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Antidiarrheal potential of *Tabernaemontana divaricata*

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Abstract

The antidiarrheal activity of hydroalcoholic and aqueous extracts of *Tabernaemontana divaricata* leaves were evaluated in rats. Studies were carried out on castor oil induced diarrhea and gastrointestinal motility. The hydroalcoholic and aqueous extracts of *Tabernaemontana divaricata* leaves (100, 200 and 300 mg/kg, p.o.) causes a dose dependent protection against castor oil induced diarrhea and decreased markedly gastrointestinal motility. A preliminary phytochemical screening of extracts of *Tabernaemontana divaricata* leaves revealed the presence of alkaloids, tannis, resins, proteins, amino acids, flavonoids, saponins, phenols, glycosides, steroids, triterpenoids, fixed oils and fats. The results obtained showed that the hydroalcoholic and aqueous extracts of *Tabernaemontana divaricata* leaves showed a significant activity against diarrhea and so it can be useD traditionally for gastrointestinal disorders.

Keywords: *Tabernaemontana divaricata;* diarrhea, castor oil; tannins

Introduction

Diarrhea is one of the main water-borne diseases endemic in many regions of the world and considered to be the major health threats to the world populations, both in tropical and subtropical poor countries (Damiki & Siva, 2011). The disease is majorly responsible for morbidity and mortality in developing countries leading to the death of millions of people each year (Carlos and Saniel, 1990). It may be defined as a situation in which an adult daily stool exceeds 200 g and contains 60-95% water (Weber, 1976).

The major causative agents of diarrhea in human beings include various enteropathogens like *Shigella flexneri, Escherichia coli, Staphylococcus aureus, Salmonella typhi*, and *Candida albicans* (Robert et al., 2001; Anne et al.,2002).For the treatment and management of diarrhea in developing countries, the world health organization (WHO) has constituted a diarrhea disease control programme (DDC) which includes studies of traditional medicine practices together with the evaluation of health education and prevention approaches (Syder

& Merson, 1982). About 80% of the world's population relies on the use of traditional medicine, which is predominantly based on herbal products (WHO, 1993).

T. divaricata belongs to the Apocynaceae family. The generic synonym of *T. divaricata* is *Ervatamia coronaria* and widely distributed in tropical countries as a garden plant. *T. divaricata* is a shrub or small tree, usually glabrous, found in the Konkan, North Kanara, Western ghats in Malabar, throughout North India and Travencore upto 3000 ft. (Nadkarni, 1954; Sharma & Mehta,1969; Kirtikar & Basu,1975).

Leaves of *Tabernaemontana divaricata* contain indole alkaloids stapfinine, (Atta - Ur - Rahman et al.,1985; Atta - Ur - Rahman et al.1986) dimeric indole alkaloids -conophyline and conophyllidine (Kam et al.,1993), minor alkaloid-voaharine (Kam et al.,1992). Flowers of *E. coronaria* contains α- amyrin acetate, β- amyrin acetate, lupeol β–sitosterol and stigmasterol, flavone, apigenin ,four indole alkaloids harmine, heyneanine, voacristine and apparicine, phenolic acids namely salicylic acid, syringic acid and vanillic acid (Joshi,2004). Stems of *E. Coronaria* contains bis indole alkaloid 19,20-dihydro ervatanine A, other alkaloids coronidine, heyneanine, voacristine, voacamine, descarbomethoxy voacamine and five phenolic acids namely vanillic, gentisic, syrengic, α-hydroxy benzoic and salicylic acid. (Henriques et al.,1996).

Root bark of *T. divaricata* contains α- amyrin acetate, lupeol acetate, α- amyrin, lupeol ,cycloartenol, β-sitosterol, campesterol, benzoic acid, aurantiamide acetate, coronaridine, coronaridine hydroxyindolenine, ibogamine, 5-hydroxy-6-oxocoronaridine, 5-oxo- coronaridine, 6-oxocoronaridine, (±)19- hydroxycoronaridine and 3- oxocoronaridine and voacamine (Kamesh et al.,1980).

In traditional medicine *Tabernaemontana divaricata* (L.) R.Br.is used to treat various diseases like diarrhea, abdominal tumours, arthralgia, asthma, epilepsy, eye infections, fever, fractures, headache, inflammation, leprosy, mania, oedema, paralysis, piles, rabies, rheumatic pain, skin diseases, urinary disorders, strangury, toothache (Ghani,2003), ulceration and vomiting. It is also used as anthelmintic, antihypertensive, aphrodisiac, diuretic, emmenagogue, hair growth promoter, purgative, remedy against poisons and tonic to the brain, liver and spleen (Hoernle, 1983; Khan , 2010).Regardless of the reasonably wide use of the plant as popular medicine for its antidiarrheal properties, amazingly no research has been carried out to evaluate the antidiarrheal activity of leaves extract. The present study was thus carried out to investigate the antidiarrheal effect of hydroalcoholic and aqueous extract of *Tabernaemontana divaricata* leaves evaluating the traditional folklore medicinal use of the plant.

