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PHYTOCHEMICAL INVESTIGATION & IN VIVO ANTI-INFLAMMATORY EFFECT OF ETHANOLIC & AQUEOUS EXTRACT OF NYMPHAEA ALBA LINN.

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Abstract:

Preliminary phytochemical screening of plant extracts of Nymphaea alba, showed the presence of alkaloids, flavonoids, tannins, terpenes, phenolic acid, and glycosides. This study was intended to evaluate the anti-inflammatory activity of ethanol & aqeous extract of fresh flowers of Nymphaea alba, experimentally by carrageenan induced rat paw odema method. Ethanolic and aqueous extracts which showed; best in vitro *anti-inflammatory activity was screened at the dose level of 100 and 200 mg/kg p.o. Diclofenac Sodium at the dose level of 10 mg/kg was used as reference standard drug. Both the extracts showed a dose dependent significant ($P < 0.05$) reduction in paw edema when compared to the control, at all the time intervals and comparable to Diclofenac Sodium treated group. There is a significant ($P < 0.05$) percentage inhibition of paw edema, at doses of 100 and 200mg/kg, respectively, at 4th hour by aqueous extract of Nymphaea alba. Therefore, it can be inferred that the inhibitory effect of aqueous extract of Nymphaea alba on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis. The results of the present study demonstrate that aqueous extract of Nymphaea alba possess significant anti-inflammatory potential. These findings support the use of the extract in traditional.*

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INTRODUCTION:

Inflammation is a part of the complicated biological reaction of vascular tissues to harmful stimuli, including pathogens, damaged cells or irritants. It is characterized via redness, swollen joints, joint pain, its stiffness and lack of joint characteristic. Inflammation is presently treated via NSAIDs. Unfortunately these capsules motive elevated danger of blood clot ensuing in heart assaults and strokes [1]. Inflammation is a normal, protective reaction to tissue damage caused by physical trauma, noxious chemical compounds or microbiological marketers. Inflammation is a stereotyped reaction, inherent to vascularized tissues, which has the goal of reestablishing tissues homeostasis. The inflammatory process has cell and humoral additives, such as leucocytes (neutrophils, macrophages, eosinophils, mast cells and lymphocytes) and the humoral proteolytic structures (complement, kinins and coagulation), respectively. These components paintings synergistically and concurrently, inflicting vascular changes and leukocyte recruitment to the lesion [2]. Nymphaea alba, also known as the European white water lily, white water rose or white nenuphar, is an aquatic flowering plant of the family *Nymphaeaceae*. [3]. *Nymphaea alba* is rich in tannic acid, gallic acid, alkaloids, Sterols, flavonoids, glycosides, hydrolyzable tannins and high-molecular-weight polyphenolic compounds[4]. It is used as an aphrodisiac, anodyne, antiscrophulatic, astringent, cardiotonic, demulcent, antioxidant, sedative and anti-inflammatory. It also produces calming and sedative effects upon the nervous system, and is useful in the treatment of insomnia, anxiety and similar disorders. It is also used in treatment of diaphoresis. In combination with slippery elm (*Ulmus rubra*) or flax (*Linum usitatissimum*) it is used as a poultice to treat boils and abscesses [5].

MATERIAL & METHODS:

Plant Material:

The plant material used in this study was Fresh flowers of *Nymphaea alba*, collected from local area of Bhopal, Madhya Pradesh, India and was authenticated from Department of Botany, Safia College of Science and Education Bhopal (MP) India. After due authentication, flowers of *Nymphaea alba*, was collected in bulk quantities and rinsed thoroughly with distilled water for removal of adhered dust particles and then was shade dried for 24 h. The shadow dried flowers were roughly powder by a mechanical grinder and kept in a nylon bag inside a deep freezer, till further use.

Extraction of Plant Material:

Accurately weighed 500gm of the powder resources was firstly defatted with petroleum ether and was extracted with ethanol and aqueous solvents in a soxhlet extractor. The percentage yield of petroleum ether, ethanol and aqueous extract were found to be 8 gm, 18 gm and 20 gm, respectively. The standard extracts obtained from Nymphaea alba were packed in an air tight container and then kept in a refrigerator at a temperature of 4°C to use next for phytochemical investigation and pharmacological tests [6].

Phytochemical investigation:

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents by slandered procedures [7].

Total Phenolic content estimation:

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard:

10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol.

Preparation of Extract:

10 mg of dried extracted dissolve in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenols.

Procedure: 2 ml of extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [8].

