ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 8, Issue 2, 2015

ISSN - 0974-2441

Research Article

EVALUATION OF DIURETIC ACTIVITY OF *MUSSAENDA FRONDOSA*

SREELAKSHMI R[1], SHAN P MOHAMMED[1], JYOTI HARINDRAN[2], SRIGANESAN P[2]

[1]Department of Pharmacology, Mahatma Gandhi University, Kottayam, Kerala, India. [2]Department of Pharmaceutics, Mahatma Gandhi University, Kottayam, Kerala, India. Email: sreelakshmiunni11@gmail.com

Received: 17 November 2014, Revised and Accepted: 23 December 2014

ABSTRACT

The present study deals with the evaluation of diuretic activityof the plant *Mussaenda frondossa* Linn. ,using Lipschitz method. Wistar albino rats was divided into 4 groups of 6 animals in each. Frusemide (20 mg/Kg p.o) used as a standard diuretics. Two doses of plant extract was used for the study. In urine the volume and concentration of electrolytes was measured at the end of 24 hrs. Result shows significant increase in diuretic activity.

Keywords: *Mussaenda frondosa*, Lipchitz method, Alcoholic extract, Diuretic activity.

INTRODUCTION

Mussaenda frondosa Linn. (Rubiaceae) found throughout India and has historically been used to treat a wide assortment of diseases. Plant is known by various names in different languages as "Bedina" in Hindi, "Sriparnah" in Sanskrit, and "Nagavalli" in Telungu. Traditionally *M. frondosa* Linn. is reported to possess number of medicinal properties. The whole plant is used as an astringent, expectorant, in jaundice, hyperacidity, ulcers, leprosy, diuretic, wound healing, swelling, antimicrobial, protective, and asthma. The plant is also found to possess hypolipidemic effect, hepatoprotective activity, fever and cough [1-9]. The diuretic activity of alcoholic extract of *M. frondosa* was evaluated by Lipschitz method in albino rats.

METHODS

Collection and extraction of plant material

Whole plant of *M. frondosa* was collected from different parts of Kottayam district in January 2014 and identified by Prof. Rogimon P Thomas, Department of Botany C.M.S. College, Kottayam. Dried whole plant were ground to a coarse powder, passed through sieve no. 24 and stored in air tight container and used for further extraction.

Preparation of ethanol extract

The powdered material was exhaustively extracted with 95% ethyl alcohol using a soxhlet apparatus. The extract was concentrated in vacuum to a syrupy consistency.

Preliminary phytochemical screening

The extracts were screened qualitatively for the phytochemical constituents by utilizing standard methods of analysis.

Acute toxicity study

This was performed for the extracts to ascertain safe dose by the acute oral toxic class method by the Organization of Economic Cooperation and Development (OECD) 423 guidelines.

Swiss Albino mice weighing between 20 and 25 g and between age 8 and 12 weeks were procured for the experimental trial. The selected animals were fed with standard feed and drinking water and monitored on a regular basis. The animals were selected and grouped, three animals per group. They were kept fasting 4 hrs prior to the treatment, and the test substance was administered in a single dose by the oral route. A single administration of starting dose of 2000 mg/kg body weight/p.o. of the extract (ethanolic extract was suspended in 1% tween 80 solution) was administered to three female mice. They were noted individually after dosing, at least once during the first 30 minutes, with special attention is given during the first 4 hrs and thereafter for a total of 14 days. The mice were observed for 3 days to evaluate considerable changes in body weight and other signs of toxicity. There was no considerable change in body weight before and after treatment, and no sign of toxicity was observed. When the experiment was repeated again with the same dose level 2000 mg/kg body weight/p.o. of plant extract for 7 more days and observed for 14 days, no change was observed from the experiments.

Animals

Wistar Albino rats of both sex (120-200 g) and female albino mice (20-25 g) were procured from Animal House, Department of Pharmacology, UCP, RIMSR. The experiments were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee. Throughout the experimental period, the animals were housed in departmental animal house at 26±2° and relative humidity 44-56% and were exposed to 12 hrs light: Dark cycle. The animals were allowed to acclimatize to the environment for 7 days. They were fed with standard pellet diet collected from Hindustan Lever Ltd., Bangalore and water given *ad libitum*. All procedures and experiments were conducted in the day time according to specification of the Indian National Science Academy. The experiments were carried out after obtaining the permission of Institutional Animal Ethics Committee.

