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# EVALUATION OF ANTI-DIURETIC ACTIVITY USING A COMBINATION OF ETHANOLIC ROOT EXTRACTS OF ECLIPTA ALBA AND OCIMUM SANCTUM

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**Abstract:**

*The present study aimed to investigate the antidiuetic potential of ethanolic root extracts of Eclipta alba(EA) and Ocimum sanctum(OS) , two plants used in ayurvedic formulations. The extracts were obtained using the maceration technique with ethanol as the solvent. Primary phytochemicals screening revealed the presence of various bioactive compounds, including alkaloids, tannins, flavonoids, saponins, glycosides,carbohydrates,coumarins and polyphenols. The investigation utilized in vivo and in vitro methodologies to assess antidiuretic activity,employing Albino Wistar rats as the experimental model. The animals were randomly assigned to nine groups, including a normal control , a standard group administered vasopressin (0.13 mg/rat), and multiple test groups treated with either individual or combined doses of EA and OS. Urine volume, electrolytes excretion (sodium, potassium and chloride), and biochemical markers were measured to assess antidiuretic modulation. The fusion of EA and OS at 400 mg/kg is important in reducing urine output, showing comparable efficacy to vasopressin. These findings suggest that the extracts possess strong antidiuretic properties, likely due to their phytochemical fingerprinting. The results support the herbal use of these herbs and suggest their potential application in managing disorders related to fluid imbalance. The EA & OS at 400 mg/kg significantly reduce urine output compared to the control and furosemide groups ($p < 0.01$).* ***Keywords:*** *Eclipta alba, Ocimum sanctum, Antidiuretic activity, Ethanolic extract, Furosemide, Vasopressin, Wistar rats, Phytoconstituents.*

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## INTRODUCTION:

**DIURETIC ACTIVITY**

Diuretic activity refers to the promotion of urine production by the kidneys, leading to increased excretion of water and electrolytes such as $Na^+$, $K^+$ and $Cl^-$.The term is derived from Greek:
"diurus" = urination "diuresis" = increased urine formation. Diuretics are agents that stimulate diuresis & are commonly used to manage conditions related to fluid overload/hypertension.

**ANTIDIURETIC ACTIVITY:**

Antidiuretic activity refers to the ability to reduce urine formation, thereby promoting water reabsorption in the kidneys and conserving body fluids. It plays a vital role in fluid and electrolyte balance and blood pressure regulation.
The term "anti-diuretic" comes from:
"anti" = against
"Diuresis" means increased urine production.

**PLANT PROFILE:**

**Eclipta alba, Ocimum Sanctum**

**Eclipta alba**

Figure 1: Eclipta Alba

**Botanical Name :-** Eclipta prostrata.
**Synonyms :-***Eclipta alba* (L.) Hassk., *Eclipta erecta* L., *Eclipta punctata*
**Common names :-** False Daisy, Whitehead, Bhringraj, Galagara, Bhringraja
**Phylum:-** Magnoliophyta
**Family:-** Asteraceae (Daisy family)
**Genus:-** Eclipta
**Species:-** Eclipta prostrata

**Ocimum sanctum**

Figure 2: Ocimum sanctum

**Botanical Name :-** Ocimum tenuiflorum.
**Synonyms :-** *Ocimum sanctum* L., Ocimum *album* Vahl.
**Common names :-** Holy Basil, Sacred Basil, Tulsi, Thiruneetru Pachai.
**Phylum:-** Magnoliophyta
**Family:-** Lamiaceae (Mint family)
**Genus:-** Ocimum
**Species:-** Ocimum tenuiflorum.

## METHODOLOGY:

**COLLECTION AND AUTHENTICATION OF PLANT MATERIALS :**

The dried roots of Eclipta alba & Ocimum sanctum were collected from medicinal plant repository.

**CHEMICALS**:

Furosemide, Vasopressin, Ethanolic root extracts of Eclipta alba & Ocimum sanctum (100, 200, 400 mg/kg), Ethanol.

**EXTRACTION BY MACERATION:**

Powdered roots of *ECLIPTA Alba* were used. 500 ml of ethanol was added to the powdered roots in a porcelain container. The jar was kept in a sterile, darker place, covered with an alum sheet. Approximately 120 ml of methanol was administered twice daily. The mixture was stirred with a glass rod for 1 week. After a week, the extract was filtered using a muslin cloth. Filtration is carried into a wide-mouthed porcelain dish. Then it is filtered, and the filtrate is collected and evaporated in an open area to evaporate ethanol and obtain a concentrated extract. A thick, creamy residue was obtained. This extract is used for animal testing as per the dose determined by acute toxicity testing. Normal saline is used for reconstituting the extract at the time of dosing to animals.

