

EVALUATION AND COMPARISON OF DIURETIC ACTIVITY OF LEAF AND ROOT EXTRACT OF PLANT VITEX NEGUNDO L.

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ABSTRACT

The purpose of the present study is to scientifically evaluate and compare the diuretic effect of plant *Vitex negundo* L. in experimental animal model by following standard procedure. The ethanolic extracts of leaf (EEVN-Leaf) and root (EEVN-Root) were administered to wistar rats orally at a dose of 300mg/kg and 600mg/kg and compared with furosemide (20mg/kg). The treated animals were observed for total urine volume, urine concentration electrolytes such as sodium (Na⁺), potassium (K⁺), and chloride (Cl⁻) and pH of urine. Diuretic index and natriuretic potential were also calculated. The Plant *Vitex negundo* L. increased the urine output in a dose-dependent manner and also affect the urinary electrolyte concentrations in both leaf and root extract. In the present study, it can be concluded that both ethanolic extracts of root and leaf shows diuretic activity and a comparatively root extract was more potent than leaf. The study supports the folklore use of *Vitex negundo* L.

Keywords: *Vitex negundo* L., Ethanolic Extract, Diuretic, Natriuretic.

INTRODUCTION

Diuretics mainly inhibit absorption of electrolyte from the lumen of nephron which increases osmolarity and enhances water and electrolyte excretion¹. These are effective to cure oedema, heart failure, renal failure, nephrosis, hypertension, etc. Though the use of herbal plants in traditional medicine as diuretic have increased recent year and might be a useful tool in the treatment of hypertension,

which is considered to be one of the dangerous complication of diabetes mellitus^{2,3}.

From the family Lamiaceae, *Vitex negundo* is a large, deciduous, aromatic shrub or small, slender tree up to 4.5 m tall. Branchlets quadrangular, densely whitish-tomentose. Bark thin, gray. Leaves are compound, opposite decussate, exstipulate, long petioles, digitately 3-5 foliate. Common petioles 2.5-3.8

cm long. Leaflets lanceolate, apex and base acute, terminal leaflets 5-10 cm long and 1.6-3.2 cm wide with a petiolule 1-1.3 cm long, lateral leaflets smaller with a shorter petiolule, margins entire or rarely crenate, nearly glabrous above, white-tomentose beneath. It found throughout India and Sri Lanka to an altitude of about 1500 m in the outer Himalayas⁴. Commonly *Vitex negundo* is known as Nirgundi⁵, Sinduka⁶ (Sanskrit/ Hindi) and Chaste tree (English)⁷. The whole plant is traditionally used and reported as trichogenic, ophthalmic⁸, diuretic⁹, antiparasitic¹⁰, antipyretic¹¹, CNS depressant¹², anti-inflammatory¹³ and analgesic^{14,15}. *Vitex negundo* is a commonly available medicinal plant in India and it's referred to as "sarvaroganivarini" – remedy for all disease, of Indian traditional circle¹⁶. It is the single plant species used for variety of disorders in tradition and folklore medicine, some of them are experimentally reported like antioxidant¹⁷, antibacterial activity¹⁸, antimicrofilarial¹⁹, antitumor & hepatoprotective²⁰, etc.

On the basis of the literary survey, it has been deduced that *Vitex negundo* L. is traditionally used as diuretic in Garhwal (Uttarakhand) and some related species- *Vitex doniana*, *Vitex heterophylla*, *Vitex leucoxydon*, *Vitex trifolia*, *Vitex polygama* have been used worldwide in the countries like Brazil (states of Bahia, Minas Gerais and Rio de Janeiro)^{21,22}. The traditional claims of a possible diuretic action in *Vitex negundo* are proposed to be ascertained here through the pharmacological model. The diuretic action in these species would pave a way for its possible action as an antihypertensive drug.

