

Research Article

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Phytochemical screening and anti-diabetic efficacy of stem of *Hiptage benghalensis* (L) Kurz.

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Abstract

Our present studies were focused on the phytochemical study and probable anti-diabetic activity of the stem of *Hiptage benghalensis* in laboratory animals and the statistical significance of such effect. Qualitative phytochemical analysis of ethanol extract of *Hiptage benghalensis* for identification of steroid, carbohydrate, flavonoid, alkaloid, tannins, phenol, mangiferin and terpenoids compounds were carried out using proper reagents. The stem extract was subjected to anti-diabetic study through six segment method which was performed to assess the amount of sucrose remaining in the GIT at six different positions. The amount of sucrose unabsorbed in different GIT segments were evaluated in control rats vs. rats fed with 500mg/kg extract at 30 minutes, 1hour, 2hour and 4 hour. The phytochemical screening of ethanol extract of *Hiptage benghalensis* exhibited the presence of several phytochemical components including the presence of flavonoids and terpenoids which have been reported to possess anti-diabetic properties. The extract caused a significant ($p < 0.05$), dose dependent inhibition of glucose absorption and showed hypoglycemic effects in Long-Evans rats weighing from 80-200 gm. The anti-diabetic effects were estimated by measuring the amount of glucose in the samples collected after the experiment. In conclusion, these observations provide evidence and possible mechanisms of action for the anti-diabetic properties of stem of *Hiptage benghalensis* claimed in Ayurveda medicine.

Keywords: Anti-Diabetic, *Hiptage benghalensis*, Hypoglycemic, Glucose, Phytochemical

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 anti-diabetic property of stems of *H. benghalensis*.

Diabetes is a major threat to global public health that is rapidly getting worse and biggest impact in adult of working age in developing countries. There are an estimated 246 million people with diabetes in the world, whom about 80% reside in developing countries.³ It is ranked seventh among the leading causes of death and third when it's fatal complications are taken into account.⁴

On the basis of the etiology, Type 1 may be due to immunological destruction of pancreatic β cells resulting in insulin deficiency. Its pathogenesis involves environmental triggers that may activate autoimmune mechanisms in genetically susceptible individuals, leading to progressive loss of pancreatic islet β cells.⁵ Many of the acute effects of this disease can be controlled by insulin replacement therapy, but there are long-term adverse effects on blood vessels, nerves and other organ systems. Type 2 DM is associated with both impaired insulin secretion and insulin resistance. Type 2 DM is more prevalent form of the disease and common in individuals over 40 years of age. It is often associated with obesity and hereditary disposition.⁶ Delayed carbohydrate digestion and absorption is already a recognized therapeutic approach in the management of diabetes mellitus. The delay of the appearance of glucose in the circulation over a long period, the delayed absorption technique enables pancreatic β -cells of diabetic subjects to adapt to the metabolic load after meal.⁷ However, the effectiveness of drug therapy is limited and is often associated with complications and side-effects including hypoglycemia.⁸

Hiptage benghalensis (L) Kurz belongs to the family Malphiaceae. The plant has strong therapeutic potential thus occasionally cultivated for medicinal purposes in the alternative medicine practice Ayurveda. According to some researches the therapeutic actions of this plant may be due to the presence of mangiferin, in the present study flavonoids have been extracted from dried and powdered samples of stems of *Hiptage benghalensis* by well-established method. Scientific report show ant-diabetic activity of the authenticated stem of *H. benghalensis*. Hence, the present studies were undertaken to find out the possible actions of ethanol extract of *Hiptage benghalensis* for its anti-diabetic activity through six- segment method in Long Evans rat.

