

Anti-Diuretic Impact of *Aerva Lanata* Extracts and their Active Compounds in Furosemide Administered Diuretic Rats

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Abstract

Bioactive compounds of aromatic and medicinal plants showed remarkable activity against bacteria and fungi. Medicinal plants were used to inhibit the pathogens and cure most of the diseases like diuretics, diabetics etc., Hence, in the present study the antidiuretic activity of the *Aerva lanata* was analysed and discussed. In this study, total urine output in normal animals was 1.32 ± 0.22 ml/kg of animal per day, but diuretic animal had 1.76 ± 0.26 ml/kg/day. It was increased to 44% and stimulated the action of diuretic 1.5 fold in this study. Furosemide (Standard diuretic drug) induced percentage secretion of saline was 8.52 ± 0.56 and urine excretion was 7.38 ± 0.53 . The active compound of *Aerva lanata* was showed to reduce the remarkable and reach the control value and showed antidiuretic efficiency. Total urine output in diuretic induced animal treated with 2-Decyl -1-tetra decanol was 1.30 ± 0.27 ml/kg of animal per day compared to control (1.32 ± 0.22 ml/kg of animal per day) it was decreased 2% and stimulated the action of diuretic 1 fold. The active compound of *A. lanata*, 2-Decyl -1-tetra decanol induced percentage of secretion of saline was 8.76 ± 0.52 and urine excretion was 7.49 ± 0.48 . The standard antidiuretic hormone also showed similar value of active compounds of *Aerva lanata*. From the investigations the *Aerva lanata* is to be used effective cheap plant based drug for diuretic diseases.

Keywords: *Aerva lanata*; Furosemide; Diuresis; Creatine; Urine

Introduction

In the pharmacological analysis, the antibiotics were used to cure diuretics but it created the side effects like increase plasma, changes in edema, hypertension and heart diseases. In earlier days, the diuretics/hypertension was cured with five types of antibiotics. These kinds of antibiotics also affected the glucose level in patients and created side effects. Additionally, there is a concern about excess mortality associated with diuretic therapy and diabetes mellitus. The alcoholic extract of *Aerva lanata* was tested for diuretic activity. Manoj Goyal et al., reported that the alcoholic extract at a dose of 800 mg/kg acted as a diuretic, with respect to control [1].

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They confirmed that the medicinal plant *A. lanata* is used an alternate for chemotherapy against diuretics. Hence in the present investigation, we estimated the diuretic efficiency of *A. lanata*.

Materials and Methods

In the present study rats (*Wister albino*) were used to screen the diuretic assay. The rats were purchased from CPCSEA approved suppliers and transferred to the laboratory for accommodation. During accommodation, animals were not disturbed and were provided an adequate amount of pellet food and water ad libitum in standard condition (12 hrs light and 12 hrs dark). After accommodation, the animals were divided into nine groups (each group contains 6 animals) and used for diuretic analysis as per the method of Ganegamage et al. [2]. Diuretic induction drug, furosemide, and a compound of *A. lanata* lethal dose were calculated by Sprague method [3]. From the lethal dose, calculate one-tenth of LD50 concentration value for treatment.

Experimental design

Group I: Normal animal without any treatment.

Group II: Diuretic animals, injection of furosemide (10 mg/kg bw) through intra peritoneal region for induction of diuresis.

Group III-VII: Animals were received a sublethal concentration of hexane, butanol, ethanol, chloroform and water extract of *A. lanata* (1g/kg bw) for treatment of diuresis animal.

Group VIII: Animals received an active compound of *A. lanata* (2-Decyl -1-tetra decanol: 0.5 g/kg) for treatment of diuresis animal.

Group IX: Animals received standard antidiuretic hormone drug (10 mg/kg) after inducing diuretics.

Diuretic/antidiuretic assay

Diuretic activities of normal and treated animals were estimated using an estimation of difference between the output of urine and consumption of liquids. The percentage of variance in between saline administered and test animals were calculated [4]. From the normal and test animals, a blood sample was collected through tail cut methods and kept it without disturbance for the separation of serum. The serum samples were used to screen the urea and creatinine by colorimetric methods using Randox kit [5].

Biochemical estimation

Biochemical parameters were like ALT, AST and ALP were estimated in normal and treated animal samples of blood and liver homogenates.

Alanine aminotransferase (ALT) estimation

Transamination is the process in which an amino group is transferred from one α - amino acid to another α - keto acid [6]. The amount of pyruvate formed was measured by means of 2, 4 trinitrophenyl hydrazine of pyruvic acid. The color of which is read at 520 nm. The activity of ALT was expressed as IU/dl.