Material and Methods

Plant collection- Fresh and disease free leaves and roots of plant *Vitex negundo* L. were collected from Dharchula (Village Tapovan) Uttarakhand during September, 2014. The collected material was identified and authenticated by Dr. K.S Negi (Principle Scientist), National Bureau of Plant Genetic Resources Regional Station, Niglat, Bhowali- 263132

Nainital, Uttarakhand with a Herbarium specimen No.KRJ-01 has been deposited in Department of Pharmaceutical Sciences, Kumaun University, Bhimtal Campus, Nainital, Uttarakhand.

Animals- Wistar Albino female rats weighing about 150-180g were used for experimental work. The rats had free access to food and water *ad libitum* and housed in polypropylene cages, which is maintained under standard condition husbandry, room temperature $24 \pm 2^\circ\text{C}$, relative humidity 30-70%, 12h dark/light cycle in an animal house approved by CPCSEA (Committee for the Propose of Control and Supervision of Experiments on Animal). The animals have been obtained and approved by the Institutional Animal Ethics Committee (IAEC) of the Institute for Industrial Research and Toxicology, Ghaziabad (IIRT) for the present study, IAEC approval No. IIRT/IAEC/2015/45.

Drug and chemicals- Furosemide tablets (Aventis Pharma Ltd.), tragacanth mucilage 0.5%w/v, distilled water, 0.9% sodium chloride solution, potassium ferricyanide, trichloroacetic acid & ferric chloride obtained from IIRT, Ghaziabad & Patanjali Research Foundation. DPPH was obtained from Himedia Laboratories, Mumbai, India.

Preparation of extract- The leaves and roots of *Vitex negundo* were thoroughly checked and freed from foreign matter, leaves were separated from stems, roots were washed under running water to remove soil and other foreign matter. Leaves and roots were then cut into small pieces and separately shade dried. To obtain ethanolic extract (EtOH), the air dried leaves and roots were coarsely powdered separately by using laboratory grinder mixtures. 200g powdered leaves were soaked in 500ml of ethanol (70%) in an airtight conical flask for 72 hrs at room temperature and then it was first filtered through double layered muslin cloth and then filtered through Whatman No. 1 filter paper, the residue was evaporated to dryness by using a rotary evaporator under reduce pressure at $40-50^\circ\text{C}$ to get the dark brown

extract and similar process was repeated with powdered roots²².

Preliminary Phytochemical Analysis- The ethanolic extracts of leaf and root of *Vitex negundo* were subjected to qualitative phytochemical tests to identify phytoconstituents for alkaloids, flavonoids, saponins, amino acids, carbohydrates, tannins, glycosides and phenols²³.

In vitro free radical scavenging activity

Reaction with DPPH radical

The scavenging effect of EEVN-Leaves and EEVN-Roots (20–200 µg/ml) against DPPH stable radical was determined using ascorbic acid (ASC, 1–5 µg/ml) as standard. Plotting the percentage for DPPH scavenging against ASC concentration gave the standard curve²⁴.

Determination of reducing power ability

The reducing power of EEVN-Leaves and EEVN-Roots (25–200µg/ml) was determined according to the method previously reported²⁴. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, the absorbance of which is measured at 700 nm. Increase in absorbance of the reaction mixture indicates the reducing power of the samples. Ascorbic acid was used as standard. Phosphate buffer (pH 6.6) was used as blank solution. The absorbance of the final reaction mixture of three parallel experiments was taken and is expressed as mean±S.E.M.

Pharmacological Evaluation

Dose selection- All drugs and vehicle (0.5% w/v Tragacanth in distilled water) were administered in the volume of 10mL/kg and doses were calculated according to the body weight of animals. The doses of ethanolic extract of *Vitex negundo* selected in the present study were, according to the reported LD₅₀ value 7580 mg/kg per oral dose (p.o)²⁵. 1/13th and 1/26th approximate of LD₅₀ were selected in the present study as high and low dose, i.e. 600mg/kg and 300mg/kg body weight of animal respectively. Furosemide (Lasix) administered at a dose of 20mg/kg along with the vehicle.

