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The reducing effect of white *nymphea lotus* (Water lily) on pain sensation in mice

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ABSTRACT

*Nymphealotus*is a perennial and herbaceous aquatic plant, whose leaves has be reportedly used for various therapeutic purposes (such as aphrodisiac, antioxidant and antibacterial purposes) including the treatment of fever, diarrhea, urinary difficulties, enteritis, dyspepsia, bowel problems tumours, gastric ulcers and abnormal heart beat. The present study investigates the effect of the ethanolic extract of *Nympheae lotus* on pain sensation in mice. The hot plate and formalin test was used to evaluate the effects of this plant on pain. Before the neurobehavioural parameters were assessed, the $LD_{50,}$ Phytochemical and acute toxicological screenings of the plant were determined. The median lethal dose was above 5000 mg/kg orally in mice for the ethanol extract. Thirty (30) Swiss mice were randomly assigned into 3 groups; control (normal saline), low dose (25mg/kg) and high dose (50mg/kg) for twenty one days (21). The results showed that the frequency and duration of hind paw licks were significantly lower inmice administeredlow dose and high dose of the plant when compared to the control ($P<0.05$).Similarly, the frequency and duration of paw attention in the low dose and high dose treated mice was also significantly lower when compared to control ($P<0.05$).The latency of jump was significantly higher for the low and high dose groups compared to control ($p<0.05$). In conclusion, these findings suggest that the ethanolic extract of *Nympheaelotus* decreases pain sensation in mice and therefore may be useful in the treatment of pain disorders.

INTRODUCTION

Nymphaea lotus belongs to Nymphaeaceae family, It is a perennial plant that grows up to 45cm in height; it is herbaceous aquatic plant, whose leaves float or submerged in water [1]. It grows in various part of east Africa and Southeast Asia. Several varieties of Lotus plants have been identified and these varieties have very keen resemblance that without proper identification, one might mistake one for the other. These lotus varieties include the white *Nymphea lotus*, the famous Egyptian blue lotus called *Nympheacaerulea*and the *Nympheathermalis*. *Nympheae lotus* variety *thermalis*is a tertiary relict variety, endemic to the thermal waters of Europe, for example the Petariver in Romania or Heviz Lake in Hungary. *Nympheae lotus* contains quinolidizine alkaloids (nupharin), tannins, flavonoids, , anthraquinones, saponins, terpenes, cardiac glycosides and phenolics. White *Nymphea lotus* is also considered effective in the treatment of fever, diarrhea, urinary difficulties, enteritis, dyspepsia, and abnormal heart beat [2]. [3]reported the significance of the leaves in the treatment of bowel problems and tumours by the traditional Sudanese. *Nymphealotus* has also been reported to have protective effects against gastric ulcers [*4*].Despite the immense technological advancement in modern medicine, many people still rely on traditional medicine and healing practices for their daily health practices. This is owned to the fact that modern medicine leave resultant side effects coupled with the high cost in acquiring synthetic drugs. The side effects of many these drugs have left many individuals and patients with irreversible neurological damage. This debilitating situation has led to the investigation of the anxiolytic effect of *Nymphealotus.* The validation of the therapeutic efficacy of *Nymphea lotus* will provide novel means and ways of treating pain disorders.

MATERIALS & METHODS

PLANT MATERIAL

Nympheae lotus (water lily) Leaves were collected and identified in the Department of Biological sciences, Ahmadu Bello University, Zaria, and a voucher specimen was deposited in the herbarium of the Department (V/N 1689)

PREPARATION OF EXTRACT

Nympheae lotus leaves were first separated from the stalk, rinsed with water to remove dirt, the air-dried leaves 200g of N. *lotus* were pulverized and soaked in ethanol (2L) for 72 hrs.The solutions were filtered and the filtrate concentrated with a rotary evaporator to give a semisolid residue. The product was kept in the refrigerator for further use.

CARE AND MANAGEMENT OF ANIMALS

Thirty Swiss mice weighing between 14g and 17g were purchased and used for this experiment. The rats were housed in the animal holding of the Department of Anatomy and physiology, Abia state University, Abia state. Animals were housed in groups of 3 (control, low dose and high dose) in plastic cages, maintained under standard dark-light cycle Food and water was available ad libitum. All rules applying to animal safety and care were observed.

