



DIURETIC ACTIVITY OF HYDRO-ALCOHOLIC EXTRACTS OF NYCTANTHES ARBORTRISTIS IN ALBINO RATS

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ABSTRACT

In the present study hydro-alcoholic extract of *Nyctanthes arbortristis* was prepared using soxhlet's apparatus. Albino rats were divided into 5 groups of 6 rats each. Group-I (Control) received distilled water 25ml/kg orally. Group-II (Standard). received Furosemide 20mg/kg orally. Group-III received hydro-alcoholic extract of *Nyctanthes arbortristis* 100 mg/kg, Group-IV received hydro-alcoholic extract of *Nyctanthes arbortristis* 200 mg/kg and Group-V received hydro-alcoholic extract of *Nyctanthes arbortristis* 400 mg/kg. The urine samples were collected for all the groups upto 5 hours after dosing and urine volume was measured. Urine was analysed for electrolytes (Na⁺, K⁺ and Cl⁻). ANOVA, Dunnet's test and p-values were measured and data was analysed. hydro-alcoholic extract of *Nyctanthes arbortristis* exhibited significant diuretic activity by increasing urine volume and also by enhancing elimination of Sodium (Na⁺), Potassium (K⁺) and Chloride (Cl⁻) at doses of 100 and 200mg/kg. hydro-alcoholic extract of *Nyctanthes arbortristis* possesses significant diuretic activity.

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INTRODUCTION

Cardiac failure is a syndrome resulting from impaired pumping capacity of the heart which occurs because of genetic or acquired abnormality in cardiac structure and/ or function. This results in symptoms and signs of cardiac failure such as dyspnea, fatigue, oedema, rales leading to frequent hospitalization, a poor quality of life and a shortened life expectancy [1]. In spite of high rates of morbidity and mortality, the pathophysiologic mechanisms and treatment of heart failure is poorly understood.

Body hydration status is of remarkable importance. Diuretics elevate the rate of urine flow and sodium excretion and are used to adjust the volume and/ or composition of the body fluids and this includes forced diuresis. Diuretics can adequately control fluid retention and restore and maintain normal volume status in patients with congestive symptoms (dyspnoea, orthopnoea, oedema) or signs of elevated filling pressures (rales, jugular venous distension, or peripheral oedema). Diuretics adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis [2]. The currently used diuretics are usually associated with many adverse reactions.

Various plant extracts used in traditional medicine have shown significant diuretic activity when tested in animal models. In this current study an attempt has been made to evaluate the diuretic activity of hydro-alcoholic leaves extract of *Nyctanthes arbortristis*.

Nyctanthes arbortristis (N. arbortristis) is a valuable medicinal plant which belongs to the family Oleaceae. The plant generally grows in tropical and subtropical region. N. arbortristis commonly known as Night jasmine, Harsinghar & Parijat. The flowers start falling after midnight and by the day break, the plant appears dull. The generic name 'Nyctanthes' has been coined from two Greek words 'Nykhta' (Night) and 'anthos' (flower) [3,4]. It is usually a shrub or a small tree having brilliant, highly fragrant flowers, which bloom at night and fall off before sunrise, giving the ground underneath a pleasing blend of white and red. Thus, during the day the plant loses all its brightness and hence is called "Tree of sadness" (arbor - tristis). It is also known as arsinghar, Coral Jasmine, Parijat, queen of the night and night flowering jasmine [5]. It is a *Nyctanthes arbortristis* of India, distributed in sub - Himalayan region and also found in Indian garden as ornamental plant. In India, it grows in the outer Himalayas and is found in tracts of Jammu and Kashmir, Nepal to East of Assam, Bengal, Tripura extended through the Central region up to Godavari in the South. Flowering usually occurs from July to October. N. arbortristis prefers a secluded and semi-shady place to grow [6].

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N. arborescens is one of the well known medicinal plant. It is a common wild hardy large shrub or small tree. Different parts of this plant are used in Indian systems of medicine for various pharmacological actions like as anti-leishmaniasis, anti-viral, anti-fungal, anti-pyretic, anti-histaminic, anti-malarial, anti-oxidant [7], anti-inflammatory [8] and many more activities.

Herbs have been always the main principle form of medicine since traditions in India and now a day it becomes most popular throughout the world. Important large shrub of tropical and subtropical regions of the world that has been traditionally used to provoke menstruation, for treatment of scabies and other skin infections as hair tonic [9]

MATERIALS AND METHODS

Animals

Albino rats (150-200g) of either sex were randomly selected from central animal facility, Modern Institute of Pharmaceutical Sciences, Indore. Animals were housed into groups of six per cage at a controlled temperature (23 ± 2°C). Light: dark cycle of 12:12 was followed. The rats had free access to standard pelleted feed and water ad libitum. The Institutional Animal Ethical Committee approved the protocol of this study.

Preparation of the extract and isolation of active principle

The plant material is made up of the leaves of *Nyctanthes arborescens*. The leaves were collected from medicinal garden of Modern institute of Pharmaceutical Sciences in August 2016. The sample of plant was identified and authenticated at Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, College of Agriculture, Indore. A weighed quantity (200g) of the powder was then subjected to continuous hot extraction in Soxhlet apparatus with hydro-alcoholic. The extract was filtered through a cotton plug, followed by Whatman filter paper (No.1) and dried at 40-50°C to get a blackish green semisolid mass, which was taken for final use.

hydro-alcoholic leaves extract of *Nyctanthes arborescens* at doses of 100, 200 and 400mg/kg respectively, all of which were administered orally immediately prior to the test in this acute study. The hydro-alcoholic leaves extract of *Nyctanthes arborescens* extracts of the test drug were suspended in distilled water for oral administration.

