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Diuretic Effect of Hydro Alcoholic Extract of *Allium sativum and Occimum Basilicum* and its Phytochemical Studies

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Abstract

Introduction: *Diuretics can help with some medical disorders, including as hypertension, decreased fluid retention, edema, inflamed ankles, and pain. Due to the global trend towards the use of natural plant remedies, information about the features and applications of medicinal plants is in great demand.*

Aim: *For the aforementioned reasons, the present investigation's objective is to assess the diuretic efficiency of the hydroalcoholic extract of* ***Allium sativum and Occimum basilicum.***
Methodology: *Five grams of dried plant were coarsely pulverized and extracted with ethanol and water. After filtration, each aqueous extract was used. The hydro-ethanolic extract of* ***Allium sativum (bulb) and Occimum basilicum (leaves)*** *is investigated in terms of histopathology and diuretic efficacy.*

Result: *When compared to zero days, the aforementioned parameters—creatinine, urea, and glucose—do not change during the seven-day treatment. Smaller biochemical changes were observed between the 15th and zeroth days. The hydroalcoholic extracts of* ***Allium sativum and Occimum basilicum*** *also enhanced urinary volume. At 200 mg/kg & 400 mg/kg body weight, the increment was 18% ($p < 0.01$) & 41% ($P < 0.001$), respectively, compared to the control group.*

Conclusion: *This current study will be extremely useful in assessing the diuretic effect of the* ***Allium sativum and Occimum basilicum*** *extracts since it will use ion-selective channel blockage to detect the ions and electrolytes that the animals evacuate.*

Keywords: *Diuretics,* ***Allium sativum, Occimum basilicum,*** *hydro-ethanolic extract.*

Introduction

Diuretics are substances that work on the kidneys to encourage the elimination of water and salt in the urine. To maintain water and ionic equilibrium, diuretics help the body rid itself of excess water, salts, toxins, and stored metabolic products. In addition to changing the excretion of Na+, diuretics may also change how the kidneys handle uric acid, other cations (K^+, H^+, Ca^{2+}, & Mg^{2+}), & anions (Cl^-, HCO^{3-}, & $H2PO^{4-}$). Furthermore, diuretics may indirectly change the activation of renal hemodynamics. They help the body get rid of extra fluid (edema) that builds up in the tissues as a result of several illnesses. Diuretics are used to treat several pathological conditions, including high blood pressure, pulmonary and systemic edema, cardiac arrest, increase blood

pressure, liver cirrhosis, kidney diseases, edema, swollen ankles, & pain and burning sensations related to cystitis.

As a result of the increased excretion of NaCl (natriuresis), they reduce the reabsorption of Na^+ & (typically) Cl^- from the filtrate, leading to greater water loss. This can be accomplished by: directly affecting the nephron's cells; and indirectly, by altering the filtrate's composition. Diuretic classes, For example, carbonic anhydrase inhibitors. Dichlorphenamide with Acetazolamide Loop Diuretics, for instance. Ethacrynic acid with furosemide Thiazides, such as benzothiazide and chlorothiazide Diuretics that spare potassium, for example. Spironolactone Osmotic, for instance. Glycerol with Mannitol.

There is a huge demand for knowledge on the characteristics and applications of medicinal plants due to the global trend toward the usage of natural plant treatments. Plant materials constitute the mainstay of Indian traditional medicine, including Ayurvedic, Siddha, and Unani. The safety, efficiency, and affordability of herbal medications have made them more significant and well-liked in recent years. In certain situations, the medicinal properties of medicinal plants are also influenced by their associations with other plants in their environment. Several clinical conditions have been shown to benefit from the well-established use of plants and their formulated polyherbal products as diuretics, including lowering hypertention, reducing fluid retention, edema, and inflamed ankles, as well as relieving the pain and burning sensation associated with cystitis, heart failure, and high BP, Liver Cirrhosis, Kidney Disorder, Pulmonary & Systemic Edema.

MATERIALS AND METHODS

Plant Collection

Fresh Occimum basilicum L. leaves and Allium sativum bulbs were gathered from the Morena region. At room temperature (20ºC+/-2ºC), the gathered plant material (leaves or dried bulb) was allowed to air dry in the dark. Until it was needed, the plant material (dry) was chopped up & kept in dark, tightly sealed receptacles.

