SHORT COMMUNICATION

Antidiarrheal and antispasmodic activities of *Trillium govanianum* rhizomes extract: involvement of calcium channel blockade

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**ABSTRACT**

The antidiarrheal effect of methanolic extract of *Trillium govanianum* Wall. ex D. Don (Melanthiaceae alt. Trilliaceae) was studied at doses of 12.5, 25, and 50 mg/kg in different animal models of diarrhea including castor oil (6 mL/kg), magnesium sulfate (2 gm/kg), sodium picosulfate (2 mL/kg) and lactitol (0.25 mL/kg). The antispasmodic effect of *T. govanianum* was studied on isolated rabbit’s jejunum, using acetylcholine as tissue stabiliser and verapamil as calcium channel blocker. *T. govanianum* attenuated the diarrhea by producing a significant decrease in the number and weight of stool, and an increase in stool latency time. *T. govanianum* completely inhibited both spontaneous as well as high potassium induced contractions of isolated rabbit’s jejunum, which was analogous to verapamil. Moreover, *T. govanianum* produced a right shift in calcium concentration response curve, confirming its calcium channel blocking activity. These findings provide scientific ground to its medicinal use in diarrhea and gut spasms.
1. Introduction

Diarrhea is still a major health problem particularly in children, and recently there has been an increase in the research underlying characterisation of novel pathways in intestinal fluid transport, increase awareness of naturally derived therapies, and development of new tools and models for efficient screening of potential anti-diarrheal therapeutic remedies (Thiagarajah et al. 2015). The traditional use of natural products including medicinal plants has immensely improved the pharmacotherapy of different diseases and provided lead compounds that possess significant therapeutic value in various pathological conditions (Petrovska 2012; Islam et al. 2015; Shahid et al. 2017).

*Trillium govanianum* Wall. ex D. Don (Melanthiaceae alt. Trilliaceae) is a perennial herb, distributed in South Asia, especially in India, China, Pakistan and Bhutan (Rani et al. 2013). The rhizome of *T. govanianum* is used in the traditional system of medicine in Indian subcontinent and China for the treatment of different ailments. In folk medicine, the rhizome is used to cure dysentery, backache, healing of wound, skin boils, menstrual and sexual disorders (Mahmood et al. 2012; Shah et al. 2015; Sharma et al. 2018).

Previous phytochemical studies revealed that *T. govanianum* rhizomes are a rich source of secondary metabolites like fatty acid (compound 1), phytoecdysteroids (compound 2, 3), steroids (compound 4, 5) and saponins (compound 6, 7, 8) (Figure S3) (Ur Rahman et al. 2015, 2017; Singh et al. 2020). Furthermore, analgesic, anti-inflammatory, antifungal, antibacterial, cytotoxicity, anti-leishmanial and anticancer activities were documented (Ur Rahman et al. 2015, 2016, 2017; Khan et al. 2018, 2020). However, to the best of our knowledge, studies concerning the therapeutic efficacy of *T. govanianum* as an antidiarrheal agent have not been carried out. Therefore, the present study, we evaluated mechanistically the crude methanolic extract for antidiarrheal potential using *in vitro* and *in vivo* animal models.

2. Results and discussion

2.1. Antidiarrheal activity of *Trillium govanianum* extract

The effect of *T. govanianum* methanolic extract at various doses (12.5, 25, and 50 mg/kg) in the castor oil induced diarrhea is shown in Table S1. The tested doses
significantly increased ($p < 0.001$) the latency to diarrhea and reduced the number and weight of stools, as compared to the control group.

The effect of *T. govanianum* methanolic extract at various doses (12.5, 25, and 50 mg/kg) in the magnesium sulfate induced diarrhea is shown in Table S1. The tested doses exhibited significant reduction in the number ($p < 0.001$) and weight ($p < 0.01$) of stool as compared to the control group.

The effect of *T. govanianum* methanolic extract at various doses (12.5, 25, and 50 mg/kg) in the sodium picosulfate induced diarrhea is shown in Table S2. The lowest dose (12.5 mg/kg), significantly increased the latency time ($p < 0.001$).

The effect of *T. govanianum* methanolic extract at various doses (12.5, 25, and 50 mg/kg) in the lactitol induced diarrhea is shown in Table S3. The total number of stool significantly decreased at doses of 12.5 mg/kg ($p < 0.01$), and 25–50 mg/kg ($p < 0.001$). This effect of *T. govanianum* was analogous to the positive control drug loperamide (4 mg/kg) in all tested models.

2.2. Effect of Trillium govanianum extract in the charcoal meal transit test

The effect of *T. govanianum* methanolic extract at various doses (12.5, 25, and 50 mg/kg) on the gastrointestinal transit time of charcoal meal is shown in Table S4. *T. govanianum* showed a similar gastrointestinal motility inhibitory action like that of loperamide.

2.3. Effect of Trillium govanianum extract on KCl-induced contractions

The mechanism underlying the reduction in the frequency of bowel movements by *T. govanianum* extract was investigated. The effect of *T. govanianum* extract on the isolated rabbit jejunum is shown in Figure 1A. The extract completely inhibited both spontaneous as well as high K$^+$ induced contractions of the isolated preparations (rabbit’s jejunum) at concentrations of 5 and 3 mg/mL, and the effect was comparable to the standard drug, verapamil, which inhibited the high K$^+$ induced as well as spontaneous contractions at concentrations of 3 and 1 μM, respectively, as shown in Figure 1B. The isolated rabbit jejunum is a spontaneously gut contracting model (Gilani et al. 2010), allowing to examine the relaxant effect, without inducing contraction. *T. govanianum* inhibited high K$^+$ induced and spontaneous contractions in the rabbit’s jejunum.

2.4. Effect of Trillium govanianum extract on calcium channels

The calcium channel blocking mediated myorelaxant effect was investigated by drawing the calcium chloride curves in the presence and absence of extract, and these were then compared with calcium channel blocker, verapamil. The extract at concentrations of 0.1–0.3 mg/mL caused a rightward shift of the calcium concentration response curves (Figure 2A) and exhibited the suppression of the maximum contractile effect that was comparable to the verapamil at tested concentrations of 0.03–0.1 μM (Figure 2B).
As the tested doses of *T. govanianum* relaxed the high K⁺ induced contractions in an analogous pattern to verapamil, it can be argued that the antidiarrheal effect of *T. govanianum* primarily involves inhibition of calcium channels. This effect (Ca⁺⁺ antagonist) was further confirmed from the shift of Ca⁺⁺ concentration response curves to the right with inhibition of the maximum response, in a similar trend to verapamil.

3. Experimental
Provided as supplementary material.

4. Conclusion
In conclusion, the methanolic extract of *T. govanianum* displayed a potent *in-vivo* antidiarrheal and *in-vitro* anti-spasmodic effects. Our findings showed that *T. govanianum* possesses promising Ca⁺⁺ antagonists-like constituents(s), which provides scientific basis to its medicinal use in diarrhea and gut spasms.

Disclosure statement
No potential conflict of interest was reported by the authors.

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