Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Pharmacia Sinica, 2011, 2 (6):17-22

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Comparative study of *Acacia nilotica* and *Acacia sinuata* for diuretic activity

Ramya Krishna. P. S*, Bhaduri Lavanya, Pulla Sireesha, S. Nagarjuna and Y. Padmanabha Reddy

Division of Pharmacology, Centre for Pharmaceutical Research (CPR), Raghavendra Institute of Pharmaceutical Education and Research, Chiyyedu, Anantapur, Andhra Pradesh, India

---

ABSTRACT

Diuretics increase the rate of urine outflow and sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephritic syndrome and cirrhosis. Some naturally used diuretic plants are Abrus precatorius, Centella asiatica, Allium sepa, Allium sativum, Aloe barbidensis, Cinnamomum verum etc. Traditionally Acacia nilotica is used as anti-cancer, astringent, antioxidant, antispasmodial, diuretic and antidiarrhoeal. Acacia sinuata is used in the treatment of skin disease, burning sensation, constipation, calculi, hemorrhoids, vitiligo, eczema and malaria fever. The present study was undertaken to investigate diuretic effect of ethanolic and methanolic extracts of stem bark of Acacia nilotica and pods of Acacia sinuata in wistar rats. The extracts were administered orally at a dose of 300mg/kg. Furosemide (20mg/kg) was used as reference standard and normal saline (25 ml/kg) was used as control. Total urine volume and concentration of Na^+, K^+ and Cl^- excreted in urine were estimated. It was concluded that both ethanolic and methanolic extracts of Acacia nilotica and Acacia sinuata showed significant diuretic activity. Acacia nilotica showed more potent diuretic activity than Acacia sinuata and of these, ethanolic extract showed more significant activity than methanolic extract.

Key words: *Acacia nilotica*, *Acacia sinuata*, Diuretic, Furosemide, Urine volume.

INTRODUCTION

Since the time immemorial, our traditional system of medicine and folklore claiming that medicinal plants as a whole or their parts are being used in all types of diseases successfully[1]. India has a rich heritage of using medicinal plants and hosting several thousands of medicinally valuable plants belonging to hundreds of families. One cannot assure that all of these plants possess a long recorded history, although they have been reported to contain medicinally valuable phyto-pharmaceuticals and subjected to formulate, ayurvedic, unani, siddha, Chinese system of medicine[2]. Diuresis is an increase in the production of urine by the kidneys, which typically results in a corresponding increase in urine expelled by the body, diuresis without an

accompanying increase of urination can cause severe medical problems [3]. Drugs that induce diuresis are known as diuretics [4]. Diuretics relieve pulmonary congestion and peripheral edema. This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure. Thus, diuretics play an important role in hypertensive patients [5], in situations of fluid overload like acute and chronic renal failure, hypercalciuria, cirrhosis of liver and also as an antihypertensive agent. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na+ reabsorption and alcohol in beer, wine and mixed drinks, which inhibit secretion of ADH [6] .A number of diuretics like mannitol, thiazides, furosemide and ethacrynic acid are used in practice [7].

Furosemide, a potent diuretic agent that induces a powerful diuresis, followed by the loss of sodium, potassium, and chloride into urine, by acting on thick ascending limb of the loop of henle [8].

Acacia nilotica is a medium-sized, thorny, evergreen tree with spreading crown; thorns straight, 2-5 cm long, white. Leaves 2-pinnate, pinnae 6-12, leaflets small, 20-40. Flowers yellow, in globose axillary heads. Fruits moniliform, compressed, constricted at the sutures between the seeds [9].

Acacia sinuata is a perennial, woody, large climbing shrub grows on big trees. Leaves are bipinnate, leaflets small, sessile, flowers small heads, fruits thin pods with 6-10 seeds per pod.

The stem bark of *Acacia nilotica* and pods of *Acacia sinuata* were well-known for its diuretic properties. So far no scientific evidence was observed for the claimed activity of this product. In this regard we have aimed to identify the diuretic effect of both ethanolic and methanolic extracts of *Acacia nilotica* and *Acacia sinuata.*

MATERIALS AND METHODS

Plant materials

The stem bark of *Acacia nilotica and* pods of *Acacia sinuata* belonging to family Mimosaceae were collected from local area of Anantapur district (India) and was identified and authenticated by Dr.J.Ravindra Reddy, M.Pharm, Ph.D, Department of Pharmacognosy, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur and voucher specimen (riper- 10/11) is preserved in Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur.