Materials and Methods

Surgical apparatus, Ice cold saline, Mortar-pastel, Insulin syringe, Syringe 5ml and 10ml, Ketamine/ Pentobarbital, Screw cap test tube, H₂SO₄ (2N), NaOH (1N), Sucrose solution, Homogenizer, Water-bath, Vortex, Centrifuge and Glucose kit

Medicinal plants (extracts)

Extract were examined in one concentration of 500mg/kg body weight of animal

Reagents, Control & Positive Control

Phytochemical Study

Reagents and chemicals- Wagner Reagent, concentrated HCL, 0.1% Ferric chloride, Molish reagent, conc. H₂SO₄, α -naphthol, chloroform

Anti-diabetic activity

Control- Sucrose solution

Reagents- NaOH (1N), H₂SO₄ (2N), Ketamine, Ice-cold saline, 80% ethanol

Experimental animal

Long Evans rats (male and female), weighing 80-200g of either sex, bred in NSU and ICDDR,B and grown in the animal house of the Department of Pharmacy, NSU were used. All the animals were acclimatized one week prior to the experiments. The animals were housed under standard laboratory conditions (relative humidity 55-65%, room temperature 25.0 \pm 2 $^{\circ}$ C, and 12 hours light dark cycle). The animals were fed with standard diet from ICDDR, B and had free access to filtered water.⁹

Plant Extraction method

Collection-

The plant sample of *H. benghalensis* was collected from Ayurvedic Institution 'Back to Nature' during 18.06.2012 in the form of stem shavings. The stem of the plant was collected and washed with water several times.

Drying and grinding-

The collected plant stems were washed with water, separated from undesirable materials or plant parts, partially dried by fan aeration and then fully dried in the oven at below 40 $^{\circ}$ C for 2 days. The fully dried leaves was then grinded to a powdered form and stored in there refrigerator at +4 $^{\circ}$ C for a few days.

Cold extraction (Ethanol extraction)-

103gm of powdered material was taken 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 (Eyela n 1000, Tokyo Rikaki Kai Co.Ltd, Rotary vacuum, Japan) at 4 to 5 rpm and at 65 $^{\circ}$ c 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 *H. benghalensis*. It was then dried in the freeze drier and preserved at +4°C for two weeks.

Phytochemical Analysis

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. The freshly prepared extract of *H. 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 conc. 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 α -naphthol and sulfuric acid and terpenoids with chloroform and conc. HCl.

Anti-Diabetic activity

Study design-

Plant extract (500mg/kg) along with sucrose solution (2.5g/kg body weight) were administered orally to 24 hours fasted rats. Control group was given equal volume of sucrose only. Ketamine hydrochloride was injected intra-peritoneally 15 minutes prior to dissection of rats of each hour (30min, 1hr, 2hr & 4hr) to elicit acute anesthetic effect and eventually death. For 30, 60, 180 and 360 minutes respective rats were sacrificed. After sacrificing,

Table 1: Result of Phytochemical Screening of Plant Extract

the whole GIT was excised into six segments. The segments being – (A) Stomach, (B) Upper 20 cm of small intestine, (C) Middle part of small intestine, (D) Lower 20 cm of small intestine, (E) Caecum and (F) Large intestine. Each segment was then washed with 10 ml of ice cold saline. The solution was then centrifuged for 15 minutes at 3000 rpm. The supernatant was then collected and to this solution, 2N H₂SO₄ (2ml) was added to acidify the solution. These mixtures were then boiled for 2 hours in paraffin oil to hydrolyse the sucrose. After 2 hours, to these mixtures, 1N NaOH was added drop by drop to neutralize the mixture and the pH was set at 6.9-7. Then the concentration of glucose was obtained by the use of GOD-PAP method and ELISA reader. Blood glucose and the amount of glucose liberated from residual sucrose in the gastrointestinal tract were measured. The gastrointestinal sucrose content was calculated from the amount of liberated glucose.

