

Original Article

Phytochemical Screening and Antidiarrhoeal Activity of *Hyptis suaveolens*

Md. Zeshan Hashib Shaikat¹, Md. Taleb Hossain² and Md. Golam Azam^{3*}

¹Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh.

²Department of Pharmacy, Northern University Bangladesh, Dhaka-1215, Bangladesh.

³Department of Pharmacy, North South University, Basundhara, Dhaka-1229, Bangladesh.

Summary. *Hyptis suaveolens* leaf has been used in conventional therapies for various disease conditions, including diarrhea. However, some of the therapeutic potentials of the plant have not been scientifically evaluated. Hence, the present study was aimed to evaluate the antidiarrhoeal activity of ethanol extract of *Hyptis suaveolens* leaf against an experimental model of castor oil-induced diarrhea in mice. Phytochemical screening of the plant extracts for their active constituents was also performed following standard procedures. Oral administration of the said ethanol extract (250 and 500 mg/kg) showed significant ($P < 0.01$) and dose-dependent inhibitory activity against castor oil induced diarrhoea. The onset of diarrhoea induced by castor oil was significantly delayed by administration of the plant extract. The results were comparable to those of standard antimotility drug, loperamide (50 mg/kg). Preliminary phytochemical screening shows the presence of alkaloid, glycoside, saponin, tannin and flavonoid as major constituents. The results indicate the presence of some active principles in the plant extract possessing anti-diarrhoeal effect and justify its traditional use in the treatment for diarrhoea.

Industrial relevance. Diarrheal diseases pose a leading cause of morbidity and mortality worldwide. Moreover, in developing countries, the cost of modern synthetic medicines is out of reach of the common man, especially those in rural areas. A large population of the Indian subcontinent depend on traditional system of medicine for their physical and psychological health needs. Hence there is an intensive search for natural products which are biologically active against diarrhea. In this study, the antidiarrhoeal activity of H. S. was studied and the results revealed that the ethanol leaf extract of *Hyptis suaveolens* extracts significantly reduced induction time of diarrhoea and number of diarrhoeal episodes in the orally treated mice. The results scientifically establish the efficacy of the plant extracts as antidiarrhoeal agents. Isolation and characterization of active principle(s) from this extract could lead to the development of herbal drug more effective in the treatment of diarrhea with less side effect, less costly affordable compared to synthetic drugs.

Keywords. *Hyptis suaveolens*; Castor oil-induced diarrhea; Anti-diarrhoeal activity; Phytochemical screening; Traditional medicine.

INTRODUCTION

Diarrhoea is one of the serious health problems especially for infants and children in the developing countries (Snyder & Merson 1982; Shobha & Thomas 2001). It accounts for more than 5 million deaths worldwide each year in infants and children of less than 5 years. Medicinal plants are potential sources of antidiarrhoeal drugs (Almeida *et al.* 1989; Maikere-Faniyo *et al.* 1989). For this reason, international organizations, such as WHO have encouraged studies for treatment and prevention of diarrhoeal diseases using traditional medicinal practices (Atta & Mouneir 2004). Currently, a number of medicinal plants with antidiarrhoeal and antimicrobial properties are being used in traditional

herbal practice in many Asian countries including India and Bangladesh (Chopra 1956; Mamoon *et al.* 2012). So, it is important to identify and evaluate commonly available natural drugs that could be used against any type of diarrhoeal diseases.

The plant, *Hyptis suaveolens* (L) Poit belonging to the family Lamiaceae or the Mint family is a medium aromatic shrub or woody herb distributed in the tropical and subtropical regions. It is a bushy erect plant with fragrant hairy cordate, opposite leaves, hairy stems, small blue or purple flowers in axillary and terminal cymes and small black mucilaginous seed. The leaves of the plant have been shown to contain alkaloids, terpenes and volatile oils (Gills 1992). Reported pharmacological activities of

*Corresponding Author.

