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INVESTIGATION OF *IN-VITRO* THROMBOLYTIC AND *IN-VIVO* ANTIPYRETIC, ANTIDEPRESSANT POTENTIALITY AND ANALGESIC ACTIVITY WITH PHYTO-CHEMICAL NATURE OF METHANOLIC EXTRACT OF *NYMPHOIDES HYDROPHYLLA*

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Keywords:

Nymphoides hydrophylla, Thrombolytic activity, Antipyretic activity, CNS depression, Analgesic effect

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ABSTRACT: *Nymphoides hydrophylla* (NH) is mainly an aquatic herb of Menynthaceae family and traditionally used to treat fever, insect bites, different ulcers, and also to wash skin, where parasitic affection occurs. In this study, methanolic extract of *Nymphoides hydrophylla* (NH) plant was examined for the determination of thrombolytic, antipyretic, Central Nervous System (CNS) and analgesic activities. The evaluation of thrombolytic potential, antipyretic, CNS and analgesic activities of the plant extract occurred by their clot lysis, temperature reducing, and depression-inducing and pain sensation decreasing ability respectively. The result from the study showed the thrombolytic activity 3.05 ± 0.70% to 26.45 ± 2.18% indicating the better thrombolytic activity at a lower dose. The maximum pyrexia suppression of 91.07% is observed for 400 mg/kg NH extract. In hole cross and open field tests, maximum 71.42% and 66.80% suppression of locomotor activity were observed with the methanolic NH extract (400 mg/kg) whereas suppression of the locomotor activity of the standard drug diazepam was 68.57% and 51.20% respectively. From the analgesic data, about 91.72% inhibition was observed for 400 mg/kg of NH extract. Statistical calculation was carried out by using one-way ANOVA followed by Dennett's multiple comparisons tests, where statistically significant values from control *P<0.05, **P<0.01, ***P<0.001. Finally, this investigation indicates that due to having a potent bioactive compound in case of CNS disorder and pain sensation, *N. hydrophylla* could be a natural alternative medication of depressant and analgesic agent.

INTRODUCTION: Human and plants are inalienable parts of nature. This is assumed that in the health care of every individual and community, medicinal plants have greater importance, although about 4000 million people of the world are entirely dependent on herbal medicine till today.

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In recent years, medical science has experienced dramatic changes, and surprisingly, every year the global traditional herbal medicine market is growing, and it is anticipated that within 2050 this market will reach to 5 trillion dollars [1, 2]. Human beings are affected by various diseases throughout their whole life, such as neurological disorder, fever, blood clotting, inflammation, *etc*. Depressants are referred to as downers, which reduce stimulation by reducing neurotransmission levels [3]. Fever or pyrexia occurs when body temperature exceeds the normal range of body temperature [4].

Again in case of stopping bleeding from the body (if cut, for example), blood clotting is good and lifesaving, but this is very unnecessary when it causes a stroke or heart attack [5]. Moreover, inflammation is a biological or protective response of the body, where immune cells, molecular mediators, and blood vessels are involved.

By inflammation, necrotic cells, damaged tissues, injured cells, *etc*. are cleared out from the body, resulting in pain, swelling, redness and heat production [6]. Although understanding inflammation has always been an enigma for mankind, it is considered that inflammation is characterized by a biological response of tissues in the body. This response may involve a complex array of activation of cellular enzymes followed by labialization as well as disruption of cell membranes.

Therefore, stabilization of cell membrane could be a significant target for anti-inflammatory action [7]. Different medicines are used for the treatment of this disease, but they cause different side effects after administration [8, 9, 10, 11, 12]. Again different synthetic drugs - antidepressant, anticoagulant and antipyretic, which are used for the treatment of neurological diseases, heart attack and fever respectively, have some side effects as well [13, 14, 15, 16, 17]. That's why for finding a better treatment of those diseases with fewer side effects, plants could be the most reliable sources.

