

VOLTAGE-DEPENDENT Ca²⁺ ENTRY BLOCKING ACTIVITY OF *HEDERA HELIX* LEAF EXTRACT EXPLAINS ITS MEDICINAL USE IN HYPERACTIVE GUT DISORDERS

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Abstract

Hedera helix L. (Araliaceae Family) has traditional reputation in gastrointestinal disorders, such as diarrhoea and spasms. This work investigated the pharmacological base of *H. helix* leaves in these disorders. In a castor oil-induced diarrhoeal model in mice, the aqueous methanolic extract from *Hedera helix* leaves (Hh.Cr), has provided 18 - 84% protection, similar to verapamil. In isolated rabbit jejunum preparations, Hh.Cr was found more potent against high K⁺ pre-contractions than spontaneous, similar to verapamil. This suggests that Hh.Cr mediates this effect through possible voltage-dependent Ca²⁺ channel blockade. This hypothesis was further strengthened when pre-incubation of the intestinal tissue with Hh.Cr (0.3 - 3 mg/mL) caused a rightward shift in the Ca²⁺ concentration-response curves, similar to verapamil. The extract was found safe in mice (5 g/kg). Hh.Cr possesses antidiarrheal and spasmolytic effects, mediated, possibly, through voltage-dependent Ca²⁺ entry blockade and provides pharmacological reasons for its medicinal use in diarrhoea and spasm.

Rezumat

Hedera helix L. (familia Araliaceae) este cunoscută în medicina tradițională pentru tratamentul afecțiunilor gastro-intestinale, cum ar fi diareea și spasmul. Această lucrare a investigat argumentele farmacologice pentru utilizarea frunzelor de *H. helix* în aceste tulburări. Într-un model de diaree indus cu ulei de ricin la șoareci, extractul hidrometanolic din frunzele de *Hedera helix* (Hh.Cr), a oferit un grad de protecție de 18 - 84%, similar cu verapamilul. Pe fragmente de jejun, izolate de la iepuri, Hh.Cr a prezentat un efect mai intens asupra pre-contracțiilor induse cu K⁺, față de cele spontane, similar cu verapamilul. Acest lucru sugerează că efectul antispastic al Hh.Cr se poate obține printr-o posibilă blocare a canalelor de Ca²⁺ voltaj dependente. Această ipoteză a fost consolidată când țesutul intestinal a fost pre-incubat cu Hh.Cr (0,3 - 3 mg/mL) și s-a înregistrat o deplasare spre dreapta a curbei concentrație de Ca²⁺ - răspuns, similară cu verapamilul. Extractul nu a prezentat efect letal la șoareci (5 g/kg corp). Hh.Cr a prezentat efecte antidiareice și spasmolitice, mediate, eventual, prin blocarea canalelor de Ca²⁺ voltaj dependente și oferă o justificare farmacologică pentru utilizarea în tratamentul diareei și spasmelor.

Keywords: *Hedera helix* leaves; antidiarrheal; spasmolytic; Ca²⁺ channel blockade; acute toxicity; castor oil model

Introduction

Hedera helix L. (Araliaceae Family), commonly known as “ivy” and locally as “palool” [1], is a popular ornamental plant in many countries and found in the northern areas of Pakistan. In folk medicine, *Hedera* leaves have a reputation in hyperactive gut diseases, such as diarrhoea and gut spasm [1, 2]. The biologically active compounds isolated from *H. helix* are triterpene saponins, glycosides, monodesmoside α -hederin, phenols, amino acids, steroids, vitamins, oils and polyacetylenes [3]. Previously, saponins isolated from *H. helix* leaves such as α -hederin showed contractile effects on rat stomach strips [4]. However, it potentiated isoprenaline induced bronchodilatation of bovine bronchial smooth muscle [5]. Hederacoside C did not affect smooth muscle tone

either of rat stomach strips [4] or of bovine bronchial muscle [5]. Limited studies are available on the leaf extract of *H. helix*; its aqueous extract possesses gastric ulcer preventive activity [6]. In isolated guinea-pig ileum, leaf extract, fractions, phenolic compounds and saponins showed antispasmodic activity against acetylcholine pre-contractions [7]. However, the authors were unable to explore the underlying mechanism of anti-spasmodic activity. The leaves of *H. helix* being spasmolytic in nature and having medicinal importance in hyper-active gut disorders, it was not explored in the past in diarrhoea and spasm. This investigation aimed to explore the potential usefulness of *H. helix* leaf extract in hyperactive gut disorders and to probe the underlying mechanism of action using *in vivo* and *in vitro* approaches.

