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PHARMACOLOGICAL EVALUATION OF DIOSCOREA BATATAS AND URTICA DIOICA ETHANOLIC ROOT EXTRACTS FOR ANTIDIURETIC ACTIVITY IN FUROSEMIDE INDUCED ALBINO WISTAR RATS

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ABSTRACT

This study provides an extensive evaluation of the pharmacological effects of two herbal extracts, Dioscorea batatas and Urtica dioica, on kidney function and oxidative stress in albino Wistar rats. The investigation focused on their influence on saluretic activity, carbonic anhydrase activity, electrolyte regulation, blood pressure, oxidative stress biomarkers and renal clearance. Experimental rats were divided into six groups, receiving either plant extracts or controls and multiple physiological and biochemical parameters were recorded. Statistical analyses were carried out using standard deviation (SD) and standard error of mean (SEM) to determine significant differences between groups. The findings demonstrate that both extracts affect renal physiology, with Dioscorea batatas showing stronger activity on electrolyte handling, oxidative stress reduction and clearance mechanisms compared with Urtica dioica. The outcomes indicate a need for additional exploration into the therapeutic potential and safety profile of these extracts in renal health management.

KEYWORDS

Saluretic activity, Dioscorea batatas, Urtica dioica, Renal function and Oxidative stress.

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INTRODUCTION

Diuretics are agents that promote an increase in urine output, thereby enhancing the elimination of excess fluid from the body. Their primary mode of action involves altering the handling of electrolytes such as sodium and chloride by the kidneys. Depending on the class of diuretic, this effect may occur at different sites of the nephron, leading to increased excretion of sodium and water. Conversely, antidiuretic agents, such as

vasopressin, limit water excretion and promote fluid retention. The kidneys play a central role in fluid and electrolyte balance, regulating sodium, chloride and water reabsorption. As sodium and chloride retention is directly proportional to water conservation, disturbances in this mechanism directly influence fluid homeostasis. Understanding these pathways is vital for identifying natural agents with diuretic or antidiuretic potential.

MATERIAL AND METHODS

Chemicals

Induction of Diuresis: Furosemide
Standard Antidiuretic Agent: Vasopressin
Extraction: Ethanol, Normal Saline
Sample Preservation for Histopathology: Formaldehyde solution

Plant Collection and Authentication

Roots of Dioscorea batatas and Urtica dioica were collected, identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor of Botany, Department of Pharmacognosy, Sri Venkateshwara College, Tirupathi.

Extraction Procedure (Maceration Method)

The powdered roots of both plants were separately soaked in 500mL of ethanol using porcelain jars. Each mixture was covered with aluminum foil and stored in a dark, sterile environment. Approximately 120mL of ethanol was replenished daily, with stirring twice a day for one week. On the final day, the mixtures were filtered using sterile muslin cloth and the filtrates were evaporated at room temperature until a thick paste was obtained. The residues were weighed and stored in airtight glass containers.

Standard Drug Preparation

Commercially available C-Pressin injection (vasopressin) was used as the standard drug. A solution of 0.13mL/rat was prepared in distilled water and administered intramuscularly.

Experimental Animals

Adult male albino Wistar rats (100-200g) were used. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC-04/SES-2023/41/104), Shadan Educational Society, Hyderabad. Rats were fasted overnight but allowed free access to water until the start of the experiment. During the 5-hour experimental period, food and water were restricted.

Animals were divided into six groups (n=6 each). All rats were pretreated with 25mL/kg of 0.9% saline intraperitoneally. Groups were assigned as follows:

Urine samples were collected in metabolic cages free from fecal contamination and total urine volume was recorded after 5 hours.

Acute oral toxicity study

Acute toxicity was evaluated following OECD guideline 425. Rats were administered a single oral dose of 2000mg/kg and observed closely for the first 3-4 hours, then periodically up to 48 hours. No signs of toxicity were observed, enabling further experimentation with graded doses.

Screening Methods

In vitro: Isolated tubule preparation, Carbonic anhydrase inhibition, Patch-clamp technique. *In vivo*: Lipschitz test, Saluretic activity in rats, Stop-flow technique, Clearance method, Micropuncture technique.

FLAME PHOTOMETRY

Compounds are determined in Spectrometer in the configuration of particles which is also referred to as Flame Atomic Emission Spectrometry. Photoelectric flame photometer measures the amount of ions like Sodium, Potassium, Lithium etc. This methodology was introduced by Bunsen and Kirchoff in the 19th century confirmed that various elements discovered in the flame are in-charge for the emission of radiation from the flames. The Wavelength of the flames tells us about the sort of element present and strength of the color of flame also indicates the aggregate of constituent present.

RESULTS AND DISCUSSION

The study includes a wide range of physiological and biochemical parameters measured in rats subjected to various experimental conditions. These parameters offer valuable insights into the impact of

different treatments on renal function and oxidative stress. In this discussion, we will analyze and interpret the data, considering the implications of each parameter on renal health and overall physiological well-being.

Saluretic and Natriuretic Activities

The saluretic activity (measured as the excretion of Na+ and Cl−) and natriuretic activity (Na+/K+ ratio) provide insights into the renal handling of electrolytes. Group-2 shows the highest saluretic and natriuretic activities, suggesting an enhanced ability to excrete sodium and chloride ions. Conversely, Group-1 exhibits the lowest values, indicating reduced electrolyte excretion.

The ion quotient Cl/(Na + K) is an important index that reflects the balance between sodium, potassium and chloride ions. Groups 2, 4, 5 and 6 exhibit higher ion quotient values, indicating a potential disturbance in the balance of these ions. These findings may be indicative of altered renal tubular function.

Carbonic Anhydrase Activity

Carbonic anhydrase is an enzyme involved in acid-base balance and ion transport. Lower carbonic anhydrase activity in Group-2 could affect the renal handling of bicarbonate ions and pH regulation. This may contribute to the observed differences in saluretic and natriuretic activities in this group.