Nymphoides hydrophylla (NH) is mainly an aquatic herb of Menynthaceae family, and it is found in Bangladesh, India, Nepal, Bhutan, Myanmar, Malaysia, Indonesia, Cambodia, *etc*. It is commonly or locally known by different names in Bangladesh, such as Panchulli, Chandmona, Chandmala. NH contains crude fat, protein, carbohydrate, fiber, *etc.*, along with gallic acid, catechin, ferulic acid, *etc*. Its useable parts are leaves and stalks, which are pulverized with oil and used for various purposes [18, 19, 20].

In our present study, after considering the traditional use and phytochemical constituents of NH, we have determined the neuropharmacological, thrombolytic, antipyretic and membrane stability or anti-inflammation effects of a phytochemically screened extract of NH leaves.

MATERIALS AND METHODS:

Collection of Materials: For this present study, NH was collected from Noakhali Science & Technology University, Noakhali, Bangladesh in the year of 2017. By the experts of Bangladesh National Herbarium, the stem was identified, which is situated in the Mirpur, Dhaka, Bangladesh. Diazepam, Streptokinase vial, Diclofenac, Aspirin was collected from Incepta Pharmaceuticals Ltd, Bangladesh. All other analytical grades reagents were collected from the Pharmacy department of Noakhali Science and Technology University (NSTU).

Experimental Mice: From the Pharmacy Department of Jahangirnagar University, both sexes Swiss albino mice (25-30g) were obtained. At 20 ± 5 ºC, they were kept in metal cages. Mice were placed in the suitable environment (relative humidity 55-65%, room temperature 23.0 ± 2.0 °C and 12 h light-dark cycle) throughout the experimental period and the study has been conducted following the guideline of The National Institutes of Health (NIH). The experimental mice were fed with Rodent diet and pure water.

Preparation of NH Extract: Collected plant part (stem) was separated from undesirable materials. It was then washed and dried in the sun. The plant stem was ground into a coarse powder with the help of a suitable grinder and stored in a conditioned room. In this process powered plant materials are submerged in methanol for several days, with irregular stirring and shaking. Then the desired extracts were taken through separation and evaporation. Powered material of NH was soaked in distilled methanol for 20 days. The whole mixture then filtered through Whatman filter paper after coarse filtration. Then it was evaporated until dried. The brown granular was obtained as a crude extract of methanol.

Experimental Design: For CNS activity, 24 mice, for analgesic test 12 mice and anti-pyrexia test, 20 mice totaling 56 mice were selected and then for all the test, separately allocated them into 4 groups, where each group was formed by either three or five mice. For both the tests, Group-1 served as controls, Group-2 for standard and Group-3, Group-4 received experimental NH extract.

Test Method for Evaluating Different Activities of NH Extract: Thrombolytic potential activity, antipyretic activity, CNS activity and analgesic activity of the NH plant extracts were examined here. Thrombolytic potential tests were done by the *in-vitro* method, whereas antipyretic activity, CNS activity, and analgesic activity tests were carried out into mice in a quiet laboratory at 20-22 °C.

Assessment of Thrombolytic Potential: A method developed by Prasad *et al*., was used for the assessment of the *in-vitro* thrombolytic activity of *Nymphoides hydrophylla* extract using Streptokinase (at 15000 and 30000 I.U) as a positive control with minor modifications [21]. 5 ml blood had drawn from healthy volunteers (n = 3) and transferred to the micro-centrifuge tube (1 ml/tube) for incubation for 45 min at 37 °C. After clot formation, measured the weight and 100 µl of the plant extract with various concentrations (2, 4, 6, 8 & 10 mg/ml) suspended overnight was added to the tubes accordingly. 100 µl of streptokinase as a positive control and 100 µl of sterilized distilled water as a negative non-thrombolytic control were added to the control tubes. All tubes were incubated again for 90 min at 37 °C and observed for clot lysis. Finally, the differences in weight taken before and after clot lysis were expressed as a percentage of clot lysis following the under beneath equation.

$$\% \text{ of clot lysis} = \text{Wt. of released clot/clot wt.} \times 100$$

$$= W_2 - W_3 / W_2 \times 100$$

Here, W_2 = Weight of clot after 45 min incubation (gm), W_3 = Weight of lysised clot after 90 min incubation (gm).

