Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2016, 6(1):4-8

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Comparative study of diuretic potential of aqueous and alcoholic extracts of *Fumaria indica* in experimental rodents

Ramesh Kumar Gupta[1,2*], Sudhansu Ranjan Swain[1], Jagannath Sahoo[2], Padala Narasimha Murthy[3], Pramod Kumar Sharma[4] and Umakant Bajaj[5]

[1]*Sherwood College of Pharmacy, Barabanki, Uttar Pradesh, India*
[2]*Department of Pharmaceutics, S. R. M. S. College of Engineering and Technology, Bareilly Uttar Pradesh, India*
[3]*Department of Pharmaceutics, Royal College of Pharmacy and Health Sciences, Berhampur, Orissa, India*
[4]*School of Medical & Allied Sciences, Galgotias University, G.B. Nagar, Uttar Pradesh, India*
[5]*KIET School of Pharmacy, Ghaziabad, Uttar Pradesh, India*

ABSTRACT

Fumaria indica Linn. (Syn: Fumaria parviflora, Fumariaceae), commonly known as fumitory, that has been traditionally used for a number of ailments. The plant is indigenously used as anthelmintic, antidyspeptic, blood purifier, cholagogue, diaphoretic, diuretic, laxative, stomachic, hepatoprotective and anti-inflammatory. The aim of present study to evaluate the comparative study of diuretic activity of Fumaria indica extract of different fractions. The diuretic activity of extracts of Fumaria indica was examined by treating different groups of wistar albino rats with single (200 mg/kg) oral doses of alcoholic and aqueous extract/fractions. Furosemide (10 mg/kg) was used as positive control in the study. Out of the different fractions and extract, the ethanolic fraction (200 mg/kg) significantly increased the urine output ($p< 0.001$) and urinary electrolyte (Na^+, $^{K+}$ and Cl^-). The pattern of diuresis induced by the ethanolic fraction was almost similar to that produced by the furosemide. The results of this study strongly indicate the ethanolic fraction of Fumaria indica showed the grater diuretic effect of which may be attributed to its diuretic potential, and there by scientifically support its traditional use.

Key words: *Fumaria indica*, Diuretics, Electrolytes, Furosemide, Terpenoid.

INTRODUCTION

Diuretics are capable of increasing the flow of urine and are useful in the treatment of diseases related with the retention of fluids [1]. Clinically useful diuretics also increase the rate of excretion of Na^+ (natriuresis) and an accompanying anion, usually Cl^-. Most clinical applications of diuretics aim to reduce extracellular fluid volume (oedema) by decreasing total body NaCl content [2]. Drug-induced diuresis is helpful in many life-threatening conditions such as congestive cardiac failure (CCF), nephritic syndrome, cirrhosis, renal failure, toxemia of pregnancy, premenstrual tension, and hypertension [3]. The study of plant species with diuretic effects is still a fruitful research in search of new diuretics.

Herbal and natural products of folk medicine have been used forcenturies in every culture throughout the world. Sci entist's andmedical professionals have shown increased interest in this field as they recognize the true health benefit s of these remedies. Diuretics that enhance the rate of urine flow and sodium excretion are used to maintain the volume and composition of body fluids in a variety of clinical situations. *Fumaria indica* Linn. (Syn: *Fumaria parviflora*, Fumariaceae) commonly known as fumitory, is an annual herb, which grow wild in plains of India and Pakistan [4]. In traditional medicine the plant is reputed for its anthelmintic, antidyspeptic, blood purifier, cholagogue, diaphoretic, diuretic, laxative, stomachic, sedative, tonic property [5], abdominal cramps [6], hepatoprotective [7], anti-inflammatory and anti-nociceptive activities [8] and psychopharmacological activity profile of one such extract has been reported [9]. No systematic studies have been reported for its diuretic activity. Therefore, an attempt has been made to evaluate comparative study of diuretic potential of *Fumaria indica*.

