

Title: Phytochemical Screening and Investigation of the Central and Peripheral Analgesic and Anti-Inflammatory activity of ethanol extract of Hiptage Bengalensis (L) Kurz

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ABSTRACT

Aims: Hiptage Bengalensis is used in the traditional system of medicine. The leaf is considered one of the important plant organs for the treatment of various diseases such as rheumatism, leprosy, wounds, ulcer, burning sensation, scabies, inflammation and cough. Hence, the present study has been undertaken to evaluate chemical constituents of the leaf with pharmacological activities.

Study Design: Our present studies were focused to evaluate probable analgesic and anti-inflammatory effect and its mechanisms of ethanol extract of Hiptage Bengalensis in laboratory animals and its statistical significance.

Place and Duration of Study: The experiments were carried out in Pharmacology lab of Department of Pharmacy North South University Dhaka, Bangladesh during the period of June 2012-February 2013.

Methodology: Carrageenan induced Hind Paw Edema test in Long Evans rat was the experiment for anti-inflammatory activity of the ethanol extract of Hiptage Bengalensis while Hot Plate test and Acetic Acid induced Writhing method were was carried out to assess its

analgesic activity in Swiss albino mice. At two different doses of 250 and 500 mg/kg body weight, the analgesic test was evaluated on mice and the anti-inflammatory test was evaluated on rats by the ethanol extract of the leaf.

Result: Phytochemical analysis of ethanol extract of *Hiptage Bengalensis* has indicated the presence of steroid, carbohydrate, flavonoid, alkaloid, tannin, phenol and, mangiferin and terpenoids-compounds.

The experimental activities for the ethanol extract of *Hiptage Bengalensis* exhibited statistically significant ($p < 0.05$) anti-inflammatory activity in Carrageenan induced Hind Paw Edema in Long Evans rat and analgesic activity by Hot Plate and acetic acid induced writhing method in Swiss albino mice.

Conclusion: In conclusion, these observations provide evidence and possible mechanisms of action for the anti-inflammatory and analgesic properties of leaf of *Hiptage Bengalensis* claimed in Ayurveda medicine.

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Keywords: Analgesic, Anti-inflammatory, Carrageenan, Hiptage Bengalensis, Phytochemical

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1. INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of which based on their use in traditional medicine. It has been noted that the original source of many important pharmaceuticals currently in use have been plants used by indigenous people [1]. Herbal medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes [2]. In this paper, we analyzed the analgesic and anti-inflammatory property of leaves of *H. Bengalensis*.

Pain has been defined by The International Association for the Study of Pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [3]. This process enables an individual to take protective measures, by providing with rapid awareness about threatening or potentially threatening injury [4]. However, if the painful sensation remains after removal of the detectable stimulus, it calls for a regimen for pain management [5].

Hiptage Bengalensis (L) Kurz belongs to the family Malphigiaceae. The plant has strong therapeutic potential thus occasionally cultivated for medicinal purposes in the alternative medicine practice *Ayurveda*. The leaves of *H. benghalensis (L.) Kurz* are used in treating skin diseases in Burma and the bark is used to heal wounds in Indonesia. In India, *H. benghalensis (L.) Kurz* is widely used to treat cough, asthma, leprosy and also to quench thirst. According to some researches the therapeutic actions of this plant may be due to the presence of mangiferin, which is known to be anti-inflammatory, hepatoprotective, antioxidant, and antimicrobial.

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2. MATERIAL AND METHODS

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Hot Plate (Model – 35100, UGO BASILE, ITALY), Electronic Balance (Ohaus manufacturer, Canada), Refrigerator (Butterfly Marketing Ltd, LG), Rotary evaporator (Eyela n 1000, Tokyo Rikaki Kai Co. Ltd, Rotary vacuum, Japan), Beakers, Petri dishes & glass wrought, Safety rat handling gloves, Mortar & pestle., Hypodermic Syringes, Holder & test tube, Plethysmometer (Ugo Basline SLR model-7140, Italy)

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67 **2.1 Medicinal plants (extracts)**

68 Ethanol extract of *H.Benghalensis* were examined in two concentrations of 500mg/kg and
69 250mg/kg body weight of animal.