Calculation of sucrose from glucose-

Amount of sucrose in certain volume = C x V x 0.342
Here, C= Conc. of glucose (mmol/ l), V= Total volume of solution

Results

Phytochemical screening

Phytochemical screening of the ethanol extract of *H. benghalensis* stem revealed the presence of various bioactive components such as tannins, flavonoids, saponins, gums, steroids, alkaloids, reducing sugar and terpenoids (Murugan M and Mohan V.R 2011) The result of phytochemical test has been summarized in the table below-

<i>Hiptage benghalensis</i> Extract	Stem						
	Tannins	Saponins	Flavonoids	Gums & Carbohydrates	Alkaloids	Reducing Sugars	Terpenoids
80% ethanol	+++	++	++	+++	+++	+++	+++

Antidiabetic Activity

Table 2: Anti-diabetic activity of *Hiptage benghalensis* (500mg) in Upper Intestine

Group	30mins	1hr	2hrs	4hrs
Control	23.19±1.68	17.21±1.17	12.71±1.06	6.28±1.35
Hiptage 500mg	36.50±1.15***	26.14±1.05***	19.37±0.73***	10.75±0.47**

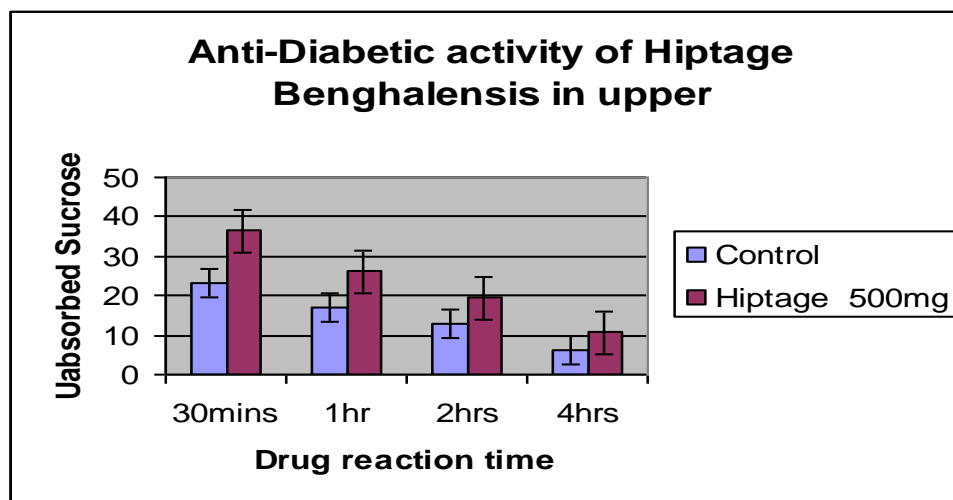


Figure 1: Anti-diabetic activity of *H. benghalensis* in Upper Intestine

Table 3: Anti-diabetic activity of *Hiptage benghalensis* (500mg) in Middle Intestine

Group	30mins	1hr	2hrs	4hrs
Control	18.98±0.97	13.00±.52	10.02±0.70	6.28±1.35
Hiptage 500mg	26.14±1.42***	19.60±0.78***	16.04±0.97***	10.75±0.47***