Drugs and chemicals

Furosemide (Aventis Pharma Ltd), Ethanol (Merck Pharmaceuticals Pvt Ltd), Methanol (Merck Pharmaceuticals Pvt Ltd).

Animals

Wistar rats of 200-250g were used to carry out the diuretic activity. The animals had free access to standard commercial diet and water *ad libitum* and were housed in cages under standard laboratory conditions i.e., 12:12 hour light/dark cycle at 25 ± 2^0C. The Institutional Animal Ethics Committee (878/ac/05/CPCSEA/011/2011**)** has approved the experimental protocol at Post Graduate Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh, India.

Preparation of ethanolic extract of *Acacia nilotica*

The stem bark of *Acacia nilotica* was extracted by soxhlet extraction by using 400ml of ethanol. In the extraction procedure a total amount of 50 gm powdered material were used. The extract was distillated and then it was concentrated. The percentage yield of ethanolic extract of *Acacia nilotica* was found to be 13% (Table-1).

Preparation of methanolic extract of *Acacia nilotica*

The stem bark of *Acacia nilotica* was extracted by maceration process by using 300ml of methanol. In the maceration procedure a total amount of 100 gm powdered material were macerated for three days. The extract was filtered and then it was concentrated .Then it was dried by rotary evaporator. The percentage yield of methanolic extract of *Acacia nilotica* was found to be 10% (Table-1).

Preparation of ethanolic extract of *Acacia sinuata*

The pods of *Acacia sinuata* was extracted by soxhlet extraction by using 400ml of ethanol. In the extraction procedure a total amount of 50 gm powdered pods were used. The extract was distillated and then it was concentrated .The percentage yield of ethanolic extract of *Acacia sinuata* was found to be 12% (Table-1).

Preparation of methanolic extract of *Acacia sinuata*

The pods of *Acacia sinuata* was extracted by maceration process by using 300ml of methanol. In the maceration procedure a total amount of 100 gm powdered pods were macerated for three days. The extract was filtered and then it was concentrated .Then it was dried by rotary evaporator. The percentage yield of methanolic extract of *Acacia sinuata* was found to be 14% (Table-1).

Phytochemical analysis

Ethanolic and Methanolic extracts of stem bark of *Acacia nilotica* and pods of *Acacia sinuata* were subjected to phytochemical screening [10].

Diuretic activity

Healthy Wistar rats of either sex weighing around 200 -250g taken. The rats were divided into six groups of six animals each. Ethanolic and methanolic extracts of both stem bark of *Acacia nilotica* and pods of *Acacia sinuata* were evaluated for diuretic activity. Furosemide (20mg/kg) was used as reference standard. Before the experiment, the rats were fasted for 18 hours with free access to water. On the day of experiment, the animals of group 1 were administered saline (25ml/kg, p.o.) [11] and this group served as control. Group 2 rats were administered with standard drug furosemide (20mg/ kg, p.o.). Group 3 and Group 4 rats received ethanolic and methanolic extracts (300mg/kg, p.o.) of *Acacia nilotica* respectively. Similarly the Group 5 and Group 6 rats received ethanolic and methanolic extracts (300mg/kg, p.o.) of *Acacia sinuata* respectively. Immediately after the treatment the rats were placed in metabolic cages and the urine samples were collected for 5h, measured using a standard measuring cylinder. The amount of urine (in ml) collected for 5 h was compared and tabulated [12]. The parameters measured were total urine volume, urine concentration of Na^+, K^+ and Cl^-. Concentration of Na^+ and K^+ were determined using flame photometer while Cl^- concentration was estimated titrimetrically using 0.02N $AgNO_3$ with 5% potassium chromate as an indicator. Appearance of brick red precipitate was taken as the end point [13].

Statistical Analysis

The results were expressed as mean ± S.E.M. The differences were compared using One Way Analysis Of Variance (ANOVA) and subsequently followed by Bonferroni's test.

