IJP (2018), Vol. 5, Issue 7 **(Research Article)**

Received on 06 March, 2018; received in revised form, 24 March, 2018; accepted, 30 March, 2018; published 01 July, 2018

EVALUATION OF ANTI-DIARRHEAL AND DIURETIC ACTIVITY OF *TABERNAEMONTANA DIVARICATA* LINN. LEAVES

Sangita Kumari [*1, 2], A. Mazumder [1] and S. Bhattacharya [3]

Noida Institute of Engineering and Technology [1], Institute of Pharmacy, Greater Noida - 201306, Uttar Pradesh, India.
Uttarakhand Technical University [2], Dehradun - 248007, Uttarakhand, India.
Department of Pharmacy [3], College of Medicine and Health, Wachemo University, Ethopia.

Keywords:

Anti-diarrheal, Diuretic, *Tabernaemontana divaricata*, MELTD, Defecations

Correspondence to Author:
Sangita Kumari

Noida Institute of Engineering and Technology, Institute of Pharmacy, Greater Noida - 201306, Uttar Pradesh, India.

Email: sangi.mph04@gmail.com

ABSTRACT: The aim of the present work was to investigate the anti-diarrheal and diuretic potentials of *Tabernaemontana divaricata* Linn. leaves of the family Apocynaceae. In almost all tropical developing countries of the world, diarrhoea is one of the most common and serious disease, the principal cause of morbidity and mortality among children under five years. The methanolic extract of the leaves of *T. divaricata* L. was evaluated for anti-diarrheal properties in castor-oil induced diarrhoea. In the castor oil induced diarrhoea, MELTD significantly inhibited the number of defecations. The diuretic activity of the methanolic extract of leaves of *T. divaricata* (MELTD) was assessed on the basis of Lipschitz value and diuretic index. The standard diuretic drug furosemide exhibited a significant increase in the urinary excretion of water and electrolytes (Na^+ and K^+), which is comparable with the extract. The diuretic index at the dose of 200 mg/kg (MELTD) was calculated as 1.55, significant as compare to the standard drug having diuretic index of 1.70.

INTRODUCTION: Diarrhoea is kenned as an incrementation in the number of stools (3 or more per 24 h), an incrementation in the fluidity of the stool and the presence of mucus or/and blood, with Neutrophils and polymorphs in the stool. Due to severe loss of fluid and electrolytes in the stool, it leads to dehydration, hyponatremia and hypokalaemia. Annually diarrheal diseases caused several million of deaths in the world [1]. Diarrhoea accounts for more than 8 million deaths worldwide each year in < age 5, especially in developing countries [2, 3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.IJP.5(7).409-413

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(7).409-13

The impact of diarrheal infection is exacerbated by lack of adequate and affordable healthcare in developing countries, particularly in rural and marginalized areas [4]. The WHO has initiated the Diarrhoea Disease Control Program, in which traditional medicinal practices and the evaluation of health education and prevention approaches were included [5, 6].

These plants and their active constituents have been experimentally screened for their gastroprotective properties [7]. The Asian traditional medicine systems have identified several herbs and spices to treat GI tract disorders. There are many herbal plants which are listed in the indigenous system for their anti-diarrheal activity with lesser side effects. Diuretic drugs increase the rate of urine flow, sodium excretion and adjust the volume and composition of body fluids in a variety of clinical situations.

Diuretic agents have very wide application in the treatment of various chronic diseases associated with edema. They are prescribed for the treatment of hypertension, congestive heart failure, nephritic syndrome glaucoma, diabetes insipidus, liver aliments and pregnancy toxaemia [8]. The herbal plants used in traditional medicine as diuretics have increased recent years and used in the treatment of hypertension. Natural diuretics increase the urine output and urinary electrolyte concentration [9]. Naturally occurring diuretics inhibit Na^+ reabsorption and also inhibit secretion of anti-diuretic hormones [10]. The wide ranges of phyto-constituents were responsible for diuretic activity includes alkaloids, glycosides, tannins, phenolics coumarins and triterpenoids *etc*. [11, 12].

Tabernaemontana, commonly known as Tagar belongs to the family Apocynaceae is a beautifully shaped evergreen shrub distributed throughout India, Bangladesh and other parts of the South East Asia [13, 14]. The genus of Tabernaemontana includes approximately 100 species, commonly distributed in tropical countries of the world, including India, Brazil, Egypt, Sri Lanka, Vietnam, Malaysia and Thailand. *Tabernaemontana divaricata* (synonym-*Ervatamia coronaria*) commonly called as Crepe Jasmine, a glabrous, evergreen, dichotomously branched shrub mostly grown in tropical countries.