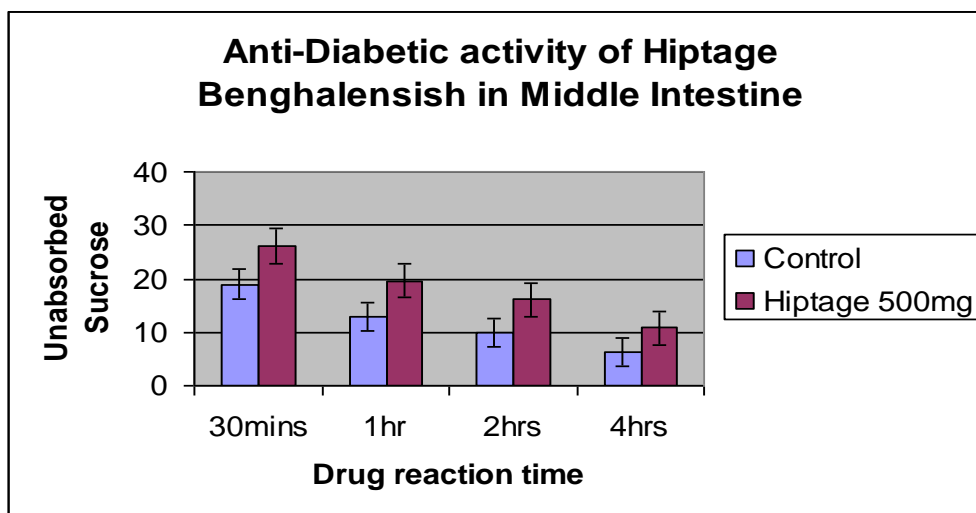


Figure 2: Anti-diabetic activity of *H. benghalensis* in Middle Intestine

Table 4: Anti-diabetic activity of *Hiptage benghalensis* (500mg) in Lower Intestine

Group	30mins	1hr	2hrs	4hrs
Control	7.75±0.96	16.33±1.18	10.34±0.56	5.91±0.51
Hiptage 500mg	8.35±.46	17.45±1.84	12.87±1.48	9.42±0.76***

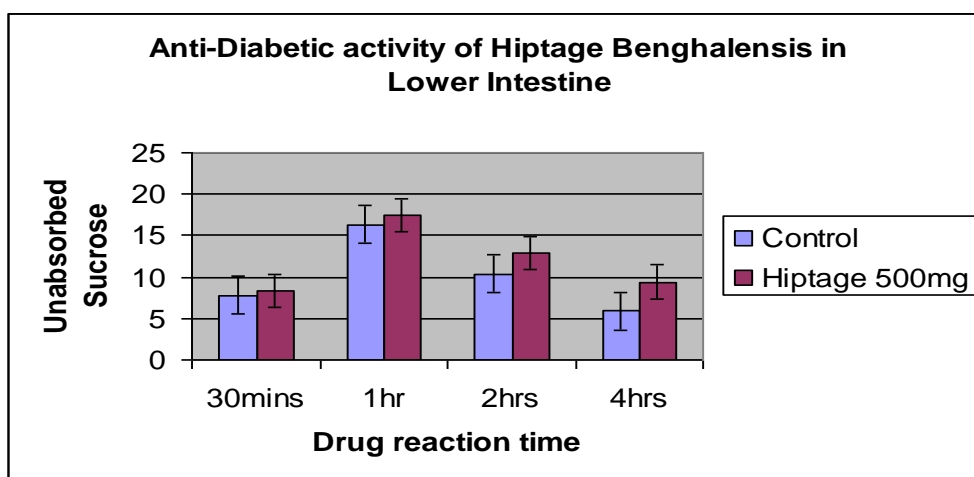


Figure 3: Anti-diabetic activity of *H. benghalensis* in Lower Intestine

Table 5: Anti-diabetic activity of *Hiptage benghalensis* (500mg) in Stomach

Group	30mins	1hr	2hrs	4hrs
Control	31.08±1.93	25.53±1.52	15.75±.61	6.32±1.73
Hiptage 500mg	51.95±2.73***	42.05±2.77***	34.50±1.64***	16.10±3.29**