Extraction of plants

A cylinder crusher was used to coarsely crush about 5 grams of dried plant material into little pieces ranging in size from 2 to 5 mm. The material was then extracted using ethanol and water, respectively. For each infusion, 5gm of dry herbal tea was combined with 200 ml of distilled water (one teacup's worth) and heated to about 100ºC+/-1ºC for 10 minutes. After passing through a paper filter, each aqueous extract was refrigerated in glass flasks tapered with a screw-on plastic top. Several publications have reported the solvent starting temperature and infusion period utilized in this study as effective conditions for extracting phytochemical components, including flavonoid and phenolic compounds, from herbs.

Test of Phytochemistry

Using established protocols, chemical tests were conducted on extracts and powder specimens to screen for and identify phytochemical ingredients in the investigated medicinal plants.

Maeyer reagent

60 milliliters of pure water were used to dissolve 0.355 grams of mercuric chloride. Twenty milliliters of distilled water were used to dissolve five grams of potassium iodide. After mixing the two solutions, distilled water was added to get the volume up to 100 ml.

Dragendorff reagent

Solution A: 80 milliliters of distilled water were used to dissolve 1.7 grams of basic bismuth nitrate & 20 grams of tartaric acid. Solution B: 40 milliliters of distilled water were used to dissolve 16 grams of potassium iodide. A 1:1 mixture of solutions A and B was made.

Check for alkaloids

Eight milliliters of 1% HCl were combined with 0.5 to 0.6 grams of the methanolic plant extract, heated, and filtered. The two reagents (Dragendorff's and Meyer's) were applied individually to 2 milliliters of the filtrate.

Check for steroids.
Two milliliters of acetic anhydride and two milliliters of sulfuric acid were combined with around 0.5 grams of each plant's methanolic extract fraction.

Check for terpenoids
In a test tube, 0.5 millilitres of methanolic extract and 2 ml of $CHCl_3$ were combined in an aliquot. To create a layer, 3 millilitres of concentrated H_2SO_4 were carefully mixed.

Check for flavonoids
A two drops of strong HCl & a few magnesium turnings were added to the alcohol-based compound & boiled for five minutes.

Test for tannins
The 0.5 gm of powdered sample of each medicinal plant leaf was boiled in 20 ml of distilled water in a test tube & then filtered. The filtration method used here was normal.

Experimental Design
Wistar rats of either sex (200-250 g) were obtained from the central animals of Shriram College of Pharmacy, Banmore, and kept in polypropylene cages on rodent pellet conditions of controlled temperature (22±2°C) and acclimatized to 12/12 h light/dark cycle. The LD50 was calculated using adult female Swiss mice weighing between 20-30 g. Food and water were freely available until two hours before to the trial. The animals were maintained and cared for by the "Committee for Control and Supervision of Experiments on Animals (CPCSEA)" approved requirements. Two hours following the trial, food and water were made available. The project proposal no. states that all animal experiments were carried out in compliance with the establishment's ethical committee on animal experimentation.

SRCP/IAEC/70/23-24.
Acute Toxicity Study
Following OECD 423 recommendations, an acute oral toxicity investigation of the aqueous extracts of Allium sativum and Occimum basilicum L was conducted. Four groups of adult female Swiss mice (n = 6) were kept fasting and given only water for three to four hours. Three groups had gastric intubations to administer extract at 200 and 2000 mg/kg body weight, whereas one control group received the vehicle (distilled water). For the first four hours following medication, the rats were routinely monitored for behavioral and neurological signs. After 24 hours, the number of surviving was recorded, and the animals were kept for an additional 14 days while daily observations were performed. The dose given was classified as hazardous if two of the three mice died. The hazardous dose was confirmed by repeating the same dose if one mouse showed signs of mortality. Up to 2000 mg/kg, no death or morbidity was seen in rodents treated with extract. For effectiveness tests, 200 mg/kg and 400 mg/kg were thus administered. Following a 16–19 hour overnight fast, blood samples were taken from each animal at intervals of 0, 7, and 15 via retroorbital puncture under diethyl ether anesthesia. Heparinized tubes were used for hematological parameters after the blood samples were placed in EDTA tubes for biochemical parameters.

