CHAPTER 4:

RESULTS

CONTENTS

4. Results:

4.1. Percentage of yield of extracts of *TT* dried fruits: The extractive values of each extract in individual solvents are expressed in Table 18.

Table 18: Extractive values of *TT* dried fruits in various solvents

Sl. No.	Type of extract	Percentage Yield	
1	CAE	9.2 %	Crude Extraction
2	Petroleum ether extract	8%	Successive Extraction
3	Chloroform extract	6.5%	
4	Methanolic extract	5%	
5	Aqueous extract	0%	

Discussion: In this study, CAE (traditional preparation) yielded the highest extraction value. Among the successive extracts, the petroleum ether extract was high in yield.

4.2.Identification of the extract with the highest diuretic potential:

The diuretic activity of CAE and other successive extracts of *TT* tested at low, medium, and high doses revealed that the high dose of CAE was the most potent diuretic among the tested extracts. There was a statistically significant ($p < 0.05$) increase in urine output volume and urinary sodium output among the animals treated with the high dose of CAE compared with those in the other groups (Table 19). The extract scored a value of 2.5 in the *Lipschitz* test for urine output, indicating a potent diuretic effect (≥ 2 potent diuretic activity). They also scored 1.4 on the *Lipschitz* test for sodium, indicating a positive diuretic property (≥ 1 positive effect) (Figure 9). As the CAE had a superior diuretic effect, it was selected for further phytochemical analysis and diuretic evaluation.

Table 19: Comparison of the diuretic action of the various extracts of *TT* dried fruit

Sl. No.	Groups	Average Urine Output (mL)	Average Na^+ Output (mmol/L)
1	CAE - Low Dose (100 mg)	8.83±4.11	110.11±10.18
2	CAE - Medium Dose (200 mg)	6.5±4.63	128.51±6.96 £, ά, έ, ή, ί, ΰ, λ, ρ
3	CAE - High Dose (400 mg)	32.66±2.33 ¥, Ά, ά, έ, ή, ί, ΰ, κ, λ, ξ, ρ	220.25±7.50 ¥, Ά, ά, έ, ή, ί, ΰ, κ, λ, ξ, ρ
4	Methanol Extract - Low Dose (100 mg)	8.83±3.81	102.01±11.75
5	Methanol Extract - Medium Dose (200 mg)	9.66±4.08	97.1±8.08
6	Methanol Extract - High Dose (400 mg)	11.33±3.20	101.35±6.88
7	Chloroform Extract - Low Dose (100 mg)	7.66±2.25	92.1±10.57
8	Chloroform Extract - Medium Dose (200 mg)	9.66±3.26	94.06±11.19
9	Chloroform Extract - High Dose (400 mg)	12.16±3.18	113.4±10.25
10	Petroleum Ether Extract - Low Dose (100 mg)	7.33±1.96	105.66±8.91
11	Petroleum Ether Extract - Medium Dose (200 mg)	8.83±2.04	110.31±9.71
12	Petroleum Ether Extract - High Dose (400 mg)	10.66±1.63	106.76±8.75

¥, ¤, £, ά, έ, ή, ί, ΰ, κ, λ, ξ, ρ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

¥ = Vs CAE - Low Dose (100 mg), ¤ = Vs CAE - Medium Dose (200 mg), £ = Vs CAE - = Vs High Dose (400 mg), ά = Vs Methanol Extract - Low Dose (100 mg), έ = Vs Methanol Extract - Medium Dose (200 mg), ή = Vs Methanol Extract - High Dose (400 mg), ί = Vs Chloroform Extract - Low Dose (100 mg), ΰ = Vs Chloroform Extract - Medium Dose (200 mg), κ = Vs Chloroform Extract - High Dose (400 mg), λ = Vs Petroleum Ether Extract - Low Dose (100 mg), ξ = Vs Petroleum Ether Extract - Medium Dose (200 mg), ρ = Vs Petroleum Ether Extract - High Dose (400 mg)

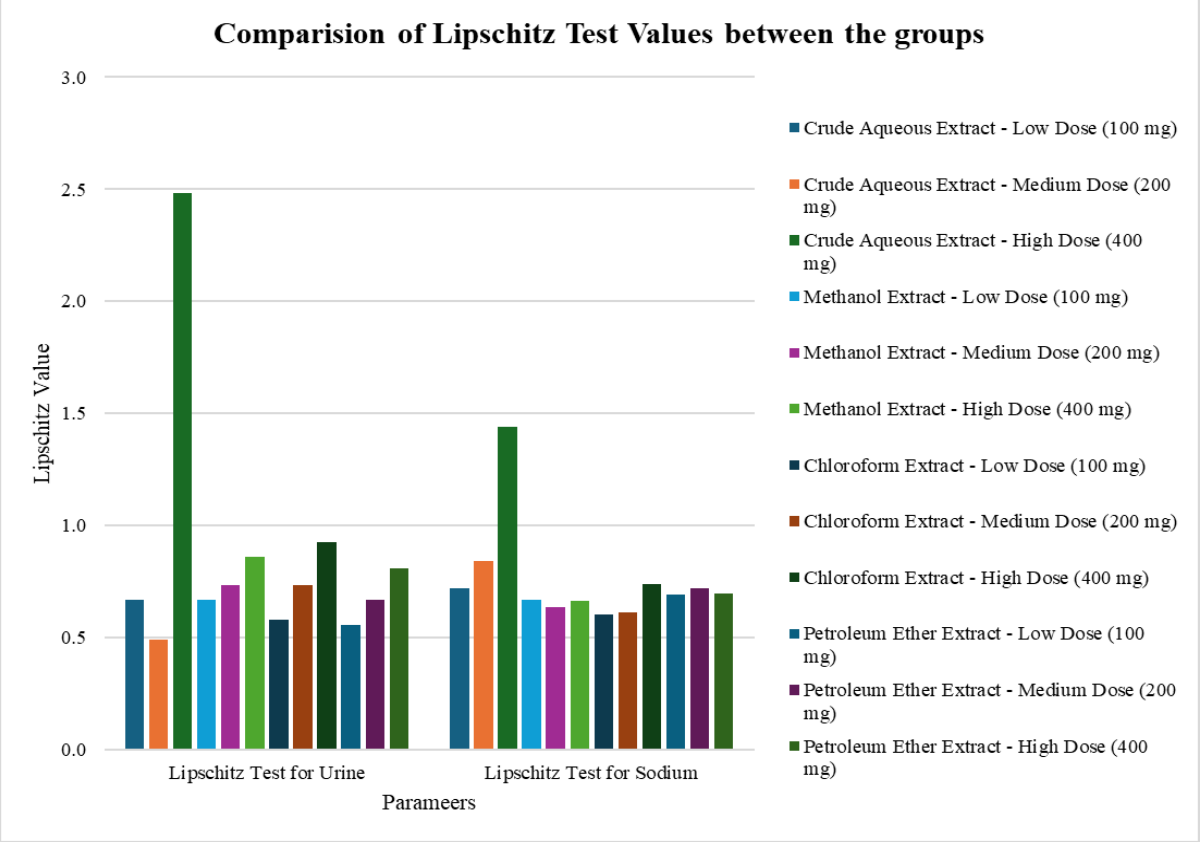


Figure 9: Comparison of *Lipschitz* values of urine and sodium between various extracts of *TT*-dried fruit

***Lipschitz* value: ≥ 1 positive effect; ≥ 2 potent diuretic activity**

Discussion:

There have been numerous studies on various extracts of *TT* and their effectiveness as diuretic agent. However, no studies have compared the effectiveness of different types of extracts. In our research, we employed a bioactivity-guided approach to identify which type of extract demonstrates better diuretic potential. To achieve this goal, we quantified the amount

of urine excretion, the urine sodium level, and the *Lipschitz* test for urine and sodium, which is considered the gold standard for evaluating the diuretic activity of any substance. Our findings indicated that the CAE has better diuretic potential than the other extracts do. Consequently, we focused our further studies on this extract, and further fractionation and subfractionation were discontinued.

In traditional medicine, such as Siddha, formulations of *TT* (*Nerunjil*) are given in the form of Kashaya, which contains the CAE of *TT*, along with other herbal formulations. Traditionally, any Kashaya is prepared by boiling the plant parts or herbs in water, yielding a final product that is considered *Satvik* in nature and that is pure, nontoxic, and balanced. CAEs closely resemble traditional preparations, ensuring holistic extraction of active phytoconstituents, such as saponins, flavonoids, glycosides, and tannins, which are therapeutically useful (167). However, successive extraction leads to a reduction in diuretic activity, probably due to the loss of phytoconstituents during the process of successive extraction. CAE contains more phytoconstituents, which might be collectively responsible for the high diuretic action of the plant.

4.3.Phytoconstituents of the CAE:

GC–MS analysis revealed that the extract contained steroids and terpenoids, amines and nitrogen-containing compounds, ketones and lactones (flavonoid-related compounds), carboxylic acids and their derivatives, alcohols and polyols, and various other compounds. A total of 26 compounds were present, with the major compounds being dodecanoic acid, 1,2,3-propanetriyl ester (21.08%), alpha-methylmannofuranoside (18.48%), smambutoxin,2-mederivative (12.14%), 1,2,3,4-butanetetrol,[S-(R*,R*)]- (11.02%), glycerin (8.45%), 1,5-diphenyl-1,5-pentanedione (1.85%), and sorbitol (1.45%). The GC–MS chromatogram is shown in Figure 10.

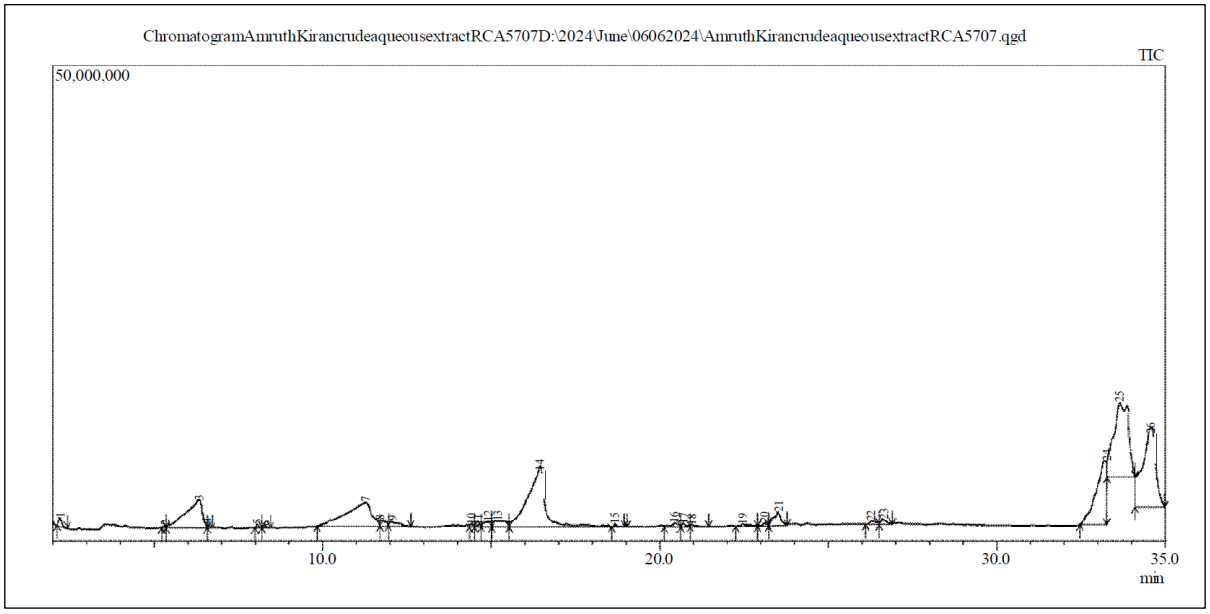


Figure 10: GC–MS chromatograms of CAE

Note: Total ion chromatograms (TICs) showing the peaks of the compounds present in the CAE.

The bioactive compounds present in the CAE, along with their retention time (RT), percentage areas, molecular weights, molecular formulas, and chemical abstract service (CAS) numbers, are given in Table 20.

Table 20: GC–MS data of CAE

Sl. No.	RT	Compound Name	Area %	GC-MS library match scores	Chemical Formula	Molecular Weight (g/mol)	CAS Number
1	33.652	Trilaurin	21.08	84	$C_{39}H_{74}O_6$	639	538-24-9
2	34.59	Trimyristin	19.15	53	$C_{45}H_{86}O_6$	723.2	555-45-3
3	16.45	Alpha-d-Mannofuranoside, methyl	18.48	78	$C_7H_{14}O_6$	194.18	4097-91-0
4	33.26	Sambutoxin,2Mederivative	12.14	25	$C_{30}H_{43}NO_4$	481	1306714-73-7
5	11.278	1,2,3,4-Butanetetrol,[S-(R*,R*)]-	11.02	96	$C_4H_{10}O_4$	122.12	2319-57-5
6	6.331	Glycerin	8.45	97	$C_3H_8O_3$	92.09	56-81-5
7	23.5	1,3-Dibenzoylpropane	1.85	72	$C_{17}H_{16}O_2$	252.31	6263-83-8
8	15.21	Sorbitol	1.45	71	$C_6H_{14}O_6$	182.17	50-70-4
9	12.047	Alpha-Acetobutyrolactone	0.87	75	$C_6H_8O_3$	128.13	517-23-7
10	14.925	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	0.71	62	$C_7H_{10}O_2$	126.15	21835-01-8
11	11.745	Alpha-Terpineol	0.65	71	$C_{10}H_{18}O$	154.25	98-55-5
12	26.629	Stigmasterol	0.57	62	$C_{29}H_{48}O$	412.7	83-48-7
13	20.448	Linoleic Acid	0.54	94	$C_{18}H_{32}O_2$	280.4	60-33-3
14	2.195	2,3-Butanediol	0.492	98	$C_4H_{10}O_2$	90.12	513-85-9

15	26.314	Diosgenin	0.45	71	$C_{27}H_{42}O_3$	414.6	512-04-9
16	22.472	Alpha-Tocopherol	0.36	77	$C_{29}H_{50}O_2$	430.7	59-02-9
17	20.705	Stearic Acid	0.29	92	$C_{18}H_{36}O_2$	284.5	57-11-4
18	20.944	Cedrol	0.25	66	$C_{15}H_{26}O$	222.37	77-53-2
19	23.105	Testosterone Cypionate	0.25	75	$C_{27}H_{40}O_3$	412.6	58-20-8
20	6.565	Furaneol	0.17	89	$C_6H_8O_3$	128.13	3658-77-3
21	14.635	Hydroxyproline	0.17	71	$C_5H_9NO_3$	131.13	51-35-4
22	14.397	Quinoline,2-ethyl-	0.16	85	$C_{11}H_{11}N$	157.21	1613-34-9
23	8.061	4H-Pyran-4-one, 2,3-dihydro-3, 5-dihydroxy-6-methyl-	0.14	95	$C_6H_8O_4$	144.12	28564-83-2
24	8.28	Benzoic Acid	0.12	93	$C_7H_6O_2$	122.12	65-85-0
25	18.681	Pentadecanoic acid	0.11	86	$C_{15}H_{30}O_2$	242.4	1002-84-2
26	5.317	Acetic Acid	0.09	67	$C_3H_6O_2S$	106.15	2365-48-2

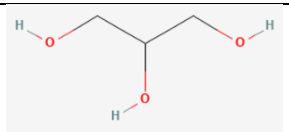
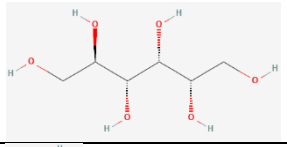
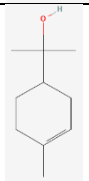
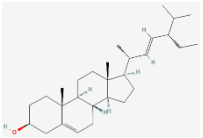
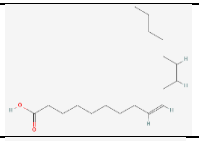
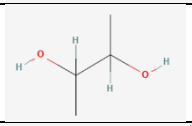
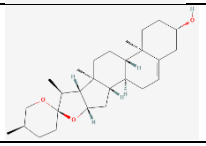
Discussion:

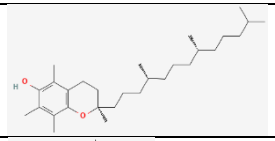
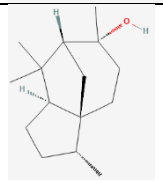
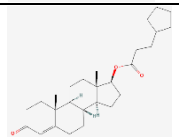
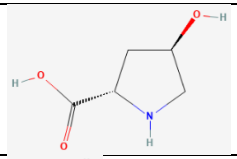
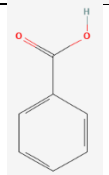
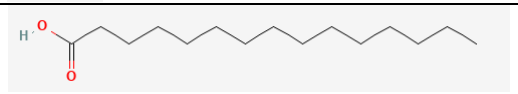
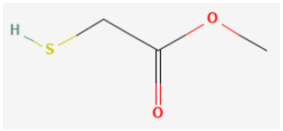
In the present study, CAE was analyzed using GC–MS, resulting in the identification of putative compounds by matching the experimental spectra with the NIST and WILEY libraries to determine and confirm the identities of the compounds. The bioactive components of CAE are all natural flavonoids, saponins, and phytosterols, which have antioxidant, antimicrobial, diuretic, laxative, neuroprotective, and anticancer properties (92,168).

Several compounds, such as 2,3-butanediol, benzoic acid, and alpha-terpineol, exhibit antimicrobial activities, making them potentially useful in treating infections. Compounds such as Cedrol and Alpha-Tocopherol provide protective effects against oxidative stress. Compounds such as linoleic acid and pentadecanoic acid are linked to managing conditions such as metabolic syndrome and type 2 diabetes. Diosgenin and alpha-terpineol demonstrate neuroprotective properties, suggesting their potential in treating neurodegenerative conditions. Testosterone cypionate is used for androgen replacement therapy in clinical settings, indicating that the extract has a potential influence on hormonal health. However, among the 26 phytoconstituents, only glycerin and sorbitol are known to have diuretic properties.

A few of the compounds identified in our study were also found in another study by Urkude et al. (2024), which reported similar compounds, including diosgenin and stigmasterol. Furthermore, a previous study revealed that diosgenin has nephroprotective effects (119,169). Overall, there is a lack of scientific evidence towards the diuretic activity of the extracts and their phytoconstituents of *TT*. Table 21 presents an overview of some of the phytocompounds found in the CAE, along with their chemical formulas, potential pharmacological properties, and uses.

Table 21: Pharmacological properties of individual phytocompounds found in the CAE

Sl. No.	Compound Name	Chemical Formula	2D Structure	Pharmacological Properties/Uses
1	Glycerine	$C_3H_8O_3$		Osmotic diuretic (72,170), laxative (171)
2	Sorbitol	$C_6H_{14}O_6$		Diuretic activity (172,173)
3	Alpha-Terpineol	$C_{10}H_{18}O$		Antimicrobial (174,175), neuroprotection, and reduces oxidative stress (176)
4	Stigmasterol	$C_{29}H_{48}O$		Anticancer, Anti-Osteoarthritis, Anti-Inflammatory, Immunomodulatory, Neuroprotective, Antimicrobial (177,178)
5	Linoleic Acid	$C_{18}H_{32}O_2$		Coronary artery disorder, type 2 diabetes (179–182)
6	2,3-Butanediol	$C_4H_{10}O_2$		Antimicrobial (183–185)
7	Diosgenin	$C_{27}H_{42}O_3$		Neuroprotective, Anticancer, Antiatherosclerosis, Hepatoprotective (186,187)

8	Alpha-Tocopherol	$C_{29}H_{50}O_2$		Antioxidant (188)
9	Cedrol	$C_{15}H_{26}O$		Anti-inflammatory and antioxidant effect (181,189), sedation (190)
10	Testosterone Cypionate	$C_{27}H_{40}O_3$		Androgen replacement therapy (191)
11	Hydroxyproline	$C_5H_9NO_3$		Maintain the stability of collagen (192,193)
12	Benzoic Acid	$C_7H_6O_2$		Antimicrobial (194,195)
13	Pentadecanoic acid	$C_{15}H_{30}O_2$		Metabolic syndrome (196)
14	Acetic Acid	$C_3H_6O_2S$		Topical antifungal agent (197)

4.4.Network Pharmacology:

Identification of Overlapping Targets between Disease, Phytochemical Targets, and FDA-Approved Drug Targets:

A total of 167 disease-related targets were identified by integrating the results from GeneCards (relevance score > 10) and removing duplicates. Phytochemical targets were predicted using Super-PRED, and their UniProt IDs were standardized to official gene names, resulting in 408 unique targets. Using the Venny 2.1 tool revealed that 27 CTs were common between the disease-associated targets and those predicted for phytochemicals (Figure 11). Similarly, Food and Drug Administration (FDA)-approved standard diuretic drug targets were compiled (n = 275 after deduplication). Twenty-nine CTs were identified between these drugs and disease-associated targets (Figure 12), accounting for 7% of the compiled dataset. These CTs reflect somewhat higher overlap than phytochemicals and may correspond to well-established pharmacological targets already leveraged in conventional therapies. This subset of targets may represent potential molecular intermediaries through which phytochemicals exert diuretic or disease-modulating effects.

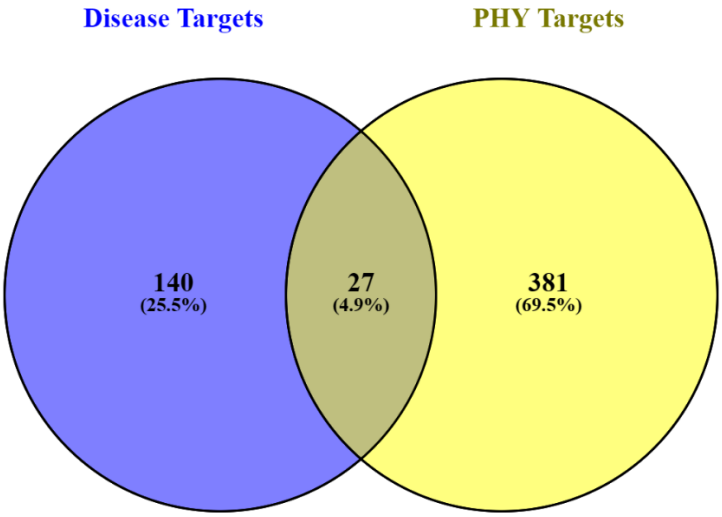


Figure 11: Venn diagram showing the overlap between disease-associated targets and predicted phytochemical (PHY) targets. Twenty-seven CTs (4.9%) were identified, representing potential shared molecular mechanisms relevant to diuretic activity.

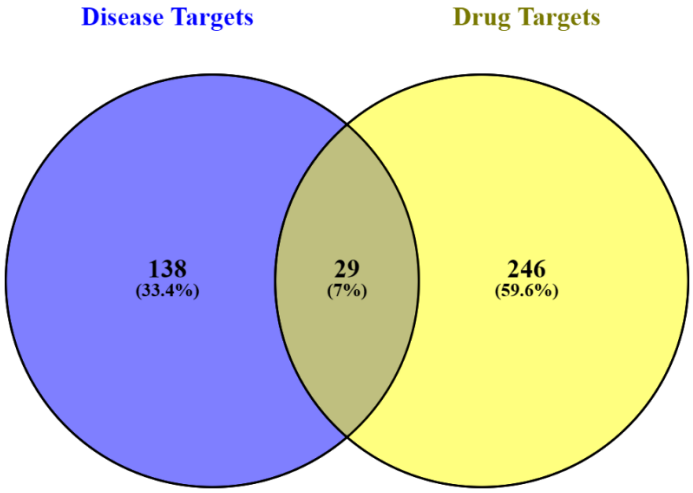


Figure 12: Venn diagram illustrating the intersection between disease-associated targets and standard diuretic drug targets. Twenty-nine CTs (7%) were identified, highlighting known therapeutic targets involved in diuresis-related conditions.

Protein–Protein Interaction (PPI) Network Analysis:

PPI networks were constructed using the STRING database and visualized with Cytoscape to explore the interactions among the CTs between disease-associated genes and therapeutic agents. These PPI networks provide insight into the molecular mechanisms by which phytochemicals and existing drugs may exert therapeutic effects, and they highlight potential hub proteins for further experimental validation.

A. PPI Network of Disease–Phytochemical Overlapping Targets:

The STRING database was run, and the PPI network comprised 27 nodes and 82 edges (Figure 13). The node size is proportional to the degree centrality, indicating the number of connections a node has within the network. Targets such as REN, ESR1, PTGS2, HIF1A, SERPINE1, and ADRB1 exhibited high degree values, suggesting their central role in network connectivity. Closeness centrality, reflected by the node fill color (red to green), highlights nodes such as REN, ESR1, and PTGS2 as topologically influential. Similarly, betweenness centrality, indicated by the node border color (magenta to cyan), identified REN and SERPINE1 as critical bridging nodes. These highly central nodes may represent key regulators or bottlenecks in the phytochemical-mediated modulation of disease pathways.

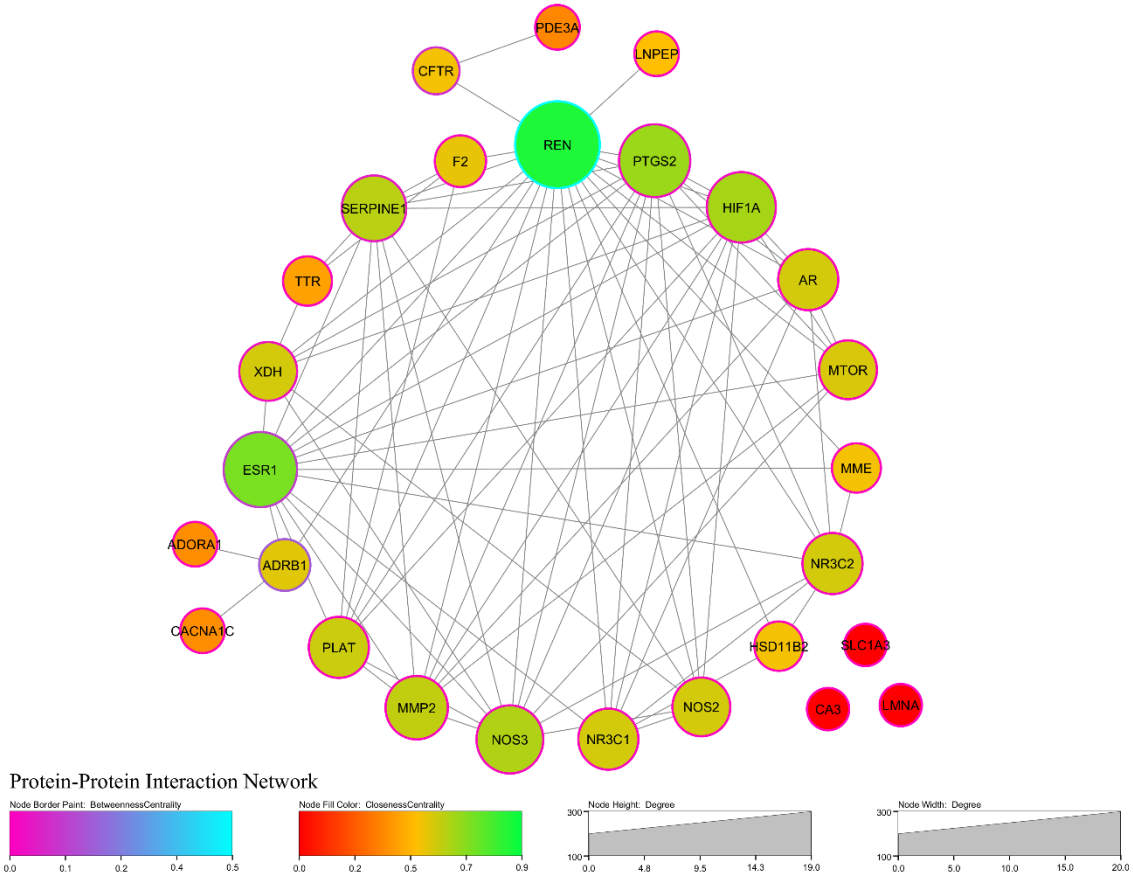


Figure 13: Protein–protein interaction network of 27 CTs between disease-related and phytochemical targets. The network was constructed using the STRING database and visualized using Cytoscape; the network consists of 27 nodes and 82 edges. Node size reflects degree centrality; node fill color indicates closeness centrality (from red to green), and node border color represents betweenness centrality (from pink to cyan).

B. PPI Network of Disease–Drug Overlapping Targets:

A separate PPI network was constructed from 29 CTs shared between disease-associated genes and FDA-approved drug targets. This network included 29 nodes and 49 edges (Figure 14). Among these, HIF1A, CA2, CA9, and CA12 emerged as the top nodes with high degree centrality, suggesting their involvement in multiple interaction pathways relevant to therapeutic action. As in the previous network, closeness and betweenness centrality analyses revealed HIF1A and CA12 as key targets with significant topological influence. Notably, many carbonic anhydrases (CA family) appeared in this network, indicating their potential as pharmacological targets in disease intervention. However, less interconnectedness was observed in the overall network.