MATERIALS AND METHODS

Drugs and Chemicals

All the chemicals used were of analytical grade and procured from (Lasilix, Pharma 5, and Morocco), USA and Qualigens fine Mumbai, India. Furosemide a high-ceiling loop diuretic was used as the reference drug.

Preparation of plant extract

The fresh plant sample of *F. indica* was collected from local market of Lucknow, India in July 2014. The plant materials were cleaned, shade drying, and coarsely ground. The powdered material was soaked in 80% aqueous ethanol for three days with occasional shaking. It was filtered through a muslin cloth and then trough a filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on a rotary evaporator under reduced pressure to a thick, semi-solid mass of dark brown colour; i.e. the crude extract. The same procedure was observed in order to obtain the water and methanol extracts. The yield was 10.4% (w/w), 12.2% (w/w) and 10.9% (w/w) for the methanol, water and ethanol extracts, respectively. The extract obtained was further subjected to Pharmacological investigation.

Animals

Wistar rats weighing (150-250 g) of either sex were procured from animal house of S.R.M.S. Bareilly, Uttar Pradesh. They were kept in departmental animal house in well cross ventilated room at 22 ± 2 °C with light and dark cycles of 12 h for 1 week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18-24 h before the experiment though water was given *ad libitum*. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India (Reg. No. 1029S/06/CPCSEA).

Acute toxicity and gross behavioural study

Acute oral toxicity of FI was evaluated in Swiss strain albino mice of either sex (20–25 g), as per OECD guideline. Forty animals were equally divided into four groups. The extract was administered in 0.3% carboxy-methyl cellulose (CMC) suspension at doses of 1, 2.5 and 5 g/kg, whereas the control group received the CMC suspension only. Mice were closely observed for the initial 4 h after the administrations, and then once daily during the following days. The behavioural changes closely observed for were: hyperactivity, ataxia, convulsions, salivation, diarrhea, lethargy, sleep and coma. Total observation period for eventual mortality was 14 days [10].

Evaluation of diuretic activity

The screening was performed according to the method described by Lipschitz et al. [11]. Male healthy wistar albino rats (150-170 g) were divided into different groups of 5 animals each and kept in standardized environmental conditions. Animals were deprived of food 18 hr before the experiment. The first group of animals, serving as control, received normal saline (25 ml/kg body weight, p.o.); the second group received furosemide (10 mg/kg, p.o.) and 3rd to 5th groups received aqueous, methanolic and ethanolic fractions of extract of *F.indica* (200 mg/kg each), in normal saline. Immediately after administration, the animals were placed in metabolic cages, specially designed to separate urine and faeces, kept at 20±1 °C. The volume of urine collected was measured at the end of 5 hr and the total urine volume, and concentrations of Na^+, K^+ and Cl^- in the urine were determined.

Analytical procedures

Determination of Na^+ and K^+ in urine sample

Na^+ and K^+ concentrations were determined by flame photometer (Model: PFP7/C Company: Jenway, England). The instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+.

Determination of Cl^- in urine sample

In the urine sample Cl^- concentration was estimated by (Mohr Method) titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator[12].

Preparation of 5% K_2CrO_4 indicator

1.0g of K_2CrO_4 was dissolved in20 mL of distilled water.

Preparation of standard $AgNO_3$ solution

9.0 g of $AgNO_3$ was weighed out, transferred to a 500mL volumetric flask and made up to volume with distilled water. The resulting solution was approximately 0.1 M. This solution was standardized against NaCl. Reagent-grade NaCl was dried overnight and cooled to room temperature. For different groups NaCl were weighed into Erlenmeyer flasks and dissolved in about 100mL of distilled water. In order to adjust the Ph of the solution, small quantities of $NaHCO_3$ were added until effervescence ceased. About 2 mL of K_2CrO_4 was added and solution was titrated to the first permanent appearance of red $Ag_2Cr_2O_4$. **Table.2**