Materials and Methods

Plant material

The leaves of *Tabernaemontana divaricata* (TD) were collected in January, 2010, from Bhopal, M.P., India. The plant was identified and authenticated by Dr. D. V. Amla, Deputy Director, National Botanical Research Institute, Lucknow, India, and a voucher specimen No. Tit/NBRI/CIF/141/2009 was deposited in Department of Pharmacognosy and Phytochemistry, TIT-Pharmacy, Bhopal.

Preparation of extract

The leaves were dried in shade and stored at 25°C., powdered, passed through sieve no.40. The dried powdered leaves of TD (500g) was first defatted with Petroleum Ether (60-80°C) and later extracted with water: ethanol (50:50) and distilled water separately by maceration for 5 days. After completion of the extraction, the solvent was removed by distillation and concentrated *in vaccuo* (40°C) to yield hydroalcoholic (26.85% W/W) and aqueous extract (28.8 % W/W) respectively.

Preliminary phytochemical screening of TD

The preliminary phytochemical investigation was carried out with hydroalcoholic and aqueous extracts of leaves of *T. divaricata* for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods (Khandelwal, 2006; Kokate ,1996).

Animals

Male wistar rats weighing 200±20g were provided by the animal house of TIT Pharmacy, Bhopal, from the stock originally purchased from, National Institute of Nutrition, Hyderabad, India. Animals were made available with the standard animal feed and water supply *ad libitum* before the experiments. The animal studies were approved by the Institutional Animal Ethics Committee (Reg. no. 831/bc/04/CPCSEA), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India. For each experimental study rats were starved for 18h with access to water only.

Chemicals

Loperamide hydrochloride (Torrent Pharmaceuticals Ltd., Baddi), castor oil(S. D. Fine Chem., India), normal saline solution, 0.9% NaCl and charcoal meal (10% activated charcoal in 5% gum acacia) were used.

Acute toxicity study

Acute toxicity study was carried out for the extracts of TD following Organization of economic co-operation and development (OECD) guidelines (OECD guideline, 2001). The extract was dissolved in distilled water in a dose of 2 g/kg body weight and orally administered to overnight-fasted, healthy rats (n = 6).The animals were observed continuously for 24 h for mortality.

Antidiarrheal activity

Castor oil induced diarrhea

Wistar rats were divided into eight groups comprising of six animals each. The first group received 2% gum acacia and served as control, while the second group received the

standard drug, loperamide (5 mg/kg, p.o.).The plant extract viz. Hydroalcohlic and aqueous (100, 200, and 300 mg/kg, p.o.) were administered to groups III-VIII, respectively. One hour later, all the animals received castor oil (2 ml/rat, p.o.). The rats were then housed singly in cages lined with white blotting paper. The papers were changed every hour. The total number of both dry and wet droppings was counted every hour for a period of 4 h and compared with the control group. The total number of droppings of the control group was considered as 100% (Awouters et al., 2008).

Gastrointestinal motility test

Wistar rats were divided into eight groups comprising of six animals each. Castor oil was administered orally to all animals to induced diarrhea, 1 h after the administration of Castor oil, Group I received 2% gum acacia and served as control. Group II received standard drug (loperamide 5 mg/kg p.o.) and Group III-VIII received hydro alcoholic and aqueous extracts (100, 200 and 300 mg/kg, p.o.). 1 h later all animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia, p.o.). After 1 h all animals were sacrificed and the distance traveled by the charcoal meal from pylorus to caecum was measured and expressed as a percentage of the total distance of the intestine (Pazhani et al., 2001)

Statistical analysis

The results are expressed as mean ± S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) after Tukey's multiple comparison tests. $P<0.05$ was considered statistically significant in all the cases.

Results

Effect of T.divaricata extracts on castor oil-induced diarrhea

One-way ANOVA followed by Tukey's test revealed that acute treatment with hydroalcoholic and aqueous extracts of *T. divaricata* leaves (100, 200, 300 mg/kg, p.o.), 60 min prior, dose dependently reduced the number of wet feces in rats ($P<0.05$) when compared to control group (Table.1). Further, post hoc test revealed that treatment with loperamide (5 mg/kg, p.o.), 60 min prior, reduced number of wet feces in rats ($P<0.05$) when compared to control group (Table.1).