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71 **2.2. Control & Positive Control**

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73 **2.2.1. Analgesic activity**

74 1. Control – distilled water

75 2. Positive control – Diclofenac sodium (Beximco Pharma, Bangladesh)

76 Administered dose – 50mg/kg body weight animal

77 **2.2.2. Anti-inflammatory activity**

78 1. Control –Distilled water

79 2. Positive control – Diclofenac sodium

80 Administered dose – 50mg/kg body weight animal

81 **2.3. Experimental animal**

82 Swiss albino mice (male and female), weighing 20-30g bred in International Centre for Diarrheal
83 Diseases and Research, Bangladesh(ICDDR,B) and grown in the Animal House of the
84 Department of Pharmacy, North South University (NSU). Long Evans rats (male and female),
85 weighing 100-170g of either sex, bred in NSU and ICDDR, B and grown in the animal house of
86 the Department of Pharmacy NSU. All the animals were acclimatized one week prior to the
87 experiments .The animals were housed under standard laboratory conditions (relative humidity
88 55-65%, room temperature $25.0 \pm 2^{\circ}\text{C}$, and 12 hours light dark cycle). The animals were fed
89 with standard diet from ICDDR, B and had free access to filtered water [6, 7].

90 **2.4. Plant Extraction method**

91 **2.4.1. Collection**

92 The plant sample of *Hiptage Benghalensis* was collected from Ayurvedic Institution 'Back to
93 Nature' on 18.06.2012 in the form of leaf shavings. The leaves of the plant were collected and
94 washed with water several times.

95 **2.4.2. Drying and grinding**

96 The collected plant leaves were washed with water, separated from undesirable materials or
97 plant parts, partially dried by fan aeration and then fully dried in the oven at below 40°C for 2
98 days. The fully dried leaves were then grinded to a powdered form and stored in the refrigerator
99 at +4°C for a few days.

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102 **2.4.3. Cold extraction (Ethanol extraction)**

103 103gram of powdered material were taken in a clean, flat bottomed glass container and soaked
104 in 500 ml of 80%ethanol, sealed and kept for a period of 2 days with occasional shaking and
105 stirring. It was then filtered first by cotton material and twice through whatman filter paper to
106 obtain a finer filtrate. The filtrate (Ethanol extract) obtained was evaporated by Rotary
107 evaporator at 4 to 5 rpm and at 65°C temperature. The separated filtrate was found to be a
108 precipitate of dark green color and the gummy concentrate was designated as the crude
109 ethanol extract of the leaves of *Hiptage Bengalensis*. It was then dried in the freeze drier and
110 preserved at +4°C for two weeks.

111 **2.5. Phytochemical Analysis**

112 **2.5.1. Study Design**

113 Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, gum and
114 carbohydrates, reducing sugar, saponins, tannin and terpenoids were carried out for the plant
115 extract by the method described by Harborne and Sazada [8,9]. The freshly prepared extract of
116 *Hiptage Bengalensis* was qualitatively tested for the presence of chemical constituents.
117 Phytochemical screening of the extract was performed using the following reagents and
118 chemicals: Alkaloids with Wagner reagent, flavonoids with the use of concentrated HCl, tannins
119 with 0.1% ferric chloride, and saponins with ability to produce suds. Gum was tested using
120 Molish reagents and concentrated sulfuric acid, steroids with sulfuric acid, reducing sugar with
121 the use of α -naphthol and sulfuric acid and terpenoids with chloroform and conc. HCl.

122 **2.6. Analgesic activity of Hiptage Bengalensis**

123 **2.6.1. Study design**

124 Experimental animals were randomly selected and divided into four groups denoted as group-I,
125 group-II, group-III, group-IV consisting of 6 mice in each group individual weighing was done to
126 adjust individual doses. Here, distilled water was given to group-I, 50 mg/kg Diclofenac sodium
127 for group II, 250 mg/kg for group III and 500mg/kg for group IV of the crude extract of *Hiptage*
128 *Benghalensis*.