Materials and Methods

Plant materials and extractions

Fresh leaves of *Hedera helix* were collected from the forest in Madyan, Swat, KPK, Pakistan, in 2007. The plant materials were identified and authenticated by Dr. Hassan Sher, Director Institute of Plant Sciences and Biodiversity, University of Swat, Swat, Pakistan. A voucher specimen (Hh-L-07) was deposited at the *herbarium* of the same Institute.

Leaves were shade-dried, coarsely ground and about 3 kg was soaked in 70% aqueous-methanol for three days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. This procedure was repeated three times and the combined filtrate was evaporated on a rotary evaporator at 37°C under reduced pressure (-760 mm Hg) to a thick, semi-solid mass of dark green colour, the crude extract (Hh.Cr), yielding approximately 55% (w/w).

Animals

Procedures and experimental protocols were in compliance with the European Communities Council (ECC, 2010) [8]. BALB/c albino mice (20 - 25 g) and local rabbits (1.5 - 2 kg) of either sex used in the study were given tap water *ad libitum* and a standard diet.

Drugs and standards

Chemicals and standards used were: acetylcholine chloride, potassium chloride, calcium chloride, verapamil (Sigma Chemical Company, St. Louis, MO, USA) and castor oil (Karachi Chemical Industries, Karachi, Pakistan). Stock solutions of all the chemicals were made in distilled water and the dilutions were prepared *ex-temporae* in normal saline on the day of the experiment.

Castor oil-induced diarrhoea

Antidiarrheal investigation was carried out as described [9, 10]. BALB/c Albino mice were fasted for 18 hrs and were divided in nine groups of five mice each. The first group received normal saline (10 mL/kg, p.o.) and served as negative control. The second group received castor oil, while groups 3rd to 5th received different doses (100, 300 and 1000 mg/kg) of the Hh.Cr administered orally through an intra-gastric feeding needle. Groups 6th to 8th received different doses (3, 10 and 30 mg/kg) of verapamil and served as positive control (calcium channel blocker, CCB). One hour after treatment, each animal received 10 mL/kg of castor oil and were observed for defecation. Four hours later, the presence of diarrhoeal droppings was noted on blotting sheets. The protection percentage against the castor oil-induced diarrhoea was calculated from the number of dry faeces.

Isolated rabbit jejunum preparations

As previously described [10, 11] rabbits had free access to water but were fasted for 24 hrs before the

experiment. The jejunal portion was isolated, cut into strips of 2 - 3 cm and mounted in 10 mL tissue baths containing normal Tyrode's solution, maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen. Composition of the normal Tyrode's solution was (mM): KCl 2.7, NaCl 136.9, MgCl₂ 1.1, NaHCO₃ 11.9, NaH₂PO₄ 0.4, glucose 5.6 and CaCl₂ 1.8 (pH 7.4). A preload of 1 g was applied to each tissue and kept undisturbed for 30 min.

Investigation of calcium antagonist activity

Potassium, as KCl, was used, because it produces sustained contractions and allows studying possible inhibitory effects on voltage-dependent Ca²⁺ channels (VDCs) [10, 12]. The extract of *H. helix* and verapamil, were then added cumulatively to obtain concentration-dependent inhibitory responses [13] which was expressed as percentage *vs.* control. Responses were analysed and recorded using force transducer (MLT 0201) coupled with bridge amplifier and Powerlab data acquisition system (ADInstruments, Australia).

For the confirmation of possible Ca²⁺ antagonist activity, the tissue was allowed to stabilize in normal Tyrode's solution, which was then replaced with Ca²⁺-free Tyrode's solution containing EDTA for 20 - 30 min in order to remove Ca²⁺ from the tissues and surroundings. This solution was further replaced with K⁺-rich and Ca²⁺-free Tyrode's solution, having the following composition (mM): KCl 50, NaCl 91.04, MgCl₂ 1.05, NaHCO₃ 11.90, NaH₂PO₄ 0.42, glucose 5.55 and EDTA 0.1. After 30 min of incubation, Ca²⁺ concentration-response curves (CRCs) were constructed in the absence and presence of different concentrations of the crude extract and verapamil (n = 5 - 7 in each case).

Acute toxicity test

As described previously [14], mice were divided in groups of 5 each. The test was performed using increasing doses of Hh.Cr (1 - 5 g/kg body weight), given orally, in 10 mL/kg to the different tested groups. Another group of mice was administered saline (10 mL/kg) and served as negative control. The mice were allowed food *ad libitum* and kept under regular observation and lethality was recorded after 24 hrs.