Antipyretic Activities Test: Antipyretic activity on albino rats was studied with fever induced by 10 mg/kg DNP [22]. Fever was induced by intraperitoneal injection (IP) of 20% 2, 4 – dinitrophenol (DNP) suspension at a dose of 20 ml/kg of body weight. The animals were divided into five groups each of five mice. All groups were fasted overnight but allowed free accesses to drink water. The body temperature was measured by a digital clinical thermometer, and after 18 h of DNP injection, mice showed a rising temperature of at least 0.5 °C which was taken for the study. Group, I received saline (10 ml/kg) as a negative control, Group II received acetylsalicylic acid (100 mg/kg) as a standard drug while the remaining groups III and IV received 200 and 400 mg/kg of NH extract respectively. After drugs administration, rectal temperature was again recorded periodically at 1, 2, 3, 4 and 5 h of drugs administration. The percent reduction in pyrexia was calculated by the following formula [23].

$$\text{Movements inhibition } \% = B - C_n / B\text{-}A \times 100$$

Where, B represents temperature after pyrexia induction; C_n temperature after 1, 2, 3, 4 and 5 h and A, normal body temperature.

Assessment of CNS Depressant Activity:

Open Field Test (OFT): This method was followed by Gupta *et al*., 1971 [24]. The test group received NH extract at the doses of 200 and 400 mg/kg whereas the control group received water 10 ml/kg and the standard group received Diazepam 1 mg/kg. The floor of an open field of half square meter was divided into a series of squares each alternatively colored black and white. The apparatus had a wall of 40 cm in height. Mice were placed in the middle of the open field. Then the number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90, and 120 min after the administration of the standard and test drugs. Percentage inhibition of movements was calculated using the following formula:

$$\text{Movements inhibition } \% = [\text{Mean of Movements (Control)} - \text{Mean of movements (Test)} / \text{Mean of Movements (Control)}] \times 100$$

Hole Cross Test (HCT): This method was described by Takagi *et al*., 1971 [25]. A partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at the height of 7.5 cm in the center of the cage. Mice were treated with control, standard or extract and were placed in one side of the cage. On one side, mice were placed after treatment. Through the hole, regular movement of every mouse was observed for 3 min and the time interval was 0 min, 30 min, 60 min, 90 min, and 120 min. Percentage inhibition of movements was calculated by using the same formula of the open field test.

Analgesic Activity: Analgesic activity of the methanolic extract of selected plants was tested using the model of acetic acid-induced writhing in mice [26]. Test samples, control, and diclofenac were

given intraperitoneally. A thirty minutes interval was given to ensure proper absorption of the administered substances.

Then the writhing inducing chemical, an acetic acid solution (0.6%, 0.1 ml/10 g body weight) was administered intraperitoneally to each of the animals of a group. After five minutes, which was given for absorption of acetic acid, a number of squirms (writhing) was counted for 15 min. Each mouse of all groups was observed to count the number of writhing that they had made in 15 min. The percentage of analgesic activity was calculated as follow [27].

% of analgesic activity = Control mean – Treated mean / Control mean × 100

Phytochemical Analysis: Small amount of newly prepared methanolic extracts of *N. hydrophylla* was separately subjected to preliminary qualitative phytochemical investigation for the detection of phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins and phenols using the following standard methods [28, 29, 30, 31].

Test for Alkaloids: 0.2 g of extracts were mixed and shaken with 1 % HCl for two minutes. After filtering the mixture, a few drops of Dragendorff's reagent was added. Formation of a precipitate indicated the presence of alkaloids.

Test for Saponins: 0.2 g of extracts was shaken with 5 ml of distilled water in a test tube. Frothing which persists on warming was taken as evidence for the presence of saponins.

Test for Tannins: 0.2 g of extracts was stirred with distilled water and filtered. A blue-black, green or blue-green precipitate after ferric chloride addition was taken as evidence for the presence of tannins.

Test for Steroids (Salkowski's Test): 0.2 g of the extracts were dissolved in 2 ml of chloroform. Concentrated sulphuric acid was carefully added to form a lower layer. A reddish-brown color at the interphase indicated the deoxy sugar characteristics of cardenolides.

