

First time reported in vitro pharmacological activity of *Heliconia rostrata* (Heliconiaceae) revealed its potency in pain management over a standard analgesic drug

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Abstract

Heliconia rostrata, locally known as Jhulonto Chingri in Bangladesh, is generally found in several woodlands worldwide. Even though *H. rostrata* is mainly farmed for beautification, this plant has diverse medicinal practices around the world. Especially, the traditional medical healers are taking advantages of using several parts of this selected plant against different ailments for thousands of decades. By inspiring from those scientific validations and as a part of identifying and discovering drugs from natural sources, evaluating the analgesic property of this reported medicinal plant was the main phenomenon of this study. The in-vitro analgesic activity of the methanolic extract of *Heliconia rostrata* (MEHR) was studied in Swiss albino mice (experimental animal model) by the acetic acid-induced writhing model. Oral administration of MEHR significantly lessened 23.20, 29.00, 46.38 and 58.00% of the number of writhing respectively of an oral dose of 50, 100, 200 and 400 mg/kg. On the contrary, standard analgesic drug "Aspirin" minimized the number of writhing 39.13 and 55.06% at a dosage of 200 and 400mg/kg respectively. Interestingly, 400mg and 200mg doses respectively of MEHR were more effective to reduce the mean number of abdominal constrictions in acetic acid-induced Swiss albino mice compared to the same doses of Aspirin. For the first time ever, our present study suggests that the MEHR can be considered as a potential natural source for the development of a new analgesic drug to relieve pain. Notably, we found that the MEHR is more potential and efficacious in comparison with the standard drug Aspirin for relieving pain.

Introduction

Pain is mainly associated with our nervous system; this physiological disorder occurs due to various unexpected conditions like a sting, burn, ache, or potential tissue damage and categorized into two folds such as acute and chronic [1]. Among them, acute pain comes suddenly due to sudden injury or inflammation or disease and lasting for a little time whereas chronic pain lasting for a long time of period and considered as incurable by medical treatment. In most cases, aspirin-like non-steroidal anti-inflammatory drugs pose their analgesic activity through selective and irreversibly inactivating cyclooxygenase enzyme to put down the production of prostaglandins by an either peripheral and/or central mechanism. According to the recent statistics of "Centers for Disease Control and Prevention", more than 50 million of adult populations in the USA suffered from chronic pain in 2016 and more than 19.5 million of USA pupils are affected by high impact chronic pain which is one of the most common cause to look for medical treatments [2,3].

Natural drugs mainly originate from nature with various pharmacological effects and keep them in advance due to their lower adverse effect, high potency and efficacy, and safety use rather than pharmaceutical drug [4]. The plants are the most potent source of natural medicines and various parts of these plants are being used to treat many diseases by traditional medical practitioners not only in Bangladesh but also worldwide. Moreover, thousands of medicinal plants have identified effective because of their promising compounds that can act against cancer, diabetes, pain, inflammations, and so many diseases. For example, a number of herbs as well as spices, which are

commonly used in the Indian subcontinent, are proved to have blood glucose reducing properties [5-7]. From last few decades, a series of research works have been studied that illustrates a correlation between the phytochemicals and improved disease conditions. Not only that, plant-based vaccination is gaining its popularity day by day because of their cost-effective production or stability in room temperature. An example would be, hepatitis B virus was successfully ingested into the transgenic plant to introduce plant-based oral hepatitis B virus surface antigen vaccine in the market that has the potential over traditional vaccines [8].

H. rostrata or falsa bird paradise or hanging lobster claw usually augments in the torrid garths as an inviter flower to many host birds, howsoever; their reported medicinal properties are very rare. Locally it is known as "Jhulonto Chingri" in Bangladesh and in Bolivia, it is known as "Patuju" and considers it as their national flower. It is found

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naturally and used to treat diabetes and diabetes associated-edema by the folk medicinal practitioner at the Sylhet division of Bangladesh [9]. Another ethnobotanical survey study reported that the leaf and seed of this plant are used to treat a headache, sprains, and pain by a medical practitioner who lived in a village near Rupsha River at Bagerhat District in Bangladesh [10]. Moreover, the rhizome of this plant is used to treat jaundice, intestinal pain and high blood pressure by the traditional people of Malaysia [11]. Interestingly, some Caribbean uses the leaves of this plant for wrapping food. On the other hand, Brazilians claimed that *Heliconias'* roots and seeds can be used against various diseases and they used this plant traditionally for centuries [12].