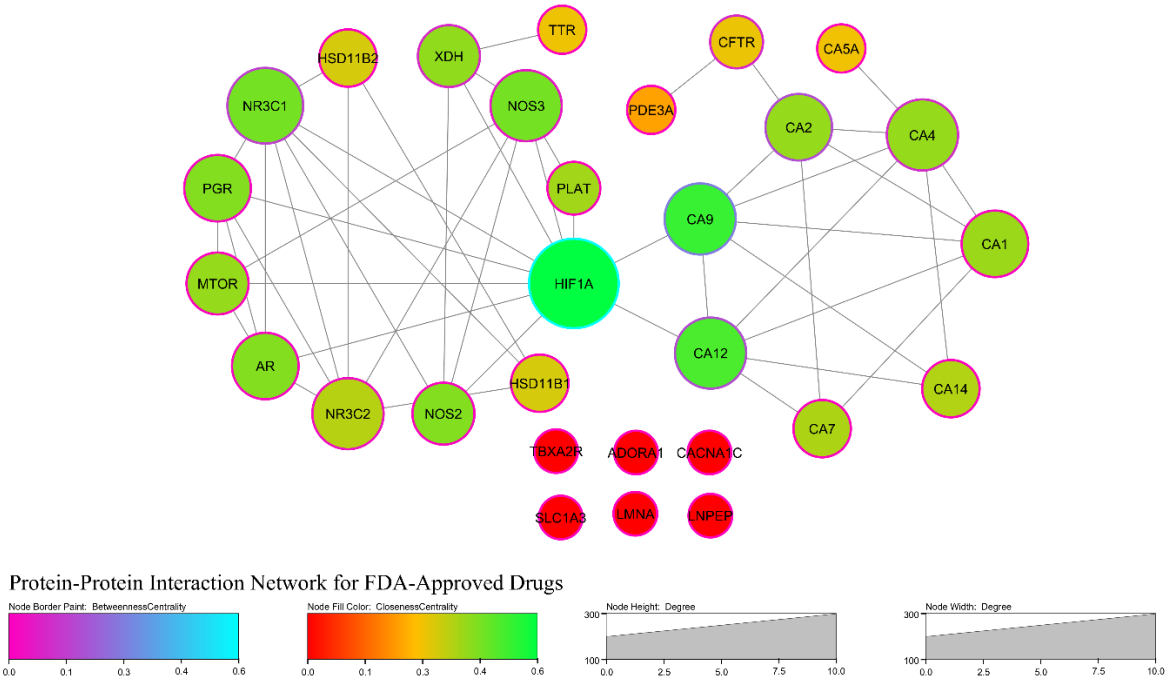


Figure 14: Protein–protein interaction network of CTs between disease-related targets and standard diuretic drug targets. The network consists of 29 nodes and 49 edges, where the node size reflects degree centrality, the node fill color indicates closeness centrality (from red to green), and the node border color represents betweenness centrality (from pink to cyan).

Pathway–Target Interaction Network and Enrichment Analysis:

To elucidate the biological relevance of the 27 CTs of the disease-phytochemical targets, GO and KEGG pathway enrichment analyses were performed using the DAVID database. A p-value cutoff of <0.05 was used to filter statistically significant pathways, leading to the identification of 25 enriched pathways. From these, 11 disease-relevant signaling pathways were selected based on target count >3. The most statistically significant pathways included (Figure 15) the renin–angiotensin system (hsa04614), renin secretion (hsa04924), the HIF-1 signaling pathway (hsa04066), apelin signaling (hsa04371), and cGMP–PKG signaling (hsa04022). These pathways are known to play pivotal roles in vascular remodeling, the hypoxia response, fibrosis, and inflammation. Notably, several pathways, such as proteoglycans in cancer (198–200) and diabetic cardiomyopathy (201,202) may reflect systemic interactions beyond renal tissue, suggesting the broader therapeutic relevance of these compounds.

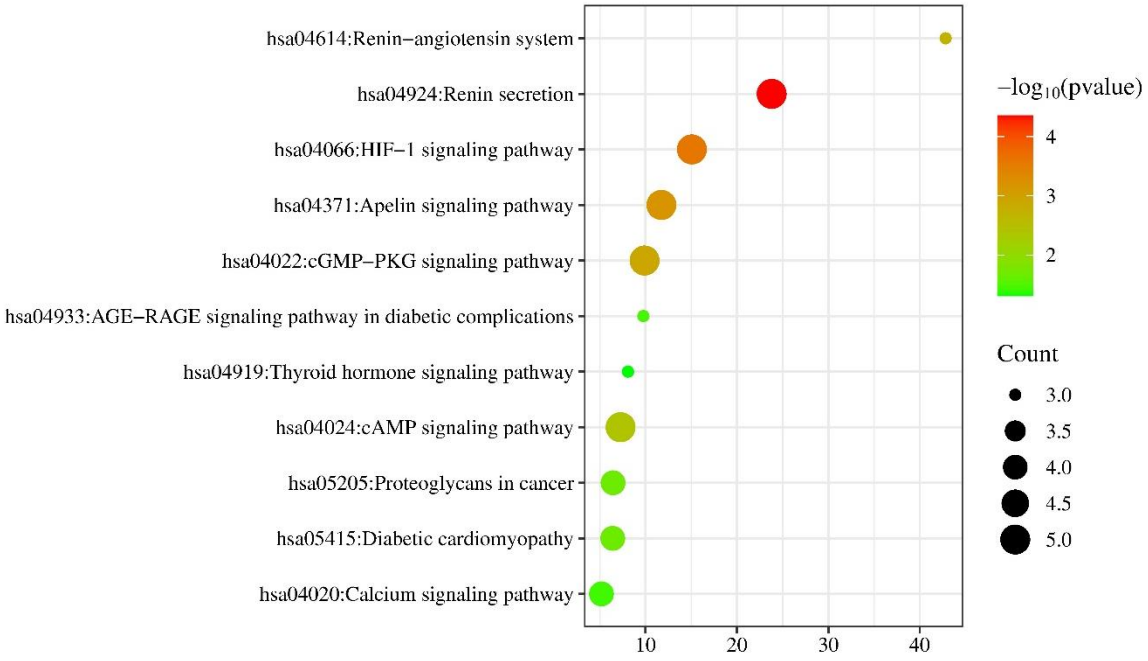


Figure 15: Bubble plot of the top 11 enriched KEGG pathways associated with phytoconstituent-related overlapping targets. The circle size represents the number of genes involved in each pathway; color indicates enrichment significance ($-\log_{10}$(p-value)).

The PaTr network for phytochemicals (Figure 16) and FDA-approved standard diuretic drugs (Figure 17) illustrates diamond-shaped nodes, while their corresponding protein targets are represented as circular nodes. Node size is proportional to the degree of connectivity, emphasizing highly interactive targets and pathways. Node fill colors represent closeness centrality, with green indicating high centrality, suggesting a greater role in the network's communication flow. Similarly, the magenta-to-cyan node border gradient represents betweenness centrality, highlighting nodes that serve as critical bridges within the network. Compared with FDA-approved standard diuretic drugs, the PaTr network for phytoconstituents shows distinct pathway–target relationships, with several overlapping but also some unique targets. Pathways like hsa04024 (cAMP signaling) and hsa05415 (prostate cancer) are prominent here, alongside key targets such as NOS3, REN, and MTOR, suggesting that phytoconstituents may exert multitarget effects across signaling cascades.

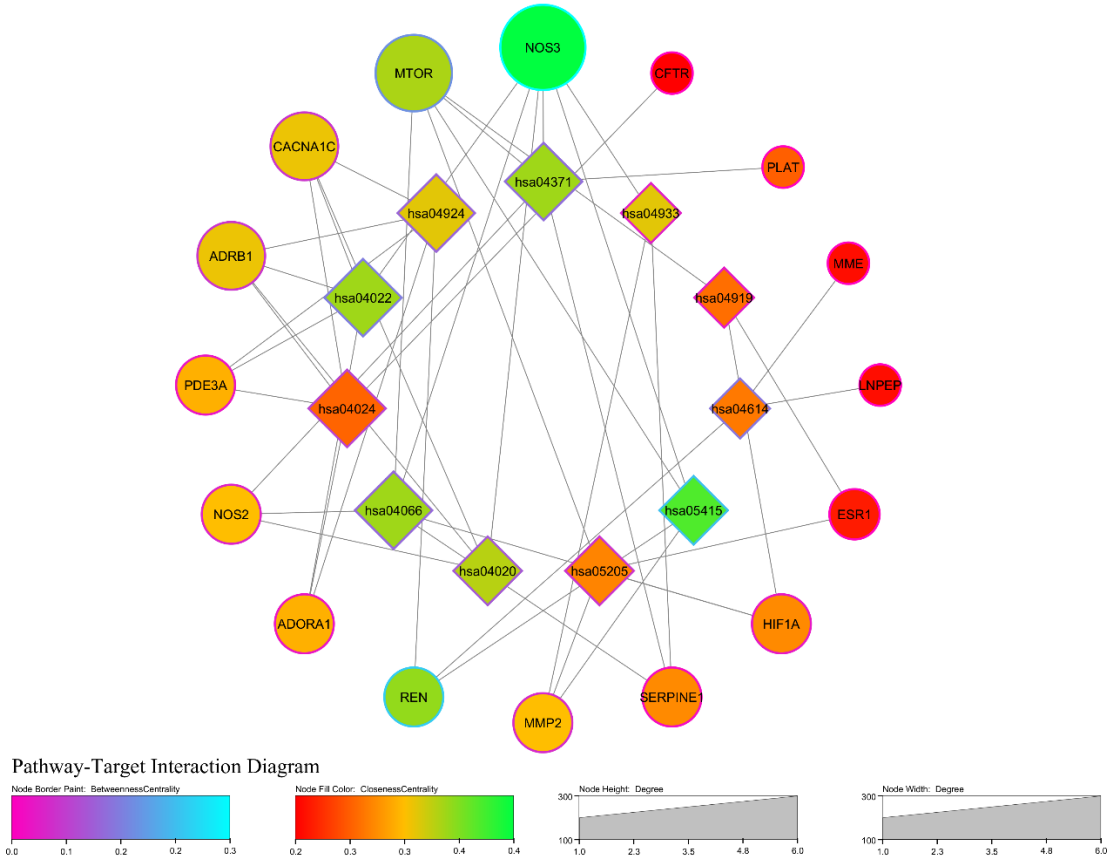


Figure 16: Pathway–target interaction network of phytoconstituents. Diamond nodes represent KEGG pathways (e.g., hsa05415, hsa04371), and circular nodes represent targets. The node fill color indicates closeness centrality, whereas the border color reflects betweenness centrality. Larger nodes have higher degree values, indicating greater connectivity within the network. Key central nodes include MTOR, NOS3, and REN, highlighting their functional significance for phytochemical interventions.

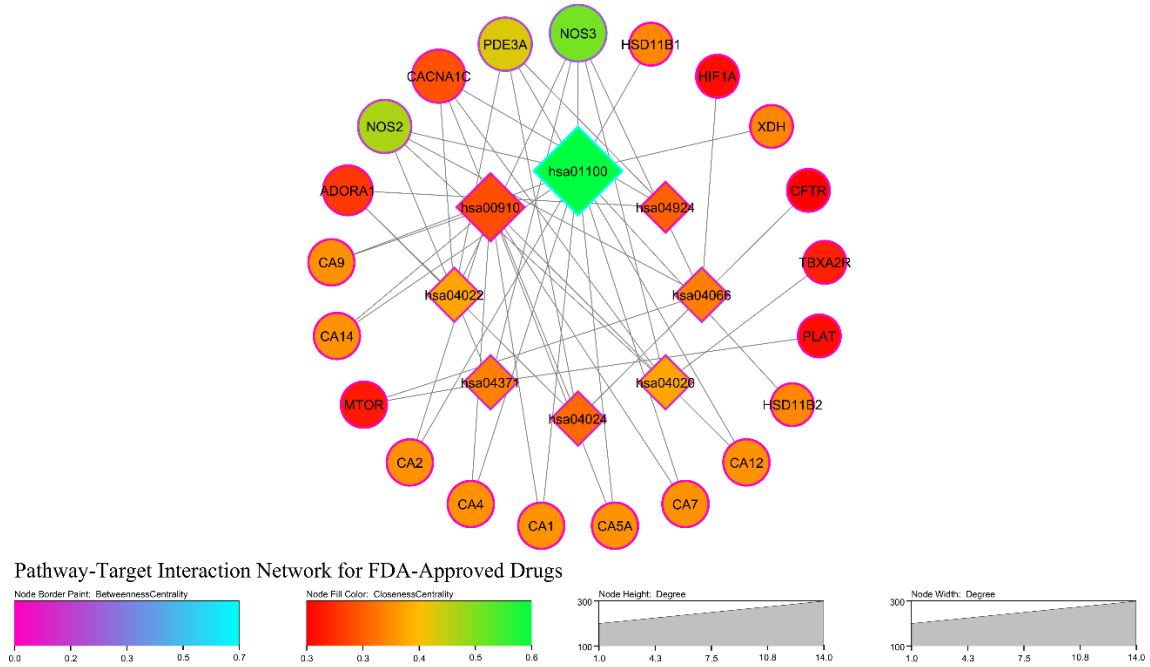


Figure 17: Pathway–target interaction network of standard diuretic drugs. Diamond nodes represent KEGG pathways (e.g., hsa00910, hsa01100), and circular nodes represent targets. The node fill color indicates closeness centrality, whereas the border color reflects betweenness centrality. Larger nodes have higher degree values, indicating greater connectivity within the network.

In addition, GO enrichment analysis (Figure 18) further classified the CTs of phytochemicals into biological processes, cellular components, and molecular functions. Among the enriched biological processes, signal transduction (GO:0007165) and proteolysis (GO:0006508) were predominant, alongside stress responses such as response to hypoxia and positive regulation of gene expression. For cellular components, the plasma membrane and cytosol were the most enriched, indicating that many targets are membrane-bound or cytoplasmic in terms of localization. In molecular function terms, protein binding (GO:0005515) dominated, followed by transcription factor binding and various enzymatic activities, suggesting that these targets are heavily involved in signaling and regulatory mechanisms.

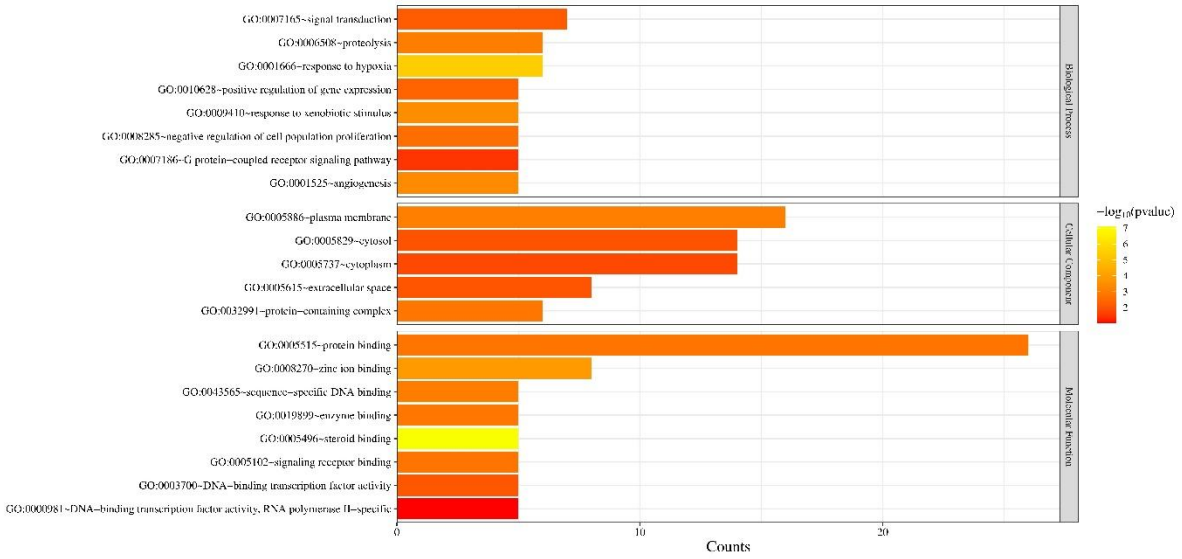


Figure 18: GO enrichment bar plot of overlapping targets. GO terms are grouped by biological process, cellular component, and molecular function. The bar length indicates the number of enriched genes; the bar color represents statistical significance ($-\log_{10}$(p-value)).

Phytochemical pathway–target and drug pathway–target interaction networks:

The integrated PyPaTr network illustrates the multitarget and multipathway interactions of bioactive phytochemicals derived from *TT* in relation to diuretic activity. This network comprises 50 nodes and 153 edges, integrating phytochemicals, their predicted protein targets, and significantly enriched KEGG pathways ($p < 0.05$, target count > 3).

In this visualization (Figure 19), triangular nodes represent individual phytochemicals, circular nodes depict protein targets, and diamond-shaped nodes correspond to biological pathways. The node fill color gradient from red to green indicates closeness centrality, highlighting nodes that are more central and potentially more influential in signal transduction. Additionally, the node border color reflects betweenness centrality, which helps to identify potential hub elements critical for intermodule communication. Node size and shape scaling are based on degree values, revealing their interaction richness. Among the key targets identified with a high degree and centrality are MTOR, NOS3, HIF1A, SERPINE1, ADORA1, and PDE3A. These targets are strongly associated with pathways such as the Apelin signaling pathway and HIF-1 signaling, underscoring their pharmacological relevance. This network reveals the systems-level modulation enabled by multiple phytochemicals acting in synergy with interrelated diuretic pathways.

An analogous DrPaTr network was constructed to benchmark phytochemical interactions using FDA-approved standard diuretic agents: furosemide, hydrochlorothiazide,

spironolactone, and acetazolamide. This network encompasses 68 nodes and 224 edges (Figure 20), linking these drugs to their respective protein targets and enriched pathways relevant to diuresis.

In the diagram, triangles represent drugs, circles represent target proteins, and diamonds represent pathways. As in the PyPaTr network, the node fill color and border gradient correspond to closeness and betweenness centrality, respectively. The key targets with high centrality included CACNA1C, NOS2, MTOR, HIF1A, and PLAT, indicating functional overlap with the phytochemical network. The enriched pathways included metabolic pathways, nitrogen metabolism, and renin secretion regulation, all of which are central to fluid and electrolyte homeostasis. The presence of shared targets and pathways between FDA-approved standard diuretic drugs and phytochemicals, particularly MTOR, NOS3, HIF1A, and CFTR, suggests that natural compounds may exert therapeutic effects through mechanisms overlapping with those of clinically validated pharmacological agents.

Upon comparing the PyPaTr and DrPaTr networks, several shared hub genes emerged as key regulators across both systems. Notably, MTOR, HIF1A, and NOS3 consistently appear as central nodes with high degree and centrality metrics, confirming their role in modulating diuretic pathways. The cytoHubba plugin analysis confirmed these targets as among the most interconnected in the broader protein–protein and interaction networks, warranting further evaluation through molecular docking studies. Finally, to comprehensively map the interplay among phytoconstituents, FDA-approved standard diuretic drugs, molecular targets, and signaling pathways, we constructed an integrated FDA-approved standard diuretic drug–phytochemical–pathway–target interaction network (Figure 21). Central targets such as NOS3, HIF1A, MTOR, and ESR1 demonstrate high connectivity and centrality, serving as key mediators between multiple drugs, phytochemicals, and pathways. Common pathways, such as HIF-1 signaling (hsa04066), the renin–angiotensin system (hsa04614), and apelin signaling (hsa04371), act as convergence hubs. Standard diuretics such as hydrochlorothiazide and spironolactone share a target space with multiple phytochemicals, indicating the potential for synergistic repurposing or combination therapy strategies. This integrative network underscores phytoconstituents' multi-target, multi-pathway therapeutic nature, supporting their relevance in systems-based approaches to diuretic actions.

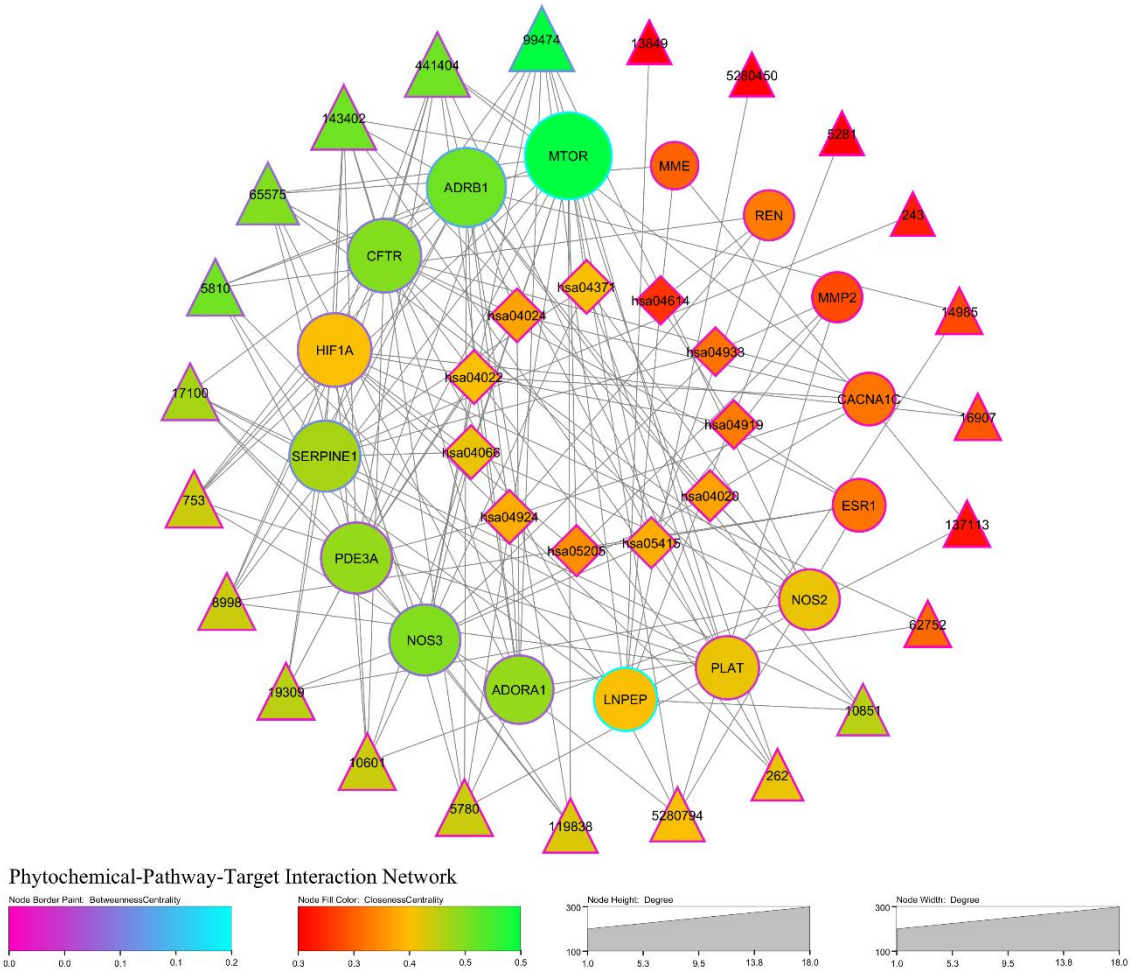


Figure 19: Phytochemical-pathway-target interaction (PyPaTr) network visualizing the interactions among phytochemicals (triangles), target proteins (circles), and enriched KEGG pathways (diamonds). The node fill color indicates closeness centrality, the border color reflects betweenness centrality, and the node size corresponds to a degree. Central targets such as MTOR, NOS3, and HIF1A are positioned at critical junctions of signaling pathways relevant to diuresis.

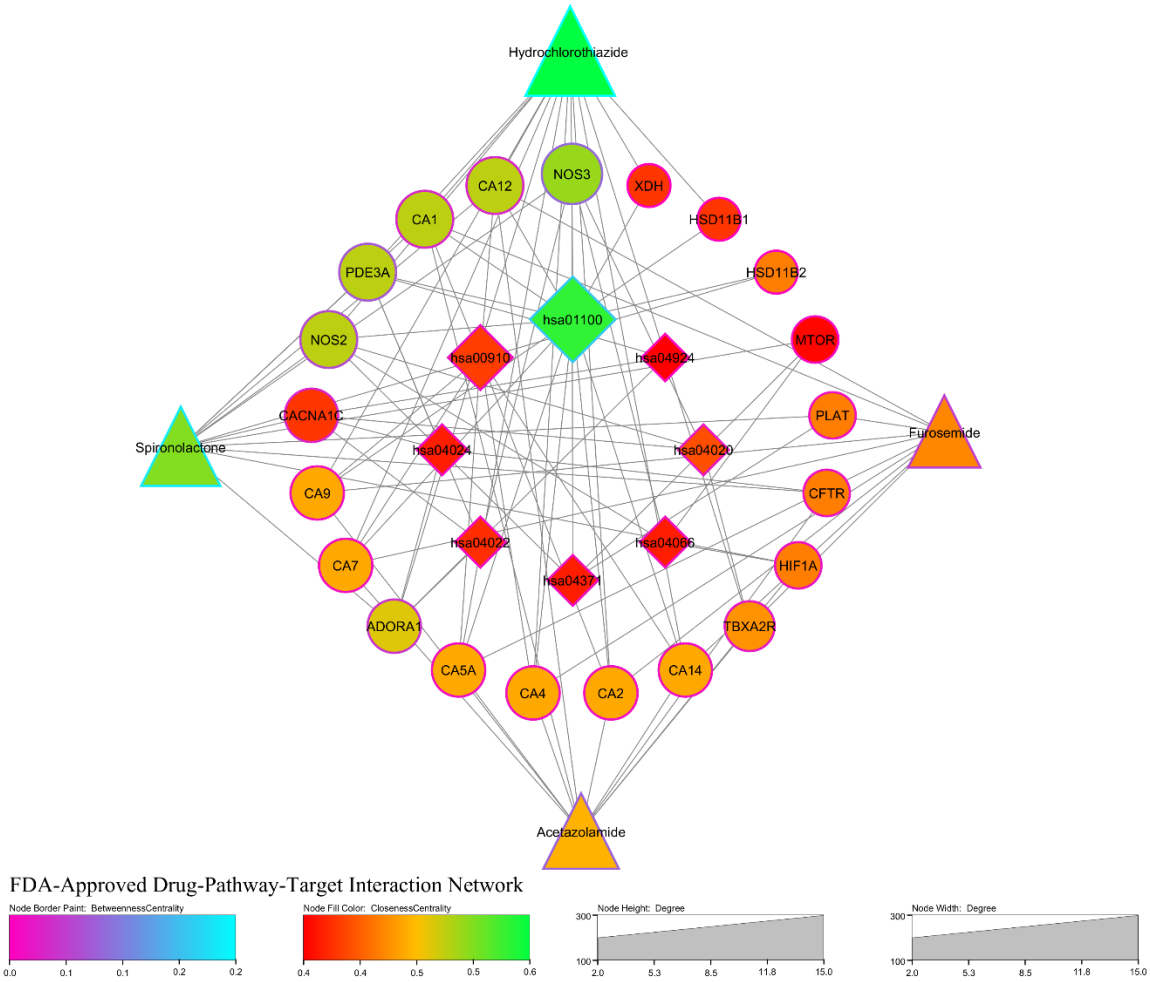


Figure 20: The standard diuretic drug–pathway–target interaction (DrPaTr) network links FDA-approved standard diuretics (triangles) with their molecular targets (circles) and corresponding biological pathways (diamonds). Centrality measures and degree-based scaling highlight key regulatory nodes shared with the phytochemical network, revealing overlapping mechanistic frameworks.

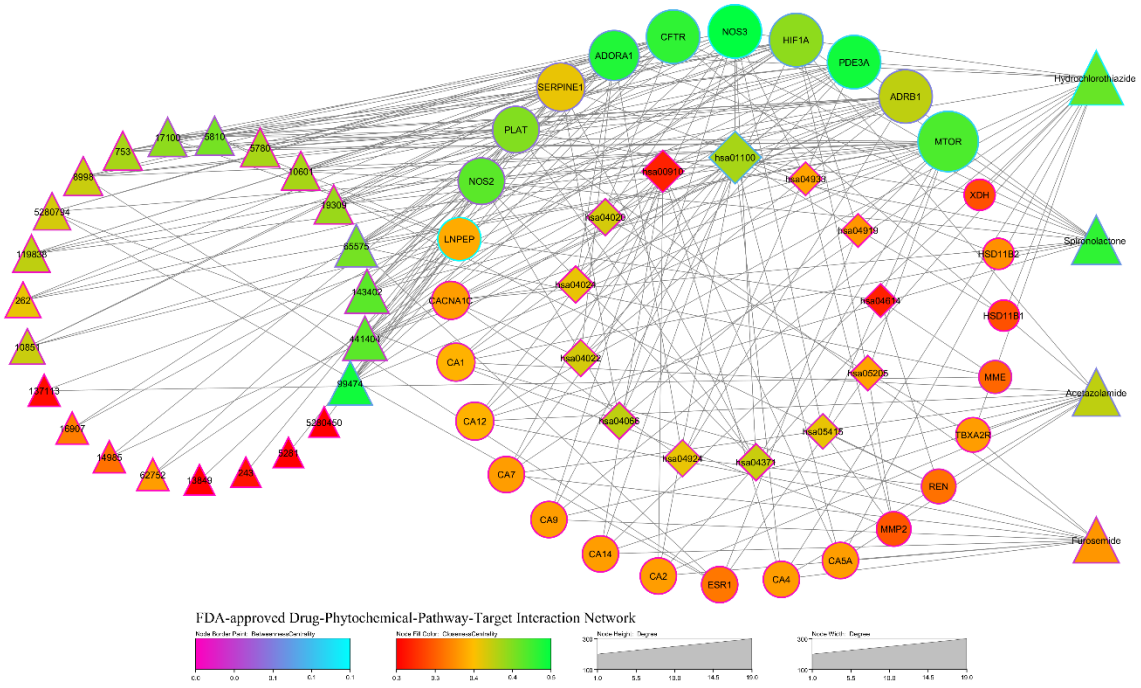


Figure 21: Standard diuretic drug–phytochemical–pathway–target interaction network. Triangular nodes (left: phytochemicals; right: drugs), circular nodes (targets), and diamond nodes (KEGG pathways). Node height and width correspond to a degree; fill color indicates closeness centrality; border color reflects betweenness centrality. Central hubs such as NOS3 and MTOR represent critical mediators across all components of the network.

Discussion:

To the best of our knowledge, no studies have been performed on network pharmacology to elucidate the possible targets and pathways responsible for their diuretic effects. The results of network pharmacology are consistent with the holistic view of TSM (203,204), which provides a systematic perspective and research pattern for predicting bioactive chemicals, probable target genes, and disease-related pathways in the extract by building a "drug-target-disease" network. In this study, we employed network pharmacology to explore the bioactive components of the extract and the possible molecular mechanisms involved in diuresis.

