CHAPTER - 4

DIURETIC ACTIVITY STUDIES
ON THREE MEDICINAL PLANTS
4.1 INTRODUCTION

Abnormalities in fluid volume and electrolyte composition are common and important clinical problems that can become life-threatening if untreated. Drugs that block the transport functions of the renal tubules are important clinical tools in the treatment of these disorders. Diuretics increase the rate of urine flow and sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations, including hypertension, heart failure, renal failure, nephrotic syndrome and cirrhosis. Technically, the term "diuresis" signifies an increase in urine volume, while "natriuresis" denotes an increase in renal sodium excretion.

By definition, diuretics are drugs that increase the rate of urine flow; however, clinically useful diuretics also increase the rate of excretion of Na\(^+\) (Natriuresis) and of an accompanying anion, usually Cl\(^-\). Sodium chloride in the body is the major determinant of extra cellular fluid volume and most clinical applications of diuretics are directed towards reducing extra cellular fluid volume by decreasing total body NaCl content.

Diuretics not only alter the excretion of Na\(^+\) but also may modify renal handling of other cations such as K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) and anions such as Cl\(^-\), HCO\(_3^-\) and H\(_2\)PO\(_4^-\) and uric acid\(^1\).
4.2 HISTORY OF DIURETICS

Calomel (mercurous chloride) had been used as a diuretic from the time of Paracelsus and it was one of the constituents of the famous 'Guy's Hospital Pill'\(^2\). Organomercurials given by injection were introduced in the 1920s and dominated for nearly 40 years. The carbonic anhydrase (CAse), inhibitors were developed in the 1950s from the observation that early sulfonamides caused acidosis and mild diuresis. The first modern orally active diuretic, chlorothiazide (1) was discovered in 1957, and by early 1960s its congeners, (thiazide diuretics) were already in common use. Availability of furosemide (2) and ethacrynic acid (3) by the mid 1960s revolutionized the pattern of diuretic use. The K\(^+\) sparing diuretics spironolactone (4) and triamterene (5) were developed in parallel to these\(^2\). Most modern diuretics were developed when side effects of antibacterial drugs were noted, which included changes in urine composition and output. Except for spironolactone (4), diuretics were developed empirically, without knowledge of specific transport pathways in the nephron.
4.3 RENAL ANATOMY AND PHYSIOLOGY

The basic urine forming unit of the kidney is the nephron, which consists of a filtering apparatus, the glomerulus, connected to a long tubular portion consisting of proximal and distal convoluted tubules, the loop of Henle and a collecting duct, that reabsorbs and conditions the glomerular
ultrafiltrate. Each human kidney is composed of approximately one million nephrons.

Urine is first formed at the glomerulus, where by ultra filtration the urinary fluid is freed from its nonfilterable cellular components, such as red and white blood cells and the plasma proteins. During its passage along the lumen of the nephron, this practically protein free fluid undergoes many alterations in its composition. Some of its components are reabsorbed almost completely into the renal blood vessels; for instance, 98 to 99% of the water together with various electrolytes (Na\(^{+}\), K\(^{+}\), Cl\(^{-}\) and HCO\(_{3}^{-}\)), glucose, and urea filtered at the glomerulus is reabsorbed in the tubule. The remainder, about 1.5 liters/day, is excreted as urine.

Table 4.1. Quantities of water, glucose and electrolytes filtered, excreted and reabsorbed by kidneys of man (Mean Normal Values).
Many diuretic agents such as loop diuretics, thiazides, amiloride, and triamterene exert their effects on specific membrane transport protein at the luminal surface of renal tubular epithelial cells. Others exert osmotic effects that prevent water reabsorption in the water-permeable segments of the nephron e.g. (mannitol), inhibit enzymes e.g. (acetazolamide) or interfere with hormone receptors in renal epithelial cells e.g. (spironolactone)\textsuperscript{5,6}.

4.4 CLASSIFICATION OF DIURETICS

Historically, the classification of diuretics was based on a mosaic of ideas such as site of action (loop diuretics), efficacy (high-ceiling diuretics), chemical structure (thiazide diuretics), similarity of action with other diuretics (thiazidelike diuretics), effects on potassium excretion (potassium-sparing diuretics)\textsuperscript{6}, etc.

4.4.1 Carbonic anhydrase inhibitors

Carbonic anhydrase is present in many nephron sites, including the luminal and basolateral membranes and cytoplasm of the epithelial cells and the red blood cells in the renal circulation. However, the predominant location of this enzyme is the luminal membranes of the proximal tubule cells, where it catalyzes the dehydration of H\textsubscript{2}CO\textsubscript{3}, a critical step in the proximal reabsorption of bicarbonate. Inhibitors of carbonic anhydrase thus block sodium bicarbonate reabsorption, causing sodium bicarbonate diuresis and a reduction in total body bicarbonate stores\textsuperscript{1}. The prototypical carbonic anhydrase inhibitor is acetazolamide (6)\textsuperscript{7}. Carbonic anhydrase inhibitors also
increase phosphate excretion by unknown mechanism but have little or no
effect on the excretion of $\text{Ca}^{2+}$ or $\text{Mg}^{2+}$

![Chemical structure](image)

The carbonic anhydrase inhibitors are unsubstituted sulfonamide
derivatives. The sulfonamide group is essential for activity. Alkyl
substitutions at this site completely block the effects on carbonic anhydrase
activity.

Carbonic anhydrase inhibition causes significant bicarbonate losses,
resulting in hyperchloremic metabolic acidosis. Because of the toxicity of this
acidosis and the fact that $\text{HCO}_3^-$ depletion leads to enhanced $\text{NaCl}$
reabsorption by the remaining tubule segments in the nephron, the diuretic
effectiveness of acetazolamide decreases significantly with use over several
days. The combination of acetazolamide (6) with diuretics that block $\text{Na}^+$
reabsorption at more distal sites in the nephron causes a marked natriuretic
response in patients with low basal fractional excretion of Na\(^+\) (< 0.2 %) who are resistant to diuretic monotherapy\(^8\).

Inhibition of carbonic anhydrase decreases the rate of aqueous humor formation, causing a decrease in intraocular pressure. This effect is of value in the management of several forms of glaucoma. Carbonic anhydrase inhibitors can also be used to prevent altitude sickness\(^9\). By decreasing cerebrospinal fluid formation and by decreasing the pH of the cerebrospinal fluid and brain, acetazolamide can enhance performance status and diminish symptoms of mountain sickness\(^10\). Carbonic anhydrase inhibitors can be useful for correcting a metabolic alkalosis, especially an alkalosis caused by diuretic induced increase in H\(^+\) excretion\(^1\).