Diuretic activity- The method of Lipschits *et al.*²⁶ also denoted in previously reported literatures^{27,28} was employed for the assessment of diuretic activity. Healthy female Wistar rats were divided into six groups of five animals in each. Furosemide (20mg/kg) was used as a standard reference drug. All the drugs were prepared by suspending in 0.5% w/v of tragacanth mucilage. Before the experiment, the rats were fasted for 18 h with free access to water. On the day of the experiment, all animals were treated with 5ml/Kg body weight (0.9% NaCl) normal saline for equal electrolyte balance. The drug treatment using oral administration route as follows:

Experimental Design

Group 1st: (Control): receives 0.5% w/v tragacanth (p.o).

Group 2nd: (Standard): receives furosemide at a dose of 20mg/kg body weight (p.o).

Group 3rd: receives EtOH of leaf (EEVN-Leaf) 300mg/kg body weight (p.o).

Group 4th: receives EtOH of leaf (EEVN-Leaf) 600mg/kg body weight (p.o).

Group 5th: receives EtOH of the root (EEVN-Root) at a dose of 300mg/kg body weight (p.o).

Group 6th: receives EtOH of the root (EEVN-Root) at a dose of 600mg/kg body weight (p.o).

Immediately after the treatment, the animals were placed in metabolic cages (one animal in one metabolic cage) provided with wire mesh bottoms and a funnel to collect the urine. Stainless steel sieves placed in the funnel to retain fecal matter and to allow the urine to pass. The urine was collected in measuring cylinder up to 2 and 6 hrs for all control and drug-treated groups. During this period no food or water was made available to the animals. The volumes of urine, electrolyte excretion (Na⁺, K⁺ and Cl⁻) was estimated in the urine for assessment of diuretic activity. Na⁺, K⁺ estimation were carried using atomic absorption spectroscopy (Delhi Test House). The chloride ion concentration was estimated by titration with 0.02N AgNO₃ using 5% potassi-

um chromate solution as indicator. The volume of urine was estimated for the assessment of diuretic activity. The diuretic action of testing drug was calculated by using the following formula:

Diuretic Activity = Diuretic action of test drug/ Diuretic action of standard drug

Statistical analysis

The values were expressed as mean \pm SEM (n=5). The statistical analysis was carried out by one-way analysis of variance (ANOVA) using Graph pad Prism 5 software followed by Dunnett's t-test & also used Origin V7 software for graph preparation & IC-50 calculation.

Results

The ethanolic extract of *Vitex negundo* was subjected to qualitative phytochemical tests to identify the phytoconstituents and it was found that the leaf possesses positive result for alkaloids, carbohydrates, flavonoids, saponins, amino acids, tannins and phenols. And root shows the presence of alkaloids, carbohydrates, saponins, terpenoids, flavonoids and phenols.

Free radical scavenging activity of EEVN-Leaf and EEVN-Root

(a) DPPH assay

The DPPH assay method is based on the reduction of the methanolic solution of colored free radical DPPH by free radical scavengers. The procedure involves measurement of the decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to the DPPH reagent solution. Reaction with DPPH radicals of EEVN-Leaf and EEVN-Root showed scavenging activity. The IC50 value for EEVN-Leaf and EEVN-Root were found in 101.87 μ g/ml and 56.63 μ g/ml, respectively, whereas IC50 values for ASC was found to be 2.87 μ g/ml, A linear correlation coefficient ($r^2 = 0.9352$) was obtained (Fig.1).

(b) Reducing power ability

Results in (Fig.2) showed that as the concentration of EEVN-Leaf and EEVN-Root (25–200 μ g/ml) was increased, the absorbance increased for each fungal fraction. This depicts that fractions have reduced power activity. The antioxidant principles present in the extracts of EEVN-Leaf and EEVN-Root the reduction of Fe^{3+} /ferricyanide complex in the ferrous form and thus proved the reducing power ability.