Total flavonoids content estimation:

Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard:

10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract:

10 mg of extract dissolved in 10 ml methanol and filter. Three (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance

was measured at 420 nm. [9].

Animals:
Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water ad libitum. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Carrageenan-induced paw edema model:
Paw edema was induced by injecting 0.1 ml of 1% w/v carrageenan suspended in 1% CMC into sub-plantar tissues of the left hind paw of each rat. Rats were divided into following groups; each group consisting of six animals.

- Group I Carrageenan control
- Group II Etanolic extract of *Nymphaea alba* (100 mg/kg/p.o.)
- Group III Etanolic extract of *Nymphaea alba* (200 mg/kg/p.o.)
- Group IV Aqueous extract of *Nymphaea alba* (100 mg/kg/p.o.)
- Group V Aqueous extract of *Nymphaea alba* (200 mg/kg/p.o.)
- Group VI Diclofenac Sodium (10mg/kg) as standard reference.

The paw thickness was measured before injecting the carrageenan and after 1, 2, 3 and 4 hour using plethysmometer. The anti-inflammatory activity was calculated as percentage inhibition of oedema in the animals treated with extract under test in comparison to the carrageenan control group. [10].

The percentage (%) inhibition of edema is calculated using the formula:-

$$\% \text{ inhibition} = \frac{T_o - T_t}{T_o} \times 100$$

Where T_t is the thickness of paw of rats given test extract at corresponding time and T_o is the paw thickness of rats of control group at the same time.

Data Analysis:
The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Dunnet's test. Differences were considered asstatistically significant at $P < 0.05$, when compared with control.

RESULT & DISCUSSION:

Phytochemical investigation:

Table No.01: **Phytochemical testing of extract**

S. No.	Experiment	Presence or absence of Phytochemical test	
		Ethanolic extract	Aqueous extract
1.	**Alkaloids**		
1.1	Mayer's reagent test	Present	Present
1.2	Wagner's reagent test	Present	Present
1.3	Hager's reagent test	Present	Present
1.4	Dragendroff test	Present	Present
2.	**Carbohydrates**		
2.1	Molish's test	Absent	Absent
2.2	Fehling's test	Absent	Absent

2.3	Benedict's test	Absent	Absent
2.4	Barfoed's test	Absent	Absent
3	**Proteins and Amino Acids**		
3.1	Biuret test	Absent	Absent
4.	**Flavonoids**		
4.1	Alkaline reagent test	Present	Present
4.2	Lead Acetate test	Present	Present
5.	**Glycoside**		
5.1	Borntrager test	Present	Present
5.2	Legal's test	Present	Present
5.3	Killer-Killiani test	Present	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
6.2	Lead Acetate test	Present	Present
6.3	Gelatin test	Present	Present
7.	**Saponin**		
7.1	Foam test	Absent	Absent
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Present	Present
8.2	Libbermann-Burchard's test	Present	Present

Total Phenolic content estimation (TPC)

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.042X+0.002$, $R^2= 0.999$,** where X is the gallic acid equivalent(GAE) and Y is the absorbance.

Table No. 2: Preparation of calibration curve of Gallic acid

S. No.	Concentration	Absorbance
0	0	0
1	5	0.194
2	10	0.422
3	15	0.637
4	20	0.848
5	25	1.035

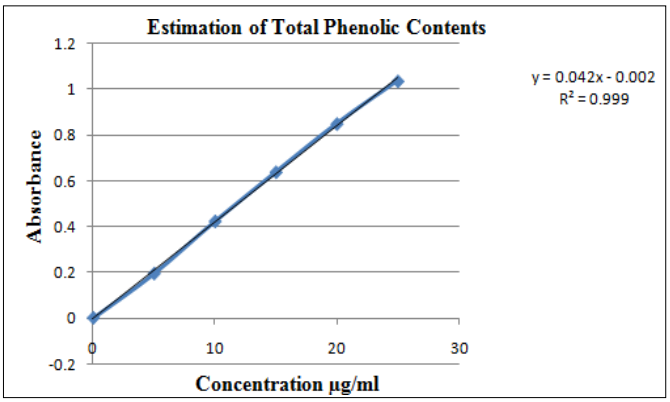

Figure .1: Graph of Estimation of Total Phenolic content

Total flavonoid content estimation (TFC):

The content of total flavonoid compounds (TFC) content was expressed as mg/100mgof quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **Y = 0.06X+0.019, R^2= 0.999,** where X is the quercetin equivalent (QE) and Y is the absorbance.