**4.4.2 Inhibitors of Na\(^+\) - K\(^+\) - 2Cl\(^-\) symport (Loop diuretics)**

Loop diuretics inhibit the activity of the Na\(^+\) - K\(^+\) -2Cl\(^-\) symporter in the thick ascending limb of the loop of Henle and thus loop diuretics selectively inhibit NaCl reabsorption in the thick ascending limb of the loop of Henle\(^11\). Inhibitors of Na\(^+\) - K\(^+\) - 2Cl\(^-\) symport in the thick ascending limb are highly efficacious, and for this reason, they sometimes are called high-ceiling diuretics. The efficacy of loop diuretics is due to a combination of two factors; (i) Approximately 25% of the filtered Na\(^+\) load normally is reabsorbed by the thick ascending limb, and (ii) nephron segments past the thick ascending limb do not possess the reabsorptive capacity to rescue the flood of rejectate exiting the thick ascending limb. Inhibitors of Na\(^+\) - K\(^+\) - 2Cl\(^-\) symport are a
chemically diverse groups. Only furosemide (2), bumetanide (7), ethacrynic acid (3) and torsemide (8) are available currently in the United states\(^1\). Furosemide (2) and bumetanide (7) contain a sulfonamide moiety. Ethacrynic acid (3) is a phenoxyacetic acid derivative and torsemide (8) is a sulfonylurea.

\[
\begin{align*}
&\text{(7)} \\
&\text{(8)}
\end{align*}
\]

Inhibitors of Na\(^+\) - K\(^+\) - 2Cl\(^-\) symporter\(^{12,13}\) bind to the Na\(^+\) - K\(^+\) - 2Cl\(^-\) symporter in the thick ascending limb and block its function, bringing salt transport in this segment of the nephron to a virtual standstill. The molecular mechanism by which this class of drugs blocks the Na\(^+\) - K\(^+\) - 2Cl\(^-\) symporter is unknown, but evidence suggests that these drugs attach to the Cl\(^-\) binding site located in the symporter's transmembrane domain\(^{14}\). They also inhibit Ca\(^{2+}\) and Mg\(^{2+}\) reabsorption in the thick ascending limb by abolishing the
transepithelial potential difference that is the dominant driving force for reabsorption of these cations.

Owing to blockade of the Na$^+$ - K$^+$ - 2Cl$^-$ symporter, loop diuretics increase the urinary excretion of Na$^+$ and Cl$^-$ profoundly. Abolition of the transepithelial potential difference also results in marked increase in the excretion of Ca$^{2+}$ and Mg$^{2+}$. All inhibitors of Na$^+$ - K$^+$ - 2Cl$^-$ symport increase the urinary excretion of K$^+$ and titratable acid.

In addition to their diuretic activity, loop agents appear to have direct effects on blood flow through several vascular beds. The mechanisms for this action are not well defined. Furosemide increases renal blood flow and causes redistribution of blood flow within the renal cortex. Furosemide and ethacrynic acid have also been shown to relieve pulmonary congestion and reduce left ventricular filling pressure in congestive heart failure before a measurable increase in urinary output occurs, and in anephric patients.

A major use of loop diuretics is in the treatment of acute pulmonary edema. A rapid increase in venous capacitance in conjunction with a brisk natriuresis reduces left ventricular filling pressures and thereby rapidly relieves pulmonary edema. Loop diuretics also are used for the treatment of chronic congestive heart failure when diminution of extra cellular fluid volume is desirable to minimize venous and pulmonary congestion. Loop diuretics are also employed in the treatment of edema and ascites of liver cirrhosis. In patients with a drug overdose, loop diuretics can be used to
induce a forced diuresis to facilitate more rapid renal elimination of the offending drug. Loop diuretics, combined with isotonic saline administration to prevent volume depletion, are used to treat hypercalcemia.  

4.4.3 Inhibitors of Na\(^+\)-Cl\(^-\) symport (Thiazide and Thiazidelike diuretics)

The thiazide diuretics emerged during efforts to synthesize more potent carbonic anhydrase inhibitors. However, unlike carbonic anhydrase inhibitors, which primarily increase NaHCO\(_3\) excretion, thiazides were found predominantly to increase NaCl excretion, an effect shown to be independent of carbonic anhydrase inhibition.

Because the original inhibitors of Na\(^+\)-Cl\(^-\) symport were benzothiadiazine derivatives, this class of diuretics are also known as thiazide diuretics\(^1\). Subsequently, drugs that are pharmacologically similar to thiazide diuretics but are not thiazides were developed and are called thiazidelike diuretics\(^1\). The nature of the heterocyclic rings and the substitutions on these rings may vary among the congeners, but all of them retain, in common with the carbonic anhydrase inhibitors, an unsubstituted sulfonamide group. Chlorothiazide (1), hydrochlorothiazide (9), chlorthalidone (10), indapamide (11), metolazone (12), quinethazone (13), etc are some of the diuretics belonging to this class.
Micropuncture and in situ microperfusion studies clearly indicate that thiazide diuretics inhibit NaCl transport in the distal convoluted tubule (DCT). Thiazides inhibit NaCl reabsorption from the luminal side of epithelial cells in the DCT\textsuperscript{22,23}.

Inhibitors of Na\textsuperscript{+} - Cl\textsuperscript{−} symport increase Na\textsuperscript{+} and Cl\textsuperscript{−} excretion only moderately and they have milder diuretic action than do the loop diuretics because approximately 90% of the filtered Na\textsuperscript{+} load is reabsorbed before reaching the distal convoluted tubule\textsuperscript{24,25}. Like inhibitors of Na\textsuperscript{+} - K\textsuperscript{+} - 2Cl\textsuperscript{−}
symport, inhibitors of Na\(^+\) - Cl\(^-\) symport also increase the excretion of K\(^+\) and titratable acid. In contrast to the situation in the loop of Henle, where loop diuretics inhibit Ca\(^{2+}\) reabsorption, thiazides actually enhance Ca\(^{2+}\) reabsorption in the distal convoluted tubule. Inhibition of Na\(^+\)-Cl\(^-\) symporter in the luminal membrane decreases intracellular Na\(^+\) levels, thereby increasing the basolateral exit of Ca\(^{2+}\) via enhanced Na\(^+\) - Ca\(^{2+}\) exchange\(^{26}\).

Thiazide diuretics are used for the treatment of the edema associated with heart (congestive heart failure), liver (hepatic cirrhosis) and renal (nephrotic syndrome, chronic renal failure and acute glomerulonephritis) disease.

Thiazide diuretics decrease blood pressure in hypertensive patients\(^{27}\) and they are used widely for the treatment of hypertension either alone or in combination with other antihypertensive drugs. They also reduce cardiovascular morbidity and mortality in hypertensive patients\(^{28}\). Thiazides have proved useful in the treatment of kidney stones caused by hypercalciuria. Approximately two-thirds of all renal stones contain calcium phosphate or calcium oxalate. Many patients with such stones exhibit a renal "leak" of calcium that causes hypercalciuria. This can be treated with thiazide diuretics, which enhance calcium reabsorption in the distal convoluted tubule and thus reduce the urinary calcium concentration\(^{26}\). Since other halides are excreted by renal processes similar to those for Cl\(^-\), thiazide diuretics may be useful for the management of Br\(^-\) intoxication.
4.4.4 Potassium–sparing diuretics

These are either aldosterone antagonist or directly inhibit Na\(^+\) channels in the distal tubule and collecting duct cells to indirectly conserve K\(^+\) ions.

