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PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL EVALUATION OF DIURETIC ACTIVITY OF COSMOS SULPHUROUS IN RATS

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Abstract: The present study investigated the diuretic activity of the methanolic extract of Cosmos sulphurous in Wistar albino rats using the Lipschitz test, with Furosemide (10 mg/kg, p.o.) as the reference standard. Animals were divided into five groups (n=6) and urine was collected for 5 hours post-dosing in metabolic cages. Diuretic response was assessed by measuring total urine volume, urinary electrolyte excretion (Na^+, K^+, Cl^-), diuretic index, saluretic index, natriuretic index, and ion quotient. The extract produced a significant, dose-dependent increase in urine output with diuretic indices of 1.61, 2.06, and 2.55 at doses of 250, 300, and 500 mg/kg, respectively, compared with control (1.0) and approaching the standard (2.9). Enhanced excretion of sodium, potassium, and chloride with mild reductions in their serum levels indicated marked saluretic and natriuretic effects. Serum total protein showed only a slight reduction within physiological limits, suggesting the absence of harmful protein depletion. Phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, phenols, saponins, tannins, steroids, and amino acids, which may contribute to the observed activity. The findings demonstrate that Cosmos sulphurous possesses significant diuretic potential comparable to the standard drug and supports its traditional use as a natural diuretic agent.

Keywords: Ampelocissus latifolia, Diuretic activity, Lipschitz test, Furosemide, Saluretic index, Natriuretic index, Urinary electrolytes.

INTRODUCTION

The kidneys serve as the principal excretory organs responsible for removing metabolic waste products from the body. In most animal species, complete loss of renal function leads to death within approximately one week. When renal function is only partially impaired, physiological disturbances occur to varying degrees depending on the amount of functioning renal tissue remaining. The term azotemia describes the accumulation of nitrogen-containing waste products in the bloodstream. Blood levels of urea and creatinine are commonly used as indicators of azotemia; however, these substances themselves are not highly toxic, even when accumulated. Animals with moderate to severe azotemia typically exhibit a range of clinical signs such as lethargy, anorexia, oral ulcers, vomiting, diarrhea, weight loss, anemia, and changes in urine output. Collectively, these manifestations are referred to as renal failure, uremia, or uremic syndrome, reflecting systemic dysfunction resulting from impaired kidney activity [1]. The kidneys play a vital role in maintaining homeostasis through multiple functions. They regulate water and electrolyte balance by conserving or excreting these substances depending on the body's needs. During periods of deficiency, they conserve essential nutrients such as glucose and amino acids, resulting in urine that is normally free of these compounds. The kidneys also maintain acid–base balance by regulating the excretion or retention of hydrogen ions, thereby keeping blood pH within a narrow

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physiological range. In addition, they eliminate nitrogenous waste products such as urea, creatinine, and allantoic acid through urine, ensuring that their blood concentrations remain stable. Beyond excretory functions, the kidneys act as endocrine organs. They produce hormones including renin, erythropoietin, and prostaglandins, and are involved in the activation of vitamin D through hydroxylation, which is essential for calcium metabolism. The kidneys also respond to several hormones such as antidiuretic hormone (ADH), parathyroid hormone (PTH), aldosterone, and thyroid hormones, which collectively regulate fluid balance, electrolyte levels, and metabolic processes [2]. From an evolutionary perspective, kidney function has adapted to the changing environmental needs of organisms. In simple unicellular and primitive organisms, diffusion was sufficient for waste removal and nutrient exchange due to their direct exposure to an iso-osmotic aqueous environment. As multicellular complexity increased, organisms developed extracellular fluid compartments that mimicked the surrounding aquatic environment, allowing basic excretory mechanisms to function effectively. However, increasing salinity in marine environments created osmotic challenges. Some species, such as sharks, adapted by retaining urea to maintain osmotic balance with seawater. Others evolved renal tubular systems capable of excreting excess salts. When certain species transitioned from marine to freshwater environments, they faced excessive water influx due to low environmental osmolarity, leading to the evolution of high glomerular filtration rates to eliminate excess water. Later, as animals moved from freshwater to terrestrial habitats, the evolutionary pressure shifted toward water conservation rather than excretion. This led to kidneys that filter large volumes of plasma but reabsorb most of the filtered water and solutes, allowing efficient waste removal while minimizing water loss [3]. This evolutionary design, while highly effective, appears inefficient from an engineering standpoint because a large portion of filtered sodium and water-about 4–6% of cardiac output-is continuously filtered and then almost completely reabsorbed, requiring significant energy expenditure. Despite this apparent inefficiency, it allows precise regulation of internal fluid and electrolyte balance.