Table No. 3: Preparation of calibration curve of Quarcetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.352
2	10	0.61
3	15	0.917
4	20	1.215
5	25	1.521

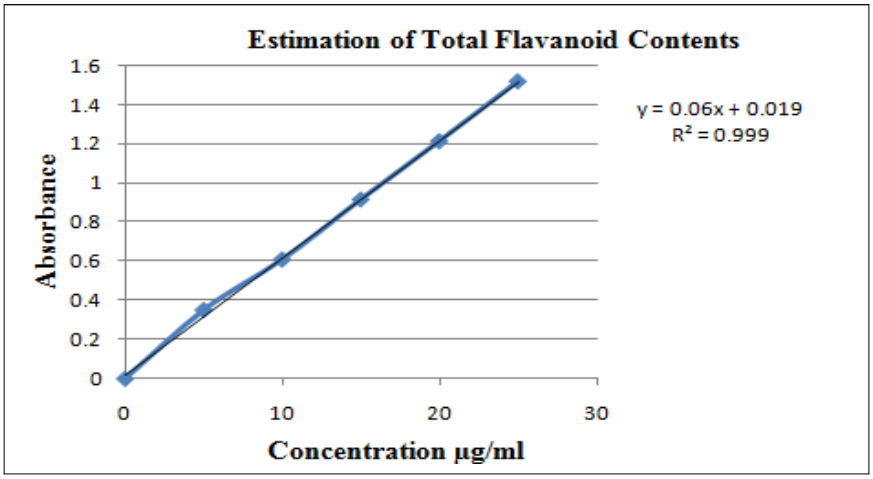

Figure 2: Graph of Estimation of Total flavonoid content

Table No. 4: Total Phenolic and Total flavonoid content

S. No.	Solvents→ Bioactive compound↓	Etanolic Extract of *Nymphaea alba*	Aqueous extract of *Nymphaea alba*
1.	Total Phenol (Gallic acid equivalent (GAE) mg/100mg)	0.675	0.456
2.	Total flavonoid (Quercetin equivalent (QE) mg/100mg)	0.832	1.091

Results of *In –Vivo* Anti-Inflammatory Activity of extracts:

Table 5 shows the effect of *Nymphaea alba* extract and standard drug as compared to carrageenan control at different hours in carrageenan-induced paw edema model using plethysmometer. Ethanolic & Aqueous extract of *Nymphaea alba* administered at a dose of 100 and 200 mg/kg p.o prevented carrageenan-induced paw edema with a percentage inhibition at 1, 2, 3,and 4 hour, respectively. Diclofenac sodium at a dose of 10 mg/kg p.o. prevented carrageenan-induced paw edema with a percentage inhibition at 1, 2, 3.and 4 hour, respectively.

Table No. 5: Effect of Ethanolic & Aqueous extract of *Nymphaea alba* at doses of 100 and 200 mg/kg, and diclofenac sodium as compared to carrageenan control group at different hours in carrageenan-induced paw edema model using plethysmometer.

Group	Treatment Dose(mg/ kg)	Change in paw thickness (mm)3 ± SD			
		1(hour)	**2**(hour)	**3**(hour)	**4**(hour)
1	Control	1.34 ± 0.1	2.35 ±0.12	3.64 ± 0.15	3.25 ± 0.15
2	Ethanolic extract of *Nymphaea alba* (100 mg/kg)	1.24 ± 0.13 (7.46%)	1.94 ± 0.22 (17.44%)	2.80 ± 0.15 (23.07%)	2.07 ± 0.16 (36.30%)
3	Ethanolic extract of *Nymphaea alba* (200 mg/kg)	1.12 ± 0.12 (16.41%)	1.84 ± 0.16 (21.7%)	1.99 ±0.10 (45.32%)	1.60±0.19 (50.76%)
4	Aqueous extract of *Nymphaea alba* (100 mg/kg)	1.16 ± 0.09 (13.43%)	1.75 ± 0.28 (25.53%)	2.12 ± 0.10 (41.75%)	1.80 ± 0.11 (44.0%)
5	Aqueous extract of *Nymphaea alba* (200 mg/kg)	0.91 ± 0.10 (32.08)%	1.40 ± 0.13 (40.0%)	1.72±0.09 (52.74%)	1.32±0.18 (59.38%)
6	Diclofenac sodium (10 mg/kg)	0.58±0.11 (55.97%)	0.8 ±0.12 (66.24%)	1.14 ± 0.12 (68.82%)	0.9 ±0.11 (72.13%)