Screening for Diuretic Activity (Lip Schitz test)
Animals required:
Species / common name: Wistar rats
Age/weight / size: 3 months / 200-250g
Gender: Male
Number to be used: 20
Number of days each Animal will be housed: 15 days

The diuretic efficacy of the hydro-ethanolic extract of Occimum basilicum and Allium sativum leaves will be assessed with minor adjustments to existing techniques. The weight of male Wistar rats will range from 200 to 250g. split up into four groups (n = 5). As a control, the animals in group I were given distilled water (a vehicle). As a benchmark, Group II was given 20 mg/kg p.o. of furosemide. The aqueous ethanolic extract of Allium sativum and Occimum basilicum in Group III is a moderate dose of 200 mg/kg, whereas the aqueous ethanolic extract of IV- is a high dose of 400 mg/kg. Standard metabolic cages will be used to house the four animals. to get urine samples at various intervals, such as five and twenty-four hours. Observe the parameters such as urine volume, PH, Urinary Na+, K+, and Cl-

Hematological parameter determination

Hematocrit (HCT), platelets (PLT), white blood cell (WBC) count, hemoglobin (Hb) levels, differential WBC count [neutrophils (N), lymphocytes (L), monocytes (M), eosinophils (E)], mean corpuscular volume (MCV), mean corpuscular hemoglobin (MH), and mean corpuscular hemoglobin concentration (MCHC) were all measured on the blood samples.

Serum biochemical parameter determination

Blood samples were centrifuged for 10 minutes at 4000 rpm to extract serum for the following tests: glucose, urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

Analysis of statistics

The mean±SEM (n=6) was used to express the experimental results. Using Graph Pad Prism software, a one-way ANOVA and the Dunnett's "t" test were used for statistical analysis.

RESULT AND DISCUSSION

Phytochemical Analysis

Following the findings of the phytochemical screening, it was determined whether the absence of turbidity and/or precipitate development indicated the absence of alkaloids. In several samples, the color shifted from violet to blue or green, signifying the presence of steroids. A favorable outcome was the formation of a reddish-brown interface when terpenoids were present. Flavonoids are shown by red coloration (Shinado's test). The test tube showed a change in hue, indicating the presence of tannins.

Table 1: Phytochemical Analysis

S.No	Phytochemicals	Allium sativum	Ocimum basilicum
1.	Alkaloids	-	-
2.	Steroids	+	+
3.	Terpenoids	+	-
4.	Flavonoids	+	+
5.	Tannins	+	+

+ : Presence , - : Absence

Acute toxicity

During the trial period, mice given oral extracts of Allium sativum and Occimum basilicum up to a level of 2000 mg/kg did not die or experience any harm. However, mice who received Allium sativum and Occimum basilicum extracts showed normal behaviour for the entire group of animals, as evidenced by haematological, biochemical, and histological monitoring. Additionally, no death was noted. According to OECD standards No. 423, oral dosages of 2000 mg/kg body weight of Allium sativum & Occimum basilicum are safe for both

medical and dietary purposes. The 200 and 400 mg/kg dosages are appropriate for diuretic activity, according to the study mentioned above.

Following OECD 423 guidelines, administer the aforementioned dosage to animals that have been fasted for three hours and are under special observation for twenty-four hours to determine the grooming, sniffing, & rearing characteristics for each group, but not the other factors (death rate, excessive urination, hair loss, convulsion, & locomotion).

Comparing the daily intake of food for all animal groups for at least 15 days to conventional clinical data of animals using the aforementioned criterion showed that the extract dosages had not caused diabetic mellitus or disorders linked to pancreatic dysfunction.

Comparing the above parameter to standard scientific animal data, water intake was normal for all mouse groups for up to 15 days, indicating that the offered medicine did not cause diabetes mellitus, disorders related to pancreatic dysfunction, or other GIT-associated ailments. Every mouse's daily body weight showed no variations across all groups when compared to scientifically standard mouse data. According to the mice's body weight results above, the extract dosages do not raise cholesterol or cause obesity.

Following the administration of extract doses, no hair loss was observed in any of the mice in any of the groups, and neither cancer nor immunological problems were induced.