Lipschitz Test

The Lipschitz test measures the composition of urine, including sodium, potassium, and chloride concentrations. Group-2 has the highest sodium and chloride concentrations, indicating increased sodium retention and altered electrolyte balance. This corresponds with the high saluretic and natriuretic activities observed in this group.

Serum Creatinine and Blood Urea Nitrogen (BUN)

Increased serum creatinine and BUN levels in Groups 2, 4, 5 and 6 suggest impaired renal function. Elevated levels of these markers are indicative of reduced glomerular filtration rates and impaired clearance of waste products, potentially due to the observed hypertension and electrolyte imbalances.

Creatinine Clearance

Creatinine clearance values are lower in Groups 2, 4, 5 and 6 further indicating compromised renal function. These findings align with the elevated serum creatinine levels observed in these groups.

Oxidative Stress Markers (MDA, SOD, CAT, GPx)

Increased malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in Groups 2, 4, 5 and 6 suggest heightened oxidative stress. This oxidative stress may contribute to renal dysfunction and may be linked to the observed hypertension and electrolyte imbalances.

Micropuncture and Stop-Flow Technique Clearance

The micropuncture data shows differences in proximal and distal tubular fluid flow rates, which may indicate alterations in tubular function. The stop-flow technique clearance data suggests impaired renal clearance in Groups 2, 4, 5 and 6 further supporting the evidence of renal dysfunction in these groups.

In conclusion, the data provides comprehensive insights into the renal function and overall physiological status of rats subjected to various experimental conditions. Groups 2, 4, 5 and 6 consistently show signs of impaired renal function, oxidative stress, and hypertension. These findings suggest a potential link between electrolyte imbalances, oxidative stress and renal dysfunction. Further research is needed to explore the underlying mechanisms and potential interventions to mitigate these adverse effects. This dataset serves as a valuable foundation for future investigations into renal physiology and pathology.

Table No.1: Experimental Animals

Groups	Drugs	Dose and route
Group-1	Normal saline	2ml/kg p.o
Group-2	Toxic control	13mg/kg p.o
Group-3	Toxic control + standard drug	0.13ml/rat p.o
Group-4	Toxic control + Test (Dioscorea Batatas)	200mg/kg p.o
Group-5	Toxic control + Test (Urtica Dioica)	200mg/kg p.o
Group-6	Toxic control + Test (Dioscorea Batatas + Urtica Diocia)	(100mg/kg)+(100mg/kg) p.o

Table No.2: Phytochemical screening

S.No	Chemical constituent	Test	Extract 1	Extract 2
1	Tannins	Ferric chloride test	+	+
		Lead acetate test	+	+
		Acetic acid sol.	+	+
		Dil. Iodine sol.	-	+
2	Alkaloids	Mayer's test	+	+
		Dragendroff's test	+	+
		Hager's test	+	+
		Wagner's test	+	+
3	Glycoside	-	-	+
4	Cardiac glycosides	Baljet's test	-	+
		Legal's test	+	+
		Keller-killiani test	-	+
		Liebermann's test	+	+
5	Steroids	Salkowski test	+	+
		Liebermann-burchard test	+	+
		Liebermann's test	+	+
6	Saponins	Foam test	+	+
7	Flavonoids	Schinoda test	-	+
		Lead acetate test	-	+
		NaOH test	-	+
8	Anthraquinones	Borntrager's test	-	+
		Modified-borntrager's test	-	+
9	Carbohydrates	Molisch test	+	+
		Fehling's test	+	+
		Benedict's test	+	+
10	Proteins	Biuret's test	-	+
		Millon's test	-	+

Table No.3: GCMS analysis of dioscorea batatas

S.No	RT Time	Chemical Constituents	Peak	Area %	Uses
1	17.93	n-Hexadecanoic acid	2.56	7.18	Palmitic acid ester Anti-oxidant, Hypocholesterolemic, Nematicide, Anti-androgenic, Hemolytic, Pesticide, Lubricant, 5-Alpha reductase inhibitor, antipsychotic
2	18.33	Hexadecanoic acid	284	13.29	Palmitic acid ester Anti-oxidant, 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 duretic
4	24.64	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	330	0.96	Amino compound Hemolytic, pesticide, flavor, antioxidant
5	24.90	Dusooctyl 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, anti-inflammatory, anti-diuretic
7	11.81	L-Glutamine	146	0.38	Amino acid building block of protein

Table No.4: GCMS analysis of urtica dioica

S.No	RT Time	Chemical Constituents	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	284	12.09	Staric acid, Anti-tumour, Antibacterial
3	18.3	Palmitic acid, ethyl ester	284	12.09	Staric acid, Anti-tumour, Antibacterial
4	20.66	Phytol	296	2.12	Diterpene Antimicrobial, Anti-inflammatory, Anticancer, Diuretic, Antifungal aganinst S-typhi, resistant gonorrhea, Joint dislocation, headache, Hermia, stimulant and antimalarial
5	21.53	9.12 Octadecadienoic acid, ethyl ester	308	3.79	Polyenoic fatty acid, Hepatoprotective, anti-histastaminic, 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, S-Alpha reductaseinhibitor, anti-histastaminic, Anti coronary, Insectifigue, Anti-eczemic, anti-acne
7	21.94	Stearic acid, ethyl ester	312	0.98	Fatty ester No activity reported
8	24.63	Hexadecanoic acid, 2 hydroxy-1-(hydroxymethyl) ethyl ester	330	0.87	Amino compound Hemolytic pesticide, flavor, antioxidant