Transamination is the process in which an amino group is transferred from one α -amino acid to a keto acid [6]. AST catalyzes the reaction in which oxaloacetate is converted to pyruvate by decarboxylation and the pyruvate is estimated colorimetrically

by the addition of 2,4 dinitrophenyl hydrazine. Two test tubes were taken and marked as test and blank. One ml of substrate was added in test and blank test tubes and incubated at 37°C for 3 minutes. To the tube marked test 0.2 ml serum was added. After incubation at 37°C added 1 ml of 2, 4 dinitrophenyl hydrazine and allowed an addition to stand for 20 minutes. Followed by the addition 10 ml of 0.4 N NaOH, the color developed was read at 540 nm using a colorimeter. Simultaneously a set of standards were prepared as follows. To 8 different test tubes added 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 1 ml of standard pyruvate solution. The buffer was added to both tubes were made up to 1.2 ml. To all the tubes add 1 ml of 2, 4 DNP, add 10 ml of 0.4 N NaOH and read at 540 nm. The activity of AST is expressed in IU/dl.

Alkaline phosphatase (ALP) estimation

The phosphate present in serum or substrate, disodium phenyl phosphate to yield phenol as a product [7]. In the presence of alkaline oxidizing agents, 4-amino antipyrine gives a purple color with phenol which is read at 520 nm using a colorimeter. Two test tubes were taken and marked as test and blank. 2 ml of buffered substrate was added to each tube and placed in a water bath at 37°C for a few minutes. To this 0.1 ml of serum was added and incubated exactly for 15 minutes. To both tubes, 0.8 ml serum was added and simultaneously a series of a standard containing 0.2, 0.4, 0.6, 0.8 and 1 ml of phenol standards were taken and made up to 1 ml with double distilled water. One ml of water considered as blank for this test. To the test, blank and standard tubes 1 ml of 4-aminoantipyrine and 1 ml of potassium ferricyanate were added and read at 520 nm after the development of color. The amount of ALP calculated and recorded in the unit of IU/dl.

Results

The lethal dose concentration of *A. lanata* was 20.4 g/kg of rat. In this concentration the animals were dead, the 50% of concentration 10.2 g/kg does not kill the animals. Hence the 1g/kg of extracts and 0.5 g/kg of active compound used for further experiments.

For the diuretic activity of the *Aerva lanata* was analysed and recorded in TABLE 1. Total urine output in diuretic induced animal was 1.76 ± 0.26 ml/kg of animal per day compared to control (1.32 ± 0.22 ml/kg of animal per day) it was increased 44% and stimulated the action of diuretic 1.5 fold. Furosemide-induced percentage secretion of saline was 8.52 ± 0.56 and urine excretion was 7.38 ± 0.53 . The active compound of *Aerva lanata* showed to reduce the remarkable and reach the control value. Total urine output in diuretic-induced animal treated with 2-Decyl-1-tetra decanol was 1.30 ± 0.27 ml/kg of animal per day compared to control (1.32 ± 0.22 ml/kg of animal per day) it was decreased 2% and stimulated the action of diuretic 1 fold. 2-Decyl-1-tetra decanol induced percentage of secretion of saline was 8.76 ± 0.52 and urine excretion was 7.49 ± 0.48 . The standard antidiuretic hormone also showed the similar value of active compounds of *Aerva lanata*. The extracts of hexane, butanol, ethanol, chloroform and aqueous also showed notable changes in the total urine output and excretion of saliva and urine were noted (TABLE 1).

TABLE 1. Diuretic/Antidiuretic analysis of animal administered with test chemicals and *A. lanata* extracts.

S. No	Test	Urine (ml)	The variance of urine (%)	Analysis		Percentage of excreted saline	Percentage of excreted urine
				Action	Potency		
G1: Control	DW, 2 mL	1.32 ± 0.22	-	-	-	8.52 ± 0.56	7.38 ± 0.53

G2:	Furosemide, 10 mg/kg	1.76 ± 0.26	Ic 44	1.33	1	13.07 ± 1.13	11.42 ± 1.11
G3:	H.E (1g/kg)	1.52 ± 0.23	Ic 20	1.15	1	11.76 ± 1.21	10.36 ± 1.08
G4:	B.E (1g/kg)	1.49 ± 0.29	Ic 17	1.13	1	10.31 ± 1.24	10.23 ± 1.08
G5:	E.E (1g/kg)	1.44 ± 0.22	Ic 12	1.09	1	10.06 ± 1.01	10.16 ± 1.02
G6:	C.E (1g/kg)	1.42 ± 0.26	Ic 10	1.08	1	11.76 ± 1.21	10.36 ± 1.08
G7:	A.E (1g/kg)	1.39 ± 0.26	Ic 7	1.05	1	11.76 ± 1.29	10.46 ± 1.07
G8:	Active compound (0.5g/kg)	1.30 ± 0.27	Dc 2	0.98	1	8.76 ± 0.52	7.49 ± 0.48
G9:	ADH, 0.13 ml/rat,	1.28 ± 0.24	Dc 4	0.97	1	8.59 ± 0.48	7.39 ± 0.51

G: Group; HE: Hexane sample; BE: Butanol Extract; EE: Ethanol Extract; CE: Chloroform Extract; AE: Aqueous Extract; Ic: Increase; De: Reduction

Aerva lanata noted all extracts and an active compound of 2-Decyl -1-tetra decanol have antidiuretic activity in diuretic animals [8]. Ratnasooriya et al., reported bark sample showed antidiuretic action and used the herbal drug in Sri Lankan traditional medicines and some antidiuretics impair GFR [9,10]. The methanol extracts of *A. lanata* diuretic activity urine output analyzed and hormonal changes were documented [2,5]. In the diuretic toxicity study, the test *Aerva lanata* (10 mg/kg) showed remarkable changes in blood samples and were recorded in TABLE 2.

