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DIURETIC ACTIVITY OF THE EXTRACTS OF *LIMONIA ACIDISSIMA* IN RATS

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ABSTRACT

Limonia acidissima (Rutaceae) is used traditionally for the treatment of constipation, vomiting, diabetic, cardiotonic and as diuretic. In the present study, the diuretic activity of methanolic extracts obtained through the Microwave assisted extraction (MAE) and Bath Sonicator extraction (BSE) of Limonia acidissima was investigated. The diuretic activity of the different extracts (obtained through the MAE and BSE) at a dose of 200mg/kg was assessed orally in rats using furosemide as a standard drug. The extract (obtained through the BSE) produced a significant increase ($P<0.001$) in urine output at the same dose. Urinary electrolyte extraction was also affected by the extract (obtained through the MAE) increase the urinary excreaction of Sodium, potassium and chloride ions. These findings support the traditional uses of Limonia acidissima leaves as diuretic agents because the extract obtained through BSE that is similar to the traditional extraction (maceration) without heating.

Keywords: Limonia acidissima leaves (Rutaceae), Katbel (Hindi), diuretic activity.

INTRODUCTION

Limonia acidissima leave (Rutaceae) is distributed throughout India. In Sanskrit, it is known as 'Kapitthah' and in Hindi it is well known as 'Katbel'. The decoction of the leaves used in the treatment of constipation, vomiting, cardiotonic, diuretic[1]. The leaves contain coumarin, triterpinoids and steroids[2]. The steam distillates were act as antimicrobial, insecticidal and antifungal activity[3]. As the plant is reported to have various medicinal uses, authors have attempted to study the pharmacognostic study of the leaves. Considering the various uses of *Limonia acidissima* in Indian folk medicine as a diuretic, this study aimed to evaluate the diuretic activity of the extracts (obtained through the MAE and BSE) of the plant.

EXPERIMENTAL

Materials

Plant material : *Limonia acidissima* (Rutaceae) leaves were collected in September 2006 from Mandsaur and identified by Prof. H. S. Chatree. A voucher specimen (No BRNCP/L/001/2006) was deposited in the Herbarium of Department of Pharmacognosy and Phytochemistry, B.R. Nahata College of Pharmacy, Mandsaur M. P. India. The leaves were separated from the stems of *Limonia acidissima*. The material was shade dried and powdered. Powdered material was sieved with sieve number 20 and was packed separately in airtight container for the extraction.

Bath Sonication Extraction (BSE) : Bath Sonication Extraction (BSE) was conducted in an ultrasonic bath. 5.0 g of exactly weighted drug powder were placed into a 100 ml volumetric flask up to volume with 50% (v/v) methanol and sonicated in a sonication bath for 20min[4]. Repeating the same procedure the

extract obtained was evaporated to dryness using a rotary evaporator at 60^0C. The percentage yield of the extract was calculated (yield:5.72%).

Microwave-assisted extraction (MAE) :Microwave-assisted extraction (MAE) was performed on a Microwave oven apparatus using a closed-vessel system and setting the microwave power at 100W. 0.2 g of powdered drug were placed into the extraction vessel, with 4ml of 50% (v/v) methanol and subjected to different temperatures for different time of irradiation. After the extraction time had elapsed, the vessels were allowed to cool to room temperature before opening[4]**.** Repeating the same procedure the extract obtained was evaporated to dryness using a rotary evaporator at 60^0C. The percentage yield of the extract was calculated (yield: 8.26%).
The aqueous MeOH extract of the *Limonia acidissima* were screened as per the procedures described in Pharmacognosy[5].

Pharmacological activity

Animals

Male albino rats (in housed bred) with weight range of 250-350g were used in all experiments. They were kept six per cage in the animal house of B.R. Nahata College of Pharmacy, Mandsaur, with 12h light dark cycle. The animals were fed with a balanced pellet diet and fresh tap water was continuously accessible. All experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care. Proposal number (65/Fac/07/IAEC/BRNCP/07-08/Mandsaur) was granted on 03/05/2007 by Institutional Animal Ethical Committee (IAEC) to perform experiments.

Acute toxicity study

Acute toxicity study was done according to OECD (Organization for Economic Co-operation and Development) Guideline 420.fixed dose method, with starting dose of 2000mg/kg body weight was adopted. Starting dose of 2000mg/kg (per oral) of each 50%v/v MeOH extracts(obtained through the MAE and BSE) was given to 5 animals (albino rats), animals were kept for observation of behavioral change and death upto 72h.

