

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF ETHANOLIC EXTRACT OF GYNURA NEPALENSIS (LEAF)

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Abstract

Objective: The present communication attempts to evaluate the Anti-inflammatory and Analgesic activities of ethanoic extract of *Gynura Nepalensis* (leaf) belongs to the family: Asteraceae, which is widely distributed in India.

Methods: Ethanolic extract of *Gynura Nepalensis* (leaf) at doses of 250mg/kg and 500mg/kg were tested for their anti-inflammatory activity against Carrageenan induced paw edema & Xylene-Induced Ear Edema test in rats and analgesic activity was tested against Formalin-induced Paw licking test & acetic acid induced writhing (in mice) models and the two phases of formalin induced paw licking models of pain.

Results: The results indicate that the ethanoic extracts at the doses of 250 and 500mg/kg body weight significantly inhibited, the carrageenan induced paw edema and Xylene-induced ear edema models of inflammation, similarly the extract at the doses of 250 and 500mg/kg significantly inhibited the acetic acid induced writhing (in mice) models and the two phases of formalin induced paw licking models of pain.

Conclusion: This study confirms that the ethanoic extract of *Gynura Nepalensis* has good analgesic and anti-inflammatory property. Further studies are necessary to isolate the compounds from the ethanoic extract of *Gynura Nepalensis* responsible for the activity.

Keywords: *Gynura Nepalensis*, analgesic, inflammation, extracts, rats and mice.

Introduction

Pain is a common and distressing feature of many diseases such as tumour, surgical procedures, physical trauma, noxious chemical stimulation etc [1]. It is mostly a warning signal and primarily protective but excessive pain can lead to other side effects such as sweating, apprehension, nausea and palpitation [2].

Drugs that are currently used for the management of pain are opioids or non-opioids and that for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapy [3]. Opioids are the commonly used drugs for the management of acute postoperative pain [4]. These make the search for new analgesic drugs a necessity and medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity [5]. *Gynura Nepalensis* is one of such plants.

Gynura Nepalensis (leaf) belonging to the Asteraceae family, local name: Terapaibi, Young stem & flower, is locally used as medicinal plant [6]. It is distributed in Tropical Asia, Pacific islands and Malaysia. A few in Tropical America and Africa and also found in Bangladesh. The plants having triterpenoids are the most widely used for the treatment of inflammation and many other life threatening diseases in the traditional medicine of different cultures [7], In India a number of medicinal plants and their formulations are widely used for the treatment of various disorders [8]. Therefore in the present study we aim to investigate whether the ethanolic extract of *Gynura Nepalensis* (leaf) has analgesic and anti-inflammatory activities.

Methods

Animals

For the experiment Swiss albino mice of either sex, 6-7 weeks of age, weighing between 20-30gm were collected from the animal research lab in the Department of Pharmacy Jahangirnagar University, Savar, Dhaka. Animals were maintained under standard environmental conditions (temperature: $27.0 \pm 1.0^\circ$), relative humidity: 55-65% and 12 h light/12 h dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

Plant Materials

The bark of plant of *Gynura Nepalensis* (leaf) was collected from Kendua under Netrokona district of Bangladesh during January 2017. The plant material was taxonomically identified by the National herbarium of Bangladesh.

Preparation of Plant Extract

The plant [leaf] material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. About 500 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 4000 ml of 96% ethanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained.

The collected mixture of active constituents with ethanol was dried with a Rota Vapor (BUCHI Rota Vapor R-114, Switzerland) under reduced pressure to get viscous substance. Then it was transferred to a beaker and taken on a water bath for further drying at room temperature. Finally a solid mass was obtained and preserved in a Petridis in the refrigerator. Extract obtained was 49 gm from 500 gm powder using 4000 ml ethanol. Yield = 9.80% w/w.

Phytochemical analysis

Desirable amount of *Gynura Nepalensis* leaf extract was solubilized in water for phytochemical analysis.

The extracted solution was tested for alkaloids, glycosides, steroids, gums, flavonoids, saponins, sugars and tannins according to the protocol described by Trease and Evans [9]

Analgesic activity

Formalin-induced Paw licking test

The method of Hunskaar and Hole (1987) was used for the study. Mice were divided into 4 groups of 6 animals each. Group I, the control group received distilled water, group II, the standard group received Diclofenac Sodium BP (100 mg/kg). Groups 3 and 4 received alcoholic extract (250 mg/kg and 500 mg/kg). After 1 hour of drug administration, 2.7% formalin was injected into the dorsal surface of the left hind paw. The time spent licking the injected paw was recorded. Animals were observed for the 5 min post formalin (acute phase) and for 5 min starting at 20th min post formalin (delayed phase) (Hunskaar and Hole, 1987) [10]