Evaluation of diuretic activity

Urine volume and diuretic activity- The result obtained from diuretic activity of ethanol leaf and root extract of plant *Vitex negundo* was shown in (Table-1). Treatment with 300mg/kg and 600mg/kg body weight of both leaf and root extracts of *Vitex negundo* shows diuretic activity in a dose dependent manner when compared to control at 2 and 6 hrs. The leaf extract at dose 600mg/kg body weight ($p < 0.01$) and root at both doses ($p < 0.001$) shows significant urine excretion at 2 hrs after dosing. High dose of root extract only shows significant urine output ($p < 0.001$) when compared to control at 6 hrs but the effect much less than standard drug furosemide. The urine output of root extract is higher in both 300mg/kg and 600mg/kg body weight when compared to leaf extract at 2 and 6 hrs after dosing (Table-2).

Effect on electrolyte excretion

Urinary sodium- Urinary sodium level of control animals was 85.5 mmol/L 6 hrs after dosing. In the standard group (20mg/kg), there was a significant ($p < 0.001$) increase in sodium (Na^+) excretion as 126.1 mmol/L and in the test group of a leaf extract (300mg/kg and 600mg/kg) the level of Na^+ was significantly greater than control group, and more significant increase in Na^+ excretion is observed in test group of root extract (300mg/kg and 600mg/kg) as 101.0 mmol/L and 111.0 mmol/L ($p < 0.001$), but much less than standard drug Furosemide as shown in (Table-3).

Urinary potassium- Urinary potassium (K^+) excretion during the 6 hrs collection in the control group was found to be 80.2 mmol/L. In standard group, there was a significant increase in K^+ excretion as 90.6 mmol/L ($p < 0.001$), no changes was observed in K^+ concentration at lower dose of leaf and root extract, though high doses of both test extract of leaf and root increases K^+ level significantly as 81.8 mmol/L ($p < 0.01$) and 85.6 mmol/L ($p < 0.001$) respectively as shown in (Table-3).

Urinary chloride- None of the extracts including standard shown much changes in Cl^- level, results are given in (Table-3).

Effect on pH of urine and weight- No significant change was observed at pH of urine in any of the group at 2 and 6 hrs of the experimental period. And the weights of animals were much less fluctuated before and after the experiment.

DISCUSSION

The preliminary phytochemical screening showed that the ethanolic leaf extract of *Vitex negundo* contained steroids, carbohydrates, flavonoids, tannins, phenols, saponins and alkaloid however root extract contained flavonoids, anthraquinones, phenol, terpenoids, saponins and alkaloids²¹. These above phytoconstituents could be fully or partially responsible for increase diuresis and moderate natriuretic activity²². An attempt has been made in the present study to evaluate free radical scavenging activity of EEVN-Leaf and EEVN-Root of *Vitex negundo* L. were evaluated for free radical scavenging activity by DPPH radical scavenging assay and by reducing power ability. Among these, two tested extracts EEVN-Root was found to be potential with the minimum IC50 value & both extracts found having reducing power ability.

Result showed an increase elimination of fluid overload with urinary hypo-osmolarity and moderate natriuretic activity. These results demonstrate that the ethanolic extract of both leaf and root of *Vitex negundo* has moderate diuretic activity. The present

study demonstrates that the urine volume of EEVN-leaf enhanced non-significantly as 0.46ml (300mg/kg) and 0.49ml (600mg/kg) at 2 hrs, after 6 hrs these values reached to 1.18ml and 1.57ml, respectively, whereas in EEVN-root extract only high dose shows significant ($p < 0.001$) increase in urine volume up to 1.96 ml at 6 hrs but in 2 hrs both dose shows significant increase as 0.51ml (300mg/kg) and 0.57ml (600mg/kg).

