

Enhanced Diuretic Effect of a Formulated Herbal Suspension -CAPAmeeta Argal^{1*}, Nishi Saxena²¹Director, Rajeev Gandhi College of Pharmacy, Bhopal, Madhya Pradesh, India²Rajeev Gandhi College of Pharmacy, Bhopal, Madhya Pradesh, India**Original Research Article*****Corresponding author**

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Abstract: A polyherbal suspension was formulated from the extracts of roots of *Cyperus rotundus* (CRR) and leaves of *Azadirachta indica* (AIL) and *Bryophyllum pinnatum* (BPL). The suspension had very good redispersibility and was very stable without agglomeration, caking or microbial growth. Study of diuretic activity was done on individual plant extract as well as formulation. There was a significant increase in the volume of urine and electrolytes Na⁺, K⁺, Cl⁻ which was similar to the standard drug furosemide. The formulation had much better activity as compared to the individual drug extracts which may be due to the synergistic effect of the herbs used. There was no significant change in pH. The loss in electrolytes Na⁺, K⁺, Cl⁻ may lead to a reduction in supersaturation of calcium in urine thereby preventing the formation of kidney stone. The PHF have good diuretic activity and can be used to reduce hypertension, kidney problems and urolithiasis.

Keywords: Diuretic activity, *Cyperus rotundus* roots, *Melia azadirachta* leaves, *Bryophyllum pinnatum* leaves, Polyherbal suspension.

INTRODUCTION

Diuretics are used in clinical disorders like hypertension, oedema, hepatic cirrhosis and renal impairment, by increasing the output of urine. They are also used in cardiac failure, acute oedema of the lung, nephritic oedema syndrome [1].

From ancient times herbs are used as diuretics but scientific dosing is essential to get efficient therapeutic effect. Plants have been explored for diuretic activity [2].

In Ayurveda combination of herbs is used to get better results. Polyherbal formulations have been reported to have efficient activity (Argal Herba polonica). Diuretic activity of polyherbal formulations has also been studied [3, 2]. Hence a polyherbal formulation as oral suspension was prepared containing three herbs by considering their therapeutic effect in kidney problem based on traditional use and scientific data. *Cyperus rotundus* L. (Cyperaceae) vernacularly called Nagarmotha is widespread in north east India and is used in spasms, arthritis, as stomachic, nervine tonic, anti-inflammatory and analgesic [4-6]. It is used in urinary problems and do not alter kidney function [7, 8]. *Bryophyllum pinnatum* Lam., (Crassulaceae) is used in diarrhea, ulcers, lithiasis [9, 10]. It has antineoplastic, analgesic, anti-inflammatory, muscle relaxant, nephroprotective and diuretic activity [11, 12]. *Azadirachta indica* A. Juss (Meliaceae) has antibacterial, antifungal, anti-inflammatory, diuretic activity [13-17].

MATERIALS AND METHODS**Plant material**

The roots of *Cyperus rotundus* (CRR), leaves of *Bryophyllum pinnatum* (BPL) and *Azadirachta indica* (AIL) were collected and authenticated from the

Department of Botany, Janata PG College, A.P.S. University, Rewa (M.P.), and Voucher Specimen Number/JC/B/PAN/054a-c. They were dried in shade and processed separately to a coarse powder.

Preparation of plant extracts & polyherbal suspension

The coarsely powdered plant material was macerated with alcohol for 7 days with occasional shaking. The menstrum was filtered and concentrated under reduced pressure to get the extracts of CRR, BPL and AIL. Their extractive values were calculated. Accurately weighed quantities of each extract were mixed in equal proportion [18] and triturated with Tween 80. Distilled water was added gradually with trituration to get a uniformly distributed suspension (CAP). The best stable formulation was selected for further studies.

Evaluation of polyherbal suspension

PHF was evaluated for organoleptic and physicochemical parameters. Particle size was determined by optical microscopy and viscosity by Brookfield viscometer type III using spindle 2 at 250 rpm. Sedimentation volume, redispersibility, density and pH were analysed Table-1.