This type of diuretics are further classified into two types.

4.4.4.1 Inhibitors of renal epithelial Na\(^+\) channels

Triamterene (5) and amiloride (14) are two nonsteroidal organic bases with identical action. Their most important action is to decrease K\(^+\) excretion, particularly when it is high due to large K\(^+\) intake or use of a diuretic that enhances K\(^+\) loss, along with a small increase in Na\(^+\) excretion\(^1\). The luminal membrane of late distal tubule and collecting duct cells expresses a distinct ‘amiloride sensitive’ Na\(^+\) channel through which Na\(^+\) enters the cell down its electro–chemical gradient which is generated by Na\(^+\) K\(^+\) ATPase operating at the basolateral membrane. The higher permeability of the luminal membrane for Na\(^+\) depolarizes the luminal membrane but not the basolateral membrane, creating a lumen – negative transepithelial potential difference. This Na\(^+\) entry partially depolarizes the luminal membrane creating a – 15 mV transepithelial potential difference which promotes secretion of K\(^+\) into the lumen through K\(^+\) channels. Though there is no direct coupling between Na\(^+\) and K\(^+\) channels, more the delivery of the Na\(^+\) to the distal nephron, greater is its entry through the Na\(^+\) channel. Because of this the luminal membrane is more depolarized as a result the driving force for K\(^+\) secretion is augmented. Carbonic anhydrase inhibitors, loop diuretics and thiazide diuretics increase the delivery
of Na\(^+\) to the late distal tubule and collecting duct, a situation that often is associated with increased K\(^+\) and H\(^+\) excretion.

Considerable evidence indicates that amiloride (14)\(^{29,30}\) and triamterene (5) block the epithelial Na\(^+\) channels in the luminal membrane of principal cells in the late distal tubule and collecting duct perhaps by competing with Na\(^+\) for negatively charged areas within the pore of the Na\(^+\) channel.

4.4.4.2 Antagonists of mineralocorticoid receptors (Aldosterone antagonists)

Epithelial cells in the late distal tubule and collecting duct contain cytosolic mineralocorticoid receptors that have a high affinity for aldosterone. Aldosterone acts on the late distal tubule and collecting duct cells by combining with an intracellular mineralocorticoid receptor which induces the formation of aldosterone induced proteins (AIPs) which promote Na\(^+\) reabsorption and K\(^+\) secretion.
Drugs such as spironolactone\(^{31}\) (4) and eplerenone\(^{32}\) (15) competitively inhibit the binding of aldosterone to the mineralocorticoid receptors. Spironolactone acts from the interstitial side of the tubular cell, combines with the mineralocorticoid receptor and inhibits the formation of AIPs in a competitive manner. Unlike the mineralocorticoid receptors – aldosterone complex, the mineralocorticoid receptor – Spironolactone complex is not able to induce the synthesis of AIPs. Since spironolactone and eplerenone block the biological effects of aldosterone\(^{33}\), these agents also are referred to as aldosterone antagonists\(^1\).

4.4.4.3 Effect of K\(^+\) - sparing diuretics on urinary excretion

Since the late distal tubule and collecting duct have a limited capacity to reabsorb solutes, blockade of Na\(^+\) channels in this part of the nephron only mildly increases the excretion rates of Na\(^+\) and Cl\(^-\) (approximately 2% of filtered load). Blockade of Na\(^+\) channels hyperpolarizes the luminal membrane, reducing the lumen –negative transepithelial voltage and thus
indirectly inhibit K⁺ excretion. The effects of mineralocorticoid receptor antagonists on urinary excretion are very similar to those induced by renal epithelial Na⁺-channel inhibitors.

Because of the mild natriuresis induced by Na⁺-channel inhibitors, these drugs seldom are used as sole agents in the treatment of edema or hypertension. Rather, their major utility is in combination with other diuretics. The ability of Na⁺-channel inhibitors to reduce K⁺ excretion tends to off set the kaliuretic effects of thiazide and loop diuretics. Consequently, the combination of a Na⁺-channel inhibitor with a thiazide or loop diuretic tends to result in normal values³⁴ of plasma K⁺. As with other K⁺-sparing diuretics, spironolactone (4) often is coadministered with thiazide or loop diuretics in the treatment of edema and hypertension. Such combinations result in increased mobilization of edema fluid while causing lesser perturbations of K⁺ homeostasis. Spironolactone(4) is particularly useful in the treatment of primary hyperaldosteronism and of refractory edema associated with secondary aldosteronism. Spironolactone is considered the diuretic of choice in patients with hepatic cirrhosis. Spironolactone, added to standard therapy, substantially reduces morbidity and mortality³⁵ and ventricular arrhythmias³⁶ in patients with heart failure.

4.4.5 Osmotic diuretics

Osmotic diuretics are agents that are freely filtered at the glomerulus, undergo limited reabsorption by the renal tubule, and are relatively inert pharmacologically. Osmotic diuretics are administered in large enough doses
to increase significantly the osmolality of plasma and tubular fluid. The four currently available osmotic diuretics\(^1\) are glycerin \((16)\), Isosorbide \((17)\), mannitol \((18)\) and urea \((19)\).

\[
\begin{align*}
\text{(16)} & \quad \text{HO} - \text{C} - \text{OH} \\
\text{(17)} & \quad \text{HO} - \text{C} - \text{O} - \text{H} \\
\text{(18)} & \quad \text{OH} - \text{C} - \text{OH} \\
\text{(19)} & \quad \text{H}_2\text{N} - \text{C} - \text{NH}_2
\end{align*}
\]

An osmotic agent that is not transported causes water to be retained in these segments and promotes a water diuresis. Osmotic diuretics limit water reabsorption primarily in those segments of the nephron that are freely permeable to water viz. the proximal tubule and the descending limb of the loop of Henle. The presence of a non reabsorbable solute such as mannitol \((18)\) prevents the normal absorption of water by interposing a countervailing osmotic force. As a result, urine volume increases in conjunction with mannitol excretion\(^3\). The concomitant increase in urine flow rates decreases the contact time between fluid and the tubular epithelium, thus reducing \(\text{Na}^+\)
reabsorption. However, the resulting natriuresis is of lesser magnitude than the water diuresis, leading eventually to hypernatremia.

Osmotic diuretics increase the urinary excretion of nearly all electrolytes, including Na⁺, K⁺, Ca²⁺, Mg²⁺, Cl⁻, HCO₃⁻ and PO₄³⁻.

A rapid decrease in glomerular filtration rate (GFR) i.e., acute renal failure (ARF), is a serious medical condition that occurs in 5% of hospitalized patients and is associated with a significant mortality rate. Acute tubular necrosis i.e., damage to tubular epithelial cells, accounts for most cases of acute renal failure. The renal protection afforded by mannitol (osmotic diuretic) may be due to removal of obstructing tubular casts, dilution of nephrotoxic substances in the tubular fluid, and / or reduction of swelling of tubular elements via osmotic extraction of water³⁸,³⁹.

