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ANTI-DIARRHOEAL ACTIVITY OF PETROLEUM ETHER EXTRACT OF VANDA TESSELLATA LEAVES ON CASTOR OIL-INDUCED DIARRHEA IN RATS

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ABSTRACT

The purpose of the present study was to evaluate scientifically the anti-diarrhoeal effects of petroleum ether extract of leaves of *Vanda tessellata Roxb.* (PVT) was studied against castor oil-induced-diarrhoea model in rats. Anti-diarrhoeal activity of petroleum ether extract of *Vanda tessellata* was investigated in this study using castor oil-induced-diarrhoea, enteropooling and Small intestinal transit models in rats. The weight and volume of intestinal content induced by castor oil were studied by enteropooling method. Standard drug diphenoxylate (5 ml/kg, p.o) was significant reductions in fecal output and frequency of droppings whereas PVT at the doses of 200 and 400 mg/kg p.o significantly ($P<0.001$) reduced the castor-oil induced frequency and consistency of diarrhoea and enteropooling. The gastrointestinal transit rate was expressed as the percentage of the longest distance travelled by the charcoal divided by the total length of the small intestine. PVT at the doses of 200 and 400 mg/kg significantly inhibited ($P<0.001$) the castor oil induced charcoal meal transit. The PVT showed marked reduction in the number of diarrhoea stools and the reduction in the weight and volume of the intestinal contents, as well as a modest reduction in intestinal transit. The results obtained establish the efficacy and substantiate the folklore claim as an anti- diarrheal agent.

Keywords: Anti-diarrhoeal Activity, *Vanda tessellata*, Castor Oil- induced diarrhoea, Enteropooling Method, Small intestinal transit.

INTRODUCTION

Vanda tessellata Roxb. (Family: Orchidaceae) plants have been used in the indigenous medicine such as Ayurveda and local traditional medical practices. The leaf juice is used for the treatment of certain inflammatory conditions. It is also instilled into the ear as a remedy for otitis. The leaves in the form of a paste are applied to the body to bring down fever. The *leaves* are used in rheumatism, dysentery, nervous problems, bronchitis, dyspepsia and fever. Unani practitioners hold it to be laxative and tonic to the liver. It is also used to treat hiccough, piles, boils on the scalp, etc. This plant *leaves* is reported to contain an alkyl perulate and beta sistosterol - D- glucoside. The dried whole herb also contains long chain alkanes and alkanol sistosterol, resin, saponin, tannins, fatty acids, colouring agents, etc. Medicinal orchids, in general, are not subjected to detailed pharmacological studies. Scientific studies on medicinal orchids can lead to the development of invaluable drugs to certain medical conditions [1]. Therefore, to justify the traditional claims the present study was undertaken to find out if petroleum ether extract of *Vanda tessellata Roxb.* leaves demonstrates the Anti-diarrhoeal Activity against Castor Oil- induced diarrhoea in rats. Hence, the present study was designed to verify the claims of the native practitioners.

MATERIALS AND METHODS

Plant collection

The leaves of *Vanda tessellata* has been collected from Sri Venketeswara University near Tirupati, Andhra Pradesh during the month of August 2011 and dried under shade. The plant was authentified by Mr. K. Madhava chetty, Assistant Professor, Department of Botany of S. V. University, Tirupati. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts

Leaves of *Vanda tessellata* were shade dried, and the dried leaves were powdered to get coarse granules. The coarse powder was subjected to continuous hot extraction in Soxhlet apparatus using petroleum ether. The solvent

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was removed by distillation under reduced pressure, which produced a greenish sticky residue (yield 10%w/w with respect to dried plant material). The concentrated crude extract were stored and used for the further study.

Animals used

Adult albino rats (Wistar strain) of either sex with weighing 150 - 180gm were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory condition for an acclimatization periods of 7 days prior to performing the experiment. The animals had access to laboratory chow and water *ad libitum*. The experimental protocols were approved by institutional Animal Ethical Committee & a written permission from Institutional ethical committee has been taken to carry out and complete this study.

Acute Toxicity Study

The acute toxicity of petroleum ether extract of *Vanda tessellata* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not mortal even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study [2].