Table-1: Evaluation of Polyherbal suspension (PHF)

S. No.	Evaluation Parameters	Inference
1.	Colour	Slightly Brownish
2.	Odour	Characteristic
3.	Taste	Acrid
4.	pH	3.77
5.	Sedimentation Volume	0.2
6.	Viscosity (cps)	48.3
7.	Average particle size	16.41
8.	Redispersibility	Easy and uniform
9.	Density (gm/ml)	1.0352

Animals

Wistar albino rats of either sex weighing between 120 to 200 gm were taken. They were kept at standard laboratory conditions with relative humidity 44–56%, temperature 25±2°C and 12 hrs. light and dark cycle. Standard pellet diet and water ad libitum was provided. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. SBRL/IAEC/2013/03).

Acute toxicity

Acute toxicity studies were done as per OECD guidelines for all extracts till a dose of 3000 mg/kg. The animals were observed for any change in behavior.

Assessment of Diuretic activity:

Six groups of rats were taken each containing six animal as follows:

- Group I - Control- administered vehicle (1ml/100gm, p.o.),

- Group II - Standard - Furosemide (10mg/kg, p.o.), only on the 1st day of experiment
- Group III - PHF - (100mg/kg p.o.) once daily for 7 days
- Group IV - CRR - (100mg/kg p.o.) once daily for 7 days
- Group V - BPL - (100mg/kg p.o.) once daily for 7 days
- Group VI - AIL - (100mg/kg p.o.) once daily for 7 days

Collection and analysis of urine

On 7th day, after administration of last dose the animals were transferred to Dolfen metabolic cages. They had free access to drinking water. After 24 hours urine was collected and measurement of volume, pH, Na⁺, K⁺ and Cl⁻ was done [19-21]. The measurement of Na⁺ and K⁺ was done by flame photometer and Cl⁻ by titration (Table-2&3).

Table-2: Effect of PHF and plant extracts on urine volume and pH

S. No.	Group	Urine volume (ml)	pH
1.	Normal control	3.3±0.33	7.03±0.2
2.	Standard	10.0±1.15***	7.43±0.08
3.	Polyherbal formulation	7.66±0.33**	7.26±0.37
4.	Extract of <i>C. rotundus</i> (CRR)	7.0±0.57*	7.0±0.11
5.	Extract of <i>B. pinnatum</i> (BPL)	6.66±0.66*	6.96±0.21
6.	Extract of <i>A. indica</i> (AIL)	6.33±0.33	6.93±0.24

N=6, All values are expressed as mean±S.E.M.

*p<0.05, **p<0.01, ***p<0.001 as compared to control

Table-3: Effect of PHF and plant extracts on electrolyte content of urine

S. No.	Group	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)
1.	Control	79.66±2.02	70.33±0.88	119.0±2.08
2.	Standard	114.33±1.76***	92.66±2.84a***	164.33±1.76a***
3.	PHF	106.0±2.08***	84.66±0.33a***	157.0±1.52a***
4.	CRR	101.66±2.4***	79.66±1.45a***	148.0±1.52a***
5.	BPL	94.66±1.76a**	75.33±1.2	140.33±1.45a***
6.	AIL	93.0±1.73a**	73.66±0.88	139.0±2.51a***

N=6, All values are expressed as mean±S.E.M.

*p<0.05, **p<0.01, ***p<0.001 as compared to control

Statistical analysis

All the values are expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and posthoc Tukey-Kramer Multiple Comparisons Test by employing statistical software, graph pad instat 3. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

The polyherbal suspension was well dispersed with desired viscosity and good dispersibility. It was observed for 18 months for stability at room temperature ($8^{\circ}\text{C} - 42^{\circ}\text{C}$). It had very good redispersibility property. There was no microbial growth, agglomeration or cake formation.

The results of animal activity show that the volume of urine increased to double as compared to normal control group. It increased significantly in all the groups except AIL when compared to control group. The volume of polyherbal suspension was more than

that of individual extracts but less as compared to standard. The pH of standard and polyherbal suspension was slightly more but there was not much change in the pH of individual extracts. The values were insignificant.