Diuretic activity

Wistar albino rat of either sex (200-250 g) was selected for this study. The method of Lipschitz *et al.*, was employed for the assessment of diuretic activity. According to this method, the animals, deprived of food and water for 18 hrs prior to the experiment, were divided into four groups (n=6). Group 1 animals received normal saline (25 ml/kg, p.o.), Group 2 received the standard diuretic, frusemide (20 mg/kg, p.o), Group 3 received *M. frondosa* (200 mg/kg, p.o.) and Group 4 ethanolic extract of *M. frondosa* (400 mg/kg,p.o.) respectively. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 5 ml/100 g body weight to impose a uniform water and salt load. All the drugs were freshly prepared prior to administration. Immediately after administration, the animals were placed in metabolic cages (each animal per cage), specially designed to separate urine and feces, kept at 20±0.5°C. The volume of urine collected was measured at the end of 24 hrs. During this period, no food and water was made available to animals. The parameters include body weight before and after test period, total urine volume, and concentration of Na^+, K^+ and Cl^- in the urine, etc. were noted [10,11].

Table 1: Effect of ethanolic extract of *M. frondosa* on urinary volume excretion

Groups	Urine volume	Diuretic index
Control	5.12±0.689	-
Standard	11.216±1.523**	2.19
Test - 200 mg/kg	6.28±0.913**	1.22
Test - 400 mg/Kg	8.15±1.042**	1.59

Values are expressed as mean±SEM, from six mice. Significant at **p<0.01 as compare to control using one-way ANOVA, followed by Tukey–Kramer test for individual comparisons. SEM: Standard error mean, *M. frondosa: Mussaenda frondosa*

Table 2: Effect of ethanolic extract of *M. frondosa* on urinary electrolyte excretion

Groups	Na mmol/L	K mmol/L	Cl mmol/L	Na/K ratio
Control	115.83±4.62	46.833±5.67	107±8.5	2.47
Standard	188.66±6.71	111.33±6.80	180.33±6.9	1.52
Test - 200 mg/kg	133.33±6.88	70.33±5.53	131.66±4.36	1.89
Test - 400 mg/kg	170.33±4.88	88.5±4.23	165.66±6.5	1.92

Values are expressed as mean±SEM, from six mice. Significant at **p<0.01 as compare to control using one-way ANOVA, followed by Tukey–Kramer test for individual comparisons, SEM: Standard error mean, *M. frondosa: Mussaenda frondosa*

Statistical analysis

The values are expressed as mean±standard error mean from six animals in each group. Results were statistically analyzed using one-way ANOVA followed by Tukey–Kramer test for individual comparisons. *p<0.05, **p<0.01, ***p<0.001 considered to be statistically significant.

RESULTS

Preparation of crude extract

The ethanolic extract was prepared, and the percentage yield of that extract was found to be 4.6% w/w.

Phytochemical screening

The preliminary phytochemical analysis of the ethanolic extract of *M. frondosa* revealed the presence of alkaloids, tannins and phenolic compound, carbohydrate, steroids, and flavonoids.

Acute toxicity studies

Acute toxicity studies for ethanolic extracts of *M. frondosa* were conducted as per OECD guidelines 423 using albino Swiss mice. Each animal was administered ethanolic extracts by oral route. The animals were observed for any changes continuously for the first 4 hrs and up to 24 hrs for mortality. There were no mortality and noticeable behavioral changes in all the groups tested. The extracts were found to be safe up to 2000 mg/kg body weight. Since no death was observed at 2000 mg/kg, it was thought that 2000 mg/kg was cut off dose and $1/5^{th}$ and $1/10^{th}$ of this dose were selected for evaluation of diuretic activity.