Test for Flavonoids: A little amount of magnesium powder and few drops of concentrated hydrochloric acid were added to 3 ml of the extracts. A red or intense red coloration indicated the presence flavonones.

Test for Phenols: 0.2 g of methanol extract was dissolved in a ferric chloride solution. A green or dirty green precipitate indicated the presence of the phenolic compound.

Statistical Analysis: The results obtained were analyzed by the SPSS software package version 20. The mean values obtained for the different groups were compared by one-way ANOVA, followed by Dunnett's test.

RESULTS:

Phytochemical Screening: Phytochemical screening showed that methanolic extract of *Nymphoides hydrophylla* contains carbohydrates, alkaloids, phytosterols, glycosides, phenols, tannins, and flavonoids. The results are summarized in **Table 1**.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE METHANOLIC EXTRACT OF *NYMPHOIDES HYDROPHYLLA*

S. no.	Phytoconstituents	Methanolic extract
1	Alkaloid	+
2	Flavonoid	+
3	Saponins	-
4	Steroids	+
5	Phenols	+
6	Tannins	+

Thrombolytic Potential: Methanolic extract of NH showed clear, visual representation and dose-dependent manner of clot lysis, which shown in **Table 2**. Minute clot lysis was observed for control, whereas significant clot lysis occurred for streptokinase and different concentration of plant extracts.

TABLE 2: *IN-VITRO* CLOT LYSIS ACTIVITY OF VARIOUS CONCENTRATION OF METHANOLIC EXTRACT OF *NYMPHOIDES HYDROPHYLLA*

Treatment	Concentration; mg/ml	% of clot lysis (Mean ± SEM)
Control	-	5.626±0.57
Streptokinase (15K)	-	26.317±1.27**
Streptokinase (30K)	-	46.60±1.83**
Sample 1	2	26.456±2.18 **
Sample 2	4	24.857±2.19**
Sample 3	6	11.558±1.23*
Sample 4	8	3.972±0.47*
Sample 5	10	3.05±0.70*

Data are reported as mean ± S.E.M. for a group of five animals. The data were analyzed by ANOVA followed by Dunnett's test. Asterisks indicated statistically significant values from control. **P<0.001, *P<0.05.

The percentage of clot lysis was 26.456 ± 2.18, 24.857 ± 2.19, 11.558 ± 1.23, 3.972 ± 0.47 and 3.05 ± 0.70 for different concentration of plant extracts such as 2, 4, 6, 8 and 10 mg/ml and at the dose of 2 mg/ml and 4 mg/ml, methanolic extract showed the highest and significant effect which are found to be almost similar effect given by Streptokinase (15,000 I.U).

Antipyretic Activities Test: The methanolic extract of NH decreased fever and showed profound activity at both doses **Table 3**. Both 400 mg/kg and 200 mg/kg NH methanolic extract showed better activity than standard paracetamol from 1st observation, whereas 400 mg/kg extract showed a better result than 200 mg/kg extract from 3rd h observation, which is significant (P<0.05, P<0.01). The percentage of Pyrexia reduction is shown in **Fig. 1**. Both extracts showed better pyrexia reduction as compared to standard whereas 400 mg/kg doses showed maximum reduction during 4th h observation.

CNS Depressant Activity:

Open Field Test: In an open field test the locomotor activity lowering effect was evident from the 2nd observation (30 min) and continued up to 5th observation period (120 min). The open field test results are presented in **Table 4**. Both doses of NH extract showed a significant effect (P<0.001) at 90 min and 120 min respectively. The percentage of inhibition was increasing with time, and in the case of 400 mg/kg of NH extract, it showed a maximum suppression of the locomotive activity of around 65%. The percentage of locomotive inhibition is shown in **Fig. 2**.