**PHYTOCHEMICAL SCREENING TEST:**

Identify bioactive constituents in the extract, such as alkaloids, flavonoids, saponins, coumarins, tannins, carbohydrates, polyphenols, glycosides, etc. Photochemical analysis was executed as per the recognised technique specified in Practical Pharmacognosy by K.R. Khandelwal, Kokate, using only analytical-grade chemicals.

**ANIMAL EXPERIMENTATION:**

Empirical research began after receiving approval from the IAEC, under the guidelines of the CPCSEA, Government of India.
The study was conducted at Shadan Women's College of Pharmacy, Khairatabad, Hyderabad.
**IAEC Approval No.: IAEC-07/SES-2025/41/110**

**ACUTE ORAL TOXICITY STUDIES:**

An acute oral toxicity study for the ethanolic root extracts of Eclipta alba and Ocimum sanctum was carried out in rats as per OECD Guideline Nos. 423 & 425. The results of these studies reveal that even at the dose of 2000 mg/kg, no rat was killed. Therefore, the different extracts of Eclipta alba and Ocimum sanctum were found to be safe and nontoxic. The LD50 range was considered greater than 2000 mg/kg. The extracts were found to be safe, as no mortality was observed even at a higher dose of 2000 mg/kg.


(a) (b)

Figure 3: Acute Toxicity

**GROUPING OF ANIMALS**

| S.NO | TREATMENT | No. of rats |
|---|---|---|
| I. | Normal Saline 2 ml | 6 |
| II. | Toxic control 13 mg/kg | 6 |
| III. | Toxic control + Standard Furosemide, vasopressin (0.13 mg/rat) | 6 |
| IV. | Toxic control + (E.E – E.A) 200 mg/kg P.O | 6 |
| V. | Toxic control + (E.E – E.A) 400 mg/kg P.O | 6 |
| VI. | Toxic control + (E.E – E.A) 200 mg/kg P.O | 6 |
| VII. | Toxic control + (E.E – O.S) 400 mg/kg P.O | 6 |
| VIII | oxic control + (E.E – E.A) 100 mg/kg P.O + (E.E – O.S) 100 mg/kg P.O | 6 |
| IX. | Toxic control + (E.E – E.A) mg/kg P.O + (E.E – O.S) mg/kg P.O | 6 |

**SCREENING METHODS:**

**In vitro methods:**

I) **Carbonic Anhydrase Inhibition:**

Carbonic anhydrase, a Zn-dependent enzyme, facilitates reversible hydration and hydroxylation of $CO_2$, forming $H_2CO_3$. This carbonic acid then spontaneously dissociates—without enzymatic aid—into $H^+$ and $HCO_3^-$.

Blockade of carbonic Anhydrase leads to depletion in bicarbonate and sodium reconsumption with the aid of the $Na+/HCO_3^-$ cotransporter, leading to reduced water reabsorption.


The results were analysed to assess relevant parameters.

**Tu** = Time/o'clock taken for colour swap without enzyme.

**Te** = Time/o'clock span for colour swap in the presence of enzymes.

Rate of enzyme = Tu - **Te**

Rate of enzyme in the presence of inhibitor = Ti

% inhibition = 1 **(Tu - Te) - (Ti - Te) ÷ (Tu - Te) × 100 II) (SOD) Superoxide Dismutase:**

**SOD** is an enzyme with therapeutic potential that neutralises damaging **$O_2$-derived** molecules within cells. It plays a vital role in protecting against diseases mediated by reactive oxygen species (ROS)

Human WBC produce $O_2$ and other ROS to destroy bacteria, using enzymes like NADPH oxidase as stimulators.

**In vivo methods:**

**LIPSCHITZ TEST/DIURETIC ACTIVITY:**

**Purpose and Rationale:**

Evaluate the anti-diuretic effects of the test substance via urine output and electrolyte excretion.

Compare urine volume and electrolyte content after treatment.

Control: Saline; Standard: Furosemide.

Animal model: Wistar albino rats (150–250 g), fasted for 18 hrs, water allowed.

Group: I – Control (Saline), II – Std (Furosemide), III – Test groups (varied extract doses)

Dosage: oral/IP route, ~25 ml/kg

Metabolic Cages: Used to collect urine separately from feces

Observation Time: Usually 5 hrs, up to 24 hrs

Parameters Measured: urine volume (ml), electrolytes: $Na^+$, $Cl^-$, $Ca^{2+}$.

Interpretation: ↑Urine + electrolytes = diuretic effect,

↓ Urine + electrolytes = anti-diuretic effect.

Figure 6: Rats are arranged in metabolic cages for the Lipschitz test.