Table No.5: PH of urine samples

S.No	Group	Mean+Sem
1	Normal Control	5.83 ± 0.4013
2	Toxic Control: Furosemide	5.83 ± 0.3073
3	Standard: Vasopressin	5.6 ± 0.401
4	DB (200mg/kg)	6.0 ± 0.33
5	DB (200mg/kg)	6.12 ± 0.307
6	DB+UD (100mg/kg)	6.24 ± 0.401

Table No.6: Flame Photometry

S.No	Constituent	Emission Wavelength	Shade of Flame
1	Potassium	764nm	Violet
2	Sodium	590nm	Yellow
3	Barium	552nm	Lime green
4	Calcium	666nm	Orange
5	Lithium	669nm	Red

Figure No.1: Chat experimental animals

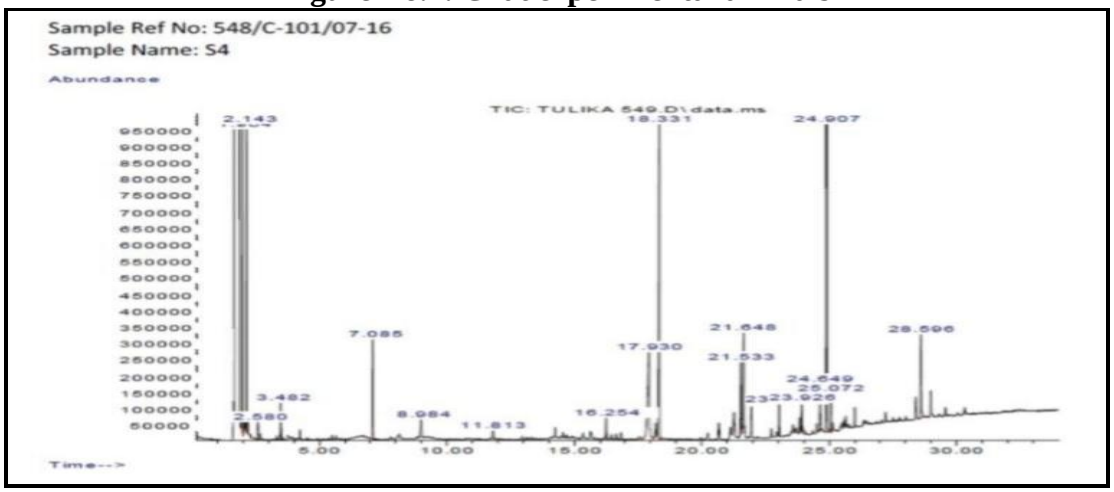


Figure No.2: Phytochemical

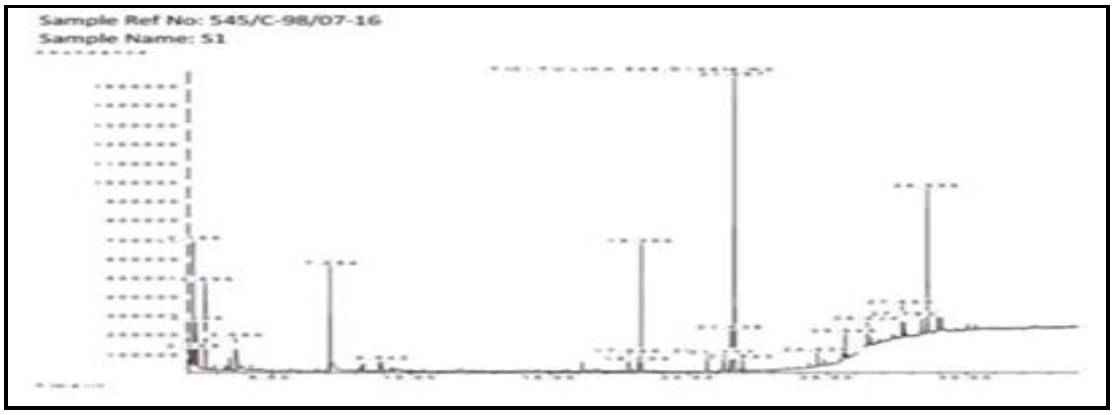
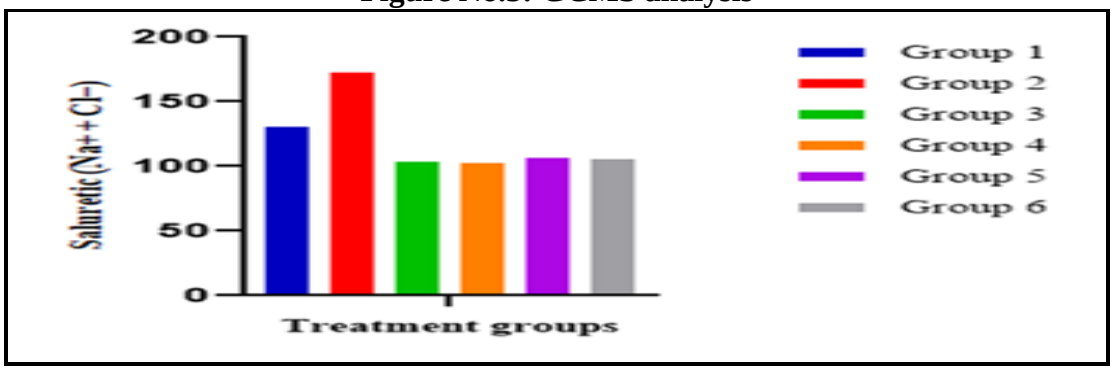
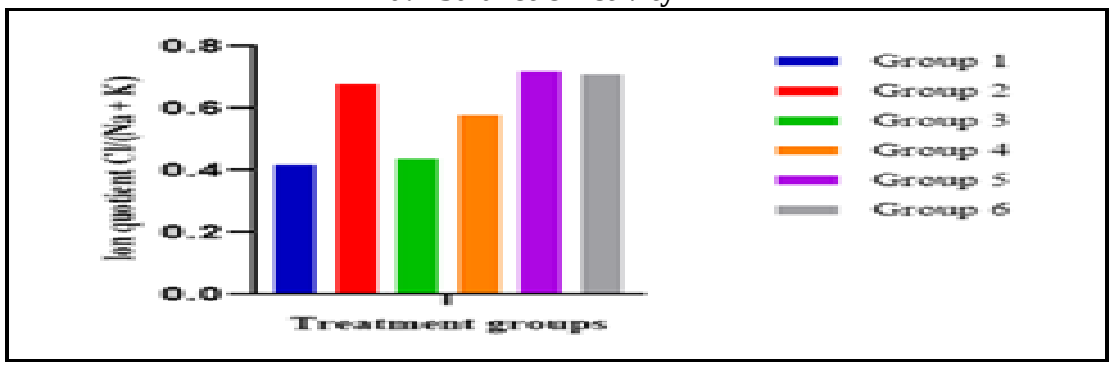