✉ mg_azam@yahoo.com

☎ + 880-1198254997

Available online <http://www.ijarnp.org>

the plant include anti-inflammatory (Grassi *et al.* 2006), antinociceptive (Santos *et al.* 2007), antiulcer (Das *et al.* 2009), antioxidant (Gavani & Paarakh 2008), insecticidal (Adda *et al.* 2011) and antibacterial (Asekum *et al.* 1999) activities. Leaves of the plant have been traditionally used as a stimulant, antispasmodic and against colds and diarrhea (Beams 1994; Kirtikar & Basu 1991). However, there is no scientific proof justifying the traditional use of *Hyptis suaveolens* in the treatment of diarrhoea. Hence, the present work was undertaken to find out scientifically the relevance of traditional use of *Hyptis suaveolens* for anti-diarrhoeal activity purposes. To fulfill this objective, a relevant experimental model of mice has been employed in this study.

MATERIALS AND METHODS

Plant material. The plant *Hyptis suaveolens* was collected from Bogra district, Bangladesh. The identification of the plant material was confirmed by the experts of Bangladesh National Herbarium (Accession. No. 33879). The collected leaves of the plant were washed repeatedly with water and finally with distilled water, sliced into small pieces and sun-dried for one week. No mold growth was observed during the period of sun-drying. The plant parts were powdered with a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place prior to extraction process (Khandelwal 1985, Sadashivam & Manikramal 1992).

Preparation of extracts. The dried powdered sample (100 gm) was extracted in 500 mL of 95% ethanol for 7 days accompanying occasional shaking and stirring. The whole mixture was filtrated through a funnel plunged with white cotton and also using Whatman filter paper number 1. The resultant filtrate was concentrated to a powdered form through complete evaporation of the extraction solvent using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK). It rendered a solid residue of greenish color (yield 3.5 %) which was designated as the ethanol extract and stored in refrigerator until further investigation (Pavia *et al.* 1976).

Animals. White albino mice (*Swiss-webstar* strain, body weight = 20-25 gm) of both sexes were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for assessing biological activity. The animals were kept in standard environmental conditions (at $24.0 \pm 4^\circ\text{C}$ temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for at least one week for adaptation and had free access to standard laboratory food and water. All animal experiments were conducted in an isolated and noiseless condition in accordance with guidelines of the Animal Ethics Committee, Khulna University, Khulna, Bangladesh (114/B/KU/2008).

Antidiarrhoeal test. Published methods (Nwodo & Alumanah 1991; Shobha & Thomas 2001) were followed for this study with slight modification. The mice were screened initially by giving 0.5 mL of castor oil and only those showing diarrhoea were selected for the experiment. The test animals fastened overnight were randomly allocated to four groups consisting of six mice in each group. The animals of group I (control) received vehicles only (distilled water containing 0.1% Tween-80). Group-II (positive control) received standard antimotility drug loperamide (50 mg/kg body weight) as oral suspension. The group-III and group-IV (test groups) were treated with suspension of leaves extract of *Hyptis suaveolens* at the oral dose of 250 and 500 mg/kg body weight, respectively. After one hour treatment with distilled water, standard drug or plant extract, each animal was given 0.5 mL of castor oil by oro-gastric polyethylene catheter and placed in separate cages having adsorbent paper beneath. The characteristic diarrhoeal droppings were noted every hour in five hours study for each mouse. At the beginning of each hour old papers were replaced with the new ones.

Phytochemical screening. The preliminary phytochemical screening with various qualitative chemical tests was performed to detect the presence of various classes of phytoconstituents in 10% (w/v) solution of the plant extract. The following reagents were used to perform tests of different chemical groups. glycoside with Fehling's solution, saponins with the capability of producing suds, steroids with Libermann-Burchard test and chloroform and sulphuric acid, flavonoids with HCl, tannins with ferric chloride test and Potassium dichromate test, gum with Molish reagents and sulfuric acid. Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagerdorff's reagents. These phytoconstituents were identified by characteristic color changes using standard procedures (Ghani 2003; Harbone 1998).