Clinically, this evolutionary background is important because renal failure affects both filtration and tubular reabsorption processes. In addition to reduced glomerular filtration leading to waste accumulation, tubular dysfunction can cause excessive loss of water and solutes. This contributes to complications such as polyuria commonly seen in renal disease [4].

NORMAL RENAL FUNCTIONS

Normal renal function is essential for maintaining homeostasis in the body through a combination of excretory, regulatory, and endocrine activities. The kidneys primarily remove metabolic waste products such as urea, creatinine, uric acid, and ammonia through urine, thereby preventing their accumulation in the blood. They regulate the body's water balance by adjusting urine volume according to hydration status and maintain electrolyte equilibrium by controlling levels of sodium, potassium, calcium, chloride, bicarbonate, and phosphate. The kidneys also play a key role in acid–base balance by excreting hydrogen ions and reabsorbing bicarbonate to keep blood pH within a narrow physiological range. In addition, they conserve essential nutrients like glucose and amino acids through tubular reabsorption. Renal function is closely involved in blood pressure regulation via the renin–angiotensin–aldosterone system (RAAS) and also includes important endocrine roles such as the secretion of erythropoietin for red blood cell production and activation of vitamin D for calcium metabolism. Furthermore, the kidneys respond to hormones like ADH, aldosterone, and parathyroid hormone to fine-tune fluid and electrolyte balance. Overall, normal kidney function is vital for maintaining internal stability and supporting overall physiological health [5-6].

Excretory Function

The excretory function of the kidneys involves the removal of metabolic waste products from the body while maintaining the composition and volume of body fluids. The kidneys filter blood through the glomeruli and eliminate nitrogenous wastes such as urea, creatinine, uric acid, and ammonia in the form of urine. This process prevents the accumulation of toxic substances in the bloodstream and maintains internal chemical balance. In addition to waste elimination, the kidneys regulate the excretion of excess water, electrolytes, and acids, ensuring stability of osmotic pressure and acid–base balance. Through

selective filtration, reabsorption, and secretion, the kidneys efficiently maintain homeostasis and support normal physiological function [6].

Regulation of Water Balance

The kidneys play a crucial role in maintaining the body's water balance by controlling the amount of water excreted in urine. They adjust urine volume according to the body's hydration status through processes of filtration, reabsorption, and secretion in the renal tubules. When the body is dehydrated, the kidneys conserve water by increasing reabsorption, producing a small volume of concentrated urine. In contrast, when excess water is present, they excrete larger volumes of dilute urine. This regulation is mainly controlled by hormones such as antidiuretic hormone (ADH), which increases water reabsorption in the collecting ducts. Through these mechanisms, the kidneys maintain fluid homeostasis and ensure proper hydration of body tissues [7].

Electrolyte Balance

The kidneys are essential for maintaining electrolyte balance in the body by regulating the levels of key ions such as sodium, potassium, calcium, chloride, bicarbonate, and phosphate in the blood and body fluids. This is achieved through selective filtration at the glomerulus followed by precise reabsorption and secretion along different segments of the renal tubules. The kidneys adjust electrolyte excretion according to the body's needs to maintain normal cellular function, nerve conduction, muscle contraction, and acid–base stability. Hormones such as aldosterone play a major role by promoting sodium reabsorption and potassium excretion, while parathyroid hormone regulates calcium and phosphate balance. Through these coordinated processes, the kidneys ensure a stable internal environment necessary for normal physiological activity.

Acid–Base Balance

The kidneys play a vital role in maintaining the acid–base balance of the body by regulating the concentration of hydrogen ions (H^+) and bicarbonate (HCO_3^-) in the blood. They help keep the blood pH within the normal physiological range by excreting excess hydrogen ions into the urine and reabsorbing filtered bicarbonate back into the bloodstream. In addition, the renal tubules can generate new bicarbonate to replace that lost during buffering of acids. These processes are particularly important in maintaining long-term pH stability, complementing the rapid buffering action of the respiratory system. Through these mechanisms, the kidneys prevent conditions such as acidosis and alkalosis, ensuring normal cellular and metabolic function [8].

Reabsorption of Nutrients

The kidneys play an important role in conserving essential nutrients through the process of selective tubular reabsorption. After blood is filtered in the glomerulus, valuable substances such as glucose, amino acids, vitamins, and small peptides are reabsorbed from the renal tubules back into the bloodstream. Under normal physiological conditions, almost all glucose and amino acids are completely reabsorbed in the proximal convoluted tubule, preventing their loss in urine. This process ensures that essential nutrients required for energy production, growth, and metabolism are retained in the body while only waste products are excreted. In this way, the kidneys help maintain nutritional balance and metabolic efficiency.