129

130 **2.6.2. Mice Screening**

131 Young Swiss-albino mice aged 4-5 weeks, average weight 25-30 gram were used for this study.
132 They were kept in standard environmental condition for one week in the animal house of the
133 Department of Pharmacy, North south University, Bangladesh for adaptation after their
134 purchase. The animals were provided with standard laboratory food and tap water *ad libitum*
135 and maintained at natural day night cycle. Mice screening was performed before Hot plate test.
136 In that experiment mice with significant response action (Licking, Shaking and Jumping) and
137 response time (at the range of 0-20 seconds) were selected.

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141 **2.6.3. Hot plate test method**

142 The Hot plate test was performed on the test subjects in a slightly modified version from the one
143 described earlier [10]. Mice were divided into four groups consisting of six animals in each
144 group. The mice of each group were placed in the Hot Plate apparatus maintained at a
145 temperature of $55^{\circ} \pm 0.2^{\circ}\text{C}$ for a maximum time of 20 second per exposure in order to obtain its
146 response to electrical heat induced pain stimulus but at the same time to prevent blister
147 formation and skin damage which might affect the result. Licking of the paws or jumping out of
148 the beaker was taken as an indicator of the animal's response to heat-induced pain stimulus.
149 The time for each mouse to lick its paws or jump out of the beaker was taken as reaction time
150 (S). Before treatment, the reaction time was taken once. The mean of this one determination
151 constituted initial reaction time before treatment of each group mice. Each of the test group
152 were thereafter treated with Distilled Water (50mg/kg of body wt), Diclofenac Sodium (50 mg/kg
153 of body wt) and ethanol extract at the doses of *H.Benghalensis* 250 mg/kg and 500 mg/kg body
154 wt. orally. Reaction time was recorded as *latency period*, when the animals licks their hind and
155 fore paws and jumped at 0, 30, 60, 120, 180 and 240 minutes after the treatment..

156 Percent analgesic score was calculated as:

157 **(1)(PAS) = $T_b - T_a / T_b \times 100$**

158 Where, T_b = Reaction time (in second) before drug administration

159 T_a = Reaction time (in seconds) after drug administration

160 **2.6.4. Acetic acid induced writhing test in mice**

161 The analgesic activity of the samples was evaluated using acetic acid induced writhing method
162 in mice. In this method, acetic acid is administered intra-peritonally to the experimental animals
163 to create pain sensation. As a positive control, any standard NSAID drug can be used. In the
164 present study as a positive control Diclofenac sodium was used to serve the purpose of
165 standard NSAID. The plant extract was administered orally in two different doses (250 and 500
166 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples and vehicle
167 were administered orally 30 minutes prior to intra-peritoneal administration of 0.7% v/v acetic
168 acid solution (0.1ml/10g) but Diclofenac sodium was administered 15 minutes prior to acetic
169 acid injection. **Animals were kept individually in glass beaker for observation.** Each mouse of all
170 groups were observed individually for counting the number of writhing they made in 15 minutes
171 commencing just 5 minutes after the intra peritoneal administration of acetic acid solution. Full
172 writhing was not always accomplished by the animal, because sometimes the animals started
173 to give writhing but they did not complete it. This incomplete writhing was considered as half-
174 writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in
175 each treated group was compared to that of a control group while Diclofenac sodium (50 mg/kg)
176 was used as a reference substance (positive control).

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182 **2.7. Anti-inflammatory Effect of *Hiptage Bengalensis***

183 **2.7.1. Preparation of inflammatory agent**

184 Preparation of inflammatory agent Carrageenan was used as inflammatory agent in this
185 experiment. It was obtained from Jahangirnagar University. Carrageenan powder was
186 suspended in 5 ml saline to make 0.1% suspension and kept in water bath for proper
187 homogenization. The tube was kept in hot water (50±2°C) containing beaker to prevent
188 transformation into a jelly like compound.

