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Evaluation of Polyherbal formulation for Diuretic activity in albino rats

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ABSTRACT

Objective: To investigate the diuretic potential of Polyherbal formulations prepared from the seeds of *Vitis venifera*, *Duranta repens* and *Centratherum anthelminticum* in Albino rats. **Methods:** Different concentrations of Polyherbal formulations (100, 200 and 400 mg/kg), furosemide (10 mg/kg) and vehicle were orally administered to rat (n = 6 animals per group) and their urine output was collected after 24h. The Urinary output, Osmolarity, pH, Na^+, K^+ and Cl^- concentrations of urine were estimated. **Results:** The Polyherbal formulations produced significant increase in Na^+, K^+, Cl^- excretion, caused alkalinization of urine, showed strong Diuretic index, saluretic index and Natruretic index. All the concentrations of Polyherbal formulation showed dose-dependent relationship when compared to control animals. **Conclusions:** These finding strongly suggests that the Polyherbal formulations have a good diuretic activity on rats in the above experimental model.

1. Introduction

Diuretics are responsible for increase the rate of urine flow, sodium excretion and to maintain the volume and composition of body fluids in a various clinical Disorders. But drug-induced diuresis is very much beneficial in such type of life-threatening disorders like CHF, hypertension, renal failure, Liver cirrhosis and often pregnancy toxaemia [1]. Naturally occurring diuretics include caffeine, alcohol and wine, which inhibit Na^+ reabsorption and inhibit secretion of ADH but have the adverse effect including impotence, fatigue, weakness etc [2,3]. Hence search for a new diuretic agent that retains therapeutic efficacy and devoid of above adverse effects.

Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system. Among the several plants, *Crataeva nurvala, Dolichos biflorus, Tribulus terrestris, Dendrophthoe falcata, Boerhaavia diffusa, Saccharum officinarum, Butea frondosa, Boerhaavia repens, Boerhaavia rependa, Homonia riparia, Centratherum anthelmintivum*, *Vitis venifera* and *Duranta repens* have shown excellent diuretic activity [4-13]. Here a Polyherbal formulation was prepared, which containing the seeds of *Centratherum anthelmintivum*, *Vitis venifera* and *Duranta repen* in the ratio of 1:1:1. The present study was planned to evaluate diuretic activity of prepared Polyherbal formulation in healthy albino rats.

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2. Materials and methods

2.1. Plant materials

The plant seeds were collected from local market of Bhopal, MP, India and authenticated at the Department of Botany, The City College, Jiwaji University Gwalior, Madhya Pradesh, India; where the plant voucher specimens number were deposited.

2.2. Preparation of Polyherbal formulation

The seed of *Vitis venifera*, *Duranta repens* and *Centratherum anthelminticum* were selected. The selected seeds were allowed to air dried and triturated to make powder form individually. The prepared powders were

mixed with the ratio of 1:1:1 and made as suspension in 8% gum acacia aqueous solution to form a Polyherbal formulation. To evaluate the dose related diuretic activity, we had chosen three doses of Polyherbal formulation Viz. 100 mg, 200 mg and 400 mg/kg of body weight as PHF-I, II and III.

2.3. Experimental animals

Healthy adult male albino rats of Wistar strain weighing between 150 and 200 g were used. Animals were housed in standard environment conditions (temperature 28-30 ℃, photoperiod; approximately 12 h natural light per day; relative humidity: 50-55%) and maintained with free access to water and ad libitum standard laboratory diet. The experimental protocol was approved by Institutional Animal Ethical Committee as per the CPCSEA guidelines, Ministry of Social Justice and Empowerment, Government of India.

