

## Antioxidant, Analgesic and Anti-Inflammatory Activities of *Nymphaea Nouchali* Flowers

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**Abstract:** *Nymphaea nouchali* leaves are used in folk medicine for the treatment of various disorders. In order to evaluate the actions of this plant, studies were performed on antioxidant, analgesic and anti-inflammatory activities. The Methanolic extract of *Nymphaea Nouchali* Flowers (MNNF) was evaluated for anti-inflammatory activity using carrageenan induced hind paw edema model whereas hot plate, writhing and formalin tests were carried out for analgesic activity. Total phenolic and flavonoids content, scavenging of 1, 1-Diphenyl-2- Picrylhydrazyl (DPPH) radical, peroxynitrate (ONOO<sup>-</sup>) as well as inhibition of total ROS generation were used to evaluate antioxidant potential of MNNF. The extract MNNF at the dose of 100 and 200 mg kg<sup>-1</sup>, produced a significant (p<0.05) increase in pain threshold in hot plate method whereas significantly (p<0.05) reduced the writhing caused by acetic acid and the number of licks induced by formalin in a dose dependent manner. The same ranges of doses of MNNF caused significant (p<0.05) inhibition of carrageenan induced paw edema after 4 h in a dose dependent manner. In DPPH, ONOO<sup>-</sup> and total ROS scavenging method, MNNF showed good antioxidant potentiality with the IC<sub>50</sub> value of 10.33±1.02, 20.16±0.61 and 31.72±0.48 µg mL<sup>-1</sup>, respectively. The findings of the study suggested that *Nymphaea nouchali* has strong analgesic, anti-inflammatory and antioxidant effects, conforming the traditional use of this plant for inflammatory pain alleviation to its antioxidant potentiality.

**Key words:** Antioxidant, analgesic, anti-inflammatory, *Nymphaea nouchali*, DPPH, Bangladesh

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### INTRODUCTION

Free radicals cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins and contribute to >100 disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (El-Hela and Abdullah, 2010; Ouattara *et al.*, 2011). Moreover, various free radicals are also responsible for the induction of short term algesia as well as play an important role in the pathogenesis of inflammation (Chung, 2004). Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, thermal and mechanical injuries (Oyedapo *et al.*, 2008). Various endogenous mediators like histamine, serotonin, bradykinin, prostaglandins, etc., are most abundant in inflammatory cell and among them prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation (Hossain *et al.*,

2011). These mediators even in small quantities can elicit pain response. Pain results in dropped muscular activities, associated with various free radicals as well as Reactive Oxygen Species (ROS) that triggers some second messengers, are involved in sensitization of dorsal horn neurons that plays a fundamentally important role in neuropathic pain (Ali and Salter, 2001; Chowdhury *et al.*, 2009; Zhang *et al.*, 2003). Medicinal plant have great value to phytochemists because of their medicinal properties so that, the study of plants that have been traditionally used as pain killers should still be seen as a fruitful and logical research strategy in the search for new analgesic drugs and pain mechanisms (Oladosu *et al.*, 2011).

*Nymphaea nouchali* Burman, (Syn. *Nymphaea stellate* Willdenow) locally known as Shapla in Bangladesh is an aquatic plant of the genus *Nymphaea*. *N. nouchali* grows abundantly as a mixed population in almost all shallow natural water bodies and has been designated as the national flower of Bangladesh. The whole plant is used for the treatment of liver disorders in