**Results:**

**PHYTOCHEMICAL EVALUATION:**

Figure 8: Phytochemical test for Eclipta alba

Figure 9: Phytochemical test for Ocimum sanctum

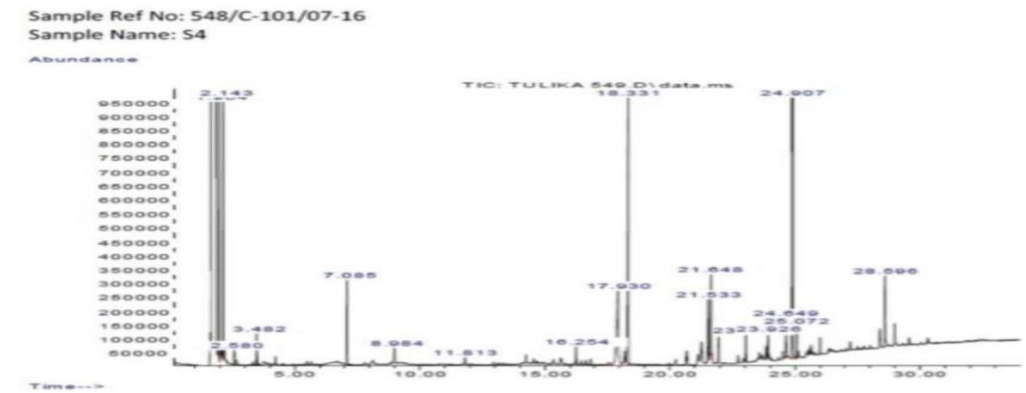
| S. NO. | CHEMICAL CONSTITUENTS | TSET | PLANT 1:EA PRESENCE | PLANT 2: OS PRESENCE |
|---|---|---|---|---|
| I | Alkaloids | Wagner's test<br>Mayer's test<br>Hagr's test<br>Dragendorff's test | +++<br>++ ++<br>+ | +++<br>++<br>+<br>++ |
| II. | carbohydrates | Molisch's Test<br>Fehling's<br>Benedict's | -<br>-<br>- | -<br>-<br>- |
| III. | Reducing sugars | Benedict's | +++ | ++ |
| IV. | Flavonoid's | Alkaline Reagent<br>$FeCl_3$ | +++<br>++ | +++<br>++ |

| | | | | |
|---|---|---|---|---|
| V. | Phenolic comp | $FeCl_3$ | ++ | + |
| VI. | Tannins | $Br_2$(aq)<br>Ferric chloride<br>Pb acetate test<br>$CH_3COOH$ acid sol | +++<br>++ ++<br>+ | ++<br>++<br>+<br>+ |
| VII. | saponins | Froth's | ++ | ++ |
| VII I. | terpenoids | Salkowski's | ++ | - |
| IX. | Sterols phytosteroids & | Liebermann–Burchard Test | ++ | +++ |
| X. | Caumarin's | Ferric chloride | ++ | ++ |
| XI. | Glycosides | Legitimate<br>Brontragr's test | -<br>- | +<br>- |

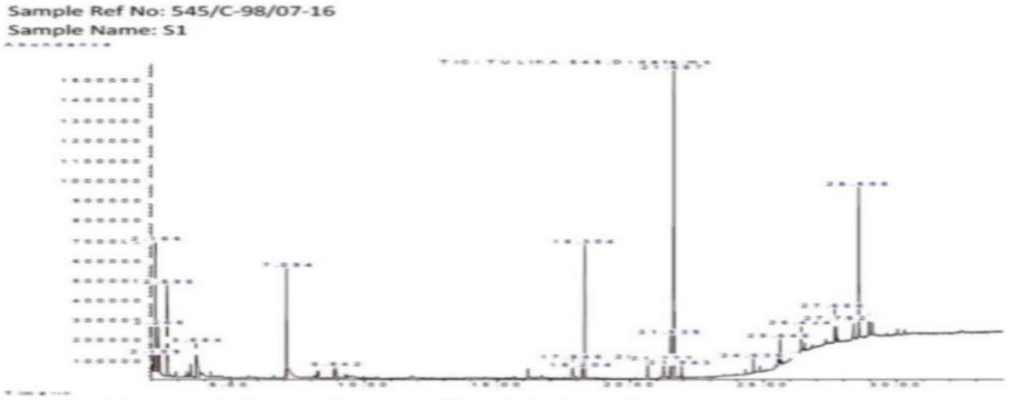
+ indicates presence, and - indicates absence