Regulation of Blood Pressure

The kidneys play a central role in regulating blood pressure by controlling blood volume and vascular resistance. They adjust the amount of water and sodium excreted or reabsorbed, which directly influences blood volume and therefore blood pressure. A key mechanism involved is the renin–angiotensin–aldosterone system (RAAS), in which the kidneys release renin in response to decreased blood pressure or reduced blood flow. Renin activates a cascade that leads to the formation of angiotensin II, a powerful vasoconstrictor that increases blood pressure and stimulates aldosterone secretion, promoting sodium and water retention. Through these coordinated actions, the kidneys help maintain stable arterial pressure and ensure adequate tissue perfusion [9].

Endocrine Functions

The kidneys function as important endocrine organs by producing and activating several hormones that regulate key physiological processes. They secrete erythropoietin, which stimulates red blood cell production in the bone marrow in response to low oxygen levels. The kidneys also release renin, an enzyme that initiates the renin–angiotensin–aldosterone system (RAAS), playing a major role in blood pressure

regulation and fluid balance. In addition, they produce prostaglandins, which help regulate renal blood flow and vascular tone. A crucial endocrine function of the kidneys is the activation of vitamin D (calcitriol) through hydroxylation, which is essential for calcium and phosphorus absorption and bone health. Through these hormonal actions, the kidneys contribute significantly to hematopoiesis, cardiovascular regulation, and mineral metabolism [10].

Osmoregulation

Osmoregulation is the process by which the kidneys maintain the balance of water and solutes in the body fluids, thereby ensuring a stable osmotic environment. The kidneys regulate the concentration and volume of body fluids by adjusting the excretion or reabsorption of water and electrolytes, especially sodium and chloride ions. When body fluids become concentrated due to dehydration, the kidneys conserve water by producing small volumes of highly concentrated urine. Conversely, when there is excess water intake, they excrete dilute urine to restore balance. This process is primarily controlled by hormones such as antidiuretic hormone (ADH) and aldosterone, which influence water and sodium reabsorption in the renal tubules. Through osmoregulation, the kidneys help maintain normal cellular function and overall fluid homeostasis [11].

Hormonal Response

The kidneys respond to several hormones that help regulate fluid balance, electrolyte levels, and blood pressure. Antidiuretic hormone (ADH) increases water reabsorption in the collecting ducts, reducing urine output and conserving body water. Aldosterone promotes sodium reabsorption and potassium excretion in the distal tubules, which helps maintain blood volume and electrolyte balance. Parathyroid hormone (PTH) regulates calcium and phosphate levels by increasing calcium reabsorption and promoting phosphate excretion. In addition, the kidneys respond to other hormones such as atrial natriuretic peptide (ANP), which promotes sodium and water excretion to reduce blood volume and pressure. Through these coordinated hormonal responses, the kidneys finely regulate internal homeostasis and maintain physiological stability.

Nephron Heterogeneity

Nephron heterogeneity refers to the presence of structurally and functionally different types of nephrons within the kidney, which allows efficient regulation of filtration and urine concentration. In mammals, nephrons are broadly classified into cortical nephrons and juxtamedullary nephrons. Cortical nephrons, which form the majority, have short loops of Henle and mainly contribute to solute reabsorption and basic urine formation. Juxtamedullary nephrons have long loops of Henle that extend deep into the medulla and play a crucial role in concentrating urine by establishing a strong osmotic gradient. This structural diversity enables the kidneys to adapt to varying physiological needs, such as conserving water during dehydration or excreting excess fluid when required, thereby maintaining fluid and electrolyte homeostasis effectively [12].

Renal Perfusion

Renal perfusion refers to the flow of blood through the kidneys, which is essential for maintaining normal renal function. Adequate renal blood flow ensures efficient glomerular filtration, delivery of oxygen and nutrients to renal tissues, and removal of metabolic waste products. The kidneys receive a high proportion of cardiac output, allowing them to continuously filter large volumes of plasma. Renal perfusion is tightly regulated by autoregulatory mechanisms, including the myogenic response and tubuloglomerular feedback, which maintain relatively constant glomerular filtration rate despite fluctuations in systemic blood pressure. Hormonal influences such as the renin–angiotensin–aldosterone system (RAAS) also help adjust renal blood flow during conditions of decreased perfusion. Proper renal perfusion is therefore critical for maintaining fluid balance, electrolyte homeostasis, and overall kidney function [13].