TABLE 2. Anti diuretic analysis of *A. lanata* extracts against diuretic animals.

Group	Biochemical and blood parameters				
	Blood Urea (mg/dL)	Blood Creatinine (mg/dL)	ALT (U/I)	ALP (U/I)	AST (U/I)
Normal	35.62 ± 2.40	0.97 ± 0.14	64.36 ± 4.20	6.09 ± 2.40	139.10 ± 19.10
Test	33.76 ± 2.90	1.22 ± 0.06	89.84 ± 7.50	7.96 ± 3.20	127.75 ± 22.00
H.E (1g/kg)	34.67 ± 2.40	1.15 ± 0.08	75.84 ± 6.20	7.58 ± 2.20	137.68 ± 18.20
B.E (1g/kg)	34.52 ± 2.60	1.04 ± 0.03	70.58 ± 6.40	7.38 ± 2.40	137.54 ± 18.40
E.E (1g/kg)	34.18 ± 2.50	1.06 ± 0.06	73.82 ± 5.60	7.23 ± 2.30	139.10 ± 18.70
C.E (1g/kg)	34.24 ± 2.40	1.04 ± 0.08	72.62 ± 8.20	7.29 ± 2.20	138.18 ± 17.90
A.E (1g/kg)	33.96 ± 2.30	1.01 ± 0.04	70.28 ± 7.20	6.95 ± 2.10	138.65 ± 18.10
Active compound (0.5g/kg)	35.26 ± 2.2	0.98 ± 0.03	66.18 ± 3.3	6.59 ± 2.0	139.70 ± 18.1
ADH, 0.13 ml/rat	35.46 ± 2.3	0.97 ± 0.04	65.28 ± 4.4	6.19 ± 2.0	139.65 ± 18.6

G: Group; HE: Hexane sample; BE: Butanol Extract; EE: Ethanol Extract; CE: Chloroform Extract; AE: Aqueous Extract; Ic: Increase; De: Reduction

The level of urea, creatine, ALT, ALP, and AST in rats maintained as control, diuretic rats treated with the effect of *A. lanata*, active compound and diuretic hormone was analysed (TABLE 2). Variation in blood urea, creatine, ALT, ALP and

AST parameters were observed during treatment periods. After treatment periods the urea content of control animal was 35.62 ± 2.4 mg/dl, Creatinine level was 0.97 ± 0.14 mg/dL, ALT was 64.36 ± 4.2 UI, ALP was 6.09 ± 2.4 UI and AST was 139.10 ± 19.1 UI. Diuretic-induced animal urea content was reduced (33.76 ± 2.9 mg/dl) but creatine level was increased (1.22 ± 0.06 mg/dl) and serum biochemical also increased (ALT was 89.84 ± 7.5 UI, ALP was 7.96 ± 3.2 UI and AST was 127.75 ± 22.0 UI). The active compound (5mg/kg) administered diuretic animal developed the antidiuretic activity, urea was 35.26 ± 2.2 mg/dl, creatine was 0.98 ± 0.03 mg/dl, ALT was 66.18 ± 3.3 UI, ALP was 6.59 ± 2.0 UI and AST was 139.70 ± 18.1 UI. The standard unit diuretic hormone-treated animal also showed urea: 35.46 ± 2.3 mg/dl, creatine: 0.97 ± 0.04 mg/dl, ALT: 65.28 ± 4.4 UI, ALP: 6.19 ± 2.0 UI and AST: 139.65 ± 18.6 UI. Extracts of hexane, butanol, ethanol, chloroform and aqueous of *Aerva lanata* also showed moderate activity on diuretic animal and were recorded in TABLE 2.

When we compared the result of active compound of *A. lanata* in combination with a diuretic drug, the combined effect showed a positive result. The result is in concordant with the reports of Chandrika et al., and Moram in animals treated with *Artocarpus heterophyllus* and *Panaxquin quefolius* respectively [11,12]. The marked increase in serum triglycerides and cholesterol observed in diabetic rats [13].

Conclusion

The active compound of *Aerva lanata* showed to reduce the remarkable and reach the control value in diuretic animals. Total urine output in diuretic-induced animal treated with 2-Decyl -1-tetra decanol was 1.30 ± 0.27 compared to control (1.32 ± 0.22). From the results healing of diuretic was improved by active compounds of *Aerva lanata*. The standard antidiuretic hormone also showed the similar value of active compounds of *Aerva lanata*. From the investigations, we can conclude that *Aerva lanata* can be used as an effective cheap plant-based drug for diuretic diseases.

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