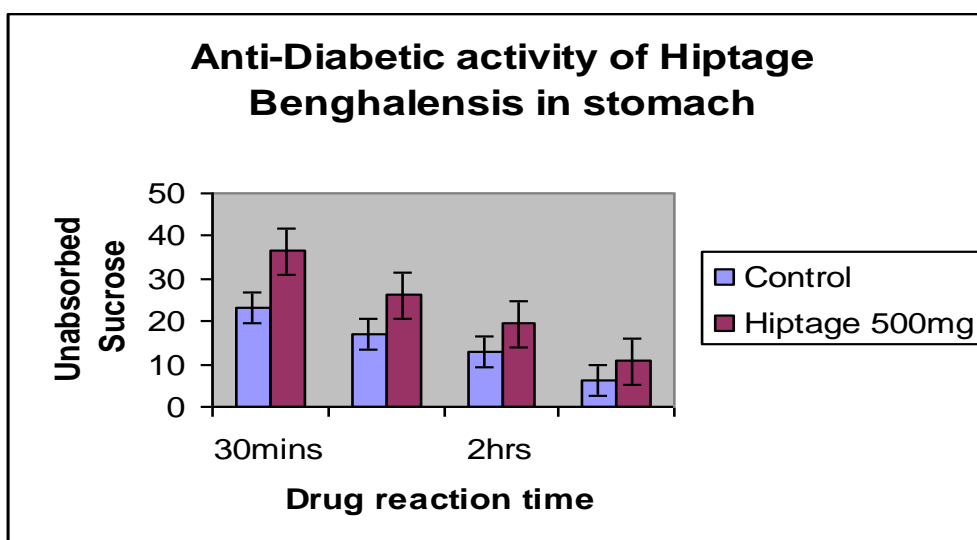


Figure 4: Anti-diabetic activity of *H. benghalensis* in Stomach

Table 6: Anti-diabetic activity of *Hiptage benghalensis* (500mg) in Cecum

Group	30mins	1hr	2hrs	4hrs
Control	7.90±.427	20.48±.92	13.92±.99	7.08±0.48
Hiptage 500mg	8.14±.62	25.57±.91***	19.72±.36***	13.12±1.49***

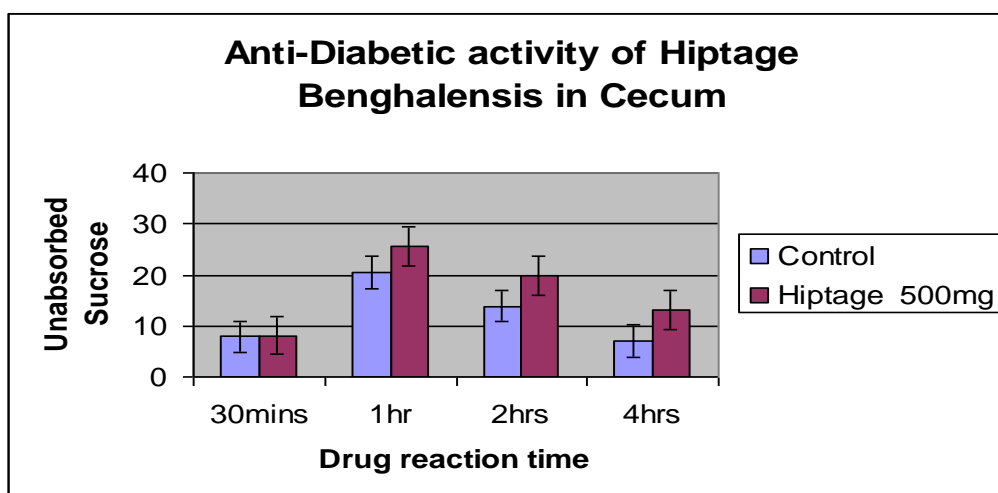


Figure 5: Anti-diabetic activity of *H. benghalensis* in Cecum

Table 7: Anti-diabetic activity of *Hiptage benghalensis* (500mg) in Large Intestine

Group	30mins	1hr	2hrs	4hrs
Control	3.43±.49	7.32±.79	12.28±1.01	8.19±0.54
Hiptage500mg	8.66±1.02***	16.95±1.82***	16.52±1.91**	12.1±0.79***