TABLE 3: EFFECT OF NH EXTRACT IN 2,4 –DINITROPHENOL (DNP) INDUCED PYREXIA

Treatment	Dose	Rectal Temperature (°C)						
		Normal (A)	After 18h (B)	C_1	C_2	C_3	C_4	C_5
Saline	10 ml	36.46±0.20	37.20±0.15	37.24±0.09	37.19±0.16	37.18±0.15	37.20±0.16	37.17±0.15
ASA	100	36.64±0.10	37.54±0.16	37.00±0.15*	36.93±0.13*	36.86±0.13	36.77±0.28*	36.82±0.05**
NH Extract	200	36.82±0.05	37.62±0.13	37.24±0.17	36.93±0.07*	36.91±0.06**	36.95±0.07*	36.97±0.05**
NH Extract	400	36.88±0.05	37.63±0.13	37.25±0.11	37.00±0.07	36.95±0.10*	36.92±0.17*	37.02±0.06**

Data are reported as mean ± S.E.M. for a group of five animals. The data were analyzed by ANOVA followed by Dennett's test. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01.

TABLE 4: EFFECT OF NH METHANOLIC EXTRACT ON OPEN FIELD TEST IN MICE

Treatment	Dose	No square travel (Mean ± SEM)				
		0 min	30 min	60 min	90 min	120 min
Saline	10 ml/kg	82.33±2.60	72.66±3.92	72.66±10.94	71.66±6.22	72.66±9.38
Diazepam	1 mg/kg	71.66±2.40*	50.66±5.14	39.33±2.91	30.33±1.85**	31.33±3.84*
NH extract	200mg/kg	70.66±2.33*	58.33±3.48	44.66±4.48*	30.33±4.70**	26.66±6.01**
NH extract	400mg/kg	71.33±2.96*	49.33±5.81*	39.33±5.48*	26.66±7.26**	17.67±10.35*

Data are reported as mean ± S.E.M. for a group of three animals. The data were analyzed by ANOVA followed by Dennett's test. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01.

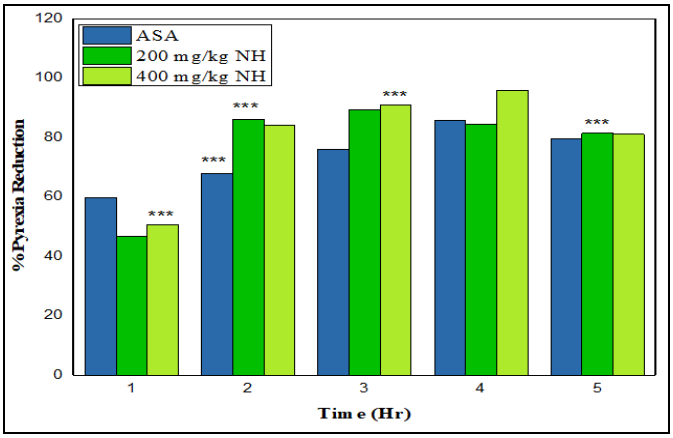

FIG. 1: ANTIPYRETIC EFFECT OF NH EXTRACT IN MICE. BAR PRESENTS THE PERCENT INHIBITION OF PYREXIA AFTER 1,2,3,4 AND 5 h OF THE TREATMENT WITH ASA (100 mg/kg) AND NH EXTRACT (200/400 mg/kg). The data was analyzed by Anova followed by Dunnett's test. Asterisks indicated statistically significant values from control. ***P<0.001. (ASA- Acetyl salicylic acid, NH- *Nymphoides hydrophylla*)

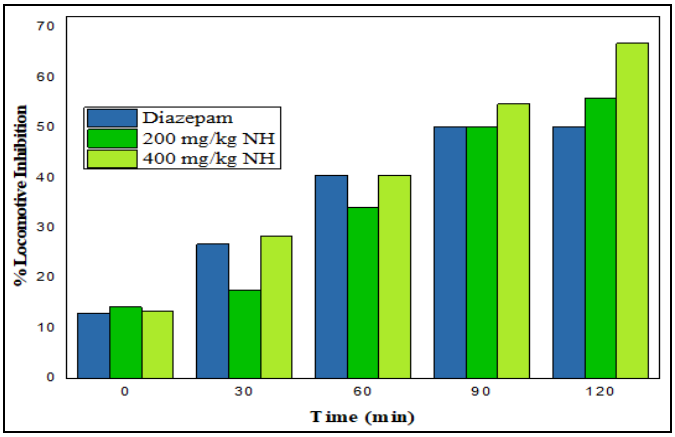

FIG. 2: CNS DEPRESSANT ACTIVITY NH EXTRACT IN MICE IN RESPECT OF OPEN FIELD TEST. Bar presents the percent inhibition of movement from 0-120 min of the treatment with Diazepam (1 mg/kg) and NH extract (200/400 mg/kg); NH- *Nymphoides hydrophylla*)