Ayurveda. Leaves, roots and flowers are used for diabetes, blood disorders, heart troubles, dysentery and as a cardiogenic, diuretic, narcotic and aphrodisiac (Deutschlander *et al.*, 2009; James, 2008; Raja *et al.*, 2011; Rajagopal and Sasikala, 2008). The flowers are used for the treatment of liver disorders in traditional medicine and has proven hepatoprotective activity (Huang *et al.*, 2010; Muthulingam, 2010). The syrup of the flower is used in cases of high fever, inflammatory diseases of the brain and also in dysuria (Karthiyayini *et al.*, 2011). Rhizomes are astringent, demulcent and used in infusion internally for chronic diarrhea as well as gargle for sore throat. Rhizomes and flowers are used as a remedy for kidney problems (Parth and Enayet, 2007; Sarma *et al.*, 2008). Different solvent extracts of the entire plant have shown the presence of sterols, alkaloids, saponins, tanins and flavonoids. Nymphaol (25, 26-dinorcholest-5-en-3 $\beta$ -ol), a new sterol has been isolated from the successive chloroform extract of the flower (Subash-Babu *et al.*, 2009). Protein, pentosan, mucilage and tanins are reported in the seeds (Rakesh *et al.*, 2010). Astragalin, corilagin, gallic acid, gallic acid methyl ester, isokaempferide, kaempferol, quercetin-3-methyl ether, quercetin, 2, 3, 4, 6-tetra-*o*-galloyl dextroglucose and 3-*o*-methylquercetin-3'-*o*-beta dextroxylopyranoside have been identified in the flowers (Nagavani and Rao, 2010; Rakesh *et al.*, 2009). The present study was carried out to evaluate the antioxidant, analgesic and anti-inflammatory activities of crude extract of *Nymphaea nouchali* flowers in different experimental model.

## MATERIALS AND METHODS

**Plant materials:** The flowers of the *Nymphaea nouchali* Linn were collected from the adjoining area of Jahangirnagar University Campus, Bangladesh during February, 2011. The plant material was taxonomically identified by the National Herbarium of Bangladesh whose voucher specimen No. JU/35334 is maintained in the laboratory for future reference.

**Chemicals:** Folin-chiocaltu phenol reagent and carrageenan were purchased from E. Merck (Germany). 1, 1-Diphenyl-2-Picryl-Hydrazyl (DPPH), ascorbic acid, quercetin and 2', 7'-Dichlorofluorescein-Diacetate (DCFH-DA), 5, 5'-Dithiobis [2-Nitrobenzoic acid] (DTNB), L-penicillamine (L-2-amino-3-mercapto-3-methylbutanoic acid), Diethylene Triamine Pentaacetic Acid (DTPA) were purchased from Sigma Co. (St. Louis, MO, USA). The 6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) The high quality 2', 7'-

Dichlorofluorescein Diacetate (DCFH-DA) and dihydrochloramine 123 (DHR 123) and ONOO<sup>-</sup> were purchased from Molecular Probes (Eugene, Oregon, USA) and Cayman (Ann Arbor, MI, USA), respectively. Nalbuphine, Indomethacin, Diclofenac-Na was collected from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals and reagents were of analytical grade.

**Preparation of plant extract:** The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve No. 40 and stored in a tight container. The dried powder material (500 g) was refluxed with MeOH for 3 h. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract (90 g).

## *In vitro* antioxidant activity

**The amount of phenolic compounds and flavonoids:** The total phenolic and flavonoid content of methanolic extract was determined using Folin-ciocalteu reagent (Yu *et al.*, 2002) and aluminium chloride colorimetric method (Chang *et al.*, 2002), respectively. The content of total phenolics in MNNF was calculated from regression equation of the calibration curve ( $y = 0.013x + 0.127$ ,  $r^2 = 0.988$ ) and is expressed as Galic Acid Equivalents (GAE) and the flavonoid contents of the extract was expressed in terms of quercetin equivalent (the standard curve equation:  $y = 0.009x - 0.036$ ).

**Free radical scavenging activity measured by DPPH:** The free radical scavenging activity of MNNF, based on the scavenging activity of the stable 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca *et al.* (2001). The percentage inhibition activity was calculated from  $[(A_0 - A_1)/A_0] \times 100$ , where  $A_0$  is the absorbance of the control and  $A_1$  is the absorbance of the extract/standard. IC<sub>50</sub> value was calculated from the equation of line obtained by plotting a graph of concentration ( $\mu\text{g mL}^{-1}$ ) versus % inhibition.

