SDI Paper Template Version 1.6 Date 11.10.2012

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

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ABSTRACT

Aims: Hiptage Benghalensis 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 Benghalensis 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 Benghalensis while Hot Plate test and Acetic Acid induced Writhing method were was carried out to assess its

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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 Benghalensis 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 Benghalensis 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 Benghalensis claimed in Ayurveda medicine.

34
35 *Keywords: Analgesic, Anti-inflammatory, Carrageenan, Hiptage Benghalensis, Phytochemical*

36

37 1. INTRODUCTION

38 Nature has been a source of medicinal agents for thousands of years and an impressive
39 number of modern drugs have been isolated from natural sources, many of which based on
40 their use in traditional medicine. It has been noted that the original source of many important
41 pharmaceuticals currently in use have been plants used by indigenous people [1]. Herbal
42 medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or
43 flowers for medicinal purposes [2]. In this paper, we analyzed the analgesic and anti-
44 inflammatory property of leaves of *H.Benghalensis*.
45 Pain has been defined by The International Association for the Study of Pain as an unpleasant
46 sensory and emotional experience associated with actual or potential tissue damage [3].This
47 process enables an individual to take protective measures, by providing with rapid awareness
48 about threatening or potentially threatening injury [4].However, if the painful sensation remains
49 after removal of the detectable stimulus, it calls for a regimen for pain management [5].
50 *Hiptage Benghalensis (L) Kurz* belongs to the family Malphigiaceae. The plant has strong
51 therapeutic potential thus occasionally cultivated for medicinal purposes in the alternative
52 medicine practice *Ayurveda*. The leaves of *H.benghalensis (L.)* Kurz are used in treating skin
53 diseases in Burma and the bark is used to heal wounds in Indonesia. In India, *H.benghalensis*
54 *(L.) Kurz* is widely used to treat cough, asthma, leprosy and also to quench thirst. According to
55 some researches the therapeutic actions of this plant may be due to the presence of
56 mangiferin, which is known to be anti-inflammatory, hepatoprotective, antioxidant, and
57 antimicrobial.
58

59 2. MATERIAL AND METHODS

60

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

66

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.

70
71 **2.2. Control & Positive Control**
72
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± 2 ^{o}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.

100

2.4.3. Cold extraction (Ethanol extraction)

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

2.5. Phytochemical Analysis

2.5.1. Study Design

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

2.6. Analgesic activity of Hiptage Benghalensis

2.6.1. Study design

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

2.6.2. Mice Screening

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

141

142 **2.6.3. Hot plate test method**

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

157 Percent analgesic score was calculated as:

158 **(1)(PAS) = Tb-Ta/Tb × 100**

159 Where, Tb= Reaction time (in second) before drug administration

160 Ta = Reaction time (in seconds) after drug administration

161 **2.6.4. Acetic acid induced writhing test in mice**

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

179

180

181

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

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 a beaker containing hot water (50±2°c) 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 Benghalensis* 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.

212
213 **3. RESULTS AND DISCUSSION**
214
215 **3.1. Phytochemical screening**

216 Phytochemical screening of the ethanol extract of H.Benghalensis 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-

220

221

222 **Table 1: Result of Phytochemical Screening of Plant Extract**
223

Hiptage Benghalensis Extract	*Leaf*						
	Tannins	*Saponins*	*Flavinoids*	*Gums& Carbohydrates*	*Alkaloids*	*Reducing Sugars*	*Terpenoids*
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**
227 **method. Statistical evaluation of the results shown in table:**
228

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***
H.Benghalensis	250mg/kg	7.68±.851	9.28±1.09	10.32±1.12**	11.28±1.07**	12.54±.912***	10.18±.747***
H.Benghalensis	500mg/kg	7.65±.312	9.22±.285	10.34±.273	11.72±.233**	12.68±.177***	10.19±.163***

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**

235

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

236
237

238

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

249
250 Values are expressed as Mean±S.E.M. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnet test.

Treatment	**Dose**	***Total Writhing Counts***					***Mean± SEM***	***% Inhibition***
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%

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*.
259

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268 **3.3. Anti-inflammatory Activity**

269 **Table 5**: **Anti-inflammatory effect of ethanol extract of *Hiptage Benghalensis on* carrageenan**
270 **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***

271
272 Values are expressed as Mean±S.E.M. Differences between groups are determined by One-Way ANOVA followed by post hoc Dunnet test.
273 *p<0.05 ,**p<0.01 and ***P< 0.001 compared to the control treated group.
274
275

276 **Table 6: Percent inhibition of the standard and two different concentrations of the extract**
277 **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

278

279 **3.3.1. Effect of plant extract on Carrageenan-induced Hind Paw Edema**

280 Table 5 and 6 shows the anti-inflammatory effect of ethanol extract of *Hiptage Benghalensis on*
281 carrageenan induced rat paw inflammation and percent inhibition of the standard and two
282 different concentrations of the extract compared with their respective means at 0 hour**.** The
283 ethanol extract of *H.Benghalensis* exhibited statistically significant ($p<0.05$) anti-inflammatory
284 activity in Carrageenan-induced Hind Paw Edema of rat. This was determined by analyzing
285 data using one way ANOVA followed by Dunnet's test. In control animals, the sub plantar
286 injection of carrageenan produced a local edema that increased progressively to reach a
287 maximal intensity four hours after the injection of the phlogistic agent. Ethanol extract of
288 *H.Benghalensis* showed a significant dose depended reduction at both 250 and 500mg/kg body
289 weight. However significant inhibition of edema was found to be 63.54% and 57.89% at six hour
290 of study at a dose of 250 and 500mg/kg body weight respectively. Further significant inhibition
291 was to be 36.61% and 29.53% at eight hour of study at a dose of 250 and 500mg/kg body
292 weight respectively.
293
294

295 **4. DISCUSSION**

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

302

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

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

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

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

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

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

355

356 ## 5. CONCLUSION

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

366 ## ACKNOWLEDGEMENTS
367
368 I express my utmost sincere gratefulness to Associate Professor and Chairman Dr. JMA Hannan,
369 Department of Pharmacy, North South University, my honorable teacher and thesis supervisor. He
370 has been a constant support and inspiration for me to accomplish this work. I would also like to thank
371 Mr. Fakruddin, Mrs. Junaida Khaleque, and Ms. Samina for providing the us with full logistical support
372 and giving advice.
373
374

375 ## COMPETING INTEREST
376
377 Authors have declared that no competing interests exist.

378
379 **AUTHORS' CONTRIBUTIONS**
380
381 Author A Shehla.U.Hridi managed the literature searches, performed the experiment and
382 statistical analysis, and wrote the first draft of the manuscript.
383 Author C MD.Fakhar Uddin Majumder assisted during the experiments and provided the logistic
384 support.
385 Author B Nafisa Ferdous managed the literature searches, performed the experiment.
386 Author D Dr.JMA Hannan designed the study, wrote the protocol, and supervised the
387 experiments.
388 All authors read and approved the final manuscript.
389
390
391
392 **CONSENT**
393
394 Not applicable
395
396
397 **ETHICAL APPROVAL**
398
399 All authors hereby declare that "Principles of laboratory animal care (NIH publication No.
400 8523,revised 1985) were followed, as well as specific national laws were applicable. All
401 experiments have been examined an approved by the appropriate ethics committee of North South
402 University.
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