Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of the leaves of *Cucumis dipsaceus* ehrenb (Cuccurbitaceae) in rats

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This is to certify that the thesis prepared by Lishan Asefa, entitled: Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of the leaves of *Cucumis dipsaceus* Ehrenb (Cuccurbitacaea) in rats and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abstract

Evaluation of the diuretic activity of the aqueous and 80% methanol extracts of the leaves of *Cucumis dipsaceus* Ehrenb (Cucurbitacaea) in rats.

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Diuretics are drugs, which increase the excretion of electrolytes and water from the body by an action on the kidney. Their primary effect is to decrease the re-absorption of salt from the filtrate and increase water loss. They are used primarily to prevent and alleviate fluid overload like edema and ascites. *C. dipsaceus* leaf is one of medicinal plants claimed to have diuretic activity in indigenous system of medicine, but has not been scientifically evaluated. Therefore, the present study was done to evaluate the diuretic activity of the aqueous and 80% methanol extracts of the leaves of *C. dipsaceus* in rats. Male rats were randomly divided into eight groups (six rats per each group): negative and positive control groups, three different groups per each extract for different doses. The volume of urine output, urinary electrolyte concentration (Na^+, K^+, and Cl^-), and urinary pH were measured and analyzed to make comparison among the groups. Both the aqueous and 80% methanol extracts significantly increased the urinary output at 200mg/kg ($p<0.01$) and 400mg/kg ($p<0.001$) doses. Comparing the urinary electrolyte excretion with negative control group: the 400mg/kg dose of aqueous extract treated groups showed significantly increased urinary excretion of Na^+ ($p<0.05$), Cl^- ($p<0.01$) and K^+ ($p<0.01$). For 80% methanol extract, K^+ and Cl^- urinary excretion also showed significantly increased at 200mg/kg (K^+; $p<0.01$ and Cl^-: $p<0.05$) and 400mg/kg (for both: $p<0.01$) doses. Similarly the saluiretic effect of both aqueous and 80% methanol treated groups were significantly increased ($p<0.01$) at 400mg/kg dose. The Na^+/K^+ ratio and pH values did not show any significant differences in all treated groups compared to control. Generally, these findings indicate that both crude extract of *C. dipsaceus* leaves exhibited significant diuretic activity, supporting the traditional claim of its use as a diuretic agent.

Keywords: *Cuccumis dipsaceus*, diuretic activity, electrolyte excretion, Na^+/K^+ ratio

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Contents

Abstract .... **i**

Acknowledgements .... **ii**

Acronyms .... **v**

List of Tables .... **vi**

List of Figures .... **vii**

1. Introduction ***1***

1.1. Renal Anatomy and Physiology **2**

1.1.1. Role of the Kidney Nephrons *3*

1.2. Development of Modern Diuretics **5**

1.3. Mechanism of Action of Conventional Diuretics **6**

1.4. Herbal Diuretics **9**

1.5. Plant under Investigation **10**

1.6. Rationale for the Study **11**

2.* Objective of the Study** ***13

2.1. General Objective **13**

2.2. Specific Objectives **13**

3. Materials and Methods ***14***

3.1. Materials **14**

3.1.1. Chemicals and Drug *14*

3.1.2. Experimental Animals *14*

3.2. Methods **14**

3.2.1. Plant Material Collection and Authentication *14*

3.2.2. Extraction and Preparation of the Plant *15*

3.2.3. Preparation of 80 % Methanol Extract *15*

3.2.4. Preparation of Aqueous Extract *15*

3.2.5. Acute Toxicity *16*

3.2.6. Screening and Dosing for Diuretic Activity ... *16*

3.2.7. Determination of Urine Excreted and Plant Extracts of Electrolyte Concentration *17*

3.2.8. Phytochemical screening ... *18*

3.2.9. Statistical Analysis ... *20*

4.* Result** ... ***21

4.1. Yield of the Extract ... **21**

4.2. Acute Toxicity Test ... **21**

4.3. Diuretic Activity ... **21**

4.3.1. Effect on Urine Volume ... *21*

4.3.2. Effect on Electrolyte ... *26*

4.3.3. Effects on pH ... *31*

4.4. Electrolyte Content of the Extracts ... **33**

4.5. Phytochemical Test ... **33**

5. Discussion ... **35**

6. Conclusions ... **39**

7. Recommendations ... **40**

8. References ... **41**

Acronyms

ADH	Antidiuretic Hormone
AQP	Aquaporin
BW	Body Weight
CA	Carbonic Anhydrase
CAI	Carbonic Anhydrase Inhibitors
DCT	Distal Convoluted Tubule
DW	Distilled Water
GFR	Glomerular Filtration Rate
NC	Negative Control
OECD	Organization for Economic Co-operation and Development
PCT	Proximal Convoluted Tubule
SEM	Standard Error of the Mean
TAL	Thick Ascending Loop

List of Tables

Table 1:-Urinary output at different time intervals after oral administration of aqueous crude extract of *C. dipsaceus* leaves in rats. …………………………………………....…..23

Table 2:-Urinary output at different time intervals after oral administration of 80% methanol crude extract of *C. dipsaceus* leavesin rats ……………...……..…………..… 25

Table 3:- Effect of aqueous crude extract of *C. dipsaceus* leaves on 5hr urinary electrolyte excretion in rats. ……………………………………….....…………… 28

Table 4:- Effect of 80% methanol crude extract of *C. dipsaceus* leaves on 5hr urinary electrolyte excretion in rats …………………..……………………..…..….…..…..…30

Table 5:- Phytochemical screenings of aqueous and hydromethanol extract of *C. dipsaceus* leaf ……………………………………………………………………...…….34

List of Figures

Figure 1:- Tubule transport system and site of action of diuretics ………….……..…...8

Figure 2:- *C. dipsaceus* Ehrenb. Ex Spach; fresh photo at its natural habitat ……….…11

Figure 3:- Urinary pH of rats treated with aqueous crude extract of *Cucumis dipsaceus* leaves…..…………………….……………….………………...…………….…………..32

Figure 4:- Urinary pH of rats treated with 80% methanol crude extract of *Cucumis dipsaceus* leaves. …..……….…….……………………………….……..…..……………33

1. Introduction

Diuretics are drugs which increase the excretion of sodium and water from the body by an action on the kidney. Their primary effect is to decrease the re-absorption of sodium and chloride from the filtrate, increase water loss being secondary to the increased excretion of salt. This can be achieved by a direct action on the cells of the nephron or indirectly modifying the content of the filtrate (Jackson *et al*., 2001). Technically, the term 'diuresis' signifies an increase in urine volume while 'natriuresis' denotes an increase in renal sodium excretion. Because the important natriuretic drugs usually also increase the water excretion, they are usually called diuretics, and the increase in sodium excretion is assumed (Ivec *et al*., 2004).

Diuretics have been used for the treatment of dropsy and effectively to treat millions of hypertensive patients as early as 16thcentury. They are used primarily to prevent and alleviate fluid overload like edema and ascites. These conditions occur in various diseases and syndromes of acute and chronic heart failure, liver cirrhosis, acute and chronic renal failure and acute pulmonary edema. They are also used in hypertension, edema of pregnancy, diabetes insipidus, brain edema, renal calculi, as well as a more general reduction of the adverse effects of salts /hypercaliuria, hypocalcaemia and/or water retention (Mitch *et al*., 1982;Ivec *et al*., 2004;Jackson, 2006).

Patients with nephropathy or heart failure may have a 10 to 30% increase in extracellular and blood volume, even in the absence of overt edema (Mitch *et al*., 1982). Using a diuretic to prevent cardiovascular complications plays an important role in the management of arterial hypertension. Diuretics increase the drainage of water and sodium into the urine, causing a reduction of blood pressure, a decrease in blood volume, and thus lowering resistance to the flow of blood (Caceres *et al*., 1987). Diuretics reduce both systolic and diastolic blood pressures in the great majority of hypertensive patients. They are as effective as most other antihypertensive drugs (Weir *et al*., 1996;Puschett *et al*., 2000). Diuretics are administered alone or in combination with other anti-hypertensive agents for the treatment of hypertensive patients (Materson *et al*., 1995). A thiazide is the usual choice, generally in combination with other antihypertensive agents. Loop diuretics are usually reserved for patients with renal insufficiency, resistant hypertension, or heart failure (Frishman *et al*., 1994). The high ceiling and osmotic diuretic agents are also used

in several poisoning condition to increase excretion of poisoning agent (Singh *et al*,. 1991, Weir *et al*., 1996).

1.1. Renal Anatomy and Physiology

The two kidneys lie on the posterior wall of the abdomen, outside the peritoneal cavity. The medial side of each kidney contains an indented region called the *hilum* through which pass the renal artery and vein, lymphatics, nerve supply, and ureter, which carries the final urine from the kidney to the bladder, where it is stored until emptied. The kidney is surrounded by a tough, fibrous *capsule and* by a layer of adipose tissue that protects its delicate inner structures from physical damage and holds them in place. If the kidney is dissected from top to bottom, the two major regions that can be visualized are the outer *cortex* and the inner region referred to as the *medulla.* The medulla is divided into multiple cone-shaped masses of tissue called *renal pyramids,* which originates at the border between the cortex and medulla and terminates in the *papilla and* projects into the space of the *renal pelvis,* a funnel-shaped continuation of the upper end of the ureter (Guyton and Hall, 2006; Saladin, 2008).

The kidneys are relatively small organs, but they receive up to 25 percent of the heart's output. The renal artery enters the kidney through the hilum, then branches progressively, and reaches the level to form *afferent arterioles,* which lead to the *glomerular capillaries,* where large amounts of fluid and solutes are filtered to begin urine formation. The distal ends of the capillaries of each glomerulus coalesce to form the *efferent arteriole,* which leads to a second capillary network, the *peritubular capillaries,* that surrounds the renal tubules, which progressively drains into the inferior venacava (Guyton and Hall, 2006; Saladin, 2008).