**3. GC-MS ANALYSIS: GC-MS of Ethanolic Extract of Eclipta Alba**:

| S.NO | RT TIME | CHEMICAL CONSTITUENTS | PEAK | AREA % | USE |
|---|---|---|---|---|---|
| 1 | 17.93 | n- Hexadecanoic acid | 256 | 7.18 | Palmitic acid ester Anti-oxidant,Hypocholesterolemic, Nematicide, Anti-androgenic, Hemolytic, Pesticide, Lubricant, 5-Alpha reductase inhibitor,antipsychotic. |
| 2 | 18.33 | Hexadecanoic acid, ethyl ester | 284 | 13.29 | Palmitic acid ester Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic. |
| 3 | 21.64 | 9,12,15-Octadecatrienoic acid, methyl ester | 292 | 2.7 | Steroid Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, anti diuretic. |
| 4 | 24.64 | Hexadecanoic acid, 2- hydroxy-1-(hydroxymethyl) ethyl ester | 330 | 0.96 | Amino compound Hemolytic, pesticide, flavour, antioxidant. |
| 5 | 24.90 | Diisooctyl phthalate | 390 | 53.84 | Plasticizer Antimicrobial, Antifouling |
| 6 | 28.59 | Stigmasterol | 412 | 2.57 | Steroid Antioxidant, hypoglycemic and thyroid inhibiting properties, precursor of progesterone, antimicrobial, anticancer,anti-arthritic, anti-asthama, antiinflammatory, anti diuretic. |
| 7 | 11.81 | L-Glutamine | 146 | 0.38 | Amino acid Building block of Protein |