Our findings revealed that the targets and pathways shared by FDA-approved standard diuretic drugs and phytochemicals, particularly MTOR, NOS3, HIF1A, and CFTR, suggest that natural compounds may exert therapeutic effects through mechanisms overlapping with those of clinically validated pharmacological agents. The phytoconstituents of the CAE might work synergistically with standard drugs to produce diuresis. The extract can act on multiple

targets and multiple overlapping targets of standard diuretics, such as NOS3, HIF1A, MTOR, and ESR1. This means that the extract has diuretic effects through multiple diuretic mechanisms. Owing to its multiple mechanistic approaches and good safety profile, the CAE of *TT* could serve as "the best diuretic" therapeutically.

4.5.Molecular Docking:

Molecular docking was performed on all 26 phytoconstituents present in the CAE against 9BWT (Human Sodium Chloride Cotransporter in the Phosphorylation State and in Complex with Hydrochlorothiazide) and 7SFL (Human NKCC1 state Fu-II), the transporters present in the nephron that regulate electrolyte reabsorption. On the basis of the docking score and the number of interactions, the top two compounds for 7SFL and the top three compounds for 9BWT were shortlisted for further analysis. The details of the shortlisted compounds are presented in Tables 22 and 23.

Table 22: Interaction analysis of shortlisted ligands of CAE with 9BWT protein

Sl. No.	Phytoconstituents	Binding Energy (kcal/mol)	No. of Interactions	Hydrogen Bonds	Hydrophobic Bonds
1	Stigmasterol	-11.2	4	-	CYS471 PHE535 (3)
2	Alpha-Tocopherol	-10.91	7	CYS471 (2)	CYS471 PHE535 (4)
3	Linoleic Acid	-8.9	6	-	CYS471 (2) PHE535 (4)

Table 23: Interaction analysis of shortlisted ligands of CAE with 7SFL protein

Sl. No.	Phytoconstituents	Binding Energy (kcal/mol)	No. of Interactions	Hydrogen Bonds	Hydrophobic Bonds
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-4.94	6	ALA1173	ALA1173 TYR1183 (3) HIS908
2	Furaneol	-4.43	4	GLY1176 (2)	TYR1183 HIS908

The study revealed that the CYS471 and PHE535 residues of 9BWT can interact with small ligands. The alpha-tocopherol was observed to form a single hydrogen bond with CYS471 and one hydrophobic interactions with CYS471 and four hydrophobic interactions with PHE535, with a binding energy of -10.91 kcal/mol. Whereas stigmasterol and linoleic

acid were observed to form no hydrogen bonds, they instead had multiple hydrophobic interactions with CYS471 and PHE535, with binding energies of -11.2 kcal/mol and -8.9 kcal/mol, respectively (Figure 22).

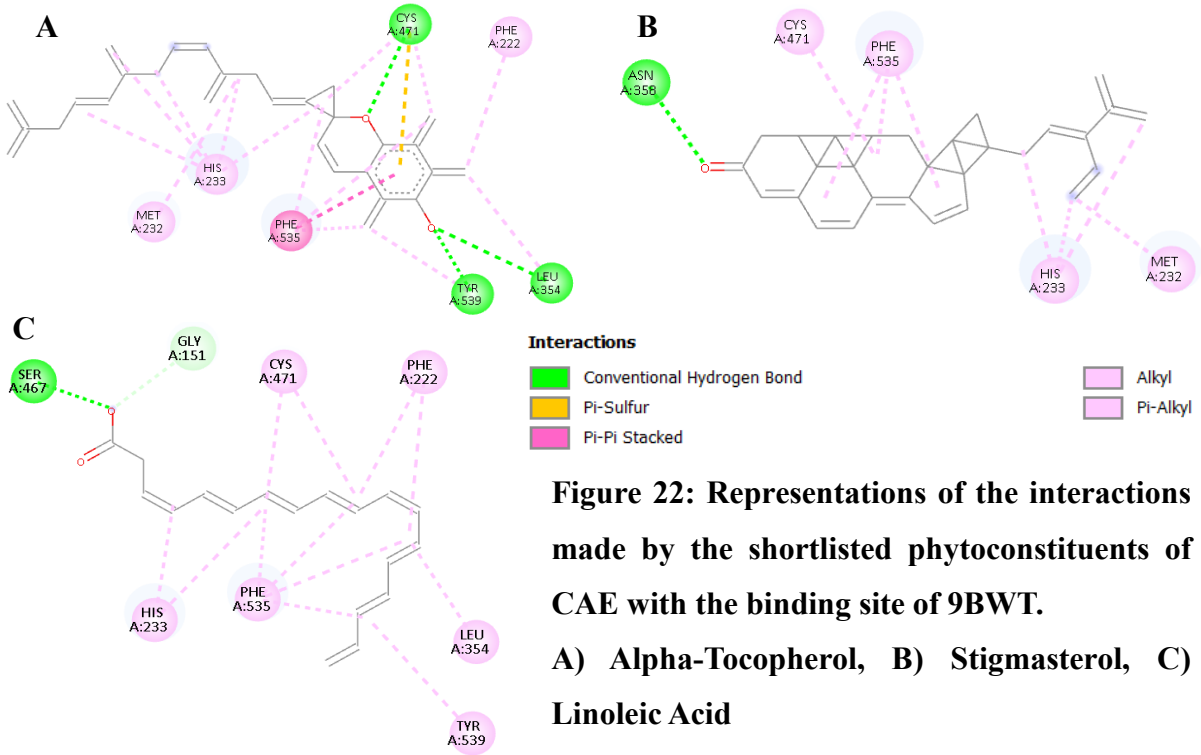


Figure 22: Representations of the interactions made by the shortlisted phytoconstituents of CAE with the binding site of 9BWT. A) Alpha-Tocopherol, B) Stigmasterol, C) Linoleic Acid

Study also revealed that the ligand 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, when interacting with the 7SFL protein, formed one hydrogen bond with ALA1173 and four hydrophobic interactions with ALA1173, TYR1183, and HIS908, with a binding energy of -4.94 kcal/mol. Similarly, another ligand, furaneol, formed two hydrogen bonds with GLY1176 and one hydrophobic interaction with TYR1183, with a binding energy of -4.43 kcal/mol (Figure 23).

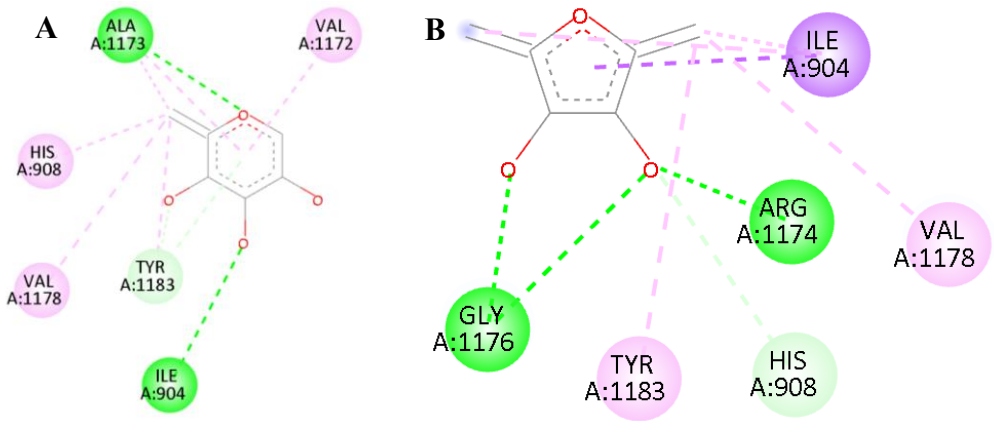


Figure 23: Representations of the interactions made by the shortlisted phytoconstituents of CAE with the binding site of 7SFL. A) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, B) Furaneol

Discussion:

Three phytoconstituents for 9BWT and 2 phytoconstituents for 7SFL were shortlisted, which showed favorable interactions with the binding site of these proteins and exhibited promising interactions with the binding surface of the proteins. However, no studies have explored the diuretic molecular mechanisms of the phytoconstituents of CAE. Our initial work on molecular docking studies needs to be conducted thoroughly all transporters and enzymes involved in the reabsorption of electrolytes in the nephrons.

The current comparative docking analysis demonstrated and supported the results of network pharmacology, which revealed that the phytoconstituents are versatile as molecular scaffolds capable of engaging structurally diverse protein binding sites through adaptive conformational strategies, hydrogen bonding networks and hydrophobic interactions. The ability to bind successfully to both the large sodium chloride cotransporter cavity and the constrained PDZ domain groove illustrates the potential of polyol-based compounds in multitarget therapeutic applications. The distinct interaction patterns observed with each protein target provide valuable insights for rational drug design efforts targeting similar binding sites while highlighting the importance of considering binding site architecture and native ligand characteristics in structure-based design campaigns. Future studies incorporating experimental validation and dynamic modeling approaches will further refine our understanding of polyol-protein interactions and their therapeutic potential.

4.6.Acute toxicity study:

Patterns observed during the study period: 2000 mg/kg of CAE did not cause any visible toxicity symptoms in experimental animals. No deaths or adverse manifestations were observed in any of the animals during the 14-day study period. The physical examination of the treated animals revealed no toxicity to their mucus membranes, eyes, fur, or further. There were no behavioral alterations, such as salivation, tremors, sleep, diarrhea, coma, or death.

Changes in BW and water consumption: The BWs of the animals decreased gradually during the study period. However, no significant changes in water intake were observed. No statistically significant differences were found in these parameters (Table 24).

Table 24: Comparison of BW, amount of water consumed in ATS of CAE

Parameters	Normal Control (n = 6)	Test (n = 5)
Increase in Body Weight (g)	30 ±3.16	30 ±2.54
Amount of Water Consumed (mL)	13.66±2.94	15.40±3.8

The analysis was conducted using a Student's t-test with a confidence interval set at 95%. The results are presented as the mean ± standard deviation (SD).

CBC: Compared with those in the normal control group, the platelet counts in the test group of animals treated with CAE were significantly greater, and no significant changes in the other parameters were detected (Table 25).

Table 25: Haematological parameter values in ATS of CAE

Parameters	Normal Control (n = 6)	Test (n = 5)
WBC (10^3/µL)	13.65±4.37	15.84±2.71
Lymphocytes (%)	56.46±4.57	57.58±2.53
Monocytes (%)	4.08±0.09	4.14±0.35
Eosinophils (%)	10.96±0.93	10.8±1.10
RBC (10^6/µL)	7.92±0.21	8.10±0.17
HgB (g/dL)	14.45±0.42	14.96±0.46
Platelet (10^3/µL)	945±27.89	1066±64.95 *

* = $p < 0.05$; The analysis was conducted using a Student's t-test with a confidence interval set at 95%. The results are presented as the mean ± SD.

Serum electrolytes and renal function tests: The results revealed no significant changes in renal function parameters, including serum electrolytes, in treated animals compared with those in normal control animals (Table 26).

Table 26: Serum electrolyte levels and renal function test values in ATS of CAE		
Parameters	**Normal Control (n = 6)**	**Test (n = 5)**
Serum electrolytes		
Sodium (mmol/L)	140.52±5.04	138.44±5.64
Potassium (mmol/L)	5.68±0.61	5.63±0.78
Chloride (mmol/L)	102.55±1.68	103.92±3.11
Calcium (mmol/L)	9.17±0.61	9.33±0.73
Phosphate (mmol/L)	5.23±0.28	5.08±0.22
Renal function test		
Urea (mg/dL)	18.65±2.00	18.89±2.76
Uric acid (mg/dL)	1.18±0.25	1.18±0.23
Creatinine (mg/dL)	0.48±0.06	0.45±0.07
Albumin (g/dL)	3.59±0.10	3.56±0.08
The results are presented as the mean ± SD. Significant difference between the groups was tested using Student's t-test, $p < 0.05$.		

Serum metabolic profile: The serum LDL and total cholesterol levels in the test group, which were administered CAE, were significantly greater than those in the normal control group (Table 27).

Table 27: Serum metabolic profile values in ATS of CAE		
Parameters	**Normal Control (n = 6)**	**Test (n = 5)**
Blood Sugar	151.83±4.49	153.2±4.43
Serum Lipid Profile		
HDL (mg/dL)	48.60±1.62	49.69±2.09
LDL (mg/dL)	20.48±2.41	23.64±0.44 *
Triglycerides (mg/dL)	58.07±2.87	61.22±2.39
VLDL (mg/dL)	11.61±0.57	12.24±0.47
Total cholesterol (mg/dL)	80.70±2.89	85.58±2.68 *

Serum liver function tests		
Direct Bilirubin (mg/dL)	0.03±0.00	0.04±0.01
Indirect bilirubin (mg/dL)	0.06±0.01	0.05±0.00
Total Bilirubin (mg/dL)	0.09±0.01	0.09±0.01
AST (U/L)	74.66±4.15	78.01±3.16
ALP (U/L)	72.47±4.38	72.58±3.41
ALT (U/L)	30.15±3.94	31.42±3.09
* = p <0.05; The analysis was conducted using a Student's t-test with a confidence interval set at 95%. The results are presented as the mean ± SD.		

Changes in urine output and urine pH: There were no significant changes in 24-hour urine output or urine pH. No statistically significant differences were found in these parameters (Table 28).

Table 28: Comparison of urine output and urine pH in ATS of CAE		
Parameters	**Normal Control (n = 6)**	**Test (n = 5)**
Urine Volume (mL)	21.33±6.97	25.00±5.00
Urine pH	7.16±0.40	7.20±0.44
The analysis was conducted using a Student's t-test with a confidence interval set at 95%. The results are presented as the mean ± standard deviation (SD).		

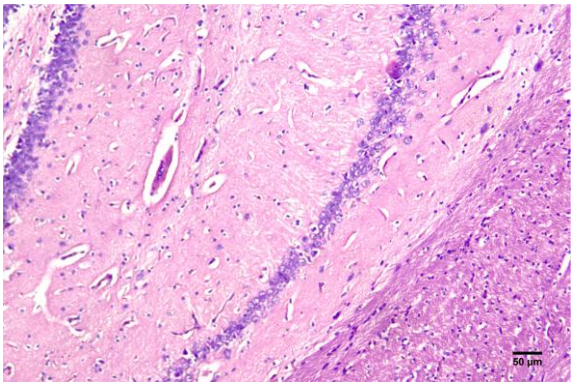
Urine electrolytes and renal function test: The urine electrolyte panel and renal function test parameters of test group animals, which were administered CAE, were similar to those of the normal control group (Table 29).

Table 29: Urine electrolyte levels and renal function test values in ATS of CAE		
Parameters	**Normal Control (n = 6)**	**Test (n = 5)**
Urine electrolytes		
Sodium (mmol/L)	129.58±5.26	130.24±5.18
Potassium (mmol/L)	6.53±1.47	6.622±1.47
Chloride (mmol/L)	93.52±3.16	92.75±3.58
Calcium (mmol/L)	9.36±1.83	10.84±1.26
Magnesium (mmol/L)	8.23±0.81	7.88±1.06

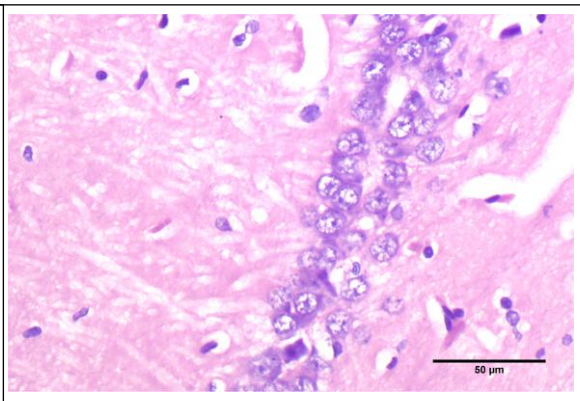
Phosphate (mmol/L)	6.77±0.72	7.11±0.82
Renal function tests		
Urea (mg/dL)	9.03±0.79	8.62±1.34
Creatinine (mg/dL)	0.76±0.13	0.77±0.09
Uric acid (mg/dL)	31.34±2.57	32.62±2.44
Albumin (g/dL)	0.54±0.04	0.57±0.02
The analysis was conducted using a Student's t-test with a confidence interval set at 95%. The results are presented as the mean ± SD.		

Histopathology of the test group:

Brain: Brain tissue sections from the cerebrum, cerebellum, and hippocampus. The neurons present in the cerebrum and hippocampus are healthy, with pale and round nuclei, well-defined nuclear boundaries, and prominent nucleoli. The slide does not show histological changes such as inflammation or degenerated and necrosed neurons (Images A and B).

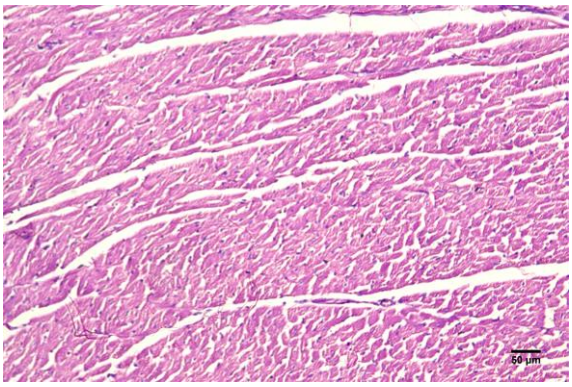


24A. CA1 Hippocampus (100X)

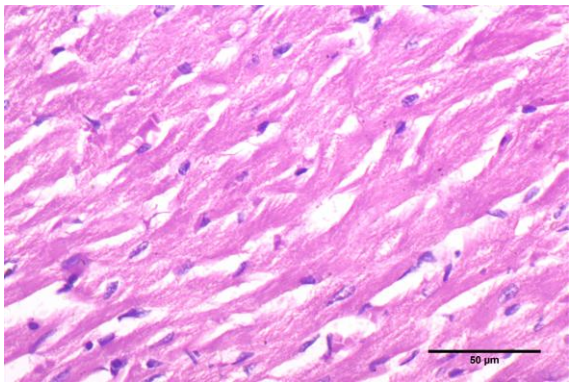


24B. CA1 Hippocampus (400X)

Heart: The slide depicts H&E-stained sections of heart tissue consisting of cardiac muscle and the myocardium. The myocardium consists of muscle cells with nuclei. The slide does not show histological changes, such as degeneration, inflammation, or necrosis, with no change in tissue architecture (Images C and D).

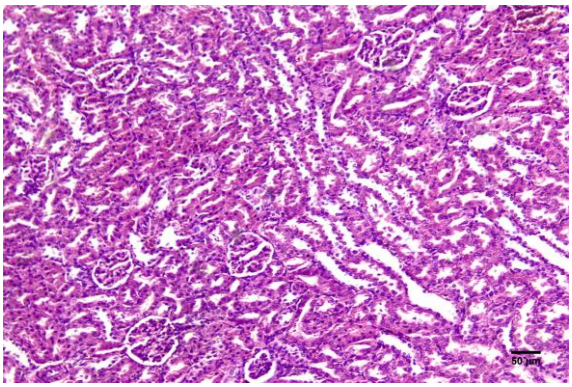


24C. Cardiac Muscle (100X)

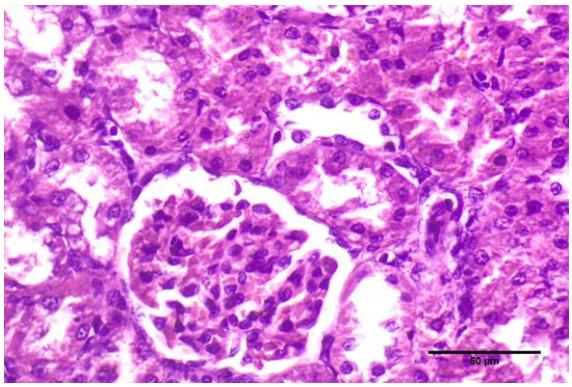


24D. Cardiac Muscle (400X)

Kidney: The slide depicts H&E-stained sections of kidney tissue consisting of the cortex and medulla. The circular structures in the cortex are corpuscles, which contain several shapes of glomerulus and tubules, including proximal and distal convoluted tubules. The slides did not show histological changes, such as degeneration, dilation of tubules, inflammation or necrosis, with no change in tissue architecture (Images E and F).

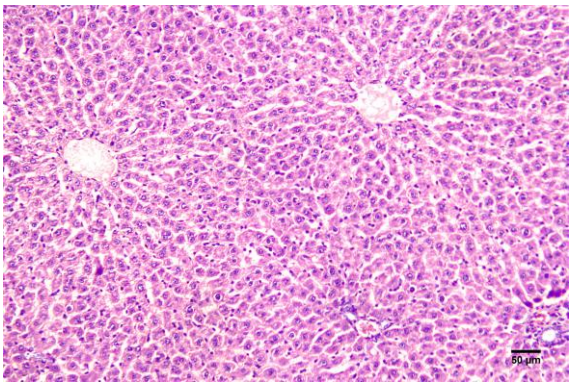


24E. Cortex and Medulla of Kidney (100X)

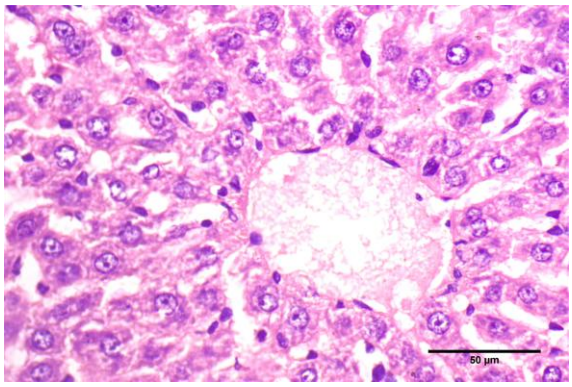


24F. Cortex and Medulla of Kidney 400X)

Liver: The slide depicts H&E-stained sections of liver tissue with a lobular arrangement, where each lobule has a central vein and a peripheral portal triad. Radial sinusoids extend from the central vein, with hepatocytes filling the spaces between them. The slides did not show histological changes, such as degeneration, cytoplasmic vacuolation, inflammation, or necrosis, with no change in tissue architecture (Images G and H).

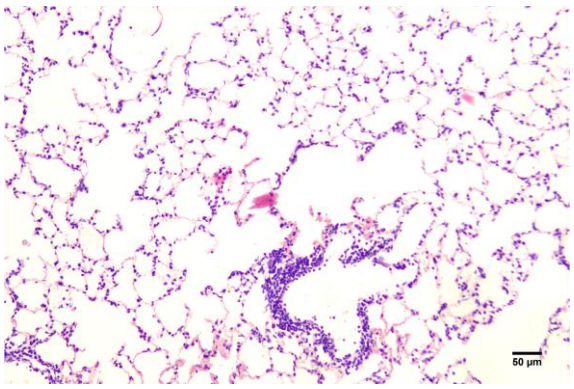


24G. Liver (100X)

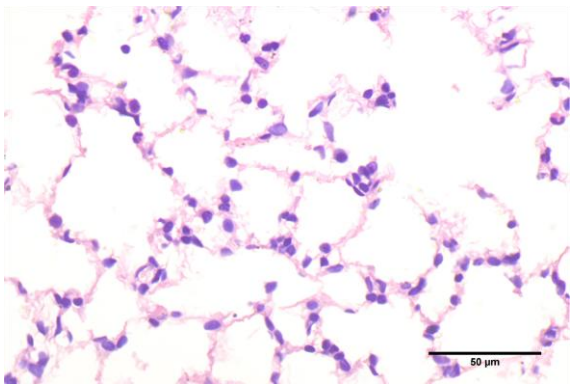


24H. Liver (400X)

Lungs: The slide depicts H&E-stained sections of lung tissue consisting of alveoli and bronchioles. The slides did not show histological changes, such as degeneration, inflammation, or necrosis, and there was no change in tissue architecture (Images I and J).

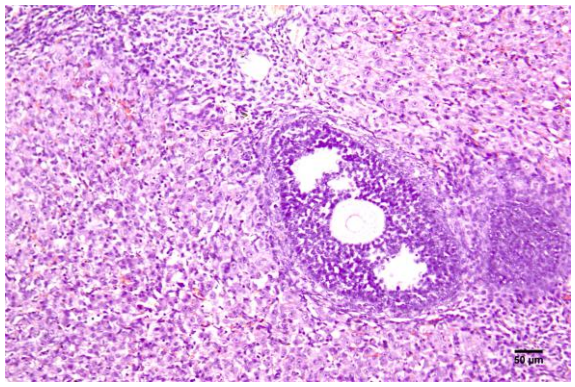


24I. Lungs (100X)

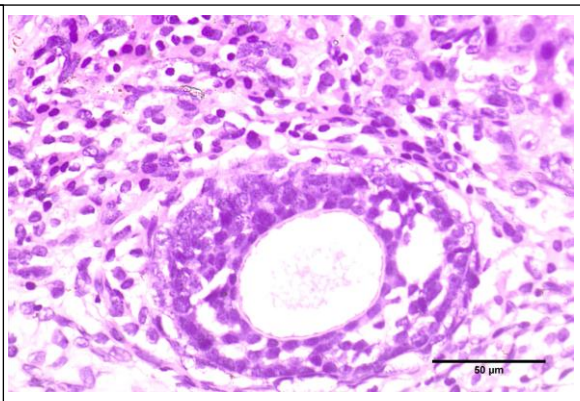


24J. Lungs (400X)

Ovaries: The slide depicts H&E-stained sections of ovary tissue with surface epithelium. The outer cortex region shows follicles, and the inner medulla shows loose fibroblastic connective tissue with blood vessels and lymphatic vessels. The ovarian follicles of the cortical region are in different stages (primary, secondary, tertiary, and graafian) of development and are embedded in the thick stroma. Very few degenerating follicles are also observed. Corpus luteum of different cycles are shown. The tissue architecture was maintained. Cysts are not observed, and there are no histological changes (Images K and L).

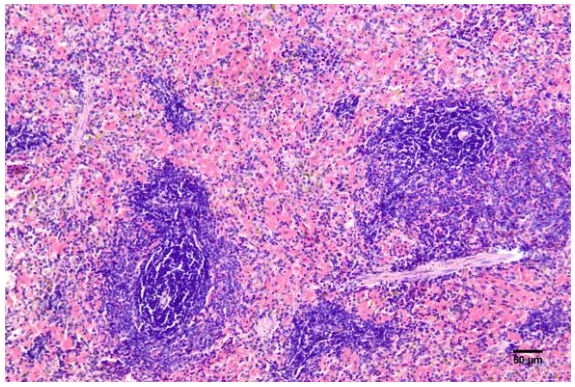


24K. Ovary (100X)

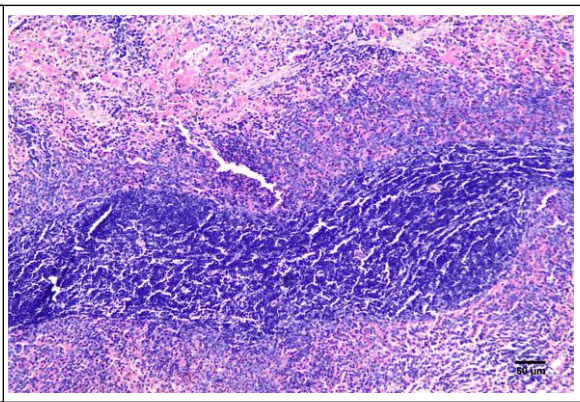


24L. Ovary (400X)

Spleen: The slide depicts H&E-stained sections of spleen tissue consisting of red and white pulp. The red pulp is vascular and has numerous sinusoids, whereas the white pulp consists of densely aggregated lymphocytes in the form of cords surrounding arterioles. Histological changes such as a reduction in white or red pulp cellularity, an increase in extramedullary hematopoiesis, and an increase in lymphocyte apoptosis are not observed (Images M and N).



24M. Spleen (100X)



24N. Spleen (400X)

Discussion:

Toxicological studies provide important insights into the safety profile of any medication and, hence, can significantly impact clinical practice. Approximately 80% of the world's population uses traditional or folk medicines (36). In developing and developed countries. *TT* decoction is prepared by traditional practitioners and is used as an aphrodisiac for benign prostatic hyperplasia, renal stones, and urinary tract infection (93,205). There are insufficient data in the literature regarding the safety profile of CAE. Since the extracts can be obtained as over-the-counter medications, the risks of misuse or overuse are high, which might

adversely affect the patient. The aim of this study was to identify the safe dose of CAE per the OECD guideline number 425 (163).

In the present study, clinical biochemistry and hematological data were used to determine the toxicity induced by the drug. There were no significant changes in the CBC, and the treated group exhibited a noteworthy increase in the platelet count compared with that of the control animals. This could indicate an immune-stimulating effect of the test drug, akin to findings in other studies where specific compounds increase platelet production, potentially owing to their influence on bone marrow activity (206). Nonetheless, this increase should be viewed carefully, as it may also reflect a stress reaction or an inflammatory process despite the absence of notable histopathological alterations in the liver or other organs (207–210).

There were no changes in the serum electrolytes or renal function tests, leading to the absence of nephrotoxicity, which is a promising finding. In addition, the absence of significant changes in urine electrolytes and renal function tests further affirms the safety of this extract in subjects with renal impairments. Moreover, there were no changes in the serum bilirubin or liver enzymes, such as AST, ALT, and ALP, reinforcing that this dosage is not hepatotoxic. However, the metabolic profile revealed a modest yet statistically significant increase in the serum LDL and total cholesterol levels in the test group compared with those in the normal control group. The increase in LDL levels and total cholesterol observed in the treated group may reflect a metabolic impact of the test medication. This could result from modifications in lipid metabolism pathways or shifts in the expression of genes related to cholesterol production and transport (211). Even with these changes, the lack of notable liver histopathological changes indicates that the drug does not lead to significant liver damage, which is commonly linked to lipid metabolism disruptions (207). These results are in accordance with those of the ATS study conducted by Abbas et al. (2022), which also revealed that the administration of the methanolic extract of *TT* at doses of 2000 mg/kg and 3000 mg/kg BW did not cause any significant changes in the renal, hepatic, and blood parameters of the treated animals Click or tap here to enter text.. Similarly, in another ATS study by Yasmeen et al. (2022), animals administered spray-dried extracts of *TT* showed no toxicity at doses of 2000 mg/kg and 5000 mg/kg BW (213).