Statistical analysis

All data expressed are mean ± standard error standard deviation and the median effective concentration (EC₅₀ values) with 95% confidence intervals (CI). Data were analysed using the Student's t-test (paired or unpaired) with p < 0.05 as significantly different.

Results and Discussion

Based on the medicinal use of *Hedera* leaves in hyperactive gut conditions, such as diarrhoea and spasm [1, 2], the crude extract of its leaves (Hh.Cr)

was tested in the castor oil-induced diarrhoeal model. The Hh.Cr inhibited significantly ($p < 0.05$) the frequency of defecation as well as wetting of faeces when compared with the untreated group. Hh.Cr and verapamil, a standard calcium channel blocker [15] reduced the wetness of the faecal droppings and provided around 18.14 - 84.75% and 39.66 - 84.45% protection, respectively (Table I). Castor oil induces diarrhoea through the action of ricinoleic acid [10], which produces changes in the transport of water and electrolytes resulting in a hyper-secretory response and contraction of the intestine [10]. Thus, a potential antidiarrheal agent may exhibit its antidiarrheal effect by inhibiting gut motility

and/or electrolyte out flux (diarrhoeal droppings) [10, 16]. The protective effect of the Hh.Cr against the castor oil-induced diarrhoea in mice, suggests inhibitory effect either on contraction or on electrolyte out flux. To see its possible inhibitory effect on gut motility, the Hh.Cr was further studied in isolated intestinal preparations. In isolated rabbit jejunal preparations, cumulative addition of Hh.Cr suppressed the spontaneous contractions, similar to verapamil (Figure 1), with EC_{50} value of 3.94 mg/mL (3.09 - 5.01), as shown in Figure 2(A), thus showing intestinal smooth muscle relaxant (spasmolytic) activity.

Table I

Effect of the crude extract of the leaves of *Hedera helix* and verapamil on castor oil-induced diarrhoea in mice

Group	Dose	Percent of total faeces in 4 h	Percent of wet faeces in 4 h	Protection (%)
Control (Saline)	10 mL/kg	17.6 ± 3.88	4.01 ± 1.84	95.99 ± 1.84
Castor oil	10 mL/kg	21.20 ± 2.40	94.33 ± 0.54	5.76 ± 0.54
+ Hh.Cr	100 mg/kg	18.40 ± 2.82	81.86 ± 1.45	18.14 ± 1.45 ^a
+ Hh.Cr	300 mg/kg	2.06 ± 2.48	53.22 ± 4.44	46.78 ± 4.44 ^b
+ Hh.Cr	1000 mg/kg	17.2 ± 2.39	15.25 ± 3.03	84.75 ± 3.03 ^c
+ Verapamil	3 mg/kg	10.22 ± 2.10	60.34 ± 4.87	39.66 ± 4.87 ^a
+ Verapamil	10 mg/kg	13.80 ± 1.72	44.51 ± 3.88	55.49 ± 3.88 ^b
+ Verapamil	30 mg/kg	18.20 ± 3.31	15.55 ± 6.12	84.45 ± 6.12 ^c

Hh.Cr, *Hedera helix* crude extract of leaves. Mean ± S.D. (n = 5): ^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ vs. control, Student's t-test.

Previously [4], the leaf extract of *H. helix* was reported as spasmolytic against acetylcholine induced contractions in isolated guinea-pig ileum. The study focused more on the chemical constituents rather than probing the nature of the spasmolytic effect [7]. Therefore, in the present investigation, the nature of the spasmolytic effect of *H. helix* leaf extract was studied. The contraction of smooth muscle preparations, including rabbit jejunum, is dependent upon an increase of the intracellular Ca^{2+} [12], which occurs either through influx *via* VDCs or release from intracellular deposits.

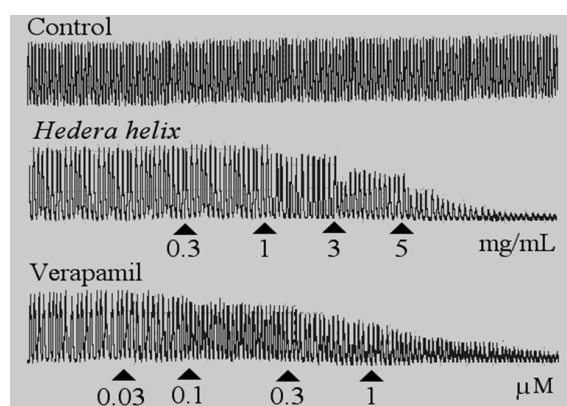


Figure 1.