Determination of Cl^- in urine sample

Urine sample of all the groups were treated at 110° C for 1 hour and cooled in desiccators. Individual sample were weighed into 250 mL Erlenmeyer flasks and dissolved in about 100mL of distilled water. Small quantities of NaHCO3 were added until effervescence ceased. About 2 mL of K_2CrO_4 was introduced and solution was titrated to the first permanent appearance of red $Ag_2Cr_2O_4$. An indicator blank was determined by suspending a small amount of chloride free $CaCO_3$ in 100 mL of distilled water containing 2 mL of $K2CrO_7$. **Table.3**

Statistical analysis

The values were represented as mean ± S.E.M. for five rats. Analysis of variance (ANOVA) test was followed by individual comparison by Newman-Keuls test using Prism Pad software (Version 3.0) for the determination of level of significance. The values of $p<0.05$ was considered statistically significant.

RESULTS

Acute toxicity and gross behavioural study

After the administration of FI extract animal did not showed abnormal behaviour. Only mild sedation observed, for initial 4 h after drug administration. No mortality was recorded during 14 days after treatment with FI extract.

Diuretic activity

In **Table 1**, the total urine volumes over 24 h were measured for the extracts, standard and control. The ethanolic extract excreted more than two fold the volume of urine as compared to control. Among the extracts, ethanol gave higher urine output followed by methanol and the least in aqueous extract. Similarly, ethanol showed more K^+ lost as compared to other extracts. The excretion of Na^+, K^+ and Cl^- ions were lower than in the control group. The effects of various extract of *F.indica* were studied on animals. Furosemide treated animals significantly ($p<0.001$) increased the urinary output (by 386.55%) and electrolyte excretion of Na^+ (by 163.68%), K^+ (by 154.46%) and Cl^- (by 187.18%) as compared to control. Ethanolic fraction of *F.indica extract* significantly ($p<0.001$) increased the urinary output (by 266.55%) and electrolytic excretion of Na^+ (by 138.35%) and K+ (by 150.83%) and Cl^- (by 175.41%) as compared to control but the methanolic fraction of *F.indica* showed the less significant effect in urinary output (by 211.03%) and electrolytic excretion of Na^+ (by 120.32%), K^+ (by 133.91.%) and Cl^- (by 131.78%) as compared to control group. While the water fraction of *F.indica extract* showed the least significant effect in urinary output (by 191.72%) and electrolytic excretion of Na^+ (by 114.15%), K^+ (by 128.0.%) and Cl^- (by 111.23%) as compared to control group. The observed Na^+/K^+ ratio for frusemide, ethanolic extract, methanolic and water fraction were 1.66, 1.44, 1.41, 1.39 respectively, as compared to 1.57 for control.

DISCUSSION

As diuretics are employed clinically in the treatment of oedema, it would appear to be most important to demonstrate effectiveness in the presence of electrolyte and water[13]. In this study, pharmacological evaluation of diuretic action of alcoholic extracts of *F.indica* were evaluated using furosemide as standard drug (a high-ceiling loop diuretic) under controlled laboratory conditions. The diuretic activities of the extracts were significant when as compared to normal saline. Diuresis has two components: increase in urine volume (water excretion) and a net loss of solutes (i.e. electrolytes) in the urine[14]. All the extracts cause increase urine volume elimination and increase in Na^+, K^+ and Cl^- excretion as compared to normal saline. These processes result from suppression of renal tubular reabsorption of water and electrolytes into the blood stream. The reference drug, furosemide, increases urine output and urinary excretion of sodium by inhibiting $Na^+/K^+/2Cl^-$ symporter (co-transporter system) in the thick ascending limb of the Loop of Henley[14], while the thiazide diuretics inhibit the Na^+/Cl^- symporter (co-transporter system) in the distal convoluted tubule, by competing for the Cl^- binding site, and increasing the excretion of Na^+ and Cl^-. However, there were significant differences between the diuretic activity of the ethanol, methanol and water extracts **(Table 1).** The extracts possibly act by the synergistic action mechanism of the $[HCO_3^-/Cl^-]$, $[HCO_3^+/H^+]$[15] exchangers and the $[N^+/H^+]$ anti-porter, to cause diuresis. In present study aqueous, methanolic and ethanolic extracts showed elevated levels of K^+ in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. Active principles such as flavonoids, saponins and terpenoids are known to be responsible for diuretic activity[16]. The ethanolic fraction induced diuresis was strong and accompanied with high natriuresis, chloruresis and kaliuresis. The increase in the ratio of concentration of excreted sodium and potassium ions indicates that the extract increases sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser hyperkalemic side effect [17]. In present study was found to be ethanolic fraction showed the grater Na^+/K^+ ratio followed by methanolic and water fraction, but the Na^+/K^+ ratio was more than that of frusemide, indicating the weak kaliuresis or K^+ saving property of alcoholic extract [18]. The above results raise the possibility of existence of diuretic activity by inhibiting tubular reabsorption of water and Na^+.

