

Pharmacognostical and pharmacological evaluation of *Andrographis paniculata* for diuretic activity

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Abstract

The effect of ethanol and methanol fractions of *Andrographis paniculata* was found to be highly potent comparison was made with the standard diuretic reference drug furosemide. But the diuretic effect observed with TEEF show the least levels of Cl⁻ output hence it show less potent diuretic action and TEEAF show moderate potent action with elimination of Cl⁻, Na and urine volume. Determination of urinary electrolyte concentration revealed that Ethanol and methanol extracts of both plants was most effective in increasing urinary electrolyte concentration for all three tested ions (Na, K, Cl⁻) While Ethyl acetate extract of *Andrographis paniculata* did not shown significant increase in either urinary volume or electrolyte concentration. The fractions Ethanol and methanol fractions of *Andrographis paniculata* significantly increases the glomerular filtration rate (GFR), it may be due to interactions of structural components of glomerular membranes as detergent and reduce the resistance of the direct effect on the arteriole wall affecting glomerular blood flow or efferent arteriole. These research results have concluded that the pharmacological evidence for the folklore claim of the plants fractions to be used as diuretic agents. On the basis of these findings, it may be inferred that seeds of *Andrographis paniculata* are diuretic agent due to the presence of flavonoids and poly phenols. The results are in agreement with the traditional use of the plants are diuretic agents.

Keywords: *Andrographis paniculata*, Diuretic, GFR, Sodium Potassium Channel, Ethyl Acetate Extract.

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1. Introduction

Diuretics are the drug that increases the rate of urine formation together with natriuresis. Diuretics are used to adjust the volume and composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome, and cirrhosis.

1.1 Role of the kidney in water homeostasis

Renal tubular re-absorption of filtered water occurs by osmosis, and, since the glomerular filtrate is essentially iso-osmotic, depends on sodium re-absorption to create an osmotic gradient. After formation of a plasma ultra-filtrate in the glomerulus, the tubular fluid enters the proximal convoluted tubule, where specific transporters reabsorb sodium, chloride, bicarbonate, glucose and amino acids [9].

About 60% of the water and most of the organic solutes are also reabsorbed in the proximal tubule. At the boundary between the inner and outer stripes of the outer medulla, the thin descending limb of the loop of Henle begins [10].

The thick ascending limb of the loop of Henle actively reabsorbs sodium and chloride from the lumen (about 35% of the filtered sodium), but unlike the proximal tubule and the descending limb, it is virtually impermeable to water. Sodium chloride re-absorption in the thick ascending limb effectively dilutes the tubular fluid, so this segment is called the 'diluting segment.' The loop of Henle therefore acts as a countercurrent multiplier producing a gradient of hyperosmolarity in the medullary interstitium.

In the distal convoluted tubule, which connects with the diluting segment, around 10% of filtered sodium chloride is reabsorbed. Like the thick ascending limb, the membrane is relatively impermeable to water, so further tubular fluid dilution ensues. The final arbiter of urine composition is the collecting duct, where 2–5% of sodium chloride reabsorption occurs. Importantly, this is where mineralocorticoids exert their influence, especially aldosterone. Sodium is reabsorbed in exchange for potassium under the influence of aldosterone and it is here that almost all diuretic-induced changes in potassium balance occur [11]. Water is reabsorbed through the action of the posterior pituitary hormone vasopressin (also known as antidiuretic hormone [ADH], although vasopressin is the preferred term) and the final urine to enter the renal pelvis is diluted or concentrated, achieved by the countercurrent mechanism that creates a concentration gradient from 50 mOsm/kg at the outer cortex to 1200 mOsm/kg at the inner medulla [12].

1.1.1 Transporters

Absolutely germane to the understanding of diuretic action is the concept of transporters. Those that operate by having to bind more than one substance to the transport protein, then facilitating the transport of these substances, together, across the membrane are called symports (or symporters). If they transport only one substance, they are uniports and others that exchange one substance for another are the antiports. The archetypal antiport is the Na^+/K^+ -ATPase, which moves three Na ions out of the cell for each two K ions that it moves into the cell [13]. From the elucidation of these mechanisms, a new and functional classification of diuretics has arisen.