189

190 **2.7.2. Carrageenan-induced Rat Hind Paw Edema test**

191 The ethanol extract of *Hiptage Bengalensis* on carrageenan induced inflammation in rat paw
192 was investigated by following the method described previously.[11] Rats were randomly divided
193 into four groups, each consisting of six animals, (weighing 150-200 gram) of which Group I was
194 kept as control giving only water. Group II was given carrageenan as inflammatory agent.
195 Group III and Group IV were given the test sample at the dose of 250 and 500 mg/kg body
196 weight respectively. Half an hour after oral administration of the test materials, 0.1ml 0.1%
197 carrageenan suspension was injected subcutaneously in left hind paw of each animal leading to
198 the formation of edema *in situ* (localized inflammation). The volume of paw edema was
199 measured at 1, 2, 3, 6, and 8 hours using water Plethysmometer after administration of
200 carrageenan. The right hind paw served as a reference non inflamed paw for comparison. The
201 average percent increase in paw volume with time was calculated and compared against the
202 control group. Percent inhibition was calculated using the formula:

203 **(2)% Inhibition of paw edema = [1- (Vt / Vc)] X 100**

204 Where Vc and Vt represent average paw volume of control and treated animal respectively

205

206 **2.8 Statistical analysis**

207 All the results were expressed as Mean ± Standard deviation (SD). Data was analyzed using
208 one-way ANOVA followed by Dunnett's t-test. The results obtained were compared with the
209 vehicle control group. The P values P<0.05, P< 0.01 and P< 0.001 were considered as
210 statistically significant. The confidence interval is 95%. All the statistical tests were carried out
211 using SPSS statistical software.

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213 **3. RESULTS AND DISCUSSION**

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215 **3.1. Phytochemical screening**

216 Phytochemical screening of the ethanol extract of *H. Bengalensis* leaf and stem revealed the
217 presence of various bioactive components such as tannins, flavonoids, saponins, gums,
218 steroids, alkaloids, reducing sugar and terpenoids [12]. The result of phytochemical test has
219 been summarized in the table below-

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222 **Table 1: Result of Phytochemical Screening of Plant Extract**

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Hiptage Benghalensis Extract	Leaf						
	<i>Tannins</i>	<i>Saponins</i>	<i>Flavinoids</i>	<i>Gums & Carbohydrates</i>	<i>Alkaloids</i>	<i>Reducing Sugars</i>	<i>Terpenoids</i>
80% ethanol	+++	++	++	+++	+++	+++	+++

224 Symbol (+) indicates presence of phytochemicals.

225 **3.2. Analgesic activity**226 **Table 2: Analgesic effect of the ethanol extract of *H.Benghalensis* using the hot –plate**

Treatment Group	Dose	Latency Period(s)					
		0 min	30min	1h	2h	3h	4h
Control	-----	10.70±.847	9.66±.937	8.00±.814	6.58±.641	5.52±.549	5.00±.443
Standard	50mg/kg	9.14±.524	11.02±1.00	12.60±.945	14.16±1.076***	15.96±.676***	12.48±.698***
<i>H.Benghalensis</i>	250mg/kg	7.68±.851	9.28±1.09	10.32±1.12**	11.28±1.07**	12.54±.912***	10.18±.747***
<i>H.Benghalensis</i>	500mg/kg	7.65±.312	9.22±.285	10.34±.273	11.72±.233**	12.68±.177***	10.19±.163***

227 **method. Statistical evaluation of the results shown in table:**

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229 Values in the results are expressed as mean ± SEM., Data was analyzed using one-way ANOVA followed by Dunnett's t-test. The
 230 results obtained were compared with the vehicle control group. The P values *P<0.05, **P< 0.01 and ***P< 0.001 were considered as statistically
 231 significant.