It has been found that there is a significant ($p < 0.001$) increase in Na^+ and K^+ excretion in both root and leaf extract at dose 600mg/kg. No significant changes were found in the chloride level at both doses. The diuretic activity of EEVN leaf is 0.56 (300mg/kg) and 0.60 (600mg/kg) at 2 hrs, after 6 hrs it reached in 0.50 (300mg/kg) and 0.67 (600mg/kg). The only high dose of leaf extract much increase to initial value. In EEVN-root, the initial values for diuretic activity are 0.63 (300mg/kg) 0.73 (600mg/kg), which after 6 hrs reaches to 0.56 (300mg/kg) and 0.83 (600mg/kg). It can be suggested that the diuretic activity of EEVN-root is more than EEVN-leaf in both dose ranges. The pH of urine is much fluctuated to the standard value and weight of animals before and after experiment remains same.

The observed pattern of test drug is much similar to the standard drug furosemide so test drug might be inhibiting the Na^+ and K^+ absorption, like loop diuretics at the distal loop of nephron²⁸. It can be suggested that the mechanism of action of test drug is quite similar to the furosemide where an increase in sodium, potassium and urine volume were observed. The EEVN-root (600mg/kg) of *Vitex negundo* showed elevated levels of K^+ in urine, which may increase the risk of hypokalemia²⁹. In the present study, in reference to the elimination of Na^+ , K^+ the extracts of *Vitex negundo* showed natriuretic effect. The Na^+/K^+ ratio define the nature of the diuretic mechanism.

CONCLUSION

The ethanolic extracts of leaf and root of *Vitex negundo* were tested for diuretic activity. From the present investigation, we can conclude that, both extracts of *Vitex negundo* show a diuretic action which is significant with ethanolic extract of root when compared to leaf extract. The phytoconstituents responsible for this activity need to be investigated. The basis of excretion pattern of water, Na⁺ and K⁺, it appears that the active principle present in these two extracts have furosemide like activity in a dose dependent manner²⁸. The diuretic activity might be due to the presence of flavonoids. The present study supports the traditional use of *Vitex negundo* for its diuretic activity.

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Table 1: Diuretic activity of test and standard drug

Treatment	Dose	Diuretic activity in 2 hrs	Diuretic activity in 6 hrs
Control	5ml/kg	0.51	0.47
Standard (Furosemide)	20mg/kg	1	1
EEVN (Leaf)	300mg/kg	0.56	0.50
EEVN (Leaf)	600mg/kg	0.60	0.67
EEVN (Root)	300mg/kg	0.63	0.56
EEVN (Root)	600mg/kg	0.73	0.83

Note: Each value in parentheses indicates are calculated by the formula, Diuretic activity = Diuretic action of test drug/Diuretic action of standard drug.

Table 2: Effect of orally administered leaf and root extract of *Vitex negundo* on urine output

Treatment	Dose	Urine vol. in 2 hrs (ml)	Urine vol. in 6 hrs (ml)
Control	5ml/kg	0.42±0.01	1.11±0.18
Standard (Furosemide)	20mg/kg	0.81±0.02***	2.34±0.16***
EEVN (Leaf)	300mg/kg	0.46±0.01	1.18±0.11
EEVN (Leaf)	600mg/kg	0.49±0.02**	1.57±0.22
EEVN (Root)	300mg/kg	0.51±0.13***	1.32±0.16

EEVN (Root)	600mg/kg	0.57±0.01***	1.96±0.05**
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Each Value in parentheses indicates Mean ± S.E.M (n=5 animals each group) urine volume in ml for 2hrs & 6hrs and *P <0.05, **P <0.01, ***P <0.001 compared control group.

Table 3: Effect of orally administered leaf and root extract of *Vitex negundo* on ionic concentration of rat’s urine

Treatment	Dose	Na ⁺ concentration (mmol/l)	K ⁺ concentration (mmol/l)	Cl ⁻ concentration (mmol/l)
Control	5ml/kg	85.5±0.73	80.2±0.31	82.9±0.22
Standard (Furosemide)	20mg/kg	126.1±1.62***	90.6±0.17***	83.2±0.31
EEVN (Leaf)	300mg/kg	91.8±1.13*	80.3±0.25	84.3±1.01
EEVN (Leaf)	600mg/kg	92.6±0.84*	81.8±0.23**	83.0±0.26
EEVN (Root)	300mg/kg	101.0±2.97***	80.6±0.26	83.2±0.21
E EEVN (Root)	600mg/kg	111.0±2.00***	85.6±0.28***	83.0±0.27

Each Value in parentheses indicates Mean ± S.E.M (n=5 animals each group) ionic concentration of rat’s urine after and *<0.05, **<0.01, ***<0.001 compared to control group.