The process involves facilitated diffusion and in the case of loop diuretics, thiazides and the carbonic anhydrase inhibitor acetazolamide, all of which are acidic, secretion into the tubular fluid, through the organic acid pathway in the proximal tubule. Amiloride and triamterene, being organic bases, enter the tubular lumen via the organic base secretory mechanism, also in the proximal tubule.

Spironolactone and other aldosterone antagonists act via a cytosolic receptor and so are delivered to their target area via the blood and the basolateral membrane. If the diuretic is very highly protein bound (96%), then glomerular filtration is limited. Even in hypoalbuminuria, there are not enough 'free' drugs at one time to get across. Other considerations apply as well and these will be examined separately, with the diuretic or disease that influences it. [2,14]

1.2 Mechanism of Diuretics:

Loop diuretics

The Loop diuretics (furosemide) and bumetanide have one common property they are all inhibitors of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ symport which transfers ions from the tubular

lumen into the tubular cells. The symport is electroneutral and is activated when all four sites are occupied. The Na that has entered the tubular cell is pumped out into the systemic circulation by the $\text{Na}^+/\text{2K}^+$ -ATPase antiport located in the basolateral membrane [15]. By its action, a very favourable electrochemical gradient for Na to enter the cell from the lumen is established because the Na concentration inside the cell is left low. A separate basolateral chloride channel (called CLCN) provides a basolateral exit conduit for Cl^- . The availability of luminal potassium limits activity of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ symport so potassium entering the cell is recycled by the ROMK (renal outer medullary potassium) channel, which is an ATP-dependent potassium channel in the luminal membrane that not only plays an important role in potassium recycling in the thick ascending limb, but also in potassium secretion in the cortical collecting ducts of the nephron.

The depolarization of the basolateral membrane sets up a transepithelial voltage difference (10 mV or so) with the lumen positive compared with the interstitial space.[16] Because of the asymmetrical stoichiometry (3 Na^+ per 2 K^+), this voltage repels cations (Na^+ , Ca^{2+} and Mg^{2+}) towards the interstitial space and drives reabsorption of these cations via paracellular paracellin-1. This region (ascending limb of the loop of Henle) is virtually impermeable to water because unlike the proximal tubule, it does not have water channels (aquaporins, AQP) which grossly facilitate the movement of water. Therefore, the actions of the symport remove Na^+ and Cl^- but not water, effectively diluting the tubular fluid, to the extent that about 25–35% of filtered sodium is reabsorbed here. If the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ symport is blocked, then about 25% of filtered sodium would not be reabsorbed, and would remain in the tubular fluid to be presented to the collecting duct, where, under the influence of aldosterone, some Na^+ would be retrieved but at the expense of exchange for K^+ , which would be lost. The other consequence of jamming this symport would be to reduce, if not abolish, the voltage difference whereby calcium and magnesium are reabsorbed. Blocking this symport would lead to a substantial natriuresis, hyponatraemia, possibly some hypokalaemia, with hypocalcaemia and hypomagnesaemia a distinct possibility. The mode of action of loop diuretics, e.g. furosemide and bumetanide is to block the $\text{Na}^+/\text{2K}^+/\text{Cl}^-$ symport (site II) and it is thought that these agents bind the Cl^- binding site that lies within the symporter's transmembrane domain. They owe their designation as loop diuretics to their site of action and they have acquired the synonym 'high ceiling' diuretics because progressive increase in dose is accompanied by an increasing diuresis (actually natriuresis because both water and solute [NaCl] are lost) they have a high ceiling to their effect shown in Figure 1.1. As they block the region of the nephron that

has the capacity to reabsorb the most sodium, they are the most potent and effective natriuretic compounds and so have claimed yet a third name - that of 'high efficiency' diuretics. These names belie the fact that they come from cosmopolitan background chemistry.