The brain, liver, heart, kidneys, and spleen are key organs examined in histopathology studies to assess acute toxicity and drug-induced damage. No lesions or alterations were found in any of the organs of the test group animals during the microscopic examination. Overall, the findings of our study support the favorable safety profile of CAE, indicating that it is a well-tolerated and safer therapeutic intervention. Since a 2000 mg/kg dose of the CAE did not show

any toxicity, 1/10 (200 mg/kg) is considered a therapeutic dosage, 1/20 (100 mg/kg) is considered a subtherapeutic dosage, and 1/5 (400 mg/kg) is considered a supratherapeutic dosage for the subsequent preclinical experiments (214–217).

4.7.Evaluation of the diuretic effect of a single oral dose of CAE:

Changes in BW and water consumption:

Among the three doses of CAE (low, medium, and high), the high dose increased the BW of the treated animals and the amount of water consumed, which was significantly greater than that of the other two doses (low and medium) of the CAE. These effects are comparable to those of standard diuretic agents such as hydrochlorothiazide and furosemide, which are statistically significant ($p < 0.05$).

The co-administration of CAE with standard diuretics did not alter the BW of the treated animals. Compared with standard diuretic drug treatments, it significantly increased water consumption ($p < 0.05$). The changes observed are mentioned in Table 30 below.

Table 30: Comparison of BW and amount of water consumed in a 24-hour diuretic study.

Sl. No.	Groups (n = 6)	Difference in the Body Weight (g)	Amount of Water Consumed (mL)
1	Normal Control	23.00±3.46	17.33±2.42
2	Hydrochlorothiazide (80.64 mg/kg/day)	24.33±4.37	25.33±1.63
3	Furosemide (12.9 mg/kg/day)	26.50±3.94	28.33±2.94
4	Spironolactone (4 mg/kg/day)	25.00±2.96	19.33±3.93
5	CAE – Low Dose (100 mg)	12.17±3.66 #, $, ¢, £	13.33±5.16 #, $, £
6	CAE – Medium Dose (200 mg)	12.83±2.32 #, $, ¢, £	15.00±12.25 #, $, £
7	CAE – High Dose (400 mg)	27.83±5.19 ¥, ɤ	33.33±2.07 ¢, ¥, ɤ
8	CAE (High dose) + Hydrochlorothiazide	25.83±2.64	34.33±4.13
9	CAE (High dose) + Furosemide	27.42±2.91	51.00±2.45 $
10	CAE (High dose) + Spironolactone	25.00±2.97	31.67±5.28 ¢

#, $, ¢, ¥, ɤ, £, §, Φ, ŧ = p <0.05; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone, ¥ = Vs CAE - Low Dose, ɤ = Vs CAE - Medium Dose, £ = Vs CAE - High Dose, § = Vs CAE (High dose) + Hydrochlorothiazide, Φ = Vs CAE (High dose) + Furosemide, ŧ = Vs CAE (High dose) + Spironolactone

Changes in urine output and urine pH:

Among the three doses of CAE (high, medium, and low), the high dose substantially increased urinary excretion compared with the low and medium doses of CAE and standard diuretics, such as hydrochlorothiazide and spironolactone, which were statistically significant ($p < 0.05$). Compared with the low and medium doses of the CAE, hydrochlorothiazide, and spironolactone, the high dose of the CAE led to an increase ($p < 0.05$) in the urine pH; this effect was similar to that of furosemide.

As the high dose (400 mg/kg) of the CAE showed better diuretic effects, this dose was selected for further investigations in combination with standard diuretics and later for chronic studies.

Compared with standard diuretics alone, the coadministration of CAE with standard diuretics, hydrochlorothiazide, and furosemide significantly ($p < 0.05$) increased urinary excretion.

The urine pH was significantly greater ($p < 0.05$) in the animals treated with the co-administration of the high dose of CAE and hydrochlorothiazide than in those treated with hydrochlorothiazide alone. The changes are illustrated in Figure 25 and mentioned in Table 31 below.

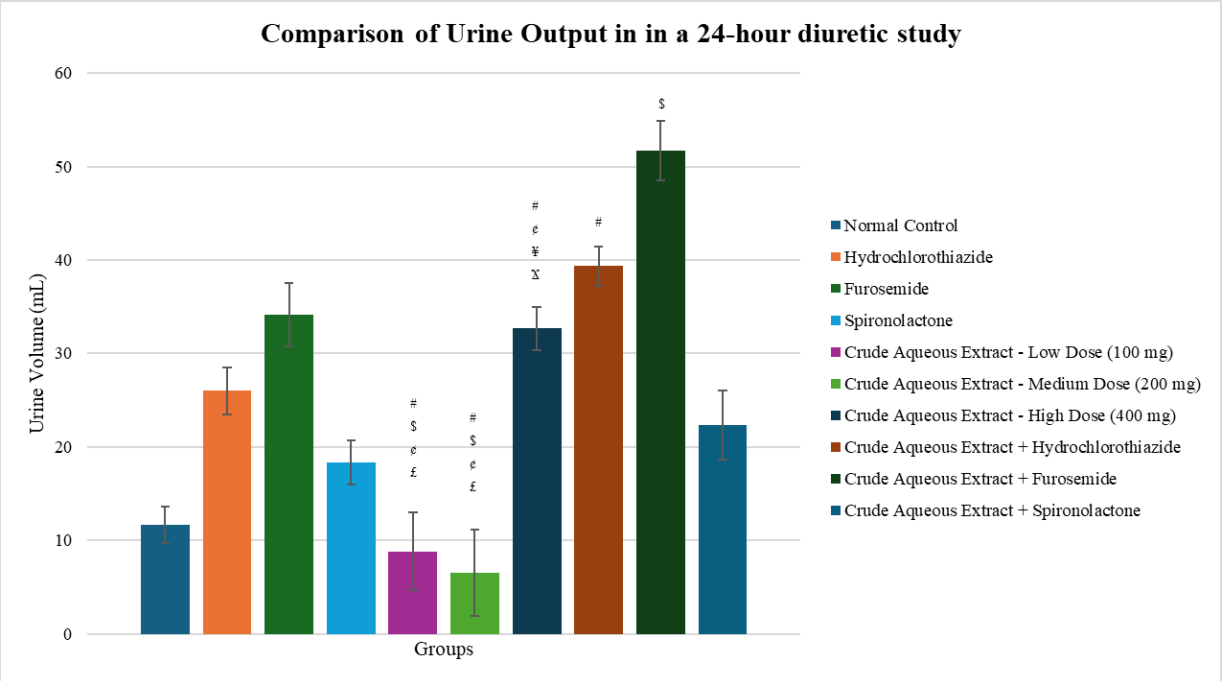


Figure 25: Comparison of the urine output among the groups in the 24-hour diuretic study of CAE alone and in combination with standard diuretics.

#, $, ¢, ¥, ¤, £ = $p < 0.05$. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone, ¥ = Vs CAE - Low Dose, ¤ = Vs CAE - Medium Dose, £ = Vs CAE - High Dose

Table 31: Comparison of urine pH in a 24-hour diuretic study of CAE.		
Sl. No.	**Groups (n = 6)**	**Urine pH**
1	Normal Control	7.00±0.00
2	Hydrochlorothiazide (80.64 mg/kg/day)	12.00±0.00
3	Furosemide (12.9 mg/kg/day)	14.00±0.00
4	Spironolactone (4 mg/kg/day)	9.33±1.03
5	CAE – Low Dose (100 mg)	8.00±0.00 $^{\#, \$, ¢, ¤, £}$
6	CAE – Medium Dose (200 mg)	10.17±1.47 $^{\#, \$, ¥, £}$
7	CAE – High Dose (400 mg)	14.00±0.00 $^{\#, ¢, ¥, ¤}$
8	CAE (High dose) + Hydrochlorothiazide	14.00±0.00 $^{\#}$
9	CAE (High dose) + Furosemide	14.00±0.00
10	CAE (High dose) + Spironolactone	10.00±0.00

$^{\#, \$, ¢, ¥, ¤, £}$ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone, ¥ = Vs CAE - Low Dose, ¤ = Vs CAE - Medium Dose, £ = Vs CAE - High Dose

Urine RFT:

There was a statistically significant ($p < 0.05$) increase in urea and uric acid excretion in the urine of the animals treated with a high dose of CAE compared with that of furosemide, spironolactone, and lower doses of CAE. No significant changes in the creatinine and albumin levels were detected ($p > 0.05$) in the animals treated with high-dose CAE compared with those treated with standard diuretics.

The co-administration of CAE with hydrochlorothiazide, furosemide, and spironolactone caused more urea and uric acid excretion ($p < 0.05$) than the individual standard diuretics themselves. This finding shows that the co-administration may produce additive effects. Similarly, there were no significant ($p > 0.05$) changes in the creatinine or albumin levels compared with those of standard diuretics. The changes observed are illustrated in Figure 26 and summarized in Table 32 below.

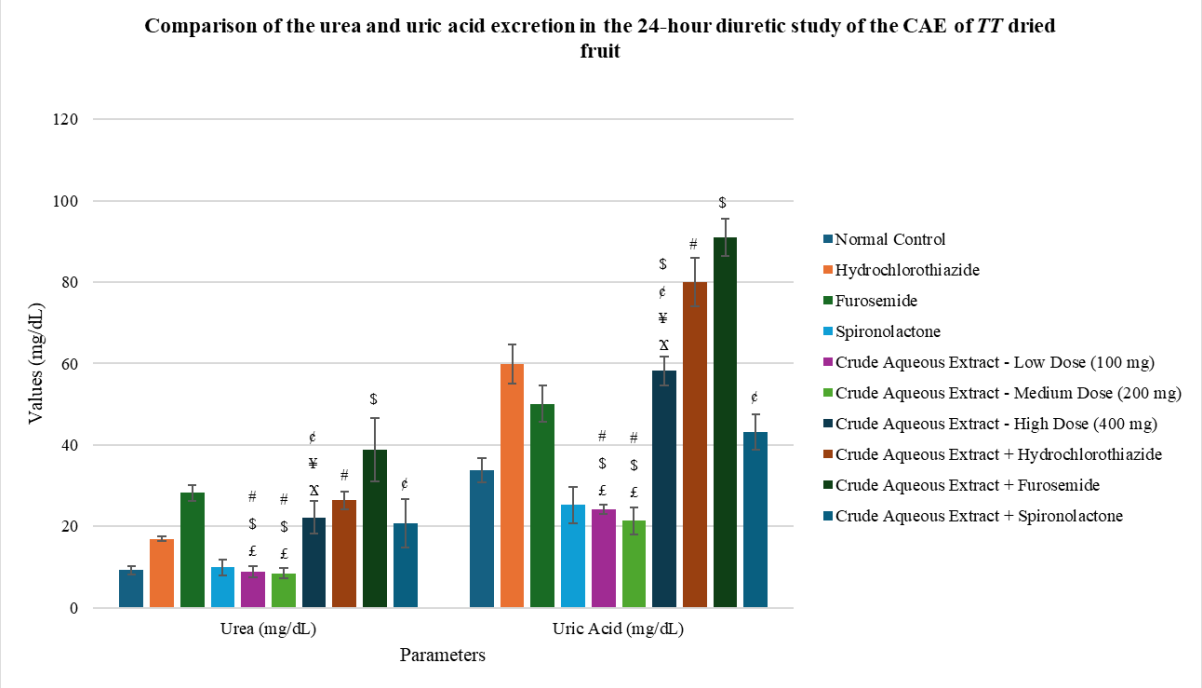


Figure 26: Comparison of urea and uric acid excretion in urine among the groups in the 24-hour diuretic study of CAE and its combination with standard diuretics.

#, $, ¢, ¥, ϫ, £, §, Φ, ϯ = $p < 0.05$. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone, ¥ = Vs CAE - Low Dose, ϫ = Vs CAE - Medium Dose, £ = Vs CAE - High Dose, § = Vs CAE (High dose) + Hydrochlorothiazide, Φ = Vs CAE (High dose) + Furosemide, ϯ = Vs CAE (High dose) + Spironolactone

Table 32: RFT values in the urine samples of a 24-hour diuretic study of CAE.

Sl. No.	Groups (n = 6)	Creatinine (mg/dL)	Albumin (g/dL)
1	Normal Control	0.5±0.06	0.55±0.12
2	Hydrochlorothiazide (80.64 mg/kg/day)	0.62±0.10	0.64±0.06
3	Furosemide (12.9 mg/kg/day)	0.89±0.22	0.63±0.19
4	Spironolactone (4 mg/kg/day)	0.49±0.16	0.58±0.09
5	CAE - Low Dose (100 mg)	0.41±0.26 $	0.57±0.06
6	CAE - Medium Dose (200 mg)	0.55±0.11	0.60±0.01
7	CAE - High Dose (400 mg)	0.57±0.10	0.52±0.06
8	CAE + Hydrochlorothiazide	0.59±0.28	0.68±0.16
9	CAE + Furosemide	0.59±0.25	0.88±0.42
10	CAE + Spironolactone	0.26±0.12	0.60±0.12

#, $, ¢, ¥, ¤, £, §, Φ, † = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone, ¥ = Vs CAE - Low Dose, ¤ = Vs CAE - Medium Dose, £ = Vs CAE - High Dose, § = Vs CAE (High dose) + Hydrochlorothiazide, Φ = Vs CAE (High dose) + Furosemide, † = Vs CAE (High dose) + Spironolactone

Urine electrolytes:

There was a statistically significant ($p < 0.05$) increase in the urine sodium and potassium outputs in the animals treated with the high dose of the CAE compared with those in the low and medium doses of the CAE, hydrochlorothiazide, furosemide and spironolactone. It also increased the urinary excretion of chloride ($p < 0.05$) compared with the low and medium doses of the CAE and spironolactone.

The high dose of CAE significantly increased the urinary excretion of calcium (calcium wasting effect) and magnesium ($p < 0.05$) compared with the low and medium doses of CAE, hydrochlorothiazide and spironolactone. It also increased the urinary excretion of the phosphate to a statistically significant level ($p < 0.05$) when compared to the low and medium doses of CAE, furosemide, and spironolactone.

Compared with hydrochlorothiazide alone, the co-administration of CAE and hydrochlorothiazide significantly ($p < 0.05$) increased the urinary excretion of sodium, potassium, calcium, magnesium and phosphate.

The co-administration of CAE with furosemide and spironolactone significantly ($p < 0.05$) increased the urinary excretion of sodium, potassium, chloride, calcium, magnesium and phosphate when compared to the furosemide and spironolactone alone. The changes observed are illustrated in Figure 27 and summarized in Table 33 below.

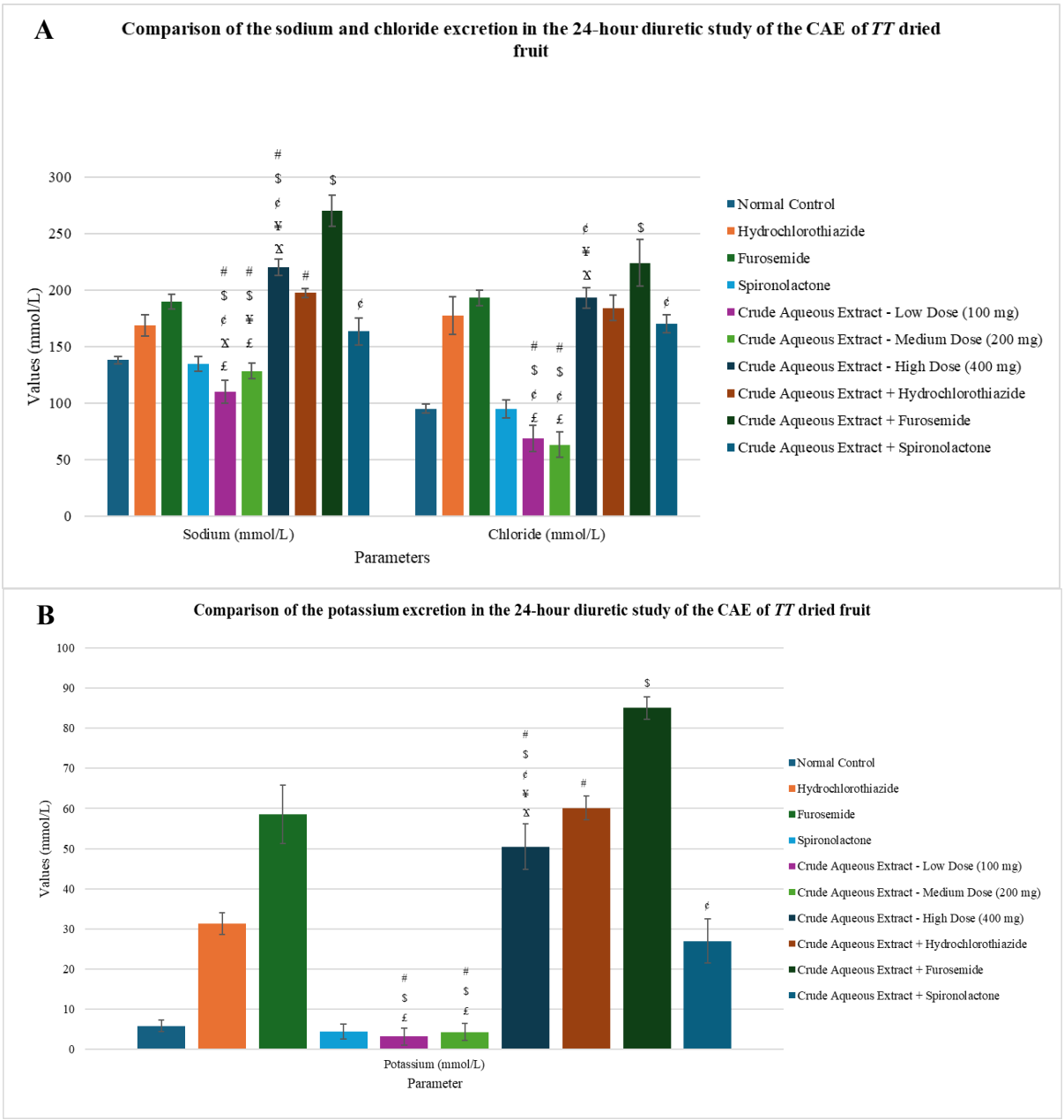


Figure 27: Comparison of sodium, chloride (A), and potassium (B) excretion in urine among the groups in the 24-hour diuretic study of CAE alone and in combination with standard diuretics.

#, $, ¢, ¥, ϰ, £ = $p < 0.05$. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone, ¥ = Vs CAE - Low Dose, ϰ = Vs CAE - Medium Dose, £ = Vs CAE - High Dose

Table 33: Urine electrolyte levels in a 24-hour diuretic study of CAE.

Sl. No.	Groups	Calcium (mmol/L)	Magnesium (mmol/L)	Phosphate (mmol/L)
1	Normal Control	7.53±0.83	7.91±0.37	7.19±0.50
2	Hydrochlorothiazide (80.64 mg/kg/day)	5.39±1.04	23.69±4.10	21.67±0.44
3	Furosemide (12.9 mg/kg/day)	22.96±0.05	33.82±3.85	11.91±0.56
4	Spironolactone (4 mg/kg/day)	6.92±1.23	8.58±1.49	5.95±1.52
5	CAE - Low Dose (100 mg)	7.88±3.88 $, £	7.18±1.05 #, $, £	0.56±0.38 #, $, ¢, £
6	CAE - Medium Dose (200 mg)	8.27±3.30 $, £	8.02±2.43 #, $, £	0.76±0.45 #, $, ¢, £
7	CAE - High Dose (400 mg)	23.10±0.26 #, ¢, ¥, ɤ	37.24±4.31 #, ¢, ¥, ɤ	22.91±0.71 $, ¢, ¥, ɤ
8	CAE (High dose) + Hydrochlorothiazide	10.89±2.55 #	31.92±0.05 #	39.79±4.36 #
9	CAE (High dose) + Furosemide	32.68±5.22 $	50.28±4.46 $	21.54±1.96 $
10	CAE (High dose) + Spironolactone	12.89±0.06 ¢	15.61±3.17 ¢	13.98±0.69 ¢

#, $, ¢, ¥, ɤ, £ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone, ¥ = Vs CAE - Low Dose, ɤ = Vs CAE - Medium Dose, £ = Vs CAE - High Dose

Indices for electrolyte excretion and diuretic activity:

The high dose of CAE, furosemide, and hydrochlorothiazide had the highest saluretic effects in terms of the excretion of sodium and chloride. All the groups showed favorable natriuretic effect. In contrast, spironolactone and medium and low doses of CAE had a potassium-sparing effect. Spironolactone and high, low, and medium doses of the CAE showed increasing carbonic anhydrase inhibitory activity.

The co-administration of CAE with furosemide showed the highest diuretic effect, followed by the combination of a high dose of CAE with hydrochlorothiazide and spironolactone, with scores of 381.6 and 333.6, respectively. Among the combinations, the high dose of CAE with spironolactone showed the highest natriuretic effect, followed by hydrochlorothiazide and furosemide, with scores of 6.0, 3.3, and 3.2 natriuretic index points, respectively. Only the combination of CAE with spironolactone did not result in carbonic anhydrase inhibitory activity, whereas the other two combinations resulted in slight carbonic anhydrase inhibitory activity. The changes observed are mentioned in Table 34 below.

Table 34: Indices for electrolyte excretion in a 24-hour diuretic study of CAE.

Sl. No.	Groups	Saluretic Effect	Natriuretic Effect	CA Inhibition Effect
1	Hydrochlorothiazide (80.64 mg/kg/day)	346.1	5.4	0.9
2	Furosemide (12.9 mg/kg/day)	382.7	3.2	0.8
3	Spironolactone (4 mg/kg/day)	229.4	30.2	0.7
4	CAE - Low Dose (100 mg)	179.0	34.4	0.6
5	CAE - Medium Dose (200 mg)	191.7	29.5	0.5
6	CAE - High Dose (400 mg)	413.4	4.4	0.7
7	CAE (High dose) + Hydrochlorothiazide	381.6	3.3	0.7
8	CAE (High dose) + Furosemide	494.2	3.2	0.6
9	CAE (High dose) + Spironolactone	333.6	6.0	0.9

Natriuretic Activity (sodium excretion): Values > 2 indicate a favourable natriuretic effect, and values >10 indicate a potassium-sparing effect

Carbonic Anhydrase Inhibition: Ratios between 1 and 0.8: No carbonic anhydrase inhibition; with decreasing ratios, slight to strong carbonic anhydrase inhibition is present

***Lipschitz* test for urine and sodium:**

Animals treated with a high dose of CAE showed a potent diuretic effect by scoring 3 in the *Lipschitz* test for urine and sodium. However, low and medium doses had positive effect by scoring 1 for both the *Lipschitz* test for urine and sodium.

Among the combinations, animals treated with a high dose of CAE in combination with furosemide and hydrochlorothiazide showed potent effects, as indicated by scores of 4 and 3 in the *Lipschitz* test for urine, whereas the CAE in combination with spironolactone showed a positive effect, as indicated by a score of 1. In the *Lipschitz* test for sodium, the CAE at a high dose in combination with furosemide showed a potent effect by scoring 2, and the CAE in combination with hydrochlorothiazide and spironolactone showed a positive effect by scoring 1. The changes observed are illustrated in Figure 28 below.

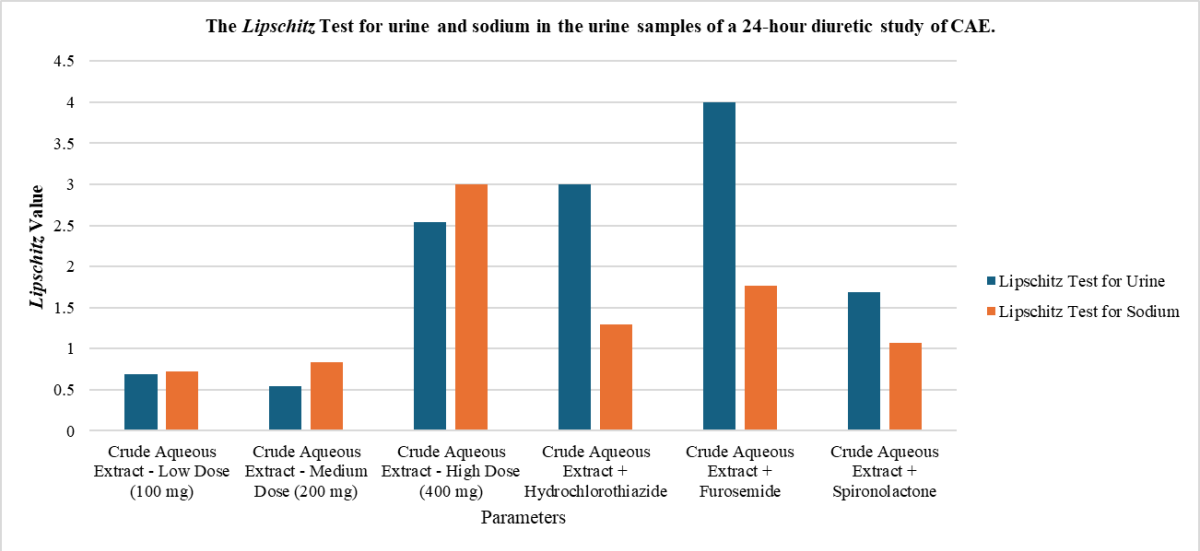


Figure 28: The *Lipschitz* test for urine and sodium in urine samples from a 24-hour diuretic study of CAE.

***Lipschitz* value:** ≥ 1 positive effect; ≥ 2 potent diuretic activity

Discussion:

In the 24-hour study, the low and medium doses of the CAE did not show potential diuretic effects compared with the high dose of the CAE. They showed a reduction in BW, urine excretion volume, and water consumption. This might reflect their low efficacy as diuretics compared with high-dose diuretics. In contrast, the high dose of CAE increased the urine output and water consumption. When a high dose of CAE is co-administered with standard diuretics, its diuretic actions are synergistically potentiated, potentially leading to improved management of health conditions requiring diuretics. This also warrants a reduction in the dose of standard diuretics, which are combined with CAE, to reduce their adverse effects.

The CAE did not exhibit dose-dependent effects on the urine output, but the variation in the urine pH was dose dependent. As the dose of CAE increased, the urine pH also increased/alkalinized. This property may be used in the management of poisoning with acidic drugs, where alkalinization of urine is needed to prevent reabsorption by ionization and to increase the excretion of the poison-causing substance. When co-administered with hydrochlorothiazide, CAE increased urine alkalinization.

A high dose of CAE increased the urinary excretion of sodium, potassium, calcium, magnesium, and phosphate. When it is co-administered with standard diuretics, the excretion of the above electrolytes was synergistically increased.

This result is in line with a previous study by M. Al-Ali et al. (2003), where researchers administered 5 g of the CAE of *TT* to *Wistar* rats as a single dose at the end of 24 hours, which increased the urine output and urinary excretion of potassium better than furosemide. It also increased the urinary excretion of sodium and chloride, making it a slightly more potent diuretic than furosemide itself (98). In another study by Nalwaya et al. (2009), researchers administered UNEX capsules containing extracts of *TT* along with *Boerhaavia diffusa* to *Wistar* rats at two doses, 600 mg/kg and 800 mg/kg. At the end of five hours, the animals showed a dose-dependent increase in the urine volume and urinary excretion of sodium, potassium, and chloride, which were significant compared with those of the normal control (125). In a study by Sudheendran et al. (2021), a single dose of CAE (8.64 ml/kg) of *TT* fruit and roots was administered to *Wistar* rats. At the end of 5 h, the extract increased urinary output in the treated animals and significantly increased the urinary excretion of potassium and chloride compared with the normal control. Compared with the fruit extract, better results were obtained for the root extract (124). These studies observed the diuretic effect only for the first 5 hours after administration.

In our study, the effects were evaluated in 24-hour urine samples after drug administration. The CAE showed increased excretion of uric acid, sodium, magnesium, and phosphate than that of furosemide. The excretion of urea and electrolytes, except for phosphate, by the CAE was higher than that of hydrochlorothiazide. The times increase or decrease in the important parameters related to the diuresis of the high-dose CAE are mentioned in Table 35 below in comparison with those of the respective groups.

Table 35: The changes (in times) produced by the CAE in the urine parameters of the 24-hour diuretic study with respect to the groups compared

Sl. No.	Parameters	NC	H	F	S	H+CAE	F+CAE	S+CAE
1	Urine Volume (mL)	2.8 ↑	1.3 ↑	1.0 ↓	1.8 ↑	0.8 ↑	0.6 ↑	1.5 ↑
2	Amount of Water Consumed (mL)	1.9 ↑	1.3 ↑	1.2 ↑	1.7 ↑	1.0 ↑	0.7 ↑	1.1 ↑
3	Urea (mg/dL)	2.4 ↑	1.3 ↑	0.8 ↓	2.2 ↑	1.6 ↑	1.4 ↑	2.1 ↑
4	Uric Acid (mg/dL)	1.7 ↑	1.0 ↑	1.2 ↑	2.3 ↑	1.3 ↑	1.8 ↑	1.7 ↑
5	Sodium (mmol/L)	1.6 ↑	1.3 ↑	1.2 ↑	1.6 ↑	1.2 ↑	1.4 ↑	1.2 ↑
6	Potassium (mmol/L)	8.6 ↑	1.6 ↑	0.9 ↓	11.3 ↑	1.9 ↑	1.5 ↑	6.1 ↑
7	Chloride (mmol/L)	2.0 ↑	1.1 ↑	1.0 ↑	2.0 ↑	1.0 ↑	1.2 ↑	1.8 ↑
8	Calcium (mmol/L)	3.1 ↑	4.3 ↑	1.0 ↑	3.3 ↑	2.0 ↑	1.4 ↑	1.9 ↑
9	Magnesium (mmol/L)	4.7 ↑	1.6 ↑	1.1 ↑	4.3 ↑	1.3 ↑	1.5 ↑	1.8 ↑
10	Phosphate (mmol/L)	3.2 ↑	1.1 ↑	1.9 ↑	3.9 ↑	1.8 ↑	1.8 ↑	2.3 ↑

Note: NC – normal control, H – hydrochlorothiazide, F – furosemide, S – spironolactone, CAE – crude aqueous extract, ↑ – increase, ↓ – decrease; CAE was compared against normal control, and standard diuretics. The combinations were compared against the respective standard diuretics.