Hole Cross Test: The validation of the experiment on CNS depressant effect of *Nymphoides hydrophylla* was carried out by measuring external signs, through hole cross test and results are presented in **Table 5**. In this test, maximum 65.71% and 62.87% suppression of locomotor activity were exhibited with the 200 mg/kg and 400 mg/kg of NH extract respectively, whereas the standard drug Diazepam displayed 68.57% suppression **Fig. 3**. This data ensures that the result is dose-dependent.

TABLE 5: EFFECT OF NH METHANOLIC EXTRACT ON HOLE CROSS TEST IN MICE

Treatment	Dose	No of hole cross (Mean ± SEM)				
		0 min	30 min	60 min	90 min	120 min
Saline	10 ml/kg	18.67±0.66	16.67±1.66	13.67±0.33	12.33±0.88	11.67±1.20
Diazepam	1 mg/kg	12.67±0.33***	8.33±1.33*	5.67±0.33***	4.667±1.20**	3.67±1.20**
NH extract	200mg/kg	14.33±1.45*	10.33±1.45**	9.67±0.33**	6.33±0.88**	4±0.57**
NH extract	400mg/kg	12.67±033***	8.33±2.33**	6.33±2.02*	5±0.57**	3.33±1.20**

Data are reported as mean ± S.E.M. for a group of three animals. The data were analyzed by ANOVA followed by Dennett's test. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01, **P<0.001

Analgesic Effect (Acetic Acid-Induced Writhing Response): The study showed that the application of different doses of NH extract had significant analgesic effects in the animals under investigation **Table 6**. The methanol extract at the dose of 400 mg/kg and 200 mg/kg body weight produced highly significant (P<0.001) reduction in the number of writhings produced by acetic acid in mice when compared to untreated group. The methanol extract at the dose of 400 mg/kg and 200 mg/kg inhibited writhing by 91.72% and 85.71% respectively which was higher than diclofenac sodium of 87.97%.

TABLE 6: EFFECT OF NH METHANOLIC EXTRACT ON ACETIC ACID-INDUCED WRITHING RESPONSE IN MICE

Treatment	Dose	No of writhing (15 min)	% of inhibition
Saline	10 ml/kg	44.33±2.40	-
Diclofenac	50 mg/kg	5.33±1.20***	87.97
NH extract	200 mg/kg	6.33±1.76***	85.71
NH extract	400 mg/kg	3.67±0.88***	91.72

Data are reported as mean ± S.E.M. for a group of three animals. The data were analyzed by ANOVA followed by Dennett's test. Asterisks indicated statistically significant values from control. ***P<0.001.

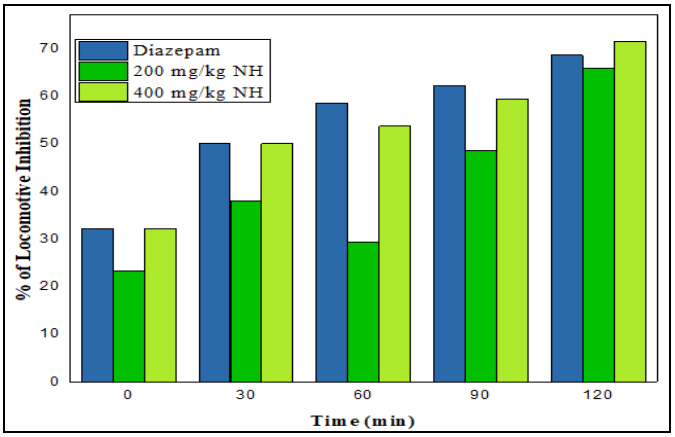

FIG. 3: CNS DEPRESSANT ACTIVITY NH EXTRACT IN MICE IN RESPECT OF HOLE CROSS TEST. Bar presents the percent inhibition of movement from 0-120 min of the treatment with Diazepam (1 mg/kg) and NH extract (200/400 mg/kg); NH- *Nymphoides hydrophylla*