**Measurement of the ONOO<sup>-</sup> scavenging activity:** The ONOO<sup>-</sup> scavenging activity was measured by monitoring the oxidation of DHR 123 by modifying the method of Kooy *et al.* (1994). The final concentration of the DHR 123 was 5  $\mu\text{M}$ . The background and final fluorescent intensities were measured 5 min after treatment both with and without the addition of authentic ONOO<sup>-</sup>. The DHR 123 was oxidized rapidly by authentic ONOO<sup>-</sup> and its final fluorescent intensity remained unchanged over time. The fluorescence intensity of the oxidized DHR 123 was measured using a microplate fluorescence reader FL 500 (Bio-Tek Instruments Inc.) with excitation and emission

wavelengths of 480 and 530 nm, respectively. The effects were expressed as the percentage of inhibition of the DHR 123 oxidation.  $IC_{50}$  was calculated from the equation of line obtained by plotting a graph of concentration ( $\mu\text{g mL}^{-1}$ ) versus % inhibition.

#### Measurement of the inhibition of the total ROS generation:

Mice kidney homogenates were mixed with or without a suspension of extracts and then incubated with  $12.5 \mu\text{M}$  DCFH-DA, at  $37^\circ\text{C}$  for 30 min. DCFH-DA is a stable compound which easily diffuses into cells and is hydrolyzed by intracellular esterase to yield a reduced non-fluorescent compound, DCFH which is trapped within the cells. The ROS produced by cells oxidize the DCFH to the highly fluorescent 2',7'-Dichlorodihydrofluorescein (DCF). The fluorescence intensity of the oxidized DCF was monitored on a microplate fluorescence spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT) with excitation and emission wavelengths of 460 and 530 nm, respectively (Label and Bondy, 1990).  $IC_{50}$  value was calculated from the equation of line obtained by plotting a graph of concentration versus % inhibition.

#### In vivo analgesic activity

**Animal:** Swiss Albino mice (25-30 g) and Wistar rats (175-250 g) of both sexes were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of 5 animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh. Animal treatment and maintenance for acute toxicity and analgesic effects were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Atish Dipankar University of Science and Technology, Dhaka, Bangladesh.

**Acute toxicity study:** Acute oral toxicity assay was performed in healthy nulliparous and non-pregnant adult female Albino Swiss mice (25-30 g) divided into different groups. The test was performed using increasing oral dose of the MNMF in water (50, 100, 200, 500, 1000  $\text{mg kg}^{-1}$  body weight), in 20 ml  $\text{kg}^{-1}$  volume to different test groups. Normal group received water. The

mice were allowed to feed *ad libitum*, kept under regular observation for 48 h, for any mortality or behavioral changes (Sanmugapriya and Venkataraman, 2006).

**Hot plate method:** The animals were divided into four groups with five mice in each group:

- Group I animals received vehicle (1% Tween 80 in water,  $10 \text{ mL kg}^{-1}$  body weight)
- Group II animals received nalbuphine at  $10 \text{ mg kg}^{-1}$  body weight
- Group III and Group IV animals were treated with 100 and  $200 \text{ mg kg}^{-1}$  body weight (p.o.) of the MNMF

The animals were placed on Eddy's hot plate kept at a temperature of  $(55 \pm 0.5)^\circ\text{C}$ . A cut off period of 15 sec, was observed to avoid damage to the paw (Malairajan *et al.*, 2006). Reaction time was recorded when animals licked their fore or hind paws or jumped prior to 0, 30, 60 and 90 min after oral administration of the samples.

**Acetic acid-induced writhing test:** The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% v/v acetic acid but Diclofenac-Na was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as writhing for the next 10 min (Ahmed *et al.*, 2004).

**Formalin test:** The antinociceptive activity of the drugs was determined using the formalin test described by Dubuisson and Dennis (1977). Control group received 5% formalin. About  $20 \mu\text{L}$  of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of MNMF ( $100$  and  $200 \text{ mg kg}^{-1}$ , p.o.) and 30 min after administration of Diclofenac Na ( $10 \text{ mg kg}^{-1}$ , i.p.). The mice were observed for 30 min after the injection of formalin and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch.