Castor oil-induced diarrhoea

Diarrhoea was induced by Nwafor *et al.,* (2005) [3]. Animals were fasted for 24 h but allowed free access to water. Rats were divided into four groups of six animals each, diarrhoea was induced by administering 2 ml of castor oil orally to rats. Group I treated as control (2 ml/kg, p.o. saline), group II received diphenoxylate (5 ml/kg p.o) served as standard and group III and IV received PVT (200 and 400 mg/kg, p.o) 1 h before castor oil administration. Then observed for consistency of faecal matter and frequency of defaecation for 4 hrs.

Castor oil-induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert *et al.,* (1976) and DiCarlo *et al.,* (1994) [4,5]. Animals were fasted for 24 h but allowed free access to water. Rats were divided four groups of six animals each. Group I received normal saline (2 ml/kg, p.o served as a control, group II received diphenoxylate (5.0 mg/kg p.o.) and groups III and IV received PVT 200 and 400 mg/kg p.o respectively 1hr before the oral administration of castor oil. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated.

Small intestinal transit

Rats were fasted for 18 h divided into five groups of six animals each, Group I received 2 ml normal saline orally, group II received 2 ml of castor oil orally with saline 2 ml/kg p.o, group III received atropine (3 mg/kg, i.p.), group IV and V received PVT 200 and 400 mg/kg p.o respectively, 1 h before administration of castor oil. One ml of marker (10% charcoal suspension in 5% gum acacia) was administered orally 1 h after castor oil treatment. The rats were sacrificed after 1h and the distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from the pylorus to caecum [6].

Statistical analysis

The data were expressed as mean ± standard error mean (S.E.M).The Significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

Results

Acute toxicity study

Acute toxicity study in which the animals treated with the PVT at a higher dose of 2000 mg/kg did not manifest any significant abnormal signs, behavioral changes, body weight changes, or macroscopic findings at any time of observation. There was no mortality in the above-mentioned dose at the end of the 14 days of observation.

Castor oil-induced diarrhoea

After 30 min administration of castor oil the diarrhoea was clinically apparent in all the animals of control group, for the next 4 h. This was markedly reduced by diphenoxylate (5 ml/kg p.o). A similar marked reduction in the number of defecations over four hours was achieved with *G.speciosa* at the doses of 200 or 400 mg/kg p.o. PVT 200 and 400 significantly inhibited the defecation PVT 200 and 400 mg/kg, p.o. dose of extract delayed the onset of diarrhoea and only 30% of animals showed diarrhoea at first hour ($P<0.001$) (Table 1).

Castor oil-induced enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop. Castor oil-induced enteropooling is not influenced by diphenoxylate (5 ml/kg p.o) in rats. PVT 200 and 400 produced a dose-dependent reduction in intestinal weight and volume. PVT 200 and 400 mg/kg, p.o dose produced inhibit the volume of intestinal content respectively with significance ($P<0.001$). The weight of intestinal content was also reduced significantly at both the doses **(**Table 2).

Small intestinal transit

The percent intestinal transit was increased with castor oil, but it was reduced in both doses of extract, and much more markedly by atropine. PVT 200 mg/kg, p.o dose of extract produced significant intestinal transit induced by castor oil respectively. Whereas, PVT 400 mg/kg, p.o dose significantly produced castor oil induced charcoal meal transit (Table 3).

Table 1. Effect of PVT on castor oil-induced diarrhoea in rats

Group	Treatment	Mean Defecation in 4hr
I	Castor oil (2ml p.o) +saline (2ml/kg p.o)	22.16±1.22
II	Castor oil (2ml p.o) +diphenoxylate (5 ml/kg p.o)	7.36±0.24**
III	Castor oil (2ml p.o) +PVT (200mg/kg p.o)	13.67±0.54*
IV	Castor oil (2ml p.o) +PVT (400mg/kg p.o)	8.28±0.44**

Effect of PVT on castor oil-induced diarrhoea in rats: PVT was administered p.o 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with Castor oil + saline-treated group.

Table 2. Effect of PVT on castor oil induced enteropooling in rats

Group	Treatment	Weight of Intestinal Content
I	Castor oil (2ml p.o) +saline (2ml/kg p.o)	2.97±0.41
II	Castor oil (2ml p.o) +diphenoxylate (5 ml/kg p.o)	1.58±0.25**
III	Castor oil (2ml p.o) +PVT (200mg/kg p.o)	1.72±0.24*
IV	Castor oil (2ml p.o) +PVT (400mg/kg p.o)	1.32±0.14**

Effect of PVT on castor oil-induced enteropooling in rats: PVT was administered p.o 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with Castor oil + saline-treated group.