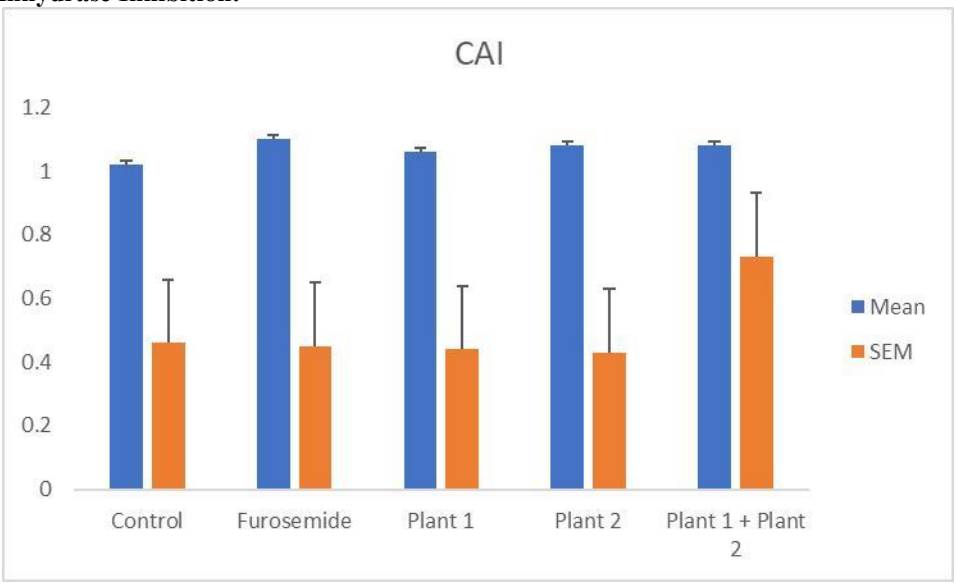

**GC-MS of Ethanolic Extract of Ocimum Sanctum:**



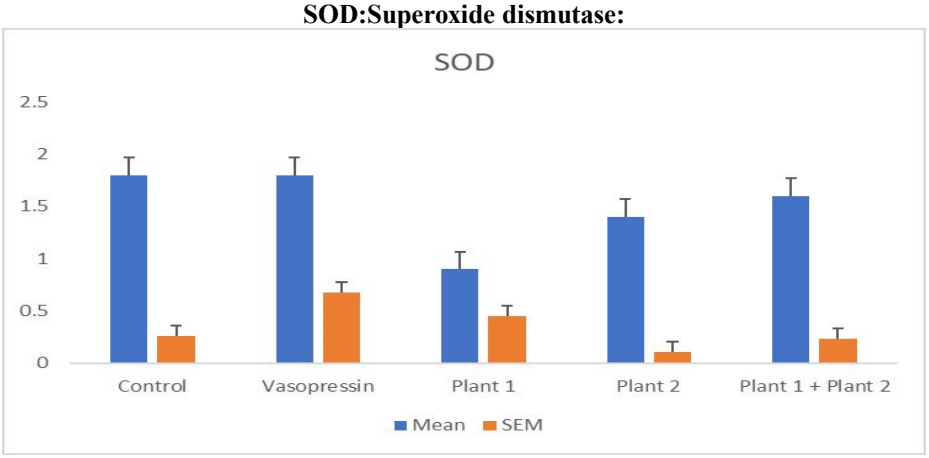

| S.NO | RT TIME | CHEMICAL CONTITUENT | PEAK | AREA % | USES |
|---|---|---|---|---|---|
| 1 | 17.93 | n-Hexadecanoic acid | 256 | 2.34 | Palmitic acid ester, Antioxidant, Hypocholesterolemic,Nematicide, Anti-androgenic, Flavor, Hemolytic , antidiuretic |
| 2 | 18.2 | E-11-Hexadecanoic acid, ethyl ester | 282 | 1.04 | Stearic acid Antifungal, Anti-tumour, Antibacterial |
| 3 | 18.3 | Palmitic acid, ethyl ester | 284 | 12.09 | Stearic acid Antifungal, Anti-tumour, Antibacterial |
| 4 | 20.66 | Phytol | 296 | 2.12 | Diterpene Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal against S. typhi, resistant gonorrhea, joint dislocation,headache, hernia, stimulant and antimalarial |
| 5 | 21.53 | 9,12 Octadecadienoic acid, ethyl ester | 308 | 3.79 | Polyenoic fatty acid Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic |
| 6 | 21.65 | Linolenic acid, ethyl ester | 306 | 26.26 | Linoleic acid ethyl ester Hypocholesterolemic, Nematicide, Anti arthritic, Hepatoprotective Anti-androgenic, Hypocholesterolemic, 5-Alpha reductaseinhibitor, Antihistaminic, Anti coronary, Insectifuge, Anti-eczemic, Anti-acne |
| 7 | 21.94 | Stearic acid, ethyl ester | 312 | 0.98 | Fatty ester No activity reported. |
| 8 | 24.63 | Hexadecanoic acid, 2hydroxy-1(hydroxymeth l) ethyl ester | 330 | 0.87 | Amino compound Hemolytic, pesticide, flavour, antioxidant. |