Effect on urine volume

There was no evidence of dehydration during the interval of 24 hrs. The standard drug frusemide shows significant (p<0.001) increase in urine volume compared to the control group. Standard drug has a diuretic index of 2.19. Test compound shows a significant increase in urine volume in a dose depended on manner, which is less than standard group (Table 1).

Effect on urinary electrolyte

As shown in Table 2 test compound shows a significant increase in the excretion of all electrolyte when compared to the control group and less than the standard group. Sodium:potassium ratio of test compound is more than that of standard group.

DISCUSSION

Diuretics have proved to be extremely valuable in the treatment of mild to moderate hypertension and also in enhancing the effect of other antihypertensive agents. Diuretics relieve pulmonary congestion and peripheral edema. These agents are useful in reducing volume over load and relieve orthopnea and paroxysmal nocturnal dyspnea in congestive cardiac failure and acute left ventricular failure. The diuretic activity of the plant may be due to the presence of active constituents such as tannins, alkaloids, etc.

Results showed that single dose administration of alcoholic extract of *M. frondosa*, 200 and 400 mg/kg and standard furosemide (20 mg/kg) have increased the urinary output along with an increase in concentration of sodium, potassium and chloride ions in urine. Alcoholic extract of *M. frondosa* 400 mg/kg produced a greater diuretic activity, which is comparable to that of standard furosemide (20 mg/kg). In traditional medicine, the plant is used for its diuretic activity.

CONCLUSION

The use of *M. frondosa* in folk medicine as a diuretic has been justified by this work, it show significant diuretic activity.

REFERENCES

1. Vaidyaratnam PS, Warier PK. Indian Medicinal Plants a Compendium of 500 Species. Madras: Orient Longman; 1993. p. 86-9.
2. Siju EN, Rajalakshmi GR, Kavitha VP, Joseph A. *In vitro* antioxidant activity of *Mussaenda frondosa* Linn. Int J PharmTech Res 2010;2(2):1236-40.
3. Patil SA, Joshi VG. Evaluation of antibacrerial and wound healing activity of leaves of *Mussaenda frondosa* Linn. Int J Res Pharm Biomed Sci 2011;2(1):147-54.
4. Sudhir NS, Patil PA, Patil SA. Hepatoprotective activity of *Mussaenda frondosa* linn extract in ethanol treated rats. Int J Drug Res Technol 2012;2(6):446-53.
5. Wesley JJ, Jeyaananathi J, Ansar AM, Ravikumar K. Hypolipidemic effect of methanolic extract of *Mussaenda frondosa* Linn. Leaves in high fat diet fed rats. J Pharm Res 2009;2(4):579-81.
6. Lakshmi DK, Girija AR, Rao DV, Rao EV. Chemical components of *Mussaenda frondosa* and *M. hirsutissima* Indian J Pharm Sci 1985;47(3):122-3.
7. Basavaraja BM, Vagdevi HM, Srikrishna LP, Hanumanthappa BC, Joshi SD, Vaidya VP. Antimicrobial and analgesic activities of various extracts of *Mussaenda frondosa* Linn. bark J Glob Pharm Technol 2011;3(2):14-7.
8. Maiti BC, Kesari A, Neelam K. Phytochemical screening of crude powder and extracts of *Mussaenda frondosa* Linn. Adv Pharm Toxicol 2011;12(2):63-6.
9. Dilip C, Ameena K, Saraswathi R, Krishnan PN, Simi SP, Sanker C. Evaluation of a new tablet excipient from the leaves of *Mussaenda frondosa* Linn. Res J Pharm Bio Chem Sci 2010;1(3):401-11.
10. Venkatesh K, Upendra Rao U, Kiranmay VN. Phytochemical screening and evaluation of diuretic activity of ethanolic extract of *Mussaenda erithrophylla* in rats. Int J Biol Pharm Res 2013;4(7):8-10.
11. Sayana SB, Khanwelkar CC, Nimmagadda VR, Dasi JM, Chavan VR, Kutani A, *et al.* Evaluation of diuretic activity of alcoholic extract of roots of *Cissampelos pareira* in albino rats. J Clin Diagn Res 2014;8(5):HC01-4.