The kidneys are highly vascularized organs that play a fundamental role in maintaining body salt, fluid balance and blood pressure homeostasis. They are designed to filter large quantities of plasma, reabsorb substances that the body must conserve like bicarbonate, glucose, amino acids etc, and leave behind and/or secrete substances that must be eliminated like metabolic wastes, excess ions, and chemicals from the blood to form urine through the actions of their nephrons (Chmielewski, 2003; Zhuo and Li, 2013).

1.1.1. Role of the Kidney Nephrons

Nephron is the basic urine-forming unit of the kidney (Jackson, 2006). Each kidney in the human contains about 1 million *nephrons*. Each nephron contains two principal parts: a renal corpuscle; a tuft of glomerular capillaries called the *glomerulus,* through which large amounts of fluid are filtered from the blood, and a long *tubule* in which the filtered fluid is converted into urine on its way to the pelvis of the kidney. The filtrate, although normally free of proteins and blood cells, contains most of the low molecular weight plasma components in concentrations similar to that in the plasma (Guyton and Hall, 2006; Saladin, 2008). These include glucose, sodium bicarbonate, amino acids, and other organic solutes, as well as electrolytes, such as Na^+, K^+, and Cl^-. The kidney regulates the ionic composition and volume of urine by active reabsorption or secretion of ions and/or passive re-absorption of water at five functional zones along the nephron. Such as: 1) the proximal convoluted tubule, 2) the descending loop of Henle, 3) the ascending loop of Henle, 4) the distal convoluted tubule, and 5) the collecting tubule and duct (Karen *et al*., 2012).

The PCT is contiguous with Bowman's capsule and located in the cortex of the kidney, almost all the glucose, bicarbonate, amino acids, and other metabolites where reabsorbed. Normally, ~65% of filtered Na^+ is also re-absorbed here, and since this part of the tubule is highly permeable to water, re-absorption is essentially isotonic(Jackson, 2006). Carbonic anhydrase in the luminal membrane and cytoplasm of the proximal tubular cells modulates the re-absorption of bicarbonate. The Na^+ is that reabsorbed is pumped into the interstitium by Na^+/K^+- adenosine tri-phosphatase (ATPase) pump; thereby maintaining normal levels of Na^+ and K^+ in the cell. The proximal tubule is the site for secretory system for a variety of organic acid such as uric acid, organic base, some antibiotics and most diuretics from the bloodstream into the proximal tubular lumen (Karen *et al*., 2012). $NaHCO_3$ re-absorption by the PCT is mediated by the action of a Na^+/H^+ exchanger, which allows Na^+ to enter the cell from the tubular lumen in exchange for H^+ from inside the cell. A metallo-enzyme, CA, which is found in the luminal and basolateral membranes, catalyzes the dehydration and rehydration of carbonic acid to provide H^+ for the exchange (Jackson, 2006; Ives, 2012).

The remaining filtrate, which is isotonic, next enters the thin descending limb of the loop of Henle and passes into the medulla of the kidney. The osmolarity increases along the

descending portion of the loop of Henle. Osmotic diuretics exert part of their action in this region. Then the filtrate enters the TAL of henle. The cells of the thick ascending tubular epithelium are unique in being impermeable to water and are responsible to reabsorb about 25% of the filtered loads of Na^+, Cl^- and K^+. The Na^+ /K^+ /2Cl^- co-transporter mediate active re-absorption of Na^+, K^+, and Cl^- (Friedman and Berndt, 1997; Jackson, 2006; Karen *et al*., 2012). Although the Na^+/K^+/2Cl^-co-transporter is itself electrically neutral, the action of the transporter contributes to excess K^+ accumulation within the cell. Back diffusion of this K^+ into the tubular lumen causes a lumen-positive electrical potential that provides the driving force for re-absorption of cations, including Mg2+ and Ca2+, via the para-cellular pathway (Ives, 2012).

The TAL empties into the DCT, its cells are also impermeable to water, where 5-10% of the filtered sodium chloride is reabsorbed via a Na^+/Cl^-co-transporter that is sensitive to thiazide diuretics (Ellison, 2000; Jackson, 2006; Reilly and Saladin, 2008).Na^+/Cl^- co-transport mediate the entry of Na^+ across the apical cell membrane (Friedman and Berndt, 1997).

Finally, the urine reaches at the collecting tubule system and the principal cells of the collecting tubule and duct are responsible for Na^+(only 2-5% of NaCl re-absorption by kidney) , K^+ and water transport, whereas the intercalated cells affect H^+ secretion. Sodium enters the principal cells through epithelial sodium channels that are inhibited by *amiloride* and *triamterene*. Mineralocorticoids exert a significant influence at this site (Ives, 2012). Once inside the cell, Na^+ re-absorption relies on a Na^+/K^+-ATPase pump to be transported into the blood. The collecting tubule, which coalesces to form the collecting ducts, performs the final adjustment of renal excretion (Range *et al*., 2012; Staruschenko, 2012). Aldosterone receptors in the principal cells of collecting tubules influence Na^+ reabsorption and K^+ secretion and two populations of intercalated cells, α and β, which secrete acid (H^+) and base (bicarbonate), respectively (Range *et al*., 2012). Aldosterone increases the synthesis of Na^+ channels and of the Na^+/K^+-ATPase pump, which when combined increase Na^+ re-absorption (Karen *et al*., 2012). The final concentration of the urine depends on the water permeability of the collecting ducts carrying the urine through the cortex and medulla (Neilsen *et al*., 1999).

ADH controls the permeability of these cells to water by regulating the insertion of pre-formed water channels AQP-2 into the apical membrane of the principal cells (Friendman and Berndt, 1997). ADH receptors promote the re-absorption of water from the collecting tubules and ducts (Karen *et al*., 2012). In the absence of ADH, the collecting tubule is impermeable to water, and dilute urine is produced. ADH markedly increases water permeability, and this leads to the formation of more concentrated final urine (Friendman and Berndt, 1997; Ives, 2012).

1.2. Development of Modern Diuretics

The history of diuretics goes back a long way. It is believed that Paleolithic man discovered the caffeine-containing plants. Beverages containing caffeine were prepared from the seeds and bark of different plants. The development and introduction of mercurial diuretics into therapy was a decisive step towards new discoveries in the field of modern diuretics. The ability of mercurial anti-syphilitic to affect diuresis was discovered by Vogel in 1919. This observation led to the development of the most effective organic mercurial diuretics; drugs that were used commonly as the mainstay of treatment until the 1960s.However, they are no longer used because of their toxicity. Other options during this period were osmotic diuretics like urea, mannitol and sucrose, acidifying salts such as ammonium chloride, xanthine derivatives (e.g. theophylline, and caffeine) and digoxin, which has a diuretic effect in addition to its inotropic effect, were also used due to their clinically important diuretic effects before the emergence of modern diuretics (Eknoyan, 1997; Wile, 2012).

In 1937, Southworth realized that patients treated with the antibiotic sulphanilamide not only breathed deeply but also produced an alkaline urine, with increased sodium and water excretion. Sulphanilamide was found to be a carbonic anhydrase inhibitor and by 1949, Schwartz had successfully treated congestive heart failure patients with sulphanilamide. Structural modification of sulphanilamide generated carboxybenzenesulphonamide, also a carbonic anhydrase inhibitor that increased sodium and chloride excretion. The carbonic anhydrous inhibitors (e.g. acetazolamide) were developed in the 1950s from the sulfonamide, following an observation that sulfonamides caused side effect and mild diuresis (Tripathi *et al*., 2003; Wile, 2012). Further molecular modification also found to increase potency, in which diuretic activity was sought rather

than carbonic anhydrase inhibition, resulted in studies of metadisulphonamide carbnonic anhydrase inhibitors analogues, and gave rise eventually to the first modern, orally active diuretic chlorothiazide in 1957, and by early 1960s its congeners (thiazide diuretics). The search for more potent classes of diuretic continued and further molecular modification led in the early 1960s to the compounds frusemide, bumetanide and later piretanide and torasemide. These agents, thought also as sulfonamide derivatives, have very few chemical features in common with the thiazides. Their mechanism of action is also different from the thiazides (Jackson *et al*., 2001).

Aldosterone antagonists, such as spironolactone, were introduced in 1962; partially satisfied this requirement, but they had several drawbacks. Numerous compounds screened and eventually amiloride and triameterene had emerged (Range *et al*., 2012).

1.3. Mechanism of Action of Conventional Diuretics

Most diuretics exert their effects by inhibiting the re-absorption of sodium at different segments of the renal tubular system or combination of these sites (Figure 1) (Mitch e*t al*., 1982). Diuretics will give net effect to increase urine volume due to sodium and water excretion (Adam *et al*., 2009). They act on specific membrane transport proteins in renal tubular epithelial cells and other diuretics exert osmotic effects that prevent water re-absorption (Mannitol), inhibit enzymes (Acetazolamide), or interfere with hormone receptors in renal epithelial cells (aldosterone receptor blockers). Naturally, occurring diuretics include caffeine, which inhabits Na^+ re-absorption and alcohol inhibit secretion of anti-diuretic hormone (ADH) (Koti and Purnima, 2008).

Osmotic diuretics are substances that are freely filtered at the glomerulus, poorly reabsorbed and are relatively inert pharmacologically (Jackson, 2006). The pharmacological activity of drugs in this group depends entirely on the osmotic pressure exerted by the drug molecules in solution, and not on interaction with specific transport proteins or enzymes. They increase the osmotic pressure in the proximal tubule fluid and loop of Henle, thereby retarding the passive re-absorption of water and impair sodium re-absorption by lowering the concentration of sodium in the tubular fluid. Mannitol is the prototypical osmotic diuretic and other agents considered in this class include urea, glycerin and isosorbide (Ellison, 2013).