#### Anti-inflammatory activity

**Carrageenan induced paw edema test in rats:** Wistar rats (175-250 g) of both sexes were divided into four groups of 5 animals each. The test groups received 100 and  $200 \text{ mg kg}^{-1}$  body weight, p.o. of the extract. The reference group received indomethacin ( $10 \text{ mg kg}^{-1}$  body weight, p.o.) while the control group received  $3 \text{ mL kg}^{-1}$

body weight normal saline. After 1 h, 0.1 mL, 1% carrageenan suspension in normal saline was injected into the subplanar tissue of the right hind paw (Winter *et al.*, 1962). The paw volume was measured at 1, 2, 3 and 4 h after carrageenan injection using a micrometer screw gauge.

**Statistical analysis:** All values were expressed as the mean $\pm$ SEM of three replicate experiments. The analysis was performed by using SPSS statistical package for Windows (version 16.0; SPSS Inc, Chicago). Results related to the reducing power activities were statistically analyzed by applying the Student t-test and  $p < 0.001$  were considered to be statistically significant. All *in vivo* data are subjected to ANOVA followed by Dunnett's test and  $p < 0.05$  were considered to be statistically significant.

## RESULTS AND DISCUSSION

**Acute toxicity studies:** The acute toxicity studies mainly aim at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. MNNF was safe up to a dose of 1000 mg kg<sup>-1</sup> (p.o.) body weight. The extract did not cause mortality in mice and rats during 48 h observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

### *In vitro* antioxidant activity

**Total phenolic and flavonoid contents:** Table 1 represents the content of both groups in MNNF extract. The content of total phenolics in the extract of *N. nouchali* was determined using the Folin-ciocalteu assay and found to be 106.33 $\pm$ 1.02 mg gm<sup>-1</sup> GAE using regression equation of the calibration curve ( $y = 0.013x + 0.127$ ,  $r^2 = 0.988$ ) and is expressed as Galic Acid Equivalents (GAE) and the flavonoid contents of the extract (52.16 $\pm$ 0.61 mg gm<sup>-1</sup> QA) was expressed in terms of quercetin equivalent (the standard curve equation:  $y = 0.009x - 0.036$ ).

**DPPH radical scavenging activity:** The percentage scavenging of DPPH radical was found to be concentration dependent with the IC<sub>50</sub> value of 10.33 $\pm$ 0.16  $\mu$ g mL<sup>-1</sup>, while IC<sub>50</sub> value of standard ascorbic acid was found to be 10.10 $\pm$ 0.02  $\mu$ g mL<sup>-1</sup> (Table 2).

**Peroxynitrite (ONOO<sup>-</sup>) scavenging activity:** The ONOO<sup>-</sup> scavenging activity was measured by monitoring the oxidation of DHR 123. The MeOH extract of MNNF exhibited significant ONOO<sup>-</sup> scavenging effects in a dose dependent manner, with IC<sub>50</sub> values of 20.16 $\pm$ 0.61  $\mu$ g mL<sup>-1</sup> whereas, penicillamine a well-known ONOO<sup>-</sup> scavenger, with IC<sub>50</sub> value of 8.20 $\pm$ 0.32  $\mu$ g mL<sup>-1</sup> (Table 2).

Table 1: Yield, total amount of plant phenolic compounds and flavonoids content of *Nymphaea nouchali* flower extract

Sample	Yield (%)	<sup>a</sup> Total phenols mg g <sup>-1</sup> plant extract (in GAE)	<sup>b</sup> Total flavonoids mg g <sup>-1</sup> plant extract (in QA)
MNNF	39.92	106.33 $\pm$ 1.02	52.16 $\pm$ 0.61

The GAE, QA and ASC values are expressed as mean $\pm$ SEM of triplicate experiments; <sup>a</sup>Galic Acid Equivalents (GAE, mg g<sup>-1</sup> of each extract) for the total phenolic content; <sup>b</sup>Quercetin equivalent (QA, mg g<sup>-1</sup> of each extract) for the total flavonoid content

Table 2: Scavenging/inhibitory effects of the *Nymphaea nouchali* flowers extract against DPPH, ONOO<sup>-</sup> and Total ROS generation