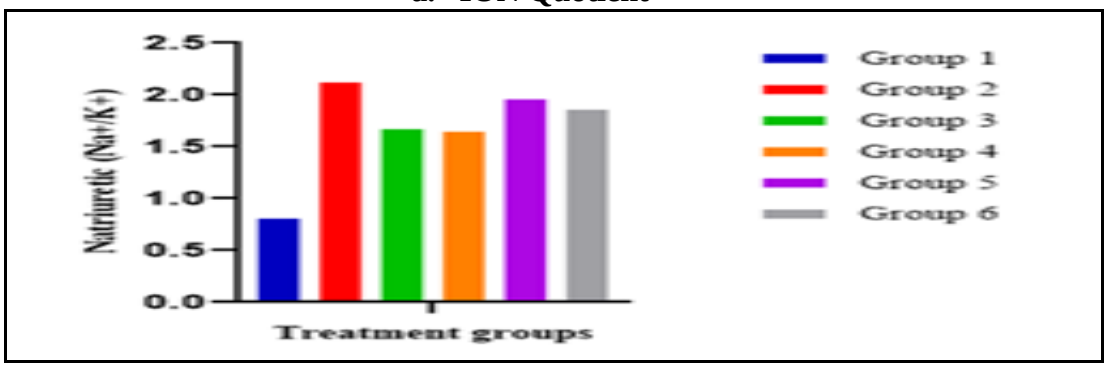
Figure No.3: GCMS analysis



a. Saluretic Activity



a. ION Quotient



1. Natriuretic Activity

Figure No.4: Saluretic Activity in rats

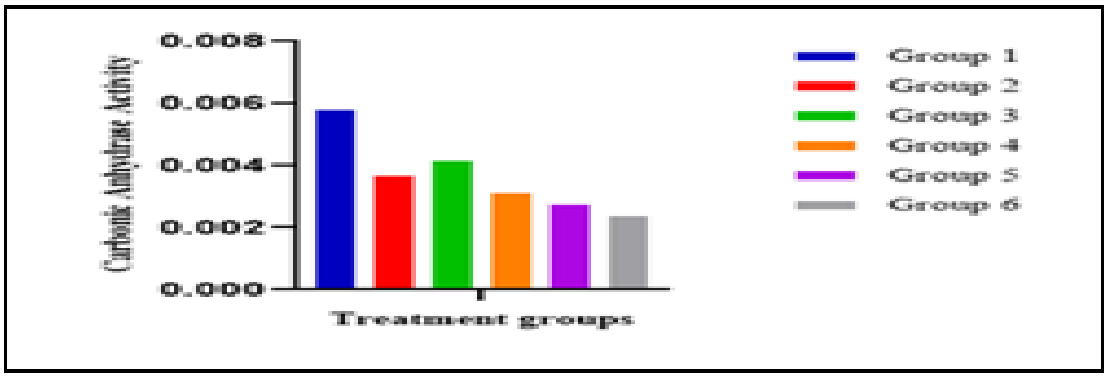


Figure No.5: Carbonic anhydrase activity

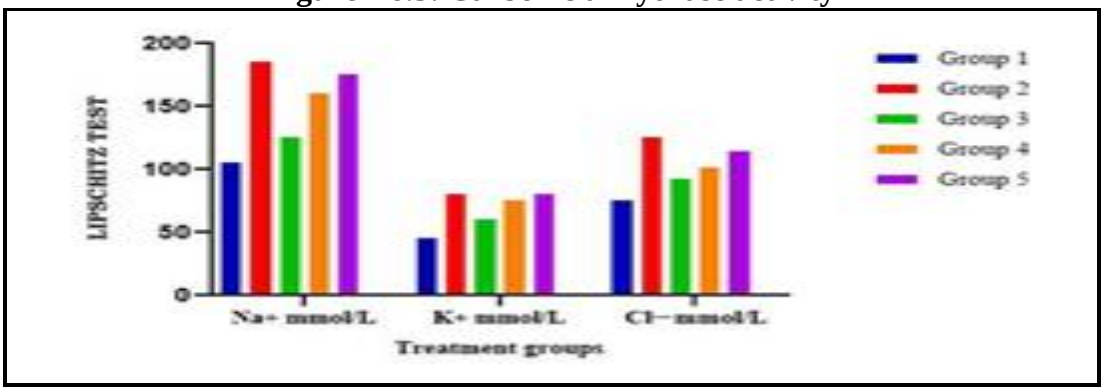


