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STUDY OF THE ANTI-INFLAMMATORY PROPERTY OF AQUEOUS EXTRACTS FROM LEAVES OF *NYMPHAEA LOTUS* L. (NYMPHAEACEA)

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ABSTRACT

In Côte d'Ivoire, the decoction of *Nymphaea lotus* leaves is traditionally used to treat inflammation. This study evaluated the anti-inflammatory activity of aqueous extracts from these plant leaves in laboratory on rat paw oedema induced by carrageenan. The doses of phytomedecines administered orally to the rats were: 840, 1400, 4200 mg/kg. The results obtained with aqueous extracts of *Nymphaea lotus* were compared, on the one hand with those of the physiological control (NaCl 0.9%) and on the other hand with those of diclofenac 25 mg/kg, reference anti-inflammatory in this study. The different comparisons were made at 1h, 2h, 3h, 4h, 5h, 6h, 12h and 24h after injection of carrageenan. The parameters considered were: percentage increase in paw circumference (%AUG) and percentage of treatment inhibition (%INH). The results showed a significant difference at $p<0.001$ between %AUG of phytomedicine treatments at different doses and those of NaCl. However, at the dose of 4200 mg/kg, the %AUG of the phytomedicine were statically similar to those of diclofenac 25 mg/kg. Comparisons of %INH showed the same results with %AUG. This study therefore reveals that *Nymphaea lotus* leaves have anti-inflammatory properties. This study therefore provides good evidence for the use of *Nymphaea lotus* leaves decoction in traditional medicine to combat inflammation.

KEYWORDS: Activity, inflammation, *Nymphaea lotus*, phytomedicine, Côte d'Ivoire.

INTRODUCTION

Inflammation is a defense mechanism of the body against aggression of physical, chemical, biological or infectious origin, essential for its integrity.[1] The role of this inflammatory response is to eliminate the aggressive agent and allow tissue repair as quickly as possible.[2] It is above all a defense mechanism, aimed at neutralizing the aggressive agent

and eliminating the damaged tissue.[3] In modern medicine, this condition is treated with pharmaceuticals called non-steroidal anti-inflammatory drugs (NSAID) and steroidal anti-inflammatory drugs (SAID) or glucocorticoids.[4] However, the use of NSAID is associated with numerous adverse effects, with a considerable prevalence of new diseases and mortality.[5] Relating to SAID, they are responsible for chronic disorders such as osteoporosis, cataracts and weight gain.[6] In view of the many side effects of NSAID and SAID, research for phytomedicines is a solution that should be encouraged. In Côte d'Ivoire, several ethnomedicinal investigations have indicated that *Nymphaea lotus* decoction is used in traditional medicine to treat anti-inflammatory deases.[7] However, no scientific study in the country has yet assessed the anti-inflammatory activity of this plant. This study provides an opportunity to verify the anti-inflammatory properties of *Nymphaea lotus*. It therefore aims to evaluate the anti-inflammatory effect of aqueous extracts from the plant at different doses in laboratory.

MATERIAL AND METHOD

Plant material

The plant material were fresh leaves from *Nymphaea lotus*. The plants were collected from a marshy pond at the University of Man in Côte d'Ivoire.

Fig. 1: *Nymphaea lotus* leaves.

Animals used and ethical consideration

In this study, the animals were used in accordance with the British law of 1986 and the OECD recommendations relating to the use of animals in experimental sciences. For compliance with these principles, the experimental protocol was validated by the ethics committee of the pharmacognosy laboratory of Félix HOUPHOUET-BOIGNY university. The experiments were performed on adult wistar rats weighing between 160 and 190 grams.

Technical equipment

To assess anti-inflammatory activity, we used an electronic calliper with a capacity of 0 to 150 mm. To prepare the extracts, we needed cotton wool, an electronic balance, a graduated cylinder, a 2CC syringe and a syringe for injecting the carrageenan.

Chemical products

The carrageenan 1% solution was used to induce oedema of the rat paw. The reference anti-inflammatory was diclofenac 25 mg/kg (Olfen-75 SR) sold in pharmacies. We also required saline (NaCl) as a control for the experiments and distilled water.

Preparation of aqueous extracts

In this study, the aqueous extracts were chosen to remain close to the traditional practitioners' model. We boiled 800g of fresh leaves from *Nymphaea lotus* during 30min in 3litres of tap water. Two litres of the decoctate were first wrung out in a clean cloth square, filtered twice on cotton wool and then on 3 mm Wattman paper. The filtrate (1l) was evaporated in a rotavapor and oven at 60°C. The crystals obtained were crushed in a porcelain mortar to obtain a fine powder weighing 10.5g. From this fine powder, we sought the saturation concentration or maximum concentration, which was 140 mg/ml. This concentration was then diluted 1/2, 1/3, 1/4 and 1/5 to give respective concentrations of : 70; 46.66; 35; 28 mg/ml. These concentrations allowed us to calculate the corresponding doses according to the recommended pharmacological standard, which is 0.6 ml per 20 grams of body weight (Doh et al., 2023). The corresponding doses in mg/kg/body weight at these different concentrations are respectively: 4200; 2100; 1400; 1050 ; 840 mg/kg administered orally. We evaluated 3 doses to assess anti-inflammatory activity. The highest dose, the medium dose and the lowest dose. These 3 doses are coded: ENL1 for 4200 mg/kg, ENL2 for 1400 mg/kg and ENL3 for 840 mg/kg.