All values are expressed as mean ± SD; P < 0.05 v/s carrageenan control

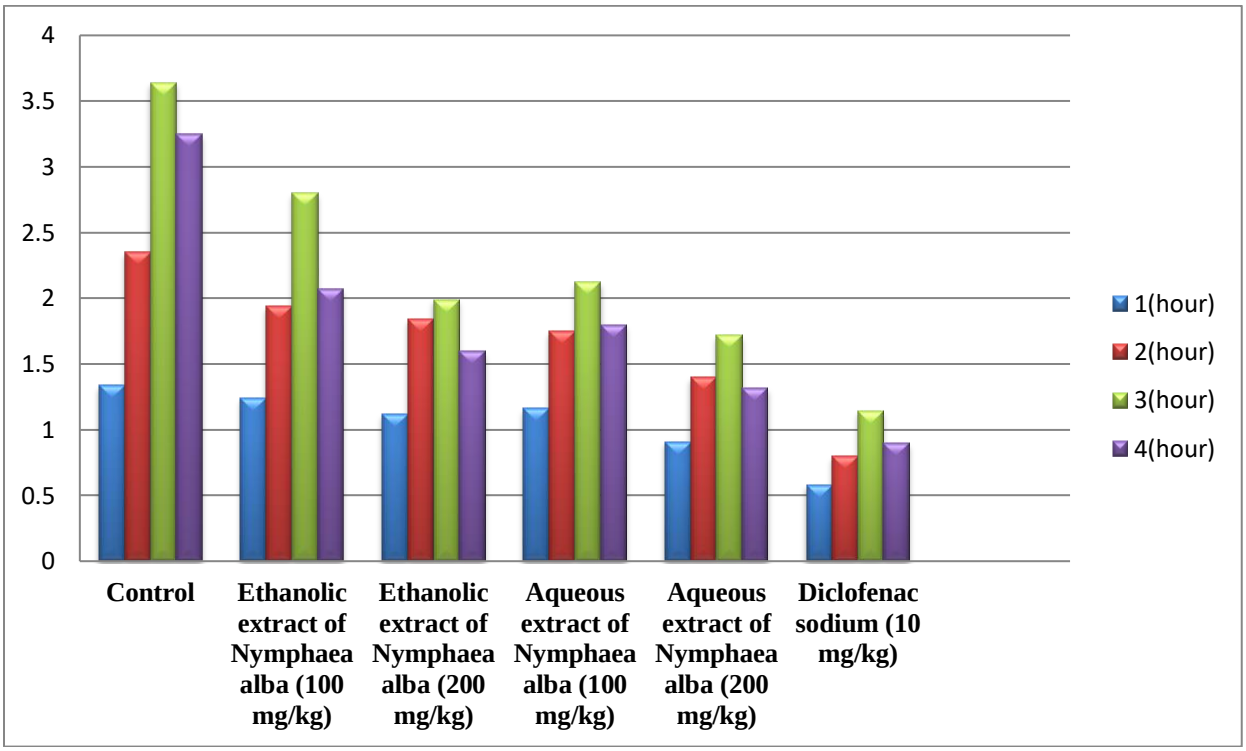

Figure 3: Effect of Ethanolic & Aqueous extract of *Nymphaea alba* at doses of 100 and 200 mg/kg, and diclofenac sodium as compared to carrageenan control group at different hours in carrageenan-induced pawedema model using plethysmometer.

SUMMARY & CONCLUSION:

Carrageenan-induced acute inflammation is one of the most suitable test procedures to screen anti-inflammatory agents. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve. The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also due to histamine and serotonin component. As shown in Table No.5**,** there was no significant inhibition of paw edema, in the early hours of study by ethanolic & aqueous extract of *Nymphaea alba* at 100 and 200 mg/kg, respectively. Hence, it can be concluded that there is no inhibition of histamine and serotonin. Carrageenan- induced paw edema model in rats is known to be sensitive to cyclo-oxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclo-oxygenase involved in prostaglandin synthesis.. As shown in the Table No.5**,** there is a significant ($P < 0.05$) percentage inhibition of paw edema, at doses of 100 and 200mg/kg, respectively, at 4th hour by aqueous extract of *Nymphaea alba*. Therefore, it can be inferred that the inhibitory effect of aqueous extract of *Nymphaea alba* on carrageenan-induced inflammation may be due to inhibition of the enzyme cyclo-oxygenase leading to inhibition of prostaglandin synthesis.Aqueous extract of *Nymphaea alba* possess significant anti-inflammatory potential. These findings support the use of the extract in traditional system of medicine for the management of inflammatory conditions.

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