Acetic acid induced writhing test

For analgesic test all mice were divided into four groups. Each group comprises of 6 mice. First group are controlled group here used to distilled water. Group two are standard group here used to Diclofenac sodium BP (100mg/kg). Group three and four received to alcoholic extract (250 & 500 mg/kg). 45 minutes later each mouse was injected with 0.7% acetic acid at a dose of 10 mL/kg body weight. The number of writhing responses was recorded for each animal during a subsequent 3 min period after 15 min of the I.P. administration of Acetic acid and the mean abdominal writhes for each group was obtained (Koster, et al., 1959).[11]

The percentage inhibition was calculated using the formula:

$$\text{Inhibition(\%)} = \frac{\left(\begin{array}{c} \text{Mean number} \\ \text{of writhes (control)} \end{array} \right) - \left(\begin{array}{c} \text{Mean number} \\ \text{of writhes (drugs)} \end{array} \right)}{\text{Mean number of writhes (control)}}$$

Anti-inflammatory action evaluation

Carrageenan induced paw edema

Pedal inflammation was produced in rats according to the carrageenan induced paw edema method [12]. 24 albino rats were divided into four groups of six each and fasted overnight for 18 hrs with water. Next day the animals were weighted and numbered. A mark was made on the right hind paw just beyond tibio-tarsal junction, so that every time the paw was dipped in the mercury (Hg) column up to the fixed mark to ensure constant paw volume. The initial paw volume was noted of each rat by mercury (Hg) displacement method. 0.1 ml of 1 % carrageenan was injected into the right hind paw of each rat under the sub plantar aponeurosis. The animals were treated orally with extracts (250 and 500 mg/kg), Diclofenac Sodium BP (100mg/kg) or Saline (10 ml/kg) 1 h before carrageenan injection. The paw volume of each rat after carrageenin injection after one hour, two hour, three hour and five hour was recorded by mercury displacement in plethysmograph.

This volume is called final volume. The anti-inflammatory activity of the extract was measured by its potential to prevent edema caused by carrageenan as against the control group which was given the vehicle only. Mean paw edema was calculated for each group as average of paw volume of individual rats belonging to that group. Since, the mean was subjected to positive and negative fluctuations hence; standard error for each group was also calculated.

$$\text{Standard error (S.E)} = \frac{\text{standard deviation}}{\sqrt{n}}$$

Where, n = number of rats in each group

Percent inhibition of paw edema was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{a-b}{a} \times 100$$

a- is the mean paw inflammation volume of control.

b- is the mean paw inflammation volume of test

Xylene-Induced Ear Edema test

The Xylene-induced ear edema test was performed as described in Dai et al. (1995). Mice were divided into 4 groups of 6 animals each. Group I, the control group received distilled water. Group II, the standard

group received Diclofenac Sodium BP [100 mg/kg]. Groups III and IV received alcoholic extract (250 mg/kg and 500 mg/kg). One hour later, each animal received 20µl of xylene on the anterior and posterior surfaces of the right ear lobe. The left ear was considered as control. Mice were sacrificed one hour after Xylene application and circular sections were taken, using a cork borer with a diameter of 3 mm, and weighed. The percentage of ear edema was calculated as inflammation based on the weight of left ear without xylene (Dai et al., 1995) [13].

Results

The results (Table 1) manifest the presence of glycoside, steroids, flavonoids, tannin, saponins and reducing sugars from ethanolic crude extract of the *Gynura Nepalensis* leaf [See Table 1]

Analgesic Studies

Formalin-induced paw licking

The ethanolic extract of *Gynura Nepalensis* at the doses of 250 and 500 mg/kg significantly inhibited the two phases of the formalin test. The summary of the result are shown in Table 2.

Acetic acid induced writhing test

The ethanolic extract of *Gynura Nepalensis* at the doses of 250 and 500 mg/kg significantly decreased the number of writhing from 10.0 ± 4.04 to 9.93 ± 1.32 . The summary of the results are shown in Table 3.

Anti-Inflammatory Studies

Carrageenan induced paw edema

The ethanolic extract of *Gynura Nepalensis* at the doses of 250 and 500 mg/kg significantly reduced the carrageenan induced edema. The results are shown in Table 4.

Xylene-Induced Ear Edema test

The ethanolic extract of *Gynura Nepalensis* at the doses of 250 and 500 mg/kg significantly inhibited the two phases of xylene induced inflammation. The summary of the result are shown in Table 5.