Conditioning, batch composition and gavage of the rats

The animals were conditioned, fasted for 16 hours and divided into 5 batches of 6 rats. The batches were formed as follows according to different treatments:

Table 1: Treatments administered based on batches of rats.

Batch Number	Number of rats	Treatments administred by oral route
01	06	Nacl at 10 ml/kg
02	06	ENL1 at 4200 mg/kg
03	06	ENL2 à 1400 mg/kg
04	06	840 mg/kg
05	06	diclofenac at 25 mg/kg

Induction of inflammation

The experiment followed the carrageenan-induced acute rat paw oedema model.[8] Thus, 1 hour after the administration of the different treatments, 0.2 ml of carrageenan solution 1% was injected under the plantar pad of the right paw of each rat. Hind paw circumference measurements were made with a caliper 1h, 2h, 3h, 4h, 5h, 6h, 12h and 24h after carrageenan injection.[9]

Parameters assessed

To assess the anti-inflammatory effect, the following parameters were evaluated: the percentage increase in the circumference of the swollen paw (%AUG) and the percentage inhibition of oedema (%INH). They are calculated according to the following formulae[10]:

$$\%AUG = 100\left(\frac{Swollen\ paw\ circumference - Initial\ circumference}{Initial\ circumference}\right)$$

$$\%INH = 100\left(\frac{\%\text{ AUG control} - \%\text{AUG treated}}{\%\text{ AUG control}}\right)$$

Statistical analysis of the study data

The percentages of increase in paw circumference (% AUG) of aqueous extracts from *Nymphaea* were calculated and compared on the one hand to those of NaCl (control solution) and on the other hand to those of diclofenac (reference pharmaceutical anti-inflammatory) by the ANOVA 1 statistical test coupled with Tukey's multiple comparisons test. The oedema inhibition percentages (%INH) of *Nymphaea lotus* extracts were compared with those of diclofenac using the same statistical tests. Graphs relating to these inhibition percentages were produced using Excel 2016 software.

RESULTS

Percentage increase in circumference of infected leg (%AUG)

Injection of carrageenan resulted in an increase in paw circumference. The percentage increase in leg circumference (%AUG) calculated for this purpose and the results of the statistical tests carried out are shown in Table 1. This table shows that there is a significant difference at P<0.001 between the percentages of increase in leg circumference (AUG) of animals that received the control treatment (Nacl) and those that received the phytomedicinal treatments (ENL1, ENL2, ENL3) and diclofenac. There was no significant difference between the AUG percentages of animals treated with ENL1 (the high dose) and those treated with diclofenac, the pharmaceutical anti-inflammatory used as a reference in this study. However, there was a significant difference between the AUG of ENL1 and those of ENL2 (the medium dose) and ENL3 (the low dose). ENL1 reacted better than ENL2 and ENL1.

Table 2 : Evolution des pourcentages d'augmentation des circonférences des pattes de rats œdèmisées (%AUG) pour les différents traitements.

Treatments and doses used (Dose/kg)	Measuring time							
	1H	2H	3H	4H	5H	6H	12H	24H
Nacl 0.9% 10 ml	54.87±7.05^{d}	75.53±9.70^{d}	81.82±10.1^{d}	85.81±7.94^{d}	84.57±8.12^{d}	82.23±5.89^{d}	79.24±2.15^{d}	73.85±12.13^{d}
Diclofenac 25 mg	4.68±9.05^{a}	5.68±10.13^{a}	8.89±9.2^{a}	10.73±8.19^{a}	9.68±5.16^{a}	9.28±9.52^{a}	8.23±4.23^{a}	8.68±9.07^{a}
ENL1 4200 mg	6.07±8.13^{a}	7.98±6.17^{a}	10.08±1.27^{a}	13.01±6.04^{a}	11.21±0.18^{a}	11.08±14.03^{a}	10.02±15.12^{a}	11.03±0.1^{a}
ENL2 1400 mg	26.57±0.98^{b}	39.02±8.03^{b}	43.23±0.56^{b}	51.98±0.68^{b}	49.62±0.31^{b}	48.91±0.15^{b}	47.39±8.21^{b}	47.82±7.14^{b}
ENL3 840 mg	38.43±9.8^{c}	51.25±13.17^{c}	62.12±7.01^{c}	68.04±4.53^{c}	65.54±0.01^{c}	61.17±10.18^{c}	59.13±3.17^{c}	60.67±11.02^{c}

Means followed by different letters in the same column are significantly different for *p<0.001*

Treatment inhibition percentages (%INH)

The treatment inhibition percentages (%INH) are shown in Figure 2. Statistical treatment of these %INH revealed that there is no significant difference between the INH percentages of the diclofenac treatment (73.86±0.38 to 81.23±0.35) and the INH percentages of the ENL1 extracts (70.41±0.38 to 75.96±0.36). The INH percentages of these two treatments are significantly different from the INH percentages of ENL2 and ENL3. The INH percentages of ENL2 varied between 40.05±1.17 and 51.41±1.67 and were significantly different from the INH percentages of EPN3, which varied between 11.12±3.17 and 33.87±3.23. Comparisons of %INH gave a similar result to %AUG.