These observed effects of the CAE in our study might be most useful in the treatment of sodium overload, hypertension, congestive cardiac failure, and edematous conditions such as glaucoma and hyperhydration.

The high dose did not have a potassium-sparing effect and a calcium-sparing effect. A high dose of CAE also increased the urinary excretion of urea and uric acid. Uricosuric property of CAE can be useful in the management of gouty arthritis and urate stones.

Overall, single-dose CAE produced a saluretic effect, a favorable natriuretic effect without a potassium-sparing effect, and mild carbonic anhydrase inhibitory activity with potent diuretic action when compared to the standard diuretics (hydrochlorothiazide and furosemide). This also reflects the usefulness of complementary medicines in conjunction with modern medicine in managing certain diseases and disorders. These results can also lead to avenues for further research into cardiac disease models to confirm the results mentioned above.

4.8.Evaluation of the long-term (28-day) diuretic effect of the CAE:

4.8.1. Baseline parameters:

Body weight and water consumption:

There were no statistically significant differences ($p > 0.05$) in the BWs of the animals in all groups, with an average of 326.56 g before the beginning of the study (we reused the animals from the 24 hours study, by maintaining a drug-free period of 4 weeks). There was a statistically significant ($p < 0.05$) increase in the amount of water consumed by the animals in the furosemide combination group compared with that consumed by the animals in the furosemide alone group. The changes observed are mentioned in Table 36 below.

Table 36: Comparison of the baseline BW and amount of water consumption values in a 28-day diuretic study of CAE.

Sl. No.	Groups	Body Weight (g)	Amount of Water Consumed (mL)
1	Normal Control	316.50±37.11	15.50±1.51
2	CAE (400 mg/kg/day)	327.83±32.10	20.33±0.81
3	Hydrochlorothiazide (80.64 mg/kg/day)	328.66±12.43	22.16±3.48
4	CAE + Hydrochlorothiazide	327.16±23.92	21.16±1.83
5	Furosemide (12.9 mg/kg/day)	328.50±10.44	22.50±2.81
6	CAE + Furosemide	328.00±19.06	28.33±2.58 $
7	Spironolactone (4 mg/kg/day)	328.16±7.88	19.00±3.57
8	CAE + Spironolactone	327.66±15.00	20.33±3.82

$ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

$ = Vs Furosemide

White blood cells (WBCs, lymphocytes, monocytes, and eosinophils):

There were no significant ($p > 0.05$) changes in the WBC count or differential counts, as the values were within the normal range. The changes observed are mentioned in Table 37 below.

Table 37: Baseline values of the White blood cells differential counts in a 28-day diuretic study of CAE.					
Sl. No.	**Parameters**	**WBC (10^3/µL)**	**Lymphocytes (%)**	**Monocytes (%)**	**Eosinophiles (%)**
1	Normal Control	12.43±4.06	56.81±2.31	3.28±0.20	1.78±0.38
2	CAE (400 mg/kg/day)	11.88±5.49	55.71±3.03	3.11±0.27	2.03±0.16
3	Hydrochlorothiazide (80.64 mg/kg/day)	10.85±2.33	56.85±2.27	3.31±0.25	2±0.16
4	CAE + Hydrochlorothiazide	11.96±1.68	57.38±3.18	3.35±0.30	1.73±0.24
5	Furosemide (12.9 mg/kg/day)	11.31±1.25	57.41±1.17	3.45±0.40	2±0.43
6	CAE + Furosemide	11.66±1.68	58±1.28	3.15±0.17	2.2±0.52
7	Spironolactone (4 mg/kg/day)	10.61±2.42	57.23±1.29	3.55±0.46	2.4±0.30
8	CAE + Spironolactone	11.21±2.13	57.28±1.36	3.7±0.35	1.81±0.17
The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.					

RBC, hemoglobin, and platelets:

There were no statistically significant changes ($p > 0.05$) in the animals in any of the groups. The changes observed are mentioned in Table 38 below.

Table 38: Baseline values of the red blood cell count, hemoglobin, and platelet counts in a 28-day diuretic study of CAE.

Sl. No.	Parameters	RBC (10^6/µL)	HgB (g/dL)	Platelet (10^3/µL)
1	Normal Control	8.01±0.51	13.5±0.65	963.33±64.18
2	CAE (400 mg/kg/day)	8.24±0.62	14.15±0.90	978.33±46.55
3	Hydrochlorothiazide (80.64 mg/kg/day)	8.95±0.67	15.58±1.75	959.83±29.33
4	CAE + Hydrochlorothiazide	8.36±0.62	15.23±0.48	963±31.87
5	Furosemide (12.9 mg/kg/day)	7.96±0.77	13.45±1.27	988.66±38.90
6	CAE + Furosemide	8.03±0.66	14.85±0.31	943.83±35.05
7	Spironolactone (4 mg/kg/day)	7.95±0.81	13.86±1.29	958.5±28.79
8	CAE + Spironolactone	8.29±0.53	14.11±0.39	945.66±32.06

The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

Blood sugar level:

There were no statistically significant ($p > 0.05$) changes in the serum blood sugar levels of any of the animals in the different groups. The changes observed are mentioned in Table 39 below.

Table 39: Baseline values of the serum sugar levels in a 28-day diuretic study of CAE.

Sl. No.	Groups	Blood Sugar Level
1	Normal Control	120.66±10.96
2	CAE (400 mg/kg/day)	113±12.50
3	Hydrochlorothiazide (80.64 mg/kg/day)	122.83±6.46
4	CAE + Hydrochlorothiazide	127±5.47
5	Furosemide (12.9 mg/kg/day)	124.5±7.47
6	CAE + Furosemide	125.33±6.40
7	Spironolactone (4 mg/kg/day)	125.33±5.60
8	CAE + Spironolactone	122.66±11.36

The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

Renal function tests in serum:

No significant changes ($p > 0.05$) were detected in the serum urea, uric acid, creatinine, and albumin levels of all the animals in the different groups. The changes observed are mentioned in Table 40 below.

Table 40: Baseline values of the renal function tests in a 28-day diuretic study of CAE.					
Sl. No.	**Groups**	**Urea (mg/dL)**	**Uric Acid (mg/dL)**	**Creatinine (mg/dL)**	**Albumin (g/dL)**
1	Normal Control	18.78±1.60	1.42±0.22	0.32±0.03	3.71±0.21
2	CAE (400 mg/kg/day)	17.32±1.35	1.58±0.34	0.40±0.04	3.71±0.11
3	Hydrochlorothiazide (80.64 mg/kg/day)	17.83±2.45	1.56±0.15	0.5±0.07	3.75±0.14
4	CAE + Hydrochlorothiazide	17.56±0.98	1.58±0.27	0.44±0.05	3.70±0.21
5	Furosemide (12.9 mg/kg/day)	18.44±2.97	1.54±0.24	0.47±0.10	3.88±0.24
6	CAE + Furosemide	19.07±0.89	1.53±0.35	0.50±0.06	4.07±0.37
7	Spironolactone (4 mg/kg/day)	20.98±1.88	1.80±0.44	0.49±0.08	3.79±0.05
8	CAE + Spironolactone	19.58±2.74	1.98±0.26	0.46±0.09	3.94±0.35
The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey’s test. The results are presented as Mean ± SD.					

Liver function tests in serum:

All the animals presented values that were well within the normal range, and no statistically significant changes were observed ($p > 0.05$) in the serum LFTs, such as direct bilirubin, indirect bilirubin, total bilirubin, AST, ALP, and ALT. The changes observed are mentioned in Table 41 below.

Table 41: Baseline values of the liver function test in a 28-day diuretic study of CAE.

Sl. No.	Groups	Direct Bilirubin (mg/dL)	Indirect Bilirubin (mg/dL)	Total Bilirubin (mg/dL)	AST (U/L)	ALP (U/L)	ALT (U/L)
1	Normal Control	0.03±0.01	0.03±0.01	0.07±0.01	63.78±2.85	70.45±3.00	31.05±0.53
2	CAE (400 mg/kg/day)	0.03±0.01	0.04±0.01	0.07±0.01	67.4±1.69	70.06±3.59	28.043±12.36
3	Hydrochlorothiazide (80.64 mg/kg/day)	0.03±0.01	0.04±0.01	0.08±0.01	69.45±1.48	71.12±2.36	31.91±2.12
4	CAE + Hydrochlorothiazide	0.03±0.01	0.04±0.01	0.07±0.01	67.81±1.60	77.48±1.22	31.03±0.32
5	Furosemide (12.9 mg/kg/day)	0.04±0.01	0.05±0.02	0.09±0.02	74.53±5.22	82.53±3.65	41.55±2.08
6	CAE + Furosemide	0.03±0.01	0.07±0.01	0.11±0.01	76.69±6.29	90.02±4.53	42.08±1.71
7	Spironolactone (4 mg/kg/day)	0.03±0.01	0.09±0.01	0.12±0.01	85.48±4.50	92.15±3.69	56.63±3.89
8	CAE + Spironolactone	0.03±0.01	0.05±0.01	0.09±0.01	91.10±5.09	96.10±8.68	56.44±4.74

The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

Serum lipid profile:

The serum lipid profile of all the groups was well within the normal limits, and no significant changes in the serum lipid profile were observed (218,219). The changes observed are mentioned in Table 42 below.

Table 42: Baseline values of the serum lipid profile in a 28-day diuretic study of CAE.						
Sl. No.	**Groups**	**HDL (mg/dL)**	**LDL (mg/dL)**	**Triglycerides (mg/dL)**	**VLDL (mg/dL)**	**Total Cholesterol (mg/dL)**
1	Normal Control	43±5.21	21.91±1.77	43.23±1.79	8.64±0.35	73.55±5.52
2	CAE (400 mg/kg/day)	43.5±2.58	21.07±3.00	49.29±1.31	9.86±0.26	74.43±3.84
3	Hydrochlorothiazide (80.64 mg/kg/day)	49.66±4.67	19.19±3.45	51.56±5.60	10.31±1.12	79.17±7.37
4	CAE + Hydrochlorothiazide	47.16±5.98	27.27±2.08	54.2±2.68	10.84±0.53	85.28±8.36
5	Furosemide (12.9 mg/kg/day)	45.16±4.79	31.59±2.58	58.33±1.25	11.66±0.25	88.42±4.92
6	CAE + Furosemide	39.66±9.13	25.82±1.96	61.31±5.15	12.26±1.03	77.75±8.66
7	Spironolactone (4 mg/kg/day)	48±9.27	28.08±3.28	66.63±2.56	13.32±0.51	89.41±8.10
8	CAE + Spironolactone	43.83±8.86	30.00±2.66	59.81±6.76	11.96±1.35	85.80±11.59
The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey’s test. The results are presented as Mean ± SD.						

Serum electrolytes:

The serum electrolyte levels of all the groups were well within the normal limits, even though statistical significance ($p < 0.05$) was observed for sodium and potassium for the animals placed under CAE and the combinations of the test drug along with furosemide. There was a significant increase in the serum potassium levels of the animals placed under the combination of the test drug with spironolactone. But all the values were well within the normal reference range (218,219). The changes observed are mentioned in Table 43 below.

Table 43: Baseline values of the serum electrolyte levels in a 28-day diuretic study of CAE.

Sl. No.	Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Calcium (mmol/L)	Phosphate (mmol/L)	Magnesium (mmol/L)
1	Normal Control	141.58±0.88	4.45±0.10	102.5±1.64	9.46±0.42	4.8±0.41	0.91±0.05
2	CAE (400 mg/kg/day)	140.53±0.68 $, ¢	4.42±0.18 $, ¢	102.33±1.63	9±0.44	5.05±0.22	0.93±0.05
3	Hydrochlorothiazide (80.64 mg/kg/day)	138.7±0.44	4.65±0.20	102.71±1.63	9.08±0.61	6.62±1.02	1.04±0.11
4	CAE + Hydrochlorothiazide	138.98±0.37	4.67±0.14	103.23±0.39	9.01±0.48	6.37±0.65	1.01±0.02
5	Furosemide (12.9 mg/kg/day)	134.55±1.60	5.54±0.69	102.37±2.44	8.55±0.99	4.72±1.50	1.02±0.07
6	CAE + Furosemide	138.1±4.65 $	4.74±0.20 $	102.73±0.64	8.75±0.81	5.68±1.14	0.95±0.13
7	Spironolactone (4 mg/kg/day)	135.73±0.84	6.16±0.55	102.03±2.13	9.45±0.57	5.41±0.60	1.02±0.08
8	CAE + Spironolactone	139.03±0.36	4.65±0.24 ¢	102.19±1.12	8.68±0.78	6.46±0.71	0.90±0.08

$, ¢ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

$ = Vs Furosemide, ¢ = Vs Spironolactone

Urine output and urine pH:

There were no significant changes ($p > 0.05$) in the urine output or urine pH of the animals in any of the groups. The changes observed are mentioned in Table 44 below.

Table 44: Comparison of the baseline urine output and urine pH values in a 28-day diuretic study of CAE.

Sl. No.	Groups	Urine Volume (mL)	Urine pH
1	Normal Control	12.33±1.96	7.33±0.51
2	CAE (400 mg/kg/day)	15.16±0.40	7.33±0.51
3	Hydrochlorothiazide (80.64 mg/kg/day)	15.33±1.63	7.00±0.00
4	CAE + Hydrochlorothiazide	15.16±0.98	7.16±0.40
5	Furosemide (12.9 mg/kg/day)	17.16±2.56	7.16±0.40
6	CAE + Furosemide	15.00±1.67	7.16±0.40
7	Spironolactone (4 mg/kg/day)	13.33±4.08	7.16±0.40
8	CAE + Spironolactone	15.00±1.09	7.33±0.51

The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

Urine electrolytes (sodium, potassium, chloride, calcium, magnesium, and phosphate):
There were no statistically significant ($p > 0.05$) differences in the urine electrolyte levels among the groups. The changes observed are mentioned in Table 45 below.

Table 45: Baseline values of the urine electrolyte levels in a 28-day diuretic study of CAE.							
Sl. No.	**Groups**	**Sodium (mmol/L)**	**Potassium (mmol/L)**	**Chloride (mmol/L)**	**Calcium (mmol/L)**	**Magnesium (mmol/L)**	**Phosphate (mmol/L)**
1	Normal Control	137.78±6.33	4.94±0.62	96.23±4.06	6.19±1.15	7.28±0.96	6.88±0.62
2	CAE (400 mg/kg/day)	137.65±8.38	4.84±1.17	97.4±4.40	7.21±0.89	7.9±0.85	6.87±0.82
3	Hydrochlorothiazide (80.64 mg/kg/day)	143.5±9.51	4.12±0.75	95±3.34	6.36±1.12	7.95±0.71	6.41±1.04
4	CAE + Hydrochlorothiazide	145.73±8.00	5.08±1.01	97.51±2.86	7.4±1.51	7.48±0.71	6.87±0.75
5	Furosemide (12.9 mg/kg/day)	135.11±12.62	5.04±0.72	94±2.89	6.76±1.16	7.20±1.15	7.22±1.00
6	CAE + Furosemide	139.90±6.73	5.03±0.80	93.1±4.84	7.06±1.51	7.02±0.87	7.74±0.85
7	Spironolactone (4 mg/kg/day)	139.15±6.09	5.14±1.19	92.83±5.45	6.73±1.06	7.48±0.87	7.13±0.54
8	CAE + Spironolactone	147.91±4.94	5.73±1.42	95±3.34	6.61±0.61	8.27±0.86	7.61±0.81
The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey’s test. The results are presented as Mean ± SD.							

Urine RFTs (urea, creatinine, uric acid, and albumin):

There were no statistically significant differences ($p > 0.05$) in the urine RFTs among the groups. The changes observed are mentioned in Table 46 below.

Table 46: Baseline values of the renal function tests in the urine samples in a 28-day diuretic study of CAE.					
Sl. No.	**Groups**	**Urea (mg/dL)**	**Creatinine (mg/dL)**	**Uric Acid (mg/dL)**	**Albumin (g/dL)**
1	Normal Control	9.83±1.36	0.61±0.18	35.13±1.12	0.37±0.06
2	CAE (400 mg/kg/day)	9.86±0.91	0.53±0.22	34.90±1.97	0.49±0.05
3	Hydrochlorothiazide (80.64 mg/kg/day)	9.26±0.82	0.33±0.11	35.71±3.86	0.54±0.11
4	CAE + Hydrochlorothiazide	9.96±0.63	0.62±0.23	33.29±1.23	0.55±0.13
5	Furosemide (12.9 mg/kg/day)	9.23±1.04	0.59±0.08	37.25±4.19	0.51±0.05
6	CAE + Furosemide	9.69±1.00	0.48±0.28	34.05±1.65	0.50±0.03
7	Spironolactone (4 mg/kg/day)	9.92±0.93	0.55±0.30	36.97±4.82	0.51±0.06
8	CAE + Spironolactone	8.99±0.75	0.70±0.15	33.73±0.89	0.47±0.05
The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey’s test. The results are presented as Mean ± SD.					

4.8.2. Long-term (28-day) Diuretic Effect of CAE:

Body weight and water consumption:

No significant changes in BW were observed among the groups. There was a significant ($p < 0.05$) increase in the amount of water consumed by the animals treated with CAE compared with the animals treated with hydrochlorothiazide and spironolactone.

When CAE was co-administered with hydrochlorothiazide and furosemide, it significantly ($p < 0.05$) increased water consumption compared with that of the animals treated with standard diuretics alone. The changes observed are mentioned in Table 47 below.

Table 47: Post-test BW and amount of water consumed values in a 28-day diuretic study of CAE.

Sl. No.	Groups	Body Weight (g)	Amount of Water Consumed (mL)
1	Normal Control	314.16±33.88	14.00±1.26
2	CAE (400 mg/kg/day)	318.16±15.91	69.66±12.02 $^{\#, ¢}$
3	Hydrochlorothiazide (80.64 mg/kg/day)	325.33±19.05	44.00±3.09
4	CAE + Hydrochlorothiazide	313.16±9.78	63.83±3.25 $^{\#}$
5	Furosemide (12.9 mg/kg/day)	313.16±10.79	61.83±2.22
6	CAE + Furosemide	312.66±21.44	83.33±2.73 $^{\$}$
7	Spironolactone (4 mg/kg/day)	333.50±17.51	38.00±4.19
8	CAE + Spironolactone	325.66±7.76	45.33±6.15

$^{\#, \$, ¢}$ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone

WBCs, lymphocytes, monocytes, and eosinophils:

Long-term administration of furosemide produced leukocytopenia ($p < 0.05$), which is a known adverse effect of furosemide, whereas CAE did not cause such an effect. After the co-administration of CAE along with furosemide, leukocytopenia was nullified ($p < 0.05$). No significant changes ($p < 0.05$) in lymphocytes, monocytes, and eosinophils were detected in any of the groups. The changes observed are mentioned in Table 48 below.

Table 48: Post-test values of the White blood cells differential counts in a 28-day diuretic study of CAE.					
Sl. No.	**Groups**	**WBC (10^3/µL)**	**Lymphocytes (%)**	**Monocytes (%)**	**Eosinophiles (%)**
1	Normal Control	15.11±0.96	54.08±3.51	3.38±0.91	1.16±0.18
2	CAE (400 mg/kg/day)	17.05±0.92 $	60.41±4.81 #, $	4.01±-0.36	1.26±0.16
3	Hydrochlorothiazide (80.64 mg/kg/day)	17.18±1.02	47.88±1.17	3.25±0.18	1.31±0.43
4	CAE + Hydrochlorothiazide	17.03±1.86	45.83±4.08	3.36±0.43	1.26±0.39
5	Furosemide (12.9 mg/kg/day)	6.68±0.79	53.63±3.28	4.08±1.04	1.3±0.41
6	CAE + Furosemide	16.51±1.92 $	46.23±1.64 $	3.3±0.12	1.28±0.20
7	Spironolactone (4 mg/kg/day)	18.66±0.83	55.96±1.88	4.1±0.34	1.28±0.27
8	CAE + Spironolactone	17.48±1.14	53.05±5.79	3.31±0.16	1.18±0.19
#, $ = p <0.05; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD. # = Vs Hydrochlorothiazide, $ = Vs Furosemide					

RBC, hemoglobin, and platelet counts:

CAE did not alter the RBC, hemoglobin or platelet counts after 28 days of treatment. The long-term administration of furosemide reduced RBC levels and produced anemia ($p < 0.05$), which was counteracted by the co-administration of CAE ($p < 0.05$). The changes observed are mentioned in Table 49 below.

Table 49: Post-test values of the red blood cell count, hemoglobin, and platelet count in a 28-day diuretic study of CAE.				
Sl. No.	**Groups**	**RBC (10^6/µL)**	**HgB (g/dL)**	**Platelet (10^3/µL)**
1	Normal Control	8.34±0.43	13.65±0.63	906.83±44.44
2	CAE (400 mg/kg/day)	8.84±0.44 $	14.9±0.50	1051.33±66.16 ¢
3	Hydrochlorothiazide (80.64 mg/kg/day)	8.475±0.25	14.06±0.36	960.66±30.57
4	CAE + Hydrochlorothiazide	9.04±0.34	14.73±0.71	977.5±45.33
5	Furosemide (12.9 mg/kg/day)	5.755±0.90 *	10.88±1.15 *	1019±107.43
6	CAE + Furosemide	8.876±0.12 $	14.6±0.56 $	923.16±65.45
7	Spironolactone (4 mg/kg/day)	8.47±0.16	14.28±0.38	883.33±28.26
8	CAE + Spironolactone	8.61±0.44	14.66±0.35	986.66±58.10

*, $, ¢ = p <0.05; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

* = Vs Normal Control, $ = Vs Furosemide, ¢ = Vs Spironolactone

Serum electrolytes:

There was no electrolyte imbalance in the animals treated with the CAE, except for a reduction ($p < 0.05$) in the serum magnesium levels (hypomagnesemia) compared with those of the normal control.

Hydrochlorothiazide and furosemide produced hyponatremia, hypochloremia, hypokalemia, hypophosphatemia, and hypomagnesemia. The co-administration with the CAE significantly counteracted ($p < 0.05$) these observed adverse effects.

The hypocalcemia and hypercalcemia produced by furosemide and hydrochlorothiazide, respectively were significantly counteracted ($p < 0.05$) by co-administrating the CAE. Similarly, the hyperkalemia produced by spironolactone was effectively reversed ($p < 0.05$) by the co-administration of CAE. The hypokalemia produced by furosemide returned to normal after co-administering CAE. The changes observed are illustrated in Figure 29 and summarized in Table 50 below.

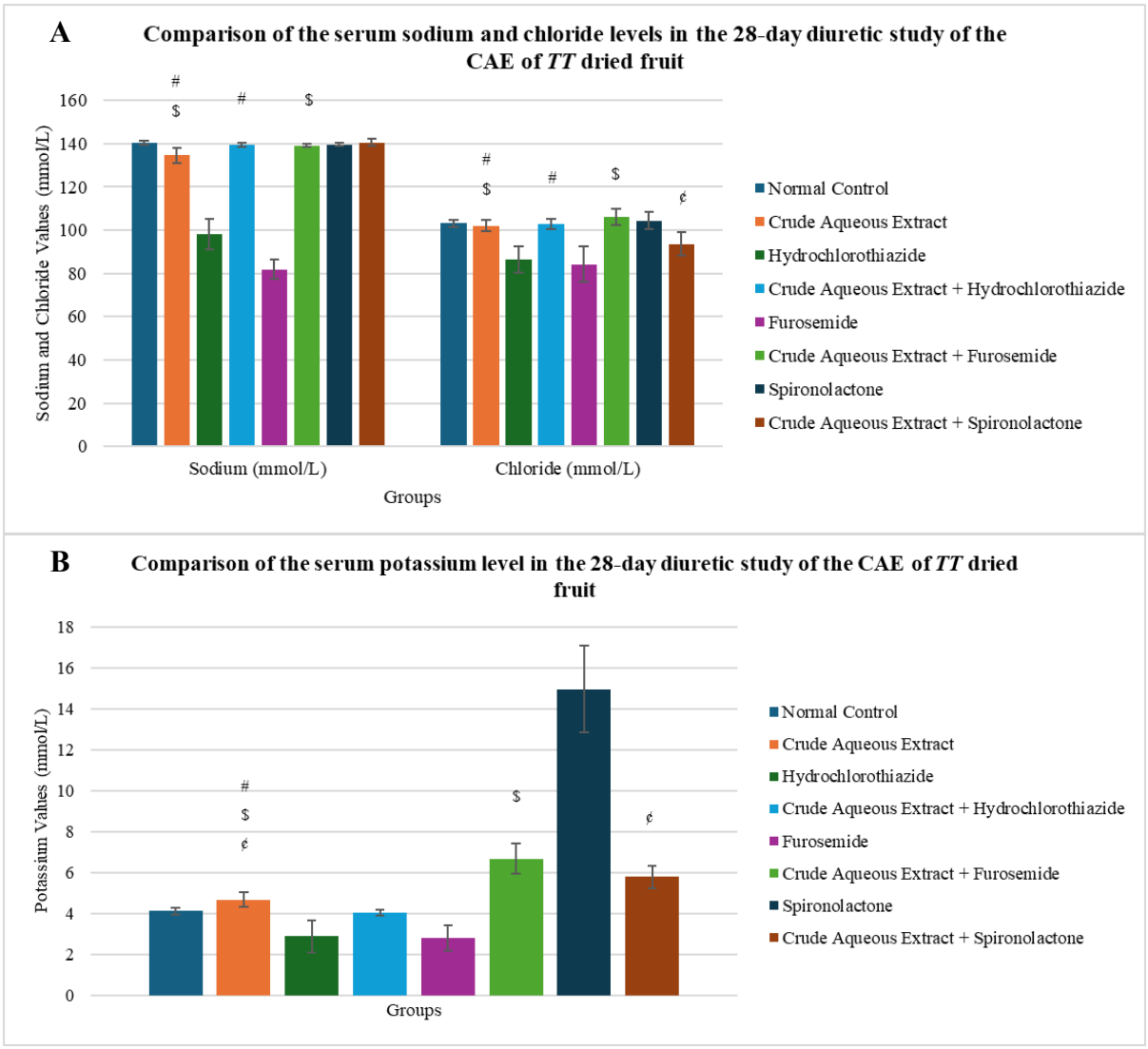


Figure 29: Comparison of the serum sodium, chloride (A), and potassium (B) levels in the 28-day diuretic study of the CAE of *TT* and its combination with standard diuretics.

#, $, ¢ = p <0.05. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone

Table 50: Post-test values of the serum electrolyte levels in a 28-day diuretic study of CAE.

Sl. No.	Groups	Calcium (mmol/L)	Phosphate (mmol/L)	Magnesium (mmol/L)
1	Normal Control	7.76±1.60	4.82±0.07	0.94±0.09
2	CAE (400 mg/kg/day)	8.59±0.56 #, $	5.39±0.44 #, $	0.55±0.28 *, ¢
3	Hydrochlorothiazide (80.64 mg/kg/day)	16.15±1.35	2.52±0.58	0.48±0.21
4	CAE + Hydrochlorothiazide	8.64±0.85 #	5.64±1.43 #	0.92±0.16 #
5	Furosemide (12.9 mg/kg/day)	5.34±0.80	1.67±0.15	0.32±0.17
6	CAE + Furosemide	8.88±0.82 $	4.37±1.29 $	0.71±0.13 $
7	Spironolactone (4 mg/kg/day)	9.91±0.74	4.62±1.08	0.92±0.16
8	CAE + Spironolactone	9.11±1.11	5.72±0.35	0.69±0.15

*, #, $, ¢ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

* = Vs Normal Control, # = Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone

Serum RFTs (urea, uric acid, creatinine, and albumin):

The CAE did not produce any changes in the serum RFTs. The standard diuretics produced uremia, hyperuricemia, and increased creatinine levels in the serum. However, the coadministration of CAE with standard diuretics, hydrochlorothiazide and furosemide, significantly ($p < 0.05$) lowered the increased urea and uric acid levels. The CAE and the combination of CAE with hydrochlorothiazide significantly reduced the serum creatinine levels. The changes observed are illustrated in Figure 30 and summarized in Table 51 below.

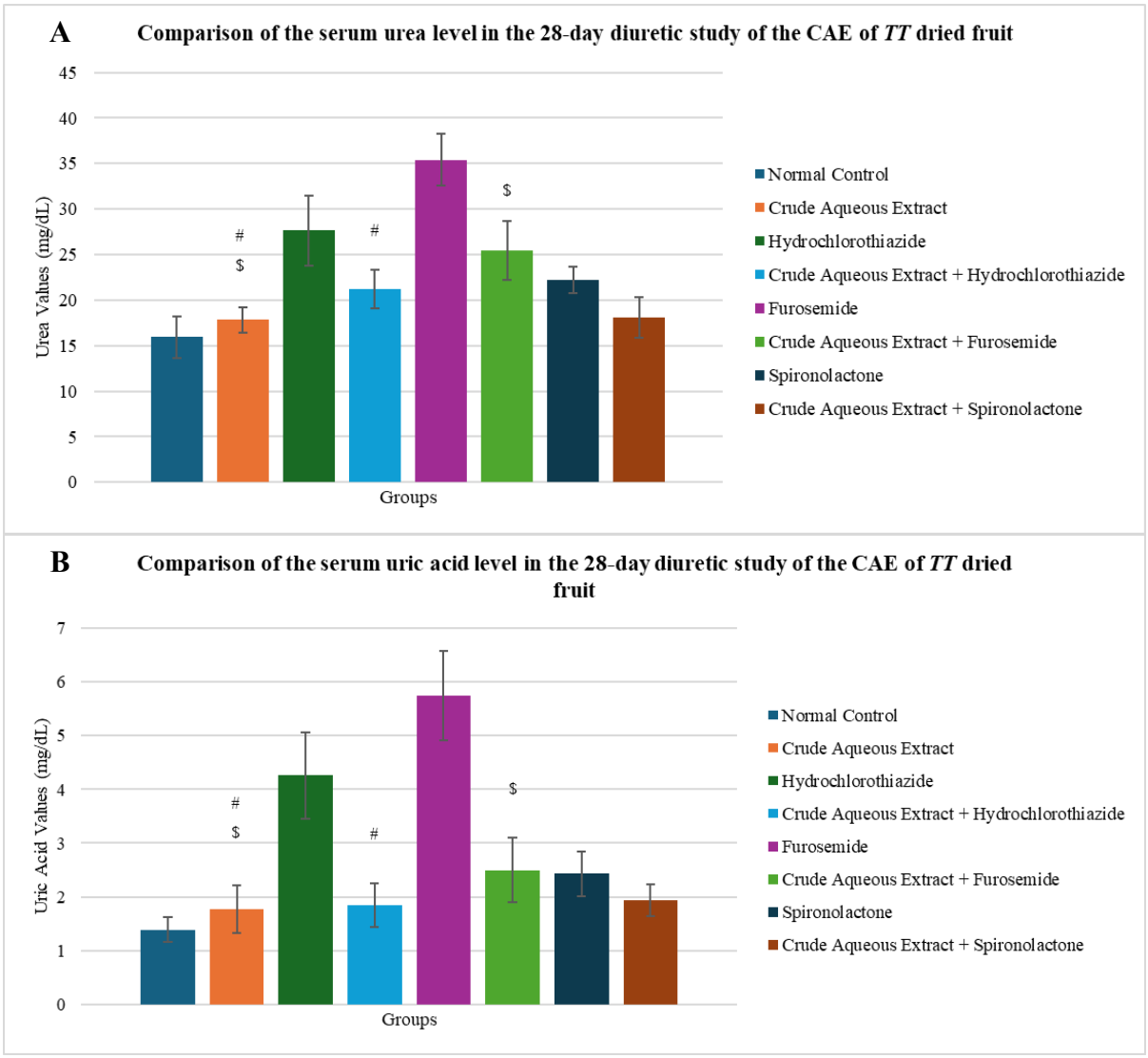


Figure 30: Comparison of the serum urea (A) and uric acid (B) levels in the 28-day diuretic study of the CAE of *TT* and its combination with standard diuretics.