Diuretic test

The method of Lipschitz was employed for the assessment of diuretic activity[6]. Four groups (each containing six rats) were fasted and deprived of water for 18h prior to the experiments. On the day of experiment animals were given normal saline orally, (25ml/kg of body weight) in which 50%v/v MeOH extracts (obtained through the MAE and BSE) were dissolved. Control animals received saline only. Immediately after dosing, the rats were placed in the metabolic cages (6 in each cage) specially designed to separate urine and faeces. Animals were kept at room temperature of 25 ± 0.5^0C throughout the experiment. The urine was collected in measuring cylinder upto 5hrs after dosing. During this period, food or water was not provied to the animals. The total volume of urine collected was measured for both control and treated groups. The parameters taken for individual rat were body weight (before and after test period), total urine volume, urine sodium and potassium concentrations were measured by flame photometry and Chlorine concentration was estimated by titration with silver nitrate solution (N/50) using 1 drop of 5% potassium chromate solution as indicator. Diuretic potential of 50%v/v MeOH extracts (obtained through the MAE and BSE) were compared with standard diuretic agents like Furosemide (10mg/kg body weight).

Statistical Analysis

Results are reported as mean ± SD, the test of significance (p<0.001) was statistically analyzed using student

RESULTS AND DISCUSSION

Acute toxicity study of (50%v/v) MeOH extracts (obtained through the MAE and BSE) of *Limonia acidissima* was done according to OECD guideline 420 by fixed dose method. Starting dose of 2000mg/kg body weight was given to the albino rats of both sexes and animals were kept under observation and no mortality was observed even after 72hrs from the treatment time. So 1/10 dose (i.e. 200mg/kg) was selected as safe dose for (50%v/v) MeOH extracts (obtained through the MAE and BSE) of *Limonia acidissima*.

Present study shows that the (50%v/v) MeOH extracts obtained through MAE *Limonia acidissima* leaves possess potent diuretic activity. The diuretic potency was comparable to that of the standard drug Furosemide. By definition, diuretics are the drugs that increase the rate of urine flow; however, clinically useful diuretics also increase the rate of extraction of Na^+ (natriuresis) and an accompanying anion, usually Cl^-. NaCl in the body is the major determinant of extracellular fluid volume and most clinical applications of diuretics are directed towards reducing extracellular fluids volume by decreasing the body NaCl content. The (50%v/v) MeOH extract showed a significant water excretor, a notable increase in the urine volume (4.2ml) confirming its use as diuretic. Concerning electrolyte excreaction, we observed something different, although an increase in ionic excretion is produced with (50%v/v) MeOH extract obtained by MAE but not with the (50%v/v) MeOH extract. Urine volume, cation and anion excreation were increased. MAE and BSE (50%v/v) MeOH extracts achieved the Na^+/ K^+ ratio of 2.047 and 2.184, respectively. The normal values for Na^+/ K^+ ratio is reported to be 2.05 –2.83 (table 1). The concentration of aldosterone is found to be dependent on Na^+/ K^+ ratio. If the Na^+/ K^+ ratio falls below the normal in plasma the aldosterone secretion will be decreased and if the ratio rises above the normal value the aldosterone secretion will be increased. Significant increase in Na^+, K^+ and Cl^- ion excreaction was observed in (50%v/v) MeOH extracts (obtained through MAE and BSE) treated animals but it was less than the standard drugs (furosemide) treated group. The diuretic effect of (50%v/v) MeOH extract obtained through the MAE was observed more than the (50%v/v) MeOH extract of BSE. The effect on electrolyte excretion is not more pronounced with regards to K^+ and the extract does not display a pronounced kaliuretic activity. The percentage of saline load excreaction and diuretic action occurs at the dose of 200mg/kg (50%v/v) MeOH extracts (obtained through MAE and BSE), which may indicate that the extracts (obtained through MAE and BSE) could have a mechanism of action may be similar to the low-ceiling diuretic agents. The diuretic activity of the *Limonia acidissima may be due to the presence of* triterpenoids, glycoside, flavonoid, polyphenols and coumarin found in the methanolic extracts (obtained through the MAE and BSE). On the basis of the above results, we can conclude that ***Limonia*** *acidissima* treatment produced a marked diuresis when rats were acutely treated. In our study, no lethality was observed at least for the dose (2000mg/kg body weight) and duration used. The best results of urine out

Treatment	Dose (mg/kg)	No of Rats used	Urine volume (ml)	Electrolyte Excretion			Total chloride μMoles/kg
				Na^+ μmole/kg	K^+ μmole/kg	Na^+/K^+	
Control	Saline 25	6	2.3±0.13	1988±39	905±32	2.196	630±27
(50%v/v) MeOH (BSE)	200	6	4.2±0.81*	3061±31*	1495±521*	2.047	1937±54*
(50%v/v) MeOH (MAE)	200	6	3.8±0.41	2896±75*	1326±22*	2.184	2366±98*
Furosemide	100	6	5.7±0.85	3431±58*	1962±315*	2.748	2893±104*

Table-1: Diuretic activities of extracts of *Limonia acidissima* leaves on rats.

*The values are expressed of the mean standard errors. * P<0.001 vs. Control. Student's "t " test.*

put observed with the methanolic extract obtained through the BSE methods. However, advanced toxicological studies remain to be performed in other animals before its recommendation to the clinical studies.

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