# Chapter 4

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* This chapter has been accepted in this format for publication in Methods & Findings in Experimental & Clinical Pharmacology.

**Antidiarrhoeal activity of aqueous extract of *Hermannia incana* Cav. leaves in Wistar rats**

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## ABSTRACT

**Objectives:** The phytochemical screening as well as the antidiarrhoeal activity of *Hermannia incana* Cav. leaf extract at 200, 400 and 600 mg/kg body weight was evaluated in Wistar rats.

**Methods:** The aqueous extract of the leaves of this plant at 200, 400 and 600 mg/kg body weight was evaluated for antidiarrhoeal activity using the castor oil-induced diarrhoea model in rats. The weight and volume of the intestinal content induced by castor oil was determined by enteropooling while the charcoal meal gastrointestinal motility was evaluated as in percentage as the ratio of the distance traversed by the charcoal to the total length of the small intestine. The phytochemical constituents of the extract were also carried out.

**Results:** The screening revealed the presence of alkaloids, tannins, saponins, phenolics, triterpenes, cardiac glycosides, flavonoids, cardenolides and dienolides. The extract significantly ($P<0.05$) prolonged the time of induction of diarrhoea and also reduced the frequency of diarrhoeal episodes and fecal parameters. The extract produced dose-dependent increase in the inhibition of defecation and intestinal content of the animals. The doses also reduced ($P<0.05$) the intestinal transit time of charcoal, masses and volumes of intestinal fluid.

**Conclusion:** These results are indications of antidiarrhoeal property of *H. incana* leaf extract with the 600 mg/kg body weight of the extract being the most effective. These findings therefore, lend scientific evidence to the use of *Hermannia incana* leaves in the management of diarrhoea by the people of Eastern Cape of South Africa.

**KEYWORDS:** *Hermannia incana*, antidiarrhoeal activity, castor oil, intestinal movement.

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## INTRODUCTION

Diarrhoea is an alteration in the normal bowel movement, characterized by increase in the water content in the intestine and or frequency of stools [(1)]. It can lead to severe dehydration and become life threatening when not treated [(2)]. Diarrhoea, which may or may not be infectious, is one of the leading causes of morbidity and mortality in developing countries [(3)]. The major causative agents in man include *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans* [(4, 5)].

Antibiotics are the major remedy of infectious diseases including diarrhoea; however, significant increase in antibiotics resistance has been observed in common human pathogens worldwide [(6)]. Similarly, oral rehydration therapy (ORT) has been widely identified as a key factor in the decline of child mortality due to diarrhoea [(7)]. However, the attack rate of the disease has remained unchanged and this treatment often fails in the high stool output state [(8)]. In view of this, there is the need to search for plants with

antidiarrhoeal activity. One of such plants used very often in the management of the disease by the people of Eastern Cape Province of South Africa is *Hermannia incana*.

*Hermannia incana* Cav. (Sterculiaceae) is known as Mavulakuvaliwe (Xhosa) and sweet yellow bells (English). It is a sparsely hairy prostrate herb with yellow flowers. It is found in grassland and marshes of the Eastern Cape Province of South Africa. *H. incana* is used as an emetic and the leaf sap extracted in cold water is used to treat stomach-ache and diarrhoea. Decoctions of the whole plant are taken to soothe coughs. However, despite the acclaimed folkloric use of *Hermannia incana* as an antidiarrhoeal agent, there is dearth of scientific evidence to substantiate such claim. The aim of this study therefore, was to evaluate the antidiarrhoeal activity of the aqueous extracts of the plant with a view to validating its acclaimed use by the traditional medicine practitioners of the Eastern Cape.

## MATERIALS AND METHODS

### Plant material

The plant samples were collected in August, 2007 from a natural population near the University of Fort Hare in the Eastern Cape Province of South Africa. The plant was identified by Prof. D.S. Grerson of the Department of Botany, University of Fort Hare, and a voucher specimen (Jaipal Med 001) was deposited in the Giffen Herbarium of the University.

**Drugs and chemicals**

The drugs used for the study include atropine sulphate and loperamide (Sigma-Aldrich, Inc., St. Louis, USA), castor oil (Appl. African Medicines (Pty) Ltd. Kempton Park, South Africa and gum acacia (BDH Chemicals Ltd., Poole, England).