2.4. Treatment schedule

Male albino rats were divided into six groups, of six animals each, in laboratory cage. The animals were fasted overnight, with free access to tap water only. Group I served as control and administered with vehicle, groups II, III and IV were given single dose of 100 mg, 200 mg and 400 mg/kg of Polyherbal formulation (as PHF-I, II and III) orally respectively, while the animals in group V as standard, were treated with furosemide in the dose of 10 mg/kg, dissolved in normal saline. All the doses were administered intragastrically by gastric canula. Food and water were withdrawn 8 h before the administration of drug [14, 15]. Immediately after dosing, all the animals were placed individually in metabolic cages and urine passed by the animals over a period of 24 h was collected in a jar. Total urine output, Urinary Osmolarity, pH was determined.

2.5. Analytical Estimation of electrolytes

Electrolyte (Na^+, K^+ and Cl^-) concentrations were estimated and expressed as mmol/L. Analytical Estimation was performed according to the procedure provided along with electrolyte estimation-standard-reagents kit (Crest Biosystems, India). The Na^+, K^+, Cl^- concentrations were measured UV spectrophotometry. Sum of Na+ and K+ was calculated as parameter for saluretic activity and the ratio of Na^+/K^+ was calculated for natriuretic activity [16]. The Saluretic index, natriuretic index and diuretic index was determined by following formulae. Saluretic index = saluretic activity in test group/saluretic activity in control group; natriuretic index = natriuretic activity in test group/ natriuretic activity in control group; diuretic index = diuretic activity in test group/ diuretic activity in control group;

2.6. Statistical analysis

Statistical work was done by using SPSS software version 11.5. Values are expressed as mean±SD. The statistical evaluation was carried out by analysis of variance (ANOVA) with post hoc test Tukey alpha (0.05). Significance was set at $P < 0.05$.

3. Results

3.1. Acute Toxicity Evaluation

The acute toxicity studies revealed that the Polyherbal formulation was nontoxic in nature. There was no lethality or any toxic reactions found at any of the doses selected until the end of the study period.

3.2. Total urine output, Urine Osmolarity and pH

The PHF II and III induced significant ($P < 0.001$) increase in urine volume, as compared to control group and producing dose dependent urine output, but maximum efficacy was obtained at 400 mg/kg dose, which was very much closer to the reference drug as furosemide. The Diuretic index of PHF-III was almost equal to std. drug as shown in Table 1. All the doses of PHF caused a gradually reduction on urine osmolarity also. At dose of 400 mg/kg, PHF showed high urinary pH which was closer to control group and std. group as shown in Table 1.

3.3. Effect on urinary electrolyte excretion

The effect of once daily dose of furosemide and different doses of PHF on electrolyte (Na^+, K^+ and Cl^-) excretion in the 24 hr urine is summarized in Table 2. All doses of PHF were enhanced the excretion of the electrolytes Na^+ ($P < 0.05$ or $P < 0.01$), K^+ ($P < 0.05$ or $P < 0.01$) and Cl^- ($P < 0.05$ or $P < 0.01$), which were greater than produced by furosemide, especially that of K^+. All the doses of PHF were significantly enhanced the excretion of the electrolytes, which was greater than those produced by furosemide.

3.4. Diuretic, Saluretic and Natruretic index

After oral administration of the doses of PHF, Furosamide and control groups, the saluretic and natriuretic index were calculated in fig 1. The saluretic, natriuretic and diuretic index of PHF were showed dose dependent manner and as closer to the standard group.

Table 1

Effects of oral administration of Polyherbal formulations (PHF-I, II, III) and Furosemide on urinary output, Osmolarity and pH.

Groups	Dose (mg/ kg p.o.)	Urinary output (ml)	pH	Urinary Osmolarity	Diuretic index
Control	–	25.39±2.06	6.3±0.3	296±0.04	1.00
Furosamide	10	36.43±2.98 [a]	6.4±0.2	305±0.06	1.43
PHF-I	100	27.8±3.21 ns	6.6±0.09	307±0.03	1.09
PHF-II	200	30.43±1.26 [a]	6.3±0.5	301±0.04	1.19
PHF-III	400	35.18±1.74 [b]	6.2±0.12	297±0.06	1.38

Values are expressed as Mean±SD, n= 6; [a] $P < 0.01$, [b] $P < 0.001$ compared with control.