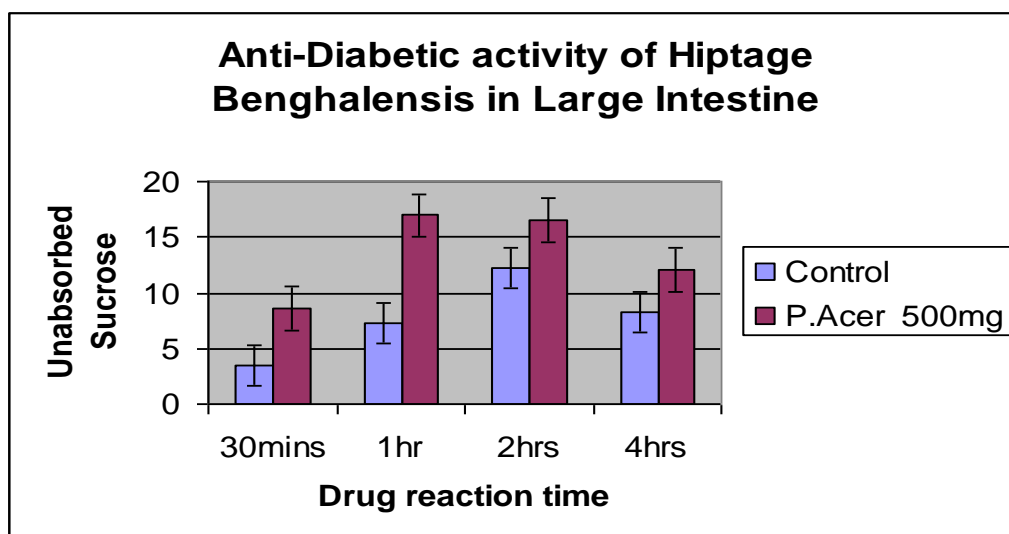


Figure 6: Anti-diabetic activity of *H. benghalensis* in Large Intestine

Table 8: Anti-diabetic activity of *H. benghalensis* (500mg) in Total GIT:

Group	30mins	1hr	2hrs	4hrs
Control	92.33±2.58	99.81±5.24	75.05±1.81	41.07±6.43
Hiptage500mg	139.68±1.698***	147.76±2.82***	119.02±4.86***	74.43±1.08**

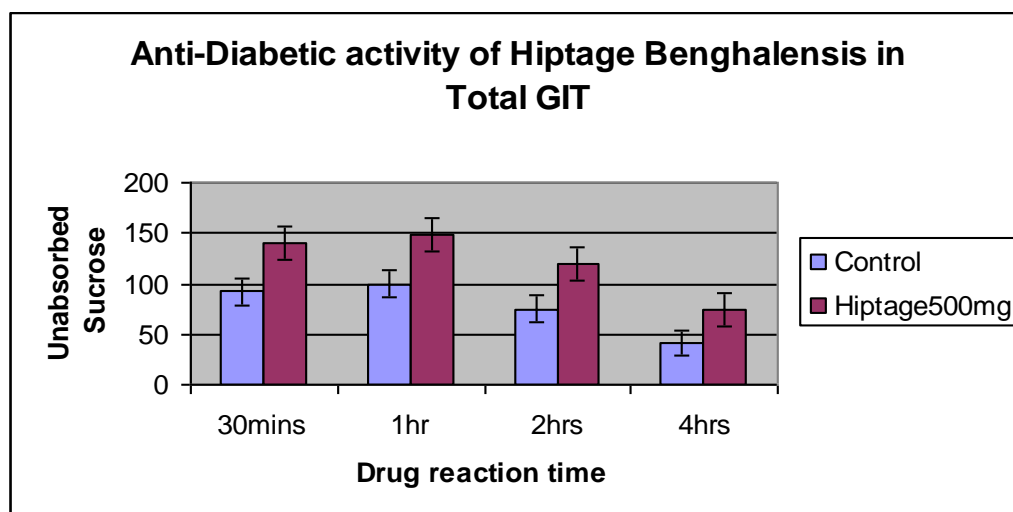


Figure 7: Anti-diabetic activity of *H. benghalensis* in Total GIT

In six segment method, the sucrose extract solution was administered to the model rat, and water and sucrose was administered to the control. Then after 30 minutes, 60 minutes, 120 minutes and 240 minutes the rats were sacrificed to observe the amount of sucrose remaining in the gastrointestinal tract. From the result we can deduce that the extract of the fruit of *H. benghalensis* was capable of causing a decrease in the absorption of sucrose solution from the gastrointestinal tract.