Renal Vasculature:

The renal vasculature is a highly specialized blood supply system that ensures efficient filtration and regulation of blood flow within the kidneys. Blood enters the kidney through the renal artery, which branches into segmental, interlobar, arcuate, and interlobular arteries before reaching the afferent arterioles. These arterioles deliver blood to the glomerular capillaries, where filtration of plasma occurs. The filtered blood then leaves the glomerulus via the efferent arterioles, which further branch into peritubular capillaries in cortical nephrons and vasa

recta in juxtamedullary nephrons. These capillary networks are essential for reabsorption, secretion, and maintenance of the medullary concentration gradient. Finally, blood is collected into venules that drain into interlobular, arcuate, interlobar veins, and ultimately the renal vein. This organized vascular arrangement supports effective filtration, tubular exchange processes, and regulation of renal blood flow.

Renal Blood Flow

Renal blood flow refers to the volume of blood delivered to the kidneys per unit time, which is essential for maintaining normal glomerular filtration and overall kidney function. The kidneys receive a large proportion of cardiac output, ensuring continuous delivery of oxygen and nutrients while facilitating efficient removal of metabolic waste. Renal blood flow is closely regulated by autoregulatory mechanisms, including the myogenic response and tubuloglomerular feedback, which help maintain a relatively constant glomerular filtration rate despite changes in systemic blood pressure. In addition, hormonal systems such as the renin–angiotensin–aldosterone system (RAAS) can adjust renal vascular tone to preserve blood flow during conditions of reduced perfusion. Proper renal blood flow is therefore vital for maintaining fluid balance, electrolyte homeostasis, and normal excretory function [14].

Autoregulation

Autoregulation refers to the intrinsic ability of the kidneys to maintain a relatively constant renal blood flow and glomerular filtration rate despite fluctuations in systemic blood pressure. This mechanism ensures stable kidney function and prevents damage to the delicate glomerular capillaries. The two main processes involved are the myogenic mechanism and tubuloglomerular feedback. In the myogenic response, the afferent arteriole constricts when blood pressure increases and dilates when it decreases, maintaining steady glomerular pressure. Tubuloglomerular feedback involves the macula densa, which senses changes in sodium chloride concentration in the distal tubule and adjusts afferent arteriole tone accordingly. Together, these mechanisms help preserve renal stability and ensure efficient filtration under varying physiological conditions.

Role of Neural Stimuli in Renal Perfusion

Neural stimuli, primarily through the sympathetic nervous system, play an important role in regulating renal perfusion, especially during stress conditions such as hemorrhage, dehydration, or shock. Increased sympathetic activity causes vasoconstriction of the renal blood vessels, particularly the afferent arterioles, leading to a reduction in renal blood flow and glomerular filtration rate. This response helps preserve systemic blood pressure by redirecting blood flow to vital organs such as the heart and brain. Sympathetic stimulation also enhances renin release from the juxtaglomerular cells, activating the renin–angiotensin–aldosterone system (RAAS), which further contributes to vasoconstriction and sodium and water retention. Through these mechanisms, neural control helps maintain overall circulatory stability during physiological stress, although prolonged vasoconstriction may impair renal function [15].

Glomerular Filtration

Glomerular filtration is the first step in urine formation, occurring in the renal corpuscle where plasma is filtered from the glomerular capillaries into Bowman's capsule. It is driven by hydrostatic pressure in the glomerular capillaries, which forces water and small solutes such as electrolytes, glucose, amino acids, and nitrogenous wastes across the filtration membrane. The filtration barrier consists of the fenestrated endothelium, basement membrane, and podocyte slit diaphragms, which prevent the passage of large molecules like proteins and blood cells. The rate of filtration, known as the glomerular filtration rate (GFR), is tightly regulated by autoregulatory mechanisms and hormonal influences to maintain stable kidney function. Glomerular filtration is essential for removing waste products while retaining essential proteins and cells within the bloodstream.

Nature of Filtrate

The glomerular filtrate is an ultra-filtrate of blood plasma that is formed as fluid passes from the glomerular capillaries into Bowman's capsule during glomerular filtration. It closely resembles plasma in composition but is almost completely free of proteins and cellular components due to the selective permeability of the filtration membrane. The filtrate contains water, electrolytes, glucose, amino acids, and small nitrogenous waste products such as urea and creatinine. Because large

molecules like plasma proteins and blood cells are retained in the circulation, the filtrate is clear and protein-free under normal physiological conditions. As it passes through the renal tubules, its composition is progressively modified by processes of reabsorption and secretion to ultimately form urine [16].