According to the best of our knowledge, no pharmacological study regarding the analgesic activity of *H. rostrata* has been carried out yet. This is why we selected *Heliconia rostrata* Ruiz and Pav. to find out new, cheap, effective and side effects free natural drugs targeting pain. We used the methanol extracts of the whole plant of *H. rostrata* to evaluate acetic acid-induced writhing test for analgesic activity in Swiss Albino mice. Notably, this test is a convenient test for measuring writhing number regarding pain management. The focus of this study was to evaluate the analgesic activity of methanolic extract of the full plant of *H. rostrata* and to make the comparison between standard analgesic drug "Aspirin" and methanolic extract of this plant. We also tried to identify the phytochemicals present in the *H. rostrata* which could be responsible for analgesic activities.

Materials and methods

Plant material collection

The whole plant of *H. rostrata* was collected from Rema Kalenga Wildlife Sanctuary in Sylhet Division, Bangladesh in November 2016. After collecting the plant, it was identified from the Bangladesh National Herbarium (BNH).

Methanolic extract preparation

First of all, the whole plants were cut into small pieces and thoroughly dried for pulverizing the whole plants into a fine powder. For preparing the extraction, the powder was mixed with methanol (1:5 ratios) for a period of more than 48 hours. At 50°C methanol was evaporated by using a water bath and then, the extract was preserved at -20°C till use. Before starting oral administration in experimental animals, the extract was dissolved in Tween 20. The percent (%) of plant yield was counted by the equation given below,

$$\text{The percent (\%)} \text{ of plant yield} = (W_2 - W_1) / W_0 \times 100$$

Where, W_2 = Weight of Plant Extract + Weight of Beaker, W_1 = Weight of Beaker, and W_0 = Weight of Crude Powder

Chemicals and drugs

Aspirin was collected from Square Pharmaceuticals Ltd., Bangladesh. Other chemicals including methanol (CH_3OH , 99%), DMSO and tween 20 (as suspending agents collected from Gaylord Chemicals, USA) and, Savlon (as an antiseptic collected from GlaxoSmithKline). An analytical grade presented in all other chemicals.

Animals

3-4 weeks old Swiss albino mice as laboratory animals were collected from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDRDB). Estimated weight of 18-25 gram mice from both sexes was used in this present study. An adaptation required for a period of more than three days, before using the mice to

actual experiments. In these periods of time, the cages were kept in well-ventilation and used to locate the animal under hygienic conditions in the animal house of the University Of Development Alternative, Dhaka, Bangladesh. According to the instruction of ICDDRDB, the room temperature was maintained at 25-27°C and the relative humidity was in the range of 50-55%. Standard mouse chow and water was given to mice as food till experiments. The approval from the Institutional Animal Ethical Committee of the University of Development Alternative, Dhaka, Bangladesh had governed the study.

Evaluation of analgesic activity

The in vitro analgesic activity of the methanolic extract of *H. rostrata* was performed in Swiss albino mice (experimental mice model) following the same protocol as previously described by Akmal Hosain Nipu *et al.* [13]. In brief, experimental mice were divided into seven groups where each group contained five mice. Group-1 was used as a control and was provided vehicle only. On the other hand, group-2 and group-3 administered standard analgesic drug "Aspirin" at a dose 200mg/kg and 400mg/kg respectively. In addition, the remaining groups (group 4-7) were received a methanolic extract of *H. rostrata* at a dosage of 50, 100, 200 and 400 mg/kg, respectively. All substances were orally administrated. After 1 hour of administration of the standard drug (group 2 and group 3) or MEHR (from group 4 to 7), 1% acetic acid was intraperitoneally injected into all mice at a dose of 10 ml per kg. Next, to ensure bioavailability and onset of chemical action for inducing pain in mice, the mice were observed for 5 minutes which was characterized by abdominal constrictions or writhes as previously described by Ahamed Ismail Hossain *et al.* [14]. After this period, the number of abdominal constrictions was counted for 10 minutes. Finally, the percentage of inhibitions of abdominal constrictions which indicate the degree of analgesic effect was calculated using the formula given below:

$$\text{The percent of inhibition} = (1 - \text{We}/\text{Wc}) \times 100$$

Where "We" represents the number of abdominal constrictions or writhings in aspirin or MEHR administrated mice (Groups 2-7),

and "Wc" represents the number of abdominal constrictions or writhings of control mice (Group 1).

Acute toxicity

Thirty mice (3-4 weeks old) from both sexes (male and female) were used to perform acute toxicity test according to the previously described manner with few modifications [15]. In brief, mice were separated into six groups, where each group contained five mice. The average weight of experimental mice was 19 mg. The methanolic extract of *H. rostrata* was administrated at a dose of 100, 200, 400, 600, 800 and 1000 mg/kg from group-1 to group-6, respectively by gavaging. Before performing acute toxicity test, experimental mice were 12 hours fasted and after administration of various doses of MEHR to the selected groups, animals were observed for 72 hours for toxicity related clinical sign like changes in behavior, alertness, restlessness, muscle convulsion, tremor, touch response, torch response, pain response, gripping, writhing, urination, salivation, skin color, food intake, water intake, grooming and mortality. During the observation period, regular food and water were given to mice on a daily basis.

Phytochemical analysis

Alkaloid test: To identify the presence of alkaloid in *Heliconia rostrata*, 0.5 gm of MEHR was dissolved in 5ml of 1% of HCl by using a

conical flask. Next, the conical flask was placed on a water bath for 3-4 minutes at 90° C. Finally, the solution was filtered followed by addition of Wagner reagent (Standard solution of I2 in KI). The formation of brown precipitation indicated the presence of alkaloid.

Flavonoid test: Small amount of methanolic extract of *Heliconia rostrata* was taken in a test tube followed by addition of a few drops of distilled water. Finally, 200 microliters of concentrated HCL acid was added to the extract solution. Quick formation of red color indicated the presence of flavonoids.

Steroid test: Liebermann-Burchard reaction was performed to carry out the steroid test. Initially, MEHR was dissolved in chloroform and wait for 2-3 hours. Next, the solution was evaporated by using a water bath at 55° C. Finally, 1 ml of concentrated H₂SO₄ was drop wisely added to the solution. The formation of red color indicated the presence of flavonoids.

Saponin test: 0.5 gm of methanolic extract was taken with water in a test tube and vigorously shaken the test tube followed by the Frothing test. Persistent frothing developed and its stability in heating indicated the presence of saponins.

Result

Plant identification & yield (%)

The scientific classification of this plant was confirmed by Bangladesh National Herbarium (Table 1) and the accession number of this Heliconiaceae family’s plant was 43730 (Figure 1). We also confirmed various ethnomedicinal properties (reported) of *Heliconia rostrata* (Figure 2) from various literature [9-12]. The final weight of the plant extract was 16.77g whereas the percentage yield of crude extract from the sample of *Heliconia rostrata* was 5.47.

Table 1. Scientific classification of *Heliconia rostrata*

Kingdom	Plantae
Subkingdom	Trachobinota
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Zingiberidae
Order	Zingiberales
Family	Heliconiaceae
Genus	Heliconia
Species	<i>Heliconia rostrata</i> Ruiz & Pav.