Discussion

It's a long and tedious process to isolate pure, pharmacologically active constituents from plants. Thus, it is necessary to have methods available which eliminate unnecessary separation procedures. Chemical screening is thus performed to allow localization and targeted isolation of new or useful constituents with potential activities. This procedure enables recognition of known metabolites in extracts or at the earliest stages of separation and is thus economically very important.¹⁰⁻¹²

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

The presence of flavonoids represents the possibility of some biological activity of the extracts of *H. benghalensis* such as it can modify allergens, viruses, and carcinogens indicating flavonoids have potential to be biological "response modifiers". It can also be used as an anti-allergic, anti-inflammatory¹³, anti-microbial and anti-cancer activities. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins.^{14, 15}

The presence of tannins represents the possibility of some biological activity of the extracts of *H. benghalensis* such as antidiarrheal, hemostatic, antihemorrhoidal, anti-inflammatory, astringent, and anti-infective. It can be used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers and as a cicatrizant on gangrenous wounds. It may have anti-viral effect which tannins have. It can also be used against poisons. There are also reports on the role of tannins in anti-nociceptive activity.¹⁶ Besides, alkaloids are well known for their ability to inhibit pain perception.¹⁷ Flavonoids and other phenolic compounds of plant origin have been reported as antioxidants and as scavengers of free radicals. Antioxidants can also exert anti-inflammatory effects.¹⁸ The flavonoids and tannins have been reported to produce anti-diabetic activity.¹⁹

The present study was undertaken to investigate the hypo-/anti-hyperglycemic activity of *H. benghalensis* extract in non-diabetic rats. Hypoglycemic activity that is found when given with a simultaneous glucose load in diabetic rats indicates that the extracts may interfere with the intestinal glucose absorption in the GI by various mechanisms.²⁰ One of the objectives of the study was to investigate whether the hypoglycemic effect is related to the inhibition of glucose absorption in the GI. From the result we can deduce that the extract of the fruit of *H. benghalensis* was capable of causing a decrease in the absorption of sucrose solution from the gastrointestinal

tract. This anti-diabetic property can be linked with the ability of the polyphenolic tannins and flavonoids (present in the fruit extract) to inhibit α -glycosidase enzyme.²¹ The ethanol extract showed significant dose dependent inhibition in activity compared with the controls.

Conclusion

The present study indicated that the ethanol extract of *H. benghalensis* may have potential use in medicine. Since glucose lowering effect of *H. benghalensis* was clearly evident from previous study reports and claims, glucose absorption inhibition could have been a possible mechanism responsible for the hypoglycemic effect.²² Our study confirms this effect as well, because when ethanol extract of *H. benghalensis* was given along with sucrose solution, it significantly increased sucrose retention in the gut compared with only the sucrose solution in control group of rats. It was found that the *H. benghalensis* extract increased glucose absorption for a certain time and after that it decreased which showed that *H. benghalensis* ethanol extract has the potency to inhibit glucotoxicity. Similar in vitro studies carried out with high concentrations of metformin also showed such inhibition of glucose absorption.²³ These observations provide evidence for the probable medicinal properties of stem of *H. benghalensis* claimed in Ayurveda medicine. Further studies should be undertaken to correlate the pharmacological activities with the chemical constituents of the stem of *H. benghalensis* and uncover specific mechanisms of action so that we may find a viable natural alternative to the traditional drugs in the market.

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Conflict of Interest

Authors have no conflict of interest.

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