Anatomic Considerations for Filtration

The process of glomerular filtration is supported by specialized anatomical structures of the renal corpuscle that ensure selective and efficient filtration of blood. Each nephron begins with the glomerulus, a tuft of fenestrated capillaries supplied by the afferent arteriole and drained by the efferent arteriole. The high hydrostatic pressure within these capillaries favors the movement of fluid into Bowman's capsule. The filtration barrier consists of three layers: the fenestrated endothelium of the glomerular capillaries, the glomerular basement membrane, and the podocyte foot process with slit diaphragms. These layers act together to prevent the passage of large proteins and blood cells while allowing water and small solutes to pass freely. Bowman's capsule collects the filtrate and directs it into the proximal tubule for further processing. The unique arrangement of the glomerular capillary network and surrounding structures is essential for maintaining selective permeability and efficient filtration.

Glomerular Filtration Dynamics

Glomerular filtration dynamics refer to the forces and mechanisms that regulate the movement of fluid across the glomerular capillaries into Bowman's capsule. Filtration is driven by Starling forces, which include the hydrostatic pressure of glomerular capillary blood that promotes filtration, and opposing forces such as plasma oncotic pressure and hydrostatic pressure within Bowman's capsule. The balance between these forces determines the net filtration pressure and the rate of glomerular filtration. Glomerular capillary pressure is maintained by the relative resistance of the afferent and efferent arterioles, allowing fine regulation of filtration rate. Changes in systemic blood pressure, renal blood flow, and plasma protein concentration can all influence these dynamics. Through this finely tuned balance of pressures, the kidneys ensure stable and efficient filtration under varying physiological conditions [17].

Filtration of Macromolecules

Under normal physiological conditions, the glomerular filtration barrier is highly selective and restricts the passage of macromolecules such as plasma proteins. This selectivity is based on size, charge, and structural characteristics of the filtration membrane, which includes the fenestrated endothelium, glomerular basement membrane, and podocyte slit diaphragms. Large molecules like albumin are generally prevented from entering the filtrate due to their size and negative charge, which is repelled by the negatively charged components of the basement membrane. As a result, the glomerular filtrate is almost protein-free. However, small peptides and low-molecular-weight substances may pass through in minimal amounts. This selective filtration is essential for maintaining plasma protein levels in the blood and preventing protein loss in urine.

Tubular Modification of Filtrate

Tubular modification of filtrate refers to the changes that occur in the composition of the glomerular filtrate as it passes through the renal tubules to form urine. This process includes selective reabsorption of essential substances such as water, glucose, amino acids, and electrolytes from the tubular fluid back into the blood, as well as secretion of certain substances like hydrogen ions, potassium ions, and organic acids into the tubules. The proximal convoluted tubule is the major site of reabsorption, while further fine-tuning occurs in the loop of Henle, distal convoluted tubule, and collecting ducts. Hormones such as antidiuretic hormone (ADH), aldosterone, and parathyroid hormone regulate these processes to maintain fluid, electrolyte, and acid–base balance. Through tubular modification, the kidneys transform the initial filtrate into concentrated urine suitable for excretion [18].

Conservation of Nutrients

The kidneys play a vital role in conserving essential nutrients by selectively reabsorbing them from the glomerular filtrate back into the bloodstream during tubular processing. Important substances such as glucose, amino acids, vitamins, and small peptides are almost completely reabsorbed in the proximal convoluted tubule under normal physiological conditions, ensuring they are not lost in urine. Sodium-dependent transport mechanisms and specific carrier proteins facilitate the efficient

recovery of these valuable nutrients. This selective conservation prevents nutrient depletion, supports normal metabolism, and maintains energy balance in the body. Only excess or waste substances remain in the tubular fluid, which is eventually excreted as urine [19].

Glucose:

Glucose is a vital energy source for the body and is freely filtered at the glomerulus into the renal tubules. Under normal physiological conditions, it is almost completely reabsorbed in the proximal convoluted tubule through sodium-glucose co-transport mechanisms (SGLT transporters) and then returned to the bloodstream. As a result, urine normally contains no glucose. However, when blood glucose levels exceed the renal threshold, the reabsorptive capacity of the tubules becomes saturated, leading to the presence of glucose in urine (glucosuria). This process highlights the kidney's role in conserving essential nutrients while also maintaining blood glucose homeostasis.