RESULTS

Table 1: %Yield, texture and colour of ethanolic and methanolic extracts of *Acacia nilotica* and *Acacia sinuate*

Plant part	Type of Extract	% Yield	Texture	Colour
Stem bark of *Acacia nilotica*	Ethanolic extract	13	Gummy	Reddish brown
	Methanolic extract	10	Gummy	Reddish brown
Pods of *Acacia sinuata*	Ethanolic extract	12	Gummy	Reddish brown
	Methanolic extract	14	Gummy	Reddish brown

Phytochemical analysis :

Both ethanolic and methanolic extracts of *Acacia nilotica* and *Acacia sinuata* revealed the presence of alkaloids, glycosides, saponins, tannins, aminoacids, steroids and terpenoids (Table-2).

Table 2 : Phytochemical analysis of ethanolic and methanolic extracts of *Acacia nilotica and Acacia sinuata*

Chemical constituents	*Acacia nilotica*		*Acacia sinuata*	
	Ethanolic extract	Methanolic extract	Ethanolic extract	Methanolic extract
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Aminoacids	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+

+ = Present

Effect of extracts on urine output and electrolyte excretion in rats:

The urine volume and concentrations of Na^+, K^+ and Cl^- in ethanolic and methanolic extracts of *Acacia nilotica* (300mg/kg) were 9.4ml, 127.8mEq/L, 87.43mEq/L, 155.8 mEq/L and 6.6ml, 113.1mEq/L, 69.17mEq/L, 120.3 mEq/L respectively (Table-3).

Table 3: Effect of ethanolic and methanolic extracts of *Acacia nilotica* on urine output and electrolyte excretion in rats

Treatment	Dose & Route	Urine volume (ml)	Electrolyte concentration in urine (mEq/L)		
			Na^+	K^+	Cl^-
Control (Saline)	25 ml/kg p.o.	4.03 ± 0.12	90.0 ± 0.61	51.73 ± 2.80	97.3 ± 1.04
Furosemide	20 mg/kg p.o.	9.267± 0.08****	136.8 ± 1.55****	94.17 ± 1.18****	163.7 ± 1.30****
Ethanolic extract	300 mg/kg p.o.	9.43 ± 0.12****	127.8 ± 1.28****	87.43 ± 1.09****	155.8 ± 2.02****
Methanolic extract	300 mg/kg p.o.	6.60 ± 0.11****	113.1 ± 1.79****	69.17 ± 0.69***	120.3 ± 2.28****

***** p <0.001 , ***p<0.01 when compared to control.*

The urine volume and concentrations of Na^+, K^+ and Cl^- in ethanolic and methanolic extracts of *Acacia sinuata* (300mg/kg) were 8.9ml, 116.5mEq/L, 73.77mEq/L, 136.9 mEq/L and 5.5ml, 104.5mEq/L, 64.67mEq/L and 114.5 mEq/L respectively (Table-4).

Table 4 : Effect of ethanolic and methanolic extracts of *Acacia sinuata* on urine output and electrolyte excretion in rats

Treatment	Dose& Route	Urine volume (ml)	Electrolyte concentration in urine (mEq/L)		
			Na^+	K^+	Cl^-
Control (Saline)	25 ml/kg p.o.	4.03 ± 0.12	90.0 ± 0.61	51.73 ± 2.80	97.3 ± 1.04
Furosemide	20 mg/kg p.o.	9.267 ± 0.08****	136.8 ± 1.55****	94.17 ± 1.18****	163.7 ± 1.30****
Ethanolic extract	300 mg/kg p.o.	8.90 ± 0.05****	116.5 ± 0.69****	73.77 ± 0.97***	136.9 ± 1.59****
Methanolic extract	300 mg/kg p.o.	5.56 ± 0.08****	104.5 ± 0.62****	64.67 ± 1.31**	114.5 ± 1.90***

***** p <0.001 , ***p<0.01 , **p<0.05 when compared to control.*

DISCUSSION

Diuretics relive pulmonary congestion and peripheral oedema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure[14].

The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles [15]. The regulation of sodium, potassium balance is also intimately related to renal control of acid-base balance. The chemical constituents may be responsible for diuretic activity include alkaloids, glycosides, saponins, tannins, aminoacids, steroids and terpenoids [16]. In the present study we can demonstrate that ethanolic and methanolic extracts may produce diuretic effect by increasing the excretion of sodium, potassium and chloride. These features suggest that the plant extract is acting in a similar way as furosemide, which increases urinary output and urinary excretion of sodium by inhibiting Na+/K+/Cl- transporter system in the thick ascending loop of Henley [17].

