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EVALUATION OF THE DIURETIC EFFECT OF ETHANOL EXTRACT OF *VITEX TRIFOLIA* LEAVES IN ALBINO RATS

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Keywords:

Vitex trifolia, Ethanol extract, Diuretic activity, Natriuretic, Percentage urinary excretion, Carbonic anhydrase

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ABSTRACT: Aims: The study was conducted to evaluate the diuretic effect of ethanol extract of *Vitex trifolia* leaves in albino rats. **Methods:** Normal rats were divided into 4 groups of 6 animal each. Group I (Control) treated with 2% Gum Acacia in distilled water orally, Group II (Standard/F20) treated with Furosemide 20mg/kg b.w. orally. Group III and IV treated with Ethanol extract of *Vitex trifolia* L. leaves at a dose of 300mg/kg b.w. and 600mg/Kg b.w. respectively. Urine collected 5 hrs after the administration of drug. Then the total urine volume was measured. Electrolyte excretion (Na+, K+, Cl-) was estimated for assessment of the various diuretic activity by Sensocare ST 200 plus electrolyte analyzer in Biochemistry Department, JNIMS. **Result:** A Significant increase in urine output was seen with ethanol extract of *Vitex trifolia* treated group of animal when compared to normal control. Diuretic activity also increases in a dose dependent manner. Significant increase in sodium, potassium and chloride was also seen in the group treated with the plant extract at both the doses compared to the normal control. **Conclusion:** The present study supports the traditional use of *Vitex trifolia* as diuretic agent. Further study at molecular and cellular level is needed to establish its safety and efficacy as diuretic agent.

INTRODUCTION: Medicinal plants have been a main source of therapeutic agents since olden times to treat human illness. Large sections of the population in developing countries still rely on traditional medicines. Herbal medicines are primarily used to manage various medical conditions [1]. Such plants should be investigated to better understand their properties, safety and efficacy. The genus *Vitex* (Lamiaceae) contains 270 species with diverse medicinal active constituents and properties.

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These species are predominantly trees and shrubs, found in tropical and subtropical regions. *Vitex trifolia* L. is a shrub which grow up to 6 m in height and found in the regions of Asia, China, India, Indonesia, Sri Lanka, Singapore and Australia. This plant has a rich history in traditional medicine for treating asthma. Most part of the plant such as the fruits, leaf, root, flower and stem reported to have medicinal values [2].

Many plants of the genus *Vitex* are used for their interesting biological activities like antimicrobial, analgesic, anti-inflammatory, antihistaminic, anticancer, *etc* [3]. The leaves of *Vitex trifolia* are used in rheumatic pain, gout, haemorrhoids, as antiviral, as antimicrobial, as mosquito repellent, anti HIV, anti cancer, antipyretic and in asthma [4, 5]. The leaves are also use to relieve pain, removing

bad taste in mouth, cure fever and also to treat hair loss [6]. Diuretics are drugs which cause increase in urinary output of electrolytes and water from the body [7]. The net loss of sodium and water in urine is due to one or more reabsorptive processes occurring at different segments of the nephron. The increased water loss is actually secondary to the increased excretion of sodium chloride. This is achieved either by directly acting on different segments of the nephron or by indirectly modifying the contents of the urinary filtrate [8]. Diuretic agents are important to promote a net loss of excessive accumulated body fluids, salts, toxins and other accumulated metabolic products [9].

Diuretics are prescribed in various conditions like oedema associated with liver cirrhosis, kidney disease, congestive heart failure, hypertension, *etc* [10]. Diuretics are also associated with several adverse effects like electrolyte imbalance, metabolic alterations, *etc*. There is a need to study new plant-based bioactive components that have effective diuretic activity with minimal side effects [11]. There are various studies and reports of diuretic activity *Vitex negundo, Vitex agnus castus*, *etc*. Even though *Vitex trifolia* used as diuretic by local healer, there is no report of study to support it. The present study is carried out to scientifically evaluate and compared the diuretic effect of ethanol extract of the plant *Vitex trifolia* L. leaves in albino rats.