**4. In vitro Studies:**

**Carbonic Anhydrase Inhibition:**

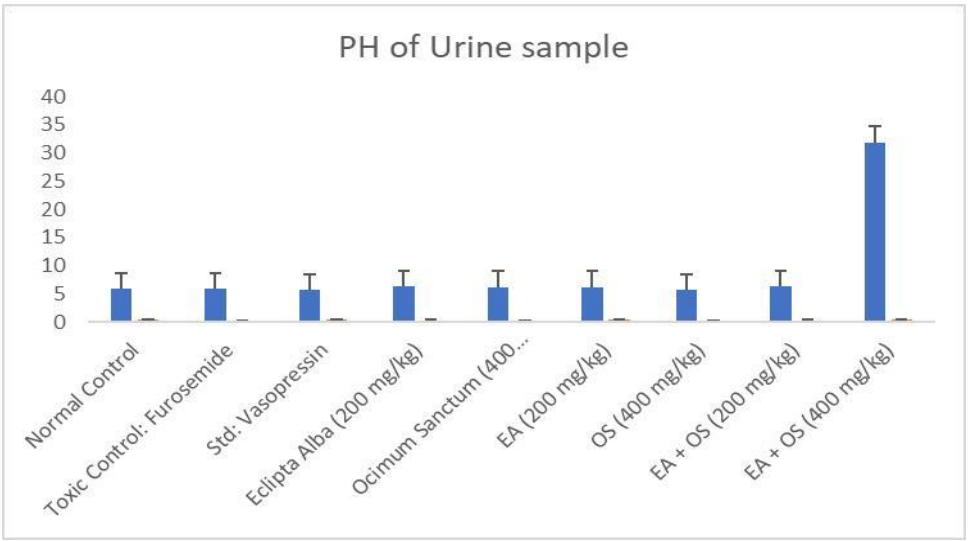



**SOD:Superoxide dismutase:**




**INVIVO RESULTS:**

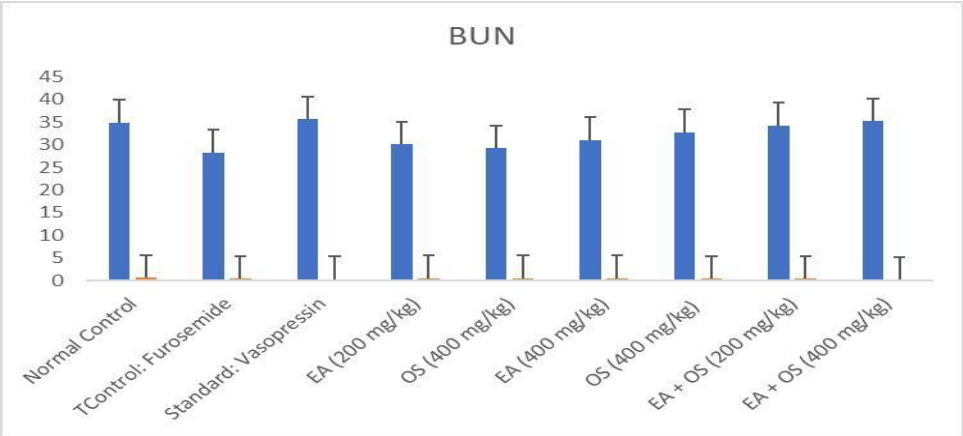
**PH OF URINE SAMPLES:**



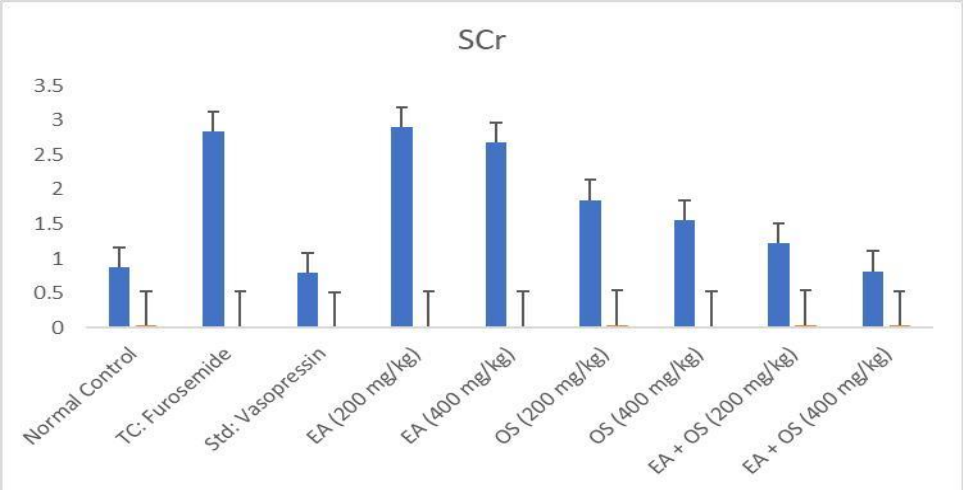
**FLAME PHOTOMETRY:**

| CONSTITUENT | EMISSION WAVELENGTH | SHADE OF FLAME |
|---|---|---|
| K+ | 765 nm | violet |
| Na+ | 591 nm | yellow |
| Ba+2 | 553 nm | Lime green |
| Ca+2 | 663nm | orange |
| Li+ | 672 nm | red |