Amino Acids

Amino acids are small organic molecules that are freely filtered at the glomerulus and are normally almost completely reabsorbed in the proximal convoluted tubule. This reabsorption occurs through specific carrier-mediated transport systems, often coupled with sodium transport, which allows efficient recovery of these essential building blocks of proteins. As a result, amino acids are typically absent from normal urine. This conservation is important for maintaining nitrogen balance, supporting protein synthesis, and preventing loss of valuable metabolic resources. Only under pathological conditions or transport defects may amino acids appear in urine (aminoaciduria [20].

Materials and methods

Collection and Authentication of plant material:

The plant Cosmos sulphurous was collected from natural habitat of Ranga Reddy District, Telangana, India. The taxonomic identification of the plant was confirmed by Dr. Madhavan Chetty, Assistant Professor, Sri Venkateshwara University, Tirupathi, and Andhra Pradesh.

The Cosmos sulphurous were cleaned and drying under sunlight.

Extraction procedure

Coarsely powdered Cosmos sulphurous were used for extraction with Ethanol by using Soxhlet method for 6-8 hours. 50g of dried powder was weighed and 250mL of solvent Ethanol is used for the extraction. The extracts were evaporated by using a rotary evaporator and dried at room temperature. The obtained crude extracts were weighed and stored at 4^{o}C for further analysis.

Screening of Diuretic Activity in Rats

Animals Stock:

Wistar albino rats of either sex weighing between 150-200 g were obtained From Jeeva Life Sciences Animals were housed in groups of 6-8 per cage at a temperature of 25±1 0C and relative humidity of 45-55%. Animals had free access to food and water; however, food and water were withdrawn 18 hours before the experiment. Jeeva life sciences The Institutional Animal Ethics Committee approved the protocol of this study.

Diuretic activity:

Animals were divided into a total of five groups (n = 6 in each group). All animals were deprived of food and water 18 hours before the experiment. On the day of the experiment, the dosing was scheduled as follows:

Animal Grouping [21]

Group I: Normal saline.

Group II: Furosemide 10 mg/kg p.o (standard drug)

Group III: Cosmos sulphurous extract 250 mg/kg p.o

Group IV: Cosmos sulphurous 300 mg/kg p.o

Group V: Cosmos sulphurous 500 mg/kg p.o

Immediately after the dosing, animals were placed in metabolic cages and urine was collected up to 5 h after dosing. Room temperature was maintained up to 25± 0.5 0C.

During this period no water or food was made available to the animals. Diuretic activity was assessed by measuring the following parameters:

- Total urine volume.
- Urine concentration of Na+, K+ and Cl-.

The effect of different extracts of Ipomoea hederifolia exhibiting diuretic activity.

Biochemical Estimation [22]

Sodium:

Principle:

The sodium and the protein in the serum are precipitated with magnesium uranyl acetate. After separation by centrifugation, the excess of uranyl ions in the supernatant reacts with potassium ferrocyanide forming a colored complex whose absorbance varies inversely to the concentration of sodium in the sample.

Sodium Assay

Step-1: Precipitation of Sodium and Serum Proteins:

Pipette into clean dry test tubes labelled as Standard (S) and Test (T)

	Standard (S)	Test (T)
R1 Precipitating Reagent	1000 µl	1000 µl
R4 Standard	10 µl	-
Serum Sample	-	10 µl

Shake vigorously and incubate at room temperature for 5 minutes. Then Centrifuge at 2000-3000 rpm for 2 minutes to obtain a clear supernatant.

Step 2: Color development

Pipette into clean dry test tubes labeled as Blank (B), Standard (S), and Test (T):

	Blank (B)	Standard (S)	Test (T)
R2 Colour Reagent	1000 µl	1000 µl	1000 µl
Supernatant from Step 1		20 µl	20 µl
R1 Precipitating Reagent	20 µl		

Mix well and allow it to stand at room temperature for 5 minutes. Then measure the absorbance of Blank (B), standard (S), and Test (T) on a photo colorimeter with green filter or on a spectrophotometer at 530nm (505 - 530 nm) within 10 minutes.

Calculations:

$$Sodium\ in\ mmole/L = \frac{Abs\ of\ B - Abs\ of\ T}{Abs\ of\ B - Abs\ of\ S} \times 150$$

Potassium: [23]

Principle:

Potassium ions in a protein-free alkaline medium react with sodium tetraphenyl boron to produce a finely dispersed colloidal suspension of potassium tetraphenyl boron. The turbidity produced is proportional to the potassium concentration in the sample.

Potassium Assay

Pipette into two clean dry test tubes labeled Standard (S) and Test (T)

	Standard (S)	Test (T)
R3 Boron Reagent	1000 µl	1000 µl
R4 Standard	50 µl	-
Serum Sample	-	50 µl

Mix well and incubate for 5 minutes at RT. Read absorbance of the Standard (Abs.S) and Test Sample (Abs.T) against Distilled water at 620 nm.