Observed parameters have indicated that the no excess urination in all the groups of animals so the doses of extract do not induce the urinary system-related disorders.

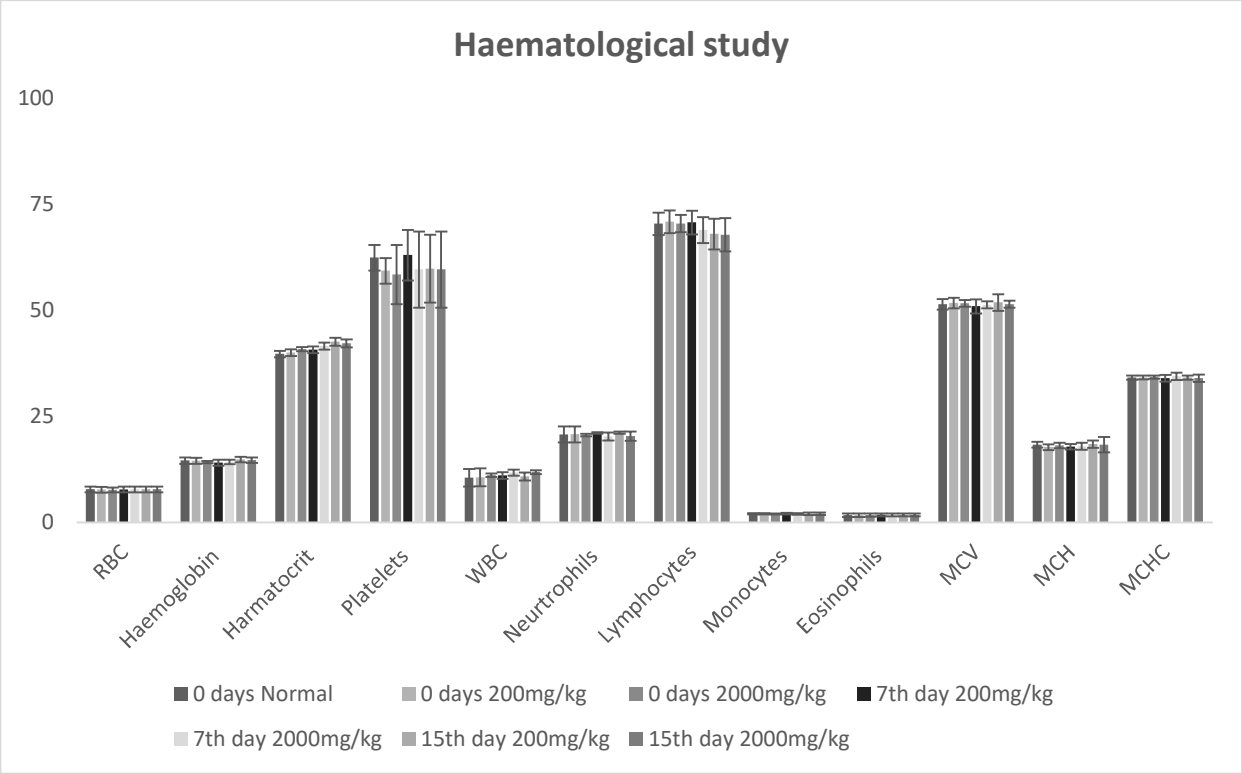
Hematological investigation

Red blood cells, hemoglobin, hematocrit, platelets, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, MCV, MCH, & MCHC were all normal following the treatment on day zero, according to the 15-day study, which was shown in Table No. When compared to zero days, the aforementioned metrics remain unchanged following the seven days of treatment. When compared to zero days, fewer differences were seen on the 15th. Comparison of the control group showed that the various time intervals of 0, 7, & 15 days above the parameters are comparable by the indication that the drug supplied has no harmful effects.