A representative tracing showing the spasmolytic effect of the crude extract of *Hedera helix* (Hh.Cr) and verapamil on spontaneous contractions in isolated rabbit jejunum preparation

The periodic depolarization and repolarization regulates the spontaneous movements of the intestine and at the peak of depolarization, the action potential appears as a rapid influx of Ca^{2+} *via* VDCs [12]. The inhibitory effect of Hh.Cr on spontaneous movements of rabbit jejunum may be due to interference either with the Ca^{2+} influxes through VDCs or Ca^{2+} releases. Our previously studies have shown that spasmolytic constituents present in different medicinal plants mediate their effect through Ca^{2+} channel blockade [10, 11, 14].

The leaf crude extract of *H. helix* also caused, in a dose-dependent manner, relaxation of high K^+ -induced contraction with comparatively less EC_{50} value of 0.89 mg/mL (0.56 - 1.39) (Figure 2(A)) than observed against spontaneous contraction, similar to verapamil (Figure 2(B)). Thus, the inhibition of high K^+ (80 mM)-induced contraction of rabbit jejunum by the Hh.Cr may reflect inhibitory effects on the Ca^{2+} entries through VDCs [15]. This hypothesis was further strengthened when pre-incubation of the jejunal preparations with Hh.Cr (0.3 - 3 mg/mL) caused a rightward shift in the Ca^{2+} concentrations-response curves (Figure 2(C)), similar to verapamil (Figure 2(D)). Calcium channel blockers are considered useful as antispasmodic and have role in the management of diarrhoea [17]. The presence of CCB constituents in the crude extract of *H. helix* may be the possible mechanism for its spasmolytic

and antidiarrheal activities. In acute toxicity study, the Hh.Cr was found safe in mice up to the dose of 5 g/kg. Mice were observed for 24 hrs and no

mortality or any other apparent behavioural abnormalities were observed.

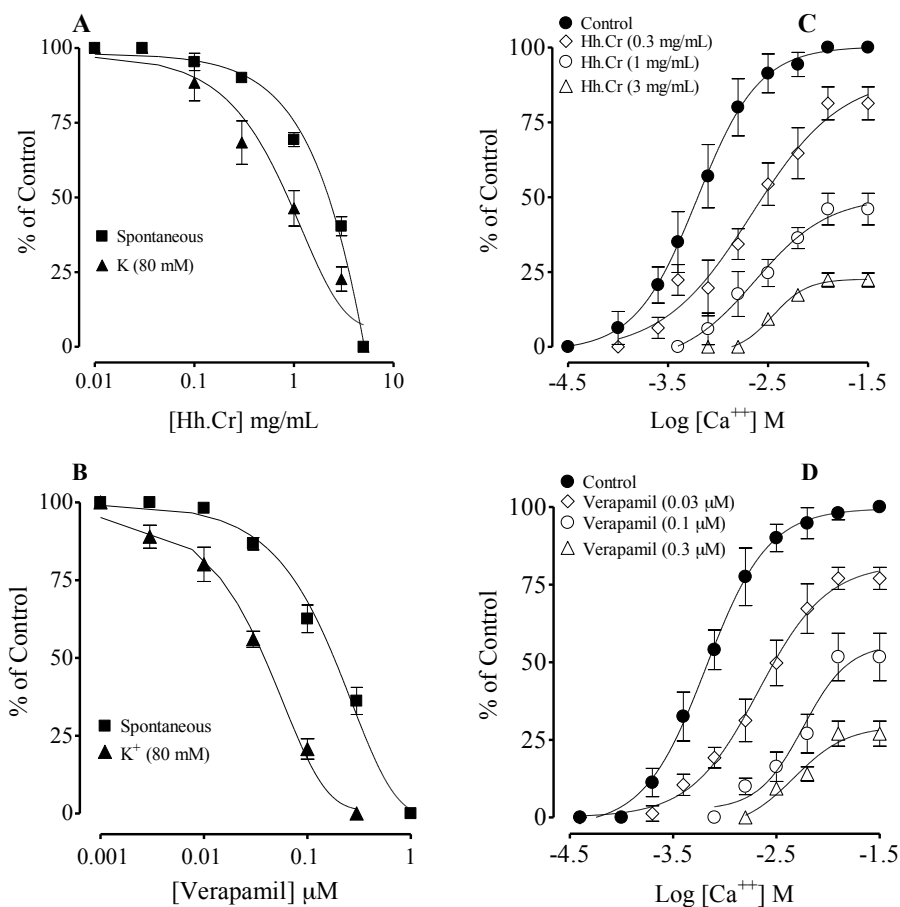


Figure 2.