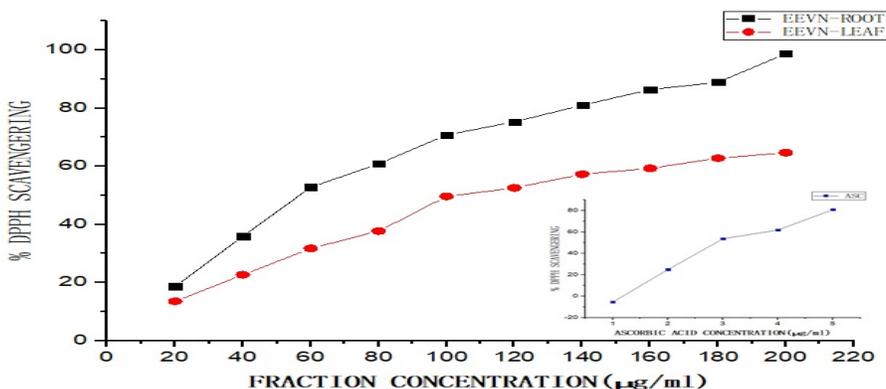


Fig. 1. Effect of EEVN-ROOT and EEVN-LEAF on DPPH radical

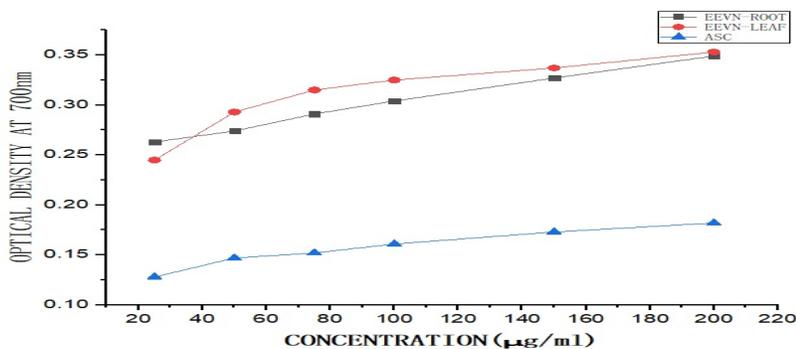


Fig. 2. Reducing Power ability of EEVN-ROOT & EEVN-LEAF.

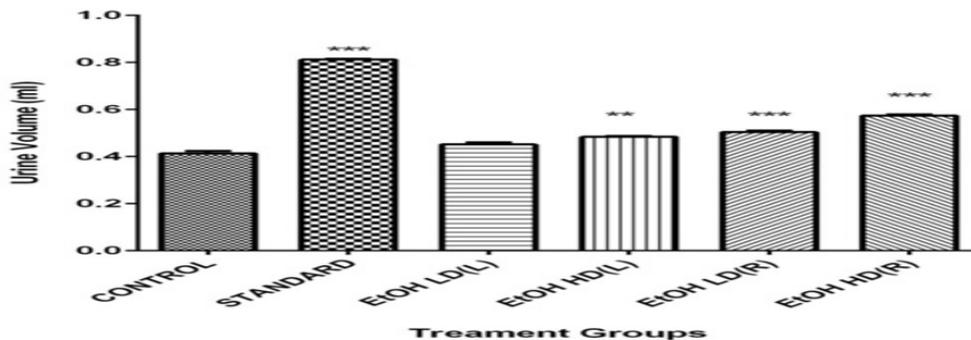


Fig.3. Effect of EEVN-ROOT and EEVN-LEAF on urine volume in 2 hrs.