232

233 **Table3: Percent inhibition of the standard and two different concentrations of the extract**
 234 **compared with their respective means at 0 hour**

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Treatment group	Dose	% Inhibition				
		30min	1h	2h	3h	4h
Standard	50mg/kg	20.56	37.00	54.90	74.61	36.54
<i>H.Benghalensis</i>	250mg/kg	20.83	34.37	46.87	63.28	32.55
<i>H.Benghalensis</i>	500mg/kg	20.52	35.16	53.20	67.75	33.20

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239 **3.2.1. Effect of plant extract on Hot-Plate test**

240 Table 2 and 3 shows the analgesic effect of the ethanol extract of *H.Benghalensis* using the hot
241 -plate method and percent inhibition of the standard and two different concentrations of the
242 extract compared with their respective means at 0 hour. The ethanol extract of *H.Benghalensis*
243 exhibited statistically significant ($p > 0.05$) analgesic effect in hot plate test of white albino mice.
244 This was determined by analyzing data using one way ANOVA followed by Dunnet's post hoc
245 test. However, the data shows that the dose dependent effect reached 67.75% at 180 minutes
246 and 63.28% at the 180 minutes at the doses of 500 and 250 mg/kg-body weight respectively.

247 **Table 4 :Result of Analgesic Activity of Hiptage Benghalensis in Acetic Acid Method**

<i>Treatment</i>	<i>Dose</i>	<i>Total Writhing Counts</i>					<i>Mean± SEM</i>	<i>% Inhibition</i>
Control	-----	23	27	29	47	32	31.6000±4.11825	
Standard	50mg/kg	14	18	16	17	13	15.6000±.92736***	50.00%
H.Benghalensis	250mg/kg	19	19	21	21	18	21.4000±1.96469***	37.66%
H.Benghalensis	500mg/kg	16	18	7	11	18	14.0000±2.16795***	55.69%

248 **Statistical evaluation of the results shown in table:**

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250 Values are expressed as Mean±S.E.M. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnet test.
251 * $p < 0.05$, ** $p < 0.01$ and *** $P < 0.001$ compared to the control treated group.
252

253 **3.2.2 Effect of plant extrat on Acetic Acid Writhing Test**

254 Table 4 shows the effects of the extracts of *H.Benghalensis* on acetic acid induced writhing in
255 mice. Both doses of the plant extract showed significant reduction ($p < 0.05$) of writhing induced
256 by the acetic acid after oral administration in a dose dependent manner. After oral
257 administration of two different doses- 250 and 500 mg/kg body weight, the percent inhibition
258 was 37.66% & 55.69% respectively.
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266 **3.3. Anti-inflammatory Activity**

267 **Table 5: Anti-inflammatory effect of ethanol extract of *Hiptage Bengalensis* on carrageenan**
 268 **induced rat paw inflammation.**

Treatment Group	Dose	Volume of Paw(ml)					
		0 min	1 h	2h	3 h	6 h	8 h
Control	----	.71±.055	.88±.077	1.18±.007	1.46±.063	1.55±.066	1.62±.065
Standard	50mg/kg	.65±.039	.85±.058	.99±.036	1.24±.046	1.02±.028***	.79±.020***
H.Benghalensis	250mg/kg	.67±.057	.99±.101	1.19±.077	1.28±.054	1.09±.053***	.92±.032***
H.Benghalensis	500mg/kg	.68±.031	1.02±.081	1.21±.056	1.29±.131	1.08±.038***	.89±.027***

269 Values are expressed as Mean±S.E.M. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnet test.
 270 *p<0.05 ,**p<0.01 and ***P< 0.001 compared to the control treated group.
 271
 272
 273

274 **Table 6: Percent inhibition of the standard and two different concentrations of the extract**
 275 **compared with their respective means at 0 hour**

Treatment	Dose	%inhibition				
		1 h	2 h	3 h	6 h	8 h
Standard	50mg/kg	29.97	51.68	89.91	56.27	20.49
H.Benghalensis	250mg/kg	48.61	77.08	91.07	63.54	36.61
H.Benghalensis	500mg/kg	48.74	77.19	88.88	57.89	29.53