#, $ = p <0.05. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide

Table 51: Post-test values of the renal function tests in a 28-day diuretic study of CAE.			
Sl. No.	**Groups**	**Creatinine (mg/dL)**	**Albumin (g/dL)**
1	Normal Control	0.33±0.02	3.55±0.71
2	CAE (400 mg/kg/day)	0.43±0.08 $^{\#, \$}$	3.68±0.96
3	Hydrochlorothiazide (80.64 mg/kg/day)	0.75±0.12	3.81±0.56
4	CAE + Hydrochlorothiazide	0.35±0.11 $^{\#}$	3.56±0.58
5	Furosemide (12.9 mg/kg/day)	0.61±0.09	3.36±0.74
6	CAE + Furosemide	0.52±0.06	3.32±0.71
7	Spironolactone (4 mg/kg/day)	0.51±0.12	3.82±0.45
8	CAE + Spironolactone	0.49±0.05	3.35±0.63

$^{\#, \$}$ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide

Serum sugar:

Hydrochlorothiazide and spironolactone significantly increased the serum sugar levels ($p < 0.05$) after chronic administration (28 days), whereas chronic administration of CAE did not alter the serum sugar levels. The co-administration of CAE with hydrochlorothiazide and spironolactone reduced the blood sugar level ($p < 0.05$). The changes observed are illustrated in Figure 31 below.

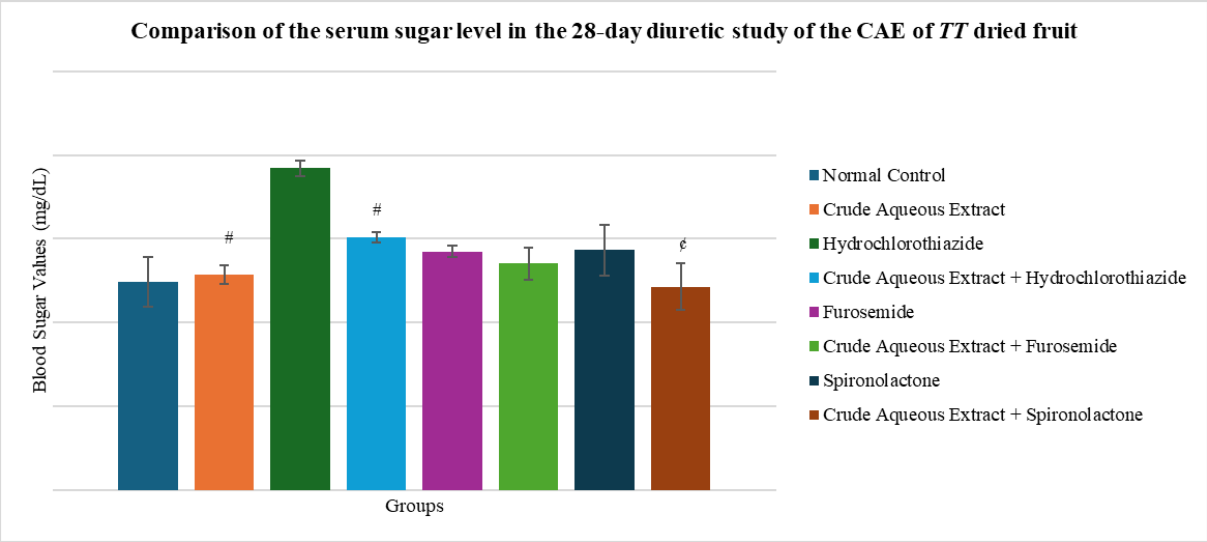


Figure 31: Comparison of the serum glucose levels in the 28-day diuretic study of the CAE of *TT* and its combination with standard diuretics.

#, ¢ = $p < 0.05$. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, ¢ = Vs Spironolactone

Serum lipid profile (HDL, LDL, triglycerides, VLDL, and total cholesterol):

The animals treated with standard diuretics showed decrease in the serum HDL and increase in the LDL, VLDL, triglyceride and total cholesterol levels ($p < 0.05$). Whereas the animals treated with CAE did not alter the serum lipid profile. Surprisingly, the altered lipids in the standard diuretic groups were brought back to normal range after the co-administration of CAE with standard diuretics ($p < 0.05$). The changes observed are mentioned in Table 52 below.

Table 52: Post-test values of the serum lipid profile in a 28-day diuretic study of CAE.

Sl. No.	Groups	HDL (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	VLDL (mg/dL)	Total Cholesterol (mg/dL)
1	Normal Control	46.38±3.58	21.92±3.13	51.80±4.24	10.36±0.84	78.67±5.83
2	CAE (400 mg/kg/day)	51.39±2.33 #, $, ¢	22.01±2.19 #, $	39.69±3.86 #, $, ¢	7.94±0.77 #, $, ¢	81.35±2.50 #, $
3	Hydrochlorothiazide (80.64 mg/kg/day)	23.62±3.40	63.67±7.29	93.76±5.34	18.75±1.06	106.05±6.77
4	CAE + Hydrochlorothiazide	45.82±5.04 #	22.77±4.49 #	42.91±6.92 #	8.58±1.38 #	77.18±6.35 #
5	Furosemide (12.9 mg/kg/day)	20.59±5.30	71.12±5.58	115.49±15.39	23.09±3.07	114.81±6.86
6	CAE + Furosemide	45.4±6.61 $	30.29±5.95 $	59.49±4.52 $	11.89±0.90 $	87.59±11.27 $
7	Spironolactone (4 mg/kg/day)	35.60±2.59	28.27±2.43	84.76±8.85	16.95±1.77	80.83±2.24
8	CAE + Spironolactone	50.13±1.34 ¢	22.6±3.45	46.43±7.48 ¢	9.28±1.49 ¢	82.01±3.79

#, $, ¢ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone

Serum LFTs (direct bilirubin, indirect bilirubin, total bilirubin, AST, ALP, and ALT):

The animals treated with standard diuretics produced significant ($p < 0.05$) increases in the serum AST, ALP and ALT levels. However, the CAE did not alter serum liver function, as no changes were detected. However, the co-administration of CAE with standard diuretics did not normalize the AST, ALP and ALT values after 28 days of administration. There are no added advantages observed after combining CAE with standard diuretics in terms of preventing liver damage during long-term usage. The changes observed are mentioned in Table 53 below.

Table 53: Post-test values of the liver function test in a 28-day diuretic study of CAE.

Sl. No.	Groups	Direct Bilirubin (mg/dL)	Indirect Bilirubin (mg/dL)	Total Bilirubin (mg/dL)	AST (U/L)	ALP (U/L)	ALT (U/L)
1	Normal Control	0.03±0.00	0.03±0.00	0.07±0.01	73.91±6.61	72.41±5.20	38.7±2.67
2	CAE (400 mg/kg/day)	0.04±0.00	0.07±0.02	0.11±0.03	73.59±18.33 #, $	86.37±5.36 #, $	42.51±6.75 #, $
3	Hydrochlorothiazide (80.64 mg/kg/day)	0.04±0.00	0.09±0.03	0.13±0.04	89.33±4.43	124.93±15.25	63.93±3.68
4	CAE + Hydrochlorothiazide	0.03±0.01	0.06±0.01	0.09±0.02	80.60±3.48	99.50±14.53 #	52.78±4.16 #
5	Furosemide (12.9 mg/kg/day)	0.03±0.01	0.08±0.02	0.11±0.04	90.08±6.40	129.45±23.51	60.79±3.82
6	CAE + Furosemide	0.02±0.01	0.06±0.02	0.09±0.01	83.81±6.88	89.68±7.71 $	59.08±6.44
7	Spironolactone (4 mg/kg/day)	0.04±0.01	0.05±0.01	0.09±0.02	75.74±5.13	97.76±11.05	46.99±7.06
8	CAE + Spironolactone	0.04±0.01	0.04±0.01	0.08±0.01	86.37±6.39	101.31±4.90	46.06±5.13

#, $ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide

Urine output and urine pH:

There was a statistically significant ($p < 0.05$) increase in the urinary excretion volume in the animals treated with the CAE compared with the standard diuretics (29.8% increase against hydrochlorothiazide, 1.87% increase against furosemide and 67.71% increase against spironolactone). There was a significant ($p < 0.05$) increase in the urine pH in the animals treated with the test drug compared with the animals treated with hydrochlorothiazide and spironolactone.

CAE when co-administered with the hydrochlorothiazide and furosemide showed a statistically significant ($p < 0.05$) increase in urinary excretion when compared with the animals treated with hydrochlorothiazide (40.42% increase) and furosemide (36.02% increase) alone, while the spironolactone combination with the CAE showed a statistically significant ($p < 0.05$) increase in urinary excretion (51.78% increase) and urine pH compared with those of those treated with spironolactone alone.

Overall, a positive diuretic effect was observed with the test drug, and an additive effect was observed with the combinations of the test drug and standard diuretics, which were statistically significant. The changes observed are illustrated in Figure 32 and mentioned in Table 54 below.

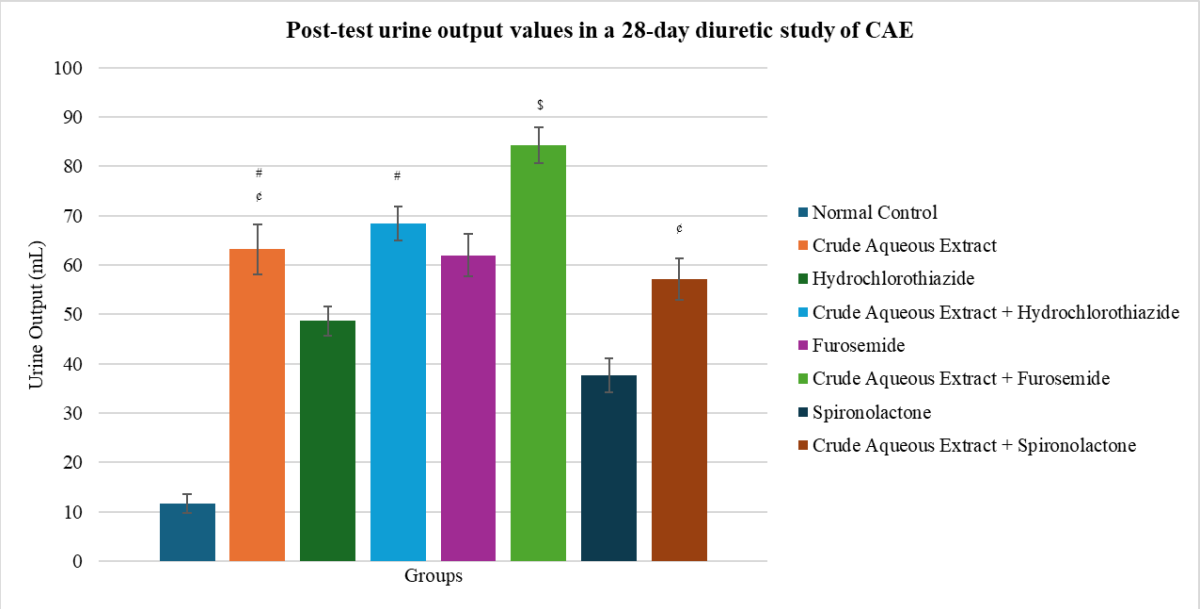


Figure 32: Post-test urine output in a 28-day diuretic study of CAE

#, $, ¢ = $p < 0.05$. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone

Table 54: Post-test urine pH values in a 28-day diuretic study of CAE.		
Sl. No.	**Groups**	**Urine pH**
1	Normal Control	7.66±1.03
2	CAE (400 mg/kg/day)	14.00±0.00 $^{\#, ¢}$
3	Hydrochlorothiazide (80.64 mg/kg/day)	12.00±0.00
4	CAE + Hydrochlorothiazide	12.00±0.00
5	Furosemide (12.9 mg/kg/day)	14.00±0.00
6	CAE + Furosemide	14.00±0.00
7	Spironolactone (4 mg/kg/day)	10.00±0.00
8	CAE + Spironolactone	11.33±1.03 $^{¢}$

$^{\#, ¢}$ = $p < 0.05$; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, ¢ = Vs Spironolactone

Urine RFTs (urea, uric acid, creatinine, and albumin):

There was a statistically significant ($p < 0.05$) reduction in urea excretion in the urine of the animals treated with CAE compared with that of the animals treated with furosemide (36.04% reduction) and hydrochlorothiazide (11% reduction). Compared with treatment with hydrochlorothiazide, furosemide and spironolactone, there was a significant increase in uric acid excretion (33.19%, 4.94%, and 69.11% increase, respectively) in the urine ($p < 0.05$).

Compared with those treated with standard diuretics alone, the urinary excretion of urea was significantly greater ($p < 0.05$) with the co-administration of CAE with spironolactone, whereas the excretion of uric acid was significantly increased with the combination of CAE with all the standard diuretics. No significant changes were observed in the creatinine or albumin levels. The changes observed are illustrated in Figure 33 and summarized in Table 55 below.

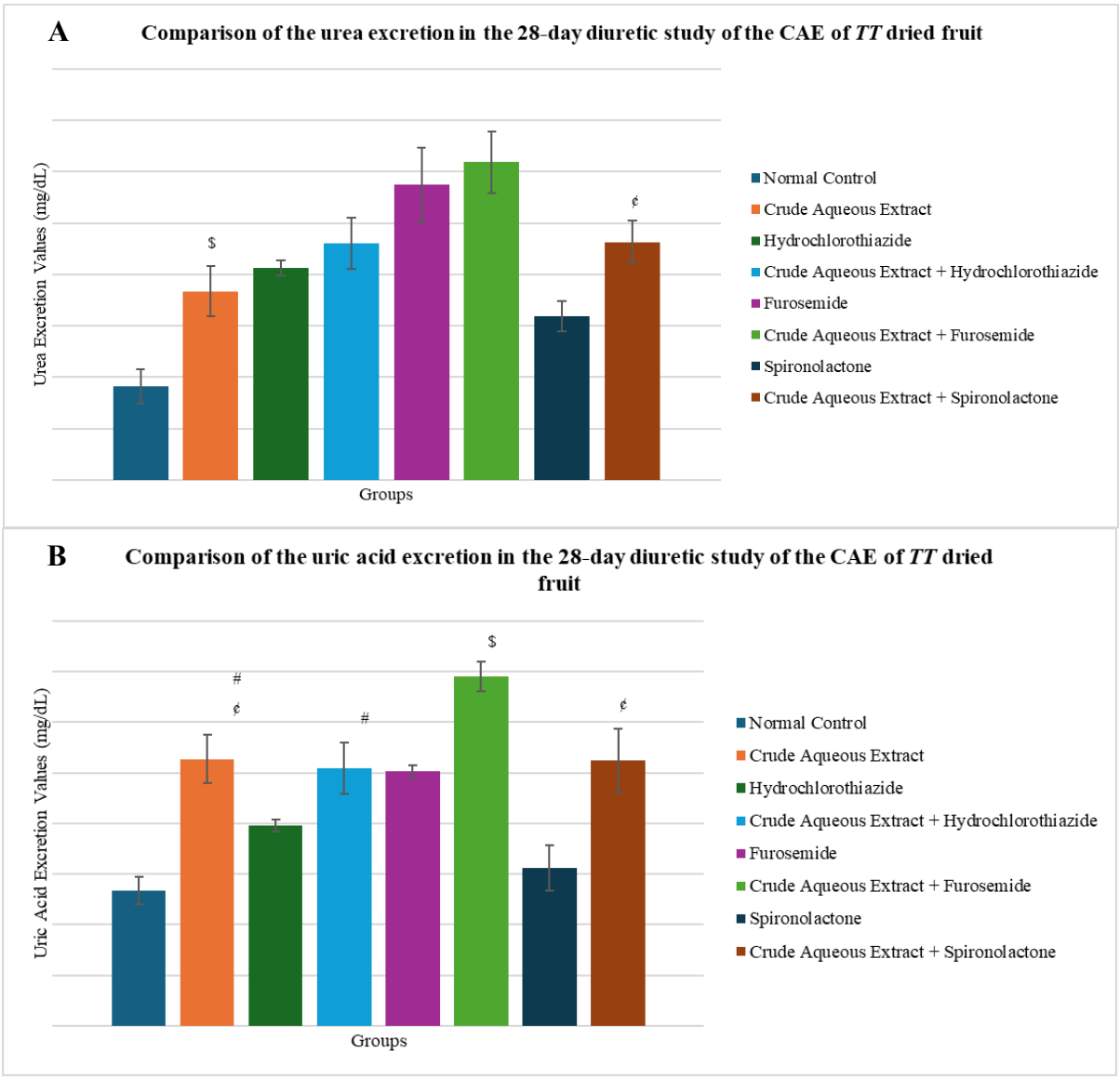


Figure 33: Comparison of the urinary excretion of urea (A) and uric acid (B) in the 28-day diuretic study of the CAE of *TT* and its combination with standard diuretics.

#, $, ¢ = p <0.05. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone

Table 55: Post-test values of the renal function test in the urine samples in a 28-day diuretic study of CAE.

Sl. No.	Groups	Creatinine (mg/dL)	Albumin (g/dL)
1	Normal Control	0.51±0.22	0.52±0.09
2	CAE (400 mg/kg/day)	0.53±0.19	0.68±0.16
3	Hydrochlorothiazide (80.64 mg/kg/day)	0.6±0.15	0.5±0.22
4	CAE + Hydrochlorothiazide	0.4±0.23	0.46±0.28
5	Furosemide (12.9 mg/kg/day)	0.58±0.18	0.71±0.29
6	CAE + Furosemide	0.45±0.19	0.47±0.22
7	Spironolactone (4 mg/kg/day)	0.56±0.17	0.44±0.37
8	CAE + Spironolactone	0.51±0.31	0.48±0.16

The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

Urine electrolytes (sodium, potassium, chloride, calcium, magnesium, and phosphate):

Compared with those of standard diuretics, there was a statistically significant increase ($p < 0.05$) in the urinary excretion of sodium (43.25% against hydrochlorothiazide, 33.33% against furosemide, and 97.98% against spironolactone), chloride (44.45% against hydrochlorothiazide, 14.13% against furosemide, and 132.35% against spironolactone) and magnesium in animals treated with the CAE. The CAE also significantly increased ($p < 0.05$) potassium excretion to 2.27 times higher than that of hydrochlorothiazide and 23.98 times greater than that of spironolactone. Compared with those in the furosemide- and spironolactone-treated groups, there was also a significant increase ($p < 0.05$) in calcium and phosphate excretion in the urine of animals administered CAE.

Compared with those administered hydrochlorothiazide alone, animals co-administered with CAE and hydrochlorothiazide had significantly greater ($p < 0.05$) urinary excretion of sodium, potassium, chloride, calcium, and magnesium. Compared with those administered furosemide alone, animals co-administered with CAE and furosemide caused a significant increase ($p < 0.05$) in the urinary excretion of sodium, potassium, chloride, calcium, magnesium, and phosphate. Compared with spironolactone alone, the co-administration of CAE with spironolactone caused a significant increase ($p < 0.05$) in the urinary excretion of potassium, chloride, and magnesium. The changes observed are illustrated in Figure 34 and summarized in Table 56 below.

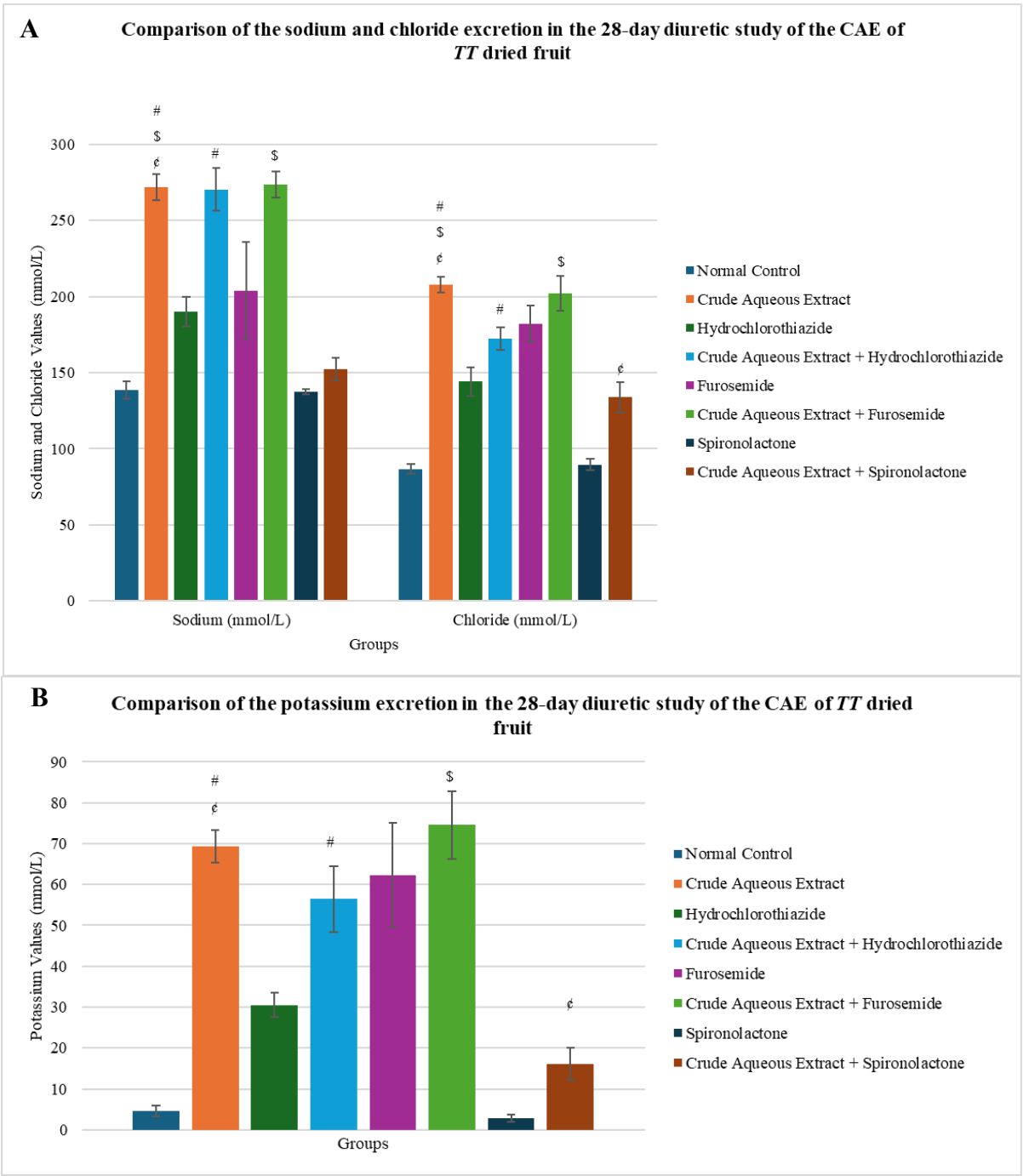


Figure 34: Comparison of the urinary excretion of sodium, chloride (A), and potassium (B) in the 28-day diuretic study of the CAE of *TT* and its combination with standard diuretics.

#, $, ¢ = p <0.05. The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone

Table 56: Post-test values of the urine electrolyte levels in a 28-day diuretic study of CAE.

Sl. No.	Groups	Calcium (mmol/L)	Magnesium (mmol/L)	Phosphate (mmol/L)
1	Normal Control	6.05±0.85	7.96±0.59	6.6±0.79
2	CAE (400 mg/kg/day)	31.68±1.66 $, ¢	53.79±0.83 #, $, ¢	30.09±4.12 $, ¢
3	Hydrochlorothiazide (80.64 mg/kg/day)	31.75±0.62	33.47±2.47	31.49±0.60
4	CAE + Hydrochlorothiazide	40.14±1.45 #	41.37±3.40 #	33.45±3.20
5	Furosemide (12.9 mg/kg/day)	4.84±1.32	40.25±4.11	8.33±0.96
6	CAE + Furosemide	18.72±2.98 $	50.15±2.30 $	12.28±1.70 $
7	Spironolactone (4 mg/kg/day)	15.24±2.28	7.49±0.87	8.15±1.07
8	CAE + Spironolactone	17.29±2.51	14.41±0.58 ¢	10.82±1.67

#, $, ¢ = p <0.05; The analysis was conducted using OneWay ANOVA followed by post-hoc Tukey's test. The results are presented as Mean ± SD.

= Vs Hydrochlorothiazide, $ = Vs Furosemide, ¢ = Vs Spironolactone

Indices for electrolyte excretion:

The animals treated with the test drug had the highest saluretic effect, followed by the combination of the test drug with furosemide, the combination of the test drug with hydrochlorothiazide and the combination of the test drug with spironolactone, in terms of excreting sodium and chloride.

All the groups showed a favorable natriuretic effect according to a score of more than 2. Compared with spironolactone alone, the test drug with the spironolactone combination did not produce a potassium-sparing effect.

All the groups except the combination of the test drug with spironolactone showed positive carbonic anhydrase inhibitory activity, and the combination of the test drug with hydrochlorothiazide resulted in the highest inhibition compared with the other groups. The changes observed are mentioned in Table 57 below.

Table 57: Post-test values of indices for electrolyte excretion in a 28-day diuretic study of CAE.

Sl. No.	Group	Saluretic Effect	Natriuretic Effect	CA Inhibition Effect
1	CAE (400 mg/kg/day)	480.0	3.9	0.6
2	Hydrochlorothiazide (80.64 mg/kg/day)	333.9	6.2	0.7
3	CAE + Hydrochlorothiazide	442.5	4.8	0.5
4	Furosemide (12.9 mg/kg/day)	386.2	3.3	0.7
5	CAE + Furosemide	475.6	3.7	0.6
6	Spironolactone (4 mg/kg/day)	226.9	47.4	0.6
7	CAE + Spironolactone	286.2	9.4	0.8

Natriuretic Activity (sodium excretion): Values >2 indicate a favorable natriuretic effect, and values >10 indicate a potassium-sparing effect

Carbonic Anhydrase Inhibition: Ratios between 1 and 0.8: No carbonic anhydrase inhibition; with decreasing ratios, slight to strong carbonic anhydrase inhibition is present

***Lipschitz* Test for Urine and Sodium:**

Animals treated with the test drug and animals treated with combinations of the test drug with hydrochlorothiazide, the test drug with furosemide, and the test drug with spironolactone showed potent diuretic effects, as indicated by *Lipschitz* test scores of 5, 5, 6, and 4, respectively, for urine as indicators of diuretic activity.

The animals treated with the test drug alone, or the combination of the test drug with hydrochlorothiazide or the test drug with furosemide presented potent diuretic activity, as indicated by a score of 2 each for sodium, which is an indicator of diuretic activity. The combination of the test drug with spironolactone had a positive effect on the *Lipschitz* test for sodium, with a score of 1. The changes observed are illustrated in Figure 35 below.

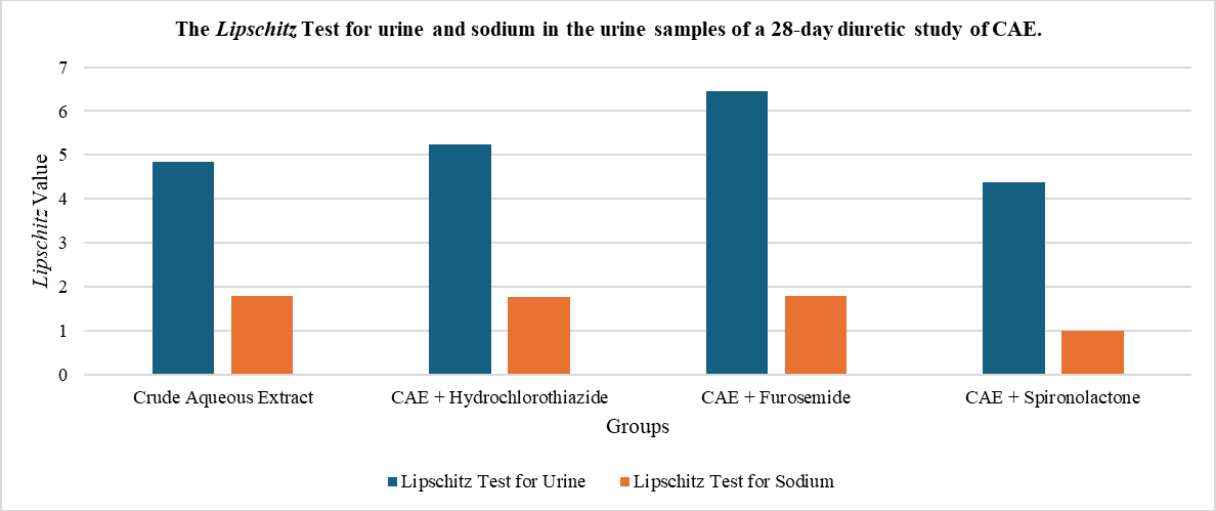


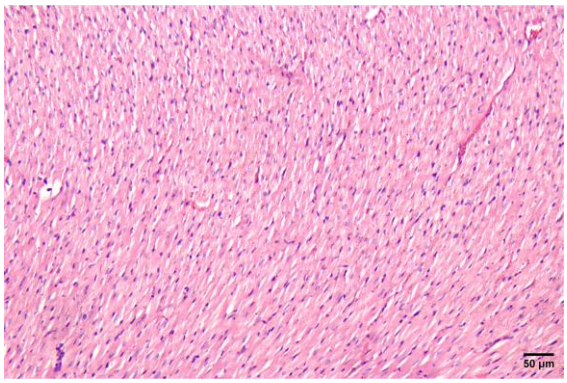
Figure 35: Post-test values of the *Lipschitz* Test for urine and sodium in a 28-day diuretic study of CAE.