The Himalayas are known for having more than 10,000 different kinds of plants, many of which are used to make medicines. The Banj oak (*Andrographis paniculata* A. Camus), which is in the family Fagaceae, is an evergreen tree that grows to about 40 m tall and is found all over the Himalayas between 800 and 2300 m latitude. The seeds of *Andrographis paniculata* have been shown to be used as a diuretic to treat high blood pressure in the past. The goal of this study was to first look at the seed of QL from a pharmaceutical perspective. Then, the active principle or principles were extracted using different solvents (methanol, n-hexane, chloroform, ethylacetate, n-butanol, and water) and tested for different physical and chemical properties, antioxidant activity, and biological activity as a diuretic in two different animal models.

The historical usage of the seeds as a diuretic has not been confirmed pharmacologically. Due to the fact that diuretics are very effective in the treatment of chronic conditions such as hypertension, edema, renal abnormalities, and urinary tract infections, the present study was conducted to explore the aforementioned action with evidence.

2. Materials and Methods

2.1 Plant material

The seeds of *Andrographis paniculata* were collected in the month of November-December from the local areas Bhopal, Madhya Pradesh and make herbarium. The plants were identified, confirmed and authenticated by Mr. Zia-Ul-Hasan, Head of the Department, Department of botany, Saifia College, Bhopal. A voucher specimen (No. NCP/PCG/06/2024) has been deposited at the RKDF College of Pharmacy, Bhopal, Madhya Pradesh.

2.2 Extraction and fractionation of seeds of traditional plants

The collected 3kg of seeds were washed with running water and shade dried for two weeks, after drying coarsely powdered using mesh No. 22. obtained power packed in to soxhlet column and extracted with methanol and ethanol (70%). The extract was concentrated under reduced pressure in rotary flash evaporator to yield crude semisolid mass at 60°C. The crude extracts of *Andrographis paniculata* seeds successive fraction with ethyl acetate, ethanol and methanol. The different fractions are completely dried under high vacuum on a rotary flash evaporator, concentrated under reduced pressure, labeled and the percentage yield was calculated. The dried fractions were stored in airtight container in refrigerator.

2.3 Preliminary phytochemical screening of the fractions

Phytochemical analysis was carried out using the extracts of both plants were qualitatively analyzed and identified the presence of 2° metabolite constituents.

2.4 Animals used

In the present study Swiss albino mice 20-25 gm and Albino Wister rats of either sex weighing between 180-200 gm were used for diuretic. The animals were maintained under standard light/dark cycle at the room temperature with free access to standard diet and water *ad libitum*. The study protocol has approved by IAEC all the animal experimental study.

2.5 Acute toxicity studies

As per CPCSEA/OECD guidelines, the study was performed according to the acute toxic method. Female albino Swiss mice were used for toxicity study. Overnight fasted animals providing only water were used, after which the test drug and fractions were dissolved in Water and it was administered orally at the dose of 2000 mg/kg and observed three days. After dose treatment observed Animals individually at least once during the first 30 min, periodically during the first 24 hr (with special attention during first 4 hr) and daily thereafter for a period of three days. Daily once cage observations for changes in skin, fur, eyes and respiratory rate and also mucous membrane (nasal), circulatory (heart rate and blood pressure), autonomic (salivation, perspiration, lacrimation, piloerection urinary incontinence, and defecation) and CNS (ptosis, drowsiness, gait, tremors and convulsion). The toxicity study carried out as per the guidelines of OECD- 421 using albino mice. The fractions were found to be safe up to 2000mg/kg bw Hence we selected 1/10th, 1/5th of LD₅₀ cut off values taken as screening dose. The doses are 100 mg, 200 mg and 400 mg/kg dose (P.O of Ethyl acetate and ethanol fractions of *Andrographis paniculata* for pharmacological screening.