Table 3: Effect PVT on castor oil-induced small intestinal transit in rats

Group	Treatment	Total Length of Intestine	Distance Travelled By Marker
I	saline (2ml/kg p.o)	90.28 ± 1.14	45.18 ± 1.17
II	Castor oil (2ml p.o) + saline (2ml/kg i.p)	85.22 ± 2.17	77.14 ± 1.13
III	Castor oil (2ml p.o) +atropine (3mg/kg i.p)	88.67 ± 2.11	35.29 ± 1.33**
IV	Castor oil (2ml p.o) +PVT (200mg/kg i.p)	85.49 ± 1.12	54.19 ± 1.14*
V	Castor oil (1ml p.o) +PVT (400mg/kg i.p)	85.27 ± 1.18	44.24 ± 1.21**

Effect of PVT on castor oil-induced small intestinal transit in rats: PVT was administered p.o 1 h before castor oil administration. Values are expressed as mean ± SEM from the experiments. *P<0.01, **P<0.001 when compared with Castor oil + saline-treated group.

DISCUSSION AND CONCLUSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by hurry, resulting in an excess loss of fluid in the faeces. At doses of 200 and 400 mg/kg, the petroleum ether extract of *Vanda tessellata* showed significant anti-diarrhoeal activity against castor oil-induced diarrhoea as compared with the control group it significantly($P<0.001$) reduced the frequency of diarrhoea and consistency of defecations (Table 1). The PVT also showed a dose related decrease in castor oil-induced diarrhoea. Several mechanisms have been supposed to be involved in the diarrhoeal effect of castor oil [7]. These include Castor oil is decreases fluid absorption, increases secretion in the small intestine and colon, and affects smooth muscle contractility in the intestine. Castor oil produces diarrhoeal effect due to its active component of recinoleic acid [8], inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption [9, 10], activation of adenylyl cyclase [11], stimulation of prostaglandin formation [12], platelet-activating factor and recently nitric oxide was contribute to the diarrhoeal effect of castor oil [13-15]. Despite the fact that number of mechanisms has been involved for the diarrhoeal effect of castor oil, it has not been possible to define its correct mechanism of action [11]. PVT may act an above any one of the mechanism.

It is also noted that PVT significantly inhibited castor oil induced intestinal fluid accumulation and the volume of intestinal content (Table 2). The secretory diarrhoea is associated with an activation of Cl^- channels, causing Cl^- efflux from the cell, the efflux of Cl^- results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea [16]. The involvement of muscarinic receptor effect was confirmed by increased production of both gastric secretion and intraluminal fluid accumulation induced by castor oil. The PVT may inhibit the secretion of water into the intestinal lumen and this effect is partly mediated by both α_2-adrenoceptor and muscarinic receptor systems. The significant inhibition of the castor oil-induced enteropooling in mice suggests that the extract of *Vanda tessellata* produced relief in diarrhoea by spasmolytic activity in vivo and anti-enteropooling effects [10].

The PVT significantly reduced the castor oil induced intestinal transit as compared with control group (Table 3). In this study, atropine increased intestinal transit time possibly due to its anti-cholinergic effect [16]. In castor oil induced diarrhoea, the liberation of ricinoleic acid results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [17] by prevents the reabsorption of NaCl and water [18]. Probably PVT increased the reabsorption of NaCl and water by

decreasing intestinal motility as observed by the decrease in intestinal transit by charcoal meal.

In conclusion, the present study has shown that *Vanda tessellata* is a potential therapeutic option in the effective management of diarrhoea, thus justifying its widespread use by the local population for these purposes. Concerted efforts are being made to fully investigate the mechanisms involved in the pharmacological activities of *Vanda tessellata* and phytochemical studies are also in progress to isolate and characterize the active constituents of *Vanda tessellata*. The isolated compound may serve as useful prototypes of anti-diarrhoeal drugs of natural origin possessing the desired pharmacological activities while lacking certain untoward effects.

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