***Lipschitz* Value:** ≥ 1 positive effect; ≥ 2 potent diuretic activity

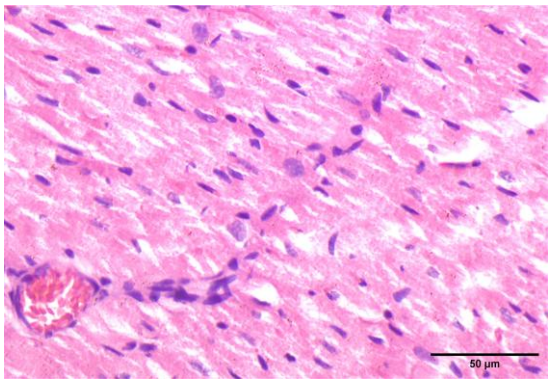
Histopathological findings of normal control, CAE, standard diuretics, and combinations of standard diuretics along with CAE groups in a 28-day diuretic study:

- **Heart:**

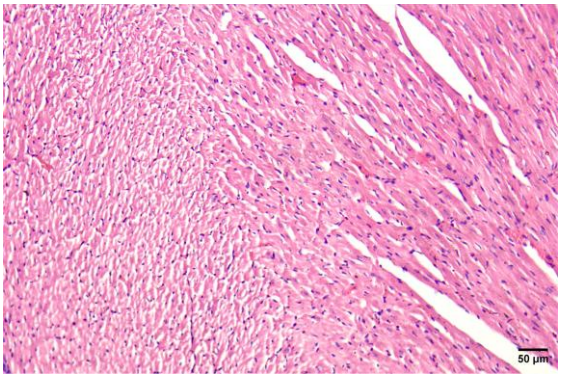
All the slides show heart tissue consisting of cardiac muscle and the myocardium. The myocardium consists of muscle cells (cardiomyocytes) with centrally placed nuclei. None of the groups showed any histological changes or changes in tissue architecture (Figures 36A to 36P).



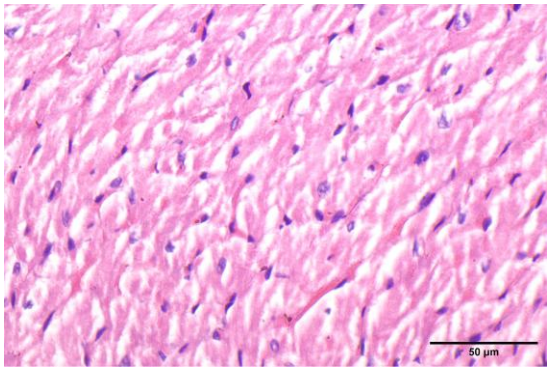
A. Normal Control (100X)



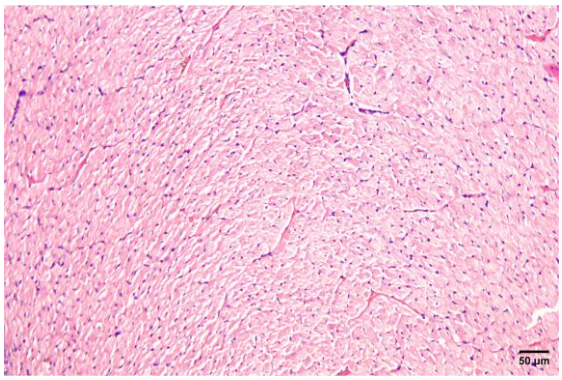
B. Normal Control (400X)



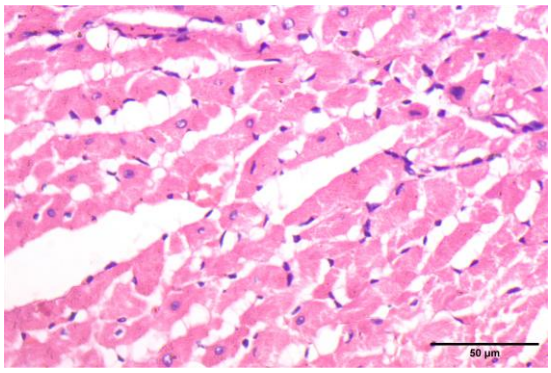
C. CAE (100X)



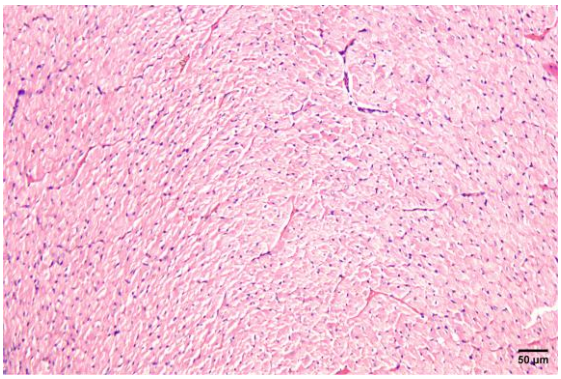
D. CAE (400X)



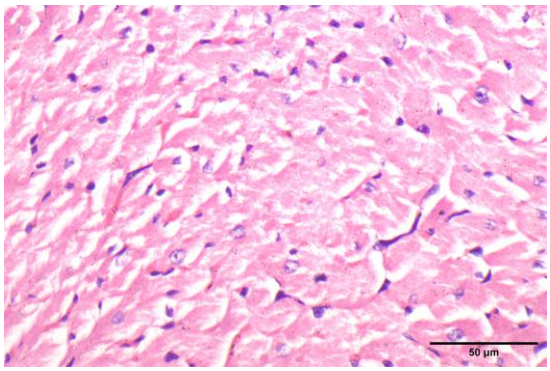
E. Hydrochlorothiazide (100X)



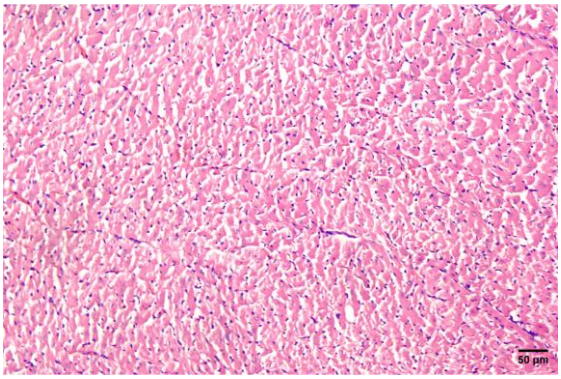
F. Hydrochlorothiazide (400X)



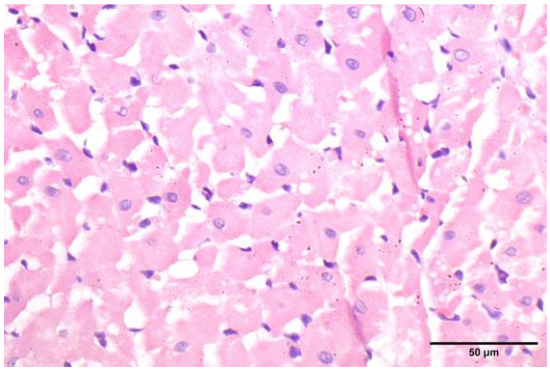
G. Furosemide (100X)



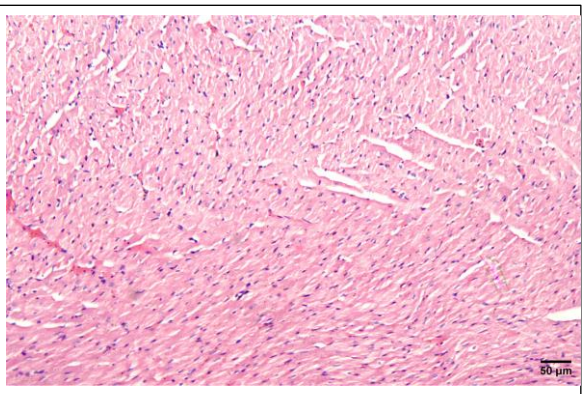
H. Furosemide (400X)



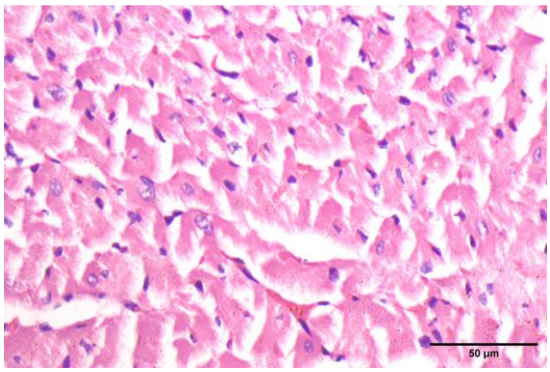
I. Spironolactone (100X)



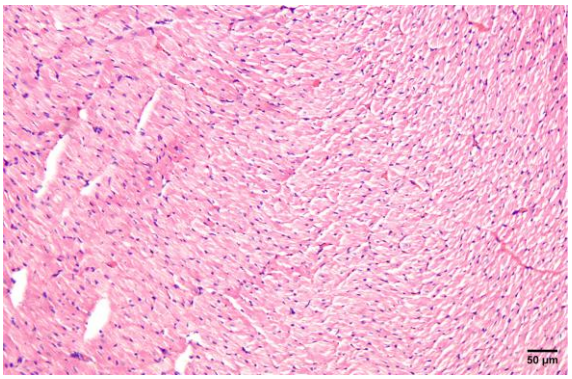
J. Spironolactone (400X)



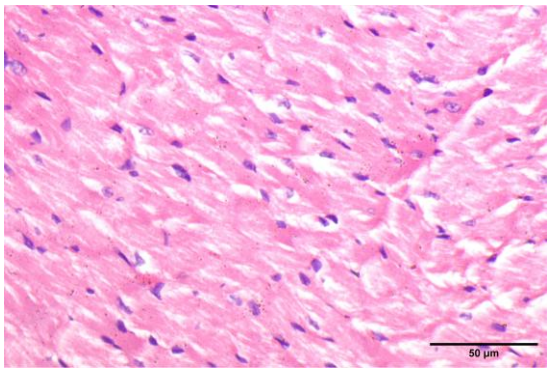
K. CAE + Hydrochlorothiazide (100X)



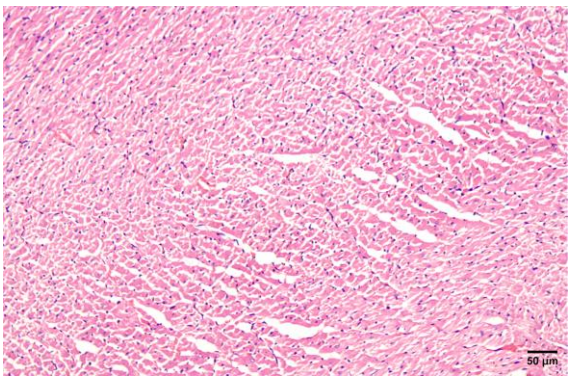
L. CAE + Hydrochlorothiazide (400X)



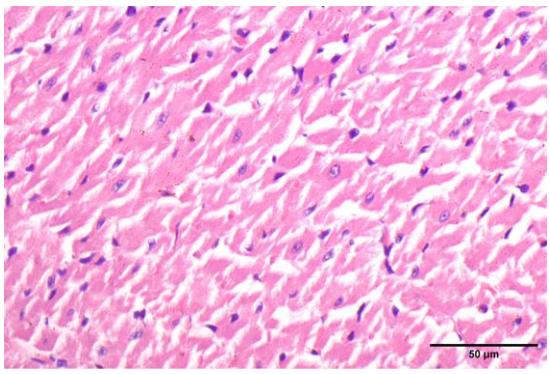
M. CAE + Furosemide (100X)



N. CAE + Furosemide (400X)



O. CAE + Spironolactone (100X)

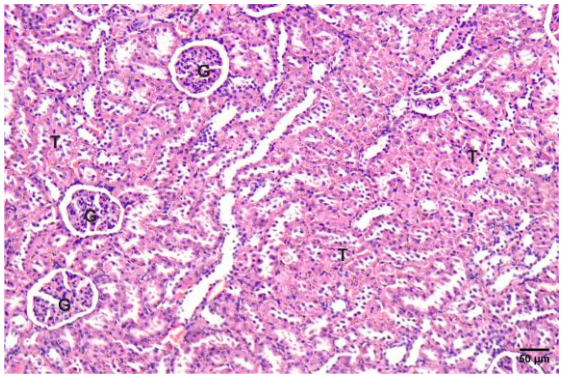


P. CAE + Spironolactone (400X)

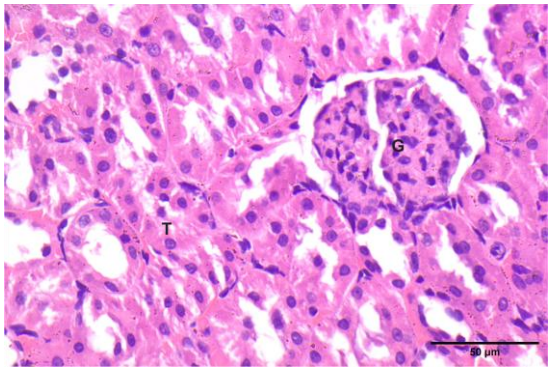
Figure 36: Histopathological findings of the heart of the normal control, CAE, standard diuretics, and combinations of CAE with standard diuretics treated groups in a 28-day diuretic study

- **Kidney:**

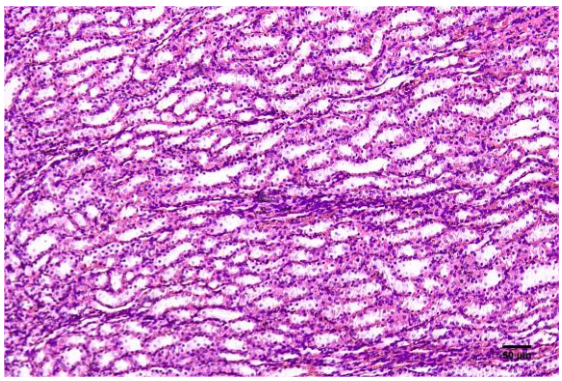
All the slides kidney tissue consisting of the cortex and medulla. The cortex consists of circular structures called corpuscles. It also contains tubules of various shapes, such as the proximal convoluted tubule and the distal convoluted tubule. Focal areas of chronic (lymphocyte) inflammatory infiltration in the cortex were observed in the animals treated with furosemide (Figure 37G), whereas the animals treated with the combination of CAE with furosemide showed dilated tubules in the medulla, mild expansion of the lumen and lined with flattened epithelium (Figure 37M). No changes in tissue architecture were observed (Figures 37A to 37P).



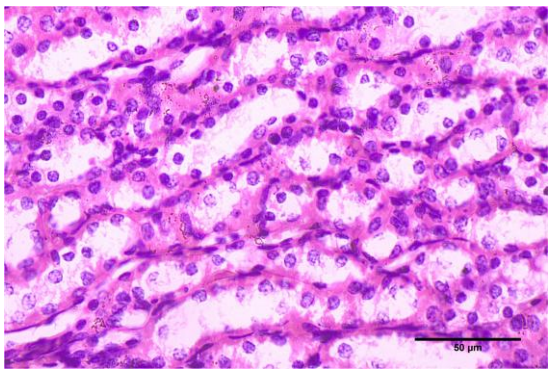
A. Normal Control (100X)



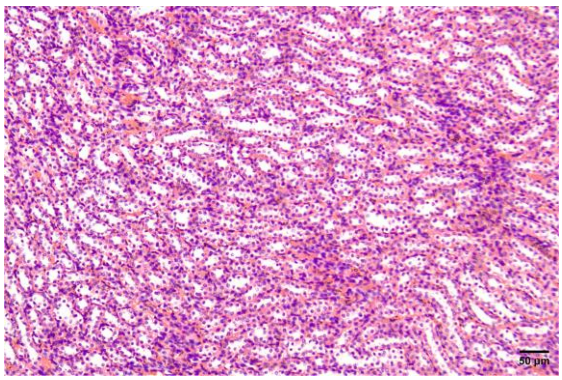
B. Normal Control (400X)



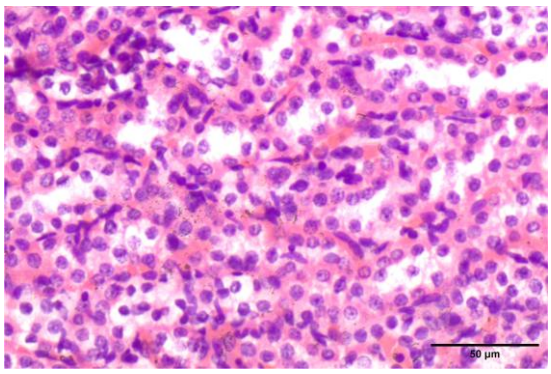
C. CAE (100X)



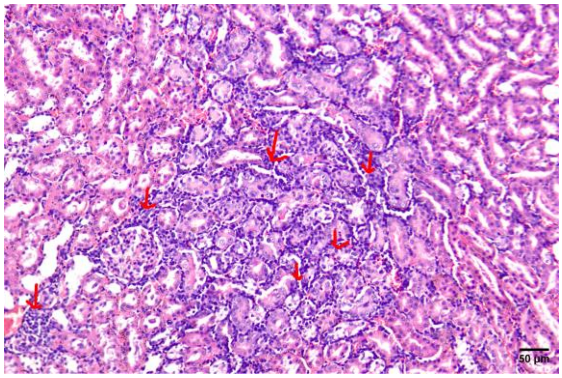
D. CAE (400X)



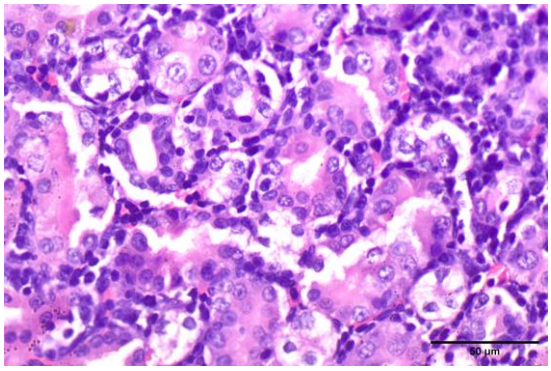
E. Hydrochlorothiazide (100X)



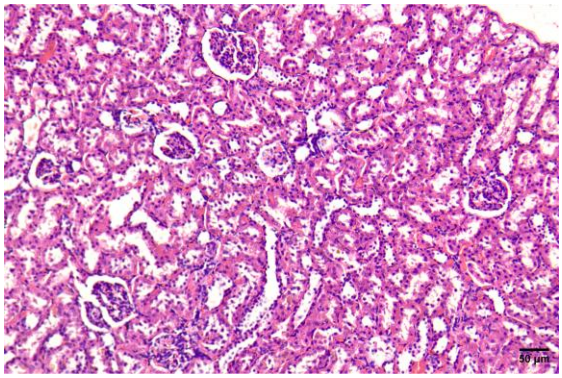
F. Hydrochlorothiazide (400X)



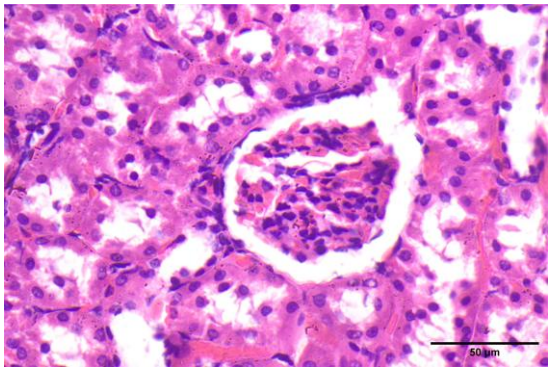
G. Furosemide (100X)



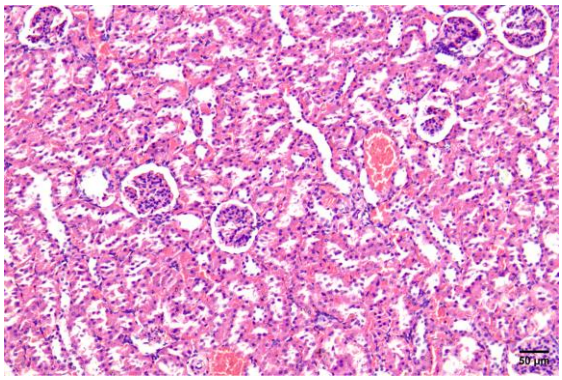
H. Furosemide (400X)



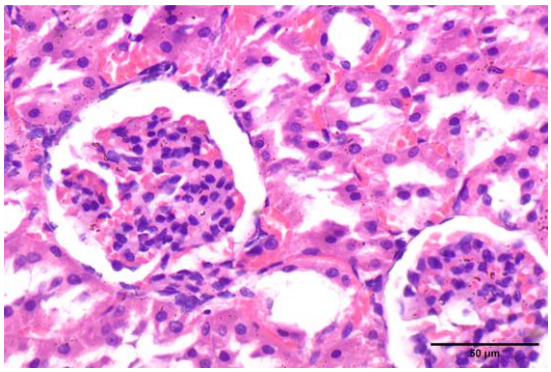
I. Spironolactone (100X)



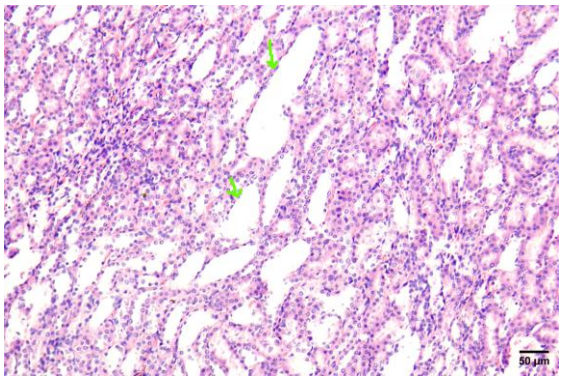
J. Spironolactone (400X)



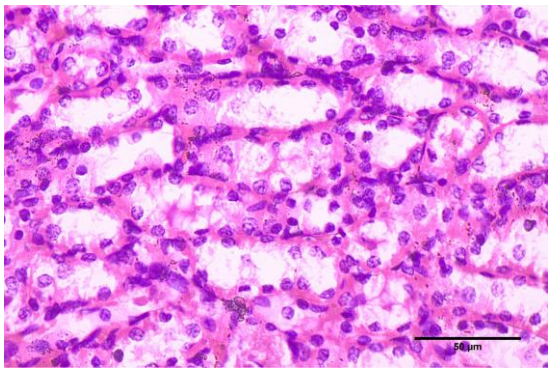
K. CAE + Hydrochlorothiazide (100X)



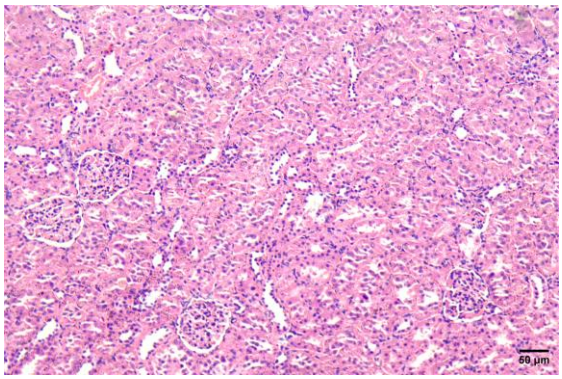
L. CAE + Hydrochlorothiazide (400X)



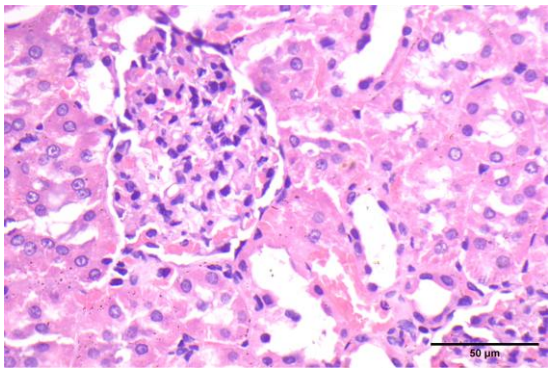
M. CAE + Furosemide (100X)



N. CAE + Furosemide (400X)



O. CAE + Spironolactone (100X)



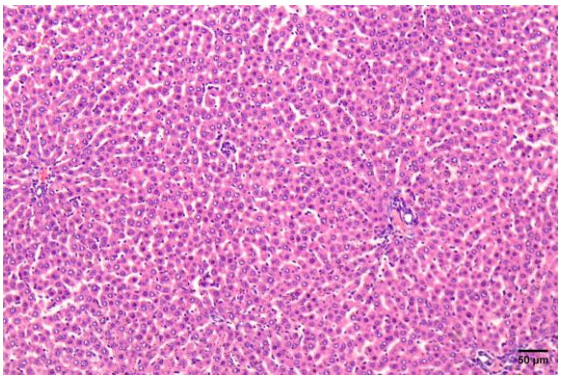
P. CAE + Spironolactone (400X)

Figure 37: Histopathological findings of the kidney of normal control, CAE, standard diuretics, and combinations of test drug with standard diuretics treated groups in a 28-day diuretic study.

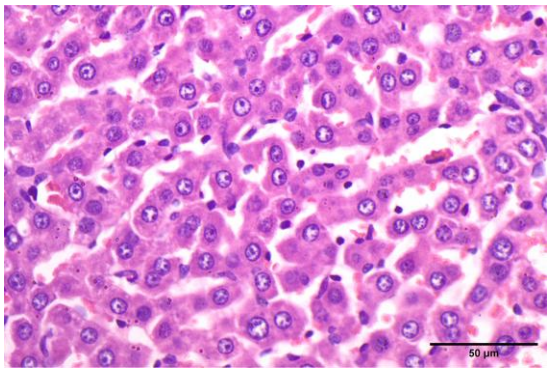
T – tubules (37A and 37B), G – glomerulus (37A and 37B), Red arrow – inflammatory infiltrate (37G), Green arrow – dilated tubules (37M)

- **Liver:**

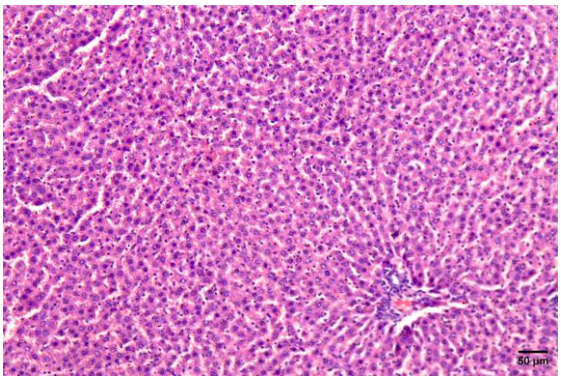
All the slides show liver tissue with a lobular arrangement. Each lobule consists of a central vein and portal triads along the periphery of the lobule. Numerous sinusoids pass radially from the central vein, and the spaces between the sinusoids contain liver cells. In the animals treated with furosemide (Figure 38G), there was a slight increase in the inflammatory infiltrate when compared with the normal control (Figure 38A). No changes in the tissue architecture were observed in any of the groups (Figures 38A to 38P).