EXPERIMENTAL DESIGN

Animals in group A received normal rat chow; group B animals received 25mg/kg and group C, received50 mg/kg of the plant extract daily for a period of 21days.The hot plate and formalin test was used to assess pain sensation in mice. The procedure for the test involved first turning the apparatus on and waiting a few minutes until the surface attained the required temperature, which was maintained at 55^0c. Mice were individually exposed to the hot plate apparatus using a plastic container. The foot pedal was tapped immediately after introducing the mouse in the apparatus to start the timer and tapped again to stop the trial when the required behavior was observed. The cut-off point for the test per mouse was 30 seconds, in case the required behavior was not observed. This was to avoid extensive tissue damage [5]. The behavior measured were the time it took for the mice to start licking their foot pad. This behavior, defined as the latency of hind paw lick, was recorded. The behavior observed, paw lick and jumping are the most common measures of pain threshold, and are considered supraspinally integrated [6-7].

Formalin-induced pain is caused primarily by peripheral tissue inflammation [8]. A central sensitization of dorsal horn neurons occurs during the inflammatory pain. In this respect, the formalin test has been regarded as being a more satisfactory model of clinical pain than hot plate tests [9].

Mice were carried into the room in their home cages. Each mouse was picked by the base of its tail and 0.2ml of 2.5% formalin was injected into the right hind paw of the mouse using a needle and syringe. The animal was placed in the observation box and observed for five (5) minutes. The animal was then returned to its cages and allowed for thirty (30) minutes before it was taken back to the observation box to be re-observed for another five (5) minutes. This procedure was repeated for each animal.

Behavior scored during the pain test included the following;

frequency of Right hind lick/scratch

frequency of Right hind paw attention

Duration of attention.

DATA ANALYSIS

The data collected during the study were presented as Mean SEM and a "P" value less than 0.05, were regarded as significant. Analysis of variance (ANOVA) and the student test were used for analysis. Also a post-hoc test (SD) was carried out. Statistical analysis was done with the aid of computer software SPSS and Excel from windows XP (Brain Series, China).

RESULTS

BEHAVIOURS SCORED IN THE HOT PLATE TEST

FREQUENCY OF HIND PAW LICKS

The frequency of hind paw licks in the hot plate test are 3.92±0.12; 2.13±0.51 and 1.20±0.23 seconds for mice fed with control, low and high dose diet of nymphaea lotus. The frequency of hind paw licks for mice administered low and high dose was significantly lower compared to control ($p<0.05$). See figure fig 1.

DURATION OF HIND PAW LICKS

The hind paw lick duration in the hot plate test was 18.82±2.31 for the control and 10.24±1.05 and 8.33±0.12seconds for the low and high dose groupsrespectively. The low and high dose fed mice were significantly lower compared to control ($p<0.05$).

LATENCY OF JUMP

The latency of jump in the hot plate test for mice fed control, low and high dose of nymphaea lotus diet were 14.46±2.09; 22.13±3.51 and 27.30±1.38 seconds. The latency of jump by the low and high dose nymphaea lotus fed mice were significantly higher compared to control ($p<0.05$).

BEHAVIOURS SCORED IN THE FORMALIN TEST FOR PAIN

FREQUENCY OF HIND PAIN LICK FOLLOWING FORMALIN ADMINISTRATION.

Figure 5 shows the frequency of hind paw licks for mice fed control, lowand high dose nymphaea lotus diets as 11.82±1.67; 7.65±0.74 and 6.63±0.12 respectively in the first trial after 5 minutes of formalin administration. The frequency of paw lick for the mice fed low dose and high dose diet of nymphaea lotus was significantly shorter ($p<0.05$) than that of the control fed mice. Similarly, in the second trial after 30 minutes of formalin administration, the frequency of paw licks was 0.86±0.21; 0.15±0.11 and 0.12±0.10 for mice fed control, low dose and high dose diet. The frequency of right hind paw lick in the low dose and high dose diet fed mice were seen to be significantly shorter ($p<0.05$) than those of the control.

FREQUENCY OF HIND PAW ATTENTION

The frequency of hind paws attention were22.00±2.05; 7.15±1.14 and 5.00±0.75 respectively in the first trial after 5 minutes of formalin administration. The frequency of hind paw attention was significantly lower in the low and high dose fed mice compared to control ($p<0.05$).In the second trial, after 30 minutes of formalin administration, the values were 1.42±0.46; 0.46±0.31 and 0.40±0.29. The frequency of hind paw attention was also significantly lower in the low and high dose compared to control ($p<0.05$).