Carbonic anhydrase inhibitors (e.g., acetazolamide and methazolamide) act by inhibiting the enzyme CA in the PCT to interfere with bicarbonate ($HCO3^-$) re-absorption which results in increased loss of Na^+, HCO_3^- and water in the urine (Snigdha *et al*., 2013). $HCO_3^-$is re-absorbed in the proximal tubule and requires the activity of carbonic anhydrase. Intracellularly carbonic anhydrase converts H_2O and CO_2 to carbonic acid that dissociates into H^+ and HCO_3^-.The HCO_3^- is transported across the basolateral membrane. H^+ is secreted into the tubular lumen in exchange for Na^+. The H^+combines with a filtered HCO_3^-(using CA) to form H_2CO_3, which immediately dissociates into H_2O and CO_2that is reabsorbed. Therefore, filtered bicarbonate is reabsorbed for every H^+ secreted. Carbonic anhydrase inhibitors, by blocking the enzyme, prevent the re-absorption of $NaHCO_3^-$ and hence diuresis occurs (Saladin, 2008).

Loop or high ceiling diuretics (e.g. furosemide, torsemide, azosemide, bumetanide and ethacrynic acid) act by blocking the $Na^+/K^+/2Cl^-$ co-transporter mainly in the medullary and TAL of Henle resulting in decreased Na^+, K^+ and Cl^- re-absorption from the urine and subsequent natriuresis and diuresis and to a lesser extent by inhibition of carbonic anhydrase (Bevevino *et al*., 1994; Shchekochikhin *et al*., 2013). They promote the excretion of a higher percentage of filtered salt. Urine that excreted is alkaline and contains excess Na^+, K^+ and HCO^-_3 (Martin-Herrera *et al*.,2008).This action inhibits electrolyte re-absorption and reduces the osmotic gradient in the renal medulla that in turn impairs both the concentrating and the diluting capacities of the kidney (Range *et al.,*2012).

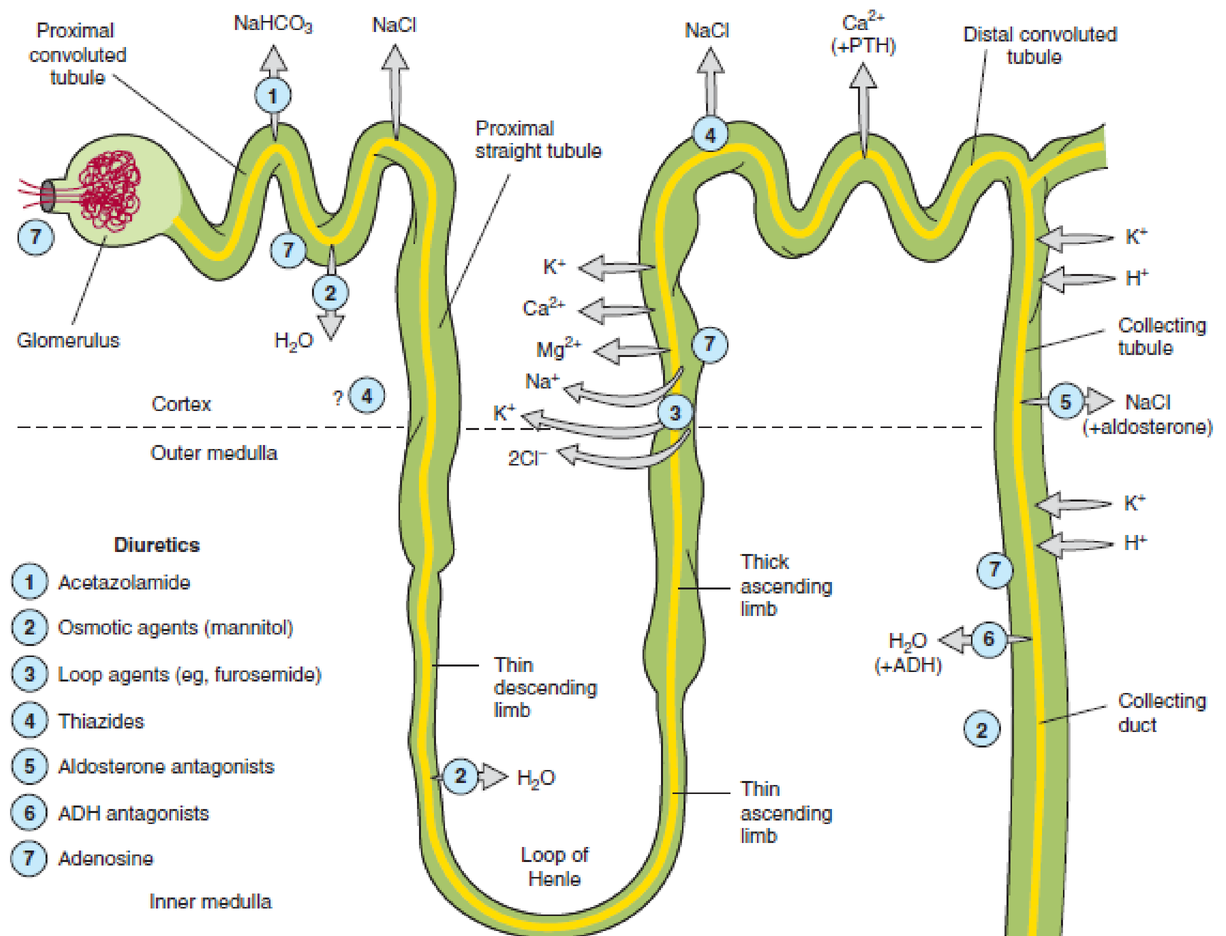


Figure 1: Tubule transport systems and sites of action of diuretics. ADH: - antidiuretic hormone; PTH:- parathyroid hormone..**Source**: (Ives, 2012, pp252, Fig. 15-1)

Thiazides exert their effect of diuretic action via inhibition of the apical Na^+/ Cl^- co-transport (NCC) in the cortical thick ascending limb and early DCT. They have a milder diuretic action than do the loop diuretics because this nephron site reabsorbs less Na^+ than the TAL. In addition, if glomerular filtration rate falls, less fluid reaches the DCT and thiazides may only have a small impact on Na^+ and water excretion. These compounds then are relatively ineffective in renal insufficiency. Use of thiazides results in an increased absorption of $Ca^{2+}$and uric acid by the proximal tubule, ultimately leading to reduced excretion of $Ca^{2+}$and uric acid (Ivec *et al*., 2004). The antihypertensive action of the thiazides may be attributed to a depletion of sodium and subsequent reduction in plasma volume and a decrease in peripheral resistance, which is thought to be due to either the loss of sodium from the arteriolar wall or a direct action on the vascular bed (Range *et al*., 12012). Thiazide or thiazide-like diuretics include hydrochlorothiazide,

chlorthalidone, indapamide, metolazone and chlorothiazide (Musini *et al*., 2012). Hydrochlorothiazide blocks Cl^- re-absorption, creating intra-luminal negative charge that impedes Na^+ reabsorption resulting in increased K^+ losses because of increase Na^+ delivery with increased aldosterone. Basically, Hydrochlorothiazide inhibits sodium and chloride re-absorption in the kidney distal tubules and produces a corresponding increase in sodium, water and potassium excretion. Hydrochlorothiazide produces alkaline urine containing increased Na^+, K^+, HCO_3^- and Cl^- (Ya'u *et al*., 2013).

Potassium sparing diuretics act primarily at the cortical part of the collecting duct and to a lesser extent in the late distal and collecting tubules either by direct blockage of mineralo-corticoid receptors (e.g., Spironolactone and eplerenone) a competitive antagonist of aldosterone; therefore it blocks aldosterone-stimulated Na^+ re-absorption and K^+ and H^+ excretion in the late distal tubule and collecting duct. Alternatively, by blocking epithelial Na+ channels in the luminal/apical membrane of the late distal tubule and collecting duct (e.g., Amiloride and triamterene). Since only a small amount of sodium is reabsorbed here, these agents are capable of limited natriuresis (Jackson *et al*., 2001; Ernst and Gordon, 2010; Musini *et al*., 2012).

1.4. Herbal Diuretics

Several countries use natural products derived from plant extracts/fractions as a best source of medicine /novel therapeutic agents for various infectious as well as degenerative diseases and most potent and powerful drugs are derived from plants (Srivastava *et al*., 1996; Uniyal *et al*., 2006). In herbal medicines, various parts of the plant (root, stem, flower, fruit, twigs exudates and modified plant organs) are used. To utilize these plants, collected on minute scale by local communities and folk healers, while to trade for herbal industries numerous other plants are collected in large amount as a raw material (Uniyal *et al*., 2006).

Medicinal plants have been traditionally used worldwide in the management of some renal diseases and have a wide application as diuretic agents since time immemorial (Pizzi, 2003; Wright *et al*., 2007; Freitas *et al*., 2011). The diuretic activities of a number of plants used in ethno-medicine have been confirmed in experimental animal models (Wright *et al*., 2007). The safety and efficacy of these plants for their claimed medicinal use, however, have not been extensively studied and remain to be in light of further

investigation. The techniques of preparation employed by traditional healers are generally not standardized and in most cases do not comply with the requirement of good manufacturing practice (Mosihuzzaman and Choudhary, 2008).

There are a growing number of studies purporting diuretic effects with traditional medicines (Wright *et al*., 2007). There were Pharmacological studies done on some traditional medicinal plants, which have supported their folkloric use as diuretic agents in Ethiopia. Some of the promising plants include: ***Carissa edulis*** *(*Nedi *et al*., 2004; Kebamo *et al*., 2015), *Rumex abyssinicus* (Mekommen *et al*., 2010), *Ajuga remota* (Hailu and Engidawork, 2014)**,** *Thymus serrulatus* (Melka *et al.,*2016) and *Moringa stenopetala* (Geleta *et al*., 2015;Fekadu *et al*., 2017).