Calculations

$$Potassium\ in\ mmol/L = \frac{Abs\ of\ T}{Abs\ of\ S} \times 5.0$$

Chloride:

Principle: When chloride is mixed with a solution of undissociated mercuric thiocyanates, the chloride preferentially combines with mercury, forming mercuric chloride. The thiocyanates that are released then combines with ferric ions present in the solution forming strongly colored ferric thiocyanates with an absorption maximum at 480 nm.

Assay procedure

Wavelength 492 (470 - 630) nm

Cuvette 1 cm

	Reagent blank	Standard (Cal.)	Sample
Reagent 1	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard (Cal.)	-	10 µl	-
Distilled water	10 µl	-	-

Mix and incubate for 5 min. at 37 0C, measure absorbance at the sample Asam and standard Ast against reagent blank.

Calculation

$$Chloride\ mmol/L = \frac{\Delta A_{sam}}{\Delta A_{st}} C_{st}$$

Cst = standard (calibrator) concentration

Diuretic activity [24-25]

Diuretic activities of normal and treated animals were estimated using an estimation of the difference between the output of urine and consumption of liquids. The percentage of variance between saline-administered and test animals was calculated.

$$Diureticaction = \frac{\text{Urinary excretion volume of the test group}}{\text{Urinary excretion volume of the control group}}$$

$$Diureticactivity = \frac{diureticactionoftest}{diureticactionofstandard}$$

Saluretic and natriuretic activity and carbonic anhydrase inhibitor [26-27]

Saluretic index, natriuretic index, and ion quotient were determined using the following formulae.

$$Salureticindex = \frac{\text{Urinary excretion of electrolytes of the test group}}{\text{Urinary excretion of electrolytes in the control group}}$$

$$\text{Natriuretic index} = \frac{\text{Urinary excretion of } Na^+}{\text{Urinary excretion of } K^+}$$

$$\text{Carbonic anhydrase inhibition} = \frac{\text{Urinary excretion of } Cl^-}{\text{Urinary excretion of } Na^+ \text{ and } K^+}$$

Statistical evaluation

All values were represented as mean values ± SEM (standard error of the mean), and the data was analyzed using one way ANOVA followed by a Dunnett's t-test using GraphPad Prism 9. The results were considered statistically significant if $p < 0.05$, $p < 0.01$, or $p < 0.001$.

RESULTS

Percentage of yield

$$\boldsymbol{Percentage\ of\ yield = \frac{Weight\ of\ crude\ extract}{Weight\ of\ dried\ material} \times 100}$$

$$Percentage\ of\ yield = \frac{50g}{250g} \times 100 = 20\%$$

50gms of drug dissolved in 250ml of solvent in 1:10 Ratio

Qualitative Chemical Analysis:

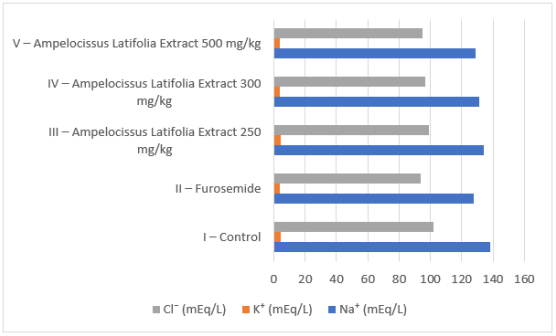
Sl. No.	Phytoconstituents	Test result
1	Alkaloid	+ve
2	Glycosides	+ve
3	Carbohydrate	+ve
4	Protein	-ve
5	Amino acid	+ve
6	Steroids	+ve
7	Flavonoids	+ve
8	Trepeniods	-ve
9	Phenols	+ve
10	Saponins	+ve
11	Tannin	+ve

+: Present; -: Absent

Electrolytes and Serum Proteins

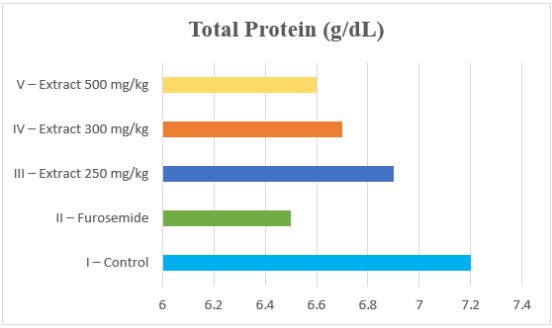
Estimation of electrolytes and serum proteins after treatment showed marked changes compared to control. The standard diuretic Furosemide produced a significant increase in urinary sodium, potassium, and chloride excretion with a mild reduction in serum protein levels. The extract of Cosmos sulphurous demonstrated a dose-dependent effect on electrolyte elimination.