MATERIALS AND METHODS: The present experimental study was conducted in the Department of Pharmacology, Jawaharlal Nehru Institute of Medical Sciences, Imphal, Manipur for a period of 2 years from April 2023 to March 2025. The present study was approved by the Institutional Animal Ethics Committee, RIMS (Registration no: 1596/GO/a/12/CPCSEA) The fresh and disease free leaves of plant *Vitex trifolia* L. were collected from Imphal East Manipur during December, 2022. The collected material was identified and authenticated by Mrs. Yumkham Sanatombi Devi, Guest faculty, Life Science Dept, Manipur University, Canchipur, Imphal. A plant sample was deposited at the Department Herbarium and allocated Acc. No. 001365 of MUH.

Preparation of Extract: The ethanolic extract of *Vitex trifolia* L. leaves obtained following the procedure described by K Anwar *et al* [12] with slight modification. The collected leaves were cleaned with water and air dried in shade for several days. The shade dried leaves powdered using a mixture grinder. 250 gm of dried and powdered leaves mixed with 70% ethyl alcohol and kept at room temperature for 36 hrs with intermittent stirring for the 1st 2 hrs. The mixture was filtered using Whatman paper and the filtrate was concentrated under reduced temperature (50°C bath temperature) and finally dried and was stored in a porcelain jar for further use. Yield obtained was 20 gram.

Experimental Animal: Healthy albino rats of either sex weighing 150 to 200gms procured from animal house of JNIMS, Imphal and kept in the departmental polypropylene cages and acclimatized for 10 days. The rats are fed with standard laboratory diet and water *ad libitum*. 12 hrs dark-light cycle maintained.

Acute Toxicity Study: Acute oral toxicity study for the test extract of the plant carried out using OECD/OCED guideline 425 [13]. The test procedure minimizes the number of animals required to estimate the oral acute toxicity. Healthy young albino rats (200- 250 g) were used for this study. Animals were fasted overnight prior to dosing.

The fasted body weight of each animal were determined, and the dose calculated according to the body weight. The drug was administered in the dose of 2000 mg/kg body weight orally to one animal. This first test animal survived. Next four other animals were administered at the same dose sequentially. Therefore, a total of five animals were tested. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 hrs (with special attention given during the first 4 hrs), and thereafter, for a total of 14 days.

Experimental Design: Evaluation of diuretic effect of ethanol extract of *Vitex trifolia* L. leaves were performed following the procedure described by Lipschitz WL *et al* [14] (1943) with slight modification. Normal rats were divided into 4 groups as given below, each group consisting of 6 rats. The drugs to be given were suspended in 2% gum acacia.

Group I (Control): 2% Gum Acacia in distilled water orally.

Group II (Standard/F20): Furosemide 20mg/kg body weight orally.

Group III (VTEE300): Ethanol extract of *Vitex trifolia* L. leaves at a dose of 300mg/kg body weight orally.

Group IV (VTEE600): Ethanol extract of *Vitex trifolia* L. leaves at a dose of 600mg/kg body weight orally.

All the rats were kept fasted for18 hrs prior to the experiment with water *ad libitum*. 1 hr before starting the experiment, all animals were treated with 10 ml per kg body weight 0.9% NaCl for equal electrolyte balance. The bladder of all the rats were emptied by gentle compression of the pelvic area and pulling of the tail. Soon after feeding of the drugs, all the rats were kept separately in metabolic cage for collection of urine. No food and water were given during the time of experiment. Urine collected 5 hrs after the administration of drug. Then the total urine volume was measured. The percentage urinary excretion was determined by the ratio of total urine output by total liquid administered multiply by 100. Diuretic action was assessed by the ratio of percentage urinary action in the test group to the control group. Diuretic activity was determined by the ratio of diuretic action of the test drug to that of the standard group. Electrolyte excretion (Na^+, K^+, Cl^-) was estimated for assessment of the various diuretic activity by Sensocare ST 200 plus electrolyte analyzer in Biochemistry Department, JNIMS.