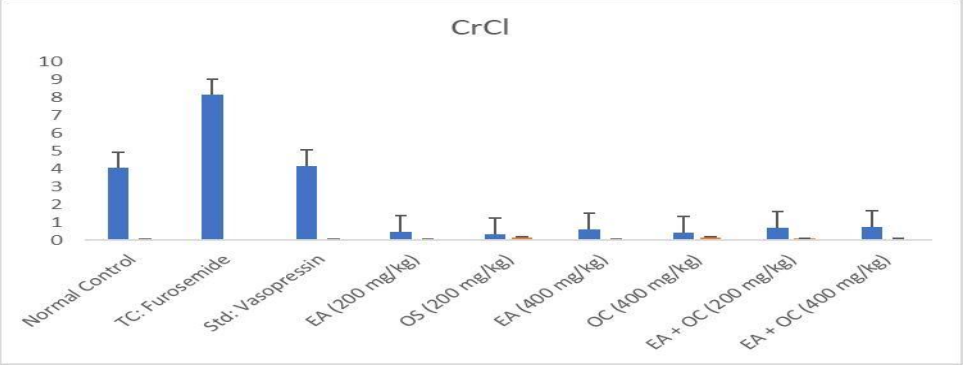
**BUN: BLOOD UREA NITROGEN:**



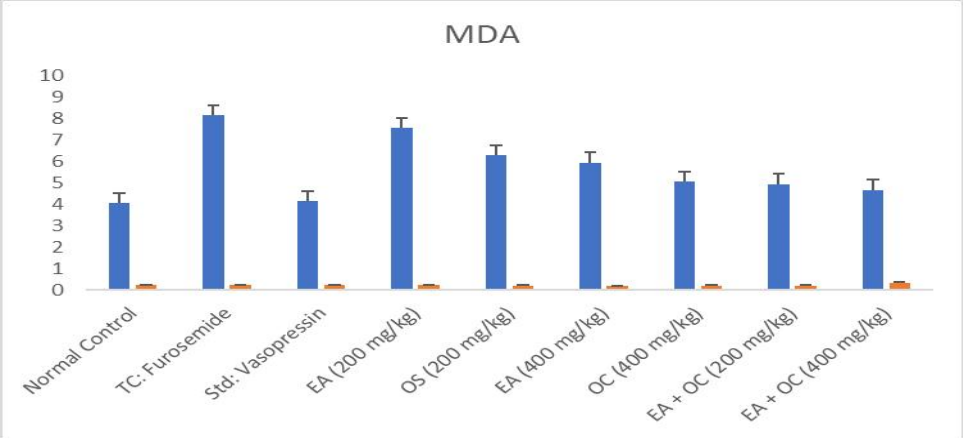

**Serum Creatanine:**



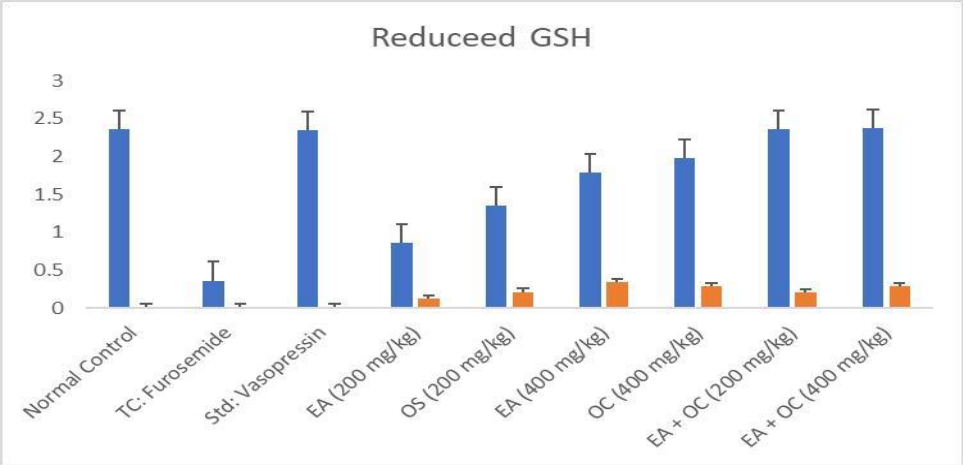

**Creatinine Clearance:**



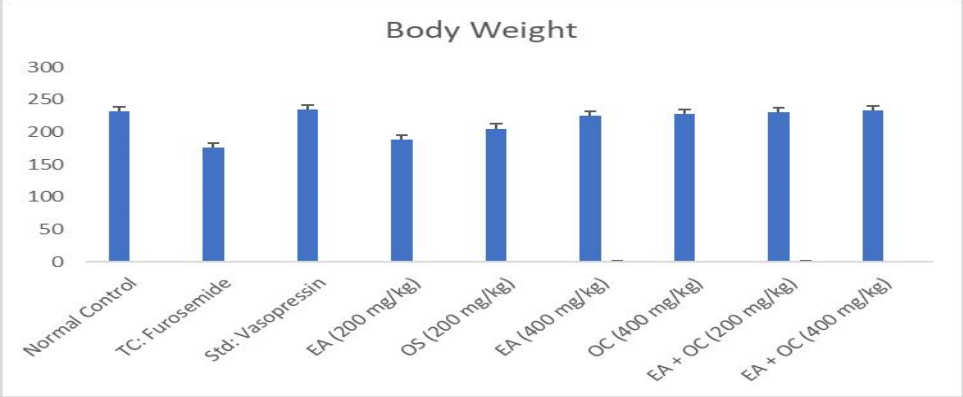

**MDA:**



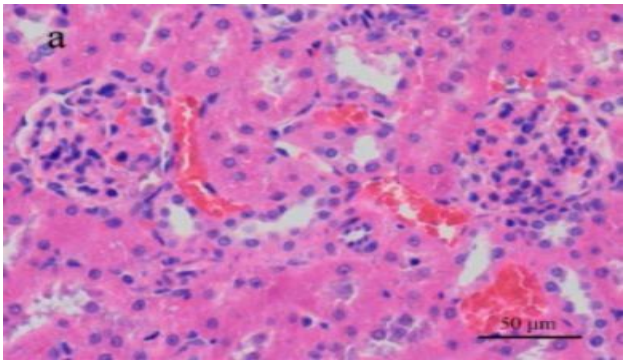

**GSH: REDUCED GLUTATHIONE:**



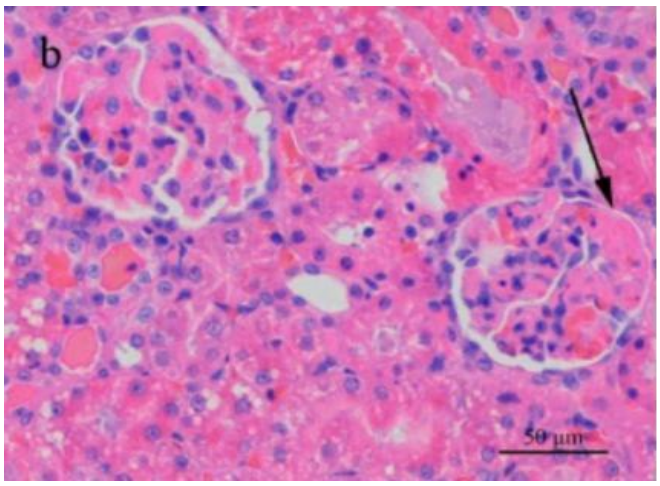

**BODY WT:**



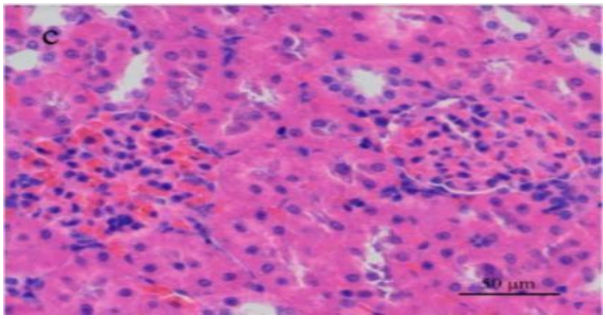

**HISTOLOGICAL RESULTS:**



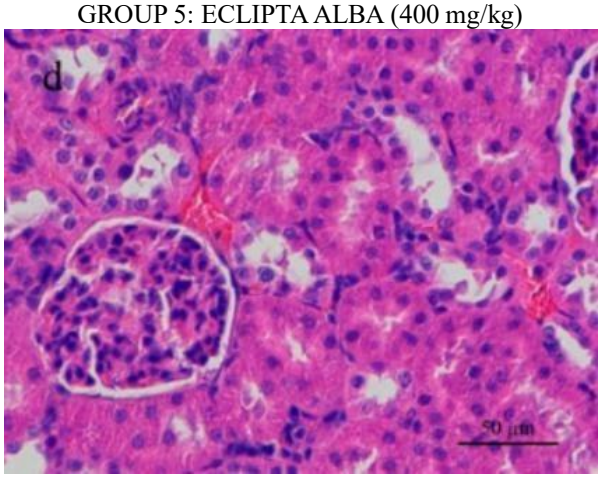

GROUP 1: NORMAL SALINE



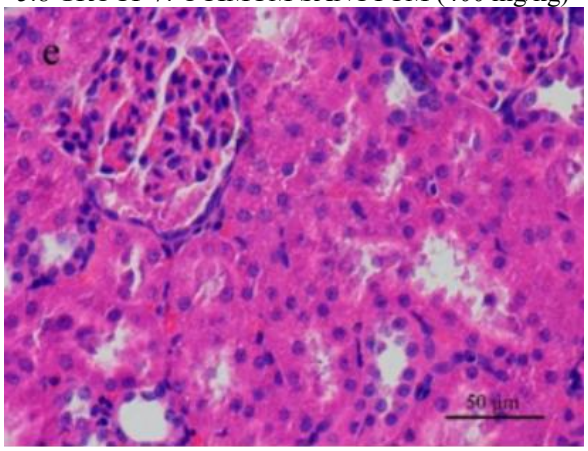

GROUP 2: FUROSEMIDE



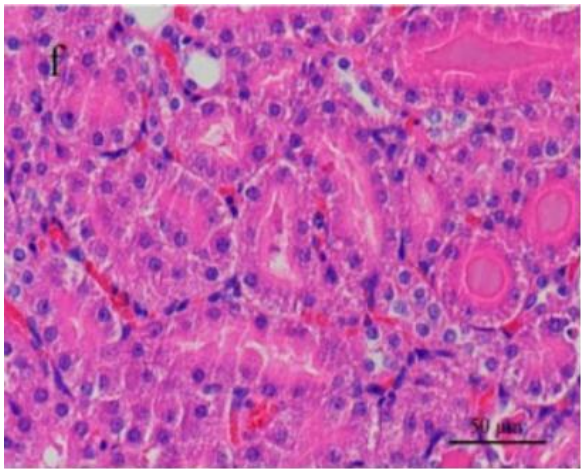

GROUP 5: ECLIPTA ALBA (400 mg/kg)




5.8 GROUP 7: OCIMUM SANCTUM (400 mg/kg)




5.9 GROUP 8: ECLIPTA ALBA + OCIMUM SANCTUM (200 mg/kg)




5.10 GROUP 9: ECLIPTA ALBA + OCIMUM SANCTUM (400 mg/kg)

## DISCUSSION:

**Herbal Therapy, Anti-diuretic Study:**

Herbal remedies such as EA & OS offer a wide range of health benefits. This research evaluated the antidiuretic effects of their ethanolic root extract. Diuretics increase urine output, aiding conditions like CHF, diabetes, and kidney stones. Antidiuretics reduce urine output, maintaining body fluids and electrolytes – vital for managing dehydration and fluid imbalance.Experimental setup: 54 male albino Wistar rats (150-200 g), 9 groups (6 rats each), 21-day period. Groups included normal saline, standard vasopressin, furosemide and varying doses of extract.

Test conducted: urine electrolytes analysis: Na+, Ca+2, K+, Ba+Li+ (flame photometry)
Lipschitz test: antidiuretic/diuretic activity; SOD assay: antioxidant activity; CAI assay: renal function support.

## CONCLUSION:

EA & OS root extract shows significant antidiuretic activity, confirmed through urine volume reduction and the Lipschitz test. It is effective in both single & combined doses; there is a reduction in urine output and enhanced fluid retention with these extracts. Electrolyte analysis supported fluid conversion.
Superoxide dismutase indicates antioxidant, and carbonic anhydrase inhibition supports the renal system.
Clinical relevance: useful for managing fluid loss in diabetes, post-surgery & vascular conditions In-depth examinations are needed to uncover functional plant metabolites and explore how they exert their effects on renal as well as hormonal pathways.

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