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277 **3.3.1. Effect of plant extract on Carrageenan-induced Hind Paw Edema**

278 Table 5 and 6 shows the anti-inflammatory effect of ethanol extract of *Hiptage Bengalensis* on
 279 carrageenan induced rat paw inflammation and percent inhibition of the standard and two
 280 different concentrations of the extract compared with their respective means at 0 hour. The
 281 ethanol extract of *H.Benghalensis* exhibited statistically significant (p<0.05) anti-inflammatory
 282 activity in Carrageenan-induced Hind Paw Edema of rat. This was determined by analyzing
 283 data using one way ANOVA followed by Dunnet's test. In control animals, the sub plantar
 284 injection of carrageenan produced a local edema that increased progressively to reach a
 285 maximal intensity four hours after the injection of the phlogistic agent. Ethanol extract of
 286 *H.Benghalensis* showed a significant dose depended reduction at both 250 and 500mg/kg body
 287 weight. However significant inhibition of edema was found to be 63.54% and 57.89% at six hour
 288 of study at a dose of 250 and 500mg/kg body weight respectively. Further significant inhibition
 289 was to be 36.61% and 29.53% at eight hour of study at a dose of 250 and 500mg/kg body
 290 weight respectively.

291 **3.4. Acute toxicity**

292 Oral administration of graded doses (250 & 500mg/kg) of the ethanol extract of H.Benghalensis
 293 to rats and mice did not produce any significant changes in behavior, breathing, cutaneous

294 effects, sensory nervous system responses or gastrointestinal effects during the observation
295 period. No mortality was recorded in any group after 24h of administering the extract to the
296 animal.

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298 4. DISCUSSION

299 It's a long and tedious process to isolate pure, pharmacologically active constituents from plants.
300 Thus, it is necessary to have methods available which eliminate unnecessary separation
301 procedures. Chemical screening is thus performed to allow localization and targeted isolation of
302 new or useful constituents with potential activities. This procedure enables recognition of known
303 metabolites in extracts or at the earliest stages of separation and is thus economically very
304 important [13,14].

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306 Preliminary qualitative phytochemical screening of *H.Benghalensis* stem extract exhibited the
307 presence of Tannins, saponins, flavonoids, carbohydrates and gums, reducing sugars, alkaloids,
308 and terpenoids. Therefore it is assumed that these compounds may be responsible for the
309 observed analgesic activity.

310 The presence of flavonoids represents the possibility of some biological activity of the extracts of
311 *H.Benghalensis*. Such as it can modify allergens, viruses, and carcinogens indicating flavonoids
312 have potential to be biological "response modifiers". It can also be used as an anti-allergic, anti-
313 inflammatory [15], anti-microbial and anti-cancer activities. Flavonoids were reported to have a role
314 in analgesic activity primarily by targeting prostaglandins [16, 17]. Flavonoids and other phenolic
315 compounds of plant origin have been reported as antioxidants and as scavengers of free radicals.
316 Antioxidants can also exert anti-inflammatory effects [18].

317 The presence of tannins represents the possibility of some biological activity of the extracts of
318 *H.Benghalensis*. Such as antidiarrheal, hemostatic, antihemorrhoidal, anti-inflammatory, astringent,
319 anti-infective. It can be used for immediate relief of sore throats, diarrhea, dysentery,
320 hemorrhaging, fatigue, skin ulcers and as a cicatrizant on gangrenous wounds. It may have anti-
321 viral effect which tannins have. It can also be used against poisons. There are also reports on the
322 role of tannins in anti-nociceptive activity [19]. Besides, alkaloids are well known for their ability to
323 inhibit pain perception [20].

324 As a result of adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and
325 dependence induced by opiates, the use of these drugs as anti-inflammatory and analgesic agents
326 have not been successful in all the cases . Therefore, new anti-inflammatory and analgesic drugs
327 lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates.
328 During this process, the investigation of the efficacy of plant-based drugs used in the traditional
329 medicine have been paid great attention because they are cheap, have little side effects and
330 according to WHO still about 80% of the world population rely mainly on plant-based drugs [21].