Table 2: Haematological values of rats in the acute toxicity study of Alcoholic Extract of *Allium sativum and Ocimum basilicum*

Parameters	**0 days**			**7th day**		**15th day**	
	Normal	200mg/kg	2000mg/kg	200mg/kg	2000mg/kg	200mg/kg	2000mg/kg
Red blood cells	7.80±0.68	7.71±0.60	7.65±0.68	7.76±0.68	7.80±0.68	7.80±0.68	7.80±0.68
Hemoglobin	14.58±0.73	14.55 ± 0.26	14.26 ± 0.31	14.07 ± 0.74	14.25 ± 0.54	14.8 ± 0.64	14.71 ± 0.64
Hematocrit	39.75±0.78	40.08 ± 0.48	40.93 ± 0.65	40.75 ± 0.77	41.65 ± 0.82	42.65 ± 0.93	42.26 ± 0.97
Platelets	62.5±2	59.40 ± 7	58.50 ± 8	63.1 ± 6	59.7 ± 9	59.9 ± 8	59.7 ± 9

WBC	10.54±2.1	10.62 ± 0.45	11.13 ± 0.88	11.06 ± 0.8	11.75 ± 0.70	10.83 ± 0.97	11.85 ± 0.45
Neutrophils	20.74±1.89	20.8 ± 0.32	20.62 ± 0.88	21.12 ± 0.2	20.28 ± 0.96	21.17 ± 0.29	20.34 ± 1.08
Lymphocytes	70.51 ± 2.66	70.98 ± 2.06	70.56 ± 2.01	70.81 ± 2.77	69.03 ± 3.07	68.1 ± 3.61	67.91 ± 3.93
Monocytes	2.03 ± 0.17	2.01 ± 0.08	1.98 ± 0.09	2.05 ± 0.18	2.00 ± 0.18	2.00 ± 0.3	2.03 ± 0.33
Eosinophils	1.7 ± 0.43	1.7 ± 0.37	1.7 ± 0.36	1.73 ± 0.34	1.73 ± 0.35	1.75 ± 0.32	1.75 ± 0.34
MCV	51.47 ± 1.25	51.8 ± 0.79	51.7 ± 0.84	51.02 ± 1.65	51.37 ± 0.86	51.92 ± 1.98	51.5 ± 0.84
MCH	18.34 ± 0.69	17.75 ± 0.63	18.15 ± 0.65	17.87 ± 0.66	17.97 ± 0.82	18.46 ± 0.88	18.33 ± 1.82
MCHC	34.17 ± 0.47	34.2 ± 0.36	34.3 ± 0.36	34.03 ± 0.77	34.46 ± 0.88	34.2 ± 0.49	34.01 ± 0.87