2.6 Pharmacological activities

Diuretic activity

The Lipschitz described method was adopted for the evaluation of diuretic activity. Normal urine output in rats is very small (1-2ml/rat/day). Hence to get the measurable quantity the animals are first hydrated. The urine output is increased after administration of diuretics like urea, hydroflumethiazide and frusemide. Increase in volume of urine was measured with the help of measuring cylinder and compared with the normal urine output.

Furosemide was used as standard diuretic agent, purchased from local pharmacy. The drug is dissolved in water for injection and administered in a dose of 20 mg/kg (i.p) to rats. Six groups of six animals in each were fasted and 15 hours deprived of water prior to the experiment. All the extracts *Andrographis paniculata* normal saline were administered orally and standard drug Furosemide by i.p

route. All the animals received priming dose of normal saline solution of 25 ml/ kg body weight. Group I (control) received normal saline, Group II received the Furosemide and Group III, IV, V, and VI received seeds extracts of *Andrographis paniculata* in normal saline. After the administration, immediately the rats (two in each cage) were placed in metabolic cages, which are specially designed to separate urine and faeces. Though the experiment cages kept at the room temperature of $25 \pm 0.5^\circ \text{C}$. up to 3 hrs the urine was collected in measuring cylinder after dosing. During this period, no feed or water is provided to the animals. The parameters taken for each individual rat was total urine volume, pH, Concentration of K^+ , Na^+ and Cl^- in urine. Finally the mean urine volumes were determined and used for their pH determinations using a pH meter (systronic Digital pH meter). Concentrations of Na^+ , K^+ , and Cl^- were measured by titrimetric method against AgNO_3 solution, and the diuretic potency was assessed by comparison of urine excretion due to the fraction with respect to the normal urine output.

Group I: Control (Normal saline 25ml/kg p.o)

Group II: Standard (Furosemide 20mg/kg i.p)

Group III: *Andrographis paniculata* Ethyl acetate fraction (100mg/kg p.o)

Group IV: *Andrographis paniculata* Ethanol fraction (200mg/kg p.o)

3. Results and Discussion

3.1 Preliminary phytochemical screening

The coarsely powered plant materials were extracted with 70% ethanol and methanol to obtain crude extracts and were further partitioned into different fractions on the basis of increasing polarity are shows the colour and percentage yield in table No.1. The preliminary Phytochemical studies result with the different solvent fractions of seeds of *Andrographis paniculata* revealed the presence of steroid, flavonoids, glycosides, terpenoids, carbohydrates and alkaloids respectively. Phytochemical investigation of both plants fractions are shown in table 2.

Table 1: Percentage yield of leaves fractions of *Andrographis paniculata*

Sl. No.	Solvent	Colour and consistency	Percentage yield
1	<i>Andrographis paniculata</i> Ethyl acetate fraction	Greenish brown sticky	5.6
2	<i>Andrographis paniculata</i> Ethanol Fraction	Dark red solid	7.3

Table 2: Phytochemical results for *Andrographis paniculata* seeds fractions

Phyto chemicals	Ethyl acetate Fraction	Ethanol fraction
Alkaloids	-	+
Carbohydrates	+	++
Flavonoids	+	++
Glycosides	++	++
Proteins	+	++
Steroids	-	++
Saponins	-	++
Tannins	-	+
Terpenoids	-	++

+ Indicates presence

++ Represents the thick presence of the tested phytoconstituent in the Sample Fraction

-- Represents the absence of the tested phytoconstituent in the sample Fraction.

3.2 Acute toxicity studies

The acute toxicity studies showed no mortality and adverse effect at 2000/kg. b.w. of ethanolic and ethyl acetate fractions of *Andrographis paniculata* seeds fractions during 24 hrs observation. In the ethyl acetate fraction of both plants showed mortality at 1000mg/kg. b.w (p. o). The doses fixed to 200 mg/kg and 100 mg/kg b.w. for Ethanol and ethyl acetate fraction of *Andrographis paniculata*. In *Andrographis paniculata* methanol and ethyl acetate fractions fixed to 400mg/kg and 100mg/kg b.w. respectively.