Table 1. Diuretic activity of Ethanolic extract and its fractions of *Fumeria indica* linn

Group	Dose	Total urine output (ml)	Electrolytes concentration (mmol/L)			
			Na^+	K^+	Cl^-	Na^+/K^+
Control	25 ml/kg	2.9 ± 0.34	72.32 ± 2.1	46.03 ± 2.4	11.39 ± 0.82	1.57
Furosemide	10 ml/kg	11.21 ±0.83†	118.38± 3.4†	71.10 ±4.8†	21.32 ± 1.2†	1.66
Water Ext.	200 mg/kg	5.56 ± 0.51^{a}	82.56 ±3.1^{a}	58.92 ±2.6^{a}	12.67 ± 0.9^{n}	1.39
Methanol Ext.	200 mg/kg	6.12 ± 0.43^{b}	87.02 ± 2.8^{b}	61.64 ±2.8^{b}	15.01 ±0.92^{b}	1.41
Ethanol Ext.	200mg/kg	7.73 ± 0.68^{c}	100.06± 3.3^{c}	69.43 ±3.2^{c}	19.98 ± 1.1^{c}	1.44

Values are mean ±S.E.M. of 5 rats in each group
n: non significant
P values: †<0.001 compared with respective control group I
P values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 compared with control group I.

Determination of Chloride by the Mohr Method

Table 2.Data regarding Standardization of $AgNO_3$:

Group	*Sample, g NaCl*	*Volume of AgNO3 used, mL*	*Concentration of AgNO3, M*	*mmole of $AgNO_3$*
Blank	-	***0.3***±.01	-	-
Control	0.2± 0.01	42.90±3.1	0.082±0.01	3.42±1.1
Furosemide	0.29±0.01†	54.20±4.3†	0.09±0.01†	4.96±1.2†
Water Ext.	0.25±0.02^{a}	46.20±3.2^{a}	0.08±0.01^{a}	3.93±1.3^{n}
Methanol Ext.	0.27±0.01^{b}	47.80±3.8^{b}	0.09±0.02^{b}	4.27±1.6^{b}
Ethanol Ext.	0.25±0.02^{c}	51.24±4.5^{c}	0.09±0.01^{c}	4.62±1.8^{c}

Values are mean ±S.E.M. of 5 rats in each group
n: non significant
P values: †<0.001 compared with respective control group I
P values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 compared with control group I.

Mmoles of AgNO3 = (g NaCl / 58.44g/mole) X (1000 mmoles NaCl / 1 mole NaCl)

Table 3: Data regarding Determination of Chloride in urine sample

Group	Wt. of urine sample	*Volume of AgNO3, mL*	Mmole of Cl^-
Blank	-	-	-
Control	2.3±1.0	138.90±5.2	11.39 ± 0.82
Furosemide	9.57±1.2†	236.88±3.2†	21.32 ± 1.2†
Water Ext.	4.7±1.0^{a}	158.37±4.3^{a}	12.67 ± 0.9^{n}
Methanol Ext.	6.6±1.1^{b}	169.77±3.8^{b}	15.01 ±0.92^{b}
Ethanol Ext.	8.14±1.3^{c}	222.0±5.1^{c}	19.98 ± 1.1^{c}

