract or standard was administered to respective group animals and temperature was recorded again at one hour interval up to 22h after yeast injection. [1,14]

Statistical analysis: The data were expressed as mean \pm SD. Results were analysed statistically by one-way analysis of variance (ANOVA) followed by Dunnet and Tukey's test. P- value <0.05 was regarded as statistically significant.

Results

Results of preliminary phytochemical screening:: Preliminary phytochemical tests were conducted, the ethanolic and aqueous extracts of *Nelumbo nucifera* were found to contain flavonoids, saponins, alkaloids, aminoacids and proteins, carbohydrates, steroids and tannins.

Results of acute toxicity study: There was no change in normal behavioral pattern of animals. No sign and symptoms of toxicity were observed during the observations which were done continuously for the first four hours and then up to twenty four hours for mortality. The extracts were safe up to a maximum dose of 2000mg/ kg b.w. The biological evaluation was carried out at doses of 200 and 400mg/kg b.w.

Anti pyretic activity: The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 18h of administration. Treatment with ethanolic and aqueous extracts of *Nelumbo nucifera* at a dose of 200 and 400mg/kg b.w., significantly decreased the rectal temperature of the rats.

Table 1: Effect of ethanolic and aqueous extracts of *Nelumbo nucifera* on body temperature in yeast induced pyrexia, in ° C.

TREATMENT	Temperature in ° c at					
	INITIAL	After 18h (0 h)	1h	2h	3h	4h
CONTROL	38.43 ±0.22	40.75± 0.20***b	41.05± 0.55***b	41.18± 0.58***b	45.23± 0.51***b	46.70± 1.50***b
Paracetamol (150mg/kg)	38.25± 0.65	40.25± 0.53***b	38.30± 0.12***a	37.63± 0.40***a	37.53± 0.39***a	38.00± 1.16***a
NNE 200	37.40± 0.41	40.25± 0.52***b	37.60± 0.57***a	37.40± 0.27***a	37.08± 0.39***a	37.13± 0.69***a
NNE 400	37.35± 0.70	39.63± 0.87***b	36.80± 0.26***a	36.70± 0.48***a	37.08± 0.39***a	38.13± 0.48***a
NNA 200	37.43± 0.32	39.30± 0.48***b	36.83± 0.91***a	38.83± 0.46***a	37.23± 1.00***a	38.38± 1.10***a
NNA 400	37.68± 0.48	40± 0.64***b	38.03± 0.84***a	38.8± 1.20***a	38.33± 0.74***a	38.15± 0.45***a

Values are the mean ±SD, n=6, where, NNA 200 and 400, NNE 200 and 400 indicates *aerial part of Nelumbo nucifera* aqueous and ethanolic extracts at doses 200 and 400 mg/kg b.w. respectively.

*Symbols represent statistical significance. *** $P < 0.001$, * $P < 0.05$. 'a' as compared with control and 'b' as compared with initial temperature.

Table 2: Percentage change of body temperature brought about by ethanolic & aqueous extracts and standard at various intervals.

TREATMENT	After 18 h (0 h)	1h	2h	3h	4h
CONTROL	↑2.33±0.19	↑2.75±0.39	↑2.83±0.34	↑6.80±0.42	↑8.28±1.61
Paracetamol (150mg/kg)	↑2.10±0.18	↓1.95±0.41	↓2.33±0.17	↓2.70±0.58	↓2.25±0.77
NNE 200	↑2.75±1.07	↓2.65±1.05	↓2.63±0.59	↓3.18±0.79	↓3.13±0.95
NNE 400	↑2.73±0.79	↓2.83±1.05	↓2.93±1.10	↓2.45±1.04	↓1.50±0.93
NNA 200	↑1.88±0.40	↓2.48±0.69	↓0.48±0.46	↓2.08±0.85	↓0.93±0.64
NNA 400	↑2.33±0.81	↓1.98±0.70	↓0.70±0.22	↓1.68±0.45	↓1.60±0.47

Administration of Brewer's yeast to the animals produced a significant ($p < 0.001$) elevation in the temperature after 18h as compared to the initial body temperature. During the first hour of the study, there was a significant ($p < 0.001$) reduction in body temperature in animals treated with extracts and standard when compared to control. NNE 400 and NNA 200 produced antipyretic effect which was comparable to that of standard. During the second hour of the study, significant ($p < 0.001$) antipyretic effect was produced in all extract treated and standard treated groups when compared to that of control. Ethanolic and aqueous extracts at both the doses produced antipyretic activity which was comparable to that of standard. During third and fourth hour also the extracts produced significant ($p < 0.001$) antipyretic activity, which was comparable to that of standard (Table 1). Maximum reduction in rectal temperature was brought about by NNE 200 at 3 and 4h (Table 2).

Discussion

Pyrogen/Fever is defined as an elevation of body temperature. It is a response due to tissue damage, inflammation, malignancy or graft rejection. Cytokines IL-1b, IL-6, interferon's and TNF- α are formed in large amount under this condition, which increase PGE₂. PGE₂ in turn triggers hypothalamus to elevate body temperature. Antipyretics produce their action by inhibiting PGE₂ synthesis.

The main constituents of *Nelumbo nucifera* are: Nuciferine, β & γ Sitosterol, isoquercitrine, quercitrine-3-glycoside in leaves, syringetin-3-O- β -d-glucoside, quercetin-3-O- β -d-glucoside, isorhamnetin-3-O- β -d-glucoside and kaempferol-3-O- β -d-glucoside in flower, nelumbine, resins, glucose, metarbin, tannins and fat in rhizome and seeds.

β -sitosterol is a plasminogen activator. Beta-sitosterol and its glycosides possess potent anti-inflammatory and antipyretic activity, by reducing the secretion of proinflammatory cytokines and TNF- α . [15]

There is much evidence accumulated that flavonoids possess important effects on various biological systems, which may explain their widespread therapeutic uses. Particularly, the quercetin derivatives are known to possess many beneficial effects on human health including cardiovascular protection, anti-cancer activity, antiulcer effects, etc. It is also reported that quercetin possesses antinociceptive effect by acting through central mechanism. The potent anti-inflammatory activity of different types of flavonoids including quercetin and kaempferol derivatives has also been reported. It is suggested that some flavonoids, including quercetin, block both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade at relatively high concentrations, while at lower concentrations only the lipoxygenase pathway is blocked. It is also reported that quercetin is an effective inhibitor of phospholipase A₂ which catalyses the hydrolysis of phospholipids to release arachidonic acid which is the precursor of the inflammatory response. [16] Ethanolic & aqueous extracts of *Nelumbo nucifera* were effective in counter acting yeast induced pyrexia. This may be a consequence of synergistic effect of the constituents present in the extracts like β -sitosterol and flavonoids (kaempferol, quercetin etc.). β -sitosterol, through reduction of secretion of proinflammatory mediators, and Flavonoids through inhibition of PGs synthesis at 2 steps;

- a) Inhibition of arachidonic acid synthesis itself by inhibiting phospholipase A₂.
- b) Inhibition of PGs synthesis by blocking COX pathway.

The present study reveals that the ethanolic and aqueous extracts of *Nelumbo nucifera* produced a significant anti-pyretic effect in yeast induced elevation of body temperature.

We can conclude that the study supports the claim of traditional system of medicine that *Nelumbo nucifera* is useful in the treatment of fever.

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