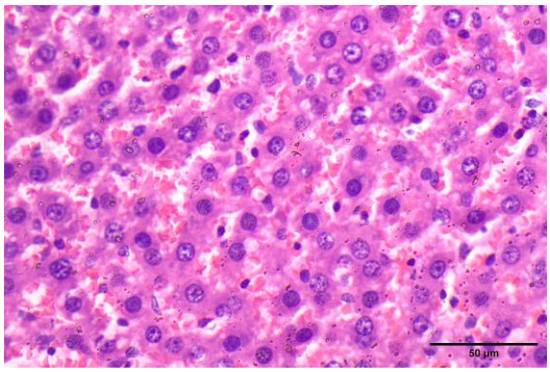
A. Normal Control (100X)



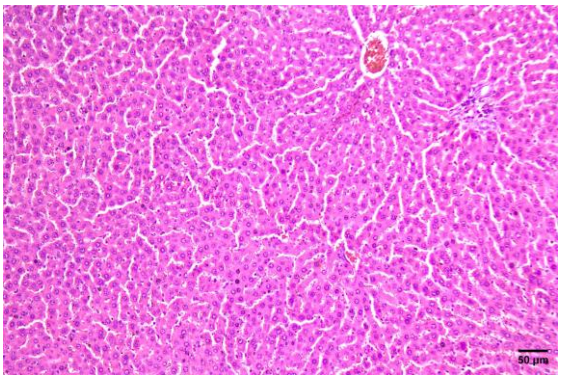
B. Normal Control (400X)



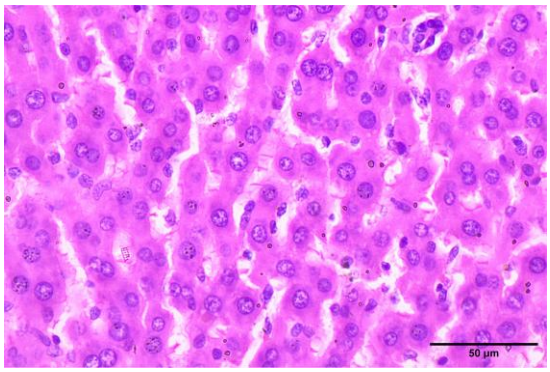
C. CAE (100X)



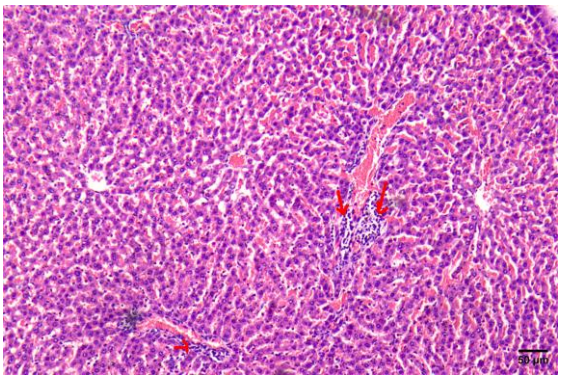
D. CAE (400X)



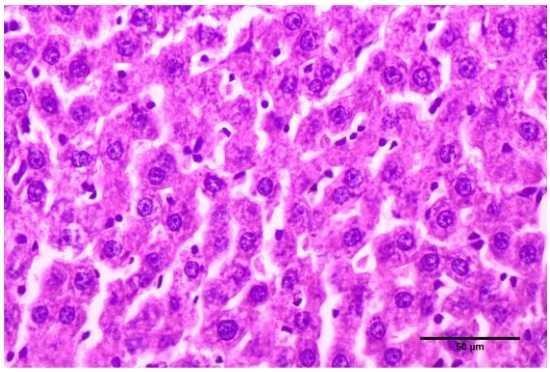
E. Hydrochlorothiazide (100X)



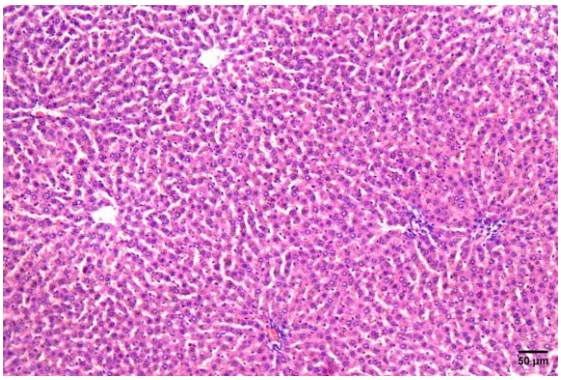
F. Hydrochlorothiazide (400X)



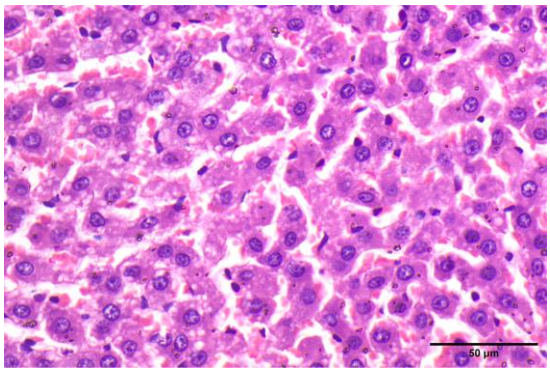
G. Furosemide (100X)



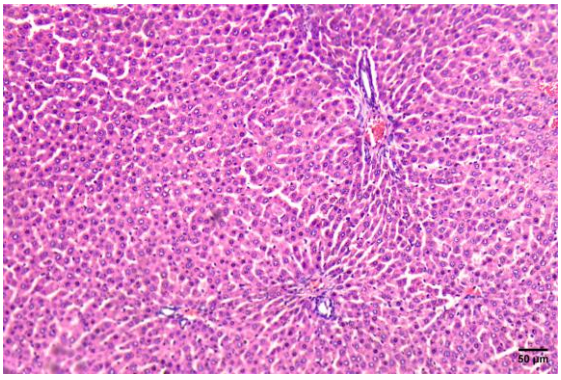
H. Furosemide (400X)



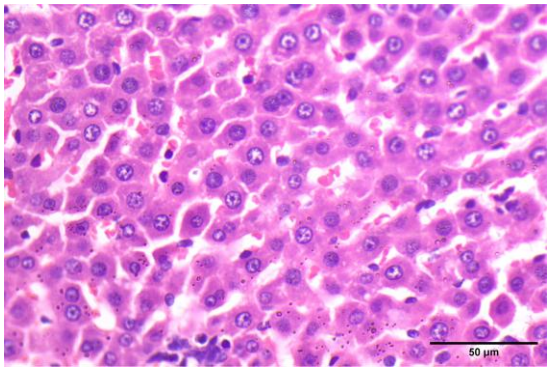
I. Spironolactone (100X)



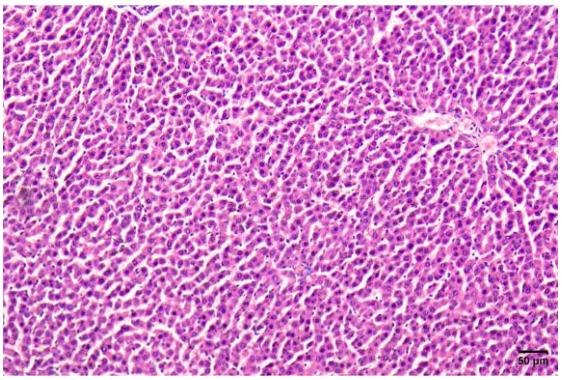
J. Spironolactone (400X)



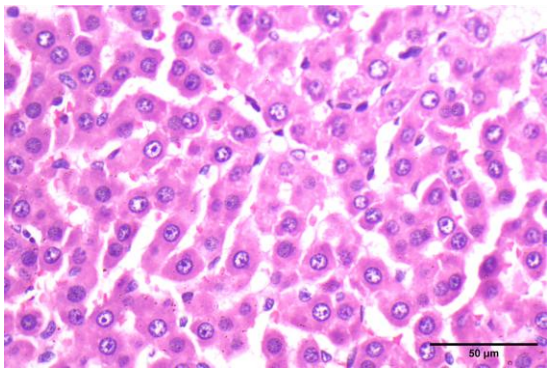
K. CAE + Hydrochlorothiazide (100X)



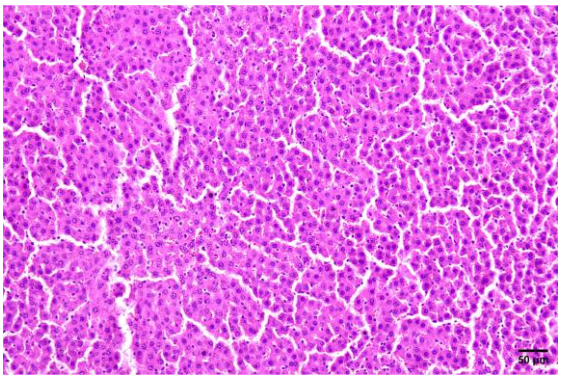
L. CAE + Hydrochlorothiazide (400X)



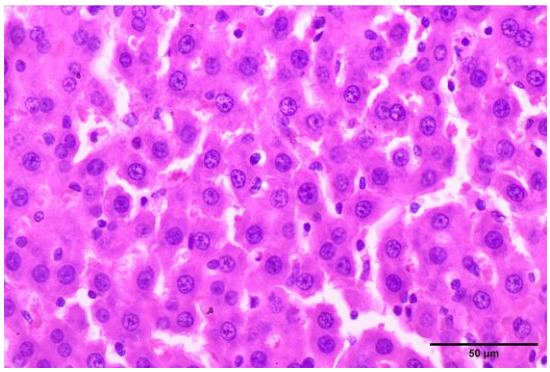
M. CAE + Furosemide (100X)



N. CAE + Furosemide (400X)



O. CAE + Spironolactone (100X)



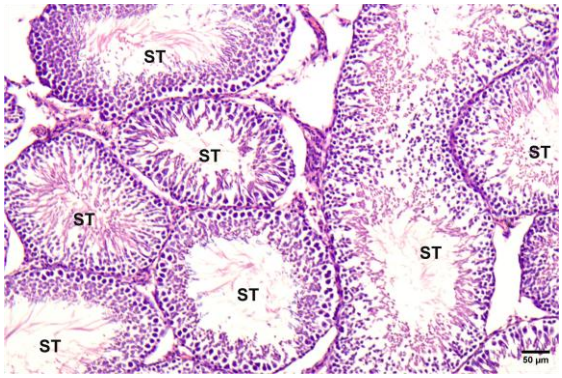
P. CAE + Spironolactone (400X)

Figure 38: Histopathological findings of the liver of normal control, CAE, standard diuretics, and combinations of CAE with standard diuretics treated groups in a 28-day diuretic study

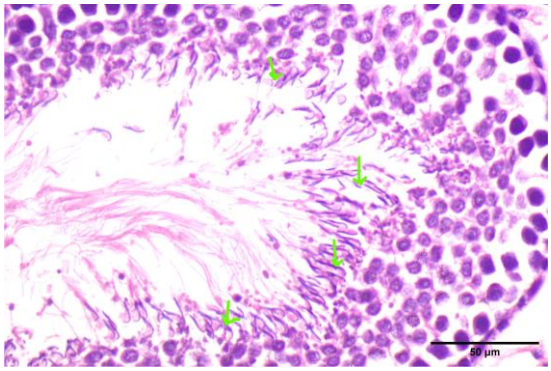
Red arrow – inflammatory infiltrate (38G).

- **Testis:**

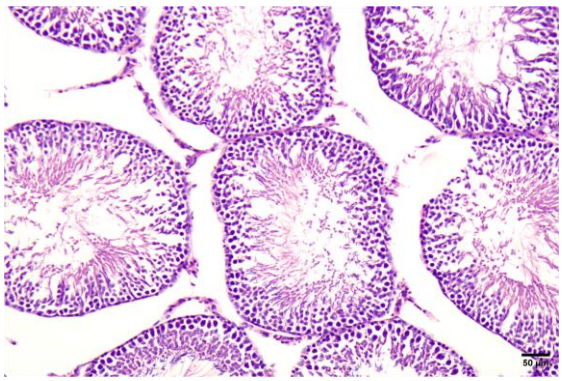
All the slides show testis tissue consisting of many seminiferous tubules at different stages. The seminiferous tubules contain different germ cells, such as spermatocytes, spermatids, and spermatozoa, which are arranged in layers. None of the groups showed any histological changes or changes in tissue architecture (Figures 39A to 39P).



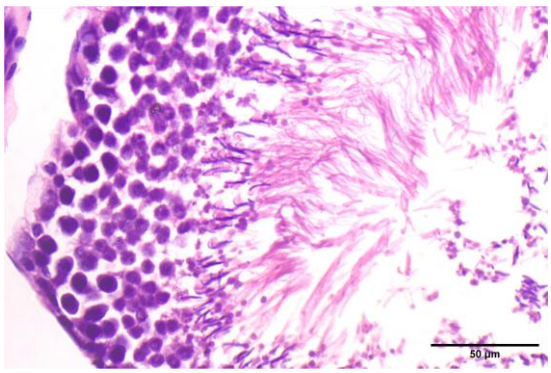
A. Normal Control (100X)



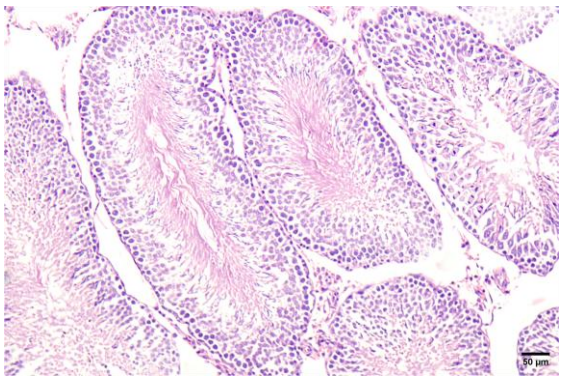
B. Normal Control (400X)



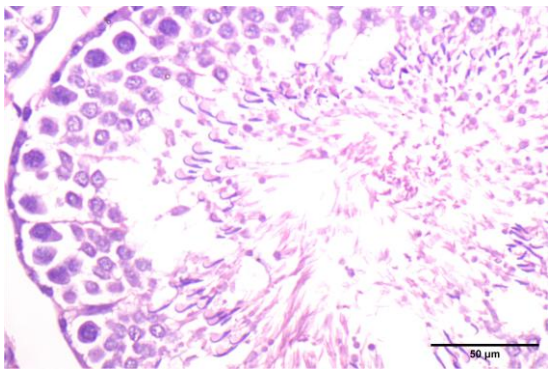
C. Test (100X)



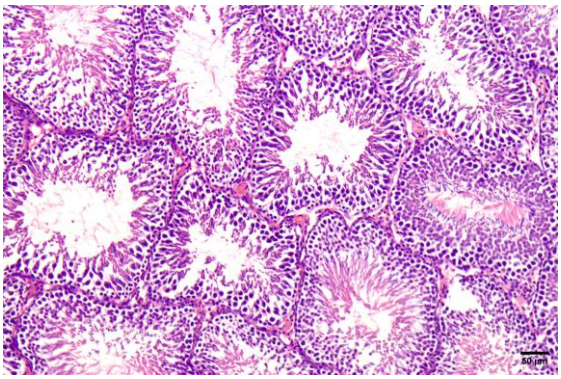
D. Test (400X)



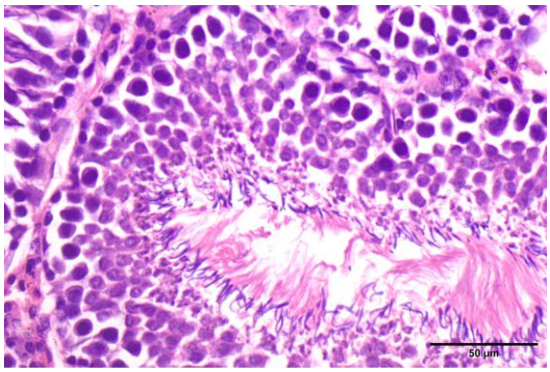
E. Hydrochlorothiazide (100X)



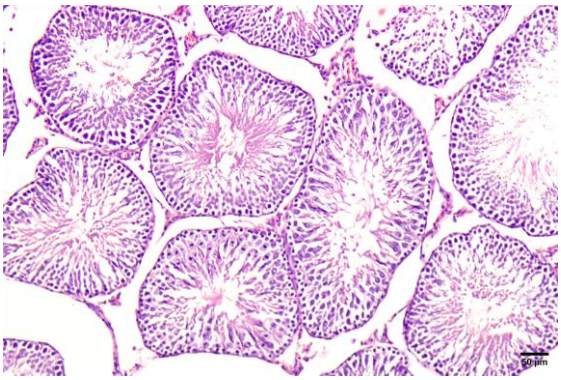
F. Hydrochlorothiazide (400X)



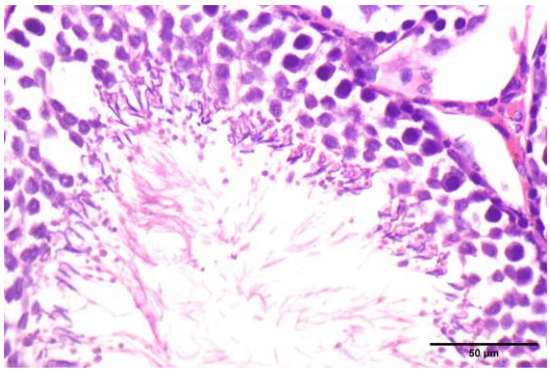
G. Furosemide (100X)



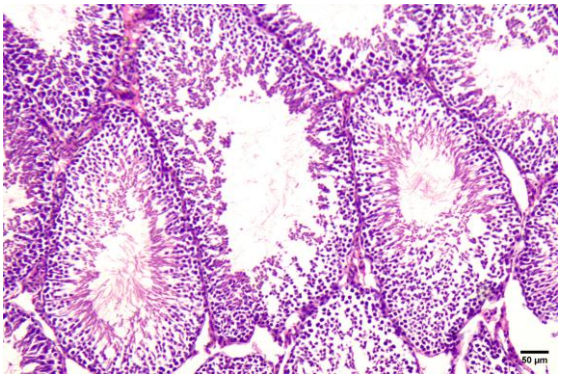
H. Furosemide (400X)



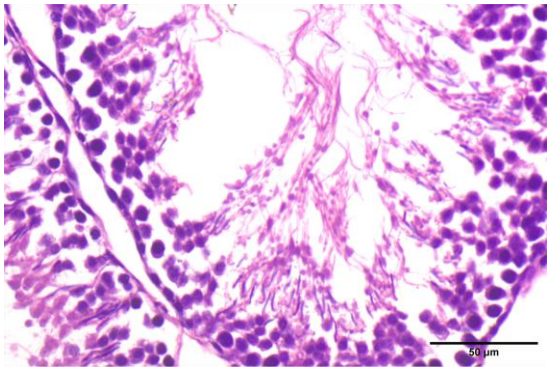
I. Spironolactone (100X)



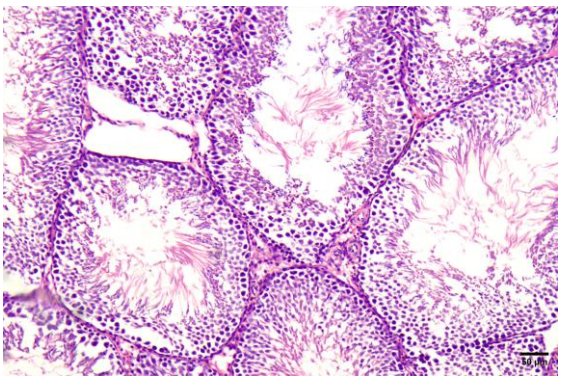
J. Spironolactone (400X)



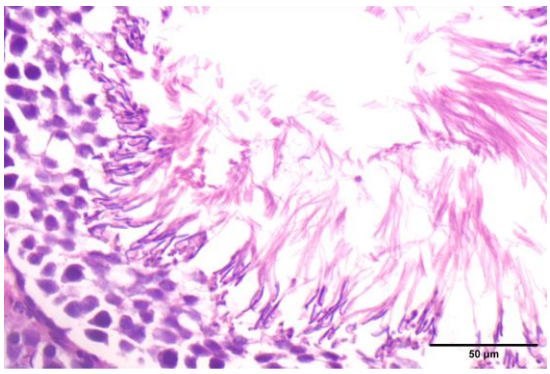
K. CAE + Hydrochlorothiazide (100X)



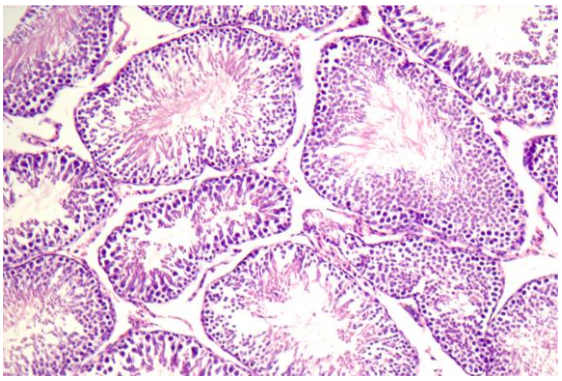
L. CAE + Hydrochlorothiazide (400X)



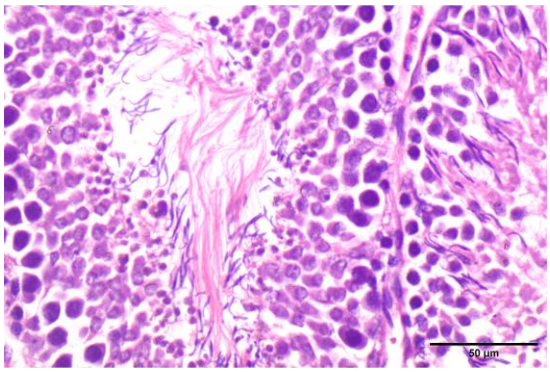
M. CAE + Furosemide (100X)



N. CAE + Furosemide (400X)



O. Test + Spironolactone (100X)



P. Test + Spironolactone (400X)

Figure 39: Histopathological findings of the testis of normal control, CAE, standard diuretics, and combinations of CAE with standard diuretics treated groups in a 28-day diuretic study

ST – Seminiferous tubules (39A), Green arrow – Spermatozoa (39B)

Discussion:

In a 28-day combination study, we tested the efficacy of combinations of CAE with standard diuretics and compared them to standard diuretics when given alone. Compared with standard diuretics, daily administration of the CAE for 28 days led to a significant increase in

urine volume, water consumption, and urine pH, indicating that the extract might be equal to or more potent than standard diuretics.

No significant changes or toxicity were observed in the CBC with CAE administration. There was lymphocytopenia in the animals treated with hydrochlorothiazide and furosemide. We observed furosemide-induced erythrocytopenia in our study, and similar observations have already been reported (61–65,67,68,76,81,82). The co-administration of CAE with standard diuretics counteracted all the hematological toxicity induced by standard diuretics. These findings suggest that the CAE does not pose any hematologic risk; instead, it completely reverses the changes caused by the standard drug.

Standard diuretics induced hyperglycemia (220–222) was not observed in CAE, but it could effectively control hyperglycemia after co-administering with standard diuretics. This property would be helpful in diabetic patients receiving long-term diuretic therapy.

The long-term administration of CAE did not alter serum electrolytes except magnesium, which is associated with hypomagnesemia. This might put susceptible patients at risk of arrhythmia. The electrolyte imbalance caused by standard diuretics was counteracted by the co-administering CAE.

CAE significantly increased the serum HDL level without altering the other lipid parameters. Standard diuretics reduced the serum HDL level and increase the serum LDL, VLDL, triglyceride, and total cholesterol levels (223–225), which are counteracted by the co-administering the CAE. This property might be helpful in patients at risk of dyslipidemia.

Standard diuretics altered the LFT and RFT parameters, whereas CAE did not alter them. The LFT changes were not reversed after the co-administration of CAE with standard diuretics. There was a significant reduction in the altered RFT values that were observed even though they were not normalized.

Animals treated with CAE showed higher urinary excretion of sodium, potassium, chloride, calcium, magnesium, and phosphate, urea and uric acid than standard diuretics. By co-administering the CAE, there was a synergistic increase in the urinary excretion of electrolytes, urea and uric acid. This property is helpful in patients with sodium overload, CCF, hypercalcemia, hyperkalemia, urinary stones, gouty arthritis and other edematous conditions. Compared with other standard diuretics, furosemide tends to have a calcium-sparing effect, but in combination with CAE, it increased the urinary excretion of calcium, leading to a calcium-wasting effect. These results revealed that the administration of CAE alone for 28 days did not alter the serum potassium level. At the same time, it brought the elevated potassium level to the normal range in the animals treated with spironolactone and, brought the reduced potassium

level to the normal range in the animals treated with furosemide. This indicates that the CAE is an eukalemic agent. Hence, this could be safely combined along with standard diuretics to prevent electrolyte imbalance in long-term treatments. The times of increase or decrease in the important parameters related to the diuresis of the high-dose CAE are mentioned in Table 58 below in comparison with those of the respective groups.

Table 58: The changes (in times) produced by the CAE in the urine parameters of the 28-day diuretic study with respect to the groups compared

Sl. No.	Parameters	NC	Hydro	H+CAE	Furo	F+CAE	Spiro	S+CAE
1	Urine Volume (mL)	5.4 ↑	1.3 ↑	1.4 ↑	1.0 ↑	1.4 ↑	1.7 ↑	1.5 ↑
2	Amount of Water Consumed (mL)	5.0 ↑	1.6 ↑	1.5 ↑	1.1 ↑	1.3 ↑	1.8 ↑	1.2 ↑
3	Urea (mg/dL)	2.0 ↑	0.9 ↓	1.1 ↑	0.6 ↓	1.1 ↑	1.2 ↑	1.4 ↑
4	Uric Acid (mg/dL)	2.0 ↑	1.3 ↑	1.3 ↑	1.0 ↑	1.4 ↑	1.7 ↑	1.7 ↑
5	Sodium (mmol/L)	2.0 ↑	1.4 ↑	1.4 ↑	1.3 ↑	1.3 ↑	2.0 ↑	1.1 ↑
6	Potassium (mmol/L)	15.1 ↑	2.3 ↑	1.8 ↑	1.1 ↑	1.2 ↑	24.0 ↑	5.6 ↑
7	Chloride (mmol/L)	2.4 ↑	1.4 ↑	1.2 ↑	1.1 ↑	1.1 ↑	2.3 ↑	1.5 ↑
8	Calcium (mmol/L)	5.2 ↑	0.9 ↓	1.3 ↑	6.5 ↑	3.9 ↑	2.1 ↑	1.1 ↑
9	Magnesium (mmol/L)	6.8 ↑	1.6 ↑	1.2 ↑	1.3 ↑	1.2 ↑	7.2 ↑	1.9 ↑
10	Phosphate (mmol/L)	4.6 ↑	1.0 ↓	1.1 ↑	3.6 ↑	1.5 ↑	3.7 ↑	1.3 ↑

Note: NC – normal control, H – hydrochlorothiazide, F – furosemide, S – spironolactone, CAE – crude aqueous extract, ↑ – increase, ↓ – decrease; CAE was compared against normal control, and standard diuretics. The combinations were compared against the respective standard diuretics.

The important observation in this study was the natriuretic action. In the single-dose study, the CAE produced higher natriuretic effect than furosemide. When CAE was combined with furosemide, the natriuretic effect increased, reaching a ceiling effect. A similar ceiling effect was observed when CAE was given alone for 28 days. This is attributed to the cumulative action of the CAE. When CAE was given alone, it produced highest natriuretic effect compared to the standard diuretics. After co-administering CAE with standard diuretics, the effect was not increased further. These findings indicate that CAE has a ceiling natriuretic effect that is greater than that of furosemide. In terms of the natriuretic effect, no advantages were observed when the CAE was combined with standard diuretics.

Ideally, a potent diuretic agent should reduce the serum sodium and potassium levels while eliminating them through the urine, which is observed with furosemide. The CAE showed higher natriuretic and potassium elimination actions than furosemide, but, did not alter the serum sodium and potassium levels. These findings indicate the activation of physiological compensatory mechanisms to maintain normal serum sodium and potassium levels in the blood following long-term administration of CAE. Maintaining serum electrolyte levels within normal limits while eliminating them through urine is possible only if the preparation naturally contains salts; CAE might not be beneficial in reducing blood pressure. Future studies in hypertensive models could provide some scientific insights.

The restoration of serum electrolyte levels during herbal diuretic therapy can be credited to the physiological and biochemical compensatory mechanisms that help the body's homeostasis (226). A key reason for maintaining homeostasis is the tubuloglomerular reabsorption, or feedback mechanism, which enables the kidney to adjust its functions based on the electrolyte levels detected in the Distal Convoluted Tubule (DCT). When diuretics cause excessive electrolyte loss, the macula densa detects these changes and triggers the release of adenosine, ultimately leading to a decrease in the glomerular filtration ratio. Over time, this feedback mechanism helps in maintaining the serum electrolyte levels (227,228).

Another possibility for serum electrolyte balance is the solvent drag mechanism. In this case, water carries the solutes from the tubular lumen to the interstitium in the Proximal convoluted tubule (PCT), a process that does not require energy. Even with a reduced state of solute reabsorption due to diuretic therapy, solvent drag continues to some extent, contributing to the restoration of serum electrolyte balance (229–231).

Nutritional intake (food and water) and cellular reservoirs, by which the body can also compensate for the electrolyte loss by increasing the water and food consumption, which enhances the gastrointestinal absorption of essential electrolytes and nutrients (232). This

adjustment helps to balance serum electrolyte levels and ensure hemodynamic stability. In our study, we observed an increase in water intake among the treated animals, which aligns with the current assumption; however, we did not measure food intake.

Cells play a crucial role as reservoirs for certain electrolytes, particularly potassium and calcium, buffering them between intracellular compartments and blood circulation (233). Additionally, bones serve as reservoirs for calcium and phosphates, releasing these minerals as needed (234,235). This adaptability is vital for the body's cumulative ability to maintain homeostatic balance during the excretion of excessive electrolytes and other substances through urine. Other contributing factors include the renin–angiotensin–aldosterone system (RAAS) (236–238) and the influence of parathyroid hormone (PTH) (239,240)-mediated bone resorption or increased intestinal absorption, which adjust and retain the levels of sodium and other electrolytes in the body. The body prioritizes maintaining extracellular fluid osmolarity and electrolyte balance (241–244).

According to a study performed by Arasaratnam et al., when the aqueous extract of *TT* was given to urolithic patients for seven days, it decreased the serum uric acid level and increased the urine output and serum Ca^{2+}, Mg^{2+}, and inorganic phosphate levels. The urinary excretion of citrate, oxalate, and inorganic phosphate also increased (126). In another study by Hanna et al., researchers provided a CAE of *Sirupeelai Samoola Kudineer* containing *TT* along with *Aerva lanata*, *Crataeva nurvala*, and *Pavonia odorata* for 21 days to *Sprague–Dawley* rats with renal calculus induced by ethylene glycol. It significantly increased the urine output and Na^{+}, K^{+}, Mg^{2+}, and PO^{3-}_{4} excretion. It also reduced plasma and urine calcium and oxalate levels; decreased calcium and oxalate deposition in the tissues of the kidneys; and decreased urinary excretion of urea, creatinine, and total proteins. The markers of renal function were normalized, and oxidative stress was reduced, leading to an effective reduction in urolithiasis (99). These results align with our current study, which focused on electrolyte excretion, a reduction in the serum urea and uric acid levels, and renoprotective effects.

Overall, 28 days of CAE administration produced better saluretic effect, favorable natriuretic effect, and mild carbonic anhydrase inhibitory effect and can be considered potent diuretic comparable to the standard diuretics. When CAE was co-administered with standard diuretics, it synergistically enhanced the diuretic property and antagonized the adverse effects. The drug–herb interaction in this case was synergistic. Therefore, these findings could be helpful in integrative medicine approaches.