Values are mean ±S.E.M. of 5 rats in each group
n: non significant
P values: †<0.001 compared with respective control group I
P values: ^{a}<0.05, ^{b}<0.01, ^{c}<0.001 compared with control group I.

mmoles of Cl^- = M_{AgNO3} x $V_{AgNO\ 3}$

CONCLUSION

In present study was found to be ethanolic fraction of *Fumaria indica* showed the grater diuretic activity as compared to methanolic and water fraction, obtained results provide a quantitative basis to explain the traditional folkloric use of *F.indica* as a diuretic agent. Thus we can say that the ethanolic fraction *Fumaria indica* used for the treatment of renal and hypertension disease. The extract has diuretic effect supporting the ethno-pharmacological use as diuretics. This effect may be explored in the use of the plant in the management of some cardiovascular diseases.

Acknowledgement
The authors are thankful to the Director of S. R. M. S. College of Engineering and Technology, Bareilly, Uttar Pradesh for providing necessary facilities throughout this research. The entire grants were provided by S. R. M. S. College of Engineering and Technology, Bareilly, (Grant No-SRMS/PH-0B).

REFERENCES

[1] Radhika B, Begum N, Srisailam K, Reddy VM. *IJNPR*, **2010,** 1(3), 353-355.
[2] Swapna B.M, Vaibhav A.J, Minal S.P, Chittam K.P, Wagh R.D. *IJDDHR*, **2011,** 1, 20-21.
[3] Sharma J, Gairola S, Sharma Y.P, Gaur R.D. Ethnomedicinal plants used to treat skin diseases by Tharu community of district Udham Singh Nagar, Uttarakhand, India. *J Ethnopharmacol*, **2014**, 158,140-206.
[4] Singh G.K, Rai G, Chatterjee S.S, Kumar V. *Chin Med*, **2012**, 3, 49-60.
[5] Rehman N, Mehmood M.H, Al-Rehaily A.J, Mothana R.A.A, Gilani A.H. *BMC CAM*, **2011,** 12, 2-8.
[6] Patil V.V, Bhangale S.C, Patil V.R. *Int J Pharm Pharm Sci*, **2010,** 2, 97-99.
[7] Jameel M, Islamuddin M, Ali A, Afrin F, Ali M. *BMC CAM*, **2014,** 14,1-9.
[8] Rao C.V, Verma A.R, Vijay K.M. *Acta Pharm*, **2007,** 57, 491-498.
[9] Singh G.K, Kumar V. *Electronic Journal of Pharmacology & Therapy*, **2010,** 3, 19-28.
[10] Singh G.K, Kumar V. *J Ethnopharmacol*, **2011**, 134, 992-995.
[11] Sayana S.B, Christina C, Medabala T, Patil P.S. *Asian J Pharm Clin Res*, **2014, 7,** 157-159.
[12] Alam M.R, Raton M, Hassan M.M, Kadir M.F, Islam S.M.A, Haque HA. *J Appl Pharm Sci*, **2012,** 2(10), 86-89.
[13] Mekonnen T, Urga K, Engida E. *J Ethnopharmacol*, **2010,** 127, 433-439.
[14] Lahlou S, Tahraoui, A, Israili Z, Lyoussi B. *J Ethnopharmacol*, **2007,** 110, 458-463.
[15] Jain S, Argal A. *JNPPR*, **2012,** 2(3), 368-371.
[16] Muthumani P, Meeral R, Devi1 P, George S, Shiek Arabath, S.A.M.S, Jeyasundari K, Babmanaban R. *IJABPT*, **2010,** 1, 1285-1292.
[17] Sandeep R.K, Vishvesh A.A, Sachin S.T, Shrinivas K.M. *Int J ChemTech Res*, **2009,** 1, 149-152.
[18] Yadav R, Yadav N, Kharya M.D, Savadi R. *Int J Pharm Pharm Sci*, **2011**, 3, 245-247.