Statistical Analysis. All the data obtained were expressed as the mean \pm standard error of mean (SEM). Statistical differences between the treatments and the controls were estimated by Statistical Package for Social Science (SPSS, version 11.5) software for Windows followed by the student's t-test. P values less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening. Preliminary phytochemical screening of ethanol extracts of leaves of *Hyptis suaveolens* revealed the presence of alkaloids, glycoside, saponin, tannins and flavonoids as major active constituents (Table 1). However, steroid and gum were absent in ethanol extract of the plant.

Antidiarrhoeal test. Castor oil (0.5 mL, p.o.) induced diarrhoea promptly within approximately

one hour in all the animals and produced a considerable amount of stool. The time for diarrhoeal induction was significantly prolonged by administration of ethanol extract of leaves of *Hyptis suaveolens* (250 and 500 mg/kg) in mice in a dose-dependent way (Table 2). None of animals treated with the plant extract showed diarrhoea up to at least one and half hour after administration of *Hyptis suaveolens*. The plant extract significantly reduced

the number of diarrhoeal episodes in comparison to control animals (Figure 1). The standard antimotility drug, loperamide (50 mg/kg) also profoundly prolonged, and reduced the onset of castor oil-induced diarrhoea and the number of diarrhoeal episodes, respectively. The anti-diarrhoeal activity of the plant extract at a higher dose (500 mg/kg) was comparable to that of loperamide at a dose of 50 mg/kg.

Table 1. Phytochemical analysis of the extracts of *Hyptis suaveolens*

Phytoconstituents	Ethanol extract
Steroid	-
Alkaloid	+
Glycoside	+
Gum	-
Saponin	+
Tannin	+
Flavonoid	+

“+” means presence and “-” means absence.

Table 2. Effect of *Hyptis suaveolens* leaves on onset of diarrhea induced by castor oil

Group	Oral treatment	Onset of diarrhoea (min)
Group I (Control)	1% Tween-80 in saline	58.8 ± 17.4
Group II (Positive control)	Loperamide (50 mg/kg)	118.2 ± 15.6*
Group III (Test group)	Plant extract (250 mg/kg)	101.4 ± 7.2*
Group IV (Test group)	Plant Extract (500 mg/kg)	111.0 ± 18.6*

Values are expressed as mean ± SEM (n = 6), *P < 0.01 vs control, Student's t-test.

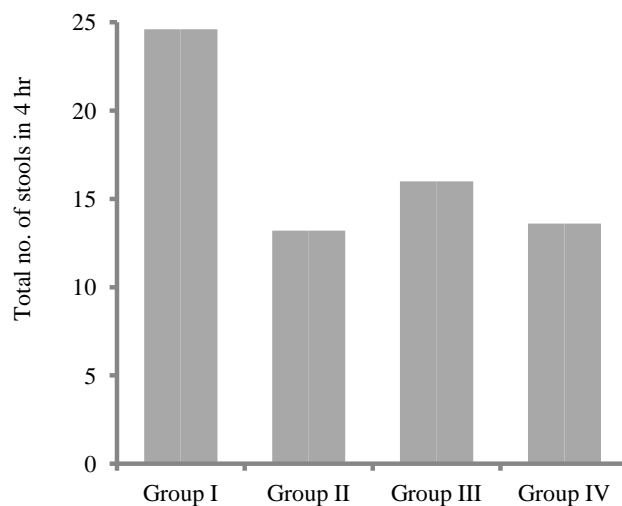


Figure 1. Effect of *Hyptis suaveolens* leaves on castor oil-induced diarrhoea in mice.

CONCLUSION

In conclusion, the present study demonstrates that the ethanol extract of *Hyptis suaveolens* contains pharmacologically active substance(s) possessing significant antidiarrhoeal activity. The present data provided a support for the traditional use of the plant

as an antidiarrhoeal remedy. However, further studies will be necessary to isolate and characterize the active principles which are responsible for the antidiarrhoeal effect and to understand exact its mechanisms of action.