Figure 1. *Heliconia rostrata* (left panel; the photo was taken from Remakalenga wildlife sanctuary in Sylhet division) and its primary identification (right panel)

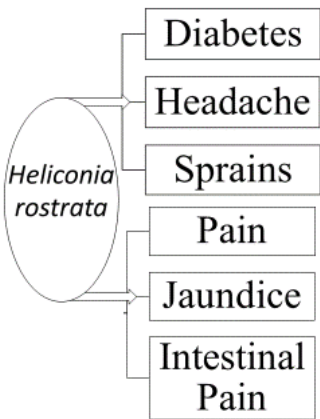


Figure 2. Folkloric uses of *Heliconia rostrata*

Analgesic activity

The analgesic effects of the methanolic extract of *Heliconia rostrata* and reference drug aspirin by acetic acid-induced abdominal writhing in Swiss albino mice is figured out in Table 2. Both MEHR (50, 100, 200 and 400 mg/kg) and standard drug aspirin (200 and 400 mg/kg) showed significant and dose-dependent analgesic activity on the acetic acid-induced abdominal constrictions in mice. In details, after administration of MEHR at a dose of 50, 100, 200 and 400 mg/kg, the reduced number of writhings were 23.20, 29.00, 46.38, and 58.00% respectively, when compared to control group. On the other hand, reference drug Aspirin, when administrated at a dosage of 200 and 400 mg/kg, down-regulated the number of writhings at 39.13 and 55.06% respectively, compared to the control group. However, 400mg/kg dose of MEHR exhibited better analgesic activity than the given doses of Aspirin (both 200 and 400mg/Kg BW). Nevertheless, 200 mg/kg dose of MEHR also showed approximately 1.2 times better result in comparison with an equal amount of Aspirin.

Acute toxicity test

Acute toxicity test was studied to evaluate the specific dose levels that can sake of mortality. Various toxicological signs including changes in behavior, alertness, restlessness, muscle convulsion, tremor, touch response, torch response, pain response, gripping, writhing, urination, salivation, skin color, food intake, water intake and grooming, and survival analysis were regarded during 72 hours of the examination period. In conformity with the result, MEHR up to 1000mg/kg dose showed no toxicity related symptoms or mortality during the entire time of acute toxicity test. Hence, the median lethal dose (LD 50) would be more than 1000mg/kg. Here, Table 3 represents the acute toxicity test data of MEHR (100, 200, 400, 600, 800, and 1000 mg/kg) in thirty Swiss albino mice (the control group did not receive any treatment); where, “✓” and “X” signs indicate normal parameter and absent parameter respectively, and zero sign “0” means Nil.

Phytochemical analysis

Medicinal plants usually use to cure many diseases due to the presence of various phytochemicals that have potential to act against several ailments. To identify why *Heliconia rostrata* shows analgesic activities, we studied a number of phytochemical analysis test. The phytochemical analysis test expressed the availability of four phytochemicals (alkaloids, flavonoids, steroids, and saponins) in the methanolic extract of *H. rostrate* as active pharmaceutical chemical

Table 2. Data chart of the analgesic study of MEHR

Treatment	Dose (mg/kg) BW	Mean Number of Abdominal constrictions	% of Inhibition
Control	10ml	6.9 ± 0.55	-
Aspirin	200mg	4.2 ± 0.55	39.13%
Aspirin	400mg	3.1 ± 0.67	55.06%
MEHR	50mg	5.3 ± 0.62	23.20%
MEHR	100mg	4.9 ± 0.61	29.00%
MEHR	200mg	3.7 ± 0.47	46.38%
MEHR	400mg	2.9 ± 0.41	58.00%

Table 3. Effect of MEHR on acute toxicity test in Swiss albino mice

SL No	Clinical Sign	Before Treatment	After Treatment					
			100mg/kg	200mg/kg	400mg/kg	600mg/kg	800mg/kg	1000mg/kg
1	Behavior	✓	✓	✓	✓	✓	✓	✓
2	Alertness	✓	✓	✓	✓	✓	✓	✓
3	Restlessness	X	X	X	X	X	X	X
4	Convulsion	X	X	X	X	X	X	X
5	Tremors	X	X	X	X	X	X	X
6	Touch Response	✓	✓	✓	✓	✓	✓	✓
7	Torch Response	✓	✓	✓	✓	✓	✓	✓
8	Pain Response	✓	✓	✓	✓	✓	✓	✓
9	Gripping	✓	✓	✓	✓	✓	✓	✓
10	Writhing	X	X	X	X	X	X	X
11	Urination	✓	✓	✓	✓	✓	✓	✓
12	Salivation	✓	✓	✓	✓	✓	✓	✓
13	Skin Color	✓	✓	✓	✓	✓	✓	✓
14	Food Intake	✓	✓	✓	✓	✓	✓	✓
15	Water Intake	✓	✓	✓	✓	✓	✓	✓
16	Grooming	X	X	X	X	X	X	X
17	Mortality	N/A	0	0	0	0	0	0