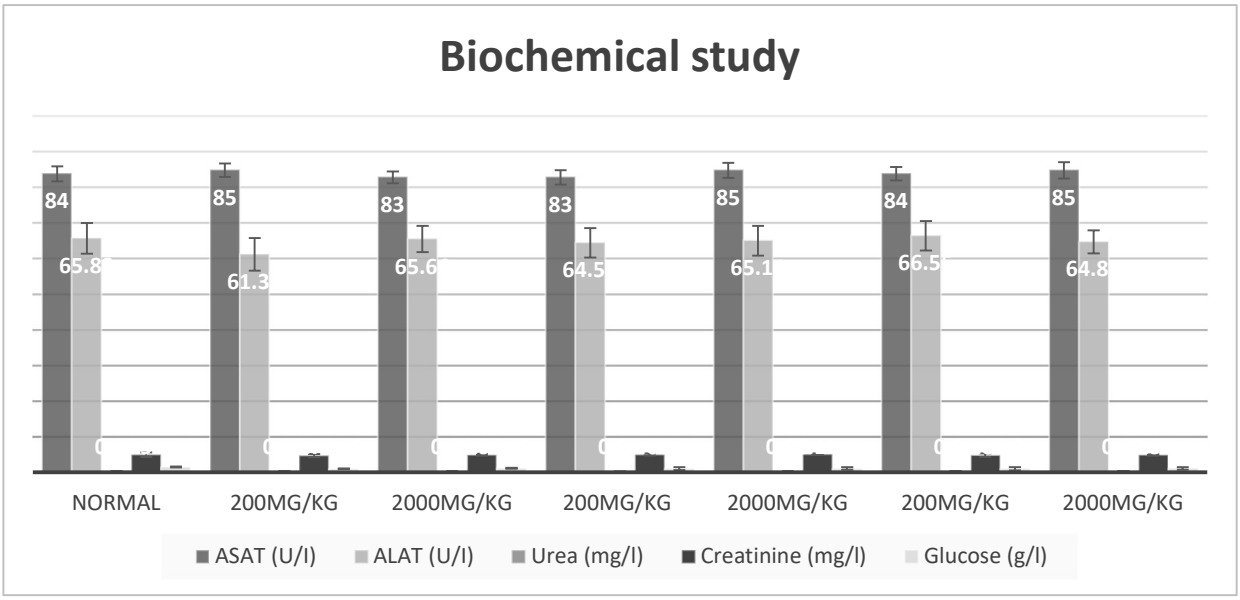
Biochemical study

According to the results of the biochemical investigation, Table No. shows that following the medicine on the day of zero, parameters such as ASAT (U/I), ALAT (U/I), urea (mg/l), creatinine (mg/l), and glucose (g/l) were all normal. When compared to zero days, the aforementioned parameters, including glucose, urea, and creatinine, do not alter following the seven days of treatment. When compared to zero days, smaller metabolic changes were seen on the fifteenth day. Comparison of the control group showed that the various time intervals of 0, 7, & 15 days above the parameters are comparable by the indication that the drug supplied has no harmful effects. ASAT (U/I) & ALAT (U/I) levels were somewhat elevated following the medication administration in comparison to the control group, suggesting that the extract possesses antioxidant qualities.

Table 3: Biochemical values

Treatment Schedule							
Parameters	Normal	200mg/ kg	2000mg/k g	200mg/ kg	2000mg/k g	200mg/ kg	2000mg/ kg
ASAT (U/I)	84 ± 3.1	85 ± 1.8	83 ± 1.7	83 ± 2.1	85 ± 2.2	84 ± 1.9	85± 2.4
ALAT (U/I)	65.84 ± 4.2	61.43 ± 4.60	65.65 ± 3.65	64.45 ± 4.13	65.26 ± 4.18	66.56 ± 4.12	64.82 ± 3.22
Urea (mg/l)	0.26 ± 0.15	0.25 ± 0.07	0.28 ± 0.05	0.25 ± 0.08	0.26 ± 0.08	0.34 ± 0.05	0.35 ± 0.07
Creatinine (mg/l)	5.0 ± 0.63	4.74 ± 0.43	4.96 ± 0.26	5.03 ± 0.29	5.11 ± 0.22	4.88 ± 0.40	4.92 ± 0.18

Glucose (g/l)	1.42 ± 0.22	1.03 ± 0.07	1.15 ± 0.12	1.08 ± 0.40	1.11± 0.38	1.07 ± 0.47	1.12 ± 0.36



Diuretics action
(Lipschitz Test)

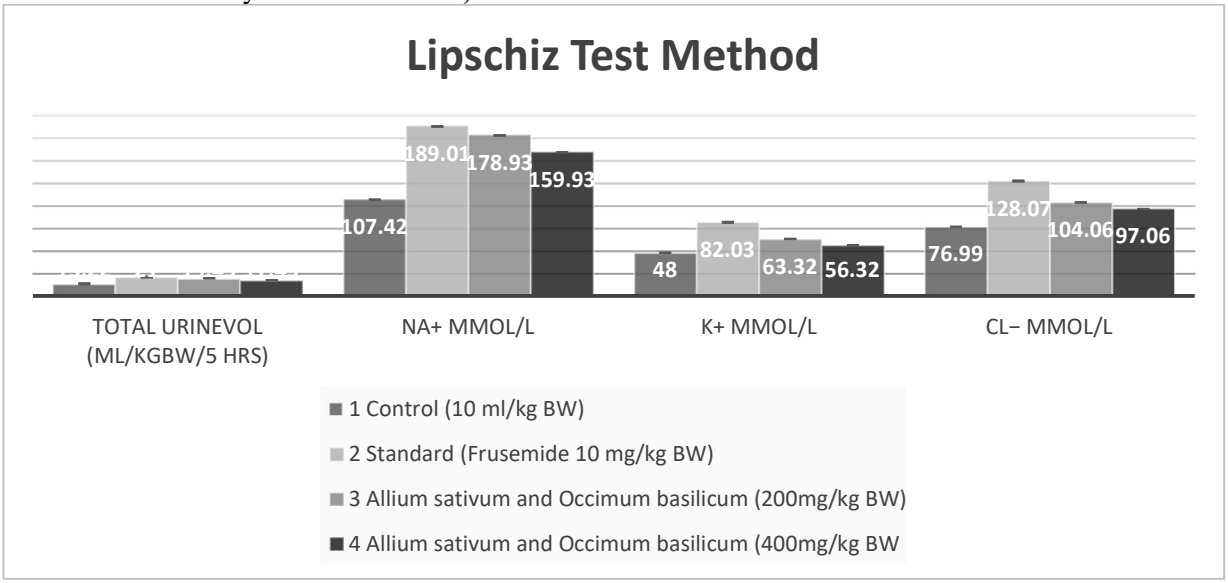
The table presents the findings from the assessments that were performed on the extracts. No. 4 displays the animals' urine volume (ml/100g/8h) & electrolyte (Na+ and K+) content (mequiv/100g/8h). urine volume. According to Table 1, HCTZ, the reference diuretic, resulted in a 54% rise in urine volume. Urine volume also increased as a result of the Alcoholic Extract of Occimum basilicum and Allium sativum. At 400 mg/kg body weight, the rise for the Alcoholic Extract of Allium sativum and Occimum basilicum was 18% ($P < 0.01$) & 41% ($P < 0.001$), respectively, in comparison to the control group.