Sample	DPPHIC <sub>50</sub> ( $\mu$ g mL <sup>-1</sup> )	ONOO <sup>-</sup> IC <sub>50</sub> ( $\mu$ g mL <sup>-1</sup> )	ROSIC <sub>50</sub> ( $\mu$ g mL <sup>-1</sup> )
MNNF	10.33 $\pm$ 1.02*	20.16 $\pm$ 0.61*	31.72 $\pm$ 0.48*
Ascorbic acid	10.10 $\pm$ 0.02	-	-
L-penicillamine	-	8.20 $\pm$ 0.32	-
Trolox	-	-	12.32 $\pm$ 0.19

IC<sub>50</sub> values are mean $\pm$ SEM (n = 3); \* $p < 0.001$  by Student t-test for values between the sample and the control

Table 3: Effects of the *Nymphaea nouchali* flowers on acetic acid induced writhing in mice

Groups	Dose (mg kg <sup>-1</sup> )	No. of writhing	Inhibition (%)
I	Vehicle	37.40	-
II	10	10.80	71.12*
III	100	21.80	41.71*
IV	200	12.07	67.72*

Values are mean $\pm$ SEM (n = 5); \* $p < 0.05$  as compared to vehicle control (One way ANOVA followed by Dunnett test); Group I animals received vehicle (1% Tween 80 in water); Group II received Diclofenac Na 10 mg kg<sup>-1</sup> body weight; Group III and Group IV were treated with 100 and 200 mg kg<sup>-1</sup> body weight (p.o.) of the MNNF

**Inhibition of total ROS generation:** The percentage inhibition of ROS generation was illustrated in Table 2 and it is observed that scavenging of ROS by the extract is also concentration dependent with the IC<sub>50</sub> value of 31.72 $\pm$ 0.48  $\mu$ g mL<sup>-1</sup> while IC<sub>50</sub> value of standard trolox was found to be 12.32 $\pm$ 0.11  $\mu$ g mL<sup>-1</sup>.

### *In vivo* analgesic activity

**Hot plate method:** Result of hot plate test is shown in Fig. 1. Both doses of the extract produced a dose dependent increase in latency time when compared with the vehicle. The result was found to be statistically significant ( $p < 0.05$ ).

**Acetic acid-induced writhing test:** Table 3 shows the effects of the extract of on acetic acid-induced writhing in mice. The oral administration of both doses of MNNF significantly ( $p < 0.05$ ) inhibited writhing response induced by acetic acid in a dose dependent manner.

**Formalin test:** MNNF (100 and 200 mg kg<sup>-1</sup>, p.o.) significantly ( $p < 0.05$ ) suppressed the licking activity in either phase of the formalin-induced pain in mice in a dose dependant manner (Table 4).

Table 4: Effect of *Nymphaea nouchali* flowers extract on hindpaw licking in the formalin test in mice

Groups	Dose (mg kg <sup>-1</sup> )	Early phase (sec)	Protection (%)	Late phase (sec)	Protection (%)
I	Vehicle	35.16±1.38	-	41.00±1.03	-
II	10	14.83±0.90*	52.82	16.83±0.70*	58.59
III	100	28.50±0.76*	18.94	23.50±0.95*	42.68
IV	200	19.00±0.65*	45.96	17.67±1.46*	56.90

Values are mean±SEM (n = 5); \*p<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test); Group I animals received vehicle (1% Tween 80 in water); Group II received Diclofenac Na 10 mg kg<sup>-1</sup> body weight; Group III and Group IV were treated with 100 and 200 mg kg<sup>-1</sup> body weight (p.o.) of the MNMF

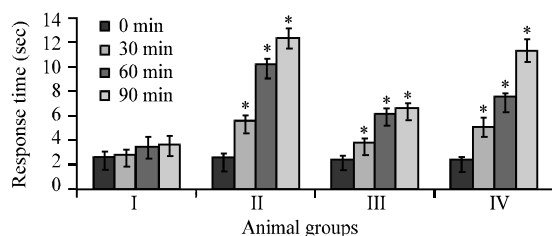


Fig. 1: Effects of the MNMF on latency to hot plate test; values are mean±SEM (n = 5); \*p<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test); Group I: animals received vehicle (1% Tween 80 in water); Group II: received Nalbuphine 10 mg kg<sup>-1</sup> body weight; Group III and Group IV were treated with 100 and 200 mg kg<sup>-1</sup> body weight (p.o.) of MNMF