Figure No.6: Lipschitz test

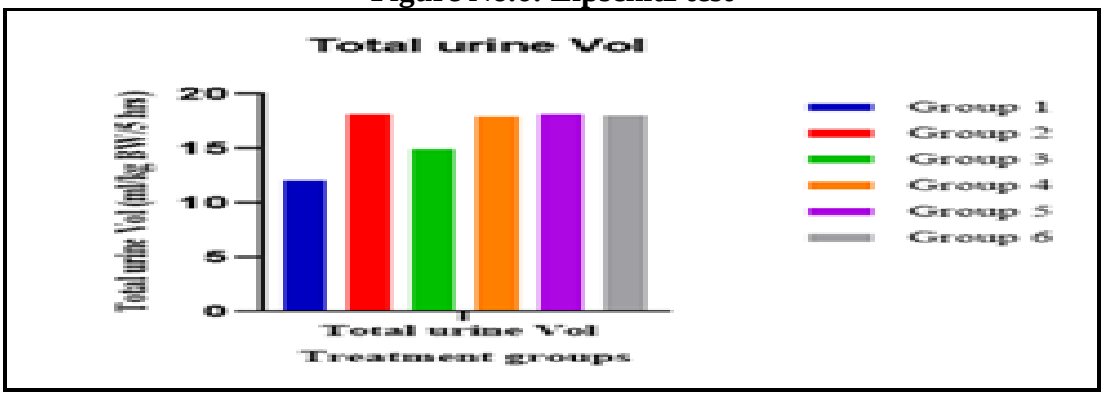


Figure No.7: Assessment of Serum creatinine level

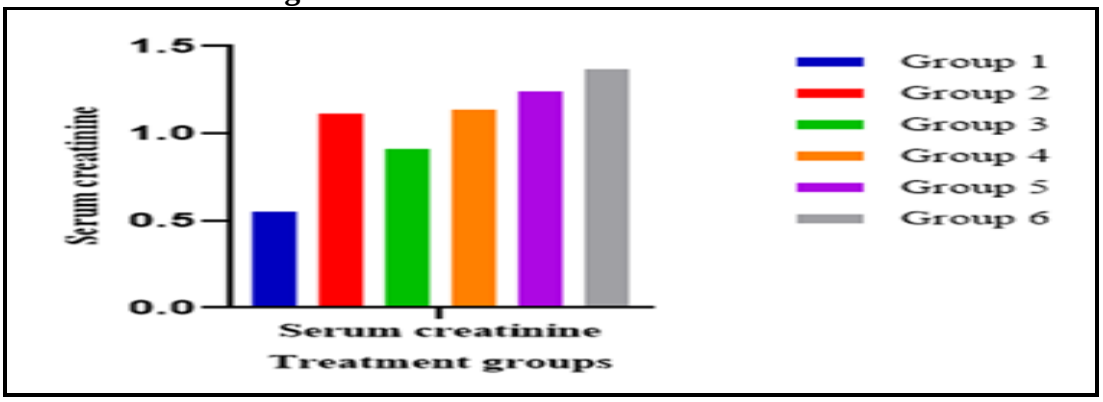


Figure No.8: Serum Creatinine Level

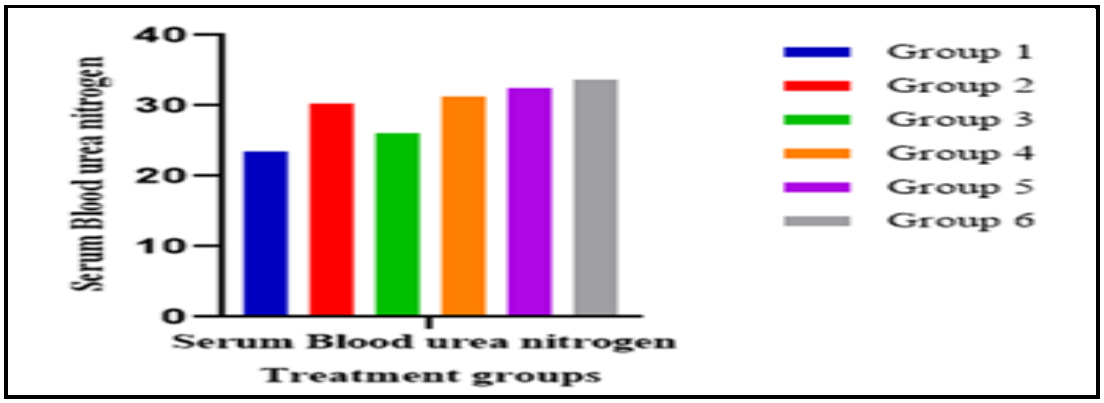


Figure No.9: Serum Blood urea nitrogen (BUN)