Another use for mannitol and urea is in the treatment of dialysis disequilibrium syndrome. Too rapid a removal of solutes from the extracellular fluid by hemodialysis or peritoneal dialysis results in a reduction in the osmolality of the extracellular fluid. Consequently, water moves from the extracellular compartment into the intracellular compartment, causing hypotension and central nervous system symptoms. Osmotic diuretics increase the osmolality of the extracellular fluid compartment and thereby shift water back into the extracellular compartment.

By increasing the osmotic pressure of the plasma, osmotic diuretics extract water from the eye and brain. All four osmotic diuretics are used to
control intraocular pressure during acute attacks of glaucoma and for short term reductions in intraocular pressure both preoperatively and postoperatively in patients. Mannitol and urea are also used to reduce cerebral edema and brain mass before and after neurosurgery\textsuperscript{1}.

4.4.6 Changes in urinary electrolyte patterns in response to diuretic drugs.

Changes in urinary electrolyte patterns and the primary site of actions are presented in Table (4.2.)\textsuperscript{40}.

Table 4.2 Changes in urinary electrolyte patterns in response to diuretic drugs.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Na\textsuperscript{+}</th>
<th>K\textsuperscript{+}</th>
<th>Cl\textsuperscript{–}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonic anhydrase inhibitors</td>
<td>+</td>
<td>++</td>
<td>(+)</td>
</tr>
<tr>
<td>Inhibitors of Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{–} Symport (Loop diuretics)</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Inhibitors of Na\textsuperscript{+}-Cl\textsuperscript{–} Symport (Thiazide diuretics)</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>K\textsuperscript{+} Sparing agent</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Osmotic diuretics</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

++ marked increase; + mild to moderate increase; (+) slight increase; – decrease
4.5 PAST WORK ON DIURETIC ACTIVITY OF MEDICINAL PLANTS

Diuretic agents are most widely prescribed categories of drugs in the world. But synthetic diuretics have serious side effects such as diabetonic effect, electrolyte imbalance, impotence and hyperuricemia. Hence the search for herbal drugs to act as diuretics are inevitable and can alter therapeutic efficacy without any adverse effects. Several indigenous drugs have already been screened by various workers for their diuretic activity. Four indigenous drugs viz. *Boerhaavia repens*, *Boerhaavia repanda*, *Pedalium murex* and *Tribulus terrestris* have been tested for diuretic activity by Krishnan Haravey. The effects of administration of *Stevia rebaudiana* extracts on renal function and mean arterial pressure in normal Wister rats have been evaluated by Melis. The extract was found to induce hypotension, diuresis and natriuresis. Traditional medicines reported to be used as anti-hypertensives or diuretics from different regions in the world have been investigated for inhibition of the angiotensin converting enzyme. Alcoholic extract of *Aerva lanata* was tested for diuretic activity in rats. The parameters measured for diuretic activity were total urine volume, sodium, potassium and chloride content. The results clearly indicated that the alcoholic extract at a dose of 800 mg / kg acted as a diuretic, with respect to control. Natriuretic and diuretic responses induced by garlic powder in anaesthetized dogs were evaluated by Pantoja et al. Garlic extract also inhibited sodium transporting epithelia and decreased ATPase activity.
Diuretic and natriuretic effects of chromatographically purified fraction of garlic *Allium sativum* have also been performed by Pantoja *et al*.49

The diuretic activity of the stem–bark extracts of *Steganotaenia araliacea* and effects on urine electrolytes in rats was studied by Agunu *et al*.50 Eighty species of vascular plants were tested for their ability to inhibit the angiotensin converting enzyme which plays an important role in the regulation of blood pressure and diuresis51. Diuretic effect of aqueous extracts of *Rosmarinus officinalis* and *Centaurium erythraea* on Wistar rats have been evaluated by Haloui *et al*.52. Hnatyszyn and co–workers have assessed the diuretic activity of aqueous extract of *Phyllanthus sellowianus*53.

*Euphorbia hirta* is locally used in Africa and Australia to treat numerous diseases, including hypertension and edema. The diuretic effect of the *E. hirta* leaf extracts were assessed in rats using acetazolamide and furosemide as standard diuretic drugs54. In the Sri Lankan Traditional Medicine, *Spilanthes acmella* flowers are claimed to possess powerful diuretic activity. The diuretic potential of *Spilanthes acmella* flowers was evaluated in rats using a cold–water extract. The extract caused marked increase in urinary Na⁺ and K⁺ levels and a reduction in the osmolarity of urine55. *Maydis stigma* (Corn silk) is a herbal drug reputed for the treatment of urinary ailments in various traditional systems of medicine. Diuretic activity of *M. stigma* has been evaluated in adult male Wistar rats for eighty days by Maksimovic *et al*.56.
The effects of *Clematis montevidensis* on urinary excretion of water, sodium and potassium were investigated in rats loaded with isotonic saline solution. The infusions of the root and aerial part of *C. montevidensis* showed a moderate diuretic activity. This effect could be due to the presence of oleanolic acid (20) isolated from this plant. The aerial parts of *Bidens odorata* are used in Mexican folk medicine to treat renal diseases. The diuretic response to aqueous extract of this plant was compared with that induced by furosemide by Camargo *et al.* Jawarish Zarooni Sada (JZS) is a polyherbal preparation containing fifteen ingredients, mainly described to be diuretic and nephroprotective and used widely in the Unani system of medicine in the management of renal diseases. Diuretic and nephroprotective effects of Jawarish Zarooni Sada have been evaluated by Afzal *et al.* Nedi *et al.* have investigated the diuretic activity of different extracts of *Carissa edulis* in rats using hydrochloro thiazide as a standard drug and the findings support the traditional use of *Carissa edulis* as a diuretic agent. Sripanidkulchai *et al.* have investigated the diuretic activity five Thai indigenous medicinal plants in rats and have come to the conclusion that only two plants viz. *Ananas comosus* and *Carica papaya* demonstrated significant diuretic activity.
Through ethnobotanical surveys in Guatemala, about 250 plants were identified for use in the treatment of urinary ailments. From 67 of these, aqueous extracts were prepared to investigate their oral diuretic activity in albino rats. The trials demonstrated that in 33 cases urinary excretion was not significantly increased, in 20 cases intermediate activity was seen and in 14 cases high diuretic activity was noted. Oral administration of the water extract of *Spergularia purpurea* at different doses produced a significant and dose dependent diuresis and increase in electrolyte excretion. Chronic treatment with *S. purpurea* decreased significantly urine osmolality, while a slight increase in glomerular filtration rate was also observed.