### Anti-inflammatory activity

**Carrageenan induced paw edema test:** Figure 2 shows the results of the anti-edematous effects of orally administered methanolic extract of *N. nouchali* on carrageenan induced paw edema in rats. MNMF showed dose dependent anti-inflammatory activity and statistically significant (p<0.05). MNMF showed remarkable anti-inflammatory effects at 200 mg kg<sup>-1</sup> dose (71.06% inhibition), whereas standard indomethacin showed 78.02% of inhibition of paw edema.

The upshots of oxidative stress are serious and sometimes manifested by increased activities of enzymes involved in oxygen detoxification (Lompo *et al.*, 2007). Therefore, the identification of new antioxidant may reduce the risk of various chronic diseases involved in free radicals. The knowledge of total antioxidant activity can be useful in the analysis of changes in plasma antioxidant activity related to oxidative stress or the understanding of structure activity relationships of pure antioxidant species. DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and is usually used as a substrate to evaluate the antioxidant activity of a compound (Nakayama *et al.*, 1993). Based on the data obtained from this study, DPPH radical scavenging activity of MNMF (IC<sub>50</sub> 10.33±0.16 µg mL<sup>-1</sup>) was similar to the standard ascorbic acid (IC<sub>50</sub> 10.10±0.02 µg mL<sup>-1</sup>).

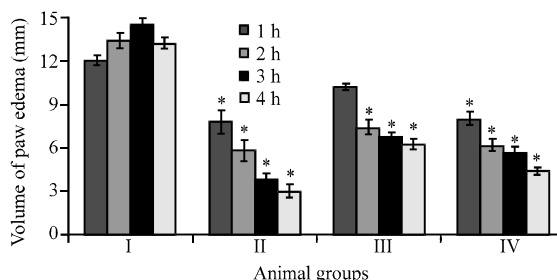


Fig. 2: Effects of the MNMF on carrageenan induced paw edema Test. Values are mean±SEM (n = 5); \*p<0.05 as compared to vehicle control (One way ANOVA followed by Dunnet test); Group I animals received vehicle (1% Tween 80 in water); Group II received indomethacin 10 mg kg<sup>-1</sup> body weight; Group III and Group IV were treated with 100 and 200 mg kg<sup>-1</sup> body weight (p.o.) of the MNMF

Moreover, it was revealed that MNMF did show the proton donating ability and could serve as free radical inhibitor or scavenger. Polyphenolic compounds, like flavonoids, tannins and phenolic acids commonly found in plants have been reported to have multiple biological effects including antioxidant activity (Kahkonen *et al.*, 1999). Phenolic compounds are understood to induce the cellular antioxidant system; increase approximately 50% cellular glutathione concentration. *Nymphaea nouchali* flowers are rich in phenol, polyphenol and tannin (Arambewela *et al.*, 2005) and may responsible for causing the paramount antioxidant effect which is supported to the previous study (Dasgupta and De, 2004; Nagavani and Rao, 2010; Rakesh *et al.*, 2009).

The hot plate method is commonly used for assessing central antinociceptive response involving higher brain functions and is a supraspinally organized response (Chapman *et al.*, 1985). Narcotic analgesics inhibit both peripheral and central mechanism of pain while non steroidal anti-inflammatory drugs inhibit only peripheral pain (Elisabetsky *et al.*, 1995; Pal *et al.*, 1999). As noted, nalbuphine the reference narcotic analgesic drug (5 mg kg<sup>-1</sup>, p.o.) exhibited significant and paramount analgesic effects in the hot plate (supra spinal) test, whereas MNMF (100 and 200 mg kg<sup>-1</sup>, p.o.) also produced a statistically significant but lesser in

degree antinociceptive response to that of nalbuphine suggesting that the plant extract may act as a narcotic analgesic.