Statistical Analysis: For descriptive analysis, mean and standard error used. For analytical statistics, ANOVA (analysis of variance) followed by Dunnett T test for statistical significance. The significance expressed by F-value and P value. P-values of less than 0.05 considered significant.

RESULTS:

Acute Toxicity Test: Acute toxicity test was done according to OECD/OCED Guidelines 425 [13]. The ethanol extract of *Vitex trifolia* L. leaves were found to be safe and no mortality was observed up to a dose of 2000 mg/kg body weight orally after 14 days.

Effect on Urine Volume, Percentage Urinary Excretion, Diuretic Action and Diuretic Activity: The effect of ethanol extract of *Vitex trifolia* leaves on 5 hr urine volume, percentage urinary excretion, diuretic action and diuretic activity in albino rats are shown in **Table 1** and **Fig. 1.**

A Significant increase in urine output was seen with ethanol extract of the plant with 2.95±0.08ml of urine volume for VTEE 300 ($p<0.01$) and 3.45±0.11ml for VTEE 600 ($p<0.001$) when compared to the normal control. Urine output decrease when compared to the standard which is significant only with VTEE 300 ($p<0.05$). Percentage urinary excretion (%UE) increases in a dose dependent manner in both the extract as shown in **Table 1.** %UE of VTEE 300 and VTEE600 is 75.25 and 88.07 respectively which is much higher than the normal control. The plant extract also shows increase in diuretic action in a dose dependent manner compared to the normal control but less than the standard. Diuretic activity also increases in a dose dependent manner as shown in **Table 1**. The diuretic activity of VTEE300 and VTEE600 was 0.76 and 0.89 respectively.

Effect on Electrolyte Excretion: The effect on electrolyte (Na^+, K^+, Cl^-) excretion is shown in **Table 2** and **Fig. 2.** The Na^+ electrolyte excretion in case of ethanol extract of the plant was 78.91±1.50mmol/L and 98.27±1.12mmol/L at 300 mg/kg body weight and 600mg/kg body weight respectively which is much higher than the normal control (48.15±0.46 mmol/L) but less than the standard drug (133.75±2.07 mmol/L) and these have been found to be statistically significant compared to both normal control and the standard.

The K^+ excretion with the ethanolic extract is shown to be increased in a dose dependent manner, 63.22±1.15 mmol/L and 85.84±1.30 mmol/L at VTEE 300 and VTEE 600 respectively which is more than the control but lesser than the standard and it is found to be statistically significant compared to the control ($p<0.001$) in both the doses but is found to be statistically significant compared to the standard ($p<0.001$) only with VTEE300. The chloride excretion increases significantly in a dose dependent manner in both the extract when

compared to the normal control ($p<0.001$). The excretion of Cl^- was found to be 100.35±1.51mmol/L and 119.94±1.17mmol/L at 300mg/kg body weight and 600mg/kg body weight respectively.

The ratio of sodium by potassium was greater than 1 in both the doses. The ratio of $Cl^-/(Na^+ + K^+)$ in a both the doses is less than that of normal control but greater than the standard drug.

TABLE 1: EFFECT OF ETHANOL EXTRACT OF *VITEX TRIFOLIA* LEAVES ON 5 HRS URINE VOLUME IN ALBINO RATS (MEAN±SEM)

Group	Urine volume(ml)	% UE	Diuretic action	Diuretic activity
Control	2.10±0.10	51.36	1	0.37
Standard(F20)	3.87±0.18	97.66	1.84	1
VTEE300	2.95±0.08*#	75.25	1.4	0.76
VTEE600	3.45 ±0.11**	88.07	1.64	0.89

F=22.58 df= 7, 40 p-value<0.001, *p<0.01, **p<0.001 compared to normal control, #p<0.05 compared to standard.