In traditional medicine, *Tabernaemontana divaricata* (L.) R.Br.is utilized in treatment of the diseases like diarrhea, abdominal tumors, arthralgia, asthma, epilepsy, ocular perceiver infections, pyrexia, fractures, headache, inflammation, leprosy, mania, oedema, paralysis, piles, rabies, rheumatic pain, skin diseases, urinary disorders, strangury, toothache, ulceration and regurgitating [15].

MATERIALS AND METHODS:

Plant Collection and Authentication: The aerial part of the *Tabernaemontana divaricata* was collected in the month of September from Greater Noida, Uttar Pradesh, India. The herbarium of the plant specimen *Tabernaemontana divaricata* (L.) R.Br.ex Roem and Schult. was authenticated by Dr. K.C. Bhatt, Senior Scientist, NBPGR, Indian Council of Agricultural Research, New Delhi with voucher number NHCP/NBPGR/2011-64/. The herbarium of this plant specimen is similarly preserved in the NIET institute.

Extraction: *Tabernaemontana divarivata* leaves were collected, dried in the shade and powdered. Dried powder was subjected to Soxhlet extraction using methanol at 40 - 60 ºC. The liquor was concentrated to recover methanol and the extracts was kept in dessicator for drying. The percentage yield was calculated as 10.8%. The dried methanol extracts of the leaf of *T. divaricata* (MELTD) was stored in tightly closed amber color glass bottle.

Experimental Animals: Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida (Reference no.1121\ac\CPCSEA\07). Adult wistar rats (150-250g) of either sex were used for the study. The animals were maintained at the Animal House of the NIET Pharmacy Institute maintained under standard hygienic conditions. They were fed with standard diet and had free access to water *ad libitum*. The animals were maintained under standard conditions of humidity, temperature and 12h light/12h darkness cycle.

Anti-diarrheal Activity: Anti-diarrheal activity of methanolic extract of leaves of *Tabernaemontana divaricata* (MELTD) was assessed by castor oil induced diarrheal method. The Wister rats of either sex (weight 150-200g) were divided randomly into groups with five animals in each group (n=5) and fasted for 18 h. Normal saline was administered to control group and the different doses of MELTD were administered in treated groups.

Loperamide was used as standard drug. One h later, all the animals were administered with 1 ml/rat of castor oil orally by gavage. The severity of diarrhoea was assessed at 1, 2, 4 h for 6 h. The total number of feces (both diarrheal and non-diarrheal) expelled was compared with the control group. The anti-diarrheal effect of the drug was calculated by considering the total number of diarrheal feces of the control group as 100% [16].

Procedure: The Wister rats were divided into fourteen groups with six animals in each group.

Group 1: - Normal saline (5ml/kg p.o) and served as control.

Group 2: - Standard drug, loperamide (5mg/kg, orally)

Group 3, 4 and 5: - MELTD (50,100 and 200mg/kg body weight, p.o), respectively.

The antidiarrheal activity was expressed as % inhibition of diarrhoea. The percent (%) inhibition of defecation was measured using the following formula:

$$\% \text{ inhibition of defecation} = \frac{A - B}{A} \times 100$$

Where,
A = mean number of defecation time caused by castor oil.
B = mean number of defecation time caused by drug or extract.

Diuretic Activity: The diuretic activity was performed by the Lipschitz method, is based on the principle that water and sodium excretion in test animals is different as compared to rats treated with a high dose of urea. The "Lipschitz value" is the quotient between excretion by test animals and excretion by the urea control. Six adult albino rats per group (150-250g) were kept in metabolic cages, provided with a funnel and wire mesh at the bottom to collect the urine. The urine excretion was recorded after 5h. The sodium and potassium content were determined by flame photometer.

Procedure: The albino rats were divided into fourteen groups with six animals in each group.

Group 1: Normal saline (20 ml/kg p.o) and served as control.

Group 2: Standard drug, furosemide (20 mg/kg i.p).

Group 3, 4 and 5: MELTD (50, 100 and 200 mg/kg body weight, p.o), respectively.

The total urine volume was collected at the end of 5h.The parameters accounted for ascertaining the diuretic activity will be the total volume of urine and the urine concentration of Na^+ and K^+. Furthermore, two important indices such as diuretic index and Lipschitz value were determined using following formula [16, 17].

Diuretic index =

$$\frac{\text{Urinary excretion volume of the test group}}{\text{Urinary excretion volume of control group}}$$

Lipschitz value =

$$\frac{\text{Urinary excretion volume of the test group}}{\text{Urinary excretion volume of treated group}}$$

The Lipschitz value of 1.0 and more are regarded as a positive effect.