The possible mechanism of action of phytochemical constituents that are responsible for diuretic action may be Saponins due to local irritation of kidney epithelium [18]. Alkaloids due to vasodilatory action on renal blood vessels [19]. Glycosides due to increased blood flow to the kidneys from enhanced cardiac contractility [20].

CONCLUSION

Ethanolic extract of *Acacia nilotica* and *Acacia sinuata* showed significant increase in urine volume and concentrations of Na^+ ,K^+,Cl^- when compared to methanolic extract of *Acacia nilotica* and *Acacia sinuata* .Of these both, *Acacia nilotica* showed more potent diuretic activity than *Acacia sinuata*.

Acknowledgements
The authors thankful to Dr. P. Ramalingam, Division of Medicinal Chemistry, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur for providing necessary facilities and Dr.J.Ravindra Reddy, M.Pharm, Ph.D, Department of Pharmacognosy, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur for authentication of plant specimens.

REFERENCES

[1] Yogesh Shivhare1, Priya Singh, Sunita Singh, Rambabu Tiwari, Pramod K Bharti, Neeraj Upmanyu; *Der Pharmacia Sinica*, **2010**, 1 (3): 40-45.
[2] Kathiresan Prabhu, Pradip Kumar Karar, Siva Hemalatha, Kathiresan Ponnudurai and Prakash Mankar; *Der Pharmacia Sinica*, **2011**, 2 (2): 131-141.
[3] Fereira IJ, Fererira AI. *Rev Esp Cardiol* **1995**; 4: 66-71.
[4] Agrawal SS, Paridhavi M. Herbal drugs technology. 1 st ed. Hyderabad; Universities press (India) Private Limited; **2007**; 545-546.
[5] Jain DL, Baheti AM, Parakh SR, Ingale SP, Ingale PL. *Pharmacognosy Magazine* **2007**;3: 116-119.
[6] Saurabh Srivastav, Pradeep Singh, K. K. Jha, Garima Mishra, Shruti Srivastava, M.S. Karchuli and R. L. Khosa, *European Journal of Experimental Biology*, **2011**, 1 (2):97-102
[7] Singh RG, Singh RP, Usha KP. *J Res Edu Ind Med* **1991**; 3:19-21.
[8] Kajal Ghosal, Subrata Chakrabarty and Arunabha Nanda; *Der Pharmacia Sinica*, **2011**, 2 (2): 152-168
[9] Medicinal plants of Bangladesh "The authentic Taxonomic information ,Description of the plant, Chemical constituents,Uses and distribution of species in Bangladesh.
[10] V.C. Mbatchou1, A.J. Ayebila1 and O.B. Apea, *Journal of Animal & Plant Sciences*, **2011**; 10(1): 1248-1258.
[11] Wiebelhaus V. D., Weinstock J., Maass A. R., Brennan F. T., Sosnowski G., and Larsen T. *J.Pharmacol. Exp. Therap*., **1965**;149 (3): 397-403.
[12] Singh GK, Dixit VK, *Journal of Pharmacy Research*; **1992**; 30(4):170-172.
[13] P. Sravani, Mohana lakshmi,Saravana kumar. *International Journal of Preclinical and Pharmaceutical Research* **2010**; 1(2):31-34.
[14] R.D. Hoeland and M.J. Mycek, Lippincott's illustrated Reviews: Pharmacology, Lippincott Willams and Wilkins, Philadelphia, **2000**;240-241.
[15] Guyton AC and Hall JE,In: Textbook of medical physiology, Singapore, 9 th ed .PA: W.B. Saunders Company.**1998**;306-308.
[16] Vivek Kumar Gupta and Vikrant Arya , *J. Chem. Pharm. Res*., **2011**; 3(1):613-620.
[17] K.K Hullatti1, M.S Sharada and I.J Kuppasth, *Der Pharmacia Sinica*, **2011**, 2 (1): 129-134
[18] David Hoffmann, Fnimh, AHG, Medicinal Herbalism,The science and Practice of herbal medicine , **2003**; 78.
[19] Margaret F.Roberts, Michael wink, Alkaloids: Biochemistry, Ecology, and Medicinal applications,**1998** New York.
[20] Dollyseye, Nclex review, October **2009**; 27.