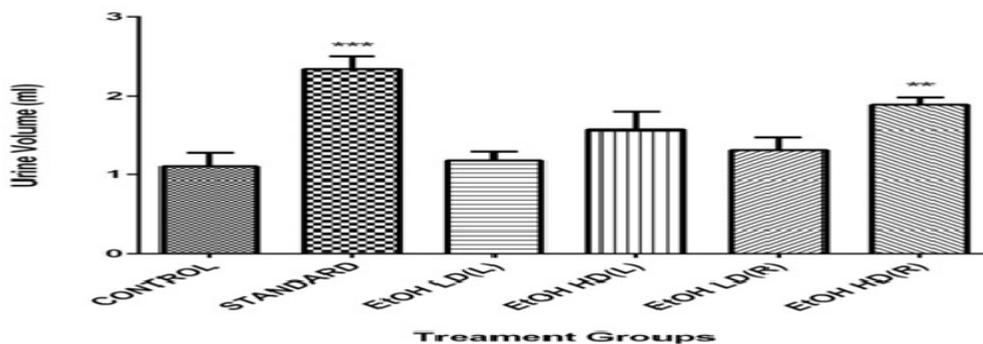


Fig. 4. Effect of EEVN-ROOT and EEVN-LEAF on urine volume in 6 hrs

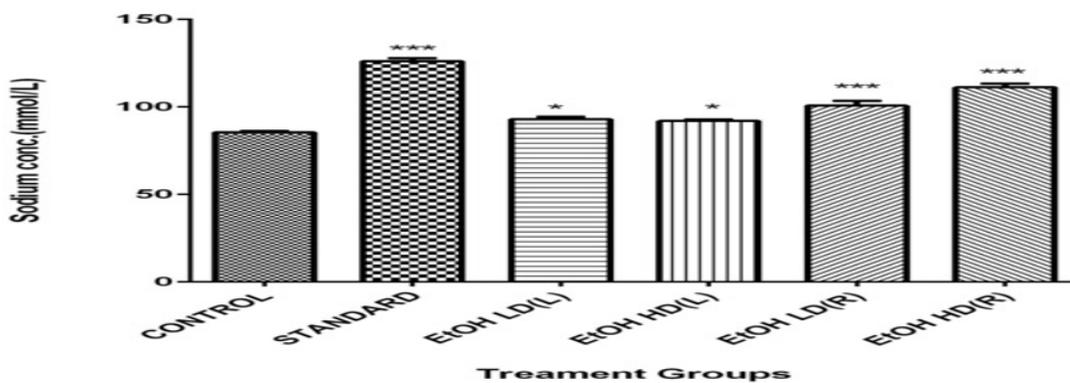


Fig. 5. Effect of EEVN-ROOT and EEVN-LEAF on sodium concentration in 6 hrs

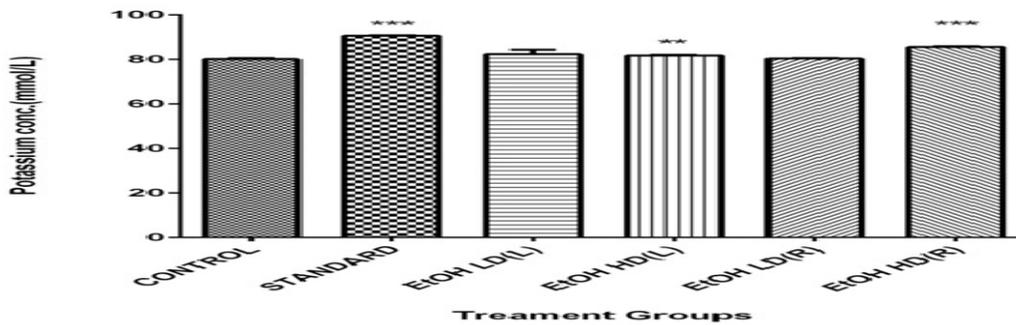


Fig. 6. Effect of EEVN-ROOT and EEVN-LEAF on potassium concentration in 6 hrs

Source of Support: Nil

Conflict Of Interest: None Declared

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