RESULT AND DISCUSSION: Diarrhoea induced by castor oil is described as an appropriate model of the complex, prolonged processes of hyper-secretion and accelerated transit that characterizes secretory diarrhoea. The induction of diarrhoea due to the action of ricinoleic acid formed by hydrolysis of the castor oil [18, 19]. Ricinoleic acid alters in the transportation of water and electrolytes resulting in a hypersecretory response [20]. Ricinoleic acid also sensitizes the intramural neurons of the gut. The neuronal sensitization promotes the removal of soft intestinal content further.

Evaluation of Anti-diarrheal Activity Using MELTD: The MELTD was evaluated for anti-diarrheal properties in castor oil-induced diarrhoea. Castor oil induces diarrhoea by increasing the volume of intestinal content by prevention of the reabsorption of water. 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. Diarrhoea was clinically apparent in all rats of the positive control group after 30 min of oral administration of castor oil for next 4 h.

TABLE 1: EFFECT OF MELTD ON CASTOR OIL INDUCED DIARRHEA

Group	Treatment	Dose	Total no. of feaces	No. of diarrheal feaces	% Inhibition
1	Normal saline	25 ml/kg	25.33 ± 0.42	17.5 ± .36	-
2	Standard	5mg/kg	9.0 ± 0.258**	5.06 ± 0.47**	71.1
3	Test Drug I	50 mg/kg	19.3 ± 0.49*	12.5 ± 0.43*	28.57
4	Test Drug II	100 mg/kg	17.8 ± 0.31**	8.8 ± .30**	49.7
5	Test Drug III	200 mg/kg	11.3 ± 0.33**	6.7 ± 0.36**	61.7

The one way ANOVA and Dunnet's Multiple comparison test was used for statistical analysis. Values are expressed as mean ± SEM (N=6), *P<0.05, **P<0.01, when compared with control group.

The methanol extract of *T. divaricata* showed a marked antidiarrheal effect in the rats as shown in the **Table 1**. The doses of 100 mg/kg and 200 mg/kg of MELTD extract produced significant ($p<0.01$) defecation. The doses of 100 mg/kg and 200 mg/kg decreases the 49.7% and 61.7% respectively of wet feces produced upon administration of castor oil, compared to the standard group administered loperamide (71.1%) at the dose of 5 mg/kg.

Evaluation of Diuretic potentials of MELTD: The determination of Lipschitz value was useful in assessment of diuretic activity [21]. The standard diuretic drug furosemide exhibited a significant increase in the urinary excretion of water and electrolytes (Na+ and K+), which is comparable with the extract. The Lipschitz value for MELTD at dose of 50mg/kg, 100mg/kg and 200mg/kg were calculated as 0.64, 0.82 and 0.91 respectively. As shown in the **Table 2**, the urinary excretion of water and electrolytes exhibited by furosemide and MELTD doses were 70%, 25.8%, 40% and 55.3% respectively, as compared to control group over a period of 5 h.

TABLE 2: EFFECT OF MELTD ON DIURETIC ACTIVITY

Group	Dose	Mean urine volume	Concentration of excreted ions		Diu. index	Lipschitz value
			Na^+(mEqL)	K^+(mEqL)		
Control	25 ml/kg	5.76 ± 0.033	68.67±0.236	45.26±0.33	-	-
Std.	20 mg/kg	9.8 ± 0.157	132.5±0.367**	116.3±0.67**	1.70	-
Test I	50 mg/kg	7.25 ± 0.067	107.28±0.49**	73.6±0.226**	1.08	0.64
Test II	100 mg/kg	8.05 ± 0.66	115.2±0.77*	79.1±0.32**	1.39	0.82
Test III	200mg/kg	8.95±0.76	123.95±0.27**	84.3±0.27**	1.55	0.91

The one way ANOVA and Dunnet's Multiple comparison Test was used for Statistical analysis. Values are expressed as mean ± S.E.M. (N=6), *P<0.05, **P<0.01, when compared with control group.