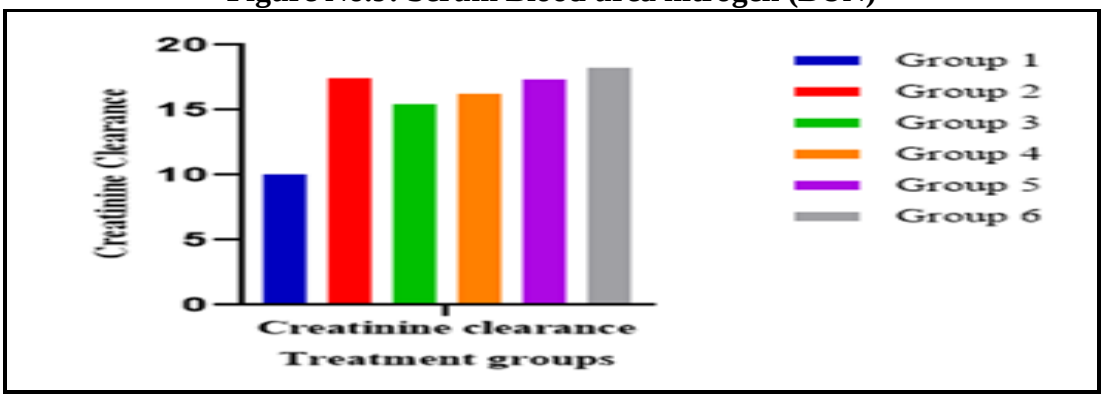


Figure No.10: Assessment of Creatinine Clearance

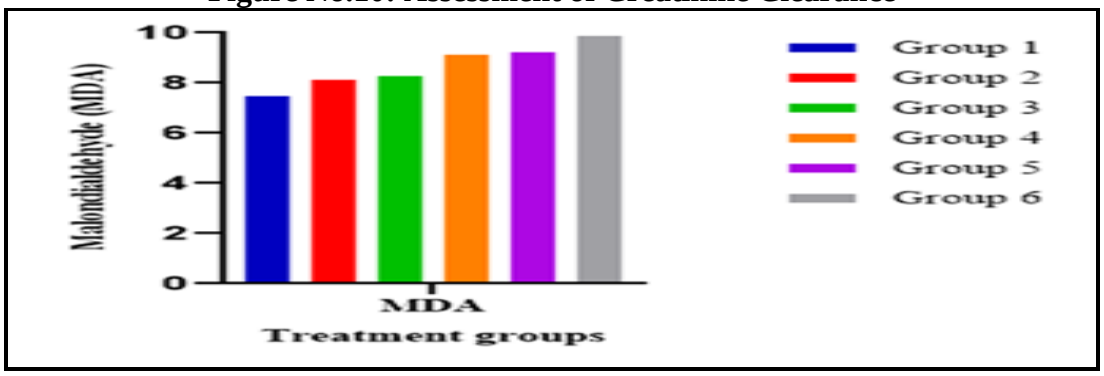


Figure No.11: Assessment of Oxidative Stress malondialdehyde (MDA) parameter

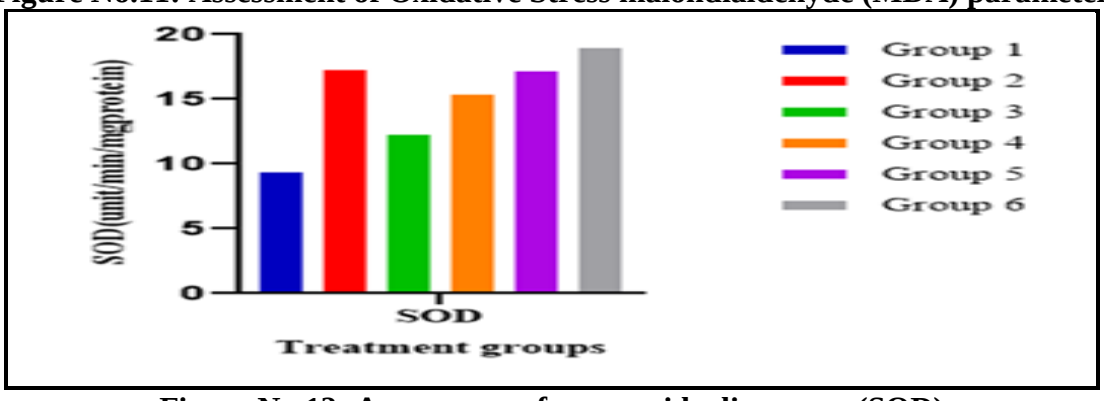


Figure No.12: Assessment of superoxide dismutase (SOD)

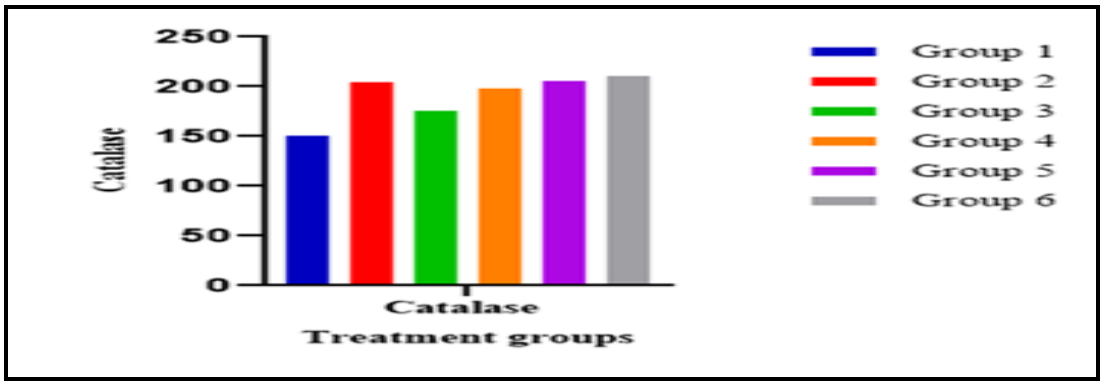


Figure No.13: Assessment of Catalase (CAT)

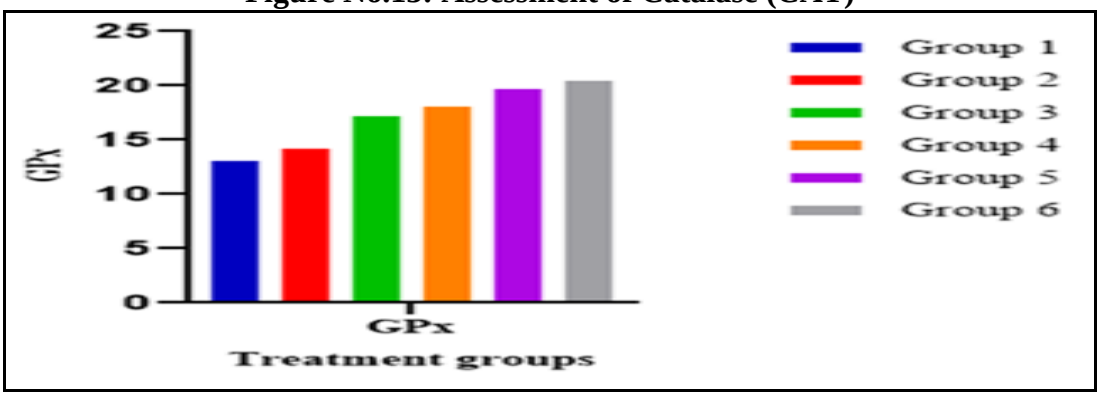


Figure No.14: Assessment of Glutathione peroxidise (GPx)