3.3 Diuretic Activity

Treatment with the different fractions of *Andrographis paniculata* (TEEAF 100, TEEF 200mg/kg). All the doses of the fractions of both plants showed increase in the elimination of total urine volume, among these doses 200mg/kg of ethanol fraction of *Andrographis paniculata* and 400 mg/kg of *Andrographis paniculata* ethanol fraction showed significant increase concentration of Na^+ , Cl^- and K^+ ion comparable to control group of rats, (Table. 3) the extract also increased the volume of urine at both dose levels, the obtained effect was comparable to that of frusemide (20 mg/kg) as reference standard drug. When orally administered doses to rats at 100mg/kg TEEAF and TEEF 200mg/kg. The ethanolic fraction of *Andrographis paniculata* produces increased urination at 200 mg/kg dose, however, there was moderate diuretic effect at 100 mg/kg dose of ethyl acetate fraction of *Andrographis paniculata*. These experimental results have established pharmacological evidence for the folklore evidence claim of the plants to be used as diuretic agents. Although these results provide a support for the traditional uses of *Andrographis paniculata* areal parts, further studies are necessary to better evaluate its safety and modes of action.

Table 3: Diuretic activity *Andrographis paniculata* of seeds Fractions

Treatment	Dose (mg/kg)	Volume	Concentration of Ions (meq/l)			pH
			Na+	K+	Cl-	
Control	25	1.95	68.4±0.81	25.98±0.61	43.11±0.67	6.7
Standard (Furosemide)	20	4.26	121.52±1.13	51.96±0.53	76.18±0.32	6.4
TEEAF	100	2.93	74.43±1.5	30.01±1.4	36.50±0.47	7.6
TEEF	200	3.45	86.46±0.26	37.28±0.17	44.16±0.83**	7.1

Values are mean±S.E.M (n=6); ***p<0.001, **p<0.01, *p<0.05. Student's 't' test

4. Discussion

The results obtained in diuretic assay of TEEAF, TEEF of *Andrographis paniculata* were shown in Table 8.3. From the results it can be observed that ethanol and methanol fractions had shown potent diuretic activity by increasing urine excretion and enhancing the elimination of potassium, sodium and chloride quantity when compared to control group. The effect of ethanol and methanol fractions of *Andrographis paniculata* was found to be highly potent effect, comparison was made with the standard diuretic reference drug furosemide. But the diuretic effect observed with TEEF shows the least levels of Cl⁻ output hence it shows less potent diuretic action and TEEAF shows moderate potent action with elimination of Cl⁻, Na⁺ and urine volume. Determination of urinary electrolyte concentration revealed that Ethanol and methanol extracts of both plants were most effective in increasing urinary electrolyte concentration for all three tested ions (Na⁺, K⁺, Cl⁻). While Ethyl acetate extract of *Andrographis paniculata* did not show significant increase in either urinary volume or electrolyte concentration. The fractions Ethanol and methanol fractions of *Andrographis paniculata* significantly increase the glomerular filtration rate (GFR), it may be due to interactions of structural components of glomerular membranes as detergent and reduce the resistance of the direct effect on the arteriole wall affecting glomerular blood flow or efferent arteriole. Diuretics are the drugs that increase the excretion of Na⁺ and water from the body by an action on the kidney; their primary effect is to increase excretion of NaCl, this can

achieved by i) A direct action on the cells of nephron. ii) Filtrate content is indirectly modified.

Since a very large proportion of the NaCl and water that passes into the tubule ion, the glomerulus is reabsorbed; a small increase in reabsorption can result in a marked increase in excretion. For example loop diuretics as furosemide, there is an increase in the excretion of Ca, Mg and decrease in excretion of uric acid. The effect of Na is beneficial in the treatment of hypocalcaemia.

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