Erva Tostao is called “Punamava” in India. It is botanically called *Boerhaavia diffusa*. This plant has a long history of use by indigenous and tribal people, and in Ayurvedic medicine in India where the roots are employed for many purposes, including liver, gallbladder, kidney, renal and urinary disorders.
The diuretic action of Erva Tostao (*Boerhaavia diffusa*) has been studied and validated by scientists in several studies which help to explain its long history of use in various kidney and urinary conditions. Researchers showed in the mid 1950’s that low dosages (10 mg/kg to 300 mg/kg) produced strong diuretic effects while higher dosages (>300 mg /kg) produced the opposite effects, reducing urine output. Other researchers who followed, verified these diuretic and anti-diuretic properties as well as the beneficial kidney and renal effects of Erva Tostao roots in animals and humans.

Extracts of 32 medicinal plants used popularly for their presumed diuretic and/or antihypertensive properties were tested for diuretic effects in conscious unrestrained rats. The most significant diuretic effect was observed with *Hedychium coronarium* sheath and leaf-blade extracts. Methanol extract of *Strychnos potatorum* seeds was evaluated for its diuretic activity in Wister albino rats. Total urine volume, excretion of Na+ and K+ ions and Cl− of drug treated groups increased significantly with respect to control group and the diuretic effect was comparable with that of the standard drug furosemide. The aqueous extract of the bark of *Raphanus sativus* has been tested for its antiuro lithiatic and diuretic activity by Vargas et al.

Chloroform extracts of *Equisetum fluviatile, E. hiemale* Var. affine, *E. giganteum* and *E. myriochaetum* were studied to determine diuretic activity in mice using hydrochlorothiazide, spironolactone and furosemide as standard drugs for comparison. It was found that the most active plant was...
*E. hiemale* Var. affine, followed by *E. fluviatile, E. giganteum* and *E. myriochaetum*, producing an effect similar to that of hydrochlorothiazide in relation to the excretion of sodium, potassium and chloride. An alcoholic extract of *Cucumis trigonus* was studied for its diuretic activity in albino rats using hydrochlorothiazide as a standard drug for comparison. The extract exhibited a dose–dependent saluretic effect reaching a peak at 4 hours. Unlike hydrochlorothiazide, the extract does not affect potassium excretion⁷². Crude aqueous extract of *Cleome rutidosperma* was investigated for diuretic and antibacterial activity by Bose *et al*⁷³. The diuretic activity was tested in rats orally and compared with furosemide as the standard and the extract was found to possess significant dose dependent diuretic activity.

Dandelion, *Taraxacum officinale* leaves have traditionally been used to increase urine production and excretion. It is one of the most effective diuretic herbs; its effect being comparable to that of the drug frusemide. In addition to its efficacy as a diuretic, Dandelion leaf has added benefits as a rich source of potassium, replacing all potassium that is flushed from the body via diuresis ⁷⁴,⁷⁵. Diuretic action of an aqueous extract of *Lepidium latifolium* has been studied by Eduardo *et al*⁷⁶. Aqueous and alcoholic extracts of *Vitis vinifera* leaves were tested for diuretic activity in rats by Shastry *et al*⁷⁷. using furosemide as a reference diuretic. The infusion and the essential oil of Juniper berries, *Juniperus communis* as well as terpinen–4–ol were tested for diuresis response in rats by Gordana *et al*⁷⁸ and they came to the conclusion that the diuretic activity of Juniper berries
couldn't be attributed only to the essential oil but also to hydrophilic drug constituents.

Intravenous administration of the aqueous extract of *Retama raetam* produced a significant increment on diuresis from the second hour to the fourth hour in normal rats\(^7^9\). The increase in diuresis was associated with an elevation of glomerular filtration rate and a significant decrease of urinary osmolarity. Diuretic activity of infusions of *Artemisiathuscula* was evaluated in saline loaded rats by Benjumea et al\(^8^0\). Urinary excretion of water, pH, density, conductivity and Na\(^+\), K\(^+\) and Cl\(^-\) content were investigated and the drug showed potassium sparing effect.

In the Moroccan traditional medicine, the ripe fruits of *Carum carvi* and the leaves of *Tanacetum vulgare*, two widely available plant materials, are used as diuretics\(^8^1\). The diuretic potential of aqueous extracts of *Carum carvi* fruit (Caraway) and the leaves of *Tanacetum vulgare* (Tansy) in normal rats after acute and sub–chronic oral administration was evaluated. Both extracts increased urinary levels of Na\(^+\) and K\(^+\), to about the same extent, while furosemide (reference diuretic) increased urinary levels of only Na\(^+\) and decreased urinary K\(^+\). The diuretic activity of an infusion and the methanol extract of *Withania aristata* was evaluated in laboratory rats by Martin Herrera et al. Both the infusion and the methanol extract showed a significant diuretic effect compared with non–treated controls, with notable increase in the rate of water and sodium excretion. There was also a potassium retention effect observed\(^8^2\).
4.6 SCOPE OF THE PRESENT STUDY

Phytochemicals have played a vital role in the treatment of diseases in the past and will continue to do so in the future. Although synthetic drugs can produce dramatic results in most cases, the side-effects associated with them are a major concern. The source of many compounds used in modern medicine today can be traced down to plant origin. Whether or not scientific justification is available for the use of most plant products, the continued use of these compounds is due to their safety profile, ease of availability and also economic reasons. Each medicinal plant that has been used in the traditional system of medicine must be scientifically tested in order to bring forth its active principle that might be effectively used as a phytomedicine. However, there should be adequate data from in-vivo and in-vitro studies to validate the therapeutic potential claimed. There is a need to establish the pharmacological activities for identifying and comparing the various preparations for potency. Quite a good number of indigenous drugs have been claimed to have diuretic effect in the Ayurvedic system of medicine and several drugs have already been screened by various workers for their diuretic activity. In the present study the diuretic activity of the methanolic extracts of the roots of Dichrostachys cinerea, Hemidesmus indicus and the thallus of a lichen, Parmelia perlata have been tested in male albino rats. The advantage of traditional systems of medicine with respect to their safety and efficacy could result in a better utilization of our herbal resources with application of the scientific methods.
4.7 RESULTS

4.7.1 Dichrostachys cinerea

The root of *Dichrostachys cinerea* is extracted successively with different organic solvents such as, petroleum ether (40°–60°C), benzene, chloroform, methanol and water. The methanolic extract was condensed and evaporated to dryness under vacuum (yield 23 g, 4.6%) and used for the present study. The diuretic activity was evaluated on male albino rats using Lipschitz’s method. The parameters measured for diuretic activity are total urine volume, sodium, potassium and chloride contents of urine samples. The results are presented in Tables 4.3 and 4.4. Urea was used as a reference diuretic. All the results are expressed as mean ± standard error. Test of significance is statistically analysed using Student’s ‘t’ test.