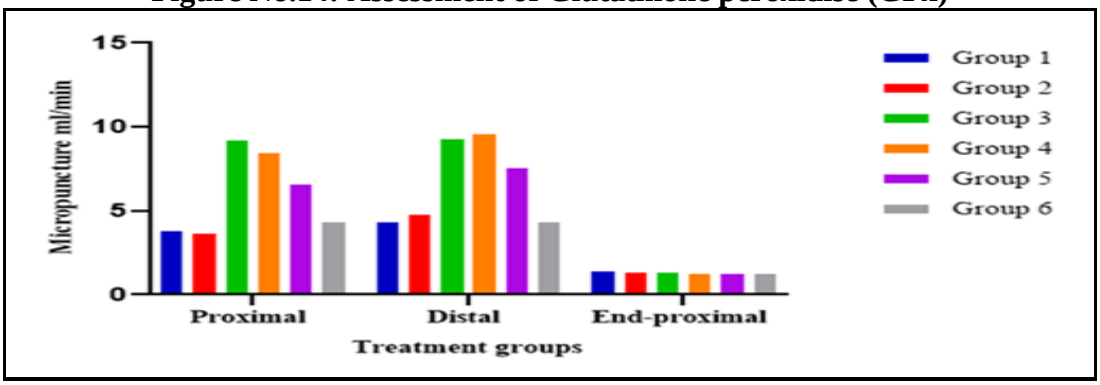


Figure No.15: Micropuncture

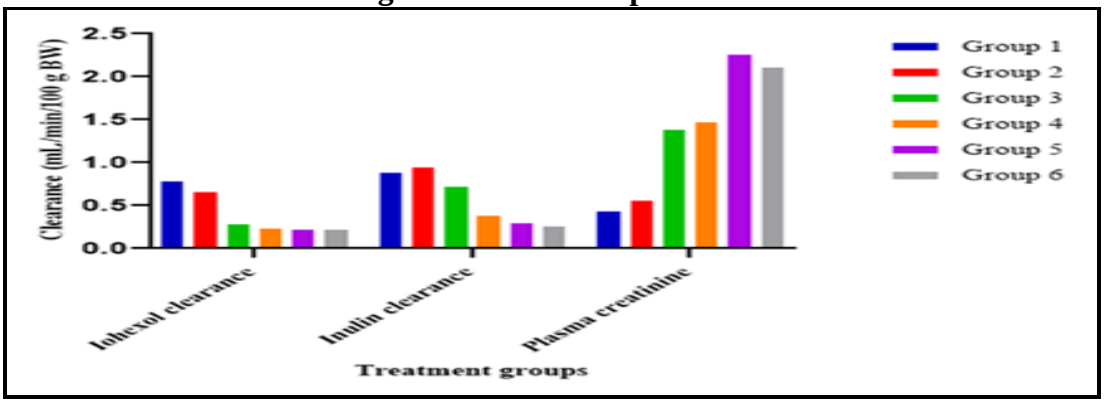
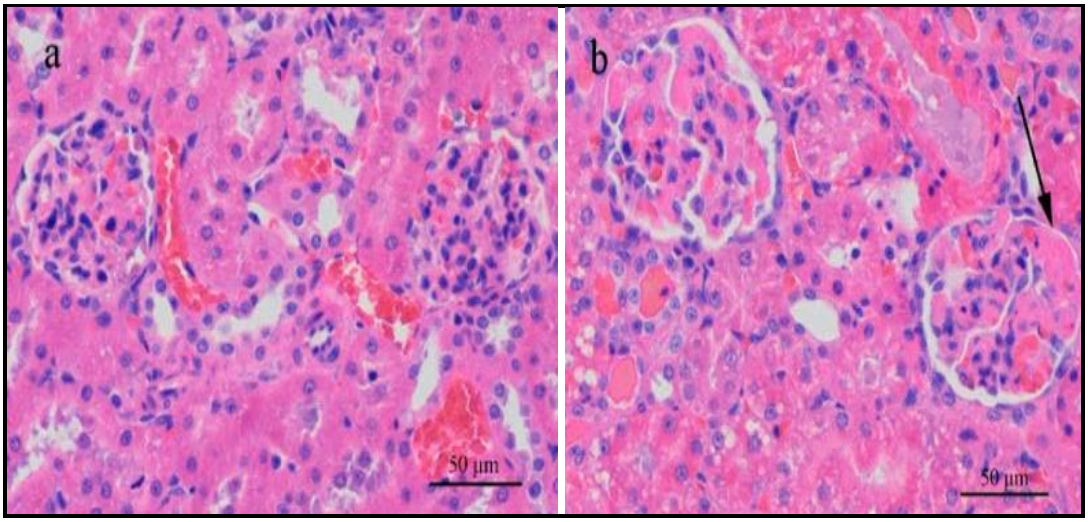
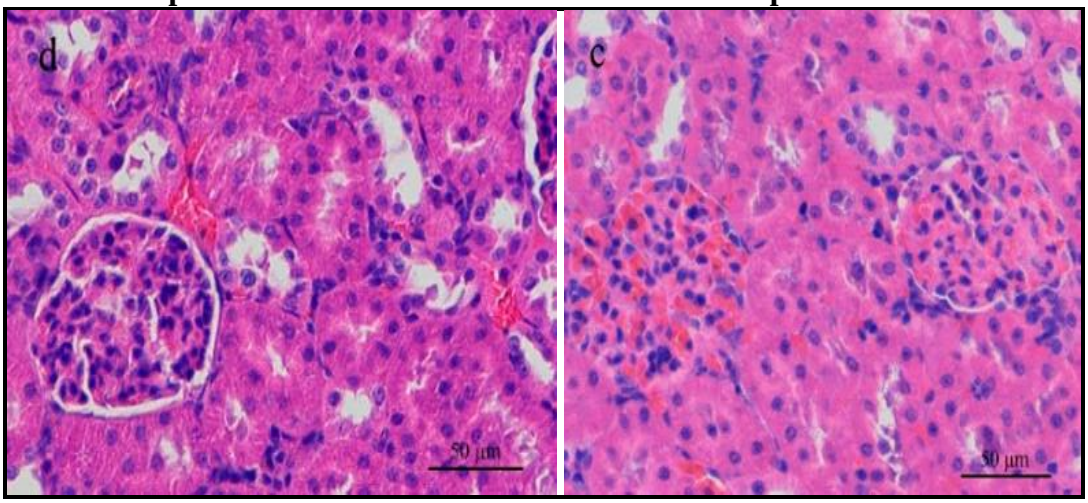