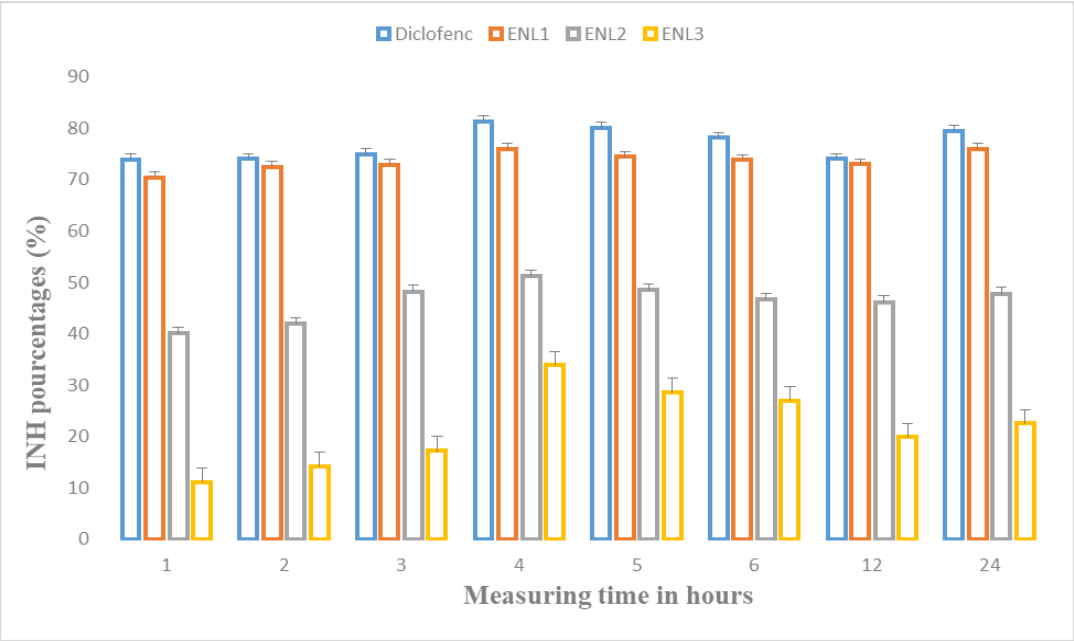

Fig. 2: Evolution of inhibition percentages during the experiment for each treatment.

DISCUSSION

The results show that *Nymphaea lotus* leaves have anti-inflammatory properties. The effect of the plant against inflammation is comparable to that of diclofenac 25mg/kg. High doses of plant extracts exert a better anti-inflammatory effect than low doses. This indicate a dose-response effect in the plant's response against inflammatory conditions. Regarding literature, *Nymphaea lotus* contains several chemical groups including polyphenols, alkaloids, and flavonoids.[11] Alkaloids and flavonoids have already been identified as chemical groups with anti-inflammatory properties.[12] Concerning polyphenols, numerous studies have shown that these chemical groups and their metabolites inhibit the enzymatic activities of arachidonic acid metabolism and reduce the production of inflammation mediators such as arachidonic acid, nitrogen monoxides, prostaglandins and leukotrienes.[13] The presence of polyphenols in aqueous extracts of *Nymphaea lotus* may therefore have a positive impact on the use of the plant in the treatment of inflammatory conditions. Furthermore, the reference anti-inflammatory drug diclofenac used in this study increases the risk of arterial thrombosis in patients.[14] The search for anti-inflammatories without side effects should focus on plants such as *Nymphaea lotus*. In addition to this study, several research studies have focused on plants that can combat inflammatory pathologies. *Buchholzia coriaceae* and *Annona reticula* have already been revealed in the same perspectives.[15] The anti-inflammatory activity of these different plants has been evaluated on measurements circumferences of paw oedema in rats induced by carrageenan as this study.

CONCLUSION

The study assessed the anti-inflammatory activity of aqueous extracts of *Nymphaea lotus* in rats. The doses of phyto-drugs administered to the animals were as follows: 840, 1400, 4200 mg/kg orally. The results obtained with the aqueous extracts of *Nymphaea lotus* were significantly different from those of the physiological control (NaCl 0.9%), which represents the control treatment. However, the results of the plant extracts were statistically similar to those of the pharmaceutical anti-inflammatory: diclofenac 25 mg/kg. The comparisons attest to the anti-inflammatory properties of *Nymphaea lotus* leaves. This study therefore provides good evidence of the traditional use of decoction from

Nymphaea lotus leaves in the treatment of inflammatory pathologies. This plant should be of interest to scientific communities in research for new anti-inflammatory solutions.

Competing interest

Authors declare that there is no conflict of interest relating to this study.

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