**Animals**

Healthy, albino rats (171.06 ± 7.64 g) of both sexes were obtained from the Animal House of the Agricultural and Rural Development Research Institute, University of Fort Hare. All the animals were housed in clean metabolic cages placed in well-ventilated house conditions (temperature 23 ± 1˚C; photoperiod: 12 h natural light and 12 h dark: humidity: 45-50%). They were also allowed free access to Balanced Trusty Chunks (Pioneer Foods (Pty) Ltd, Huguenot, South Africa) and tap water. The cleaning of the cages was done daily.

**Preparation of extract**

The leaves of *H. incana* were separated from the stem, washed under running tap and air-dried at room temperature for 4 days. The dried material was pulverized with an electric blender. Fifty grams of the powder was extracted in 500 ml of distilled water for 48 h on an orbital shaker (Stuart Scientific Orbital Shaker, UK). The extract was filtered using a Buchner funnel and Whatman no. 1 filter paper and the resulting filtrate was freeze-dried (Savant Refrigerated Vapor Trap, RV T41404, USA) to give a yield of 7.31 g. This was reconstituted separately in distilled water to give the required doses for each experiment.

**Phytochemical screening**

The screening of some chemical constituents of the extract was carried out as described for the detection of tannins and triterpenes [9], alkaloids [10], phenolics and flavonoids [11], cardiac glycosides and saponins [12], steroids, cardenolides and dienolides and anthraquinones [13].

**Castor oil-induced diarrhoea**

Diarrhoea was induced in the rats using a modified method of Sunil *et al* [14] and Gerald *et al* [15]. The test animals were starved for 18 h prior to the experiment but were allowed free access to water. The animals were grouped into controls (distilled water and loperamide) and test groups (extract at 200, 400 and 600 mg/kg body weight) containing five rats each. All the groups received 1 ml of castor oil per animal orally [16]. The control group received distilled water, the second, third and fourth groups received plant extract of 200, 400 and 600 mg/kg body weight respectively while the fifth group (positive control) received the reference drug, loperamide (2.5 mg/kg orally). Half an hour (30 min) after drug and extract treatment, each animal was administered 1 ml of castor oil orally and the time between oil administration and appearance of first diarrhoeal drop was noted. Observations for the severity of diarrhoea were assessed each hour for a period of 6 h by monitoring the diarrhoeal drop on a pre-weighed filter paper placed beneath the individual rat cages. The total number of faeces, diarrhoea faeces and the total weight of faeces excreted were expressed as average and compared with the control groups. The percentage inhibition of diarrhoeal defecation in each group was also computed.

**Castor oil-induced enteropooling**

Intraluminal fluid accumulation was determined as described by Havagiray *et al.* [3]. Briefly, the test animals were starved for 18 h prior to the experiment but were allowed free access to water. Five animals were randomly selected into each group and placed in plastic cages. The negative control group received distilled water while the positive control group received atropine sulphate at the dose of 2.5 mg/kg body weight. The test groups were orally administered with the extract at the doses of 200, 400 and 600 mg/kg body weight. Immediately afterwards, 1 ml of castor oil was administered orally to each of the rats in all the groups. After 30 min, each rat was sacrificed according to the method of Yakubu *et al* [17] and the ends of the small intestine tied (at the pylorus and the caecum). The organ was dissected out and intestinal content was collected by squeezing into a measuring cylinder. The volume and the mass of the intestinal content were obtained.

**Gastrointestinal motility test**

The methods described by Abdullahi *et al* [18] and Gerald *et al* [15] were adopted for the effect of the extract on gastrointestinal transit in rats. The test animals were starved for 18 h prior to the experiment but were allowed free access to water. The animals were grouped into controls and tests containing five rats per group. The negative control group received distilled water while the positive control group received atropine sulphate at 2.5 mg/kg body weight The test groups received the extract at the doses of 200, 400 and 600 mg/kg body weight. After 30 min, rats from each group were administered with 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia). After 30 and 45 min of the

administration of charcoal meal, the animals from the various groups were sacrificed using anaesthetic method [17]. The small intestine was removed and the length (pylorus to caecum) as well as the distance travelled by charcoal meal through the organ was measured. This distance was expressed as a percentage of the length of the small intestine.

**Statistical analysis**

Data are means of five replicates ± SD. Percentage data were transformed to arcsine before analysis. Statistical analysis was done using Student's t-test. Significant levels were tested at $P<0.05$.