Figure No.16: Stop-Flow Technique Clearance Method

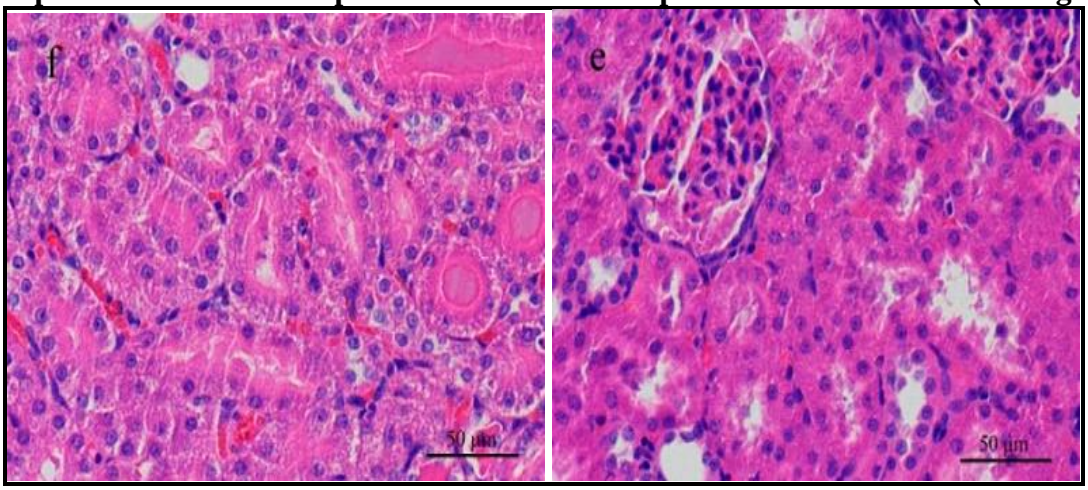
HISTOPATHOLOGICAL ASSESSMENT



Group 1: Normal Control **Group 2: Furosemide**



Group 3: furosemide+ vasopressin standard **Group 4: Dioscorea batatas (200mg/kg)**



Group 5: Urtica dioica(200mg/kg) **Group 6: D.batatas+ U. dioica (100mg/kg+100mg/kg)**

Figure No.17: Microphotograph of kidneys of albino wistar rats showing the effects of on renal function

CONCLUSION

The comprehensive analysis of the data involving two distinct plant extracts, *Dioscorea batatas* and *Urtica dioica*, provides valuable insights into their potential effects on renal function and oxidative stress in rats.

Saluretic and Natriuretic Activities

The data reveals significant variations in saluretic and natriuretic activities among the experimental groups. Notably, Groups 2 and 4 exposed to *Dioscorea batatas* display elevated saluretic and natriuretic indices. This suggests that *Dioscorea batatas* may have a pronounced impact on the excretion of sodium and chloride ions. In contrast, *Urtica dioica* appears to have a milder influence on these parameters in Groups 3 and 5.

Carbonic Anhydrase Activity

Carbonic anhydrase activity, linked to acid-base balance and ion transport, shows subtle differences between the plant-exposed groups. *Dioscorea batatas* (Group 2) displays lower carbonic anhydrase activity, which may affect pH regulation and ion handling to a greater extent compared to *Urtica dioica* (Group 3).

Lipschitz Test and Blood Pressure

The Lipschitz test and blood pressure measurements highlight potential electrolyte imbalances and hypertension in Group 2 (*Dioscorea batatas*) and Group 5 (*Urtica dioica*). These effects may contribute to renal stress and compromised overall health in these groups.

Oxidative Stress Markers

Elevated levels of malondialdehyde (MDA) and reduced antioxidant enzyme activities (SOD, CAT, GPx) in Groups 2 and 4 (*Dioscorea batatas*) suggest heightened oxidative stress, potentially linked to the observed renal dysfunction.

Renal Function Assessment

Assessment of serum creatinine, blood urea nitrogen (BUN), creatinine clearance, and clearance via stop-flow techniques consistently points to impaired renal function in Groups 2 and 4, aligning with the oxidative stress findings.

Micropuncture and Blood Volume

Micropuncture data indicates alterations in tubular function, while flow measurements provide insights into peripheral circulation. However, these parameters do not directly correlate with renal function.

In summary, the data suggests that both *Dioscorea batatas* and *Urtica dioica* may have significant effects on renal function, electrolyte balance, and oxidative stress. Notably, *Dioscorea batatas* appears to have a more pronounced impact on these parameters compared to *Urtica dioica*. These findings warrant further investigation into the mechanisms underlying these effects and the potential therapeutic or adverse implications of these plant extracts in renal health.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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