Another study, in evaluation of antioxidant activity of this plant shows both aqueous and methanol extracts of *C. dipsaceous* exhibited effective reducing capacity. In addition, both extracts were investigated for their inhibitory effects on nitric oxide production. The methanolic extract showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals than aqueous extract (Urs *et al*., 2017).

C. dipsaceus Ehrenb. Ex Spach (Belayneh and Bussa, 2014) with a vernacular name of "Harree Gogee" (Afan Oromo) or "Yewef Hareg" (Amaharic) is claimed for the treatment of gonorrhea, urinary retention and skin fungus in indigenous system of medicine. There is no scientific study on the diuretic activity of the plant to date. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of natural medicines. Hence, the aim of present study was to evaluate the diuretic activity using the aqueous and 80% methanol extract of *C. dipsaceus* leaves.

1.5. Plant under Investigation

C. dipsaceus Ehrenb. ex Spach is a species of annual climbing and flowering plant belonging to genus *Cucumis* L., family Cucurbitaceae. It has its origin in Ethiopia and the genus *Cucumis* is cosmopolitan in distribution (Africa (Ethiopia, Kenya, Somalia, Tanzania, Uganda, Sudan, Southern Egypt) and Asia: India and represented by about 52 species in the world (Mabberley, 2008; Nivedhini *et al*., 2014). *C. dipsaceus* is known by several common names like "teasel gourd", Arabian cucumber, hedgehog and locally by

Afan Oromo as “Harree Gogee”; Amaharic as “Yewof Hareg”. Medicinally the genus is very important. Usually, the leaves of *C. dipsaceus* are consumed as a leafy vegetable (Verdcourt and Trump, 1996). Its fruit juice is topically applied to prevent hair loss (Bussmann and Glenn, 2010). Poultice is prepared from leaves and tendrils for the treatment of wounds. Fruit juice act as an antidote in poisoning case (Christopher and Ruffo, 2002). Traditionally, *Cucumis* is used for different ailments. Its stems decoction used as anti-emetic. Its fruit used for gastrointestinal diseases, diarrhoea, stomach pain, constipation and meningitis. Roots are used for hepatitis, local application, snakebite, carnivore bite and gallstone. Extract of fresh leaves used for haemorrhoid and rabies. The seeds are diuretic; the fruit juice is used as a nutritive and as a demulcent in anti-acne lotions (Kumar *et al*., 2010).

Figure 2: *Cuccumis dipsaceus* Ehrenb. Ex Spach; at its natural habitat.

1.6. Rationale for the Study

About 80% of the world’s populations living in developing world rely on herbal medicinal products as the primary source of health care (Ekor, 2014). Herbs are effective in the treatment or prevention of various diseases, such as diabetes mellitus, ascites, cardiovascular disorders, renal problems and even cancer (Bijekar and Gayatri, 2014). Within the last few decades diseases like congestive heart failure, renal failure,

hypertension, syndrome of inappropriate anti-diuretic hormone (SIADH) and hypervolemic hyponatremia have been considered as the common diseases of man (Maramag, 2013). Management of these disorders is mainly dependent on increasing urinary volume and electrolyte output using diuretic drugs (Ellison, 2013). A number of diuretics like mannitol, thiazides, furosemide, ethacrinic acid are used in clinical practice. However, the most commonly used diuretics, thiazide and loop diuretics, have been associated with a number of adverse effects, like disturbance of electrolytes, acid-base and water balance, change in uric acid, carbohydrate and lipid metabolism and drug interactions (Wile, 2012; Tamargo *et al.*, 2014). To overcome these problems, there is a need to search for new diuretics, which are comparatively more effective and less toxic from local plant materials.

Many indigenous drugs have been claimed to have diuretic effect in Ayurvedic system of medicine but they were not properly investigated (Samiulla *et al.*, 2000). Hence, the present study, attempted to scientifically evaluate the diuretic activity of the crude extract of the leaves of *C. dipsaceus* plant in rats, which could help subsequent investigation to understand the precise molecular mechanisms and identify the specific chemical(s) responsible for the activity.

2. Objective of the Study

2.1. General Objective

- To evaluate the diuretic activity of C. *dipsaceus* in rats.

2.2. Specific Objectives

- To test the acute oral toxicity of the aqueous and 80% methanol crude leaf extract of *C. dipsaceus*
- To determine the effect of aqueous and 80% methanol extract of *C. dipsaceus* on urinary volume excretion
- To determine the effect of aqueous and 80% methanol extract of *C. dipsaceus* on urine electrolyte excretion (Na^+, K^+, and Cl^-).
- To determine the pH value of urine produced by different concentration of the leaf extract of *C. dipsaceus*.
- To screen the phytochemical constituents of the aqueous and 80% methanol crude leaf extract of *C. dipsaceus*

3. Materials and Methods

3.1. Materials

3.1.1. Chemicals and Drug

Furosemide (Kawasan preindustrial (Malaysia) and distilled water (Ethiopian pharmaceuitical manufacturing, Ethiopia) were used during the experiment as positive and negative control, respectively. Absolute methanol (Carlo Erba, France) and distilled water for extraction of the plant, physiological saline (NS) (Acute life Health care, India) to impose a uniform water and salt load (Kau et al. 1984).

3.1.2. Experimental Animals

Adult healthy Swiss albino rats of either sex male (for diuretic activity test) and female (for acute toxicity test) having weight range of 200-300g and aged 6–8 weeks, were used and obtained from animal house unit of School of pharmacy of Addis Ababa University. To acclimatize, the animals were kept for one week in polypropylene cages (8-10 animals per cage) under standard environmental conditions on a 12h light–dark cycle. The animals were allowed free access to standard laboratory pellet diet and tap water. Prior to the start of the experiment, all animals were fasted for 15hrs with water allowed ad-libtum. The care and handling of the animals was in accordance with the internationally accepted guidelines for use of experimental animals (Vogel, 2007; OECD, 2008).

3.2. Methods

3.2.1. Plant Material Collection and Authentication

Fresh leaves of *Cucumis dipsaceus* (Cucurbitaceae) were collected from Dengego Valley, Dengego district, Dire Dawa in April 2018. The area is located at 515 km east of Addis Ababa. For identification purpose, small amount of the plant leaves were collected in December 2017and submitted to taxonomist at the National Herbarium, College of Natural Sciences and Computation, Addis Ababa University. The plant identification and authentication was done and the plant specimens were deposited for future reference having a voucher number of 001.

3.2.2. Extraction and Preparation of the Plant

After collecting the fresh part of the plant leaves, it was thoroughly washed using distilled water. Then, dried at room temperature under shade for two weeks. The dried leaves were then powdered finely using pestle and mortar, then used for extraction.

3.2.3. Preparation of 80 % Methanol Extract

A sample of 250g powder of *Cucumis dipsaceus* leaves was macerated with 2500ml of 80% methanol for 72 h at room temperature in Erlenmeyer flask. For a proper mixing, the plant material and the solvent were shaken continuously on a horizontal orbit shaker (Stuart, UK). The extract was first filtered using cotton gauze and later with whatman filter paper, No.1 and the marcs were re-macerated once using the same volume of solvent to exhaustively extract the plant material. The methanol was removed from the extract under reduced pressure using Rota vapor (Buchi, Switherland) at 40°C. Then, the extract was dried using a lyophilizer (Operon, Korea) to remove the remaining water, the resulting dried 80% methanol extract of the plant was weighed, and percentage yield (13.50%) was calculated and finally stored in -20°C and was reconstituted with distilled water immediately prior to the experiment for oral administration.

3.2.4. Preparation of Aqueous Extract

250gram of dried powdered leaves of *Cucumis dispaceus* was cold macerated with 2500ml of distilled water in an Erlenmeyer flask. Then, allowed to stand at room temperature for a period of 72h. For a proper mixing, the mixture of the plant material and the solvent were shaken continuously on a horizontal orbit shaker (Stuart, UK). The mixture was then, first filtered with gauze and later with what man filter paper. Marcs from the plant were then re-macerated once using the same volume of solvent to exhaustively extract from the plant material. The filtrate was freeze dried in a lyophilizer (Operon, korea) to remove water. The freeze-dried extract of the plant was collected, weighed and percentage yield (8.14%) was calculated. Finally, it packed in a plastic vial and kept in a refrigerator at -20ºC. Then, used when the experiment commences. For oral administration, it was reconstituted by distilled water immediately prior to administration.

3.2.5. Acute Toxicity

The study was carried out by limit test in accordance with OECD 423 guideline (OECD, 2008). The Limit test was used to determine if the toxicity of a test substance is above or below a specified dose. In this study, female Swiss albino rats weighing between 200–300 g and age 6-8 week were divided into three groups having six animals each. Before the day of the experiment, the animals were deprived of food for 15h and water was given ad libitum. On the day of the experiment, the animals were weighted and the negative control group received distilled water, the other two groups received a limit dose of 2g/kg BW of aqueous and 80% methanol extract orally. Immediately after administration, the animals were carefully observed continuously for the first 4h for any overt signs of toxicity and death and then for the next 24h. Thereafter, they were kept under close observation up to 14 days to monitor the presence of any signs of morbidity or mortality. Then, the weight of each animal was also record at the seventh and 14th days of administration to verify any weight change that might have occurred.

3.2.6. Screening and Dosing for Diuretic Activity

Each extracts was screened for their diuretic activity according to the following described methods (Kau and Andrews, 1984). Male Swiss albino rats weighing between 200-300g were used and divided into eight groups each group containing six rats. The animals were placed in a standard metabolic cage. Food but not water was withholding15hs prior to the experiment. The test substance, distilled water and standard drug were administered based on the weight of the animal. Before administration of the extracts, both extracts were dissolved in distilled water to make the required concentrations and then administered orally. All the animals received normal saline (2ml/100g) orally prior to the commencement of experiment.