Concentration-response curves (A) show the effect of the crude extract of *Hedera helix* leaves (Hh.Cr) and (B) show the effect of verapamil on spontaneous and K⁺ (80 mM)-induced contractions in isolated rabbit jejunum preparations. (C) and (D) show the effect of Hh.Cr and verapamil on the Ca²⁺ concentration-response curves in isolated rabbit jejunum preparations, made up in Ca²⁺-free medium. Values are mean ± SD (n = 5 - 7)

Conclusions

We have provided an evidence that the crude extract *H. helix* leaves possesses Ca²⁺ channel blocking constituents, which rationalizes the traditional use of *H. helix* in gut disorders, such as diarrhoea and spasm. Further studies will be needed in order to explore the chemical constituents and the molecular nature of the effect.

References

1. Baquar SR, Medicinal and poisonous plants of Pakistan. Printas, Karachi, 1989; 213-240.
2. Duke JA, Handbook of Medicinal Herbs. CRC Press, London. 2002; 392-417.
3. Yulia L, Wiesława B, Irena M, Roman D, *Hedera helix* as a medicinal plant. *Herba Polonica*, 2010; 56: 83-95.
4. Mendel M, Chłopecka M, Dziekan N, Wiechetek M, The effect of the whole extract of common ivy (*Hedera helix*) leaves and selected active substances on the motoric activity of rat isolated stomach strips. *J Ethnopharmacol.*, 2011; 134(3): 796-802.
5. Wolf A, Gosens R, Meurs H, Häberlein H, Pre-treatment with α -hederin increases β -adrenoceptor mediated relaxation of airway smooth muscle. *Phytomedicine*, 2011; 18(2-3): 214-218.
6. Mulikjanyan K, Novikova Zh, Sulakvelidze M, Getia M, Mshvildadze V, Dekanosidze G, Ivy water extracts as gastric ulcer preventive agents. *Georgian Med News*, 2013; 224: 63-66.
7. Trute A, Gross J, Mutschler E, Nahrstedt A, *In vitro* antispasmodic compounds of the dry extract obtained from *Hedera helix*. *Planta Med.*, 1997; 63: 125-129.
8. ECC. European Communities Council Directive 2010/63/EU.
9. Awouters F, Niemegeers CJE, Lenaerts FM, Janssen PAJ, Delay of castor oil diarrhoea in rats, a new way to evaluate inhibitors of prostaglandin biosynthesis. *J Pharm Pharmacol.*, 1978; 30: 41-45.
10. Shah AJ, Bhulani NN, Khan SH, Najeebur R, Gilani AH, Calcium channel blocking activity of *Mentha*

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- longifolia* L. explains its medicinal use in diarrhea and gut spasm. *Phytother Res.*, 2010; 24: 1392-1397.
11. Shah AJ, Gowani SA, Zuberi AJ, Ghayur MN, Gilani AH, Antidiarrheal and spasmolytic activities of the *Alstonia scholaris* crude extract are mediated through calcium channel blockade. *Phytother Res.*, 2011; 24: 28-32.
 12. Karaki H, Use of tension response to delineate the modes of action of vasodilators. *J Pharmacol Methods*, 1987; 18: 1-21.
 13. van-Rossum JM, Cumulative dose-response curves. II. Techniques for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch Int Pharmacodyn Therapie*, 1963; 143: 299-330.
 14. Khan AU, Gilani AH, Airways and cardiovascular inhibitory effects of *Olea europea* and *Terminalia bellerica* aqueous fractions. *Farmacia*, 2010; 58(6): 758-763.
 15. Godfraind T, Miller R, Wibo M, Calcium antagonism and calcium entry blockade. *Pharmacol Rev.*, 1986; 38: 321-416.
 16. Croci T, Landi M, Almond-Alt X, Le FG, Maffrand JP, Manara L, Role of tachykinins in castor oil induced diarrhea in rats. *Br J Pharmacol.*, 1997; 121: 375-380.
 17. Brunton LL, Agents affecting gastrointestinal water flux and motility; emesis and antiemetics; bile acids and pancreatic enzymes. In Goodman and Gillman's *The Pharmacological Basis of Therapeutics*, Hardman JG, Limbird LE, Molinoff PB (eds). McGraw Hill: New York, 1996: 917-936.