**RESULTS**

Phytochemical screening of the extract of *Hermannia incana* revealed the presence of alkaloids, tannins, saponins, phenolics, triterpenes, flavonoids, cardiac glycosides, cardenolides and dienolides while anthraquinones and steroids were not detected.

In the castor oil-induced diarrhoea experiment, aqueous extract of *Hermannia incana* significantly prolonged the time of diarrhoeal induction in a dose dependent manner. The frequency of stooling (number of wet feaces and total number of feaces) as well as fresh weight and water content of the faeces decreased significantly (Table 1). There was more reduction in these parameters at 600 mg/kg body weight when compared with loperamide. There was also increase in the percentage inhibition of defecation. However, the highest dose (600 mg/kg body weight) produced inhibition of defecation that compared favourably with the loperamide (Table 1).

The masses and volumes of the intestinal fluid significantly decreased in dose dependent manner. Similarly, the inhibition of the intestinal content of the animals increased in dose dependent manner. However, the 600 mg/ kg body weight of the plant extract produced the highest percentage inhibition of intestinal content among the various groups (Table 2).

Compared with the distilled water control, the extract reduced the distance moved by the charcoal meal. Whereas the 400 mg/kg body weight resulted in charcoal meal transit time that was similar to the reference drug, atropine sulphate, the 600 mg/kg body weight of the extract produced the least transit time (Table 3).

## DISCUSSION & CONCLUSION

The use of herbal remedies in the treatment of diarrhoeal diseases is a common practice in many countries of the world including South Africa. A number of medicinal plants have been reported to be effective against diarrhoea and dysentry, [15, 19, 20].

The use of castor oil as diarrhoea inducer is well documented [21, 22]. The most active component of the oil is the ricinoleic acid. Ricinoleic acid causes irritation and inflammation of the intestinal mucosa. The irritation stimulates the peristaltic activity of the small intestine, causing changes in the electrolytic permeability of the intestinal mucosa. This sequence of events leads to the release of prostaglandins which stimulates motility and secretion thereby decreasing the absorption of sodium and potassium ions [20-22]. Inhibitors of prostaglandin synthesis are also known to delay diarrhoea induced by castor oil [14]. Therefore, the prolonged time of induction of diarrhoea, decreased frequency of stooling and fecal parameters (total number, fresh weight, water content and

number of wet feaces) observed with the extract in this study are indications of antidiarrhoeal potential. These observations also suggest that the antidiarrhoeal activity of the extract may be due to the inhibition of prostaglandin biosynthesis.

Atropin sulphate is known to produce anticholinergic effect in the evaluation of intestinal transit [23], while the activated charcoal is capable of preventing the absorption of drugs and other chemicals into the body [24]. The suppressed intestinal propulsive movement of the charcoal meal by the extract of *H. incana* suggests antidiarrhoeal activity of the plant. This may be due to the ability of the extract to increase the time for absorption of water and electrolytes in the manner similar to the action of atropine sulphate.

It has been shown that castor-oil causes motility and secretory diarrhoea [20]. This is achieved through its dual effects on gastrointestinal motility as well as water and electrolyte transport (decreasing $Na^+$ and $K^+$ absorption) across the intestinal mucosa [20]. The inhibition of castor-oil induced intestinal fluid accumulation (enteropooling) as well as the weight of the intestinal content may be due to the ability of the extract to increase the reabsorption of electrolytes and water. This may also be due to the ability of the extract to inhibit the induced intestinal accumulation of fluid in a manner similar to loperamide [25]. In this study, the 600 mg/kg body weight of the extract of *H. incana* showed the best antidiarrhoeal activities.

The antidiarrhoeal activities of medicinal plants have been attributed to the presence of bioactive agents such as tannins, alkaloids, saponins, flavonoids, steroids and terpenoids [3]. While the flavonoids are known to inhibit intestinal motility and hydroelectrolytic secretion [24], tannins denature proteins in the intestinal mucosa by

forming protein tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion [3]. Therefore, the antidiarrhoeal activity of *Hermannia incana* leaves observed in this study may be attributed to the presence of tannins, flavonoids, alkaloids and saponins in the aqueous extract. The prolonged onset of diarrhoea, inhibition of castor oil-induced enteropooling and the suppressed propulsive movement observed in this study are indications of antidiarrhoeal potential of *Hermannia incana* leaf extract. These findings lend support to the fokloric use of *Hermannia incana* in the Eastern Cape of South Africa as an antidiarrhoeal agent.