Urinary output of Na^+ and Cl^- was significant in all the extracts and suspension but K^+ output was non-significant in BPL and AIL. The pattern is similar to that of loop diuretics which act by decreasing the reabsorption of sodium in the distal convoluted tubule [22, 23]. The results showed that the polyherbal formulation had best diuretic activity as compared to the individual drug extract. This is due to the synergistic effect of herbs in formulation. No toxicity was observed. The suspension contains flavonoids, terpenoids, tannins and saponins. Saluretic activity may be because of saponin [24]. Presence of flavonoids, terpenoids and tannins are responsible for diuretic activity [25-27].

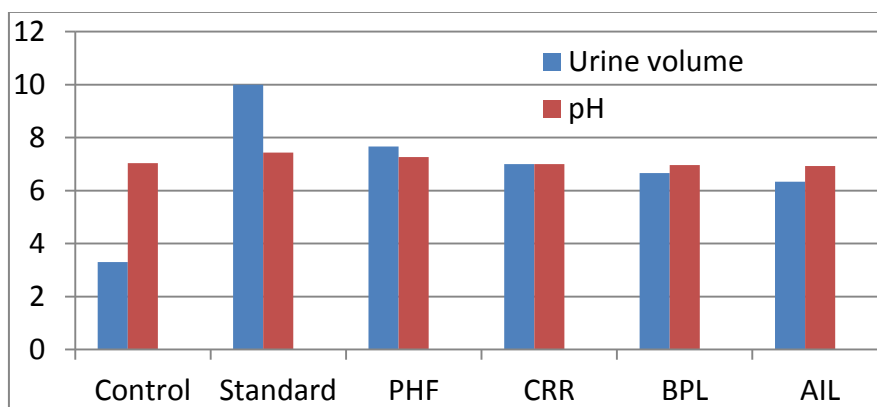


Fig-1: Effect of polyherbal formulation and plant extracts on urine volume and pH

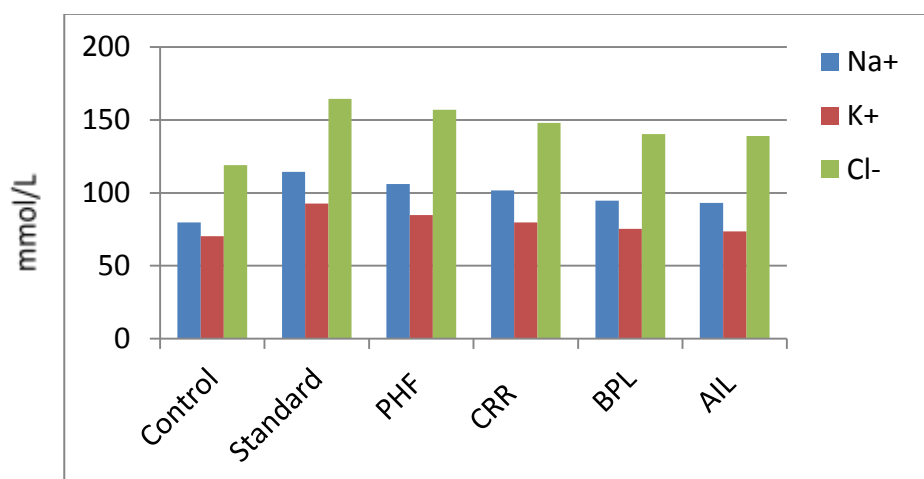


Fig-2: Effect of polyherbal formulation and plant extracts on sodium, potassium and chloride output

CONCLUSION

There was a significant increase in the output of urine and its electrolytes Na^+ , K^+ and Cl^- in individual extracts and PHF. But the results of PHF are much better which may be due to the synergistic effect

of the herbs present in it. The significant increase in urinary output and urinary electrolyte concentration of PHF confirms that it has enhanced diuretic activity. Loss in electrolytes may lead to a reduction in super saturation of calcium in urine thereby preventing the

formation of kidney stone (Argal JDDT). Hence it can be used in hypertension, kidney problems and urolithiasis.

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