Group1 received distilled water (2ml/100g) and served as negative control, Group-2 received standard diuretic drug, furosemide (10mg/kg) and served as positive control. Group-3 to 5 received aqueous extract and Group-6 to 8received 80% methanol extract test substances each at 100, 200 and 400 mg/kg dose levels, respectively. The cumulative urine excreted was measured at the end of 5 h in all groups and the collected urine stored at -20 ^{0}C until further analysis. According to Mukherjee (2002) and Durairaj *et al*.,(2007), total urine volume, diuretic action, diuretic activity, urinary excretion, saluretic

activity, natriuretic activity, urinary electrolyte concentration of Na^+, K^+ and Cl^- were the parameters considered in order to compare the effects of the test doses of the extract with negative control and standard drug, Furosemide, on diuresis. The volume of the urine excreted in 5 hours of the study period is expressed as the percent of the liquid (Normal Saline) administered giving rise to a measure of ''urinary excretion'' independent of animal weight, that is to say, the urinary excretion was calculated as the total urinary output divided by total liquid administered (Formula-1). The ratio of urinary excretion in test group to urinary excretion in the control group was used as a measure of diuretic action of the diuretics (Formula-2). The diuretic action, also called "Diuretic Index", Indices of 1.0 and more are regarded as a positive effect or potent diuretics. Since diuretic index is prone to variability, a less variable parameter known as Lipschitz value was calculated. To obtain the Lipschitz value, urine volume of the treated rats was compared to that of the group that receive the standard drug, which is called diuretic activity. The diuretic activity was also calculated as the ratio of diuretic action of the test substances to that of the standard drug (Formula-3) (Mukherjee, 2002; Somova *et al*., 2003).

$$\text{Urinary Excretion (UE)} = \frac{\text{Total Urinary output (VO)} \times 100\%}{\text{Total liquid administered (VI)}} \quad \text{……… [Formula 1]}$$

The ratio of urinary excretion in test group and control group was denoted ''Diuretic action'', which will used as the measure of degree of diuresis.

$$\text{Diuretic action} = \frac{\text{Urinary excretion in test groups(UEt)}}{\text{Urinary excretion in control group (UEc)}} \quad \text{………. [Formula 2]}$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of test drug (DAt)}}{\text{Diureticaction of standard drug (DAf)}} \quad \text{…….….. [Formula 3]}$$

3.2.7. Determination of Urine Excreted and Plant Extracts of Electrolyte Concentration

The five-hour urine electrolyte concentration (Na^+, K^+ and Cl^-) of the experimental, negative control and standard drug treated groups were analyzed. Urinalysis was done using Ion Selective Electrode (ISE) analysis method for chloride and Flame photometry for sodium and potassium (Cobas6000, Roche, Germany) described by the user instruction manual of the Biochemical kits available in the laboratory of Ethiopian Public Health Institution (EPHI). For the fresh urine sample, pH was measured using a digital automated pH meter (Sigma-Aldrich, USA).

Parameters considered in urinary Electrolyte excretion include:

Natriuretic activity (Na+/ K+ ratio)

$$Na+/K+ = \frac{Concentration\ of Na+\ in\ the\ urine\ of\ the\ group}{Concentration\ of K+\ in\ the\ urine\ of\ the\ same\ group}$$

Na^+/K^+:- Ratio of concentration of sodium ion in the urine of the test group to concentration of potassium ion in the urine of the same group. Estimate Natriuretic activity.Values greater than 2.0 indicate a favorable natriuretic effect and values greater than 10.0 indicate a potassium-sparing effect.

$$Saliuretic\ index = \frac{Concentration\ of electrolyte\ in\ the\ urine\ of\ test\ group}{Concentration\ of electrolyte\ in\ the\ urine\ of control\ group}$$

Saliuretic index: Is the ratio of concentration of electrolyte in the urine of group to concentration of electrolyte in the urine of control group. The sum of Na^+ and Cl^- excretion was estimated for saliuretic activity.

$$Ion\ quotient = \frac{[Cl-]}{[Na++K+]}$$

The ratio $[Cl^-]$ / $[Na^+]$ + $[K^+]$ (ion quotient) in the urine was derived to estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8, with decreasing ratios; slight-to-strong carbonic anhydrase inhibition can be assumed (Vogel, 2007).

3.2.8. Phytochemical screening

The aqueous and methanol extracts,were tested for the presence of phytochemicals in *Cucumis dipsaceus*leaves by using the following standard methods.

Detection of alkaloids (Hager's test)

One and half ml of 10% HCl was added to 0.5 mg of the extracts in a test tube. The mixture was heated for 20 min. It was then cooled and filtered. To 1 ml of the filtrate, five drops Mayer's and Dragendorff's reagents each was added. The formation of cream and orange colored precipitates, respectively, indicated the presence of alkaloids in the extracts (Trease and Evans, 1989).

Detection of saponins (Frothing test)

50 mg of extractwas diluted with distilled water and was made up to 20 ml. The suspension was shaken for 15 min in a graduated cylinder. A formation of 2-cm layer of foam is anindicator of the presence of saponins (Thangaraj, 2016).

Test for tannins

Crude extract of 0.5gram was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration is an indicator of the presence of tannins (Yadav and Agarwala, 2011).

Test for terpenoids (Salkowski test)

2ml of chloroform was added to 0.5g ofeach sample of the plant. Then, 3ml concentrated sulfuric acid was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids (Degu *et al*,. 2016)

Test for flavonoids

About 10 ml of ethyl acetate was added to 0.2g of each sample heated on water bath for 3minutes. The mixture was cooled and filtered. Then, about 4ml of the filtrate was shaken with 1ml of diluted ammonia solution. The layers were allowed to separate and the yellow color in the ammoniacal layer indicated the presence of flavonoids (Degu *et al*,. 2016)

Test for glycosides(keller-kilani test)

Solvent extract of 0.5g of each sample was dissolved in 2.0ml of glacial acetic acid containing one drop of ferric chloride ($FeCl_3$) solution. Each mixture was then under laid with 1.0ml of concentrated sulfuric acid. A brown ring obtained at the interface indicated the presence of glycosides (Pandith, 2012).

Test for phenols

0.5g of both extracts were treated with few drops of neutral ferric chloride solution 5%, intense color developed indicates the presence of phenols (Pandith, 2012).

Test for steroids

0.5g of both extracts were mixed with 2.0ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colorproduced in the lower chloroform layer indicated the presence of steroids (Yadav and Agarwala, 2011).

3.2.9. Statistical Analysis

The results of this study were expressed as mean ± standard error of the mean (SEM) and the experimental results were analyzed using the software Statistical Package for Social Sciences (SPSS), version 20 and statistical significance was determined by one way analysis of variance (ANOVA) followed by Tukey post Hoc test for multiple comparisons. The level of significance was also set at $P < 0.05$.

4. Result

4.1. Yield of the Extract

After drying or concentrating both aqueous and 80% methanol crude extracts, the yields were 20.34g (8.14%) and 33.76g (13.5%) respectively.

4.2. Acute Toxicity Test

In acute toxicity study, after oral administration of the test substances at the dose of 2g/kg body weight, rats were observed for two weeks to see if the extracts had toxic effect, all the rats were survived even after 14 days with progressive gaining in body weight. This indicates that both extracts were found to be safe up to a maximum dose level tested. This was evidenced by absence of major toxicity signs such as tremor, loss of weight, lethargy, paralysis, stress or adverse behaviors, no sign of diarrhea and none of the treated rats died during this period of study. This suggesting the LD50 is greater than 2g/kg**.**

4.3. Diuretic Activity

4.3.1. Effect on Urine Volume

Aqueous extract:

The effect of oral administration of aqueous crude extract of *C. dipsaceus* leaves on urinary output is shown in Table 1. All aqueous crude extract treated groups were showed increased in urinary volume excretion starting from the first hour and markedly increased at fifth hour as for AQ100(77.66%), AQ200(97.72%) and AQ400(120.94%). As showed in Table 1, AQ200 treated group had increased a significance difference at third hour (83.80%, $p<0.05$) and fifth hour (84.30%, $p<0.01$) in urinary excretion as compared to NC. In addition, as shown in Table 1, rats treated with AQ400 was produced an increased significant diuresis starting from the second hour (110.71%, $p < 0.01$) and continued to the fifth hour (132.26%, $p<0.001$) as compared to NC.

Furosemide treated rats produced markedly greater diuresis and showed a significant differences starting from the very beginning hour (316.67%, $p<0.05$) and continued until the end of the fifth hour (132.26%, $p<0.001$) as compared to NC. The standard drug SF10 had a significant diuretic effect than that of AQ100 ($p<0.05$) but had comparable

significant diuretic effect with AQ400 ($p < 0.001$) starting from third hour to the end of the fifth hour.

As shown in Table 1, the diuretic activity of AQ400 was 0.94 as compared to the standard drug SF10, this result also reveal their comparable diuretic effect; and had less diuretic activity of 0.60 and 0.76 for AQ100 and AQ200 respectively.

When the three doses of the aqueous extracts treated groups compared to each other, AQ400, produced greater diuresis which was significant at the end of the fifth hour ($p<0.01$) as compared to AQ100. In comparison of aqueous extract treated group with the standard, the SF10 was significantly increase ($p<0.01$) in diuresis starting from the second hour as compared to AQ100.

Table 1: Urinary output at different time intervals after oral administration of aqueous crude extract of *Cucumis dipsaceus* leaves in rats.