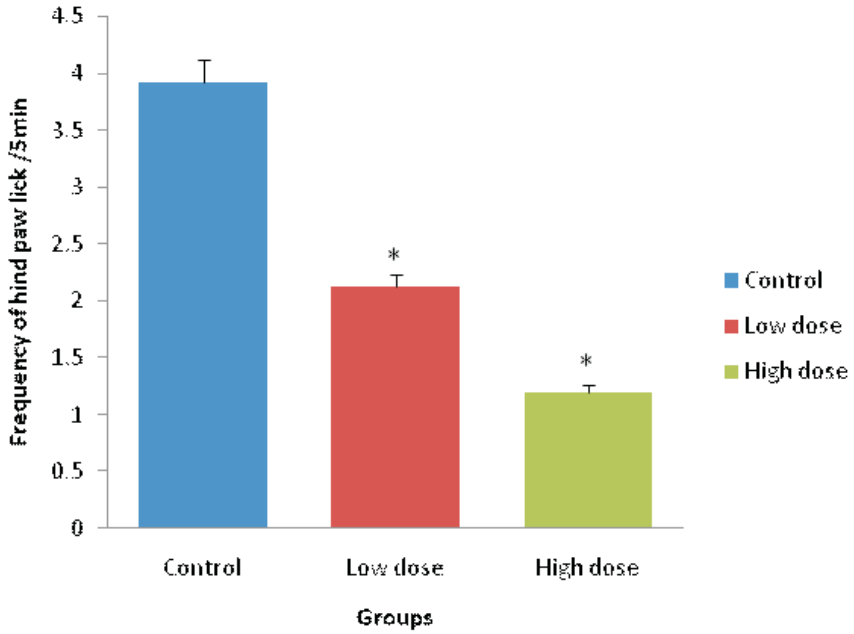

Fig 1 : Frequency of hind paws licks of the different experimental groups during the hot plate test. Values are expressed as as mean, ± SEM, n = 10,*p<0.05 vs. control.

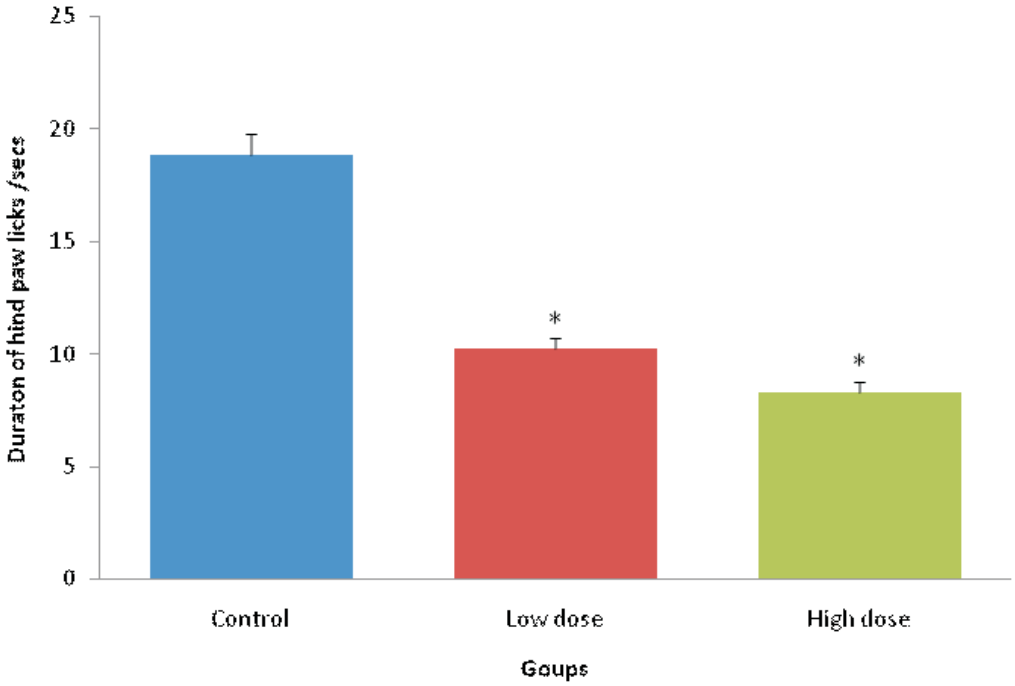

Fig 2 : Duration of hind paw licks among the experimental groups during the hot plate test. Values are expressed as mean,± SEM, n = 10,*p<0.05 vs. control.