TABLE 2: EFFECT OF ETHANOL EXTRACT OF *VITEX TRIFOLIA* LEAVES ON 5 HRS URINARY ELECTROLYTE EXCRETION IN ALBINO RATS (MEAN±SEM)

Group	Urinary Electrolyte mmol/L			Saluretic Index			Na/K	Cl/Na+K
	Na+	K+	Cl-	Na+	K+	Cl-		
D/W	48.15±0.46	40.27±0.58	70.24±1.18	1	1	1	1.2	0.8
F20	133.75±2.07	90.33±1.53	136.75±1.72	2.78	2.24	1.95	1.48	0.61
VTEE300	78.91±1.5*##	63.22±1.51*##	100.35±1.51*##	1.64	1.57	1.43	1.22	0.71
VTEE600	98.27±1.12*##	85.84±1.30*	119.94±1.17*#	2.04	2.13	1.71	1.15	0.65
F	441.825	234.669	248.847					
dF	7.4	7.4	7.4					
p	<0.001	<0.001	<0.001					

*p<0.001 compared to normal control, #p<0.01, ##p<0.001 compared to the standard drug.

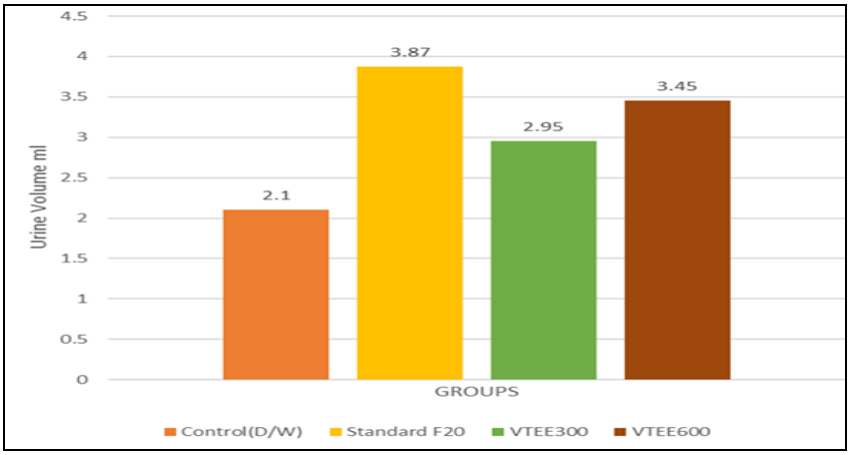

FIG. 1: BAR CHART SHOWING 5 HRS URINE VOLUME IN DIFFERENT GROUPS

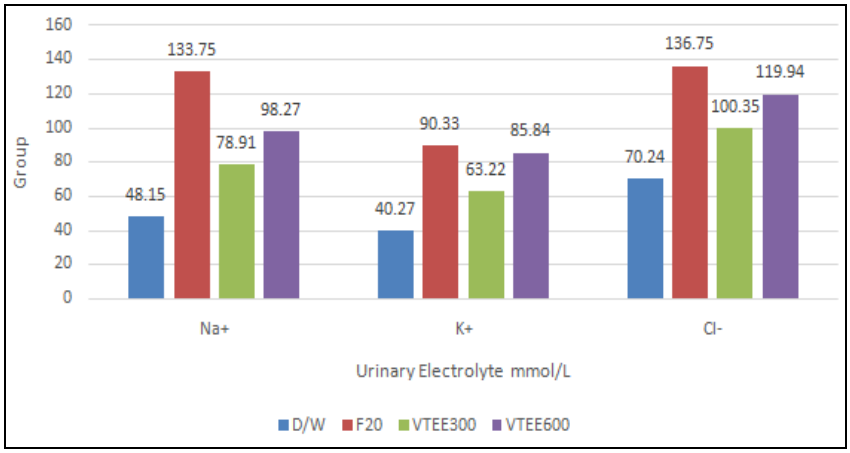

FIG. 2: BAR CHART SHOWING EFFECT ON URINARY ELECTROLYTE EXCRETION

DISCUSSION: Several medicinal plants have been found to improve conditions of volume overload resulting from retention of electrolytes and water with better safety profiles. This study reports the diuretic, natriuretic, saluretic and carbonic anhydrase inhibitory CAI) activity of ethanol extract of *Vitex trifolia* L. leaves in albino rats.