CONCLUSION: Worldwide and especially in developing countries, diarrhoea is a major cause of deaths among the children below 5 years. The entire pharmaceutical industry is looking for new chemical entity from natural source, as natural agents are devoid of side effects or have very less side effects. The methanolic extract of *T. divaricata* leaves showed promising result as an antidiarrheal agent when compared to the standard moiety loperamide. The MELTD showed significant decrease in number of wet feces produced upon administration of castor oil as compare to the control group. Recently, researchers are focusing towards safe and economic therapeutic agents. The use of herbal plants mentioned in traditional medicine as diuretics have increased recent in years. The MELTD showed the Lipschitz value and diuretic index of 0.91 and 1.55 respectively, indicating the MELTD can be beneficial in the development of new moiety having good diuretic potentials.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Field M: Intestinal ion transport and the pathophysiology of diarrhea. The Journal of Clinical Investigation 2003; 111(7): 931-943.
2. Kosek M, Bern C and Guerrant RL: The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ 2003; 81(3):197-204.
3. Almeida CE, Karnikovski MG, Foleto R and Baldisserotto B: Analysis of antidiarrhoeic effect of plants used in popular medicine. Revista de Saude Publica.1995; 29(6):428-433.
4. Syder JD and Merson MH: The magnitude of the global problem of acute diarrheoal disease: A review of surveillance data. Bull World Health Organ 1982; 60: 605-13.
5. Mukherjee J, Das R, Balasubramania K, Saha M and Pal BP: Anti-diarrhoeal evaluation of *Nelumbo nucifera* rhizome extract. Indian Journal of Pharmacology 1995; 27(4): 262-4.
6. Bhandari N, Nahl R, Mazumdar S, Martines J, Black RE and Bahn MK: Effect of community-based promotion of exclusive breastfeeding on diarrhoeal illness and growth: a cluster randomized controlled trial. Lancet 2003; 361 (9367): 1418-1423.
7. Al-Mofleh IA: Spices, herbal xenobiotics and the stomach: friends or foes? World Journal of Gastroenterology 2010; 16(22): 2710-2719.
8. Dixit PK and Mittal S: A Comprehensive review on herbal remedies of diuretics. International Journal of Research in Pharmacy and Science 2013; 3(1): 29-31.
9. Wright CI, Van-Buren L, Croner CI and Konning MM: Herbal medicines as diuretics, a review of the scientific evidence. Journal of Ethnopharmacology 2007; 114(1): 1-31.
10. Mishra S, Sharma SK, Mohapatra S, Singh L and Singh T: An overview on herbal medicines as diuretics with scientific evidence. Scholars Journal of Applied Medical Sciences 2013; 1(3): 209-14.
11. Gupta VK and Arya V: A review on potential diuretics of Indian medicinal plants, Journal of Chemical and Pharmaceutical Research 2011; 3(1): 613-20.

12. Balamurugan G, Selvarajan S, Balakrishnan D and Muralidharan P: Diuretic activity of *Abutilon indicum* linn. (Sweet) seed extract. Journal of Herbal Medicine and Toxicology 2010; 4(1): 49-52.
13. Anonymous: The Wealth of India-A Dictionary of Indian Raw Materials and Industrial Product. Council of Scientific and Industrial Research, New Delhi, Vol. 3, 2003: 192-194.
14. Pratchayasakul W, Pongchaidecha A, Chattipakorn N and Chattipakorn S: Ethnobotany and ethnopharmacology of *Tabernaemontana divaricata*. Indian Journal of Medical Research 2008; 127: 317-335.
15. Ghani A: Medicinal Plants of Bangladesh: Chemical constituents and uses. Dhaka: Asiatic Society of Bangladesh, Vol. 381, 2003; 1-16.
16. Mukherjee PK: Pharmacological Screening of Herbal Drugs; Quality Control Herbal Drugs- An approach to Evaluation of Botanicals. Business Horizons Pharma-ceutical Publishers, New Delhi, 2009: 537-579.
17. Al-Saikhan FI and Ansari MN: Evaluation of the diuretic and urinary electrolyte effects of methanolic extract of *Peganum harmala* L. in Wistar albino rats. Saudi Journal of Biological Sciences 2016; 23: 749-53.
18. Iwao I and Terada Y: On the mechanism of diarrhea due to castor oil. Japanese Journal of Pharmacology 1962; 12: 137-145.
19. Watson WC and Gordon RS: Studies on the digestion, absorption and metabolism of castor oil. Biochemical Pharmacology 1962; 11: 229-236.
20. Ammon HV, Thomas PJ and Phillips S: Effects of oleic and ricinoleic acids on net jejunal water and electrolyte movement. Journal of Clinical Investigation 1974; 53: 374-379.
21. Srivastav S, Singh P, Jha KK, Mishra G, Srivastava S, Karchuli MS and Khosa RL: Diuretic activity of whole plant extract of *Achyranthes aspera* Linn. European Journal of Experimental Biology 2011; 1(2): 97-102.

How to cite this article:
Kumari S, Mazumder A and Bhattacharya S: Evaluation of anti-diarrheal and diuretic activity of *Tabernaemontana divaricata* Linn. leaves. Int J Pharmacognosy 2018; 5(7): 409-13. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(7).409-13.