The present study reveals that the methanolic extract of the root of *D. cinerea* at the 250 mg/kg and 500 mg/kg doses possess good diuretic activity. The urine volume was found to be the maximum at 250 mg/kg dose. Since the urine excretion is found to be the maximum at 250 mg/kg dose, Na⁺, K⁺ and Cl⁻ concentrations in this particular dose have been determined. Urea increased the urine volume 2.3 fold with respect to the control (Table 4.3.) whereas methanolic extract of *D. cinerea* at 250 mg/kg increased the urine volume around 2 fold. Urea increased the Na⁺ excretion level by 1.8 fold, K⁺ excretion level by 1.2 fold and Cl⁻ excretion level by 1.3 fold when compared to that of the control. The methanolic extract of *D. cinerea* at 250 mg/kg dose
Table 4.3

Diuretic activity of methanolic extract of \textit{Dichrostachys cinerea} root on rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg bw)</th>
<th>Urine Volume (ml/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline, 25 ml</td>
<td>2.0 ± 0.08</td>
</tr>
<tr>
<td>\textit{Dichrostachys cinerea}</td>
<td>100</td>
<td>2.1 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>3.9 ± 0.16**</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>3.3 ± 0.16**</td>
</tr>
<tr>
<td>Urea</td>
<td>750</td>
<td>4.6 ± 0.33**</td>
</tr>
</tbody>
</table>

Student's t-test was performed (Fisher, 1950).
Each value is the mean ± SE of six rats weighing 250 - 300 g.
Statistically significant from vehicle control : ** P < 0.001

Table 4.4

Effect of methanolic extract of \textit{Dichrostachys cinerea} on Na\textsuperscript{+}, K\textsuperscript{+} and Cl\textsuperscript{−} concentration in the urine of albino rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Electrolyte excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg/kg bw)</td>
</tr>
<tr>
<td>Control</td>
<td>Normal Saline 25 ml</td>
</tr>
<tr>
<td>\textit{D.cinerea}</td>
<td>250</td>
</tr>
<tr>
<td>Urea</td>
<td>750</td>
</tr>
</tbody>
</table>

Student's t-test was performed (Fisher, 1950).
Each value is the mean ± SE of six rats weighing 250 - 300 g.
Statistically significant from vehicle control : ** P < 0.001
increased the $\text{Na}^+$ excretion level by 1.4 fold, $\text{K}^+$ excretion level by 1.7 fold and $\text{Cl}^-$ excretion level by 2 fold. $\text{K}^+$ and $\text{Cl}^-$ excretion levels are significantly increased to nearly 1.5 fold when compared to that of urea (Table 4.4). The sodium and potassium ratio is decreased to half fold when compared to that of urea.

4.7.2 *Hemidesmus indicus*

The root of *Hemidesmus indicus* has also been extracted with various solvents as mentioned above. Among the various extracts only the methanolic extract has been subjected to the present analysis. The methanolic extract of *H. indicus* was condensed and evaporated to dryness under vacuum (yield 21 g, 4.2%) and used. Male albino rats weighing between 250 – 300 g have been used for the study. The parameters taken for individual rat are body weight before and after test period, total urine volume, concentration of $\text{Na}^+$, $\text{K}^+$ and $\text{Cl}^-$ in urine, according to the method of Lipschitz *et al.* Urea is used as a reference diuretic and the results are presented in Tables 4.5 and 4.6. All the results are expressed as mean ± standard error. The data is analysed using Student’s ‘t’ test for statistical significance.

The present study reveals that the methanolic extract of the root of *H. indicus* at the dose of 250 mg/kg shows significant diuretic activity and the urine volume is the highest at this dose (Table 4.5.) beyond which it was reduced. Therefore in the present study, the $\text{Na}^+$, $\text{K}^+$ and $\text{Cl}^-$ concentrations at this particular dose are determined (Table 4.6.) and there is no significant
### Table 4.5
Diuretic activity of methanolic extract of *Hemidesmus indicus* root on rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg bw)</th>
<th>Urine Volume (ml/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline, 25 ml</td>
<td>2.0 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>2.0 ± 0.08</td>
<td></td>
</tr>
<tr>
<td><strong>Hemidesmus indicus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>2.6 ± 0.08*</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>1.9 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>750</td>
<td>4.6 ± 0.33**</td>
</tr>
</tbody>
</table>

Student's t-test was performed (Fisher, 1950). Each value is the mean ± SE of six rats weighing 250 - 300 g. Statistically significant from vehicle control: *P < 0.05; ** P < 0.001.

### Table 4.6
Effect of methanolic extract of *Hemidesmus indicus* on Na\(^+\), K\(^+\) and Cl\(^-\) concentration in the urine of albino rats

<table>
<thead>
<tr>
<th>Electrolyte excretion</th>
<th>Treatment</th>
<th>Dose (mg/kg bw)</th>
<th>Na(^+) (meq / l)</th>
<th>K(^+) (meq / l)</th>
<th>Cl(^-) (meq / l)</th>
<th>Na(^+)/K(^+) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Normal Saline 25 ml</td>
<td>7.39 ± 2.2</td>
<td>33.82 ± 2.8</td>
<td>18.02 ± 4.1</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>H. indicus</strong></td>
<td>250</td>
<td>7.39 ± 2.0</td>
<td>33.49 ± 2.6</td>
<td>15.17 ± 4.6</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>750</td>
<td>26.19 ± 2.8**</td>
<td>54.92 ± 4*</td>
<td>48.8 ± 4**</td>
<td>0.47</td>
<td></td>
</tr>
</tbody>
</table>

Student's t-test was performed (Fisher, 1950). Each value is the mean ± SE of six rats weighing 250 - 300 g. Statistically significant from vehicle control: *P < 0.05; ** P < 0.001.
change in these values compared to that of the control. Urea increased the urine volume by about 2.3 times when compared with control whereas the methanolic extract of *H. indicus* at 250 mg/kg dose increases the urine volume by about 1.3 times, compared to that of the control. The Na\(^+\) value is significantly increased, whereas K\(^+\) level is also slightly increased in the urea treated group, when compared to that of the control.

4.7.3 *Parmelia perlata*

The thallus of *Parmelia perlata* has been successively extracted with petroleum ether (40°–60°C), benzene, chloroform, methanol and water and the methanolic extract is used for the present study. This indigenous drug has been tested for its diuretic activity and the relative diuretic potency has been estimated and compared with urea. The method adopted during the present investigation is that described by Lipschitz *et al*\(^8\). For diuretic activity, healthy male albino rats weighing between 250-300 g are used. The results are statistically analysed using Student’s ‘t’ test\(^8\) and are presented in Tables 4.7. and 4.8.