On the other hand, acetic acid induced writhing response is a sensitive procedure to evaluate peripherally acting analgesics and represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid (Ahmed *et al.*, 2006). The response is thought to be mediated by peritoneal mast cells (Ribeiro *et al.*, 2000) acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Hossain *et al.*, 2006). The organic acid has also been postulated to act indirectly by inducing the release of endogenous mediators which stimulates the nociceptive neurons that are sensitive to NSAIDs and narcotics (Adzu *et al.*, 2003). It is well known that non-steroidal anti-inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process (Alam *et al.*, 2012). Therefore, it is likely that MNMF might have exerted its peripheral antinociceptive action by interfering with the local reaction caused by the irritant or by inhibiting the synthesis, release and/or antagonizing the action of pain mediators at the target sites. The above findings clearly demonstrated that both central and peripheral mechanisms are involved in the antinociceptive action of MNMF. Interestingly, compounds like flavonoids (Kim *et al.*, 2004) and steroids, triterpenes in part, have been shown to possess anti-inflammatory, analgesic activity and the claim made by Pritam *et al.* (2011). Based on the classes of compounds detected in MNMF extract, several mechanisms of action could be used to explain the observed activities of MNMF extract.

The formalin model normally postulates the site and the mechanism of action of the analgesic. This biphasic model is represented by neurogenic (0-5 min) and inflammatory pain (15-30 min), respectively (Hunskar and Hole, 1987). Drugs that act primarily on the central nervous system such as narcotics inhibit both as steroids and NSAIDs suppress mainly the late phase (Adzu *et al.*, 2003). The suppression of neurogenic and inflammatory pains by the extract might imply that it contains active analgesic principle that may be acting both centrally and peripherally. This is an indication that the extract can be used to manage acute as well as chronic pain. The mechanism by which formalin triggers C-fibers activation remained unknown for a relatively long time. Recently, however, McNamara *et al.* (2007) demonstrated that formalin activates primary afferent neurons through a

specific and direct on TRPA1, a member of the transient receptor potential family of cation channels, expressed by a subset of C-fiber nociceptors and this effect is accompanied by increased influx of  $\text{Ca}^{2+}$  ions. TRPA1 cation channels at primary sensory terminals were also reported to mediate noxious mechanical stimuli (Kerstein *et al.*, 2009). These experiments suggest that  $\text{Ca}^{2+}$  mobilization through TRPA1 cation channels is concomitant with noxious chemicals and mechanical stimuli as they produce their analgesic action. It is likely that the inhibitory effect of MNMF to pain response is due to inhibit the increase of the intracellular  $\text{Ca}^{2+}$  through TRPA1, presumably evoked by formalin. So, MNMF may contain substances that affect the metabolism of  $\text{Ca}^{2+}$ .

Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (Gupta *et al.*, 2006). Since, the extract significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin whose mechanism of action is inhibition of the cyclooxygenase enzyme. Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Sawadogo *et al.*, 2006). This hypothesis is strongly supported by the previous study, which has shown that *Nymphaea nouchali* possess anti-inflammatory activity due to the presence of high flavonoid content (Karthiyayini *et al.*, 2011). In addition, the release of ROS and excessive Nitric Oxide (NO) due to the activation of neutrophils during tissue damage and inflammation which is responsible a variety of disease (Koblyakov, 2001; Bhandare *et al.*, 2010).

## CONCLUSION

The results of the experiments conclude that *Nymphaea nouchali* may be used as an alternative or supplementary herbal remedy for the treatment of analgesic and inflammatory disease. Thus, the present study warrants further investigation involving

components of *Nymphaea nouchali* for possible development of new class of analgesic and anti-inflammatory drugs.

## RECOMMENDATIONS

Recent findings (Viana *et al.*, 2003) suggest that polyphenols are potent inhibitors of NO synthase activity and NO production. As MNNF showed significant free radical as well as ONOO<sup>-</sup> scavenging activity, so this can be responsible for the reduction of inflammation in the carrageenan induced paw edema in rats.

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