ingredients. The results are abridged in Table 4. In details, after adding the Wagner reagent in the filtered solution, the formation of brown precipitation indicated the presence of alkaloid. On the contrary, after dropwise adding of concentrated HCl and H₂SO₄ to the two separate solutions of flavonoid and steroid tests, the formation of red color indicates the presence of flavonoid and steroid in the MEHR respectively. Moreover, in the saponin test, persistent frothing developed and its stability in heating indicated the presence of saponins.

Discussion

In the present study, we evaluated the analgesic effects of methanolic extract of *Heliconia rostrata* and the result was beyond the expectation. Acetic acid induced abdominal constrictions of Swiss albino mice which are observed visually and useful for screening of analgesic activity as reported before [16]. The methanolic extract of *H. rostrata* was administrated at a dose of 50, 100, 200 and 400 mg/kg separately into mice by gavaging and exhibited a significant analgesic activity by decreasing the abdominal constrictions in the acetic acid-induced writhing model. According to our result, MEHR at 200 mg/Kg BW dose inhibited the abdominal constriction of 46.38% compared with 200 mg/kg BW dose of the reference drug Aspirin which inhibited 39.13%. More surprisingly, when we doubled the dose of MEHR by means of 400 mg/kg dose concentration, then the percentage of the inhibition of writhing number was raised to 58 compared to the control group. In addition, Aspirin reduced the abdominal constrictions at 55.06% at 400 mg/kg dose concentration compared to the control group. Therefore, 200mg & 400 mg/kg BW doses of MEHR showed a better analgesic activity than a standard analgesic drug “Aspirin” at the same doses respectively (Figure 3A). In addition, MEHR reduces the mean number of abdominal constrictions at 5.3, 4.9, 3.7, and 2.9 after oral gavaging

Table 4. Phytochemical analysis data

Phytochemicals	Availability
Alkaloids	Present
Flavonoids	Present
Steroids	Present
Saponins	Present

of 50 mg, 100 mg, 200 mg and 400mg of MEHR, respectively, whereas the reference drug Aspirin can downregulate the number of abdominal constrictions at 4.2 and 3.1 after peritoneal administration (Figure 3B). We found, 400 mg/kg BW dose of MEHR was 1.25 times more effective compared to the same dose of Aspirin in the experimental murine model whereas 200 mg/kg BW dose of MEHR was 1.4 times more effective than 200 mg/kg BW dose of Aspirin

In our recent study, we performed acute toxicity test and the result suggested that MEHR didn't show any toxicity even in 1000 mg/kg concentration. According to the result, we did not find any alterations in changes of behavior, alertness, restlessness, muscle convulsion, tremor, touch response, torch response, pain response, gripping, writhing, urination, salivation, skin color, food intake, water intake and grooming in comparison with control group. In spite of that, the survival rate of mice in all groups was 100% during 72 hours of study (Figure 4).

We also tried to identify some phytochemicals in this plant and found alkaloid, flavonoid, steroid, and saponin were presented in this plant samples (Table 4) where all of these photochemical had already been identified for their analgesic activity [17-20]. Be specifically, koumine is a type of alkaloids, which is responsible for reducing postoperative pain. On the other hand, Flavonoids can