Groups	1hr	2hr	3hr	4hr	5hr	UE (V_o/V_i) *100%	Diuretic action	Diuretic activity
NC	0.78±0.19	2.80±0.0.21	3.58±0.0.31	4.20±0.40.33	4.65±0.0.28	58.71	1	-
SF10	3.25±0.61*	7.33±0.59***[b]	8.95±0.52***[b]	9.83±0.91***[a]	10.80±0.68***[b]	129.34	2.20	1
AQ100	2.33±0.57	4.05±0.45	5.20±0.39	6.32±0.71	6.78±0.62	77.66	1.32	0.60
AQ200	3.08±0.55	5.12±0.55	6.58±0.47*	7.57±0.68	8.57±0.72**	97.72	1.67	0.76
AQ400	2.73±0.33	5.90±0.52**	7.85±0.44***	9.67±0.54***	10.80±0.72***[d]	120.94	2.06	0.94

Note: Each value represent the mean ± SEM (n = 6). The mean difference is significant at the levels of:

- * $p < 0.05$ against NC; ** $p<0.01$ against NC; *** $p<0.001$ against NC; a: $p < 0.05$ against AQ100.
- b: $p<0.01$ against AQ100; d: p < 0. 01 against AQ100.
- UE = Urinary Excretion; Vo = Volume of urine output, Vi = Volume of liquid administered
- NC= Negative Control group receiving vehicle
- SF = Standard drug furosemide; SF10 = Furosemide 10mg/kg receiving group
- AQ = Aqueous extract; AQ100 = Aqueous extract 100mg/kg body weight receiving group; AQ200 = Aqueous extract 200mg/kg body weight receiving group; AQ400 = Aqueous extract 400mg/kg body weight receiving group

80% methanol extract:

As showed in Table 2, the crude 80% methanol extract increased urinary volume excretion almost as effective as that of aqueous extract in the same fashion as for HM100 (90.45%), HM200 (106.32%), and HM400 (115.79%) at fifth hour; but the onset of significant diuresis appeared less than that of the aqueous extract. The diuresis was significantly increased at third hour (82.12%, $p<0.05$) and fifth hour (88.17%, $p<0.01$) for HM200, as compared to NC. In addition, HM400, produced an increased significant diuresis starting from the third hour (94.13%, $p<0.01$) to the end of fifth hour (112.90%, $p<0.001$) as compared to NC. As the 80% methanol extract treated groups compared to that of standard, SF10, HM100 treated group at the third hour was significantly ($p<0.01$) produced a lower diuretic effect. However, the three doses showed an increasing diuretic activity of 0.70, 0.82 and 0.90 in increasing their dose, respectively.

Table 2: Urinary output at different time intervals after oral administration of 80% methanol crude extract of *Cucumis dipsaceus* leaves in rats.

Groups	1hr	2hr	3hr	4hr	5hr	UE $(V_o/V_i)*100\%$	Diuretic action	Diuretic activity
NC	0.78±0.19	2.80±0.0.21	3.58±0.0.31	4.20±0.40.33	4.65±0.0.28	58.71	1	-
SF10	3.25±0.61*	7.33±0.59***	8.95±0.52***[c]	9.83±0.91***	10.80±0.68***	129.34	2.20	1
HM100	2.52±0.53	5.15±0.62	5.75±0.73	7.13±0.77	7.58±0.83	90.45	1.54	0.70
HM200	2.78±0.77	5.18±0.71	6.52±0.80*	7.32±0.97	8.75±0.90**	106.32	1.81	0.82
HM400	2.68±0.50	5.13±0.0.51	6.95±0.83**	8.75±0.86**	9.90±0.88***	115.79	1.97	0.90

Notes: Each value represent the mean ± SEM (n = 6). The mean difference is significant at the levels of:

- * p<0.05 against NC; ** p<0.01 against NC; *** p<0.001 against NC
- c: p<0.01 against HM100; UE = Urinary excretion, Vo = Volume of urine output, Vi = Volume of liquid administered
- NC= Negative Control group receiving vehicle
- SF = Standard drug Furosemide; SF10 = Furosemide 10mg/kg receiving group
- HM =80% methanol extract; HM100 = 80% methanol extract 100mg/kg receiving group; HM200 = 80% methanol extract 200mg/kg receiving group; HM400 = 80% methanol extract 400mg/kg receiving group

4.3.2. Effect on Electrolyte

Aqueous extract:

As showed in Table 3, the urine samples collected over the five hours were analyzed for the electrolyte content of Na^+, K^+, and Cl^-. AQ100 in sodium loss was equal to that of the NC, but AQ200 was increased by 55.10% as compared to NC. AQ400 showed an increased significance difference in urinary sodium loss (105.10%; $p<0.05$) as compared to NC. The standard drug treated group (SF10) was increased significantly (122.45%; $p<0.01$) and found the highest urinary Na^+ loss as compared to NC.

Urinary K^+ excretion for AQ400 had shown statistically significant kaliuresis (210.10%; $p<0.01$) effect as compared to NC. However, AQ100 and AQ200 were increased in potassium loss by the percentage of 59.58% and 76.50% respectively as compared to NC. The standard drug treated group also showed an increase in potassium loss by 41.62% as compared to NC and it found the lowest effect in potassium urinary excretion as compared to aqueous extract treated groups.

In case of urinary Cl^- losses, AQ100 and AQ200 increased by 36.68% and 85.66%, respectively and AQ400 significantly increased by 177.30% ($p<0.01$) as compared to NC. SF10 was showed a significant increase in chloride loss by 225.33 % ($p<0.001$) as compared to NC and found the highest losses as compared to aqueous extract treated groups.

As showed in table 3, the saliuretic activity were found as 16.90%, 94.44% and 138.36% for lowest, medium and highest dose compared to NC, respectively. The aqueous extract at AQ400 (138.36%, $p<0.01$) and the SF10 (169.84%, $p<0.001$) treated groups were significantly increased in salt urinary excretion as compared to the NC. The saliuretic index of Na^+ (1, 00, 1.55 and 2.05), K^+ (1.60, 1.77 and 3.10) and Cl^-(1.37, 1.86 and 2.77) found for the doses of AQ100, AQ200 and AQ400 extract treated groups, respectively. While the saliuretic index of SF10 for Na^+, K^+ and Cl^- found as 2.22, 1.42 and 3.25 respectively as compared to NC.

In comparison of urinary electrolyte excretion between the aqueous extract and standard drug treated groups, there was a significance increase in Na^+ for AQ400 ($p<0.05$), and in

Na^+ and Cl^- for SF10 ($p<0.001$) were showed as compared to AQ100. Similarly, AQ400 treated group also showed a significant increase ($p<0.05$) in K^+ urinary excretion as compared to AQ100 and SF10 treated groups.

In addition, the Na^+/K^+ ratio (Natriuretic activity) of aqueous extract treated groups were found the least (0.58) at AQ100 and highest (0.77) at AQ200, but natriuretic activity of all aqueous extract treated groups were found less than SF10 (1.29). The standard drug treated group and aqueous extract treated groups were not showed any significant differences in Na^+/K^+ ratio as compared to the NC. The CI^-/ (Na^+ + K^+) was also calculated and all groups treated by different doses of extract were comparable to each other (0.40, 0.41 & 0.41 in increasing dose, respectively), but all ion quotients were found less than SF10 (0.69).

Table 3: Effect of aqueous crude extract of *Cucumis dipsaceus* leaf on 5h urinary electrolyte excretion and other parameters in rats

Treatment	Dose (mg/kg)	Urinary electrolyte excretion			Saliuretic Index			$Na^+ + Cl^-$	Na^+/K^+	$Cl^-/(Na^+ + K^+)$
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-			
NC	20ml/kg	19.60±1.23	24.17±2.85	16.74±2.37				36.34±3.33	0.86±0.12	0.38±0.04
SF	10	43.60±5.53**[a]	34.23±1.83	54.46±4.73***[a]	2.22	1.42	3.25	98.06±10.06***[a]	1.29±0.19[g]	0.69±0.02***[h]
AQ	100	19.60±2.37	38.57±7.56	22.88±3.75	1.00	1.60	1.37	42.48±5.25	0.58±0.10	0.40±0.02
	200	30.40±3.75	42.66±8.21	31.08±7.66	1.55	1.77	1.86	61.48±11.33	0.77±0.09	0.41±0.03
	400	40.20±4.99*[b]	74.95±13.27**[c]	46.42±3.88**	2.05	3.10	2.77	86.62±7.38**[d]	0.63±0.15	0.41±0.03

Notes: Each value represent the mean ± SEM, n = 6, * $p< 0.05$ against NC; ** $p< 0.01$ against NC; *** $p<0.001$ against NC;

- a: $p<0.01$ against AQ100; b: $p < 0.05$ against AQ100; c: $p<0.05$ against SF10 and AQ100; d: $p<0.05$ against AQ100; g: $p< 0.01$ against AQ100 & AQ400; h: $p<0.001$ against AQ100 & AQ400
- NC= Negative Control group received vehicle; SF = Standard drug furosemide; AQ = Aqueous extract.
- Na^+/K^+: Ratio of concentration of sodium ion in the urine of the group to concentration of potassium ion in the urine of the same group; $Cl^-/(Na^+ + K^+)$(Ion quotient): Is derived to estimate Carbonic anhydrase inhibition; $Na^+ + Cl^-$: Estimate saliuretic activity.

The 80% methanol extract:

As showed in table 4, the urinary Na^+ excretion was observed increasing in respective of increasing dose of the extract as 9.18%, 46.94% and 57.14% for HM100, HM200 and HM400, respectively, and all doses not showed significance difference in Na^+ urinary excretion as compared to NC. In case of the K^+ urinary excretion, HM100 increased by 88.37% and it was significantly increased at HM200 (179.07%, $p<0.01$) and HM400 (184.24%, $p<0.01$), as compared to NC. In case of CI^- urinary excretion, HM100 was increased by 85.78% and HM200 and HM400 were increased significantly by 158.66% ($p<0.05$) and 202.63% ($p<0.01$) respectively, as compared to NC.