Table 4: Diuretic effect of Alcoholic Extract of *Allium sativum and Occimum basilicum*
Lipschiz Test Method

S.NO	**Groups**	**Total urine Vol (ml/kg BW/5 hrs)**	**Na^+ mmol/L**	**K^+ mmol/L**	**Cl^- mmol/L**
1.	Control (10 ml/kg BW)	13.62±0.32	106.42±0.75	48.00±0.32	76.89±0.23
2.	Standard (Frusemide 10 mg/kg BW)	21.00±0.03***	187.01+0.64***	82.03±0.44***	128.07±0.24***
3.	*Allium sativum and Occimum basilicum* (200	19.65±0.41***	180.93±0.73***	63.42±0.35***	104.16±0.06***

	mg/kg BW)				
4.	*Allium sativum and Occimum basilicum* (400 mg/kg BW	17.55±0.41***	159.91±0.73***	56.82±0.35***	97.04±0.06***

HA EMC: Alcoholic Extract of *Allium sativum and Occimum basilicum*
n = 6, values expressed as mean ±SEM. Significant at P < 0.001*** compared with the control group (one-way ANOVA followed by Dunnett's 't' test)



RESULTS

Table 4 presents the findings from an assessment of the diuretic activity of *Occimum basilicum* and *Allium sativum.* According to the results, the extract of Occimum basilicum and Allium sativum considerably raised the excretion of electrolytes (Na+, K+, & Cl-) and urine production in comparison to the control group. When compared to the usual medication furosemide, the extract's excretion of urine output increased significantly at the 400 mg/kg dosing level for both *Occimum basilicum* and *Allium sativum*.

CONCLUSION

Herbal medications are made only from medical plants, whereas traditional medicines are made from organic materials, minerals, and medicinal plants. An essential part of India's healthcare system is the long-standing practice of using plants as medication. Since the majority of medical professionals in India create and administer their recipes, this calls for appropriate recording and study. The absence of scientific & clinical evidence, as well as a greater comprehension of the safety and effectiveness of herbal products, is the main obstacle to the integration of herbal medications into contemporary medical procedures. In the past, edema was treated with herbs that activate the kidneys. Allium sativum and Occimum basilicum are two medicinal plants that are the subject of the current study's alcoholic extract. By employing ion-selective channel inhibition to identify the ions and electrolytes that the animals excrete, the current study will be very helpful in assessing the diuretic effect of extracts from Allium sativum and Ocimum basilicum leaves. Allium sativum and Occimum basilicum, two species that may not have demonstrated any toxicity but which the findings of the study could demonstrate to the human body, will undergo acute toxicity tests. Allium sativum and Occimum basilicum have diuretic action, which has some positive effects on the chosen animals. As a result, these herbs may be chosen for

additional research to demonstrate their clinical relevance.

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