The methanolic extract of *Parmelia perlata* produces significant dose dependent decrease in urinary excretion with respect to the control. The maximum decrease in urine output is observed for the higher dose ie. 500 mg/kg and the lower dose 100 mg/kg do not produce any change in urinary excretion. There is no significant change in the concentration of Na\(^+\), K\(^+\) and Cl\(^-\) in the drug treated group with respect to the control.
Table 4.7

Diuretic activity of methanolic extract of *Parmelia perlata* plant body on rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg bw)</th>
<th>Urine Volume (ml/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline 25 ml</td>
<td>2.0 ± 0.08</td>
</tr>
<tr>
<td><em>Parmelia perlata</em></td>
<td>100</td>
<td>2.0 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1.3 ± 0.08**</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0.9 ± 0.70*</td>
</tr>
<tr>
<td>Urea</td>
<td>750</td>
<td>4.6 ± 0.33**</td>
</tr>
</tbody>
</table>

Student's t-test was performed (Fisher, 1950).
Each value is the mean ± SE of six rats weighing 250 - 300 g.
Statistically significant from vehicle control: *P < 0.05, **P < 0.001

Table 4.8

Effect of methanolic extract of *Parmelia perlata* on Na\(^+\), K\(^+\) and Cl\(^-\) concentration in the urine of albino rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Electrolyte excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose mg/kg bw</td>
</tr>
<tr>
<td>Control</td>
<td>Normal Saline 25 ml</td>
</tr>
<tr>
<td><em>Parmelia perlata</em></td>
<td>250</td>
</tr>
<tr>
<td>Urea</td>
<td>750</td>
</tr>
</tbody>
</table>

Student's t-test was performed (Fisher, 1950).
Each value is the mean ± SE of six rats weighing 250 - 300 g.
Statistically significant from vehicle control : *P < 0.05, **P < 0.001
4.8 DISCUSSION

Herbs that stimulate the kidneys are traditionally used to reduce edema. Herbal diuretics do not work the same way that drugs do and thus it is unclear whether such herbs would be effective for this purpose. Solidago canadensis (Goldenrod) is considered one of the strongest herbal diuretics. Animal studies show, at very high amounts, that dandelion, Taraxacum officinale leaves possess diuretic effects that may be comparable to the prescription diuretic, furosemide. Corn silk (Zea mays) has also long been used as a diuretic, though a human study do not find that it increases urine output. Aescin (21) isolated from horse chestnut seed, has been shown to effectively reduce post surgical edema in preliminary trials. Horsetail (Equisetum spp.) has a diuretic action that accounts for its traditional use in reducing mild edema. The volatile oils in Juniper cause an increase in urine volume and in this way can theoretically lessen edema. Cleavers, Galium aparine is one of numerous plants considered in ancient times to act as a diuretic. It is therefore used to relieve edema and to promote urine formation during bladder infections. Medicinal plants are the oldest known health—care products. Medicinal plants are also important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also when they are used as basic materials for the synthesis of drugs or as models for pharmacologically active compounds.
Animal techniques for evaluating diuretics have been critically reviewed by Baer\textsuperscript{89}, Ginsburg\textsuperscript{90} and Lazarow\textsuperscript{91}. Rats are convenient animals for screening of diuretic agents because of the cost and ease in maintaining large number of animals. In the present study diuretic activities of the methanolic extracts of three plants \textit{viz.} \textit{Dichrostachys cinerea, Hemidesmus indicus} and \textit{Parmelia perlata} have been evaluated in male albino rats. Out of three drugs tested for diuretic activity only \textit{Dichrostachys cinerea} gave promising results. Urea increases the urine volume 2.3 fold whereas \textit{D. cinerea} methanolic extract at 250 mg/kg dose increases the urine volume around 2
fold. Potassium and chloride excretion levels, for *D. cinerea* are even greater than that of the reference diuretic. Since the methanolic extract of *D. cinerea* acts as a potent kaliuretic, the sodium and potassium ratio is decreased to half fold when compared to urea. Previous study on the diuretic activity of *Aerva lanata* also indicated similar results.\(^4\)

Methanolic extract of *H. indicus* root at 250 mg/kg dose shows significant diuretic activity, but there is no appreciable change in electrolyte excretion. The data reveals that the methanoilc extract of *H. indicus* acts as a good aquaretic rather than a kaliuretic or saluretic. The diuretic effect of *H. indicus* is not comparable to that of urea, which is a known osmotic diuretic. A valuable diuretic should cause marked natriuresis also. This is not observed in the case of *H. indicus*, which is more aquaretic. Therefore *H. indicus* can possibly be used as a diuretic in combination with other herbs only, which are natriuretic and kaliuretic.

The methanolic extract of *Parmelia perlata* was found to exhibit antidiuretic effect where there is dose dependent decrease in urinary excretion. The maximum decrease in urinary excretion is observed for 500 mg/kg dose. The decease in urinary excretion is unaccompanied by appreciable change in electrolyte excretion. The sustained oliguric action (reduction in passage of urine) is the action which is clinically useful in combating the polyuria of diabetes insipidus. In the absence of antidiuretic hormone (ADH), large volumes of diluted urine are excreted (diabetes insipidus). Under the influence of antidiuretic hormone, the distal tubule and
the collecting duct of the nephron become permeable to water, leading to a reduction in the total urine volume. The electrolyte pattern of the urine, however, is not altered. Absence of antidiuretic hormone causes diabetes insipidus. Drugs like morphine, nicotine (22) and barbiturates can stimulate the release of antidiuretic hormone. Alcohol and chlorpromazine (23), on the other hand, depress antidiuretic hormone release.

The antidiuretic activity may also be assayed on animals. Lindquist and Rowe have used the rabbit to assay the antidiuretic activity of vasopressin (ADH). Larson carried out studies which indicate that enzyme processes in the liver and kidney attack vasopressin and terminate its activity. However mechanism behind the antidiuretic activity of *P. perlata* is not known, the antidiuretic activity of this plant drug could be due to the phytoconstituents of the plant species.
In the case of *D. cinerea* and *H. indicus*, lower dosage (250 mg/kg) produces strong diuretic effect where as higher dosage (500 mg/kg) decreases the urine flow.

The diuretic action of Erva Tostao (Boerhaavia diffusa) has been studied and validated by Scientists in several studies, which help to explain its long history of use in various kidney and urinary conditions. Researchers showed in the mid 1950's that low dosages (10 mg/kg to 300 mg/kg) of *B. diffusa* produced strong diuretic effects while higher dosages (>300 mg/kg) produced the opposite effect, reducing urine output. Other researchers who followed, verified these diuretic and anti-diuretic properties as well as the beneficial kidney and renal effects of Erva Tostao roots in animals and humans.

In the case of *D. cinerea* maximum diuretic effect is observed in 250 mg/kg dose and it is found to decrease beyond that. In the case of *H. indicus* also maximum diuretic effect is observed in 250 mg/kg dose and an opposite effect is observed at higher dose 500 mg/kg, reducing urine output. Perhaps the higher dose used in the study may be inhibitory to the mechanism of diuresis. In *H. indicus* at 250 mg/kg dose the increase in urine volume is unaccompanied by a corresponding increase in electrolyte excretion, thus the extract behaves like a water diuretic rather than a kaliuretic or saluretic.