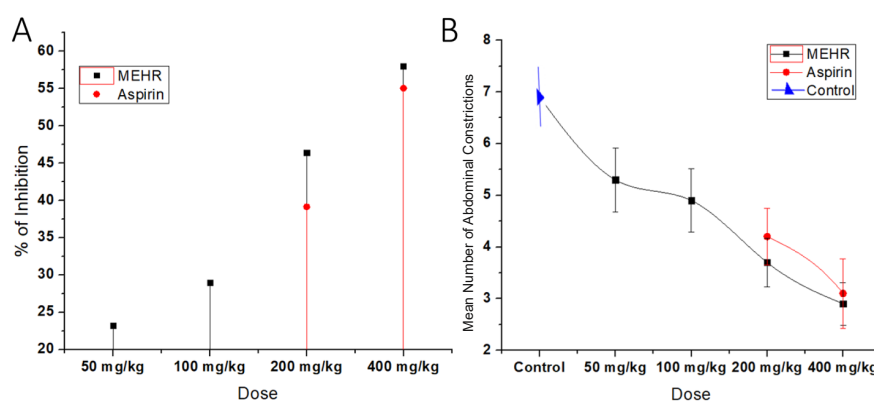


Figure 3. Comparative Analgesic Study between MEHR and Aspirin. (A. Percentages of inhibition by various doses of MEHR and Aspirin, B. Mean number of abdominal constrictions for various doses of MEHR and Aspirin)

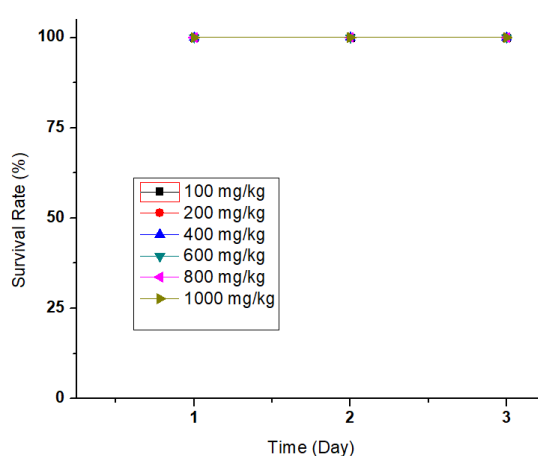


Figure 4. Survival Analysis of Swiss Albino mice with various doses of MEHR

inhibit various mediators like serotonin, histamine, nitric oxide, reactive oxygen species, which are derived from plasma of cell and reduced the inflammation as well as pain [19]. In addition, steroids are the most prescribed drug to reduce pain for cancer patients even though these types of drugs have their own limitations like severe side effects and adverse effects [20]. Their main analgesic mechanism is to downregulate the inflammation and cancer assisted edema. In this case, saponins generally can put down the synthesis of prostaglandins by inhibiting the enzymatic activities of phosphodiesterases, protein kinase C, phospholipase A2, and protein tyrosine kinases.

In our study, for the first time, we claimed that as 200 mg and 400 mg doses of MEHR exhibited a satisfactory result against pain management in the acetic acid-induced writhing model in mice. This result is very closely related to the presence of secondary metabolites such as alkaloids, flavonoids, steroids, and saponins in *Heliconia rostrata*. Importantly, our study was based on oral delivery, even; free plant extract shows very poor result due to the extreme acidic environment of GIT. Hence, as naked therapeutics MEHR exhibits very good result compare to reference drug Aspirin. In near future, we may consider MEHR as a side effect free natural potential analgesic drug.

Conclusion

The natural product is a vast topic that has a big scope to discover and develop new drug component. On the basis of drug discovery, we performed our study to identify the analgesic activity of *Heliconia rostrata* plant. For the first time, we evaluated the analgesic activity

of the methanolic extract of *H. rostrata* that revealed a significantly better result rather than analgesic drug Aspirin with no related toxicity whereas the oral LD50 of the MEHR was more than 1000 mg/kg. The presence of four phytochemicals, which are already reported for analgesic activity which are responsible for such analgesic activities. The result of our study suggested that the extract of this plant can relieve pain and further research can make this plant extract as a potential analgesic drug.

Author's contributions

SMSS and MN designed this study. SMSS, LAS, MFR, and MBV conducted these experiments according to the supervision of MN. SMSS and MFR wrote the manuscript draft which was read and edited by MN and MAR.. All authors read and approved the final version of the manuscript.

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Conflicts of interest

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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