DISCUSSION: A natural fibrinolytic agent called plasmin which helps to initiate the breakdown of fibrinogen and fibrin resulting in lysis of a blood clot. The standard SK forms a complex with plasminogen to change plasmin [32]. As the extract of *Nymphoides hydrophylla* contains flavonoids, tannin, phenols, and alkaloid in the crude extract as revealed by phytochemical screening, it could be predicted that mainly tannin and alkaloid phytochemicals may confirm its clot lysis activity. Fever means increased body temperature which is a complex response of body physiology, and it occurred due to increased prostaglandin E2 (PGE2) concentration [33]. Antipyretics such as paracetamol used in the management of fever act by reducing levels of prostaglandins acting on cyclooxygenase enzymes, enhancing antipyretic message within the brain and stimulating anti-inflammatory signals at injury site [34]. This NH extracts here reduced fever by decreasing PGE2 concentration in the brain or by increasing arginine and vasopressin production, which are the body's antipyretic substances [35]. The antipyretic activity is due to the presence of steroids [36].

Central Nervous System (CNS) excitability is measured by locomotors activity. When motor activity is increased, it means alertness, and when decreased, then this is the sign of sedative effect [37]. By HCT and OFT locomotors, an activity of NH was measured. In HCT, decreased motor activity showed from 1st to 5th observation at both doses. Different sedative-hypnotic, anxiolytic drugs give their activity through GABA. Much evidence has suggested that CNS depressant barbiturates like Thiopental sodium binds to the barbiturate binding site on the GABA receptor complex and stimulate GABA mediated hyperpolarization of postsynaptic neurons [38].

So, this NH extract may give depressant activity via hyperpolarization of the membrane through potentiating GABAergic inhibition, which decreases the firing rate in the brain [39]. Again phytochemical investigation of methanol extract *Nymphoides hydrophylla* under this study explored the presence of medicinally active secondary metabolites alkaloids, steroids, tannins, and flavonoids. The sedative and anxiolytic potential may be due to the presence of those secondary metabolites.

Acetic acid-induced writhing is a well-recommended protocol in evaluating medicinal agents for their analgesic property. Free arachidonic acid released from tissue phospholipids *via* cyclooxygenase (COX) usually produces prostaglandin specifically PGE2 and PGF2α resulting in pain sensation. This prostaglandin along with lipoxygenase products causes inflammation and pain by increasing capillary permeability. As suggested by peripheral mechanism substances having analgesic activity preferably inhibit the prostaglandin [40]. Our findings strongly recommend that *Nymphoides hydrophylla* has peripheral analgesic activity and their mechanisms of action may be mediated through inhibition of local peritoneal receptors. Some studies conducted on various medicinal plants revealed the presence of secondary metabolites like flavonoids and alkaloids had been linked to possessing analgesic, antipyretic, and other properties.

Thus, all of the medicinal activity observed in this study can be assumed due to the presence of one or several phytoconstituents detected in the plant. The various components of each extract and their bioactive compounds were not separated which remains the limitations in the study.

CONCLUSION: The *in-vitro* test for thrombolytic and *in-vivo* test for CNS depressant, antipyretic activity and analgesic activity of NH methanolic extract showed positive results.

So, it can be said that this plant can give a beneficial effect in reducing fever, decreasing neurological problem, anti-coagulating for blood and pain removal. And that's why; further investigation is needed for finding out the main active compounds, which are responsible for this significant effect.

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Consent for Publication: Not Applicable

Authors' Contributions: Sourav Roy and Md. Mobarak Hosen designed the experiment and experimented. Shovon Bhattacharjee and Abhijit Das supervised the work. Shujit Chandra Paul and Tutun Das Aka draft and composed the article. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate: The total study workout, the protocol of the study and the consent forms of the volunteers, were approved by the Institutional Ethical Review Committees of Noakhali Science and Technology University.

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COMPETING INTEREST: The authors declare that they have no competing interests.

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