Effect of *T. divaricata* extracts on *Gastro intestinal motility test*

The hydroalcoholic and aqueous extracts of *T. divaricata* leaves (100, 200, 300 mg/kg, p.o.) also significantly ($P<0.05$) dose dependently reduce the gastrointestinal distance (40.56 ± 1.38 cm and 58.20±3.04 cm) respectively traveled by the charcoal meal in the rat's gastrointestinal tract compared with the control group (Table 2). Loperamide (5 mg/kg, p.o.) produced a marked (34.31 ± 2.028 cm) decrease in the propulsion of charcoal meal through gastrointestinal tract (Table 2).

Table 1. Effect of *T. divericata* leaves extracts on castor oil–induced diarrhea in rats.

Treatment (mg/kg, p.o.)	Mean number of wet feces (0- 4 h)
Control (2% gum acacia)	7.55 ± 0.42
Loperamide 5	0.79 ± 0.08***
T. divericata hydroalcholic extract	
100	3.88 ± 0.25**
200	2.64 ± 0.22***
300	1.87 ± 0.21***
T. divericata aqueous extract	
100	5.49 ± 0.36*
200	5.18 ± 0.57**
300	5.03 ± 0.72**

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at ‴ $P<0.001$, ‟ $P<0.01$, ‧ $P<0.05$ vs. control group respectively (One-way ANOVA followed by Tukey's post hoc test).

Table 1. Effect of *T. divericata* leaves extracts on gastrointestinal motility in rats..

Treatment (mg/kg, p.o.)	Mean number of wet feces (0- 4 h)
Control (2% gum acacia)	67.68 ± 1.54
Loperamide 5	34.31 ± 2.02***
T. divericata hydroalcholic extract	
100	55.82 ± 1.65**
200	52.91 ± 1.56***
300	40.56 ± 1.38***
T. divericata aqueous extract	
100	58.82 ± 1.74*
200	57.48 ± 2.14*
300	58.20 ± 3.04*

Values are expressed as mean±S.E.M. (n = 6).Values are statistically significant at ‴ $P<0.001$, ‟ $P<0.01$, ‧ $P<0.05$ vs. control group respectively (One-way ANOVA followed by Tukey's post hoc test).

Preliminary phytochemical screening of TD

Phytochemical screening of TD revealed the presence of alkaloids, tannis, resins, proteins, amino acids, flavonoids, saponins, phenols, glycosides, steroids, triterpenoids, fixed oils and fats.

Discussion

The result indicates that the hydroacoholic and aqueous extracts of *T. divaricata* leaves possesses significant antidiarrheal activity. Numerous phytochemicals, including sugars, flavonoids, flavonol glycosides, alkaloids and terpenes have been identified in this plant (Atta-Ur-Rahman et al.,1985; Atta-Ur-Rahman et al.1986; Henriques et al.,1996; Joshi, 2004) and these may mediate the antidiarrheal properties. Vidari et.al reported in his finding that the plant *Baccharis teindalensis* consist of few flavonoids such as apigenin which may be

responsible for the antidiarrheal activity (Vidari et al., 2003). *T. divaricata* also contains apigenin (Joshi,2004) which may participate in antidiarrheal property of this plant.

Tannins are one of the major constituents of the plants which may have antidiarrheal potential (Mukherjee et al., 1998; Atta & Mouneir, 2005), and leaves of *T. divaricata* also contain tannins which is been confirmed from the positive phytochemical test although the active component has not been defined.

These tannins precipitate proteins of enterocytes, result into reduce peristaltic movements and intestinal secretion (Almeida et al., 1995). Studies on the functional role of tannins suggested that they could also carry similar functions by reducing the intracellular Ca^{2+} inward current or by activation of the calcium pumping system (which induces the muscle relaxation) (Belemtougri et al., 2006). *T. divaricata* leaves extract have inhibiting effect both in fluid secretion and gastrointestinal propulsion hence from the results it seems that the antidiarrheal effect of *T. divaricata* may be due to the similar mechanisms. In conclusion, these experimental studies validate the presence of antidiarrheal activity in *T. divaricata* leaves, which are used traditionally by humans suffering from diarrheal disorders.

In conclusion, these experimental studies validate the presence of antidiarrheal activity in *T. divaricata* leaves, which are used traditionally by humans suffering from diarrheal disorders.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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