Group	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)
I – Control	138 ± 4	4.5 ± 0.3	102 ± 3
II – Furosemide	128 ± 3	3.8 ± 0.2	94 ± 3
III – Extract 250 mg/kg	134 ± 3	4.2 ± 0.2	99 ± 2
IV – Extract 300 mg/kg	131 ± 3	4.0 ± 0.2	97 ± 2
V – Extract 500 mg/kg	129 ± 2	3.9 ± 0.2	95 ± 2



Total Protein (g/dL)

Group	Total Protein (g/dL)
I – Control	7.2 ± 0.3
II – Furosemide	6.5 ± 0.2
III – Extract 250 mg/kg	6.9 ± 0.2
IV – Extract 300 mg/kg	6.7 ± 0.2
V – Extract 500 mg/kg	6.6 ± 0.2

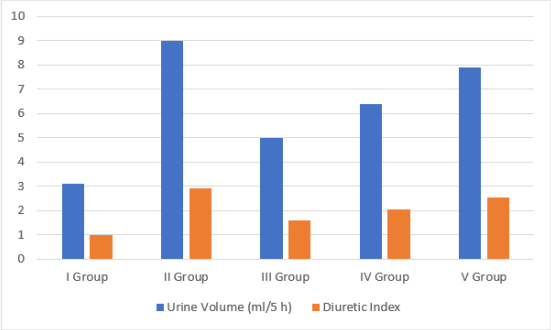


Serum total protein levels showed a mild reduction in the treated groups compared with control. The standard diuretic

Furosemide produced a noticeable decrease in serum protein concentration, which is commonly observed due to increased fluid loss and haemodilution during diuresis. The extract of Cosmos sulphurous also showed a dose-dependent, slight reduction in serum protein levels. However, the values remained within the normal physiological range, indicating that the extract did not cause harmful protein depletion. This mild change supports the diuretic effect without producing significant protein imbalance in the body.

Diuretic index

Group	Treatment	Urine Volume (ml/5 h)	Diuretic Index
I Group	Normal saline	3.1 ± 0.3	1
II Group	Furosemide 10 mg/kg	9.0 ± 0.5	2.9
III Group	Cosmos sulphurous 250 mg/kg	5.0 ± 0.4	1.61
IV Group	Cosmos sulphurous 300 mg/kg	6.4 ± 0.5	2.06
V Group	Cosmos sulphurous 500 mg/kg	7.9 ± 0.6	2.55



DISCUSSIONS

The present study evaluated the diuretic potential of the methanolic extract of Cosmos sulphurous in Wistar albino rats using Lipschitz test, with Furosemide as the standard. The extract exhibited a clear dose-dependent increase in urine output over 5 hours, reflected by rising diuretic indices (1.61, 2.06, and 2.55 at 250, 300, and 500 mg/kg, respectively) compared with control (1.0) and approaching the standard (2.9). Treated groups showed enhanced urinary electrolyte excretion with reductions in serum Na^+, K^+, and Cl^- relative to control, indicating effective saluretic and natriuretic activity. The natriuretic pattern (Na^+/K^+ ratio) suggested favourable sodium elimination without excessive potassium loss, while the ion quotient supported mild carbonic anhydrase inhibitory involvement. A slight, dose-related reduction in serum total protein remained within physiological limits, consistent with fluid loss during diuresis rather than protein depletion. Phytochemical screening revealed alkaloids, glycosides, flavonoids, phenols, saponins, tannins, steroids, and amino acids, which may underlie the observed diuretic action.

CONCLUSION

the methanolic extract of Cosmos sulphurous demonstrated significant, dose-dependent diuretic activity in Wistar rats as assessed by the Lipschitz test, with effects approaching those of the standard diuretic Furosemide. The extract increased urine output and promoted marked excretion of Na^+, K^+, and Cl^-, indicating pronounced saluretic and natriuretic actions without causing harmful protein depletion. The pattern of electrolyte elimination suggests a mechanism comparable to loop diuretics rather than primary carbonic anhydrase inhibition. The presence of bioactive phytoconstituents such as flavonoids, glycosides, saponins, tannins, phenols, and steroids may contribute to the observed activity. Overall, the study supports the potential of Cosmos sulphurous as a promising natural diuretic agent.

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