REFERENCES

- Adda C, Atachi P, Hell K, Tamò M 2011. Potential use of the bushmint, *Hyptis suaveolens*, for the control of infestation by the pink stalk borer, *Sesamia calamistis* on maize in southern Benin, West Africa. *J Insect Sci* 11:33.
- Almeida CE, Karnikowski MG, Foletto G, Baldisserotto B 1989. Analysis of antidiarrhoeic effect of plants used in popular medicine. *Revista de Saude Publica* 29. 428-433.
- Asekun, OT, Ekundayo O, Adeniyi BA 1999. Antimicrobial activity of essential oil of *Hyptis suaveolens* leaves. *Fitoterapia* 70. 440-442.
- Atta AH, Mouneir SM 2004. Antidiarrhoeal activity of some Egyptian medicinal plant extracts. *J. Ethnopharmac* 92, 303-309.
- Beams C 1994. Atlas of plants of Mexican traditional medicine, First edition, National Indigenous Institute, Mexico City.
- Chopra RN, Nayar SL, Chopra IC 1956. Glossary of Indian medicinal plants. New Delhi. Council of Scientific and Industrial Research.
- Das PK, Sahoo S, Sethi R, Nayak PS, Nayak S, Joshi A 2009. Phytochemical and pharmacological investigation of the protective effect of plant *Hyptis suaveolens* against duodenal ulceration, *J Global Pharma Tech* 1. 82-87.
- Gavani U, Paarakh PM 2008. Antioxidant Activity of *Hyptis suaveolens* Poit. *Int J Pharmacol* 4. 227-229.
- Ghani A 2003. Medicinal Plants of Bangladesh- Chemical constituents and uses. Second edition. Asiatic Society of Bangladesh, Dhaka.
- Gills LS 1992. Ethnomedicinal uses of plants in Nigeria, University of Benin Press, Benin City, Nigeria, 276 p.
- Grassi P, Urías Reyes TS, Sosa S, Tubaro A, Hofer O, Zitter-Eglseer 2006. Anti- Inflammatory activity of two diterpenes of *Hyptis suaveolens* from El-Salvador. *Z. Naturforsch*, 61. 165-170.
- Harbone JB 1998. Phytochemical methods. a guide to modern techniques of plant analysis. Third edition. Chapman and Hall, London.
- Khandelwal KK 1985. Practical Pharmacognosy, Nirali Prakashan, Pune, India.
- Kirtikar KR, Basu BD 1991. Indian medicinal plants, Singh B & Singh MP Publishers, India. Vol. 3, P. 2032.
- Maikere-Faniyo R, Van Puyvelde L, Mutwewingabo A, Habiyaremye FX 1989. Study of Rwandese medicinal plants used in the treatment of diarrhoea I. *J Ethnopharmacol* 26 . 101-9.
- Mamoon SA, Azam MG 2012. Preliminary phytochemical screening and antidiarrhoeal activity of *Derris trifoliata* Lour. *Int J Pharm Sci Res* 3. 79-100.
- Nwodo OFC, Alumanah EO 1991. Studies on *Abrus precatorius* seed II. antidiarrhoeal activity. *J Ethnopharmacol* 31. 395-398.
- Pavia D L, Lampman G M, Kriz G S 1976. Introduction to organic laboratory techniques. a contemporary approach, Second edition. Saunders Golden Sanburst Series, W.B Saunders Company, Philadelphia, London, Toronto.; Pp 599-614.
- Sadasivam S, Manickam A 1992. Biochemical methods for agricultural sciences. Wiley Eastern Ltd., New Delhi, India.
- Santos TC, Marques MS, Menezes IA, Dias KS and Silva AB, Mello IC, Carvalho AC, Cavalcanti SC, Antonioli AR, Marcal RM 2007. Antinociceptive effect and acute toxicity of the *Hyptis suaveolens* leaves aqueous extract on mice. *Fitoterapia* 78(5). 333-336.
- Shoba FG, Thomas M 2001. Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhoea. *J Ethnopharmacol* 76:73-6.
- Snyder JD, Merson MH 1982. The magnitude of the global problem of acute diarrhea disease. A review of active surveillance of data. *Bull WHO* 60:605-613.