Aminoacids and flavonoids are reported to possess calculi dissolving ability and diuretic activity. In the present study, the diuretic activity of
**D. cinerea** and **H. indicus** may be due to the presence of these polyphenolic compounds. In similar studies, the fungus **Polyporus umbellatus** is reported to possess strong diuretic action\(^97\). The major components isolated from it are proteins (which are composed of amino acids) and ergosterol. Similarly, a flavonoid isolated from **Helichrysum bracteatum** exhibited significant diuretic activity in rats\(^98\). **Lepidium latifolium** containing phenols and sterols was found to exhibit significant diuretic activity\(^76\).

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. Several double-blind trials have found that 400 mg per day of coumarin, a flavonoid found in a variety of herbs, can improve many types of edema, including lymphedema after surgery\(^99\)-\(^102\). A group of semisynthetic flavonoids known as hydroxyethylrutosides are also beneficial for some types of edema\(^103\)-\(^105\).

A combination of the flavonoids diosmin and hesperidin has been investigated for the treatment of a variety of venous circulation disorders\(^106\). Because coumarin, hydroxyethylrutosides and diosmin are not widely available in the United States, other flavonoids, such as quercetin, rutin, or anthocyanosides (from bilberry) have been substituted by doctors in an attempt to obtain similar benefits. In one study, quercetin in amounts of 30–50 mg per day corrected abnormal capillary permeability (leakiness)\(^107\), an effect that might improve edema. A similar effect has been reported with rutin at 20 mg three times per day\(^108\).
Diuretics remain the cornerstone for the treatment of edema or volume overload, particularly that owing to congestive heart failure, ascites, chronic renal failure and nephrotic syndrome.

The medicinal plants of present investigation contain several types of phytochemicals including flavonoids that are believed to be responsible for their diuretic action.

The exact site of action of these herbal diuretics is difficult to delineate in this preliminary studies. As urea was the only drug taken as the standard during the present investigation, the diuretic activity of all the drugs was tested for a maximum period of five hours only.

4.9 CONCLUSIONS

Traditional medicines are used by about 60 per cent of the world’s population. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. While the traditional medicines are derived from medicinal plants, minerals and organic matter, the herbal drugs are prepared from medicinal plants only. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. In the Indian systems of medicine, most practitioners formulate and dispense their own recipes, hence this requires proper documentation and research. The major hindrance in the amalgamation of herbal medicines into modern medical practices is the lack of scientific and
clinical data and better understanding of efficacy and safety of the herbal products. Herbs that stimulate the kidneys were traditionally used to reduce edema. In the present investigation three medicinal plants viz. *Dichrostachys cinerea*, *Hemidesmus indicus* and *Parmelia perlata* which are used by traditional medical practitioners for the treatment of urinary disorders have been screened experimentally on saline–loaded albino rats for diuretic activity. The diuretic activity has been determined with reference to urea as standard. All the three drugs exhibited varied degrees of diuretic activity. Out of the three drugs tested, *D. cinerea* gave promising results where significant increase in urine volume and electrolyte excretion were observed. *H. indicus* showed moderate increase in urinary excretion which was unaccompanied by any significant change in electrolyte excretion. *Parmelia perlata* showed anti-diuretic effect with decrease in urinary excretion was produced. However a detailed long perspective study must be performed to explore and establish these drugs into routine therapeutic practices.
4.10 EXPERIMENTAL

4.10.1 Collection of plant materials

Roots of *Dichrostachys cinerea* and *Hemidesmus indicus* were collected respectively from Thirukurunkudi and Thenmalai of Tirunelveli District of Tamil Nadu and *Parmelia perlata* was collected from Thantrikudi, Dindigul District of Tamil Nadu, India in the month of September. Plants were identified by Dr. V. Chelladurai, Research Officer (Botany), Survey of Medicinal and Aromatic Plants Unit–Siddha, CCRAS, Palayamkottai, Tirunelveli District, Tamil Nadu, India and voucher specimens have been deposited at the Department of Chemistry, Manonmaniam Sundaranar University, Tirunelveli District, Tamil Nadu, India [*Dichrostachys cinerea* (MSU 051), *Hemidesmus indicus* (MSU 052) and *Parmelia perlata* (MSU 053)].

4.10.2 Preparation of plant extracts

The plant materials were thoroughly washed with water, cut into small pieces, dried under shade for two weeks and powdered. The powdered plant materials were individually and successively extracted with petroleum ether (40°–60°C), benzene, chloroform, methanol and water. The last trace of the solvent was removed under reduced pressure distillation and the crude extract was dried in a vacuum desiccator and used for the experiments. Dried methanolic extracts were weighed exactly and used for the evaluation of diuretic activity.
4.10.3 Animals

Male Wister albino rats weighing between 250 – 300 g were used for the study. They were given standard rodent diet (Lipton India Pvt. Ltd. Mumbai) and tap water ad libitum. They were housed under standard animal husbandry conditions and had no access to water or food, during the test period. For each experiment rats were randomly selected into groups comprising of 6 rats.

4.10.4 Diuretic activity

4.10.4.1 Diuretic activity of D. cinerea

The modified method of Lipschitz et al (1943) was employed for the evaluation of diuretic activity. Rats were divided into five groups of six rats, each weighing 250–300 g and were fasted and deprived of water for eighteen hours prior to the experiment. On the day of experiment, the methanolic extract of D. cinerea was suspended in normal saline at three different doses namely 100, 250 and 500 mg/kg body weight and were administered orally to three different groups. A group of rats receiving normal saline (25 ml/kg bw) alone was taken as the control and another group of rats received urea (750 mg in 25 ml of normal saline) as a reference drug. The fluid intake was the same in all cases, i.e. 25 ml/kg of the body weight. Immediately after administration, the rats were placed in metabolic cages (3 in each cage) specially designed to separate urine and faeces. Animals were kept at room temperature of 25°C ± 0.5°C, throughout the experiment. The urine was collected in measuring cylinders up to 5 h after dosing. During this period, no food or water was made available to animals. The total volume of urine collected was measured for both control and drug treated groups. Since in
the process of feeding, the animals lose all their urine, it was considered
important at the end of the experiment to expel the urine from the bladder by
pulling the base of the tail. The parameters taken for individual rat were body
weight before and after test period, total urine volume, concentration of Na⁺,
K⁺ and Cl⁻ in urine.

Analytical procedure

Na⁺ and K⁺ concentrations were determined by using a flame
photometer¹⁰⁹ (Sistronics-128, Chennai) and Cl⁻ concentration was estimated
by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium
chromate solution as an indicator.

Statistical analysis

All the results are expressed as mean ± standard error. The data was
analysed using Student's 't' test⁸⁴ for statistical significance and P < 0.05 was
considered significant.

4.10.4.2 Diuretic activity of H. indicus

Diuretic activity of methanolic extract of H. indicus was performed
using the modified method of Lipschitz⁸³ et al) as mentioned under
D. cinerea.

4.10.4.3 Diuretic activity of P. perlata

The modified method of Lipschitz⁸³ et al was used for the evaluation of
diuretic activity of methanolic extract of P. perlata as mentioned under
D. cinerea.
4.11 REFERENCES