In comparison of urinary electrolyte excretion between 80% methanol extract and SF10 treated groups; the SF10 treated group was showed significantly increase in urinary Na^+ excretion as compared to HM100 ($p<0.01$). In case of K^+ and CI^-, there was no any significant difference between the standard and the three different doses of the 80% methanol extract. The three doses of 80% methanol extract treated groups were also not showed significance difference in urinary electrolyte loss.

As showed in Table 4, the saliuretic activity increased by 44.47% and 98.40% for HM100 and HM200, respectively. It was significantly increased for HM400 (124.16%; $p<0.01$) and SF10 (169.84%; $p<0.001$) treated groups as compared to NC. Saliuretic indices had also been calculated and results were obtained as for Na^+ (1.09, 1.47, and 1.56), K^+ (1.88, 2.79 and 2.84) and CI^-(1.86, 2.57 and 3.03) for the lowest, medium and highest doses, respectively as compared to NC. The quotient of Na^+/K^+, natriuretic activity, was calculated and values of 0.54, 0.45 and 0.46 were obtained for HM100, HM200 and HM400 respectively and the greatest result was showed for SF10 (1.29). Similarly, the value of $[CI^-] / [Na^+ + K^+]$ had been also calculated and results of 0.44, 0.45 and 0.52 were obtained for HM100, HM200 and HM400 respectively. These results were found less than that of SF10 (0.69).

Table 4: Effect of 80% methanol crude extract of *Cucumis dipsaceus* leaf on 5h urinary electrolyte excretion and other parameters in rats

Treatment	Dose (mg/kg)	Urinary electrolyte excretion			Saliuretic Index			$Na^+ + Cl^-$	Na^+/K^+	$Cl^-/(Na^+ + K^+)$
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-			
NC	20ml/kg	19.60±1.23	24.17±2.85	16.74±2.37				36.34±3.33	0.86±0.12	0.38±0.04
SF	10	43.60±5.53***[a]	34.23±1.83	54.46±4.73***	2.82	1.42	3.25	98.06±10.06***[a]	1.29±0.19[g]	0.69±0.02***[hij]
HM	100	21.40±1.81	45.53±10.01	31.10±8.09	1.09	1.88	1.86	52.50±9.62	0.54±0.88	0.44±0.04
	200	28.80±4.09	67.45±5.75**	43.30±2.83*	1.47	2.79	2.57	72.10±5.02	0.45±0.09	0.45±0.04
	400	30.80.00±4.38	68.70 ±5.25**	50.66±4.91**[b]	1.56	2.84	3.03	81.46±9.07**[e]	0.46±0.07	0.52±0.05

Notes: Each value represent the mean ± SEM, n = 6,

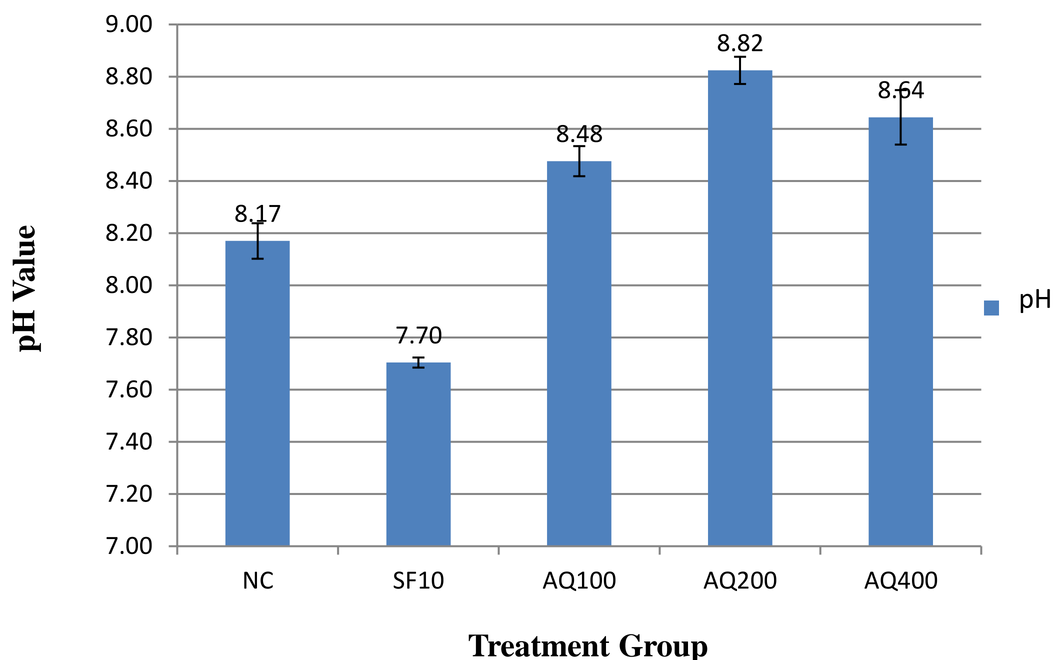
- * $p<0.05$ against NC; ** $p< 0.01$ against NC; *** $p<0.001$ against NC
- a: $p<0.01$ against HM100; b: $p < 0.05$ against AQ100; e: $p<0.05$ against AQ100. g: $p< 0.01$ against HM100, HM200 & HM400. h: $p<0.001$ against HM100;i: $P<0.01$ against HM200; j: $p< 0.05$ against HM400
- NC= Negative Control group received vehicle; SF = Standard drug furosemide; HM Hydro methanol extract.
- Saliuretic index: Is the ratio of concentration of electrolyte in the urine of test group to concentration of electrolyte in the urine of control group
- Na^+/K^+:- Ratio of concentration of sodium ion in the urine of the group to concentration of potassium ion in the urine of the same group. Estimate Natriuretic activity.
- $Cl^-/(Na^+ + K^+)$(Ion quotient): Is derived to estimate Carbonic anhydrase inhibition; $Na^+ + Cl^-$: Estimate saliuretic activity

In comparison between both extracts on urinary electrolyte excretion, the highest aqueous, AQ400, treated group showed significantly increase in Na^+ ($p<0.05$) as compared to HM100 treated group. On the other hand, the highest dose, HM400, treated group was significantly ($p<0.05$) increased in urinary excretion of Cl^- and saliuretic activity as compared to AQ100 treated group.

4.3.3. Effects on pH

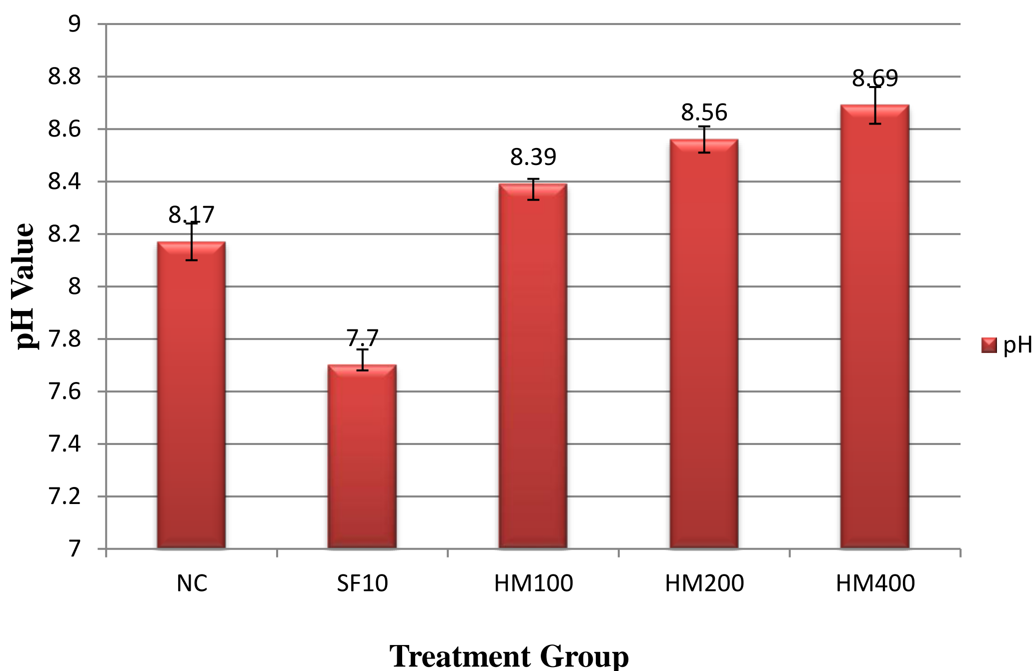
Urinary pH measurement revealed that the different treatment groups of both aqueous and 80% methanol extracts had produced relatively alkaline urine (Fig. 3 and Fig. 4). The five hour mean ± SEM urinary pH were found, 8.48 ± 0.06, 8.82 ± 0.05 and 8.64 ±0.10, for aqueous extract and 8.39±0.06, 8.56±0.05 and 8.69±0.07 for 80% methanol extract of urine sample, at doses of 100, 200 and 400 mg/kg respectively. In addition, the negative control and standard drug treated groups were found 8.17±0.08 and 7.70 ± 0.02 respectively over the five-hour urine collection. None of the treated groups showed any significance differences on the effect of urinary pH, as compared to NC. As it was shown from figure 3, the maximal pH value was produced by AQ200 and the least pH was produced by SF10 which making the urine less alkaline (Fig. 3 and 4).

Figure 3: Urinary pH of rats treated with aqueous crude extract of *C. dipsaceus* leaves



Notes: Each value represent the mean ± SEM, n = 6, NC= Negative Control group receiving vehicle; SF = Standard drug furosemide; SF10 = Furosemide 10mg/kg received group; AQ = Aqueous extract; AQ100 = Aqueous extract 100mg/kg body weight received group; AQ200 = Aqueous extract 200mg/kg body weight received group; AQ400 = Aqueous extract 400mg/kg body weight received group.