Table 2

Effect of oral administration of Polyherbal formulations (PHF-I, II, III) and Furosemide on urinary electrolyte excretion.

Groups	Na^+ (mmol/ L)	K^+ (mmol/ L)	Cl^- (mmol/ L)	Saluretic index	Natruretic index
Control	89.67±4.32	74.37 ±1.21	121.45±2.98	1.00	1.00
Furosamide	113.18 ±6.21 [b]	83 ±2.01 [b]	166.71±3.76 [b]	1.32	1.13
PHF-I	96.32 ±2.81 [a]	75.61 ±1.43 ns	134.79±4.65 [b]	1.09	1.05
PHF-II	103.41 ±5.89 [b]	79.98 ±2.38 [b]	149.53±3.43 [b]	1.19	1.07
PHF-III	109.98 ±6.93 [b]	85.46 ±1.89 [b]	155.34±4.21 [b]	1.25	1.06

Values are expressed as Mean±SD, n=6; [a] $P < 0.05$, [b] $P < 0.001$ compared with control.

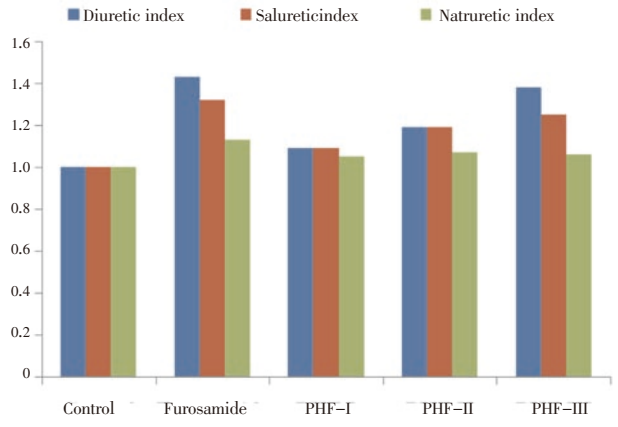

Fig 1: The Diuretic, Saluretic and Natruretic index of Polyherbal formulations (PHF-I, II, III) and Furosemide.

4. Discussions

The seed extract of *C. anthelminticum* contains flavonoids, phenolic compounds, saponins, sterols, tannins, proteins, carbohydrate etc [17]. *Duranta repen* contains flavonoids [18], Steroids, Saponins, lignans, Coumarin derivatives etc where as *Vitis venifera* contains polyphenols [19], procyanidins, anthocyanins, Flavanoids, hydroxylcinnamic acid derivatives, triterpenes, sterols, tannins, polysaccharides, monosaccharide's and nonalkaloid nitrogen containing compounds [20]. So the prepared Polyherbal formulation contains the mixture of saponins, flavonoids, steroids, terpenoids etc. The presence of saponin might be responsible for salurtic activity by modulating renal sodium excretion [21-3]. Presence of phenolic compounds, organic acids and polar compounds such as flavonoids and steroidal saponins are responsible for diuretic activity [24-26].

This study confirms that the Polyherbal formulation at different range of doses given orally in a single dose have diuretic potential. The dose related response was observed and induces high Na^+, K^+, Cl^- excretion with alkalinization of urine with increasing urinary output. These observations suggest that the prepared PHF is acting as a loop diuretic due to significant increase of excretion of water, sodium, potassium and chlorine etc. As the dose increased, slight decrease in urinary pH was also observed.

The diuretic activity of Polyherbal formulation may be due to the presence of saponins, flavonoids, steroids etc. Further research is under way in our laboratory to elucidate the exact mechanism of this diuretic effect and particularly the structure and role of the compounds which are responsible for diuretic activity. In the near future we will be able to identify these principal components using spectroscopic methods, and confirm their potential for inducing diuretic activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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