331 Effect of ethanol extract of *Hiptage Benghalensis* in hot plate method is shown in the table 2. It is
332 one of the most common tests for evaluating the analgesic efficacy of drugs/compounds. The paws
333 of mice and rats are very sensitive to heat at temperature which is not damaging to the skin. The
334 responses are shaking, jumping, withdrawal of the paws and licking of the paws. The time until this
335 response is prolonged after administration of centrally acting analgesics. *H.Benghalensis* extract at
336 the dose of 250 and 500 mg/kg showed the significant ($P<0.05$) increase in latency time as
337 compared to control. Positive control Diclofenac Na showed significant ($P<0.05$) analgesic activity
338 at the dose of 50 mg/kg. The analgesic activity was expressed as mean increase in latency after
339 drug administration \pm SEM. *H.Benghalensis* exhibited potent analgesic activity at the dose levels of

340 250 and 500mg/kg. These extracts show analgesic activity at low dose of 250mg/kg even in first
341 hour in test. These results indicate that ethanol extract of *H.Benghalensis* can produce significant
342 analgesic effect.

343 Carrageenan induced paw edema is most widely use acute inflammatory model for studying anti-
344 inflammatory activity and it includes two phases. First phase occurs within an hour of injection of
345 phlogistic agent and is mediated through release of histamine serotonin and kinin. While the
346 second phase which can be measured around 3 to 4 hours is related to release of prostaglandins .
347 Carrageenan-induced edema involves the synthesis or release of mediators at the injured site.
348 These mediators cause pain and fever [22]. Inhibitions of these mediators from reaching the
349 injured site or from bringing out their pharmacological effects normally ameliorate the inflammation
350 and other symptoms. In the present study, it has been shown that the ethanol extract of the
351 *H.Benghalensis* possess a significant anti-edematogenic effect on paw edema induced by
352 carrageenan. Slight inhibition of inflammation is observed in first phase and maximum in second
353 phase, which is mainly due to release of prostaglandins. The possible anti-inflammatory effect may
354 be due ro inhibition of cyclooxygenase enzyme which catalyzes the biosynthesis of prostaglandins
355 and thromboxane from arachidonic acid. There are reports that flavonoids possess anti-
356 inflammatory activity and some act as phospholipase inhibitors [23, 24, 25]. Such inhibitors are able
357 to decrease the inflammatory response to Carrageenan in rats [26, 27].

358

359 **5. CONCLUSION**

360 The present study indicated that the ethanol extract of *H.Benghalensis* may have potential use in
361 medicine. In our study, the ethanol extract of the plant showed significant dose dependent
362 inhibition of paw edema and significant analgesic effect. Now our next aim is to isolate the leading
363 compounds and to establish their chemical structure as well. Further studies should be undertaken
364 to correlate the pharmacological activities with the chemical constituents of the leaf of
365 *H.Benghalensis* and uncover specific mechanisms of action so that we may find a viable natural
366 alternative to the traditional NSAIDs. Thus, it is concluded that the ethanol extract of leaf of *Hiptage*
367 *Benghalensis* produce significant anti-inflammatory and analgesic activities in dose dependant
368 manner.

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378 **COMPETING INTEREST**

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380 Authors have declared that no competing interests exist.

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AUTHORS' CONTRIBUTIONS

Author A Shehla.U.Hridi managed the literature searches, performed the experiment and statistical analysis, and wrote the first draft of the manuscript.

Author C MD.Fakhar Uddin Majumder assisted during the experiments and provided the logistic support.

Author B Nafisa Ferdous managed the literature searches, performed the experiment.

Author D Dr.JMA Hannan designed the study, wrote the protocol, and supervised the experiments.

All authors read and approved the final manuscript.

CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care (NIH publication No. 8523, revised 1985) were followed, as well as specific national laws were applicable. All experiments have been examined and approved by the appropriate ethics committee of North South University.

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