DISCUSSION

In this study, the animal models of physiological pain assessment used include, formalin test and hot plate test [10]. The hot plate paw-shaking and paw-licking is a complex and supra-spinal organized behavior. The latency of jump in the hot plate (heated to and maintained at 55^{0}C to prevent excessive tissue damage) was defined as the time it takes the mice to jump after introduction into the hot plate. The hot plate procedure is believed to have an advantage over other thermal nociceptive pain model like tail flick procedure[11], because it can be applied repeatedly in the same animal over a short period of time(2-3 hours) without causing tissue damage or injury, especially if the maximum observation time is 30 seconds. It also constitutes a more global estimate of nociceptive reactivity because it represents a complex pattern of willed behavior rather than a simple reflex, like the tail flick [12]. In the hot plate test, the latencies of jump observed in

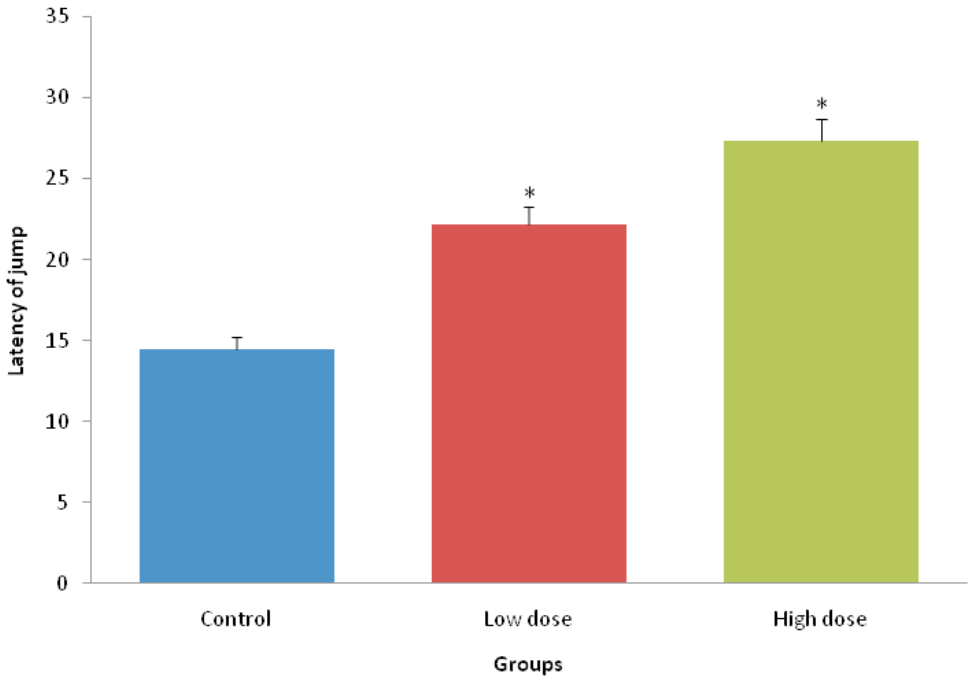

Fig 3 : Latency of jump among the different experimental groups. Values are expressed as mean,± SEM, n = 10,*p<0.05 vs. control.

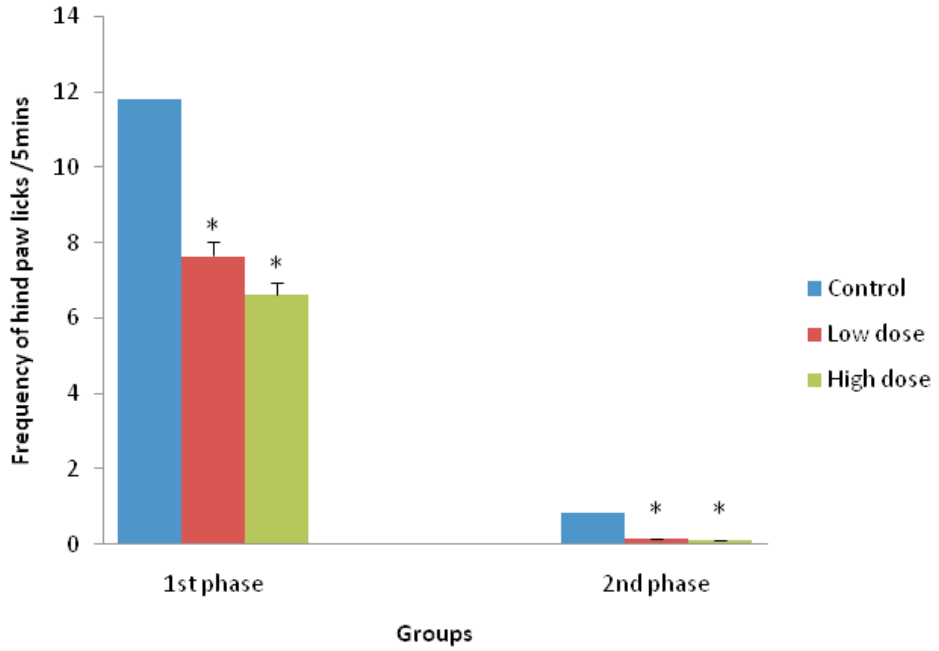

Fig 4 **:** Frequency of hind paws licks among the different experimental groups following formalin administration. Values are expressed as mean,± SEM, n = 10,*p<0.05 vs. control.