The preliminary phytochemical screening of ethanol extract of *Vitex trifolia* L. have shown it to contained alkaloids, glycosides, flavonoids, saponins, phenols, proteins and carbohydrates [15]. Terpenoids and flavonoids have been identified as the main bioactive compounds in *Vitex trifolia* [2].

With regard to urine output, the ethanol extract of *Vitex trifolia* leaves resulted in an increase in urine excretion in a dose dependent manner. Percentage urinary excretion, diuretic action and diuretic activity was seen to increase with the plant extract in a dose dependent manner compared to normal control but less than the standard drug frusemide 20mg/kg b.w. Diuretic activity is considered good if it is greater than 1.50, moderate if between 1.00 to 1.50, mild if between 0.72 to 0.99 and nil if less than 0.72 [16].

The excretion of all the electrolyte increases with the plant extract in a dose dependent manner. Ratio of Na^+/K^+ in both the doses was greater than 1 but less than 2 which indicates satisfactory natriuresis [17]. This ratio if greater than 2 and 10 indicates, favourable natriuresis and potassium sparing activity respectively.[18] The $Cl^-/(Na^++K^+)$ is used to estimate carbonic anhydrase activity(CAI). If the ratio lies between 1 to 0.8, it excludes CAI. With decreasing ratio, the carbonic anhydrase inhibitory activity can be assumed to be more stronger [19].

The ratio of $Cl^-/(Na^++K^+)$ was 0.71 and 0.65 at a dose of VTEE300 and VTEE600 respectively. This suggest that the plant extract shows carbonic anhydrase inhibitory activity which increases with increasing doses. Na^+/K^+ ratio is greater than 1in both the extract but less than 10 which excludes potassium sparing activity of the plant extract. This suggest that the plant extract might act through the same mechanism as that of loop diuretics. Loop diuretics increase the urinary flow rate and urinary excretion of sodium, potassium and chloride by inhibiting $Na^+/K^+/2Cl^-$ symporter in the thick ascending limb of the loop of Henle stimulating the production of renal prostaglandins and inhibiting carbonic anhydrase enzyme in the proximal convoluted tubule (PCT) [20].

Previous studies revealed that certain phytochemicals compounds had diuretic and natriuretic activities via several mechanisms. Flavonoids are one of the natural antagonist ligands for A1 adenosine receptors, while antagonistic activity to the receptor is known to associate with diuretic activity [21]. The adenosine A1 receptors are responsible for the reabsorption of 60–70% of filtered sodium and water in the PCT. Adenosine A1 receptor antagonists can induce diuresis and Na+ excretion by direct inhibition of Na+ re-absorption in proximal tubules [20]. The diuretic effect of the plant may be due to the presence of alkaloids, flavonoids, glycosides, phenols and the possible mechanism comparable with furosemide. Further studies at molecular level needed to established the exact mechanism of action.

CONCLUSION: The result of this study revealed that the ethanol extract of *Vitex trifolia* leaves given at a graded dose of 300mg/kg and 600mg/kg body weight possessed diuretic activity in albino rat. An increase in urine output as well as electrolytes namely sodium, potassium and chloride was seen in a dose dependent manner compared to normal control but less than that of the standard drug frusemide 20mg/kg body weight. The ratio of sodium by potassium was also greater than 1 which indicates natriuretic property. The ratio of chloride by the sum of sodium and potassium in both the doses was less than 0.8 which indicates CAI activity. This property may be mediated by the presence of active phytochemical constituents such as flavonoids, saponins, phenols, alkaloids, etc. The present study supports the traditional use of *Vitex trifolia* as diuretic agent.

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CONFLICT OF INTEREST: Nil

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