Assessment of diuretic activity

Albino rats weighing 150–200 g were used. The rats were fed with standard pellet diet and provided water ad libitum. The same was withheld 18 hours prior to the experiment. They were hydrated with 5ml/kg of distilled water prior to drug/extract administration. Immediately after dosing the animals were placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine. Stainless-steel sieves were placed in the funnel to retain feces and to allow the urine to pass. The urine was collected in measuring cylinder up to five hours after dosing. During this period, animals were deprived of food and water [11]. The volume was measured and urine sample kept in refrigerator until Na+, K+ and Cl- levels were estimated. The urine samples were kept without adding any preservatives. The concentrations of urine Na+ and K+ were determined by flame photometry and concentration of Cl- was estimated titrimetrically using 0.02N AgNO₃ with 5% potassium chromate as indicator.

Statistical analysis

The effects of hydro-alcoholic leaves extract of *Nyctanthes arborescens* were calculated by taking the Mean values and Standard Deviation of the outcome parameters. ANOVA (Analysis of Variance) was applied to compare the effects of drugs under study. The data were analysed using ANOVA followed by Dunnett’s test

RESULTS

Oral administration of single dose of hydro-alcoholic leaves extract of *Nyctanthes arborescens* significantly increased the urine output at the doses of 100 and 200 mg/kg.

Table No. 1 Effects of oral administration of hydro-alcoholic leaves extract of *Nyctanthes arborescens* on urine volume and electrolyte excretion

Treatment group	Dose (Oral)	Urine volume(ml)	Na+ (mEq/L)	K+ (mEq/L)	Cl- (mEq/L)
Control	25 ml/kg	2.93±0.20	112.00±2.52	48.75±1.20	160.50±4.32
Standard	20 mg/kg	8.43±0.89	140.33±1.86	90.70±1.44	314.50±12.48
Test- 1	100 mg/kg	5.80±0.35*	131.50±1.64*	55.61±3.15*	263.00±4.14*
Test- 2	200 mg/kg	5.23±0.40*	128.16±4.95*	66.31±3.41*	170.33±7.89*
Test- 3	400 mg/kg	5.16±0.56*	120.16±3.31	81.93±3.60*	155.50±3.21

Drugs and chemicals

Furosemide 20 mg/kg body weight (Lasix 20mg) (Sanofi Aventis India Limited), hydro-alcoholic leaves extract of *Nyctanthes arborescens* 100, 200 and 400 mg/kg body weight and distilled water.

Animals were divided into five groups (with six rats each). Animals were deprived of food and water for 18 hours prior to the experiment. Group I received 25ml/kg of distilled water and served as the control, Group II received Furosemide 20mg/kg as standard [10], Groups III, IV and V received

The effect was found to be dose dependent with more pronounced outflow at 100 mg/kg (p<0.01 vs control) and the results were not significant when the dose was increased to 400 mg/kg as indicated in [Table-1].

The effect of hydro-alcoholic leaves extract of *Nyctanthes arborescens* on the excretion of urinary electrolytes is dose dependent. The test doses of 100 mg/kg and 200 mg/kg significantly increased the excretion of all the electrolytes Na+, K+ and Cl- estimated in the study. However, the test drug at higher dose of 400 mg/kg did not induce any significant increase in the Na+ and Cl- excretion.

DISCUSSION

Fluid overload is usually observed in various pathological states such as cardiac failure and secondary oliguric states. Heart failure is the leading cause of hospitalization in people older than 65. More than 20 million people have heart failure worldwide [12]. The prevalence and incidence of heart failure in India has been estimated to range from 1.3 to 4.6 million, with an annual incidence of 0.5- 1.8 million [13]. In spite of high rates of morbidity and mortality, the pathophysiologic mechanisms and treatment of heart failure is poorly understood. Even with the advent of newer and selective agents, their side effect profile is a setback and also few cases show refractoriness to conventional treatment. The current study was aimed at evaluating the diuretic activity of hydro-alcoholic leaves extract of *Nyctanthes arbortristis* in albino rats.

Diuretics used in the treatment of heart failure act by enhancing urine outflow, decreasing plasma volume and venous return to the heart, and thereby subsequently decrease cardiac workload, oxygen demand and blood pressure. The major site of action of the loop diuretic, furosemide is the thick ascending limb of loop of Henle where it acts by inhibiting the Na⁺/K⁺/2Cl⁻ co-transport carrier in the luminal membrane. It increases the urine output along with increased urinary excretion of Na⁺, K⁺ and Cl⁻.

The present study showed that the diuretic activity of the hydro-alcoholic leaves extract of *Nyctanthes arbortristis* is relatively modest and slow in onset as compared to the reference drug, furosemide. The plant extract also caused increased urine volume and increased urinary excretion of Na⁺, K⁺ and Cl⁻ like that of furosemide

Therefore the probable diuretic action of hydro-alcoholic leaves extract of *Nyctanthes arbortristis* could be due to its interference with the Na⁺/K⁺/2Cl⁻ co-transport carrier in the luminal membrane of the thick ascending limb of loop of Henle, similar to the mechanism of action of furosemide. And, this effect of the extract may be related mainly to the sugar mannitol.

hydro-alcoholic leaves extract of *Nyctanthes arbortristis* was observed to have diuretic activity in experimentally induced diuresis in albino rats. The study explores the complementary nature of *Nyctanthes arbortristis* with conventional treatment making it comparatively safer, economical, easily available and well tolerated therapy.

CONCLUSION

We conclude from the study that *Nyctanthes arbortristis* has a beneficial role as a diuretic and thereby support the claim of traditional use of the plant as a diuretic. Further studies are indicated to identify the adverse effects, optimal treatment routes and dosage.

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