Discussion

The results obtained from the present study shows that the ethanolic extract of *Gynura Nepalensis* has analgesic and anti-inflammatory activities. The

extracts significantly inhibited the licking time in the two phases of the formalin test as well as the characteristic writhing observed following intraperitoneal injection of acetic acid. These two tests (formalin induced paw licking and acetic acid induced writhing) are used for detecting specific activities of drugs that may have analgesic activities. Drugs that inhibit the first phase of the formalin test have the ability to alleviate neurogenic pain while those drugs that inhibit the second phase of the test have the ability to inhibit inflammatory pain [14]. The acetic acid induced writhing test on the other hand is a model of visceral pain that is useful for analgesic drug development except that it gives false positives for muscle relaxant and sedatives [15, 16] Since the extract of *Gynura Nepalensis* produced significant effects in all the models of pain used in this study it show that the extract at high doses has strong analgesic activity. The anti-inflammatory activity of the ethanolic extract of *Gynura Nepalensis* was investigated using two models namely; carrageenan, and Xylene-induced ear edema test models which represent acute and chronic forms of inflammation [17]. The extract dose dependently inhibited both two types of inflammation although it was the higher doses of the extracts that were highly effective against both the two types of inflammation Above extract at concentration of 500mg/kg was found to inhibit 94.4% of edema measured after 4 hrs of injecting 0.1ml of 1% (w/v) of carrageenin, which was comparable to Indomethacin [100 mg/kg] taken as standard, showing 88.9% of edema inhibition after 4 hrs. A marked decrease in mean paw edema was observed in both the extract treated groups observed at 1hr, 2hr, and 3hr and 4hr favoring it was more explicit in the higher dose (500mg/kg) group.

Conclusion

In conclusion, this study demonstrated that the ethanolic extract of *Gynura Nepalensis* contain Glycoside, alkaloid, flavonoid, saponin, tannin and steroid type of compounds and have a significant analgesic and anti-inflammatory activity. Further studies will be necessary to establish the probable mechanism of action of anti-inflammatory and analgesic activities of different extracts of *Gynura Nepalensis*.

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Table 1: phytochemical properties of *glochidion thomsonii* crude extract

Compounds	Observation
Alkaloids	+ ve
Steroids	+ ve
Gums	- ve
Flavonoids	+ ve
Saponins	+ ve
Reducing sugars	+ ve
Tannins	+ ve

Table 2: Effects of the ethanolic extract of *Gynura Nepalensis* on Formalin-Induced Paw Licking Test.

Groups	No. of animal	Dose (mg/kg b.wt.)	Licking time(sec)a	
			Early phase	Late phase
Control	06	10 ml/kg	131.6 ± 5.8	89.60 ± 5.8
Diclofenac Sodium BP	06	100 mg/kg	68.20 ± 5.4**	27.20 ± 5.5**
Extract I	06	250 mg/kg	66.00 ± 3.5	32.60 ± 6.9
Extract II	06	500 mg/kg	55.70 ± 5.6***	26.50 ± 2.4***

Each value is the mean ± S.E.M. of 6 rats

*P < 0.05; **P < 0.01; ***P < 0.01 compared with control; student's t-test.

Table 3: Effect of the ethanolic extract of *Glochidion thomsonii* on Acetic acid induced writhing in mice.

Groups	No. of animals	Dose (mg/kg b.wt)	Writhing count	% of inhibition of analgesia
Control	06	10 ml/kg	23.80 ± 0.92	00
Diclofenac sodium BP	06	100 mg/kg	8.40 ± 1.08**	64.71
Extract I	06	250 mg/kg	10.0 ± 4.04*	57.98
Extract II	06	500 mg/kg	9.93 ± 1.32*	58.28

Each value is the mean ± S.E.M. of 6 rats

*P < 0.05; **P < 0.01; ***P < 0.01 compared with control; student's t-test.

Tables 4: Effects of ethanolic extract of *Glochidion thomsonii* on Carrageenan-induced paw edema.

Group	No. of animal	Dose (mg/kg.wt.)	Mean increase in paw volume		Inhibition (%)	
			3hr	4hr	3hr	4hr
Control	06	10 ml/kg	3.6 ± 0.60	3.4 ± 0.75		
Diclofenac Sod. BP	06	100 mg/kg	1.6 ± 0.24*	0.4 ± 0.45*	55.6	88.9
Extract I	06	250 mg/kg	0.8±0.20**	0.8 ± 0.20*	77.8	77.8
Extract II	06	500 mg/kg	0.6± 0.24**	0.2 ± 0.20*	83.3	94.4

*Each value is the mean ± S.E.M. of 6 rats

*P < 0.05; **P < 0.01; ***P < 0.01 compared with control; student's t-test.

Table 5: Effect of the methanol extract of *Glochidion thomsonii* in the xylene induced inflammation in mice.

Groups	No. of animals	% of inflammation	% of inhibition of inflammation
Control	06	149.44 ± 9.14	00
Diclofenac Sod. BP [100 mg/kg]	06	91.40 ± 7.65**	38.84
Extract I [250 mg/kg]	06	120.80 ± 14.31*	19.16
Extract II [500 mg/kg]	06	95.16 ± 11.95*	36.32

*Each value is the mean ± S.E.M. of 6 rats

*P < 0.05; **P < 0.01; ***P < 0.01 compared with control; student's t-test.