the low and high dose groups of mice were significantly longer than that of the control mice that consumed normal rat chow, indicating that it took longer time for nymphaea lotus fed mice to perceive pain than the control group of mice. This means that the leaves of the nymphaea lotus had an analgesic effect on mice. Similarly; the frequency and duration of paw lick also indicates decrease in pain sensation among the low and high group of mice fed with the nymphaea lotus diet when compared to the control.

The response of formalin-induced behavior reflects activation of C fiber primary afferent nociceptors [10]. This test was in two phases. The first 30 seconds following formalin injection is the perception of acute pain, while the later period shows chronic pain perception. Frequency of hind paw attention and hind paw-licking following injection with formalin was defined as the number of times the mice lick or shake their hind paw after injection with formalin. Lower frequencies of hind paw attention

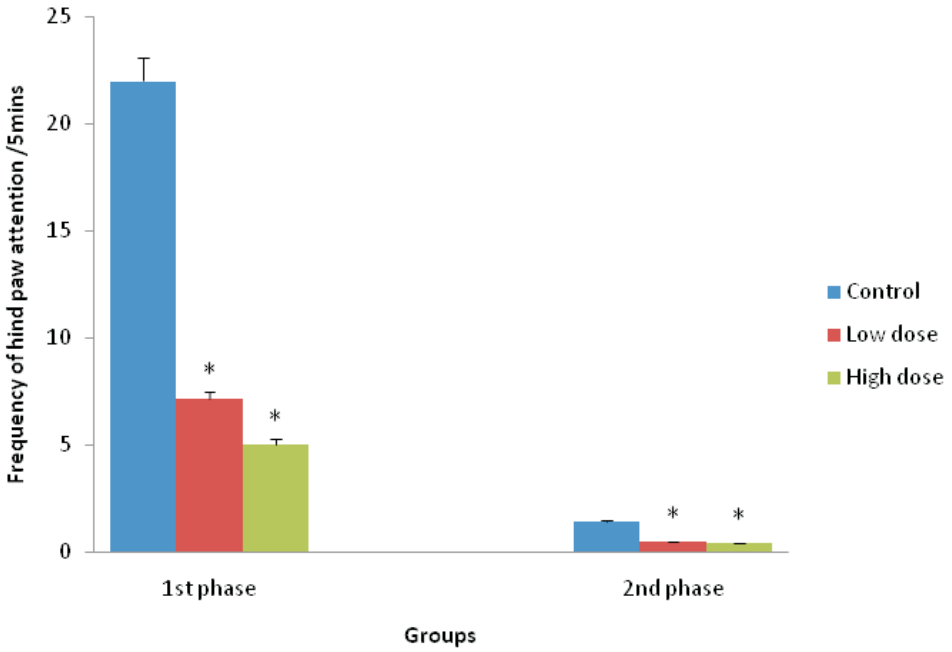

Fig 5 : Frequency of hind paws attention among the different experimental groups following formalin administration. Values are expressed as mean,± SEM, n = 10,*p<0.05 vs. control.

and hind paw licking indicate analgesic effect while higher frequencies indicate hyperalgesia.

Our finding showed that during acute and chronic phases of pain, the low and high dose diet- fed mice had significantly less pain perception compared to control, since the frequencies and durations of hind paw lick and paw attention following formalin injection was significantly lower in the low and high dose diet-fed mice than the control. Pain reduction was observed on the first and second phases of pain following chronic consumption of nymphaea lotus. The first phase was the fast or pricking pain mediated by the type A-delta fibres that release the neurotransmitter glutamate while the second phase represent slow pain where inflammation of tissues occurs. Slow or chronic pain is mediated by the neurotransmitter; substance P [13].It is therefore interesting to note that nymphaea lotus diet can be beneficial in the reduction of chronic pain if the results in mice can be extrapolated to man.

CONCLUSION

This research has shown that the ethanolic extract of *Nympheaelotus* decreases pain sensation in Swiss mice. This may probably be attributed to the presence of phytochemicals which could cause an inhibitory effect on the cerebral cortex. Further study is suggested on determining the particular phytochemical responsible for this effect and clinical trials carried out to determine its efficacy on humans subjects pain disorders.

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AUTHOR(S) CONTRIBUTION

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