**ACKNOWLEDGEMENT**

This research was supported with grants from the National Research Foundation, South Africa.

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Table 1: Effect of aqueous extract of *Hermannia incana* leaves against castor oil-induced diarrhoea in Wistar rats. n = 5, x ± SD

| | Loperamide (mg/kg body weight) | Water | Plant extract (mg/kg body weight) | | |
|---|---|---|---|---|---|
| Parameters/Doses | 2.5 | 0 | 200 | 400 | 600 |
| Onset time (min) | $102 \pm 0.04^{a}$ | $70 \pm 0.03^{b}$ | $84 \pm 0.04^{b}$ | $106 \pm 0.05^{a}$ | $142 \pm 0.05^{c}$ |
| Total number of feaces | $2.66 \pm 0.13^{a}$ | $6.50 \pm 0.64^{b}$ | $4.83 \pm 0.12^{c}$ | $3.50 \pm 0.17^{d}$ | $2.83 \pm 0.14^{a}$ |
| No of wet feaces | $1.00 \pm 0.05^{a}$ | $5.33 \pm 0.01^{b}$ | $5.16 \pm 0.13^{b}$ | $1.16 \pm 0.03^{a}$ | $0.66 \pm 0.03^{c}$ |
| Fresh weight of feaces (g) | $0.88 \pm 0.09^{a}$ | $4.58 \pm 0.71^{b}$ | $2.65 \pm 0.05^{c}$ | $1.07 \pm 0.09^{a}$ | $0.48 \pm 0.05^{d}$ |
| Water content of feaces (ml) | $0.42 \pm 0.06^{a}$ | $2.17 \pm 0.07^{b}$ | $1.35 \pm 0.04^{c}$ | $0.45 \pm 0.04^{a}$ | $0.16 \pm 0.02^{d}$ |
| Inhibition of defecation (%) | 59.08 | 0.00 | 25.69 | 46.15 | 56.46 |

Values carrying superscripts different from the controls for each parameter are significantly different ($P<0.05$).

Table 2: Anti-enteropooling activity of *Hermannia incana* in Wistar rats. n = 5, x ± SD

| | Atropine sulphate (mg/kg body weight) | Water | Plant extract (mg/kg body weight) | | |
|---|---|---|---|---|---|
| Parameters/Doses | 2.5 | 0 | 200 | 400 | 600 |
| Mass of intestinal fluid (g) | $1.82 \pm 0.09^{a}$ | $2.91 \pm 0.11^{b}$ | $2.57 \pm 0.1^{c}$ | $2.12 \pm 0.06^{d}$ | $1.14 \pm 0.16^{e}$ |
| Volume of intestinal fluid (ml) | $1.77 \pm 0.11^{a}$ | $2.87 \pm 0.14^{b}$ | $2.59 \pm 0.10^{c}$ | $2.16 \pm 0.05^{c}$ | $1.10 \pm 0.17^{d}$ |
| Inhibition of intestinal content (%) | 38.67 | 0.00 | 9.68 | 24.87 | 61.67 |

Values carrying superscripts different from the controls for each parameter are significantly different ($P<0.05$).

Table 3: Effects of aqueous extract of *Hermannia incana* on charcoal meal transit time in Wistar rats. n = 5, x ± SD

| | Atropine sulphate (mg/kg body weight) | Water | Plant extract (mg/kg body weight) | | |
|---|---|---|---|---|---|
| Parameters/Doses | 2.5 | 0 | 200 | 400 | 600 |
| 30 min Transit (%) | $28.42 \pm 0.62^{a}$ | $52.65 \pm 2.39^{b}$ | $47.78 \pm 1.07^{b}$ | $32.55 \pm 1.84^{a}$ | $21.97 \pm 1.53^{c}$ |
| 45 min Transit (%) | $37.00 \pm 0.67^{a}$ | $62.05 \pm 1.00^{b}$ | $56.44 \pm 1.01^{c}$ | $38.59 \pm 1.236^{a}$ | $30.64 \pm 1.02^{d}$ |

Values carrying superscripts different from the controls for each parameter are significantly different ($P<0.05$).