Figure 4: Urinary pH of rats treated with 80% methanol crude extract of *C. dipsaceus* leaves



Notes: Each value represent the mean ± SEM, n = 6, NC = Negative Control receiving vehicle, HM = 80% methanol extract; HM100 = 80% methanol extract 100mg/kg received group; HM200 = Hydro methanol extract 200mg/kg received group; HM400 = Hydro methanol extract 400mg/kg received group.

4.4. Electrolyte Content of the Extracts

The electrolyte content of both aqueous and 80% methanol extract had been investigated. The result of aqueous extract showed as 0.02mg of Na^+, 0.13mg of K^+, and 0.06mg of Cl^- contents were obtained per mg of the extract. Likewise, in case of the 80% methanol extract, showed as 0.02mg of Na^+, 0.14mg of K+ and 0.09mg of Cl- contents were obtained per mg of the extract. The Na+ content in both extracts were almost equivalent, but the K^+ and Cl^- contents were higher in case of 80% methanol extracts.

4.5. Phytochemical Test

The *C. dipsaceus* **leaf extract** was subjected to qualitative phytochemical tests to identify the presence or absence of phyto-constituents as showed in table 5 for saponins, tannins, flavonoids, terpenoids and cardiac glycosides.

Table 5:- Phytochemical screenings of aqueous and hydromethanol extract of *C. dipsaceus* leaf

Test for	Aqueous extract	Hydro-methanol extract
Saponin	+	+
Tannin	+	+
Alkaloids	+	+
Terpenoid	+	+
Flavonoid	+	+
Cardiac glycosides	+	+
Steroid	-	-

Key: + = present, - = absent

5. Discussion

Medicinal plants offer a natural defense against diseases and are substantial treatment for certain diseases. Numerous mono- and poly-herbal preparations are used as diuretics. There are also a number of studies that have been carried out to support the diuretic effects of many traditional herbal medicines (Dutta *et al.*, 2014). Diuresis has two components, increase in urine volume and a net loss of electrolytes in the urine (Jackson, 2006). In the present study, therefore, both the urinary volume and electrolyte excretion were measured to evaluate the diuretic activity of the plant extracts. It revealed that both aqueous and 80% methanol extract of *C. dipsaceus* leaf increase the urinary volume output and electrolyte loss as compared to the negative control. Of the two crude extracts, the aqueous extract produced a better dieresis. The minimum dose of both extracts did not show any significant diuretic activity which could be due to lack of strength. The diuretic effect of these two crude extracts appeared maximum at their highest doses. However, the maximum dose of aqueous extract resulted in slightly higher urine volume than the corresponding dose of 80% methanol extract. As a result, it is persuasive to suggest that the component(s) responsible for the diuretic effect of the plant could probably be more of polar and hence better extracted in aqueous solvent.

The highest doses of aqueous and 80% methanol crude extract produced values of diuretic activity 0.94 and 0.90, respectively. These values are closer to 1.0 of the standard drug, which indicates that the active component(s) of the plant could produce diuresis that is comparable to that of currently available clinically useful synthetic diuretics.

The diuretic activity numerical results are classified as follows: Diuretic activity is considered to be high if it is greater than or equal to 0.90; moderated if it is between 0.89 - 0.70, low if it is between 0.69 - 0.50; and null if it is less than 0.50 (Perez et al., 2011). Therefore, based on the result showed in Table1 and 2, the diuretic activity of *C. dipsaceus* leaves extracts were considered as high for AQ400 (0.94) and HM400 (0.90); as moderate for AQ200 (0.76), HM100 (0.70) and HM200 (0.82); and as low for AQ100 (0.60). Therefore, these results indicate both aqueous and 80% methanol leaves crud extracts of the plant has diuretic activity in rat.

The amount of urinary sodium, potassium and chloride were measured from the five hour collected urine, as shown in Table 3 and 4. The increases in diuresis induced by AQ and HM extract treated also reflected in similar manners with urinary ionic excretion. The AQ400 significantly increased in Na+, K+ and Cl- urinary loss and HM200 and HM400 significantly increased in K+ and Cl- urinary loss as compared to NC. The positive control group; excretion of Na+ and Cl- were significantly increased as compared to the NC and AQ100; and Na+ urinary excretion also significantly increased as compared to HM100. The effect of the extracts on water excretion was appeared to be accompanied by an increased in urinary electrolyte / salt excretion effect as compared to the NC, which supports the idea that the diuretic effect of C. dipsaceus was of the saliuretic type in contrast to aquaretic type that is feature of most diuretic agents (Martin-Herrera et al., 2007).

The Na^+/K^+ ratio can predict the nature of the diuretic mechanism (Toma *et al*., 2015). A Na^+/K^+ ratio of greater than one indicates a satisfactory diuresis without excessive urinary potassium loss (Alexander *et al*., 1977). Values greater than 2.0 indicate a favorable natriuretic effect, if the ratio exceeds 10.0, it would have potassium-sparing effect (Vogel, 2007). The ratio of Na^+/K^+ was calculated as an indicator of natriuretic activity and this observation suggests that the plant material has no potassium sparing activity.

The $Cl^-/ (Na^+ +K^+)$ ratio was calculated and showed the extent of CA inhibitory effect. CA inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios slight to strong CAI can be assumed (Vogel, 2007). Thus, the present study indicates that AQ might have strongest CA inhibitory effect with values of 0.40, 0.41 and 0.41 for the lowest, medium and highest aqueous extract treated doses, respectively; but these result in case of 80% methanol were found greater values as 0.44, 0.45 and 0.52 in increasing doses, respectively. Still the maximum doses produced the highest diuresis, even though the lowest dose had the lowest $CI^-/Na^++ K^+$ ratio for aqueous and 80% methanol extracts, thus there ought to be another mode of action which manifested at the higher doses. In determination of the urinary pH, the extracts showed a relative increased in the pH values as compared to the controls, so this strengthens that CA inhibition as one of the mechanisms of action of the plant.

One possibility for the observed diuretic properties could be due to direct action of K^+ content of *C. dipsaceus* leaves extracts caused by highest potassium ion content (Jouad *et al*., 2001). An increment of urinary output in rats might result from high potassium content in the plant infusion (Nilveses *et al*., 1989). Potassium overloading, which occurs when the kidney tubules are incapable of absorbing it, produces urinary excretion of the osmotic type (Kanias *et al*., 1979). Quantitative determinations of the electrolytes present in the AQ and HM of *Cucumis dipsaceus* revealed the presence of high amount of K^+. The leaves of *C. dipsaceus* were also analyzed and quantified, for the presence of important macro- and micronutrients, the results shows the leaf sample is found to have N, K, Na, Ca, P, and Fe in a well appreciable amount (Chandran *et al*.; 2013). This suggests that diuretic activity of the extract might seem to be an osmotic type, as K^+ content of the extract was high to account for the diuretic activity. It was confirmed that the diuretic action of most active fraction should not be attributed exclusively to the presence of their potassium content but also to other constituents (Kanias *et al*., 1979). Plant extracts may be inhibiting potassium absorption or stimulating potassium secretion, or both, leading, in either case, to more potassium retention in the lumen of the kidney tubules and osmotic water flow (Kreydiyyeh and Usta, 2002).

According to previous study, as the leaves of *C. dipsaceous* extract were analyzed and quantified it possess carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, tannins, flavonoids, cardiac glycosides, phytosterols and fixed oils and fats and confirmed that it has an antioxidant activity (Chandran *et al*.; 2013; Lata and Mittal, 2015; Pariya and Anusuba, 2018). The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant, although it is usually not attributed to a single compound but a combination of the metabolites. The results revealed that the plant has potential phytochemicals with important biological activities. These phytochemicals also indicate the richness medicinal value in leaf (Chandran *et al*.; 2013). The AQ and HM appears to have multiple mode of diuretic action, which could be due to the presence of several secondary metabolites in the plant extract that act synergistically or antagonistically to produce a resultant effect. Multiple mode of diuretic action is reported with some herbal medications (Wright *et al*., 2007).

Previous studies confirmed that there are several compounds which could be responsible for the plants diuretic effects in different plant extracts such as flavonoids, saponins,

organic acids/ ascorbic acid, carbohydrates, phenolic compounds, terpenoids/triterpenes , alkaloids, glycosides, sterols, sesquiterpenes/or lactones, amino acids and carotinoids (Sayana *et al*., 2014). Therefore, it is possible to suppose that the identified natural compounds in the crude extracts of *C. dipsaceus* might be responsible for the observed diuretic activity. The compounds may act individually or synergistically promoting an initial vasodilatation (Stanic and Samarzija 1993) thereby increasing renal blood flow (Martin-Herrera *et al*., 2008) or by other mechanism of action that enhances fluid and electrolyte flow out of the body.

6. Conclusions

The present study showed that both the aqueous and 80% methanol crude extracts of *C. dispaceus* leaves provide evidence as a diuretic agent through enhancement of salt and water excretion, with the aqueous extract being slightly more effective. The diuretic activity of the plant seemed mainly due to polar component(s) as the activity appeared more in aqueous extracted solvent. Thus, secondary metabolites that are moderately polar to more polar in nature could either individually or synergistically act by multiple mechanisms to produce the observed effect. From the data of electrolyte analysis and urinary pH, it is plausible to assume that the plant could have multiple mode of action. The larger doses of both the crude aqueous and 80% methanol leaf extracts produced a remarkable diuresis, which was comparable to Furosemide. The safe nature of the plant in addition to the evidenced diuretic effect from both extracts in the present study provides further support to conclude the etheno-medicinal use of *C. dipsaceus* as a diuretic agent.

7. Recommendations

- Further investigation is required to isolate the active ingredients responsible for the diuresis activity.
- It need further experiment to decide the precise site of action and mechanism by which the extracts of *C. dipsaceus* cause diuresis
- As diuretics are associated with electrolyte imbalances and other serious side effects, so, it needs the study of sub chronic and chronic toxicity profile of the plant, in order to verify its safety in long-term use.

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